ANNALS OF BOTANY

EDITED BY

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ASSISTED BY OTHER BOTANISTS

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Some Remarks on the Anatomy of the Osmundaceae.

BY

D. T. GWYNNE-VAUGHAN, M.A., F.R.S.E., F.L.S.

Professor of Botany in Queen's University, Belfast.

With Plate XLIV and five Diagrams in the Text.

THE investigations that have given rise to this account were begun with the intent of finding out whether any of the primitive features exhibited by certain fossil Fern stems assigned to the Osmundaceae by Dr. Kidston and myself[1] were still retained in the young plants of the existing representatives of the order. Before they were completed, however, a full description of the anatomy of *Osmunda cinnamomea* was published by Professor J. H. Faull,[2] with whose observations my own agree very closely. A short account of the facts in *Osmunda regalis* may still be justified, because I place an entirely different interpretation upon them to that advanced by Professor Faull, and in any case confirmation in a different species is not without value.

My observations were chiefly made upon serial microtome sections of sporelings of *Osmunda regalis*. A few sporelings of *O. palustris* and of a *Todea* were also examined, but without materially affecting the results.

The stem of the young sporeling curves round at once from its point of attachment to the prothallus into an obliquely erect position. The first leaf constantly departs from a point directly opposite to the foot, and the protoxylems of the diarch xylem of the first root lie in the plane running through the foot and the first leaf. The second leaf arises opposite to the first, but the subsequent leaves are arranged radially. In *Osmunda cinnamomea*, Faull found that the two-ranked arrangement is continued until the fifth or eighth leaf. He also discovered a cortical mycorhiza in the first roots of this plant which is not present in those of *O. regalis*.

Attention was especially directed to the effect of the departure of the

[1] Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae. Part I, Trans. Roy. Soc. Edin., vol. xlv, 1907, p. 759. Parts II and III, vol. xlvi, 1908–9, p. 213, and p. 651. Part IV, vol. xlvii, 1910, p. 455.

[2] Faull: The Stele of *Osmunda cinnamomea*. Trans. Canadian Institute, vol. viii, 1909, p. 515.

earlier leaf-traces upon the stele of the stem. It would, however, be unnecessary to give a detailed description of particular cases, owing to the close similarity with those described by Faull in *Osmunda cinnamomea*. An attempt was made to put together a generalized account, but it proved to be very difficult and unsatisfactory because the individual sporelings exhibit great diversity in their structure. In order to get over this difficulty, and at the same time to attain both brevity and clearness, a few diagrams have been constructed representing median longitudinal sections of characteristic cases.[1] In the diagrams the leaves are all supposed to arise in two opposite rows, but since they are really arranged radially it must be understood that the diagrams do not express all the facts as seen in the series of transverse sections from which they were constructed. The diagrams are not generalized conceptions, but each one is intended to represent at a glance, and as faithfully as possible, the nature and sequence of the events in a particular sporeling. If diagrams on the same plan be made from Professor Faull's descriptions, comparison with his results is greatly facilitated.

In every sporeling the first two leaf-traces departed in a perfectly protostelic manner, i.e. without leaving any depression in the xylem of the stem (Pl. XLIV, Figs. 1 and 2). The third leaf is nearly always protostelic, the fourth very often, and sometimes even the fifth (cf. the diagrams). Sooner or later, and sometimes as early as at the third leaf, the parenchyma of the xylem-sheath encroaches upon the xylem of the stem in the axil of the departing leaf-trace. In this way a groove or bay of parenchyma is formed which is prolonged downwards below the level of the departure to form a pocket (Fig. 3). The first parenchyma to appear in the solid xylem of the stem is often due to the formation of such a pocket (Diagrams 1 and 2). Just as often, however, the first parenchyma to appear is a true pith quite independent of the departure of the leaf-trace next above (Diagrams 3 and 4). It is continued past one or more leaf-trace departures without coming into relation with them even if they already have a pocket (Diagram 4). Cases also occur in which a pocket and a pith appear practically simultaneously (Diagram 5 and Fig. 3).

After the fifth or sixth leaf-trace the pith opens out into the xylem-sheath bay above the axils of every trace forming the so-called medullary rays. Nevertheless, the pocket in the peripheral xylem is very often still formed, and is quite independent of the pith, from which it is separated by a flange of tracheae (Fig. 4). Sometimes this flange is short and the pocket opens out into the pith below the level of the leaf-trace. More often it reaches up to the level of the leaf-trace departure, and the pocket opens out into the medullary ray. Sometimes, again, it is prolonged for some distance above this level, and the pocket opens out into the xylem-sheath (cf. the diagrams).

[1] In the diagrams the dotted area represents phloem and pericycle, the black xylem, and the white parenchyma.

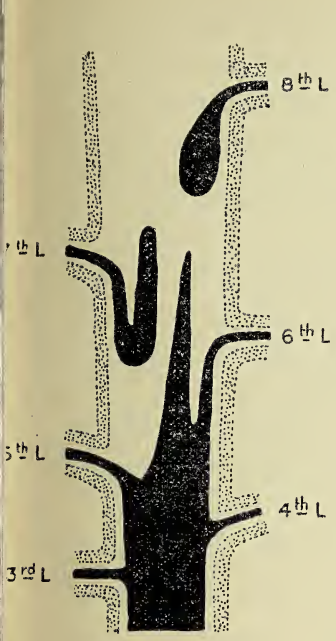


Diagram 1

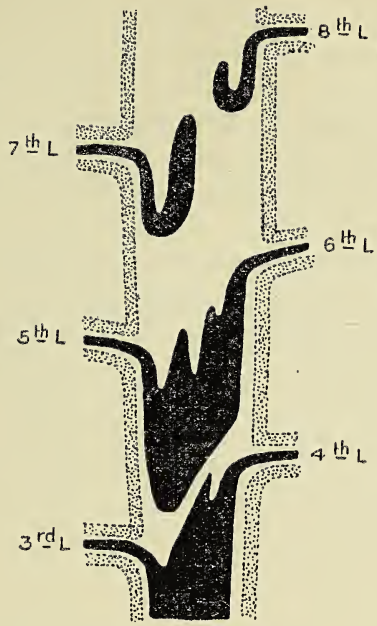


Diagram 2

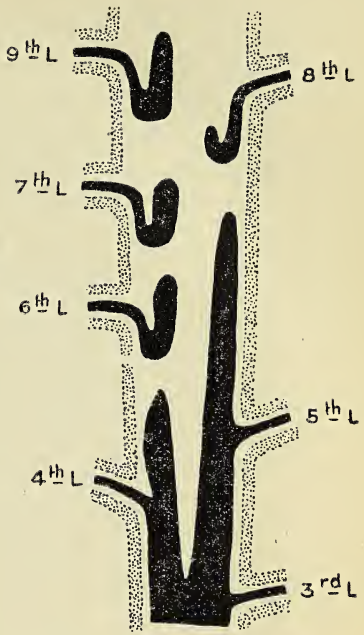


Diagram 3

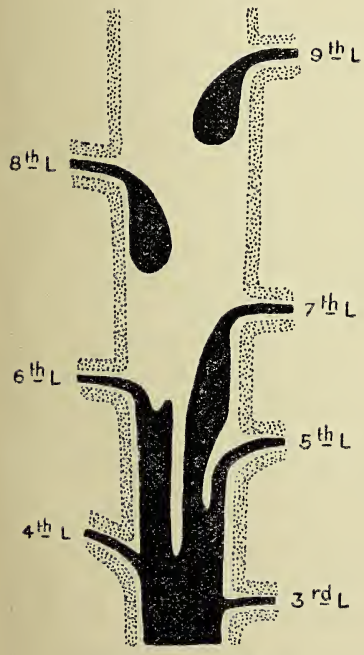


Diagram 4

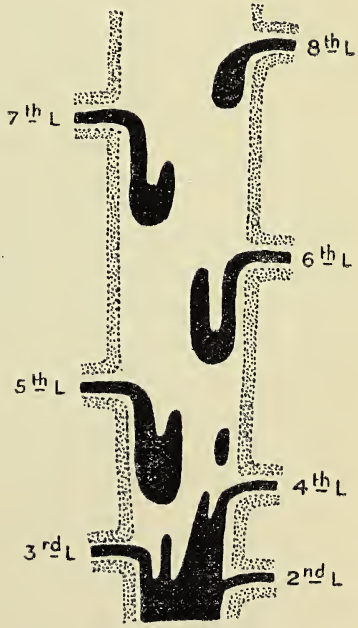


Diagram 5

Once formed the pith rarely disappears again. In two cases, however, the xylem became solid once more above the level of the seventh leaf. This seems to happen more frequently in *Osmunda cinnamomea*. Indeed, in this plant the transitional region of the sporeling seems to be much more drawn out and extended than in *O. regalis*, for Faull finds protostelic leaf-trace departures as high up as the seventh or eighth leaf, and he says that a permanent pith is not attained until about the level of the twentieth.

Detailed Structure of the Sporeling.

In both *Osmunda* and *Todea* the foot of the sporeling forms a massive protuberance and is prolonged laterally into two wings which are wrapped round the stem like stipules. In sporelings of some age the cells of the central tissue of the foot become quite thick-walled. The endodermis of the stele usually shows a slight projection on the side towards the foot.

The amount of xylem in the stele varies considerably with the strength and vigour of the individual sporeling. Just below the first leaf it consists on an average of about eighteen tracheae, below the second about 20–30 are present, and below the third it may contain as many as forty. The largest mass of solid xylem without any trace of parenchyma amounted to fifty tracheae. The xylem-sheath is relatively stout in the Osmundaceae as an order, and it is already well developed in the sporeling, being from two to four cells wide in the protostelic region. The phloem is well formed from the first and consists of one, rarely two, rows of rather large sieve tubes. No porose layers were found in the earlier formed regions of the stem. The pericycle is at first a single layer which is sometimes interrupted so that a sieve tube may be in direct contact with the endodermis. The endodermis is clear and distinct from the very first, but it is not differentiated beyond the simplest type of endodermis; that described by Kraemer as a 'primary endodermis'.[1] It is interesting to note that according to Rumpf[2] and Bäsecke[3] the endodermis remains in the primary condition in this order, in the Ophioglossaceae and Marattiaceae, while in the other Filicales it differentiates into a more advanced type of structure. Caspary's band is usually well formed, but is sometimes badly delimited, extending also over the tangential walls. It does not appear to be cutinized or suberized, but gives all the reactions usually associated with the presence of lignin. The contents of the endodermal cells take up certain stains such as methylene blue and iodine green with especial avidity, apparently owing to the

[1] Karl Kraemer: Wurzelhaut, Hypodermis und Endodermis der Angiospermwurzel. Bibliotheca Botanica, 1903, Heft 59, p. 87.

[2] Georg Rumpf: Rhizodermis, Hypodermis und Endodermis der Farnwurzel. Ibid. 1904, Heft 62, p. 25.

[3] Paul Bäsecke: Beit. z. Kennt. der physiologischen Scheiden der Filicineen-Achsen, &c., p. 12. Inaug. Dissert., Marburg, 1908.

presence of some tannoid substance which acts as a mordant, for they are coloured blue by cupric acetate and reddish-brown by bichromate of potash.

Cells containing tannoid substances, and in consequence staining exactly the same as those of the endodermis, also occur in the very earliest formed pith, and at first sight might easily be taken as endodermal cells (Pl. XLIV, Fig. 5). They are scattered about irregularly and are also found in the medullary rays and in the xylem-sheath, both in the axil of the leaf-trace and for some distance out into the free petiole. In the later formed regions of the sporeling these cells are no longer present. The earliest formed cells of the pith are considerably elongated, almost as long as the tracheae, but above they soon become approximately isodiametric. The isodiametric cells, however, give no indication of having been derived from the septation of elongated cells in the apical meristem. No especially short tracheae were found towards the centre of the xylem, such as might have been expected from *Thamnopteris* and *Zalesskya*, either before or after the appearance of a pith. The cortical tissue of the stem is all more or less sclerotic below the first leaf. Above this level it consists of 4–7 layers of heavily sclerotic outer cortex surrounding a single layer of thin-walled inner cortex.

Structure of the Leaf-trace.

The size of the earlier leaf-traces varies so much from one seedling to another that the following must be regarded as an average account only. The xylem of the first leaf-traces is always very scanty and often no definite protoxylem could be distinguished. Probably the tracheae differentiated all simultaneously or else quite irregularly. Whenever a definite protoxylem could be determined in these small traces it was usually endarch. In a number of traces, however, the protoxylem was unmistakably mesarch (Fig. 5–8). In all, thirteen undoubtedly mesarch traces were found which belonged to seven different sporelings. Several other traces also showed a strong tendency to mesarchy. The majority of the mesarch traces belonged to leaves lying between the third and the sixth, two belonged to the second leaf and one to the eighth. Most of the mesarch traces were fairly large, containing from fourteen to twenty tracheae, but two contained only eight tracheae. All the mesarch traces eventually became endarch if followed out far enough into the free petiole (Fig. 9). The mesarch condition sometimes continued all the way from the stele far out into the stipular leaf-base, or it was only present while passing through the cortex of the stem, or sometimes only in the basal region of the stipule. The mesarch traces are more or less oval in transverse section, and it should be noted that the departure of such a leaf-trace does not leave a pocket in the xylem of the stem because it is not hollowed out adaxially at the point of its departure.

All the earliest leaves, even the very first, have fully developed stipules, and in the upper region of the stipule of the later leaves (from about the seventh or eighth) a conspicuous patch of protophloem is to be seen at each side of the leaf-trace on its abaxial surface. Three groups of mucilage sacs also appear in the same region of the stipule; one just outside each of the above-mentioned protophloems and one median in the adaxial concavity of the trace. These features are characteristic of the stipular base of mature leaves, but they do not appear to occur in any part of the stipule of leaves below the sixth. The special tannin-containing cells that occur in the xylem-sheath of the leaf-trace in the base of the stipule of the early leaves have already been mentioned. It is to be noted that the xylem-sheath of the petiole of the fossil *Bathypteris rhomboidea* contains scattered sclerotic elements (Kidston and Gwynne-Vaughan, Part IV, Fig. 53).

Discussion.

The study of the sporelings of *Osmunda regalis* shows that in addition to and independent of the pithing of the stele, there is also a pocketing of the xylem-sheath in the axils of the leaves into the peripheral region of the xylem-ring. These xylem-sheath pockets account for the formation of the leaf-gaps or medullary rays in the originally continuous ring of xylem. In fact they break through the ring to meet the pith. In our paper on the Fossil Osmundaceae, Dr. Kidston and I did not fully appreciate the importance of the axillary pockets in the formation of the medullary rays. We regarded the medullary rays rather as directly due to the removal of the elements of the xylem-ring by their passage outwards into the departing trace, and we looked upon the pockets more as subsequent downward prolongations of the medullary rays into the xylem of the ring. I now regard the formation of xylem-sheath pockets as the initial cause of the medullary rays.

The interpretation of the vascular structure of the sporeling as indicating the succession of the stages passed through in the phylogeny of the race is by no means a simple straightforward matter, but is one that requires the greatest caution in its treatment. It seems to me that each new modification in a series of progressive changes in the evolution of any vascular system would appear first of all in the mature regions of the fully adult plant only, as a new and an additional stage in its ontogeny. If it may be held that the longer any given modification has been in existence in the race the earlier it will appear in the ontogeny of the individual, it follows, theoretically, that it will eventually appear as the earliest stage of the sporeling. This is only possible, however, if the stele of the sporeling is able to become bulky enough to express the modification in question. If it is not, there must be a lower limit to the downward penetration of the modification into the sporeling stem which will be determined by the minimum number of

elements necessary for its physiologically effective construction. Below this limit the preceding stages will continue to exist undisturbed. If now a still later modification should arise requiring no more elements for its expression than the one immediately preceding it, it will eventually catch the latter up at its lower limit of penetration. There the two modifications will be superposed and may coexist, or, if their coexistence at the same level in the stem is impossible, the later modification will replace the earlier and eliminate it from the ontogeny altogether.

A series of modifications will appear in orderly succession in the sporeling stem only when the lapse of time has not yet been great enough for the successive stages to have caught each other up, or when each stage requires a successively increasing number of elements for its effective expression. Otherwise, in course of time the ontogeny will become telescoped and the sporeling will begin with the latest modification. In the case of a new modification that has caught up and overlapped its predecessor, the structure of the sporeling stele in this region will depend upon the effect of the superposition of the new modification upon the older one. This will vary according to the nature of the two modifications. They may be able to coexist without serious interference with each other at the same level in the stem, or the later one may so affect the earlier that it is no longer recognizable as such. Again, it is conceivable that a new modification should require even fewer elements for its expression than its predecessor. In this case it might pass by its predecessor and appear at a lower level in the sporeling than the really more primitive stage that phylogenetically preceded it, provided the latter is not deleted by the overlapping. All these and other factors still more obscure related to the special conditions of existence of the sporeling itself must, I believe, be taken into account when considering the phylogeny of the Osmundaceae in the light of the vascular structure of the sporeling. On these grounds the early appearance of the xylem-sheath pockets in the sporeling of *Osmunda regalis* is in no way incompatible with the true intrastelar nature of the pith. It seems clear that they are two entirely independent phenomena.

From this point of view also the criticism of the reduction theory put forward by Dr. Kidston and myself on the grounds of lack of sporeling evidence is not altogether valid. The term 'reduction' is taken to include simplification in structure consequent upon decrease in size, and it now seems to me that a sporeling could only be expected to provide evidence of such a reduction upon the supposition that it had an effect upon every stage in the plant's life. It is conceivable, however, that the reduction should only affect the later more complex stages in the development of a plant in such a manner that they are no longer formed. Such a reduction would be without influence upon the sporeling, which would still repeat the ascending series of changes up to the latest stage of reduction.

From the above considerations, again, it is evident that the structure of the sporeling will not conclusively decide whether the pockets or the pith were the first to appear in the phylogeny of the Osmundaceae. Some facts bearing upon this question may be obtained from the fossil representatives of the order. In the first place *Thamnopteris* and *Zalesskya*, which have a solid xylem with a central mass of short thin-walled tracheae, show no trace of pocketing whatever. It must not be taken, however, that this fact excludes the possibility of the appearance of pockets before the formation of a pith. In both these genera the leaf-trace at its actual point of departure is rounded or elliptic in section, and it is easily seen that there would be no encouragement to form a pocket in the axil of a leaf-trace of such a form. The mesarch traces in the sporeling of *Osmunda regalis* are similar in section, and in this case also a pocket is never present. *The formation of axillary pockets is clearly related to the departure of leaf-traces which are adaxially concave at their very point of origin.* If, indeed, such a thing ever existed as an Osmundaceous stem with a solid xylem and leaf-traces gutter-shaped at the very point of their departure, it is quite to be expected that there will also be xylem-sheath pockets in the periphery of its xylem. In *Osmundites Dunlopi*, the nature of the pith of which is unknown, the pockets are very small, and rarely, if ever, break the continuity of the xylem-ring. On the other hand, in *Osmundites Kolbei*, which has a mixed pith, the pockets are so deep that they break up the xylem-ring into separate strands. At the same time they are not so well developed as in the modern Osmundaceae, for the interruption of the xylem-ring is seldom completed at the actual level of the departure of the trace, but only at a point some distance above.[1] It would appear, therefore, that the relative development of pithing and of pocketing was not uniform, but probably varied in different lines of evolution.

Two conclusions as to the nature of these nodal pockets are possible. The one that I would bring forward is that the xylem-sheath pockets are a relatively primitive feature in the order, and that they were primitively associated with protostelic leaf-traces, which departed without leaving a gap in the xylem-ring. Such protostelic departures with pockets are still to be found in the sporelings of *Osmunda regalis* and *O. cinnamomea*, and in the latter they sometimes even occur in adult plants.[2] I have also described similar cases in *Todea*.[3] The accuracy of this statement has since been called into question by E. W. Sinnott.[4] My observations were made, it is true, from hand-cut sections and not from microtome series, but in the light of Faull's results I still venture to think they are correct.

[1] Kidston and Gwynne-Vaughan, l. c., Pt. IV, p. 459, Fig. 3.
[2] Faull, l. c., pp. 517 and 523.
[3] Kidston and Gwynne-Vaughan, Pt. I, p. 775.
[4] Sinnott, E. W.: Foliar gaps in the Osmundaceae. Annals of Botany, vol. xxiv, 1910, p. 109.

The other interpretation of the nodal pockets advanced by Faull[1] is that they do not indicate a primitively gapless departure of the leaf-trace (cladosiphony), but a 'cladosiphony secondarily produced', and that they afford strong proofs of an evolution that tends *towards* cladosiphony. He holds that they owe their existence to an evolutionary tendency on the part of the xylem to increase in the direction of the pith. In fact, they are portions of the central ground tissue that have been closed in as a result of a centripetal proliferation of the xylem. As instances of this tendency to centripetal proliferation, Faull mentions cases in *Osmunda cinnamomea* in which he found xylem-strands of considerable size on the inside of the xylem-ring and separated from it by a varying amount of parenchyma; also cases in which isolated tracheides, each surrounded by a ring of endodermal cells, occurred in the central ground tissue *inside the internal endodermis*. The latter he regards as having been 'pinched off' from the stele. If this is really the case they should be connected at some point with the main xylem-ring, and they would then have some analogy to the internal accessory strands in *Dicksonia adiantoides* and *D. rubiginosa*, and to me they would suggest an increase rather than a reduction of the vascular tissue in the stele. I consider all these cases to be very interesting, but I regard them as indicating a readiness on the part of the cells of a true pith to revert to the type of element from which they were derived, in fact, as partial reversions to a mixed pith. The existence of stems with a parenchymatous pith containing scattered tracheae, isolated or in groups, such as occur in the Lepidodendreae, Zygopterideae, and Osmundaceae, seems to me to be a very serious obstacle in the way of the acceptance of Professor Jeffrey's statement that all piths are extra-stelar.[2] It is possible to account for them on the lines of this theory by using Faull's conception of a centripetal proliferation of the xylem into the enclosed extra-stelar ground tissue. But this is putting a heavy strain upon the idea and does not appear to me at all satisfactory. In particular, as regards the Osmundaceae, I find it difficult to reconcile a proliferation of xylem with the theory advocated by both Jeffrey and Faull, that the vascular system of this order has undergone a simplification of structure owing to reduction in size.

The Zygopterideae, again, present a further obstacle to those who deny the existence of a true intrastelar pith. For, so far as I am aware, no Zygopterid stem has yet been found with either a leaf-gap or a branch-gap in the stele, whereby the extra-stelar tissues could get in.

The presence of short relatively thin-walled tracheae occupying the position of a pith in *Thamnopteris*, *Zalesskya*, and *Diplolabis Römeri*,[3] fits

[1] l. c., p. 524.

[2] Jeffrey: The Pteropsida. Botanical Gazette, vol. L, 1911, p. 401.

[3] Gordon: On the structure and affinities of *Diplolabis Römeri* (Solms). Trans. Roy. Soc. Edin., vol. xlvii, 1911, p. 711.

in well with the theory of the intrastelar origin of the pith in their respective orders, but would be meaningless if the pith be extra-stelar.

It is recognized that, if the Osmundaceous pith is intrastelar, the presence in the pith of an internal phloem in *Osmundites skidegatensis* and *Osmunda cinnamomea* and of an internal endodermis in *Todea hymenophylloides* has still to be accounted for, although this question may be taken as apart from, and as having no direct bearing upon, the intrastelar origin of the pith itself. As a matter of fact, several possibilities are open. In the first place Dr. Kidston and I have suggested that the phloem and endodermis may have been decurrent through the branch-gaps into the pith. It must be understood that the word 'decurrent' is here used in a special sense, and of course does not imply any actual motion. It is meant to imply that the stimulus to produce internal phloem and endodermis originated at the margins of a branch-gap, and phylogenetically was gradually transmitted to lower levels in the stem. To this idea Faull objects that he has found internal phloem in plants of *Osmunda cinnamomea* that have not yet branched. I do not see that this affects the question. If the internal phloem and endodermis in their present state of development are decurrent for some distance below the point of branching, the apical meristem must form them in this region some time before it branches.

A second alternative, and a very probable one, is that the internal phloem and endodermis may have arisen entirely *de novo* in the intrastelar pith, and that subsequently connexions were established with the corresponding external tissues.

A third possibility is that some time after the formation of an intrastelar pith the outer phloem and endodermis were invaginated into the same through the leaf-gaps. Faull[1] figures a case in *Osmunda cinnamomea* in which the two endoderms are in continuity through a leaf-gap. To me, however, his figure suggests that the internal endodermis has reached outwards to meet the external rather than vice versa.

As matters stand, however, it is in no way incumbent on a supporter of the intrastelar theory to pin his faith to any one of these suggestions.

The fossil evidence in the Osmundaceae I regard as distinctly in favour of the intrastelar nature of the pith, since it has brought to light *Osmundites Dunlopi*, the mixed pith in *Osmundites Kolbei*, and also the short tracheae in *Thamnopteris* and *Zalesskya*. Dr. Kidston and I believe that in *Osmundites Dunlopi* the pith is surrounded by an uninterrupted ring of xylem, but even if this be not so it would still represent an important transitional stage between a continuous ring and well-defined leaf-gaps which in any case must have existed. Both Faull and Sinnott have laid more stress upon the imperfection of this fossil than it really deserves, for in our description of it we have been far from lenient. It is true that the interpretation of fossil

[1] l. c., Fig. 11

structures is often to some extent a matter of personal opinion, and all that can be said is that, after making every allowance for lack of preservation, Dr. Kidston and I still adhere to our original statement, that 'if medullary rays actually were present in the living plant they must have been extremely narrow and very rare'.[1]

As regards the lack of transitional forms, the supporters of the extra-stelar pith theory in the Osmundaceae are very badly off indeed. As I understand it, they regard all the known forms, except those with solid protosteles, as stages in a series of reductions. In consequence they have not a single form to show of all the advancing stages that must have occurred from the first pocketing into the solid protostele up to the production of the perfectly dictyostelic form that is supposed to have antedated *Osmundites skidegatensis*.

As regards evidence of pocketing in the sporeling, I maintain that a distinction must be drawn between the pocketing of the peripheral tissues of the stele into the xylem and the pocketing of the external tissues of the stem into the stele. It is clear that the former must precede the latter. That the outer tissues of the stem have, in many cases, a tendency to invade the central tissues in the axils of the leaf-traces is well known, *and these xylem-sheath pockets represent the initial and the simplest possible expression of this tendency.* The next step is represented by the *Lindsaya* type of stele in which the phloem has followed the xylem-sheath, but the pocketing is still intrastelar. The simpler stages of extra-stelar pocketing are met with in certain Gleichenias and in *Davallia pinnata*, leading on to the formation of solenosteles and dictyosteles. Finally, even the epidermis and the surrounding atmosphere may join in the invasion and penetrate into the central tissues of the stem, as is shown in *Onoclea*, *Cystopteris*, and *Aneimia*. In the sporeling of *Osmunda*, however, there is no evidence whatever of the pocketing of the external ground tissue or of the endodermis into the stele. The xylem-sheath pockets are intrastelar and nothing else. It is only by first of all assuming that the reduction theory is true that they can be imagined even as indicating the position of previously existing extra-stelar leaf-gaps.

The arguments brought forward by Sinnott[2] in favour of the primitive existence of leaf-gaps in the stele of the Osmundaceae based on the existence of gaps in the petiolar meristele made by the departure of the pinna-traces necessitate the acceptance of the preliminary assumption that the leaf is equivalent to an axial branch system. Even if this be granted, there is no reason to expect that the caulome and the phyllome should undergo the

[1] Professor Faull (l. c., p. 530), referring to the presence of scale-leaves in *Osmundites Dunlopi*, represents us as admitting that this plant is reduced. This is obviously a misunderstanding. The scale-leaves occur in regular succeeding zones as they do in *Osmunda regalis*, and no doubt they served the same purpose of protecting the apex during adverse seasons. We do not regard either of these plants as reduced.

[2] l. c., p. 113.

same evolutionary changes, but much to the contrary. No one, I take it, doubts the extra-vascular nature of the ground tissue in the concavity of the gutter-shaped leaf-trace. The way in which a leaf-trace originally elliptic in section becomes hollowed out adaxially is beautifully shown by that of *Thamnopteris* in its course through the cortex of the stem.[1] This change in form is no doubt due to the bilaterality and dorsiventrality of the leaf as a whole, and can have nothing to do with the gaps made in the already gutter-shaped meristele by the far-away pinnae. Incidentally in the earlier leaves of the sporeling of *Osmunda regalis* the pinna-traces do not leave gaps of any kind. They are supplied by strands that are nipped off from the extremities of the *already* gutter-shaped meristele.

Summary.

1. The early appearance of axillary pockets of xylem-sheath parenchyma in the xylem of the sporeling stele of *Osmunda* is confirmed.

2. The intrastelar origin of the pith in the Osmundaceae is adhered to.

3. The medullary rays are due to the breaking through of the xylem-ring by the xylem-sheath pockets, and are in consequence also intrastelar in origin.

4. The mesarchy found in the basal region of the leaf-trace in *Thamnopteris* and *Zalesskya* is still occasionally retained in the early leaves of *Osmunda regalis*.

DESCRIPTION OF THE FIGURES IN PLATE XLIV.

Illustrating Prof. Gwynne-Vaughan's paper on the Anatomy of the Osmundaceae.

Figs. 1–4 are made from drawings, Fig. 5 from an untouched photograph, and Figs. 6–9 from under-exposed prints used as camera lucida outlines.

Abbreviations : *l. t.* = leaf-trace xylem ; *prx.* = protoxylem ; *M.* = pith ; *pkt.* = xylem-sheath pocket.

Figs. 1 and 2. *Osmunda regalis.* Xylem of sporeling showing leaf-trace xylem departing in a protostelic manner. × 210.

Fig. 3. *Osmunda regalis.* Xylem of sporeling showing simultaneous appearance of the pith and of a xylem-sheath pocket. × 210.

Fig. 4. *Osmunda regalis.* Xylem of young stem; the xylem-sheath pocket on the left never comes into direct contact with the pith. × 130.

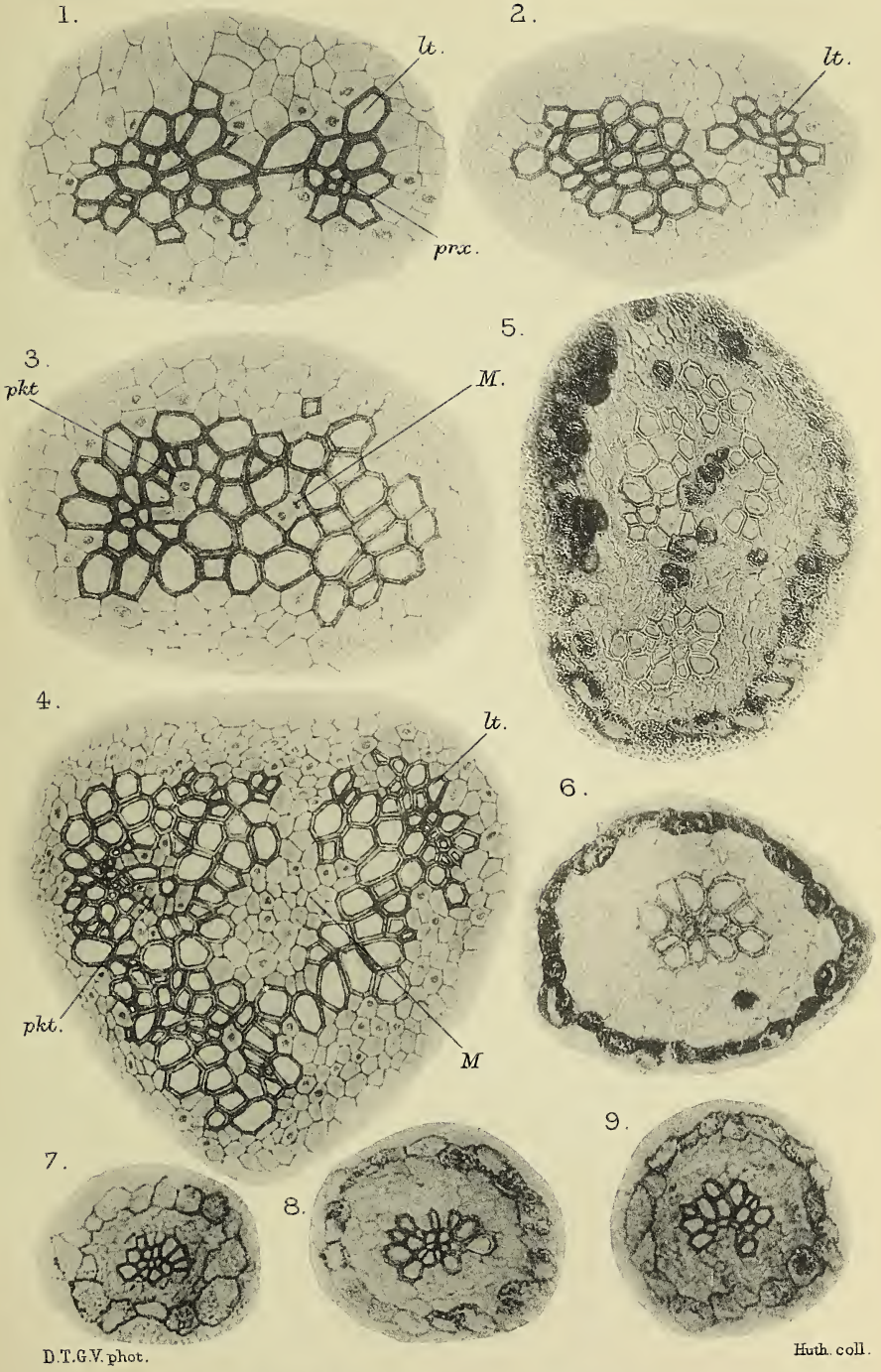
Fig. 5. *Osmunda regalis.* Xylem of sporeling showing a mesarch leaf-trace and the tannin-containing cells in the early pith. × 150.

Figs. 6, 7, and 8. *Osmunda regalis.* Meristeles of sporeling leaves with mesarch protoxylem. × 150.

Fig. 9. *Osmunda regalis.* A mesarch leaf-trace becoming endarch. × 150.

In the leaf-traces the adaxial side is towards the bottom of the page.

[1] Gwynne-Vaughan : On the Origin of the Adaxially Curved Leaf-trace in the Filicales. Proc. Roy. Soc. Edin., vol. xxviii, Pt. VI, No. 29, 1908, p. 433.



D. T. GWYNNE-VAUGHAN —— OSMUNDA REGALIS.

On the Primary Xylem, and the Origin of Medullation in the Ophioglossaceae.

BY

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With Plates XLV and XLVI.

BOTRYCHIUM is the only genus of living Ophioglossaceae which shows secondary thickening of its axial stele. The greater part of the vascular tissue of the axis owes its origin to the cambium, so much so that in some cases 'the wood is practically all secondary, as may be learned from the radial arrangement of its matured elements'.[1] This prevalence of the secondary tissues has diverted attention from the primary tissues, which are apt to be ignored in the descriptions which are given of the structure of the axis. But for purposes of comparison with other genera of the family, and with other Pteridophytes, it is desirable that the primary tissues should be recognized, so far as they are present in *Botrychium*. It is the object of this paper to bring them more clearly forward than has hitherto been done.

In the mature axis of all the genera of this family there is a central pith, and the xylem as seen in transverse sections forms a more or less interrupted ring surrounding it. The interruptions are caused by the departure of the leaf-traces, each consisting of a single strand, excepting in the more specialized species of *Ophioglossum*.[2] In the pith an endodermal structure has been observed, but not with any high degree of constancy. This internal endodermis was first described by Van Tieghem,[3] in young plants of *Ophioglossum vulgatum*: it was also seen in young plants of *Botrychium Lunaria*, and its origin traced by involution of the outer endodermis at the leaf-gap, the foliar strand being there detached with its special pericycle and endodermis. Van Tieghem described[4] how 'the endodermis of the central cylinder, thus open, folds its two margins inwards, where they extend and rejoin around the pith, which is thus incorporated

[1] Jeffrey, Gametophyte of *Botrychium virginianum*, 1898, p. 22.
[2] Bower, Annals of Botany, vol. xviii, p. 215, and vol. xxv, p. 278.
[3] Journal de Botanique, 1890, p. 405. [4] Loc. cit. p. 407.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

with the cortex.' This was probably the first example of encroachment of the cortex upon the stele to be observed or described in vascular plants. The same author further pointed out that between the invaginated endodermis and the internal margin of the wood one or two layers of parenchyma intervene, which are continuous at their margins with the pericycle. So, according to his view, the pericycle is invaginated like the endodermis. Further up the axis the internal endodermis separates completely from the external, and henceforth there are two distinct layers of endodermis, one external, the other internal. This structure is continued upwards, but above the fourth leaf the internal endodermal characters were gradually lost, and in the adult plant there is only a single endodermis, i. e. the external. An internal endodermis was not found in *B. ternatum*, *virginianum*, or *daucifolium*, but it was clearly seen in *B. boreale*.

There were no illustrations of the data thus described by Van Tieghem, but subsequently M. G. Poirault, to whom the observation appears to have been in the first instance due, delineated the internal endodermis for *B. Lunaria*, and also for *Ophioglossum Bergianum*, in both cases finding it present only at the base of the plant.[1] An internal endodermis has since been found, and described in the old stocks of *Helminthostachys zeylanica*.[2] A current view which has been based upon these descriptions is that the pith of the Ophioglossaceae is to be ranked as intrusive cortex, with a lining of endodermis and pericycle. In the case of *Botrychium* the primary wood would then lie, such as it is, between the parenchyma which Van Tieghem described as internal pericycle and the zone of secondary wood.

It seemed desirable, in view of the advances in stelar investigation in other plants, to look again into the facts relating to the Ophioglossaceous stele. There is a general opinion that here, as elsewhere, the stelar condition has been ultimately derived from the solid protostele. Van Tieghem describes this structure for *Ophioglossum vulgatum* (loc. cit., p. 405) and *Botrychium Lunaria* (loc. cit., p. 407). In *Ophioglossum vulgatum* Van Tieghem states that 'in the whole region below the first leaf there is a central cylinder which is slender and without pith' (loc. cit., p. 405). I do not find this to be constant in plants sprung from adventitious buds on the roots. A pith may be present in these from the very first. The same medullated condition is found to be the case in some specimens of *O. reticulatum*, but others show a solid xylem up to a point immediately below the departure of the first leaf-trace. In *O. palmatum* such young plants as were observed were medullated from the first (Pl. XLV, Fig. 1). It appears, therefore, that variability exists within the genus *Ophioglossum* in respect of the medullation of the individual in the young state. There is some reason for believing that the existence or non-existence of a pith from the first

[1] Poirault, Ann. Sci. Nat., 1893, pp. 169, 170.

[2] Farmer and Freeman, Annals of Botany, vol. xiii, p. 421, Pl. XXII, Fig. 11.

depends upon the nourishment of the young plant. If it be well nourished the non-medullated phase is omitted, and pith is present from the first. But it is in ill-nourished or depauperate plants that the start is made with a protostele, which subsequently becomes medullated.

It will be important to observe the exact method of origination of the pith as it first appears in the protostele, in order to form an opinion on its morphology. The first appearance of the pith in a plant with a solid protostele at the base has been followed in *O. reticulatum*, and it is illustrated in the Figs. 2–5. At first the solid xylem-core is without parenchyma, being composed only of tracheides. But a little below the origin of the first leaf-trace, single cells, or small nests of them, retaining their thin walls and protoplasmic contents, appear among the mature tracheides (Fig. 2). Passing upwards, these merge into a central parenchymatous pith, which, however, at first has no clear limit from the xylem. Tracheides and parenchyma are intermixed (Figs. 3, 5). This continues only for a short distance, the pith consisting subsequently of pure parenchyma, which is then continued without interruption upwards. The surrounding ring of tracheides very shortly opens, throwing the pith into communication with the outer conjunctive tissue of the stele. This is the first step in the segregation of the leaf-trace (Fig. 4).

There can be no doubt that the parenchyma nests which precede the first establishment of the pith, and merge with it, are not only intrastelar, but also intraxylic, in origin. A proportion of the pith may, however, owe its origin to an intrusion of the outer conjunctive tissue: but this source also is intrastelar. The similar origin of an intrastelar pith to be described below for *Botrychium Lunaria*, where the endodermis is clearly defined, confirms this conclusion.

The well-known imperfection of the endodermis in *Ophioglossum* is certainly a difficulty in the interpretation of the development of the young plant in most species of the genus. But on the other hand, the small species, *O. Bergianum*, has been quoted by Poirault (loc. cit., p. 170, Fig. 12), as having not only an external, but also an internal endodermis, the latter in the pith. I have cut the stock of my only specimen into transverse sections, but with negative results. No endodermis of the axis was found to take the safranin stain, though that of the roots, wherever present, showed up well in the same sections, with the same stain, and on the same slides. The structure was, however, conformable otherwise to that shown by Poirault, and I have no reason to doubt his observations, which were probably made on more favourable material. But it appears that the development of the endodermis is variable in the species. The above plant was sent to me by Mr. H. Bolus from the Cape in 1894. With it was sent a distinct plant, which I recognize as one of the small Cape forms of *O. lusitanicum*. This was also cut into sections, and showed the

disposition of its weak vascular system on the Ophioglossaceous plan, and with the endodermis variable in clearness of characters. Endodermal markings were seen outside the meristeles and also in the pith, in positions generally corresponding with what is shown in Poirault's drawing of *O. Bergianum*. Further, the outgoing leaf-trace was in some cases surrounded by a special endodermis of its own, as will be shown to be the case in *Botrychium*. These conditions are perfectly compatible with the arrangements more clearly seen in *Botrychium*, which will be described below. The conclusion which I draw from these imperfect observations is, that Poirault's observations are to be upheld; further, that the small species of *Ophioglossum* which show, however inconstantly, an internal endodermis, repeat on a reduced and incomplete scale the same type of structure as is seen more fully developed in *Botrychium*. It will be desirable that this be tested by some observer with more ample material at his disposal.

A similar variability to that found in the genus *Ophioglossum* in respect of the medullation of the individual in the young state, is seen also within the genus *Botrychium*. Already Jeffrey has shown that in *B. virginianum* the sporeling is medullated from the first, a fact which is probably in relation to its nutrition by the large storage prothallus, which serves as an effective nursing mother (loc. cit., pp. 21–2). The fact is corroborated by sections of my own sporelings collected in Jamaica. No internal endodermis was seen in them. In order to compare with this the sporeling structure in *B. Lunaria*, and at the same time to give the most pointed antithesis by selecting weak plants instead of those highly nourished by large storage prothalli, serial sections were cut from sporeling plants taken from the rocky ledges of the Breadalbane Hills, about 3,000 feet up. The plant most exhaustively treated bore one leaf, with a spike on which were only four sporangia. The axis was about $\frac{1}{2}$ inch in length, and very thin, with several roots. As sections showed, a number of leaves had preceded that which was expanded. Following the series of sections of this plant from below upwards, the stele appears from the first to be composed of tracheides with occasional parenchyma cells intermixed, while it is circumscribed by an endodermis, which is well defined considerably below the point of departure of the first leaf-trace (Pl. XLV, Fig. 6). Three roots were attached at this region. Already the cells around the primary xylem show activity of division, thus initiating the secondary development. At first the parenchyma cells in the xylem are not located specially at the centre of the stele; but as the level of departure of the first leaf-trace is approached, a concentration of the parenchyma occurs internally, and a central pith is thus formed. An essential point is that intraxylic parenchyma exists before the appearance of a definite pith, and contributes to its formation. Very soon the pith is put in connexion with the outer conjunctive tissue by the opening of the xylem-ring. This

prepares for the departure of the first leaf-trace (Fig. 7), one margin of which is defined by the gap in the xylem-ring; but for some time the leaf-trace strand remains connected by the other margin with the xylem of the stele, as it is seen to be in Fig. 7. Meanwhile, the endodermis remains continuous all round, but it shows some irregularities in position of its cells in the neighbourhood of the leaf-trace. These lead to an involution, first on the side on which the opening of the ring began (this is indicated in Fig. 7), and subsequently on the other side. These involutions of the endodermis approach one another as the foliar strand moves outwards, and meet, as seen in Fig. 8. The endodermal limit of the stele is thus never interrupted, and there is no direct communication between the cortex and the stele. After a time the xylem-ring closes again, completely surrounding the central pith. But very soon it opens on the opposite side, for the issue of the second leaf-trace, which is also effected without any interruption of the continuity of the endodermis. The stele meanwhile assumes again a solid xylem-core, except for a few parenchyma cells irregularly scattered as at first among the tracheides. But soon the pith is reconstituted as before, and the process may be repeated at the exit of later leaf-traces. One point worthy of special notice is that as the leaves of the sporeling are successively larger, and their traces stronger, the incurving of the endodermis into the pith (which also becomes more massive as the plant strengthens) becomes more marked, indicating gradual steps towards that degree of encroachment upon the pith, and formation of that internal endodermis, which was first suggested by Van Tieghem. This is shown in slight degree in Fig. 9, and higher leaves of the same plant showed still deeper intrusion. Since, however, the degree of intrusion of the foliar pocket thus formed appears to depend upon the size of the leaf, it would not be expected to be large in so weak a plant as this sporeling from the Breadalbane Hills.

Another of the same batch of weak plants from the hills, which was cut into sections by Dr. Lang, showed the following interesting features. Passing upwards from the base, the vascular structure begins with a solid stele, in which, however, a distinct pith appears at the departure of the first leaf-trace. But there is no communication with the cortex, the endodermis being continuous, as in the previous case. The trace with its surrounding parenchyma is nipped off from the stele by involution of the endodermis, as in the specimen already described (compare Fig. 8). But the next two leaf-traces differ slightly in their behaviour, for in their departure they show the formation of a band of endodermis, which passes internally to the leaf-trace, before the actual separation takes place. The stelar sheath is thus completed before the outer band surrounding the stele and the departing trace is interrupted. This band of endodermis appears to be a new formation, not directly referable to involution, as in the case of

the first leaf: a similar origin applies also for the corresponding tissue opposite the later leaves of the plant previously described (compare Pl. XLV, Fig. 9).

At the departure of the next leaf-trace of the plant cut by Dr. Lang, which is the fourth leaf in succession from the base, scattered tracheides appear in the pith, as seen in Fig. 10. This leaf-trace, as well as those of all the subsequent leaves, departs, as was found by previous authors to be the case in mature plants, leaving an interruption in the external endodermis. But in this case it is plainly the result of that imperfect formation of the endodermal characters which is general in the older regions of these plants. The fading out of them may be clearly traced by passing upwards through the series of sections; for at the first separation of the strand from the stele the endodermis is complete: as the trace passes out the endodermis yields, and remains complete for a time, but the staining reaction of the radial walls fails, and the failure is first seen externally to the trace itself, as shown in Fig. 10 for the fourth leaf. No internal endodermis extending into the pith was observed in any part of this plant.

The structure thus seen in the two plants above described appears to be quite incompatible with any theory of intrusion of the cortex to form the pith, for the pith is present in both cases for at least four internodes below the first interruption of the internal endodermis, and structurally its origin is fully accounted for in relation to the intrastelar tissues. Moreover, the appearance of the scattered tracheides in the pith, at the level of the fourth leaf-trace in the second plant, appears to indicate a local reversion of the pith towards its original condition of xylem. This points to its phyletic origin having been, in part at least, by degradation of tracheides to parenchymatous cells.

An examination of other young plants of *B. Lunaria* shows that in respect of their medullation and in the behaviour of the endodermis in relation to the exit of the leaf-traces, they conform in essentials to what has been described; but there may be considerable variety in the exact details. The further elucidation of these will be necessary for a full understanding of the stelar condition of the genus, and of the family at large.

These observations on weak sporelings of *B. Lunaria* require comparison with Van Tieghem's results. They correspond in the main features. The chief difference lies in the question of the interruption of the endodermis. According to my own observations on young plants of *B. Lunaria* the endodermis is not interrupted at the exit of the traces of the lower leaves. It remains continuous, and all the changes seen are strictly intrastelar. In the stronger and older state the endodermis fades out. It is believed that greater reliance may properly be placed upon the earlier data, and especially those seen in weak plants, than on those derived from

old and strong plants. Accordingly it is not stated that Van Tieghem's observations of the interruption of continuity of the endodermis were erroneous, or that the endodermal connexions are always such as to constitute a complete barrier. What is stated is that such a barrier does occur in certain cases, and that still in them a stelar pith is present. Therefore, the establishment of the continuity between the cortex outside the endodermis and the pith within the stele is not a necessary condition for the formation of a pith in the Ophioglossaceae. Moreover, as the condition where the endodermis forms a complete barrier is the state of the young plant, the ontogeny indicates that this was the primitive condition in the family.

There remains the question of the real nature of the pith in these young plants where it originates without any direct connexion with the cortex, the stele being completely shut off by the continuous ring of endodermis. It appears to originate partly from the intraxylic parenchyma, which masses together to produce it; partly from the conjunctive tissue outside the xylem (or xylem-sheath as it is sometimes called), with which the intraxylic parenchyma is connected at the foliar gap. The arrangement of the cells as shown in Figs. 7 and 8 may be held to suggest intrusive growth of the conjunctive tissue. But the conjunctive parenchyma is itself of stelar origin, as is also the intraxylic parenchyma. Thus the pith is in fact of intrastelar origin in these young plants, though there is some evidence of intrusion of an outer upon an inner stelar region.

Returning now to the examination of the primary xylem in *Botrychium*, an inspection of Figs. 6–9 shows that while there are in all cases signs of cambial activity outside the xylem-tract, and though some of the more peripheral tracheides may have had a cambial origin, this is certainly not the source of the majority of them, for their arrangement is not in radial rows. A comparison of these drawings with those of Professor Jeffrey for the seedling of *B. virginianum* (loc. cit., Pl. IV, Figs. 62, 63) shows clearly the difference. Whereas in that species primary xylem is virtually absent, here it is present in considerable quantity. It became accordingly a matter of interest to see the condition not merely at the base of the plant, as shown in Figs. 6–9, but also towards the apex, in a case where numerous leaves had been formed. This is shown in Fig. 11, which represents a section just below the apex of *B. Lunaria*, where the secondary thickening is beginning, but does not appear to have produced any tracheides as yet. All those here shown are regarded as primary xylem. It is to be noted that the tracheides are associated with parenchyma, which constitutes a pith at the centre. Some of the primary tracheides shown in Fig. 11 are seen to lie isolated in the pith. They may be held to represent vestigial remains of a more extensive primary xylem, which is in course of substitution by a development of secondary wood.

It becomes thus a question of interest to inquire what evidence there is of the existence of such vestigial remains of primary xylem in the genus, and what is its distribution in the different species. Frequently no such isolated tracheides exist in the pith of *B. Lunaria*. But where they do occur, they may be found to occupy positions which would be difficult to harmonize with a theory of intrusive origin of the whole pith. That is particularly the case in the section represented in Pl. XLV, Fig. 12. This shows in a weak plant the separation of a leaf-trace from the xylem-ring. The gap has opened, and parenchymatous continuity is established between the central pith and the outer conjunctive tissues. If the connecting tract were intrusive tissue of cortical origin, it should contain no tracheides. But here a tracheide is found occupying a central position in the connecting tract. A similar difficulty arises in the case of Fig. 10, where a great part of the medulla at the level of the fourth leaf takes the character of a mixed pith with scattered tracheides, though lower down it was purely parenchymatous.

Passing to *B. virginianum*, which has already been quoted as having virtually no primary xylem in the seedling, this condition is usually maintained in the older axis. But occasionally isolated tracheides occur. One of these is shown by Professor Jeffrey in his Pl. IV, Fig. 62, while Atkinson has also delineated two isolated tracheides in the pith of the same species ('Biology of Ferns,' Fig. 152), which can only be held as representing vestigial remains of a primary xylem. In my own sections, which were all from young plants, such a state is only occasionally met with in *B. virginianum*, but an example is shown in Fig. 13.

Very interesting conditions are seen, however, in *B. ternatum*. I have not had the opportunity of examining young sporelings of this species, but by cutting the base of the stock of fairly advanced plants collected in Jamaica, I was able to obtain results which show that the species retains the vestigial xylem more firmly than *B. virginianum*. Examples are seen in Figs. 14–16 of isolated tracheides, or groups of tracheides, lying in the pith: these, as they cannot be referred to a cambial origin, can only represent the vestigial primary wood. But since *B. ternatum* is thus seen to be more prone to such vestigial characters in its xylem, it was a matter of special interest to observe in one specimen of that species the consequences of an injury to the stock, brought about probably by the attack of some insect, or it may have been by some mechanical injury. Professor Jeffrey has lately brought forward evidence in support of the view that traumatic changes are apt to be reversionary, the tissues affected by the injury showing ancestral characters. This gives additional interest to the study of this case in *B. ternatum*.[1]

The stock was cut into serial sections. The structure at the base of

[1] Wound Reactions of *Brachyphyllum*, Ann. of Bot., vol. xx, p. 383 ; Traumatic Ray-Tracheids *Cunninghamia sinensis*, Ann. of Bot., vol. xxii, p. 593.

the plant was found to be normal, and the stock of moderate size. Pl. XLVI, Fig. 17, shows its condition, with a large pith, slightly compressed. But quite suddenly, owing no doubt to the damage of which signs are seen penetrating deeply from the outside, the stele contracted to a much smaller size (Fig. 18), while the pith, which hitherto had been free from isolated tracheides, now showed many of them interspersed throughout the parenchyma. The central region of the stele is shown on a larger scale in Fig. 19, from which it is seen that tracheides lie at the very centre of the pith. Presently, as the damaged region was left behind, the stele again expanded, the pith enlarged and gradually became clear of tracheides. In fact a normal structure was resumed (Fig. 21), though of smaller size than that of the original stock. The interest of this case lies in the light it sheds on the relation of the primary wood of *Botrychium* to the pith. If by traumatic conditions the plant, as Professor Jeffrey holds, is liable to reversionary changes, then, since in this special case the parenchymatous pith has many of its cells replaced by tracheides right through to its centre, that would appear to indicate that the pith, right through to its centre, was originally of xylem origin.

The condition shown by the pith in this injured stock of *Botrychium ternatum* may be compared with that of the centre of the stele in a small rhizome of *Helminthostachys*, which was described by Farmer and Freeman.[1] The structure which is there shown follows naturally on that of the still younger stems represented by Lang.[2] A photograph of one of Professor Farmer's sections is shown in Fig. 22. In this, as in the injured stock of *B. ternatum*, the centre of the stele is occupied by a tissue composed of tracheides and parenchymatous cells. It has in fact the character of what is known as a 'mixed pith'. This condition is represented in greater or less degree in the young plants observed from the other genera of the Ophioglossaceae. It is shown in the young protostelic plant of *O. reticulatum* in Pl. XLV, Fig. 2, and for *Botrychium Lunaria* in Fig. 6. In each of these cases there is a tract of tissue where parenchyma and tracheides are intimately associated, though it extends only a short distance along the axis. But in *Helminthostachys* that condition may be continued for a long distance. Whether it be short or long, such a mixed condition exists as the progression takes place from the solid protostele to the medullated state. Thereafter, by gradual increase in the proportion of the parenchyma cells, and a decrease leading to complete absence of the tracheides, a substantial part, or it may be the whole, of the central medulla is established. It appears that owing to injury *Botrychium ternatum* has, in the case quoted, returned structurally to the phase of mixed pith commonly passed over rapidly in the individual life, but represented more effectively in young plants of *Helminthostachys* than in other members of the family. This interesting example of the principle of traumatic reversion, suggested by Professor

[1] Ann. of Bot., vol. xiii, p. 421, Pl. XXIII, Fig. 23. [2] Ibid., vol. xvi, Pl. III, Figs. 65–70.

Jeffrey, has its important bearing as indicative of the intrastelar origin of a substantive part of the pith in the Ophioglossaceae.

Comparing the three genera of Ophioglossaceae, it is seen that the structure of all of them is referable in origin to the protostele, in which the xylem may be composed at first entirely of tracheides, as seen in the various species of *Ophioglossum*, and it appears to be the case also in some specimens of *Helminthostachys*.[1] Or a few parenchyma cells may be intermixed with the tracheides, as is more common in *Helminthostachys*, and in *Botrychium*. But, alternatively, in all the three genera the stele may be medullated from the first. In this respect there is inconstancy even in the single species. Whether initially medullated or not, a pith of some sort is formed in all cases before the departure of the first leaf-trace. Ontogenetically it is of intrastelar origin, and its source is by accumulation of the parenchyma of the xylem at the centre of the stele, added to, when the xylem-ring opens, by some degree of intrusion of the conjunctive tissue; but this is certainly not the only source. Its original source appears to be the former, and the occurrence of isolated tracheides, or groups of them, about the margin of the pith (Pl. XLV, Figs. 14–16), as well as the changes following on injury seen in *B. ternatum*, support this conclusion.

In *Ophioglossum* and *Helminthostachys* there is no cambium. But in *Botrychium* a secondary activity appears early, and most of the wood is so produced. The primary wood is accordingly vestigial in most species, though it may be fairly represented in *B. Lunaria*. The resulting condition may be compared with that seen in the fossil *Botrychioxylon*, recognized and briefly described by Scott.[2] The full description of this stem with figures will be awaited with increasing interest. Dr. Scott kindly showed me the specimens for the purpose of this comparison. The stele has centrally a core composed of tracheides scattered through a parenchymatous matrix. This represents the primary wood, and compares with that of the young stem of *Helminthostachys* (Pl. XLVI, Fig. 22), or of *Botrychium* (Pl. XLV, Figs. 10, 11). A broad band of secondary wood surrounds it, which corresponds to the secondary wood of *Botrychium*. The similarity between the fossil and the traumatic condition of *B. ternatum* (Pl. XLVI, Figs. 18–20) is specially striking, while, on the other hand, the comparison may be drawn with certain of the Botryopterideae, and especially with the stem of *Zygopteris*, which shows a central differentiation of the xylem along somewhat similar lines.

The course of development of the stock in the Ophioglossaceae shows a further factor, absent in the first steps of medullation, but increasingly important as the plant becomes established and the individual leaves attain larger size. It is the intrusion of foliar pockets. As Pl. XLV, Fig. 8, shows,

[1] Lang, Ann. of Bot., vol. xvi, p. 42 : 'The xylem is in some cases a solid central strand.'

[2] Journ. Roy. Micr. Soc., 1906, p. 519; and somewhat more fully in Progressus Rei Botanicae, Bd. i, 1906, p. 181.

the leaf-trace in the young plant of *Botrychium* may pass off without intrusion of a foliar pocket, there being a clear demarcation by endodermis of the parenchymatous pith, which is already present, from the parenchyma associated with the leaf-trace. But at the insertion of the stronger leaves the endodermal barrier encroaches inwards, as suggested by Fig. 9, and it appears still more clearly in sections from other plants. Thus an addition may be made to the already existent pith. But as the plant becomes established, the barrier between the two components of the ultimate pith column is no longer maintained, for the intrusive endodermis is often imperfect, while there is also some evidence of formation of endodermis *de novo* in relation to the departure of the leaf-trace. It is probably this intrusive endodermis, whether originated simply by involution, or by formation *de novo*, which was observed by Van Tieghem, and the drawings of *B. Lunaria* and of *O. Bergianum* by Poirault apparently show the imperfect remains of that morphological barrier. The ontogeny is to be taken here as a true guide to the origin of the condition seen in the mature plant. From it we learn that an intrastelar pith was first initiated, and that subsequently foliar pockets, with ultimately a cortical intrusion, encroached upon the pith, and added to its bulk.

A comparison of the results thus obtained for the Ophioglossaceae with the description given by Faull for the seedlings of *Osmunda cinnamomea* shows an essential similarity.[1] In the first place he records (loc. cit., p. 525) the occurrence of isolated tracheides in the pith within the internal endodermis of *O. cinnamomea*. Unfortunately he does not figure them, but states that they are surrounded by a ring of endodermal cells, and suggests that they have been 'pinched off' from the stele. But are they not more probably to be compared with the medullary tracheides of *Botrychium*, and especially of *B. ternatum* (Pl. XLVI, Figs. 18–20)? In that case they would appear to be vestigial evidences of degeneration of the xylem. Their existence seems clearly to favour a view involving intrastelar medullation. All Faull's observations of medullation in the seedlings of *O. cinnamomea* up to the twelfth leaf deal with changes which are purely intrastelar. The ring fence of the outer endodermis is uninterrupted, and shuts off the cortex from any connexion with those parenchymatous tracts which occupy the stele. The twelfth leaf of the seedling (Faull's Fig. 14) still shows the barrier complete. In point of fact, his drawings and descriptions furnish a demonstration of the origin of an intrastelar pith, and would never have been recognized as anything else, but for the influence of Professor Jeffrey's comprehensive theory of intrusive pith, with which I propose to deal later.

The drawings and description of Faull show further in the seedling of *O. cinnamomea* that the pith originates partly from intraxylic parenchyma, partly from the conjunctive parenchyma with which the intraxylic pith

[1] Trans. Can. Inst., vol. viii, 1909, p. 515, &c.

unites at the leaf-gaps. The question may be raised, What is the real nature of that external conjunctive tissue? It is to be noted in *Osmunda*, as it is also in the Ophioglossaceae, that the connexion is not with the parenchyma immediately within the endodermis, which might be held to be really a sister layer with the endodermis, and so be ultimately cortical in its origin; but with the conjunctive parenchyma internal to the phloem, which is truly stelar. This being so, the origin of the whole pith in the Osmundaceous seedling up to the twelfth leaf (and even further, as shown by Faull's drawings, Pl. VI, Figs. 13–17) appears to be truly intrastelar, but derived partly from intraxylic parenchyma, partly from the conjunctive tissue. Divested of all theory, this appears to be the actual fact.

Faull states specifically (loc. cit., p. 527) that in seedlings of *O. cinnamomea* 'internal phloem, and wide open leaf-gaps, through which cortex and pith communicate, were not found'. He goes on to suggest that 'their absence may be indicative of reduction expressed in the seedling stage'. But this seems an unnecessary perversion of the evidence from ontogeny in the interests of a favoured theory. It would seem better to question the truth of the theory of the reduction of the Osmundaceous vascular system from an 'amphiphloic siphonostele', than by its acceptance to be driven to construct a second hypothesis of 'reduction expressed in the seedling stage', in order to support it. If, however, the up-grade view of the structure of the Osmundaceae be accepted, the facts of the ontogeny will be found to run parallel with the facts for *Botrychium*. We may conclude from the ontogeny that an intraxylic pith originated first in all these cases, and that it is subsequently put into connexion with the conjunctive parenchyma (or xylem-sheath) at the gaps in the xylem-ring.

Further, we may see from Faull's description of sporelings of *Osmunda*, and less clearly in my weak plants of *Botrychium Lunaria*, that the purely stelar pith, once established, is liable to be almost or even entirely obliterated as it passes upwards, and that it may be again reconstituted, until it finally settles down to permanence. This is to be compared with the behaviour of the intrastelar pith in the roots of certain Leguminosae, in which Flaskaemper was able experimentally to control the disappearance and reappearance of pith, and to show that the formation of an intrastelar pith depends upon nutrition.[1]

A comparison of the structure of the sporeling in the two families suggests an interesting relation of it with the early nutrition. In the Osmundaceae the nutrition of the sporeling is by the green prothallus, which lives as it were hand to mouth. The sporeling which it produces is at first protostelic, and the vascular changes as described by Faull are entirely intrastelar for many internodes. In the Ophioglossaceae, on the other hand, there is a storage prothallus, which in *B. virginianum* is of

[1] Flora, 1910, p. 205.

large size and massive structure. It presents at once to the sporeling an ample supply of nutritive material. The sporeling in this species is, as might be expected, relatively complex in its early structure. The protostelic state is not represented in it, but it is medullated from the first. In *B. Lunaria* the prothallus is described by Bruchmann[1] as being 1 mm. to 2 mm. long, and 1 mm. wide. It is very much smaller than that of *B. virginianum*, and will offer a correspondingly smaller nutritive supply to the sporeling. We should expect from this that its sporeling would be structurally less complex. It is seen to be commonly though not always protostelic. The medullated state is certainly not the primitive condition. It may safely be concluded that the young plant of *B. virginianum*, like the offspring of the rich, is saved from early struggles. It omits the elementary structural stages, and springs at once into a condition of structural advance. If then we desire to see in their fullness the early steps of ontogeny, it is not to the plethoric sporelings we should go, but to those that are less well nourished, or it may be even starved. In them we may expect to see the steps of preliminary structure most gradually taken, and in extreme cases drawn out almost like the joints of a telescope. This was in fact found to be the case in the sporelings of *B. Lunaria* from the high ledges of the Breadalbane Hills. It is believed that in such depauperate plants the successive steps of the ontogeny serve as a safe guide in studying the origin of the vascular structure seen in the mature plant.

In the first place it is to be noted that in the early stages of these plants, though the external endodermis is well represented, an internal endodermis is absent. Sometimes it does not appear at all: in other cases it appears relatively late, in more or less direct relation to the intrusion of foliar pockets. But these are entirely absent from the first stages of the young plant. The medullation precedes them. Plainly, therefore, the pockets cannot cause the medullation. The converse is the more probable line of causality, viz. that the prior existence of soft internal tissue will favour the formation of intrusive pockets. From the study of these plants it is seen that the pith is in the first instance intrastelar, but it may subsequently be added to by the intrusion of foliar pockets. Sometimes the limits of these two components of the ultimate pith may be maintained, as shown by Pl. XLV, Fig. 9, and indicated by Poirault (Figs. 11, 12, p. 169, &c., loc. cit.). But, as is well known from the time of Van Tieghem's description of it, the endodermal limits become less definite in fully matured plants of *Ophioglossum* and *Botrychium*, while in *Helminthostachys* an internal endodermis is only known in strong and mature rhizomes.

It appears that a like condition has ruled in the Osmundaceae, but it is attained only in a more advanced state of the young plant. Here the sporeling is from the first less bulky, perhaps in relation to its assimilating

[1] Flora, 1906, p. 205.

prothallus, so different as a nursing mother from the bulky storage prothallus of the Ophioglossaceae. And the first ontogenetic stages are accordingly shown in more extended sequence. Faull points out the entire absence of foliar pockets in the young plants of *Osmunda cinnamomea*. Similarly Kidston and Gwynne-Vaughan have shown their absence from the earlier related fossils, such as *Zalesskya* and *Thamnopteris*.[1] But later in the development of the seedling of *O. cinnamomea* narrow foliar pockets make their appearance. And similarly in the later fossil forms, such as *Osmundites skidigatensis* and *Kolbei*, foliar pockets are found. Finally, in older plants of *O. cinnamomea* a continuity is established between the internal endodermis and phloem and the external, through the ramular, and less completely through the foliar gaps.[2] But this connexion only occurs in the mature plant. The ontogeny thus indicates that it is a late and derivative state. A like state is found in the relatively late fossil *O. skidigatensis*. The parallelism which thus rules between the ontogeny of the living forms of Osmundaceae and the story as based upon the study of the sequence of the fossils gives mutual strength and conviction to the evidence derived from each. From both sources the facts indicate that an up-grade development has occurred, involving a formation of intrastelar pith, and a progressive formation of leaf-pockets. But the latter seem never to have attained to a large size in the Osmundaceae, or to have contributed in the same measure as in the Ophioglossaceae to the sum of the central parenchymatous mass. This is the structural expression of the fact that in the Osmundaceae the leaves are numerous and individually smaller in proportion to the axis, while in the Ophioglossaceae, as a rule, only a single leaf is matured in each season, but it is of relatively large size.

Summary.

1. The young plant in all the genera of Ophioglossaceae is variable in its state of medullation. In some cases there is at first a solid xylem-core; in others the pith is present from the first.

2. This difference in medullation may be found between specimens of the same species, and there is reason to believe that it is determined by nutrition, the best nourished plants having a pith from the first.

3. Where the axis in *Ophioglossum* and *Botrychium* is at first protostelic, the pith is initiated below the departure of the first leaf-trace, and is referable in origin partly to intraxylic parenchyma, partly to the conjunctive parenchyma of the xylem-sheath; the two tissues being put into communication at the first leaf-gap. Meanwhile, in *Botrychium* the endodermis forms an uninterrupted barrier, shutting off the cortex; thus the medullation is wholly intrastelar.

[1] Fossil Osmundaceae, Parts II and III.

[2] Faull, Bot. Gaz., vol. xxxii, p. 417.

4. Subsequently in *Botrychium* there is a further intrusion of foliar pockets, delimited from the primary pith by endodermis. The definitive pith column is thus derived from three distinct sources: (*a*) intraxylic parenchyma, (*b*) conjunctive parenchyma, and (*c*) intrusive parenchyma of the leaf-pockets.

5. The endodermal limits in *Botrychium* are usually maintained in the lower part of the stock, and the inner endodermis may be held to indicate (as the internal endodermis of Van Tieghem and Poirault) the distinction between the intrastelar and the intrusive pith. But the endodermis is gradually obliterated in the upper region. In *Ophioglossum* the endodermis is less distinct from the first. The obliteration is probably connected with the fact that the whole parenchymatous system of the stock serves the function of storage, and physiological barriers are not required.

6. The primary xylem in *Botrychium* is in part or almost wholly replaced physiologically by the secondary xylem, which originates from the cambium. In the mature stock it is vestigial, and may be represented by the innermost tracheides of the xylem, together with occasional isolated tracheides, or groups of them, which are found about the periphery of the parenchymatous pith.

7. But in *Botrychium ternatum* a traumatic condition in a certain specimen showed tracheides scattered throughout the pith, even to its centre. If this be held to be a reversionary state, the facts would indicate that in this species the pith to its centre was originally stelar. A somewhat similar state has been observed in a sporeling of *Botrychium Lunaria*.

8. A comparison of this traumatic state of *B. ternatum* with the fossil named by Scott *Botrychioxylon* shows substantially the same structure. A close similarity exists on the other hand with young plants of *Helminthostachys*, supposing the secondary thickening of *Botrychium* to be absent.

9. The conclusion from these observations is that the pith in the Ophioglossaceae is primarily, though not always wholly, of intrastelar origin, and that the pith is in part at least intraxylic in origin.

10. A comparison of the sporeling of *Osmunda cinnamomea*, as described by Faull, shows that, up to the twelfth leaf at least, the origin of the pith is intrastelar also, and corresponds to that of *Botrychium* before any intrusion of foliar pockets has occurred.

11. As the ontogenetic history for the two families runs parallel with the history of the fossil Osmundaceae, as disclosed by Kidston and Gwynne-Vaughan, it is concluded that an intrastelar pith was formed first in the descent both of the Osmundaceae and of the Ophioglossaceae, and that an intrusion of foliar pockets may have followed, varying in extent according to the proportion of leaf to axis in the individuals of the two families.

DESCRIPTION OF FIGURES IN PLATES XLV AND XLVI.

Illustrating Prof. Bower's paper on Primary Xylem, and the Origin of Medullation in the Ophioglossaceæ.

PLATE XLV.

Fig. 1. Transverse section through the base of a root-bud of *Ophioglossum palmatum*, showing that the stock is medullated from the first. The elongated tracheides belonged to the parent root: the ring of tracheides marks the base of the axial stele. × 125.

Fig. 2. Transverse section of the base of the stock of *Ophioglossum reticulatum*, showing a protostelic state. A few parenchyma cells are present in the otherwise solid xylem-core. × 144.

Fig. 3. A section a little higher up, showing an initial state of a parenchymatous pith. × 144.

Fig. 4. A section still higher, showing the xylem-ring open, and the pith in communication with the conjunctive tissue. × 144.

Fig. 5. A drawing of the central region of Fig. 3 on a higher scale, showing that there is no exact limit between the parenchyma nests of the xylem and the pith.

Fig. 6. Transverse section of the base of the stock of a weak plant of *Botrychium Lunaria*, from the Breadalbane Hills. The endodermis is already well marked: a root is passing off to the right. The stele is a protostele, but parenchyma cells are scattered through the otherwise solid xylem-core. × 125.

Fig. 7. Section of the same plant higher up, showing a pith connected through a gap in the xylem-ring with the conjunctive tissue. The endodermis shows irregularity of its cells, and a slight involution opposite the gap in the xylem-ring. The narrow arm of tracheides which projects outwards is the first leaf-trace, of which the gap defines one margin, while it is still connected with the xylem of the stele by the other margin. × 125.

Fig. 8. Section of the same plant at the departure of the first leaf-trace. The xylem-ring is still open, and thus the pith communicates with the conjunctive tissue. But the endodermis, which is involuted but not interrupted at the passage outwards of the leaf-trace, still forms a complete barrier, shutting in the stele. The cells of the pith to the right are clearly sister cells with the tracheides surrounding them. × 125.

Fig. 9. Departure of the trace of a larger and higher leaf of the same plant. The trace consists of more numerous tracheides, the xylem-gap is wider, and the pith more massive. But the endodermis is still a complete sheath, and has shown no interruption from the base of the plant. It surrounds still the outgoing leaf-trace, but a band of endodermis is seen internal to the leaf-trace, and it is specially to be noted that it arches strongly inwards into the wide xylem-gap. × 125.

Fig. 10. Transverse section of the stock of a small plant of *B. Lunaria* from the Breadalbane Hills, from a series cut by Dr. Lang. It shows the departure of the fourth leaf-trace. Up to this point the endodermis has been a complete barrier, but now it appears interrupted for the first time, at points outside the departing trace. The pith contains scattered tracheides, showing the condition of a 'mixed pith'. It is to be noted that where tangential divisions have doubled the cells of the endodermis it is sometimes the inner, sometimes the outer of the sister cells that bears the characteristic marking. There is no internal endodermis. × 125.

Fig. 11. Transverse section of the stem of a plant of *B. Lunaria* which has produced numerous leaves, cut at a level just below the apex. The secondary thickening is beginning, but the tracheides already matured are regarded as representing the primary wood. The limit of the pith is here very ill defined. × 125.

Fig. 12. Part of a transverse section of a stem of *B. Lunaria*, showing the departure of a leaf-trace. An isolated tracheide lies in the middle of the parenchymatous tract connecting the pith with the outer conjunctive tissue. × 200.

Fig. 13. The inner margin of the xylem in a sporeling of *B. virginianum*, showing two tracheides surrounded by pith, and almost detached from the xylem-tract. × 200.

Figs. 14–16. Transverse sections of the inner margin of the xylem in plants of *B. ternatum*, showing isolated tracheides or groups of them lying free in the parenchymatous pith. × 200.

PLATE XLVI.

Fig. 17. Transverse section of the stele of an injured plant of *B. ternatum*, in the region below the injury, showing the slightly compressed stele with a large parenchymatous pith. × about 50.

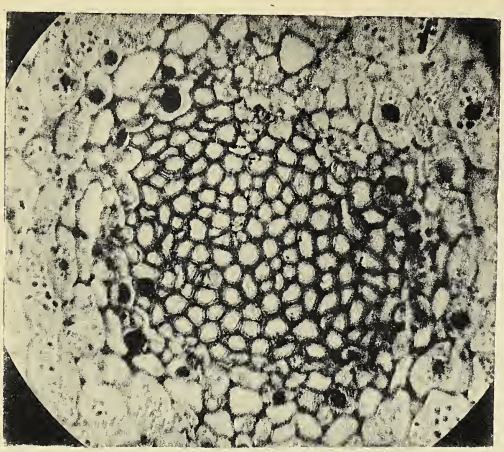
Fig. 18. A section from the same plant at the level of the injury. The stele is seen greatly contracted, with irregular xylem outside it. Internally the contracted pith contains isolated tracheides. × about 50.

Fig. 19. The central part of the above, showing more in detail the relation between the tracheides and the parenchyma cells. × 144.

Fig. 20. Another section of the same, showing isolated tracheides lying centrally in the pith. × 144.

Fig. 21. A section above the damaged region, showing the normal structure resumed, though the tissues are rather less bulky than those of the original stock below the injury. × about 50.

Fig. 22. Photograph of one of Professor Farmer's sections of a small rhizome of *Helminthostachys zeylanica* for comparison with Figs. 19 and 20 of *B. ternatum*. × 144.



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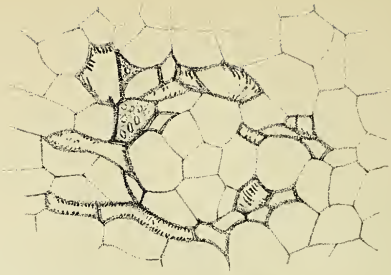

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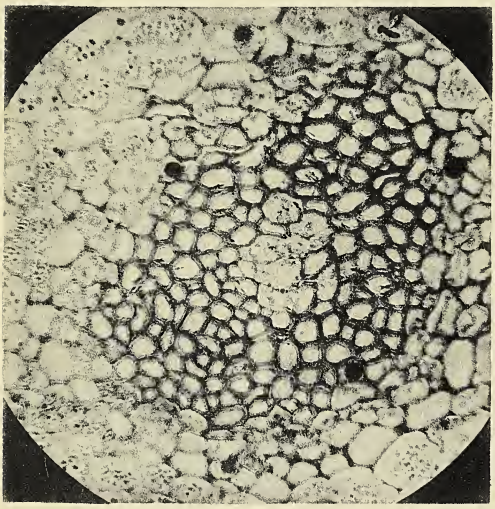

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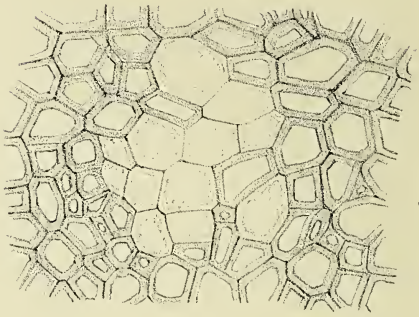

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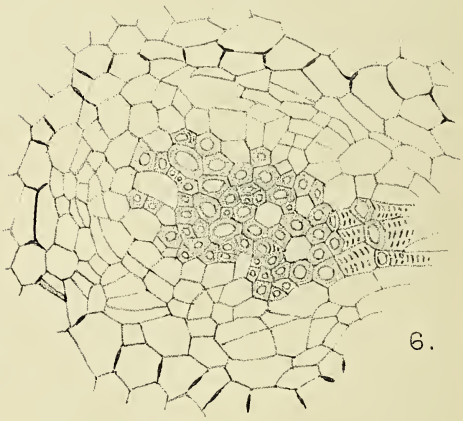

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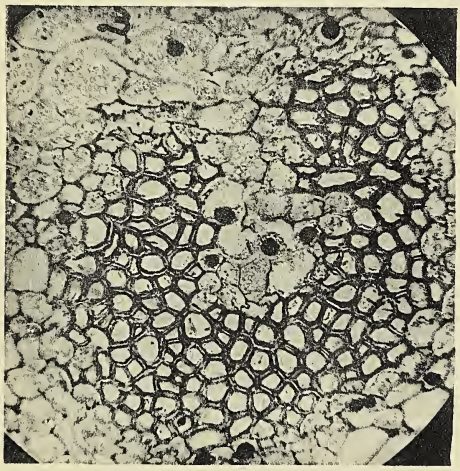

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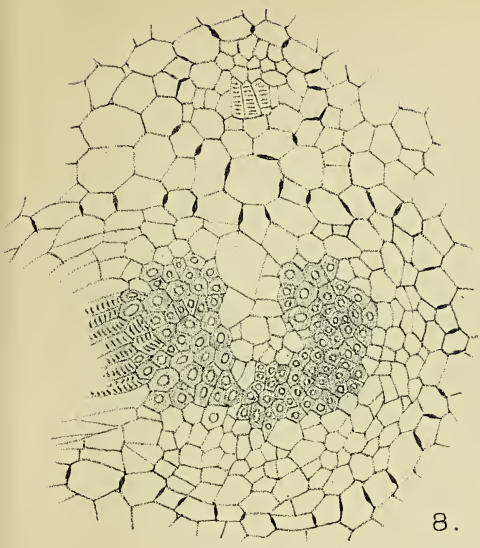

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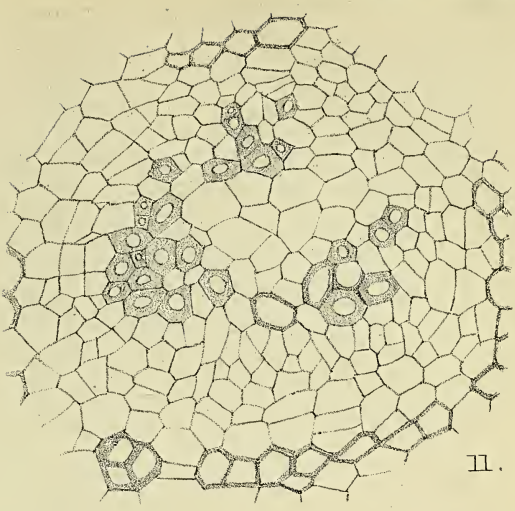

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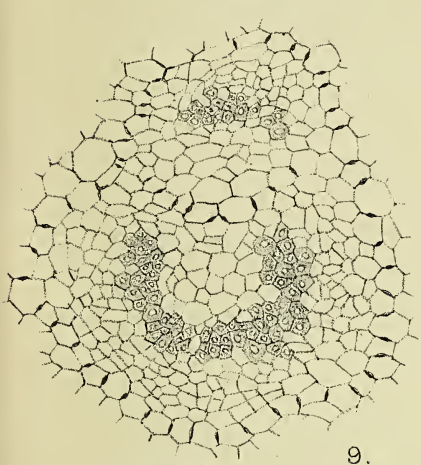

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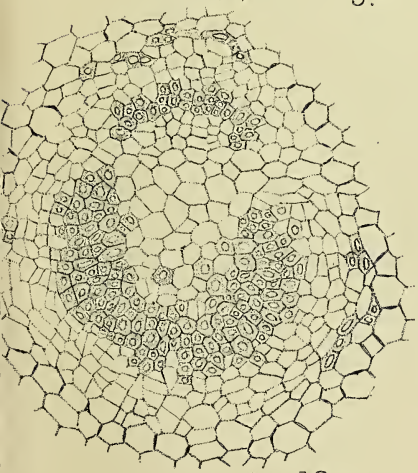

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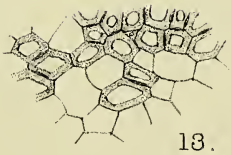

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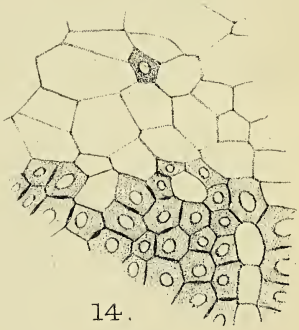

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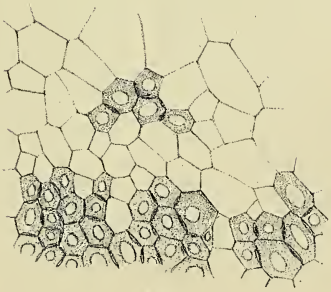

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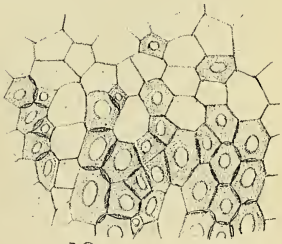

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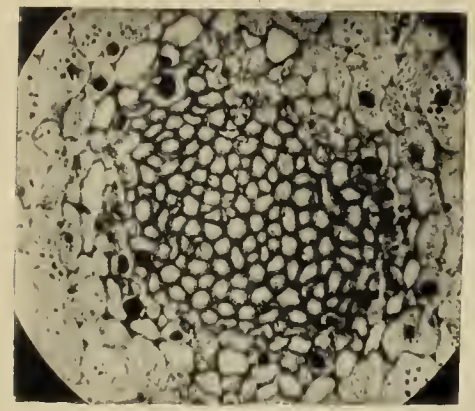

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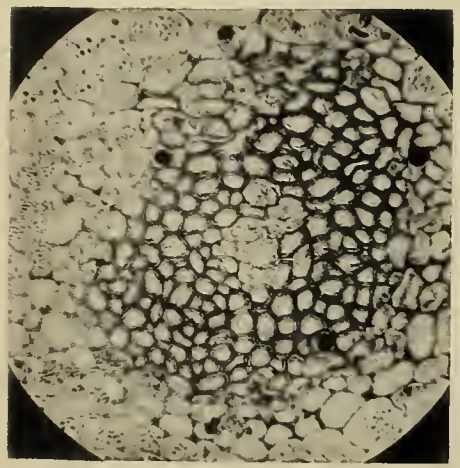

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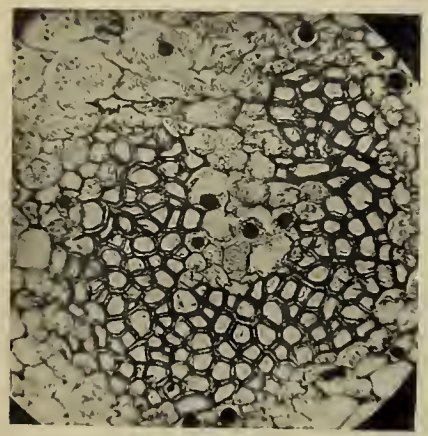

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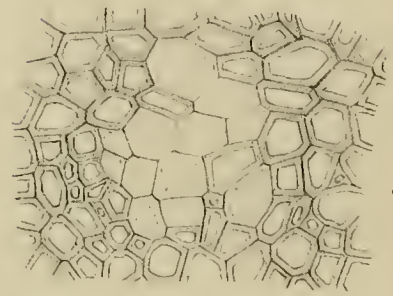

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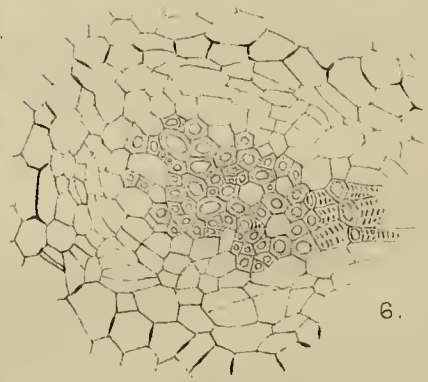

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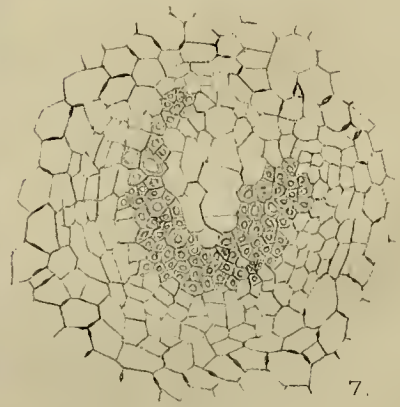

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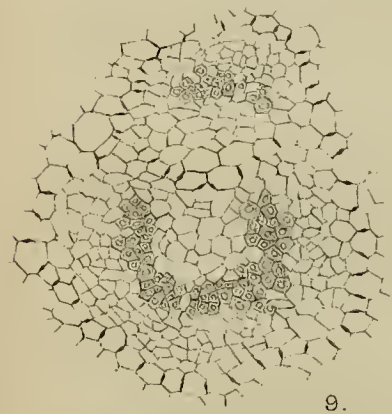

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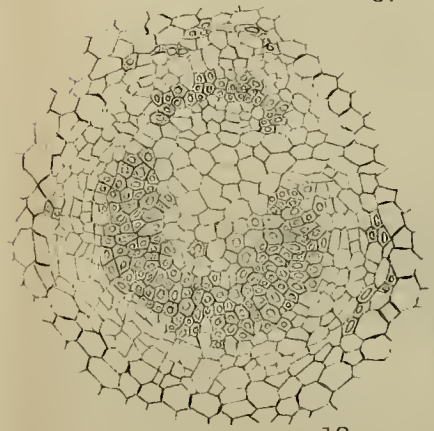

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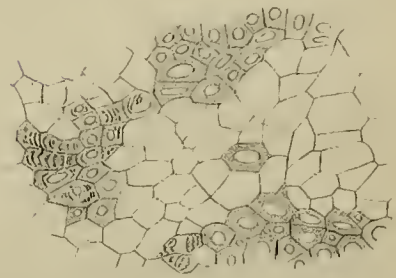

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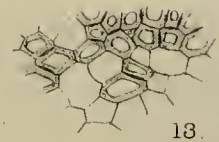

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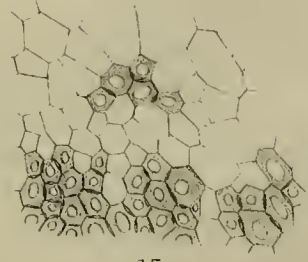

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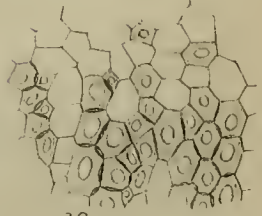

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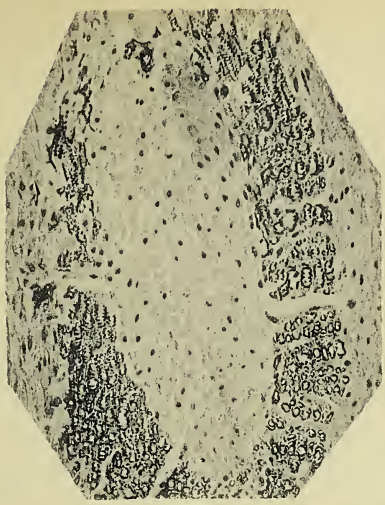
BOWER — OPHIOGLOSSACEAE.



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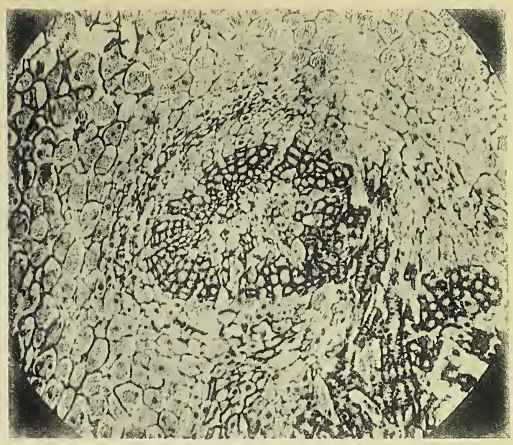

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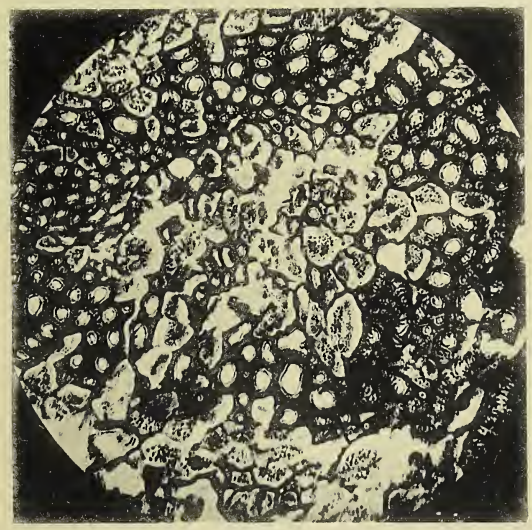

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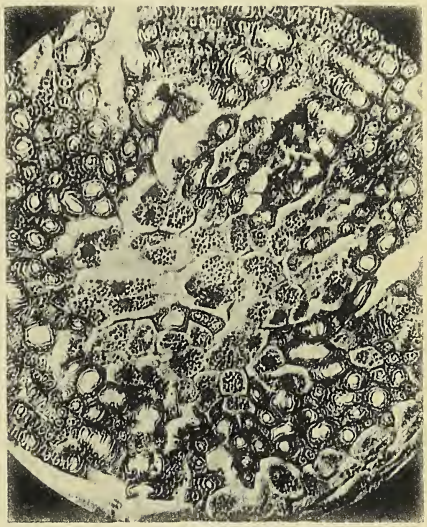

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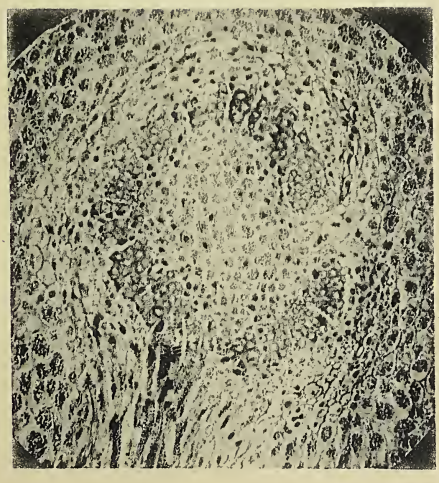

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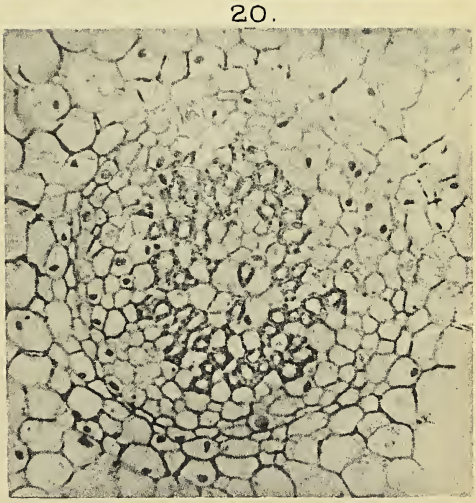

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BOWER — OPHIOGLOSSACEAE.

On Medullation in the Pteridophyta.

BY

F. O. BOWER, Sc.D., F.R.S.

Regius Professor of Botany in the University of Glasgow.

With Plate XLVII.

I WOULD willingly have left the facts and reasoning detailed in the preceding paper to take their place without further remarks in the general sum of evidence, now so rapidly growing, which relates to the origin of the pith in Vascular Plants. And I should certainly have done so, had it not been that in a recent number of the Botanical Gazette (Dec., 1910, p. 401) Professor Jeffrey has restated his position on this question with increasing decisiveness. He first enunciated his views on stelar morphology at the meeting of the British Association in Toronto, in 1897. Speaking on the original paper, I then pointed out that, in my view, a fundamental fallacy underlay the whole of his statement, and that the fallacy arose from assuming that all Vascular Plants developed their tissues along the same lines. Dr. Scott, in reviewing Professor Jeffrey's three memoirs, in which his theory had subsequently been developed, wrote later in almost equivalent terms.[1] He remarked that 'The impartial critic will probably take the view that similar vascular structures have originated in different ways in various lines of descent, and that no one rigid scheme can be applied to all'. Now, after the lapse of thirteen years, Professor Jeffrey still maintains a rigid scheme, and as regards medullation affirms for Vascular Plants that 'the pith must in all cases be regarded as a derivation of the cortex, which has become more or less completely sequestered within the stele'.[2] In his summary he adds a saving clause, stating that 'the pith of the Vasculares, in all cases where definite evidence is available, is an inclusion of the fundamental tissues of the cortex on the part of the stele'.

In the years that have elapsed since 1897 I have seen no reason to alter my first opinion as to the invalidity of the views of Professor Jeffrey on stelar morphology, and especially on the origin of pith. But as the grounds for my dissent were not put into print at the time of his first statement, the present seems a suitable occasion for doing so, while certain facts will be added which bear upon the question. Primarily my objection is the out-

[1] New Phytologist, i, p. 212.

[2] Bot. Gaz., Dec., 1910, p. 412.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

come of general morphological experience. At once the words 'in all cases' and 'must' in his latest statement will attract the critical attention of morphologists. They can quote abundant cases of parallel development and homoplasy as shown in the external form of plants. There is no reason to hold that the evolution of internal structure differed in such respects fundamentally from that of form. We learn from comparative study that plants are opportunists in their internal as well as in their external evolution, developing along lines of least resistance, and dominated neither by logic nor by rule. Consequently, in the study of their tissues, strong and consistent antitheses are not to be expected, and so we shall, from prior experience, be prepared for divergences of detail and of method in the formation of such a tissue as pith in the several phyletic lines. Generalizations in terms of 'must in all cases' require to be based upon exhaustive knowledge of detail in order to meet this general probability, and will have to be reconsidered in the light of even a single discordant fact. Thus the mode of statement adopted by Professor Jeffrey, as a dogma rather than as a working hypothesis, at once challenges prior morphological experience.

We may, however, pass from this general objection to the statement itself. It must first be premised that there are those who hold that the limits of the stele are not constant; in fact that the endodermis is not an immutable phyletic barrier between the stele and the cortex. I have no wish to prejudge this question, and myself hold it as open to demonstration. But for the moment we may here proceed on the presumption that the limits of the stele have remained constant. For us the endodermis will be a convenient indicator of tissue-locality, and we may adopt 'without prejudice' the consequent terminology. There are, in that case, three possible sources of the pith, as it is seen in Vascular Plants at large.

(i) It may be always extrastelar in origin. Subject to the saving clause already quoted, Professor Jeffrey holds that this is the constant and only source of the pith in all Vascular Plants.

(ii) It may be always intrastelar in origin. With regard to the upholders of this view we learn from Professor Jeffrey,[1] that 'the English Anatomists . . . regard the pith as in all cases a specially differentiated portion of the fibro-vascular tissue itself'; but he adds that 'of late the extremity of the English view appears to be modified somewhat by the admission that in certain instances the pith may be derived from outside the stele'. Professor Jeffrey neither names these 'English Anatomists' who hold the extreme intrastelar view nor quotes from their works. As I do not share this view, it may be dismissed without further words from the present discussion.

[1] l. c., p. 402.

(iii) It may originate sometimes in one way, sometimes in another: indeed, it may be contemplated as possible that the pith may be partly intrastelar and partly extrastelar in origin in the same individual organism. This is the view which appears to me to be in accordance with the known facts. It commends itself as being in harmony with general morphological experience, and especially in that it provides for possible cases of parallel development, which on the experience of external morphology may be expected to occur.

As the extreme intrastelar view drops out of the discussion, the question which concerns us here will be whether the pith of Vascular Plants is constantly extrastelar or whether it originates in various ways. In discussing this alternative, it must first be remarked that Professor Jeffrey appears entirely to misapprehend what will naturally be expected of him in support of his assertion of a constantly extrastelar origin. For he says[1] that, 'although it is not possible to prove in all cases that the pith may be derived from outside the stele, it is fortunately only necessary to demonstrate this in a few instances, in order to invalidate the position of those who claim that the pith is differentiated from the stele itself.' This may be a satisfactory reply to such anatomists as are said to take the extreme intrastelar view, but it is no answer to those who are prepared to see a varied origin of the pith. To show that their position is erroneous and that Professor Jeffrey is right 'in all cases', the phyletic story of the pith must be known 'in all cases', and a single discrepancy destroys his generalization. He has made his statement in rigid and comprehensive terms, and the *onus probandi* lies with him. Nor will the establishment of the phyletic history of the pith in any one line of descent have any direct bearing upon that in any other, except as an interesting analogy, *unless it can be proved that the origin of the pith antedated the phyletic segregation of those stocks from a common ancestry.* As regards the Pteridophyta, it is not yet known for certain that any two of the recognized phyla, viz. Filicales, Equisetales, Sphenophyllales, or Lycopodiales, had an origin from a common ancestor, much less do we know whether that ancestor was pithed or not. But we do know that more than one of those phyla includes protostelic types, while comparison indicates that the vascular system in all of them is probably referable to protostely as the original condition. Moreover, protostely is present in examples which on other grounds are held to be relatively primitive. Such considerations clearly indicate that the pith in each phylum, where present, has originated phyletically distinct from that of the rest. Consequently, each separate phylum in which pith appears will present its own problem of medullation, which must be solved independently of the others. There is no common rule which can be established by observations made 'in a few instances'. Professor Jeffrey must

[1] l. c., p. 403.

be prepared to demonstrate the extrastelar origin of the pith in each phyletically distinct series of Medullated Plants if his statement is to hold. The plain fact is that a statement has been made 'for all cases' before 'all cases' have been adequately investigated, and it is not put forward as a working hypothesis, but as a rigid law. Whether Professor Jeffrey's generalization be ultimately substantiated or disproved, his handling of the origin of pith cannot be quoted as a model of scientific method.

I do not propose to enter here upon any critical examination of the facts for or against the stelar or cortical origin of the pith in all the several phyla of Vascular Plants which show medullation. This will be left to the professed anatomists. But two extreme examples may be taken from the Pteridophyta, which illustrate, to my mind satisfactorily, that the origin of the pith has not been uniform. The one case provides an example of extrastelar pith, the other of pith of intrastelar origin. The first example is taken from the Filicales. No one who has followed the evidence based upon the endodermal barriers would now deny that the tissue filling the cavity in rhizomatous solenostelic Ferns is of extra-endodermal, that is of cortical, origin, certainly in the greater part, and probably in most cases altogether. The suggestion of this came first from Van Tieghem in 1890, and the idea was taken up and amplified by Professor Jeffrey in 1897. But as Mr. Tansley pertinently remarks in his 'Lectures on the Evolution of the Filicinean Vascular System' (p. 132), 'Professor Jeffrey has nowhere discussed in detail the origin of the siphonostele (solenostele) from the protostele, though he himself stated the view of the primitiveness of protostely.' The most exact demonstrations of the steps of the progression in distinct phyla of Ferns, from the protostele to the siphonostele, and of the modifications which the latter undergoes, are due to Professor Gwynne-Vaughan, Mr. Tansley, and Mr. Boodle, while by far the best comprehensive account of the process and its results is that given by Mr. Tansley.[1] It may be taken that, speaking phyletically, the cortical origin of the pith in such cases has been demonstrated by these writers. It is, however, to be clearly understood, as pointed out by Mr. Tansley,[2] that such siphonostely is characteristic of Ferns belonging to the middle grades of evolution, though it also occurs in several of the lower and a few of the higher types. Stratigraphically it has repeatedly been found in the fossils of the secondary and more recent rocks, and persists in many present forms. It remains yet to be shown that this stelar structure figured largely, or even at all, in the really primitive types of vascular construction.

The second example, where the pith appears to have been clearly intrastelar in its origin, is in the Lepidodendreae. This is indicated by a comparison of early protostelic types such as *Lepidodendron esnostense* and *L. rhodumnense* from the Culm, with the condition seen in *L. vasculare*

[1] l. c., 1908. [2] l. c., p. 56.

(= *L. selaginoides*) from the Coal Measures, where the centre of the stele is occupied by a 'mixed pith' composed of parenchyma and tracheides. A further step is seen in *L. Harcourtii*, also from the Coal Measures, where there is a bulky parenchymatous pith. It is true that Professor Jeffrey, in his Memoir on *Equisetum*,[1] states in connexion with his view of the extrastelar origin of pith, that where the pithed Lepidodendroid axis branches, the pith and the cortex are in continuity. But he does not appear to have gone further into detail on the question of the origin of the pith in the Lepidodendreae. It is to be remarked that such continuity, where it exists at the branchings, is only one condition necessary for the intrusion of the cortex into the stele. It does not demonstrate that it occurs, and it would be equally consistent with extrusion of the pith into the cortex, or with no such changes at all. In the absence of more cogent evidence than that which Professor Jeffrey has hitherto brought, the reiteration of the statement that the origin of the pith is extrastelar does not weigh against the comparison of the structure of forms in stratigraphical sequence such as those quoted. Palaeontological anatomists generally have accepted the stelar origin of the pith in the Lepidodendreae, which seems to me to be the only reasonable reading of the facts.

In presence of this evidence of the origin of an intrastelar pith in the Lepidodendreae, the next natural step in testing the truth of the conclusion should have been to see whether in their nearest modern correlatives there is any evidence of a like process, viz. in *Isoëtes* and *Selaginella*. I do not think that these comparisons have yet been made, though they would seem to be a necessary preliminary to any statement in terms of 'must in all cases'. In *Isoëtes* the anatomical and physiological conditions are so complicated and peculiar that it would not be well to lay much stress upon the structure of its primary stelar xylem. It appears to have the character of a 'mixed pith' with tracheides and parenchyma intermingled. But a better basis for comparison is in *Selaginella*, and it is not to be sought in the more specialized dorsiventral types of the genus, but in those of upright habit, with radial shoots, which comparison shows to be the more primitive, and therefore more directly comparable with the Lepidodendreae.[2] Such a species is *S. spinulosa*. Harvey Gibson[3] has long ago described the structure of its stele, and shown how it is first centroxylic, and that as it continues upwards the protoxylem strands pass to the periphery of the solid xylem-core. But he did not follow it upwards into the erect unbranched strobilus, and it is there that the point of structure appears which has a bearing upon our present question. If sections be cut through the axis below the strobilus, the stele may be found with a solid xylem-core as already described by Harvey Gibson, the protoxylem groups being peripheral.

[1] Mem. Bost. Soc. Nat. Hist., 1899, Pl. XXVII, Fig. 3.

[2] Land Flora, pp. 300, 356.

[3] Ann. Bot., vol. viii, p. 171.

But sections about the base of the strobilus show occasional thin-walled cells irregularly interspersed among the tracheides (Pl. XLVII, Fig. 1). The protoxylems are still numerous and peripheral and project slightly, while the xylems of the leaf-traces on entering the stele fuse directly, though often obliquely, each with a protoxylem which descends from a higher region of the axis. In sections taken at a higher level the thin-walled elements increase in number, forming a considerable though irregular tract towards the centre of the stele. This is, however, continuous outwards as irregular, thin-walled rays; these connect with the outer conjunctive tissue (Fig. 2). Occasionally an isolated tracheide may be found embedded in the soft central tissue (Fig. 4), or more than one of them (Fig. 6). Higher up (but still in a region where the tissues are already matured and the sporangia are in an advanced state, bearing megaspores with thick, dark-coloured walls) the central thinner-walled tissue predominates, and the xylem is divided into distinct strands, with broad thin-walled rays intervening between them. Figs. 3 and 6 show this. In Fig. 4 it is seen that the insertion of a leaf-trace impinges slightly obliquely upon one of the strands. Longitudinal sections show that the softer elements are elongated, with protoplasm and nucleus. Occasional transverse walls occur, but there is no conspicuous shortening of the cells, as is the case in the pith of most plants. This tissue may accordingly be held to represent degraded xylem.

It is thus seen in a living plant that a change of structure appears, involving the formation of a soft, pith-like intrastelar tissue, and it is to be noted that it occurs in a case where there is no branching of the axis, nor are there any foliar gaps to provide that continuity with the cortex without which cortical intrusion cannot take place. Moreover, the trabecular zone intervenes between the stele and the cortex uninterruptedly. It may of course be asserted that the softer central tissue is not really a pith because the cells are elongated. They are, however, thin-walled, and retain their protoplasm, and form a central living tract of tissue which is not vascular. These are the essential features of a pith. It may also be urged that the soft tissues are merely the result of imperfect development of tracheides, and that they would mature into tracheides later. Against this it is to be remarked that the tissues have the appearance of maturity, while the condition of the sporangia and of the strobilus as a whole shows that further development is not to be expected. This intrastelar tissue in *S. spinulosa* may in fact be held as comparable with the pith seen in certain Lepidodendreae. And a very remarkable parallel is seen between the slender cone of this *Selaginella* and the massive cone of *Lepidostrobus Brownii*, in respect of the internal structure.[1] I have shown elsewhere[2] how in this

[1] It may be noted also that a pith exists in the stele of the cone of *Spencerites* (Miss Berridge, Ann. Bot., xix, p. 274, Fig. 2). Doubtless other examples may be found in the cones of fossil Lycopodiales.

[2] Ann. Bot., vii, p. 347, Pl. XVI, XVII.

fossil there are air-spaces comparable with the endodermal space in *Selaginella*, though not in exactly the same place in the tissues. There is also a tendency to breaking up the xylem of the stele into separate strands, as in *S. spinulosa*, and, lastly, in both of them there is a mass of softer tissue occupying the centre of the stele. This I regard in both cases as an intrastelar pith, resulting from degradation of the central region of the xylem-core, a conclusion which is supported in both cases by the existence of intermediate conditions between the indurated tracheide and the thin-walled cell. Facts such as these seem to me to be incompatible with the statement of Professor Jeffrey that 'the pith must in all cases be regarded as a derivative of the cortex'. But, further, there is already at hand evidence that the formation of an intrastelar pith can be artificially determined by experiment. This has been shown by Flaskaemper in the roots of certain Leguminosae.

The medullation of roots has not hitherto figured in these discussions. But as a central medulla is frequently present in them, it would be interesting to see how it originates, and then to draw the analogy with what is seen in the shoot. No doubt the pith of the root may frequently be in continuity with the pith of the axis. It certainly is so in many seedlings, and in that case it would be open to those who hold that its origin is always cortical to regard it as a further involution from the pith of the axis, and thus be cortical in origin, though, in a sense, at second hand. But recently experiments have been described by Flaskaemper,[1] which demonstrate in the root that pith may be formed *de novo*, within the stele, where no possibility of involution exists. He found [2] that, if after a seedling of *Vicia* or *Phaseolus* had germinated and the radicle had begun to elongate the cotyledons were removed, the plant was checked, and the root, previously pithed, lost its pith in the parts subsequently formed. But when the plant had recovered strength by continued exposure to favourable conditions, the pith was reconstituted in the later parts of the same root. In a typical case, where the seedling had formed its root 6 cm. long, the cotyledons were removed: the seedling was then kept under careful cultivation for six weeks, during which time the root grew on to 26 cm. An examination of its stele from the base onwards showed in the first 5 cm. a normal tetrarch structure with many pith-cells. At 6 cm. the pith appeared reduced, and at 7 cm. pith was absent. The pithless state continued till about 20 cm. from the base, but in the distal part of the root, formed after the plant had recovered the loss of its cotyledons, pith appeared again in the transverse section. Here, then, is a clear case of the origination of an intrastelar pith in a root, without the possibility of any involution of the cortex from without. This analogy, which has a very close parallel with what has been described by Faull in his seedlings of *Osmunda*, and with my own observations on

[1] Flora, 1910.

[2] l. c., p. 205.

a weak *Botrychium* above, p. 548, has its value as bearing on the question of origin of the pith in the axis, and is a further indication of the invalidity of Professor Jeffrey's generalization.

It seems to me useless to bring forward any further considerations in order to rebut the rigid assertion of Professor Jeffrey that the pith 'must in all cases be regarded as a derivative of the cortex'. But criticism without construction is arid and unprofitable. Assuming on the basis of such examples as those quoted that the pith is not uniform in its origin, I shall now suggest a relation which appears to subsist between the form and position of the shoot and the type of its medullation, where that structure occurs. It is first necessary to show that such a relation exists, and the causes of it may be considered later. The two examples above, taken to illustrate respectively a stelar and an extrastelar origin of the pith, show strong antithesis to one another, both in the position and in the proportions of the shoot. The Lepidodendreae were upright, with columnar axis and relatively small, closely set leaves, as is also the cone in the living *Selaginella spinulosa*. On the other hand, the solenostelic, rhizomatous Ferns, such as the Dennstaedtiinae, the Matonineae, and certain Pterideae, are of creeping habit, with relatively large leaves, seated singly at intervals along an elongated rhizome. In the one case the upright axis is the dominant factor in the shoot, and the pith, where present, is intrastelar in origin. In the other the leaf is a dominant factor over the weak and prostrate axis, and the pith is extrastelar in origin. I suggest that *the position of the axis, together with the proportions of the appendages to the stem, at the time when the medullation was phyletically initiated, have been determining influences upon the method of origin of the medullation.* Once the method of the medullation was stamped in any phylum it might be continued on the same plan, with a high degree of conservatism, even though the position of the axis or the proportion of the parts might be altered. It is, in fact, suggested that in the first instance an upright microphyllous stock has favoured intrastelar medullation, while a creeping megaphyllous shoot has favoured extrastelar medullation. A test whether this relation is a real and valid one will be found in those plants in which mixed conditions occur; for instance, where the axis is and has phyletically been upright, but the leaves relatively large; or, again, where the stock is a creeping one, but the leaves only small. It will presently be shown that the mode of origin of the pith in cases where such mixed conditions occur accords generally with the suggestion, while they supply such various states of the tissues of the axis as may lead to a knowledge of the underlying principles which govern its structure.

In Chapter XVI of the 'Land Flora' I have advanced strong reasons for holding that the primitive symmetry of the Archegoniate sporophyte was radial. We know also for the Lycopodiales, Equisetales, Ophio-

glossales, and Marattiales, that the axis of the embryo is vertical, and that it grows directly into the upright seedling. But in the Leptosporangiate Ferns the embryo is prone at first. Nevertheless the radial upright stock is established at once in many Ferns. In fact, in plants which are radial in the mature state the initial condition of the recumbent embryo does not impress dorsiventrality on the seedling.[1] This is the case for *Osmunda* and *Todea*, so that for all practical purposes in the development of the stele these genera may be held to have had vertical axes from the start. It is uncertain what may have been the condition of other Leptosporangiate Ferns as regards their phyletic history, but it is significant that those great parent stocks, the Gleicheniaceae and Schizaeaceae, include prostrate forms among their most primitive types. This question will be discussed more fully on a later occasion.

It has been seen that those Lycopodiales which are pithed were upright microphyllous forms, with intrastelar pith. The same may be said for the Equisetales, though here the formation of pith and degeneration of the primary wood has gone much further.[2] But the Ophioglossaceae and Marattiaceae, together with the Osmundaceae, are megaphyllous forms with upright stocks. The question will be, What is the nature of their medullation? Of these the Marattiales, with their very complex dissolution of the vascular system into separate strands, present a peculiarly complex problem of their own, and may for the present be left on one side.[3] The most interesting cases of megaphylly with a vertical stock among forms which may be held as primitively upright are then the Osmundaceae and the Ophioglossaceae.

The Osmundaceae are still the centre of keen discussion in respect of the source of their medullation. The one view is that the stelar condition seen in the living examples of the family is the result of reduction from a more complex condition, which was that of the 'amphiphloic siphonostele'. This view is based on the study of the current representatives of

[1] l. c., p. 214.

[2] For the conclusions of a discussion of the stelar condition in the Equisetales, see Land Flora, p. 391. I see no reason to change the opinions there expressed, though the subject has been again opened by Eames (Ann. of Bot., 1909, p. 587).

[3] It may be noted that Farmer and Hill (Ann. of Bot., xvi, Pl. XVI, Figs. 11–13) have illustrated the origin of a pith, which can only be intrastelar, in *Angiopteris*. Brebner (Ann. of Bot., xvi, p. 550) points out that *Danaea* differs from *Angiopteris* and *Marattia* in not passing through a medullated stage. Further, since this paper was drafted, Miss Charles's paper 'On the Anatomy of the Sporeling of *Marattia alata*' has appeared (Bot. Gaz., Feb., 1911, p. 81). A comparison of her Fig. 8, Pl. IX, with my Fig. 8 for *Botrychium Lunaria* shows a substantial similarity. In both cases there is intraxylic parenchyma, and apparent intrusion of parenchyma lying more externally. But after this the parallel seems to cease, and *Marattia* takes its own divergent way. Thus the evidence, so far as it goes, indicates that in the Marattiaceae a pith may be absent altogether: or it may appear temporarily before the peculiar break-up of the stelar system into meristeles. But in any case it is partly, or perhaps even wholly, intrastelar, and shows some analogy with what is seen in the Ophioglossaceae and Osmundaceae.

the family, together with reference to one relatively recent fossil. It is held by Professor Jeffrey and his pupils. But they have not advanced any evidence in support of it from early fossils. It would strengthen their case if they were to produce specimens of the reputed ancestors, showing the 'amphiphloic siphonostelic' structure, from early strata. But this has not yet been done. The other view is that the present condition shows the approximate limit of an up-grade development, which arose from a protostelic state, without the amphiphloic siphonostele having figured in the earlier stages at all. It has been based upon the study of a stratigraphical sequence of related fossil forms carried out by Kidston and Gwynne-Vaughan. This view would ascribe the origin of the pith to direct medullation of the protostele. The alternative view would be that the pith originated wholly by intrusion of the cortex through the foliar gaps, according to Professor Jeffrey's generalization. If the present Osmundaceous structure has really been reduced, the fossil correlatives should indicate progressively a nearer approach to a condition of amphiphloic siphonostely. But, with the exception of the relatively late *Osmundites skidigatensis*, the reverse has been shown to be their general trend. The facts and arguments of Kidston and Gwynne-Vaughan are now so well known that they need not be recapitulated here: nor yet the rejoinders with which they have been met by the other side.[1] It will suffice to consider the facts as stated by Kidston and Gwynne-Vaughan for the Osmundaceae from the point of view of the hypothesis above propounded. What we should anticipate, if it were true, would be that the stele should show intrastelar pith in accordance with the constant and phyletically erect position of the axis. Also that there should be some degree of formation of foliar gaps in accordance with the megaphyllous character. Kidston and Gwynne-Vaughan have shown, and illustrated by photographs of sections, and by diagrams, the existence of imperfect foliar pockets in *Osmundites Kolbei* and *skidigatensis*, two of the more recent fossil types. They are comparable in essentials with those relatively small pockets found in the

[1] The Fossil Osmundaceae: Parts I–IV, Trans. Roy. Soc. Edin., 1907–10; Jeffrey, Bot. Gaz., Jan., 1908, p. 67, and Nov., 1908, p. 395; also Bot. Gaz., 1910, pp. 401, 476; Faull, Trans. Can. Inst., vol. viii, p. 515, &c.; Sinnott, Ann. of Bot., 1910, p. 107. It is interesting to note how, in the last-named work, doubts of the accuracy of the observations of Kidston and Gwynne-Vaughan grow in the course of a few pages to full assurance of their error in the interpretation of a fossil which the critic has never seen. On p. 110 Sinnott, writing of *O. Dunlopi*, remarks: '*it seems entirely possible* that very narrow rays . . . *might have* occurred.' This suggestion is based on 'the present indifferent state of preservation' of a fossil he has never personally examined. On p. 111 his statement on the same fossil is strengthened thus: 'On the whole *it seems very probable* that we have here to deal with a form . . . where foliar gaps were *always* present.' At the conclusion of the paper the general statement which covers *O. Dunlopi*, together with the rest, is couched in still stronger terms (p. 116) thus: 'From such fossil evidence as is available therefore . . . *it seems quite clear* that the presence of foliar gaps is a primitive feature in the Osmundaceae.' The italics are mine. Readers will form their own estimate of the value of evidence thus accumulated in support of a favoured theory.

living genera of Osmundaceae. But such pockets are absent from the more ancient forms. This suggests that there has been a progressive formation of such pockets. On the other hand, in the earlier types there is merely a differentiation of the massive xylem-core into a centrally-lying region composed of short, thinner-walled elements, and a peripheral ring of firmer wood (*Thamnopteris*). In later forms, such as *Osmundites Kolbei*, the centre was occupied by a 'mixed pith', containing tracheides, while the remaining xylem consisted of separate strands.[1] This leads naturally to the condition of the modern types, with their central pith of parenchyma, but occasionally containing tracheides, as shown by Faull.[2] The intrastelar pith is foreshadowed in the earlier fossil types, and the stratigraphical sequence illustrates its origin along lines in accordance with the theory. Thus the actual structure of the Osmundaceae, ancient and modern, is such as to accord with what might have been anticipated, supposing the suggested relation of habit to internal structure to be valid.

Similarly, in the case of the Ophioglossaceae, probably their stock was primitively upright, and they are not only megaphyllous, but they develop commonly one leaf only in each season, and that of a large size (monophyllous). Supposing the suggested relation to hold, they should have an intrastelar pith in accordance with their upright stock, together with intrusive pockets in accordance with their megaphyllous character. Since in *Ophioglossum* the endodermis is less perfectly developed than in *Botrychium*, it is from the latter that the best evidence may be derived.

It has been seen in the preceding paper (p. 543) that a pith, which is at first entirely intrastelar, arises partly from the intraxylic parenchyma, partly from intrusion of the conjunctive parenchyma. In weak plants this condition may be continued for some distance, and appears to correspond in origin and in structure with that demonstrated by Faull in young plants of *Osmunda cinnamomea*. But the intrastelar pith thus formed is liable to be encroached upon by intrusive foliar pockets, which become successively larger with the enlargement of the leaves. The endodermis, yielding

[1] Professor Jeffrey (Bot. Gaz., Dec., 1910, p. 476, &c.) meets the evidence adduced by Kidston and Gwynne-Vaughan from *Osmundites Kolbei* with the remark that 'the unprejudiced anatomist would scarcely admit the accuracy of their statements on the evidence they submit'. Doubtless the authors may have something further to say on this. Meanwhile, the 'unprejudiced anatomist', of whom we have heard so much in these discussions that we begin to doubt his existence, will draw his own balance between the criticisms of a writer at a distance from the specimens, who has a very rigid statement to defend, and the carefully considered conclusions of two experienced observers, whose study of the fossils themselves has been the basis of their statement. Moreover, he will take fully into account the coherent and consecutive features of their evidence, of which the facts for *Osmundites Kolbei* are only a fragment. Faull and Sinnott also take the same line of defence of their theoretical position as Professor Jeffrey by discounting the evidence from the fossils (which they have never seen) on ground of their imperfect preservation. Those who are accustomed to the interpretation of fossil structure will probably be able to assess at their proper value these criticisms *in absentiâ*. See Faull, l. c., p. 530, and Sinnott, l. c., p. 107, &c.

[2] l. c., p. 525.

apparently to encroachment of the cortex on the stele, projects convexly into the pith, and constitutes that internal endodermis recognized by Van Tieghem and Poirault. Thus the medullation in *Botrychium* corresponds to the theoretical anticipation, being both intrastelar and extra-stelar in origin. It arises from three distinct sources: (*a*) intraxylic parenchyma, (*b*) conjunctive parenchyma (both of which are intrastelar), and (*c*) intrusive parenchyma of the foliar pockets (extrastelar).

The details as regards the origin of the pith in *Helminthostachys* are not yet to hand. It should afford an interesting example, being megaphyllous, but having a vertical position of its axis only in the earlier state of the individual. In its older stages the rhizome becomes prostrate, a condition which, on our hypothesis, should lead to the formation of large pockets in a plant with leaves of so large a size.

The Osmundaceae and Ophioglossaceae are thus seen to accord structurally, though in varying degree, with the theoretical anticipation. Both of them show an intrastelar pith in relation to their primitively vertical axis, and leaf-pockets may also be formed of varying size in relation to their megaphyllous character. The converse must now be examined. We shall inquire whether in any microphyllous shoots of rhizomatous nature there is evidence of a pith of extrastelar origin.

It may be said at once that the large majority of creeping microphyllous forms do not illustrate the anticipated structure. But this might be expected, for, judging from other experience, it is only in cases where the axis had already the creeping habit at the time when the medullation was phyletically initiated that the effect would be likely to appear. In cases where an intrastelar medullation preceded the adoption of the creeping habit of the axis, the course of medullation primarily initiated appears to have been continued, even though the creeping habit might subsequently be assumed. This may be illustrated by the Equisetales. In them the embryogeny is vertical and the primary shoot is upright. We may presume that an intrastelar pith was phyletically initiated in the primary axis, as it is structurally seen in the primary axis of the embryo to-day. The creeping habit being only assumed subsequently by branches of a higher order, these maintained, with the conservatism already recognized, their primitive type of medullation, that is the intrastelar. But in other cases medullation may be entirely absent, as in the creeping axes of the more advanced types of *Lycopodium*. This is also the case for the straggling species of *Selaginella*. But within that genus there is the well-known case of *S. laevigata*, var. *Lyallii*, which illustrates the point in question, though with details which plainly indicate homoplasy as compared with the Filicales. Considering the constancy of the vertical embryogeny in the microphyllous forms, and the fact that the upright radial axis is their primitive type, the rarity of the reaction in them cannot be held as a valid objection.

Selaginella laevigata, var. *Lyallii*,[1] has a short creeping rhizome, sharply differentiated from the upright 'foliage' shoots. The latter show a number of steles arranged with no definite order. But in the creeping rhizome the stelar arrangement closely resembles that of a typical Filicinean solenostele, with gaps formed here not by the departure of leaf-traces, but of the aerial branch-traces. Centrally there may be an accessory vascular strand, or more than one, behaving just as do the central strands in certain rhizomes of solenostelic Ferns. The similarity is so great that it has repeatedly been the subject of remark, and it has even been suggested that it might have a phylogenetic bearing.[2] This would seem to be an erroneous view, for *S. laevigata* is one of the more highly specialized and most definitely rhizomatous species of the genus, in which its type of structure is rare, if not actually unique. Moreover, the structural comparison is with such Ferns as the Pterideae and Matonineae, which are not specially primitive types among the Leptosporangiates, and phyletic comparisons can only properly be suggested between the most primitive types of such distant phyla, if indeed at all. It seems to be an example, and a very remarkable one, of homoplasy, of which the stimulating cause has been the horizontal position of the rhizome in each case. But the stelar gaps in the rhizomatous Ferns are foliar, in *Selaginella laevigata* they are ramular. In both cases the inserted organ is relatively large as compared with the rhizome itself, and this proportion has probably been also a factor leading towards the similar structural result. The existence of such a structure in a microphyllous type with creeping rhizome provides an example in accordance with our second theoretical anticipation. This plant, in accordance with the creeping habit of the rhizome, has by adjustment of the stelar masses enclosed a pith-like mass of ground tissue, which was plainly of extrastelar origin.

The structure thus seen in these converse test cases indicates that the suggested relation is really existent. It appears that the position of the axis and the proportion of the appendages relatively to the axis have been determining factors in the formation of the pith respectively from an extra- or an intrastelar source. It will, however, be at once objected that the Dicksonieae and the Cyatheae, and a host of other Leptosporangiate Ferns, are megaphyllous, have upright axes, and still their pith is of extrastelar origin. At first this would seem to be a fatal objection. But it is to be remembered that *according to the theory the determining influence is effective at the time when the medullation is first initiated, and once started, the same type of medullation is apt to be retained, even after a change of position of the axis has occurred.* At the British Association at Sheffield (1910) I brought

[1] See Harvey Gibson, Ann. Bot., viii, p. 187, Pl. IX, Fig. 76, and Pl. X, Figs. 77, 83–93; also Jeffrey, Mem. Bost. Soc. Nat. Hist., vol. v, No. 5, p. 160; also Tansley, Lectures, p. 135.

[2] Harvey Gibson, l. c., p. 192.

forward evidence to show that in the Cyatheaceae the erect habit is secondary, and that the family are derived from a rhizomatous ancestry, probably of near kinship with the creeping Gleicheniaceae. They appear to have become erect subsequently to the formation of a pith by intrusion through the leaf-gaps. The same is probably the case for the Dicksonieae and for *Plagiogyria*, and my own comparative studies already indicate that a like probability will emerge in the case of other upright Leptosporangiate Ferns. The point is that in such cases the extrastelar tissue had already penetrated through the foliar gaps into the stele of the rhizomatous ancestry before the erect habit was assumed, and that that type of medullation, once initiated, was perpetuated with minor modifications when the race later assumed the upright habit. It is this conservatism more than anything else which has concealed the factors that have been at work. Examining Ferns at large, without any clear phyletic ideas regarding them, we find the pith extrastelar in many upright as well as creeping forms. This apparent inconsistency has obscured the relation which seems to have subsisted in the first instance between the prostrate position and the initiation of an extrastelar pith.

Two leading factors have been mentioned in the preceding paragraph as influencing the medullation of Vascular Plants, viz. the position of the axis at the time when the medullation was initiated, and the disturbing influence of the insertion of the appendages. Other factors not yet recognized may also have had their effect. Pending their discovery, it may now be inquired what is the balance of influence of these two factors in cases where both may have been effective, and whether that balance is constant or variable. It is in such plants as the Osmundaceae and Ophioglossaceae, where the two factors are, in a sense, pitted against one another, that the striking of the balance may be witnessed in its structural results. For in them the axis is upright, which might be expected to encourage the formation of an intrastelar pith, while the relatively large leaves would encourage the intrusion of foliar pockets, leading to an extrastelar pith.

A further circumstance, which evidently had its effect (and may indeed be regarded as a mechanical consequence of the creeping or upright habit of the shoot), is the relative size of the axis compared with the area of the leaf insertion. Megaphylly is merely a term indicating vaguely a large size of the leaf. But the proportion of leaf to axis may vary even in types accepted as megaphyllous. And this fact is also illustrated by a comparison of the Osmundaceae and Ophioglossaceae. In the stratigraphically older forms of the Osmundaceae, which had a very bulky axis, but no leaf-pockets (e.g. *Thamnopteris*), the large xylem-core already shows differentiation at its centre in the direction of a pith formation. Leaf-pockets appear in the later forms of the phylum (*Osmundites Kolbei*), but being small they never seem to have influenced the general structure so greatly as did the intrastelar pith,

which appears as a 'mixed pith' in *O. Kolbei*. Finally, in the more modern Osmundaceae the pith is fully formed, while the leaf-pockets are never seen to be so large as in other Filicales. It is thus seen that where, as in the Osmundaceae, the axis is upright and relatively bulky, and the leaf insertion relatively narrow, the intrastelar pith is dominant, while the leaf-pockets remain relatively small. But in the Ophioglossaceae the proportion of the upright shoot is habitually different. They are commonly monophyllous, producing only one leaf in each season, but that leaf is larger proportionately to the axis than are the numerous leaves of a season in the Osmundaceae. We should then expect that the intrastelar medullation in them would be proportionally smaller, and the foliar pockets be relatively more prominent. This result is clearly indicated by the observations on the seedlings of *Botrychium* above detailed.

But if the balance of size of the parts really does influence the internal structure, as suggested, an interesting condition should be seen in the seedling of *Botrychium Lunaria*, where the first leaves are all minute scale-leaves. If the size of the leaf-pocket is in any way proportional to that of the leaf itself, the pockets of the earliest leaves of the seedling of this species should be small. That is exactly what is shown by the series of sections of the young plant of *B. Lunaria* described above. There is indeed no intrusive pocket at all in relation to the first minute scale-leaves of this seedling, and the barrier of endodermis is unbroken, so that there is not any communication at all between the cortex and the pith. The latter is present, however, though of relatively small dimensions, and is thus intrastelar in its origin. But in the larger leaves the endodermis curves convexly inwards, forming a pocket that encroaches on the pith. There is thus indication that in the Ophioglossaceae there is fluctuation of the balance between the two factors, and that it may even vary in the individual plant. The facts strongly suggest that the pith is here of two distinct origins, partly intrastelar and partly extrastelar, a condition which was contemplated as possible at the opening of this essay. But the original endodermal limit between the two constituent parts is often obliterated by the loss of its endodermal characters, partially or altogether. This is found in the upper regions of *B. Lunaria*, and it appears to be the constant condition in *B. ternatum* and *virginianum*, as it is also for most of the species of *Ophioglossum*. In this genus, however, the obliteration of the endodermis has gone still further, and involved commonly the external endodermis also. In the family as a whole the irregularity of occurrence of the endodermis is probably due to there being no physiological need for the maintenance of the barrier between the various parenchymatous masses, which are used for storage purposes. That being their prime function, the less the barriers to physiological transit the better. Thus the imperfection of the endodermis finds a physiological explanation. Nevertheless, the two components of the

Ophioglossaceous pith may have been, and probably were of distinct origin, and in the first instance have been regularly delimited by endodermal sheaths, vestiges of which were first recognized as present in them by Van Tieghem and Poirault. But these are more clearly seen in the seedling than in the mature state. Thus the ontogeny supports the conclusion.

In drawing this discussion to a close, its results may be briefly summarized as follows:—

1. The evidence, whether from anatomy of the mature plant, or from the development of the seedling with its ontogenetic bearings, or from the stratigraphical sequence of the fossils such as the Osmundaceae and Lepidodendraceae, converges in the support of the conclusion that the origin of the pith in the Pteridophyta has not been uniform. Its source may vary even in the individual according to its stage of development, or its physiological condition.

2. It may be derived in the following ways:—

(i) Wholly from an intraxylic source, by degeneration of the vascular tissue: as in the Lepidodendraceae, the primitive Osmundaceae, and probably also in the Equisetales:—or partly so, as in *Selaginella spinulosa*, the Ophioglossaceae, the modern Osmundaceae when young (Faull), and *Marattia* (Charles).

(ii) The conjunctive parenchyma outside the xylem, but still intrastelar, may contribute to it: as in the Ophioglossaceae when young, and the seedling *Osmunda* (Faull). In *Selaginella spinulosa* also it appears to have played some part.

(iii) The inner layer of the double endodermis may be a source of intrusive parenchyma: this appears to have occurred in the *Marattia* seedling (Charles), and possibly also in some other cases.

(iv) The endodermis and cortex form intrusive leaf-pockets: as in the Ophioglossaceae after the first stages of the young plant are passed, and in the Leptosporangiate Ferns at large.

(v) The steles may be adjusted so as to surround a centrally lying tract of cortical origin (*Selaginella laevigata*, var. *Lyallii*).

3. It has been possible to suggest hypothetically certain conditions which have been influential in determining from what source the medullation shall originate in any given case: viz. that in the first instance an upright microphyllous stock has favoured intrastelar medullation, while a creeping megaphyllous stock has favoured extrastelar medullation.

4. It has been found that in upright stocks which are megaphyllous, the medullation may be partly intrastelar, partly extrastelar, and that the balance between the two factors may be approximately forecast from a knowledge of the proportions and position of the shoot.[1]

[1] Professor Gwynne-Vaughan suggests as a further factor the form of the leaf-trace itself.

5. It has been found that in a microphyllous stock which is of creeping habit an extrastelar medullation may be attained by adjustment of the stelar tissue, so as to resemble the condition seen in rhizomatous Ferns. This is the case in *Selaginella laevigata*, var. *Lyallii*.

6. Many facts are found to indicate that when once either intrastelar or extrastelar medullation has been initiated in a phylum it is apt to be retained, even after a phyletic change of position of the axis has occurred.

This account of medullation in the Pteridophyta is based upon such data as have been quoted in the text. Other factors than those recognized may emerge as affecting the origin of the pith, and the summary is open to amendment and modification. But it is believed that it more adequately expresses the essential features of medullation in the Pteridophytes than the rigid statement of Professor Jeffrey that 'the pith must in all cases be regarded as a derivative of the cortex'.

The work of the future relating to medullation should in the first place be to allocate among such sources as those named, or others that may be disclosed, the origin of the pith in any specific case; rather than to discuss whether or not the pith 'must in all cases be regarded as a derivative of the cortex'. It is high time that the study of medullation, and of stelar morphology, as a whole, should be freed from a form of controversy which has in its later phases resembled the contests of scholasticism rather than the more elastic discussions of modern biological science.

Tissues of various character and origin have been seen to contribute, either by degradation or by intrusion, to the central parenchymatous column commonly found in axes of the more advanced types of structure. We have seen that even the pith existing in a single individual may be referred to more than one distinct regional source. Its formation may be initiated by degradation only, without any intrusion at all, in which case it is strictly intraxylic. Or the formation may be aided, or even initiated by intrusion from without: and according as the intrusion is less or more marked it affects first the more internal and later successively the outer-lying tissues. It is thus seen that the factor of involution from without is only one of those which contribute to the general result. In point of fact the pith column, with its chief function of storage of water and of nutritive materials, is not referable to any single source in the Pteridophyta at large.

It has been pointed out that the extent of the intrusion of more superficial tissues, whether intrastelar or extrastelar, is closely connected with the relation of the leaves to the axis in point of size. Where the axis is bulky and the leaves small there is no intrusion; where the axis is relatively less bulky and the leaves large there is apt to be intrusion, and it is more or less extensive according to the preponderant size of the leaf is greater or less. This balance of the parts of the shoot is, however, itself related to habit. An upright axis must for mechanical reasons be relatively short and

bulky to secure stability, and its leaves are crowded. This type of shoot favours intrastelar medullation, which is thus biologically related to the upright habit. In the creeping forms the mechanical requirements are not so urgent, and the axis may be long and relatively thin in proportion to the size of the leaves. It may be suggested, further, that the *causa causans* of the creeping habit itself is the weight of the relatively large leaves which the axis cannot itself otherwise support. This then leads to extrastelar medullation, which is thus biologically related to the creeping habit. According as the balance is struck between extreme conditions, the intrusion involves, it may be, only the more superficial tissues of the stele, as in the seedlings of *Osmunda cinnamomea* and weak seedlings of *Botrychium*; or the inner endodermis, as appears to be the case in the young *Marattia*; or the extrastelar tissues as well, as in the older Ophioglossaceae, and in Leptosporangiate Ferns at large.

But the most extreme state of involution hitherto recorded is that seen in certain Ferns, in which a basket-like structure is assumed. Here the involution has extended to the outermost tissues of the axis, and deep pockets, lined by the superficial tissue of the stem itself, burrow far into the central column of the pith. This has been described by Gwynne-Vaughan for *Onoclea*, *Cystopteris*, and *Aneimia*,[1] and it has been again referred to by Jeffrey, but without bringing any new instances.[2] A further example may be mentioned in *Plagiogyria*,[3] though here the involutions of surface do not extend so deeply as to reach the pith. It may be remarked that all these examples come from Ferns which are closely related to forms with a creeping habit, and may be understood to have adopted relatively recently an upright habit with tufted leaves, and a massive axis for their support. Several of them indeed show the prior creeping phase and the later tufted state in their individual lives (*Onoclea*, *Plagiogyria*), while others belong to genera containing species with a creeping habit (*Cystopteris*, *Aneimia*). It may be that the basket-like structure of the stock in such cases results from the inability of the plant to meet the sudden demand of material for the construction of a more bulky axis, in species in which the adoption of an upright habit has made a broad and massive stock mechanically necessary for the insertion of the crowded leaves.

From the facts and comparisons above given, which thus lend themselves to a reasonable biological interpretation, the final result is that there is no rigid law of medullation, by which 'the pith must in all cases be regarded as a derivative of the cortex'. The only law appears to be that of organic life at large, that organisms accommodate themselves along lines of least resistance to their surroundings. That in so accommodating themselves their habit is seen to vary, and especially the proportions and attitude

[1] New Phyt., 1905, p. 211. [2] Bot. Gaz., Dec., 1910.
[3] Ann. of Bot., 1910, p. 429.

of the parts of the shoot. The consequences of this are reflected structurally even down to the pith itself, which, according to the habit and proportions of the shoot in question, may arise from more deeply seated, or successively from more superficial sources. That is the result which emerges from this study of medullation in the Pteridophyta.

The medullation of the Seed Plants, and ultimately of the Higher Flowering Plants, has not been touched upon here. But it is plain that any broad conclusion as to the origin of the pith in the Pteridophyta must affect the theoretical position as regards them. And those who hold that in the Pteridophyta the pith may be either intrastelar in origin or extrastelar will be prepared to extend such views to the Seed-bearing Plants. It has, however, been pointed out above that the establishment of the phyletic history of the pith in any one line of descent cannot have any direct bearing upon that of any other, unless it can be proved that the origin of the pith antedated the phyletic segregation of the stocks in question from a common ancestry. Applying this in the case of Seed Plants, the fact of solenostelic structure in such a Fern as *Pteris aquilina* can have no direct bearing upon questions of their medullation, unless it can be shown that the Pterideae had a common ancestry with the Seed Plants compared, and that that ancestry was already medullated and solenostelic before the two phyla were segregated. The present drift of investigation indicates that it is not in the direction of any solenostelic type of Ferns, as we now know them, that we shall look for the phyletic origin of any Seed Plants. It would be more to the point to inquire how the medullation was initiated in the Pteridosperms and other primitive Seed Plants, for their structure would throw a more direct light on the question. Moreover, what is required as a foundation for an opinion on the medullation of Seed Plants would be not merely the observation of isolated sections, but, so far as the material permits, a comparison of stems of different ages, in *Lyginodendron* for example, and of allied plants from different geological horizons. A knowledge of the origin of their pith arrived at in this way would provide a necessary test of the theoretical position of Professor Jeffrey on the stelar morphology of the Higher Flowering Plants.[1]

Finally, it must be said again that there is no wish to prejudge the question whether the endodermis is or is not an immutable barrier between the external and internal systems of tissue. Certain examples of irregular, and even of sporadic development of endodermal characters in tissues apart from the usual positions for endodermis, would seem to indicate that endodermal structure may sometimes originate *de novo*. The whole discussion has proceeded on the understanding that this question is left open for future demonstration. Here the endodermis itself has served as a con-

[1] Jeffrey, The Morphology of the Central Cylinder in the Angiosperms. Reprinted from the Trans. Can. Inst., 1900.

venient indicator of tissue locality, and it has been seen that not only the tissues outside it, but also those interior to it may take their varying share in the process of medullation.

DESCRIPTION OF THE FIGURES IN PLATE XLVII.

Illustrating Professor Bower's paper on Medullation in the Pteridophyta.

Selaginella spinulosa, A. Br.

Fig. 1. Transverse section of the stele of *Selaginella spinulosa*, taken from the base of the strobilus, showing the central xylem-core composed of tracheides, with projecting protoxylem-groups. Occasional thin-walled elements are present in the xylem. × 72.

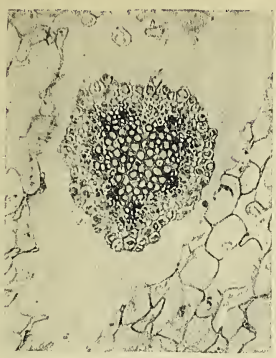
Fig. 2. Transverse section from rather below the middle of the strobilus, showing the general arrangement as before, but there are now considerable but irregular tracts of thin-walled elements, especially towards the centre of the stele, which are connected irregularly by thin-walled rays with the outer conjunctive tissue. There is, however, no evidence of intrusion shown in the arrangement of the cells. × 72.

Fig. 3. Transverse section from about two-thirds up the strobilus, showing the general arrangement as before, but the whole of the central region is occupied by thin-walled elements, constituting a massive pith. The xylem-core is now broken up into a number of separate strands, with broad parenchymatous rays intervening between them. × 72.

Fig. 4. A stage corresponding approximately to that of Fig. 2, on a larger scale, and showing a single tracheide isolated in the centre of the parenchymatous tract. × 144.

Fig. 5. A stage corresponding approximately to that of Fig. 3, on a larger scale, showing the relation of the isolated xylem-strands to the central pith. × 144.

Fig. 6. Part of a stele from a level about half-way up the strobilus, showing isolated tracheides suggestive of the degradation of the xylem-core. × 325.



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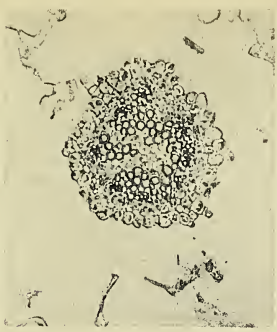

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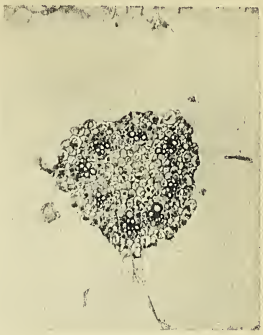

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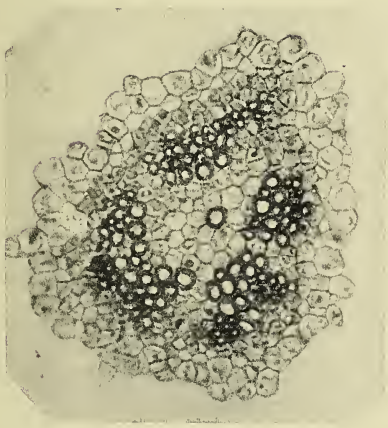

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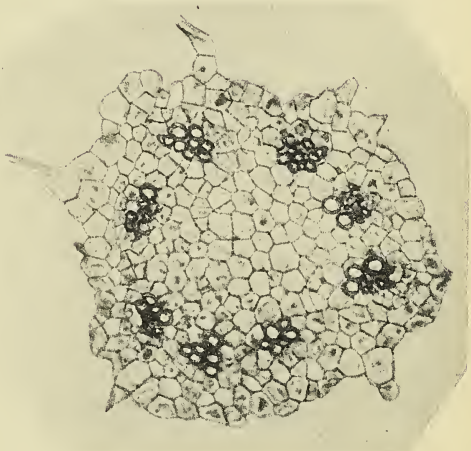

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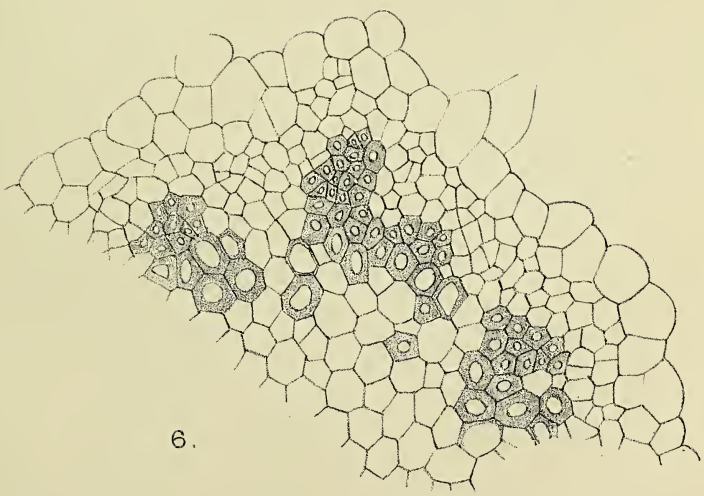

6.

Huth, London.

BOWER — SELAGINELLA SPINULOSA. A. Br.

A Review of the Genera Erythrococca and Micrococca.

BY

D. PRAIN, C.I.E., F.R.S.

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Introductory.

In a paper 'Sur le *Mercurialis alternifolia*, Desr., et sur les limites du genre *Mercurialis*' (Adansonia, iii. 167–76) Baillon, in 1862, essayed to prove that Lamarck's treatment of this plant,[1] on which Bentham based the genus *Micrococca* (Hook., Niger Flor., 503), is valid, and that, as a consequence, the genera *Adenocline*, Turcz. (Bull. Soc. Imp. Nat. Mosc. 1843, 59), *Seidelia*, Baill. (Étud. gén. Euphorb., 465, t. 9), *Claoxylon*, A. Juss. (Tent. Gen. Euphorb., 43, t. 14) and *Erythrococca*, Benth. (Hook., Niger Flor., 506) are also referable to *Mercurialis*. These Euphorbiaceous types undoubtedly belong to a definite natural group, and our instinctive reluctance, even when the necessity is more obvious than in this case, to include in one genus annual herbs like *Micrococca*, *Adenocline*, *Seidelia*, and shrubs or trees like *Claoxylon* and *Erythrococca* is shaken by the logic of Baillon, who shows that characters based on the number and arrangement of the stamens and of the glands on the receptacle so usually associated with the stamens in this assemblage of types are not of generic significance. Were these the only differential characters available here, Baillon's conclusion is hardly to be questioned.

But while all that Baillon urges with regard to the relative characters afforded by the androecium may be admitted, his reasoning involves an assumption that certain differences in the stamens themselves are unimportant. This assumption does not affect the existence of these differences; the fact that they exist leaves room for another opinion.

In the species referable to *Mercurialis*, Linn., in the usual restricted sense, the anther cells at first are pendulous or divaricate. In all those types which

[1] Lamarck, Encyc. Meth., iv. 120 (1796); the plant had already been dealt with by Linnaeus in 1753 (Sp. Pl., 980) as a *Tragia* (*T. Mercurialis*). Baillon attributes the name *M. alternifolia* to Desrousseaux, but the authorship of the article *Mercurialis* is not credited by Lamarck, in the text, to that botanist. Mueller (DC. Prodr., xv. 2, 790) is certainly under a misapprehension in attributing the name to Desvaux.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

Baillon would include in his widened *Mercurialis* the anther cells at first have an erect position. This character, superadded to those others which Baillon admits to be of sectional value, makes it as easy as it is convenient to treat *Mercurialis*, Linn., as a distinct genus. But if an intrinsic staminal character may be used for one genus we can legitimately employ such a character in connexion with another. In this way the separation of *Seidelia*, Baill., is also simple, for in *Seidelia* the anther cells open longitudinally both in front and behind so that, when their pollen is shed, the anthers are cruciately 4-valved, whereas in *Adenocline*, *Micrococca*, and *Claoxylon* the anther cells open, as Baillon states that they do in *Erythrococca*, along one side only, ' par une fente longitudinale dont les bords s'écartent beaucoup et se réfléchissent en dehors, de manière à donner à la loge ouverte la forme d'un cornet' (Étud. gén. Euphorb., 437). A criterion from the same source might be used to distinguish the genus *Adenocline*, Turcz., because in *Adenocline* the empty anther cells assume the downward position characteristic of those of *Mercurialis* while still in bud. In this instance, however, the need to rely on a staminal character does not arise ; *Adenocline* is readily separable from the other types under discussion, because the calyx in the male flower is not closed in bud as it is in *Mercurialis, Seidelia, Mirococca, Erythrococca*, and *Claoxylon*. At this point, however, staminal characters cease to be effective because the anthers of *Micrococca*, *Claoxylon*, and *Erythrococca* cannot be distinguished at any stage.[1] Had staminal characters alone been legitimate in the discrimination of the genera with which Baillon deals, the reduction of both *Micrococca* and *Erythrococca* to the older genus *Claoxylon* could be effected without further discussion. In the case of *Erythrococca*, no one has hitherto suggested that such reduction is necessary ; in that of *Micrococca* it has been effected by Thwaites (Enum. Pl. Zeyl., 271), whose action has been accepted by Mueller (DC. Prodr., xv. 2, 789) and confirmed by Hooker (Flor. Brit. Ind., v. 412).

But even if we should grant that it is necessary to merge both *Erythrococca*, Benth., and *Micrococca*, Benth., in *Claoxylon*, A. Juss., this would fall short of accepting the position which Baillon postulates. What Baillon asks us to admit is that in deciding that *Micrococca* is congeneric with *Claoxylon* and *Erythrococca* we must be guided solely by the fact that as regards their stamens the three are indistinguishable ; whereas, in deciding that *Micrococca* is congeneric with *Seidelia, Adenocline*, and *Mercurialis* we must treat their staminal differences as negligible. This position has not been accepted by Mueller (DC. Prodr., xv. 2, 775) or by Bentham (Gen. Plant., iii. 309) ; it is

[1] Mueller (DC. Prodr., xv. 2, 789) has stated that in *Micrococca* the anther cells open introrsely, whereas in *Erythrococca* (l. c., 790) and in the sections *Adenoclaoxylon, Athroandra, Gymnoclaoxylon*, and *Euclaoxylon* of *Claoxylon* (l. c., 775, 776, 780, 781) they open extrorsely : he is silent as regards *Discoclaoxylon*, where also they open extrorsely. But, as Bentham has already pointed out (Gen. Plant., iii. 309), there is here some error in observation. An examination of the anthers of *Micrococca* shows that the cells do not open introrsely.

a position the acceptance of which seems impossible. But the fact that we are unprepared to adopt his conclusions does not lessen our appreciation of the insight which has led Baillon to realize the crucial importance, in the study of this particular group of types, which attaches to the position of the genus *Micrococca*. If, in regard to this, the treatment by Bentham be sound, the maintenance of the other allied genera is practically assured, whereas, if that of Thwaites be warranted, the suppression of *Erythrococca* becomes a logical corollary. But the exhaustion of one line of inquiry does not debar us from taking up another. We are no more bound in this case to concede that staminal characters alone count than compelled to accept the contrary view. It is, therefore, open to us to examine whether *Micrococca*, *Erythrococca*, and *Claoxylon* may not be distinguishable by characters other than staminal.

A detailed revision of the genus *Claoxylon*, A. Juss. (1824), forms, it should be explained, no part of the purpose of this paper. At the same time, owing to the fact that *Claoxylon* is older by a quarter of a century than the other two genera concerned, it is necessary to undertake such a general review of this genus as is required for an exact appreciation of the relationship which it bears to its two allies.

In the only monograph of *Claoxylon* that has yet been attempted, Mueller in 1866 (DC. Prodr., xv. 2, 775–90) subdivided the genus into six sections, characterized as follows :—

§ 1. **Adenoclaoxylon,** Muell. arg.: Dioecious shrubs with perulate buds; stamens mixed with interstaminal glands, and surrounded by an extrastaminal urceolum ; anthers opening extrorsely.

§ 2. **Athroandra,** Hook. f.: Dioecious shrubs with perulate buds; stamens mixed with interstaminal glands, but not surrounded by an extrastaminal urceolum ; anthers opening extrorsely.

§ 3. **Discoclaoxylon,** Muell. arg.: Dioecious trees with non-perulate buds; stamens surrounded by an extrastaminal urceolum, but not mixed with interstaminal glands.

§ 4. **Gymnoclaoxylon,** Muell. arg.: Dioecious trees or shrubs with non-perulate buds; stamens neither mixed with interstaminal glands nor surrounded by an extrastaminal urceolum ; anthers opening extrorsely.

§ 5. **Euclaoxylon,** Muell. arg.: Dioecious trees or shrubs with non-perulate buds; stamens mixed with interstaminal glands, but not surrounded by an extrastaminal urceolum ; anthers opening extrorsely.

§ 6. **Micrococca,** Muell. arg.: Monoecious annual herbs with non-perulate buds; stamens mixed with interstaminal glands, but not surrounded by an extrastaminal urceolum ; anthers opening introrsely.

Bentham in 1880 (Gen. Plant., iii. 309) declined to follow Mueller in accepting the suppression of *Micrococca* proposed by Thwaites. At the same time Bentham refused to recognize the rest of Mueller's sections because the

receptacular glands in the male flower, the disposition of which is employed in defining the sections, happen to vary somewhat, and to be, as Mueller himself has admitted (DC. Prodr., xv. 2, 775), small and hard to see. This treatment is too severe. Mueller did not rely exclusively upon the disposition of these glands in arriving at the limits of his sections. His primary subdivision is based on the presence or absence of perulae, a character which enabled him to separate his two first sections from the other four; *Micrococca*, which is one of these non-perulate sections, has been distinguished from the remaining three by characters unconnected with these glands. Mueller has, indeed, employed the character derived from the buds with considerable restraint; Hooker, who first called attention to it, in 1862 expressed the opinion (Journ. Linn. Soc., vi. 20) that the character probably has a generic significance. But whatever the precise value of the character may be, the fact that Bentham has not alluded to it does not render it negligible. Pax, in 1890 (Nat. Pflanzenf., iii. 5, 48), while agreeing with Bentham as regards the treatment of *Micrococca*, has not followed the latter in ignoring Mueller's sections. But in considering Mueller's arrangement sufficiently practicable to warrant its retention, Pax has hardly been so critical as he might; the truth lies between his view and that of Bentham. The glands on the receptacle do supply quite valuable characters. But the evidence which these glands afford does not always justify the conclusions arrived at by Mueller, and even when allowance is made for the fact that these glands were not, as Bentham states, used primarily (*imprimis*) in delimiting Mueller's sections, there is no doubt that undue reliance has been placed upon them for this purpose.

The fact that the suppression of *Micrococca* has been proposed while no such suggestion has been made as regards *Erythrococca* renders it desirable to deal with the former before discussing the latter. This necessity therefore entails our discussing first those sections of *Claoxylon*, proposed by Mueller, which agree with *Micrococca* in having buds that are not provided with coriaceous bud-scales.

The Non-perulate Claoxyla.

The section *Discoclaoxylon*, Muell. arg., which includes three species that are confined to West Africa and Central Africa, is extremely natural and distinct; the treatment accorded to it by Mueller has been fully confirmed by the more complete material reported since it was proposed. In this case the character derived from the receptacular glands appears to possess all the weight which Mueller has attributed to it.

The section *Gymnoclaoxylon*, Muell. arg., which includes two Polynesian species that agree in facies with the bulk of those referred to *Euclaoxylon*, illustrates on the other hand the justice of Bentham's criticism. One of

these species, *C. fallax*, Muell. arg., from Fiji, has been referred to *Gymnoclaoxylon* because Mueller could not find glands on the male receptacle. The male flowers of the original specimens (Seemann, 394) have, however, just as many glands, hirsute at the apex with viscid hairs,[1] as there are stamens. In the other species, *C. sandwicense*, Muell. arg. (Linnaea, xxxiv. 165), there are no glands with viscid apical hairs. But associated with the fully developed stamens are many smaller and imperfect ones. These imperfect stamens show every transition from a filament bearing two free or nearly free empty anther cells, to a glabrous receptacular gland. The receptacle in *C. sandwicense* does possess glands, and the circumstance that these glands are glabrous does not exclude the species from the section *Euclaoxylon*. That section contains other species with glabrous glands, among them being *C. parviflorum*, A. Juss. (Tent. Gen. Euphorb., 43), the plant on which the genus *Claoxylon* was based. While, therefore, the section *Discoclaoxylon*, Muell. arg., stands, the section *Gymnoclaoxylon*, Muell. arg.,[2] is an integral part of *Euclaoxylon*.

The section *Euclaoxylon*, Muell. arg., to which, in 1866, thirty species were referred, but which is now believed to include over forty, has been found since then to be susceptible of some readjustment. Hooker f., in 1887, when revising the Indian species of *Claoxylon* (Flor. Brit. Ind., v. 410–14), was under the necessity of recognizing two sections within what corresponds to *Euclaoxylon*, and under the further necessity of removing from *Euclaoxylon* another species, *C. oligandrum*, Muell. arg. (Linnaea, xxxiv. 164), from Ceylon. With the necessity for subdividing *Euclaoxylon* we do not here require to deal; this can only be appropriately discussed in connexion with a critical revision of the genus *Claoxylon* as a whole. The transfer of *C. oligandrum* from *Euclaoxylon* does, however, immediately concern us, because of the fact that Hooker has found it most convenient to place this species in *Micrococca*. Following Thwaites and Mueller, Hooker has treated *Micrococca* as a section of *Claoxylon*; to the consideration of this section, or genus, we may now turn.

[1] In *C. fallax* these glands are thin and flaccid; each gland is closely applied to and partially embraces the base of the filament to which it corresponds. When a flower is examined under a simple microscope the appearance presented is practically that described by Mueller of stamens with short silky filaments (DC. Prodr., xv. 2, 780). But if a flower which has been softened in water be kept under observation, as it becomes dry the glands are seen to separate from the filaments, which are perfectly glabrous and are organically free from the glands.

[2] The recognition of a section limited in accordance with the definition which Mueller has provided for *Gymnoclaoxylon* is not, on this account, rendered impossible; there are several species which since 1866 have from time to time been referred to *Claoxylon*, where there are no receptacular glands. But even if we assume that the reference of these species to *Claoxylon* be correct and that the recognition of such a section be necessary, that section will not include either of the plants on which *Gymnoclaoxylon*, Muell. arg., was based, and therefore will not be Mueller's section so named.

Historical Review of Micrococca.

The section *Micrococca*, as understood by Mueller, is monotypic and corresponds with Bentham's genus of the same name. This genus *Micrococca* was established by Bentham in 1849 (Hook., Niger Flor., 503) to accommodate the tropical weed of cultivated ground, widely spread in the Eastern Hemisphere, which supplies Baillon with a title for the paper alluded to in our opening paragraphs.[1] This plant is so strikingly like *Mercurialis perennis*, Linn., that in certain states, were its flowers not examined, it might, as Mueller points out (DC. Prodr., xv. 2, 790), be easily mistaken for that species. As early as 1692 Plukenet did indeed treat it (Phytogr., t. 205, fig. 4) as a species of *Mercurialis*; *M.* maderaspatensis *tricoccos acetabulis destituta.* Linnaeus chose not to accept this position for the plant, but referred it to *Tragia*, in 1747 (Flor. Zeyl., 334) as *T. foliis ovatis*, and again in 1753 (Sp. Pl., 980) as *T. Mercurialis.* This treatment is, however, less satisfactory than that of Plukenet, to which Lamarck independently reverted when, in 1796 (Encyc. Meth., iv. 120), he described the plant as *Mercurialis alternifolia.* But Lamarck's view, though more natural than that of Linnaeus, was not adopted by subsequent authors, and the Linnaean treatment was followed until 1849, when Bentham solved the difficulty by treating *Tragia Mercurialis*, Linn. (*Mercurialis alternifolia*, Lamk.), as the type of a distinct genus. But Bentham's proposal, though much more satisfactory than those of Linnaeus and Lamarck, has not been generally accepted; twelve years later Dalzell and Gibson (Bomb. Flor., 227), Thwaites (Enum. Pl. Zeyl., 271), and Baillon (Adansonia, iii. 167) independently and simultaneously refused to adopt it. In each of these works a different conclusion was arrived at. Dalzell and Gibson, in 1861, while recognizing that the plant in question is not a *Tragia* when that genus is restricted to its natural limits, were influenced by Linnaeus's arrangement to such an extent as to believe that it was nevertheless congeneric with *Tragia Chamelaea*, Linn. (Sp. Pl., 981), a plant which had been shown by A. de Jussieu (Tent. Gen. Euphorb., 39), in 1824, to be a member of another genus, *Microstachys*, A. Juss. The transfer of *Micrococca Mercurialis*, Benth., to this genus *Microstachys*, suggested in the Bombay Flora, is therefore no great improvement on the Linnaean arrangement. This criticism

[1] It would appear that in 1858 Baillon was of opinion that *Micrococca Mercurialis*, Benth., differed from *Mercurialis alternifolia*, Lamk.: in his Étude générale, p. 436, he accepted *Micrococca* as defined by Bentham, while in the same work, p. 490, he treated *Mercurialis alternifolia* as the basis of a new section (*Erythranthe*, Baill.) of *Mercurialis.* But Baillon's *Micrococca* is not the same as Bentham's; it is made to include *Mercurialis tricocca*, E. Mey., which is in reality the basis of the distinct genus *Leidesia*, Muell. arg. (DC. Prodr., xv. 2, 792). In 1862, however, Baillon had become satisfied as to the identity of *Mercurialis alternifolia* with *Micrococca Mercurialis* and had become aware that *Mercurialis tricocca* is quite distinct. This last species he now referred to his own genus *Seidelia*, which he, as already explained, also now treated as a section of *Mercurialis*, at the same time merging his own *Mercurialis* § *Erythranthe* of 1858 in *Mercurialis* § *Trismegista.*

does not apply to the action of Thwaites in 1861 or to that of Baillon in 1862, because both of these authors have fully recognized the real affinity of Bentham's genus. Guided, however, by somewhat different considerations, they have arrived at different conclusions. There is but one species in Ceylon with which *Micrococca Mercurialis* may readily be compared; the field in which Thwaites had to work was therefore a very restricted one. Knowing that this other Ceylon species had been identified by Baillon with a plant from Java which is undoubtedly a *Claoxylon*, and finding that as regards their stamens, interstaminal glands, and hypogynous scales this plant and *Micrococca Mercurialis* entirely agree, Thwaites was left with no option in the matter. His decision to reduce *Micrococca* to *Claoxylon* was probably strengthened by the fact that he had, through some inadvertence, formed the impression that *Micrococca Mercurialis* is a dioecious plant; the fact that it is an annual one has appeared to him too unimportant for mention. Baillon, on the other hand, as we have already seen, satisfied that a striking difference as regards the position of their anther cells is of no real consequence, reverted to the conclusion of Lamarck, and replaced *Micrococca* in *Mercurialis*, Linn.

Mueller in 1866 mentioned, but could not accept (DC. Prodr., xv. 2, 775) the conclusion of Baillon; that of Thwaites he adopted in 1865 (Linnaea, xxxiv. 166), notwithstanding his belief that the stamens of *Micrococca* differ as regards their dehiscence from those of *Claoxylon*.[1] But the action of Mueller is less natural than that of Thwaites. In addition to making use of the staminal character in question, Mueller noted that in *Micrococca Mercurialis* male and female flowers may occur on the same rachis, so that the plant is not dioecious as Thwaites has stated; he further laid stress on the fact, not alluded to by Thwaites, that *Micrococca Mercurialis* is an annual. Guided by these considerations, Mueller kept the two plants which form the genus *Claoxylon*, as understood by Thwaites, rather widely apart: *Micrococca Mercurialis* he treated as the type of a distinct section; the other, which he now recognized as perfectly distinct from the Java plant (*Erythrochilus longifolius*, Bl.) with which it had been identified by Baillon, he nevertheless placed in his own section *Euclaoxylon*, with which, except for possessing interstaminal glands in the male flower, it has wonderfully little in common. It is hardly surprising, therefore, that in 1880, Bentham (Gen. Plant., iii. 309), while he agreed with Mueller in regard to Baillon's conclusion, should have felt unprepared to accept the view, either as stated by Thwaites or as modified by Mueller, that the genus *Micrococca* should be merged in *Claoxylon*.

Yet when we examine the arguments employed by Bentham in favour of the maintenance of *Micrococca* we find them as little convincing as those

[1] This belief turns out to be without foundation; the anther cells of *Micrococca* open precisely as those of *Claoxylon* proper do.

which induced Mueller to endorse its suppression. Bentham separates *Claoxylon* and *Micrococca*, taken together, from the allied genus *Erythrococca*, because in both the stigmas are entire, whereas those of *Erythrococca* are plumosely multifid, and then distinguishes *Micrococca* from *Claoxylon* because the former is an annual monoecious herb with few stamens, while the latter is composed of dioecious shrubs with usually numerous stamens. Now this stigmatic character is ineffective; the stigmas in *Claoxylon*, as Bentham has limited that genus, are not always entire; when *Claoxylon* is restricted to its natural limits the stigmas never are entire. The statement that the stigmas of *Micrococca* are entire is incorrect; they have been described by Hooker (Flor. Brit. Ind., v. 412) as 'fimbriate'; they may, if we prefer the term, be described as 'plumosely multifid', because they are exactly like the stigmas in most of the species of *Erythrococca* and in every species of *Claoxylon*.

Mueller and Bentham are therefore in agreement as regards the characters on which they rely in separating *Micrococca* from *Claoxylon*; in both cases the only characters employed which are real are that *Micrococca* is an annual and is monoecious. In this respect they are at variance with Thwaites, who has not alluded to the first character, and as regards the second laboured under a misapprehension corresponding to the misapprehension of Mueller as regards the nature of the stamens, and to that of Bentham as regards the stigmas. The difference between Bentham and Mueller resolves itself into one of opinion; Mueller believed the two valid characters to be only of sectional value, Bentham considered that they justified the maintenance of *Micrococca* as a distinct genus.

Matters remained in this *impasse* until, in 1887, Hooker threw an entirely new light on the character and composition of *Micrococca* (Flor. Brit. Ind., v. 412, 413), by pointing out that this section or genus, so far from being monotypic, includes a number of species that are frutescent and dioecious. Besides transferring *C. oligandrum*, Muell. arg., from *Euclaoxylon* to *Micrococca*, and thereby amply vindicating the action of Thwaites in having treated this plant and *Micrococca Mercurialis* as members of one genus, Hooker added to *Micrococca* three new forms, *C. Wightii*, *C. Beddomei*, and *C. hirsutum*, all natives of Southern India. Of these, *C. hirsutum* is now believed to be really only a distinct variety of *C. Wightii*; the others are certainly well characterized and valid species which agree with each other, with *C. oligandrum*, and with *Micrococca Mercurialis*, and at the same time differ very markedly from every species of *Claoxylon* proper examined by Hooker, in the character afforded by the scales of their hypogynous disc on which Thwaites laid stress. All these species further differ, as Hooker has pointed out, from every true *Claoxylon* in having long, filiform, interrupted racemes.[1]

[1] In *Euclaoxylon* and in *Discoclaoxylon* the racemes when young are substrobilate, when fully

The only species thus transferred by Hooker to *Micrococca* which was known to Mueller is *C. oligandrum*. But Mueller was acquainted with another species which belongs to the same group, but which did not come within Hooker's purview. This species is *C. capense*, Baill. (Étud. gén. Euphorb., 493), a South-East African plant of which neither Baillon nor Mueller knew the male flowers. Now that these are known, it is seen that they are borne on long, filiform, interrupted racemes, and that *C. capense* is a *Micrococca* and not, as Mueller imagined, a *Euclaoxylon* (DC. Prodr., xv. 2, 786).[1] More recently three other species of the same group have been described; these are *C. Humblotianum*, Baill. (Bull. Soc. Linn. Par., 996), from the Comoros, with *C. Volkensii*, Pax (Engl. Pflanzenw. Ost-Afrik., C, 238), and *C. Holstii*, Pax (Engl. Bot. Jahrb., xxxiv. 372), both from East Africa. The accession of these additional species shows that the arguments in favour of the widening of *Micrococca* are stronger even than they were in 1887, for in *C. Volkensii* the terminal flower of each raceme is a female one, and this species, while agreeing with all the others except *Micrococca Mercurialis* in being perennial, agrees with *Micrococca Mercurialis* in being monoecious.

A feature in *Micrococca* which differentiates it sharply from *Euclaoxylon* is to be found in the interstaminal glands. The presence of these bodies in *Euclaoxylon* is constant. In *Micrococca*, on the other hand, while glands are present in the male flowers of *C. oligandrum*, *C. Wightii*, *C. hirsutum*, and *Micrococca Mercurialis* itself, there are no such glands in the male flowers of *C. Beddomei* which Hooker, with perfect justice, has associated with the other species mentioned. The same thing is true of *C. capense*, *C. Humblotianum*, *C. Volkensii*, and *C. Holstii*, all of which have eglandular receptacles. Whatever the value of the character derived from the presence or absence of these glands may be in *Claoxylon*, in *Micrococca* that character is merely a specific one.

Another striking peculiarity in *Micrococca* which is without a parallel in *Claoxylon* proper has been pointed out by Hooker. In *C. oligandrum* and in *C. Beddomei* the male flowers are borne on minute distant spikelets, closely covered with imbricating bracteoles, whereas in *C. Wightii*, *C. hirsutum*, and *Micrococca Mercurialis* itself, the male flowers are glomerulate. The

expanded the rachis is continuously floriferous from the top of the peduncle to the apex of the raceme. In 1692 Plukenet suggested that the plant figured by Rheede as *Pee-Cupaméni* (Hort. Malab., x, t. 82) might be the same thing as *Micrococca Mercurialis*. In 1753 Linnaeus accepted this suggestion as an established fact. In this Linnaeus has been followed by Dennstedt, Dillwyn, and Hasskarl in their respective works on the *Hortus Malabaricus*. But in *Pee-Cupaméni* the racemes are continuously floriferous, and we therefore know that, whatever *Pee-Cupaméni* may be, the identification of Linnaeus and of those who have followed him is incorrect.

[1] Specimens of *C. oligandrum* sometimes bear so close a resemblance to those of *C. capense* that it is only by dissecting their flowers that the two can be definitely distinguished. The stamens of *C. oligandrum* are always accompanied by interstaminal glands; there are no such glands in the male flowers of *C. capense*.

same variability is met with among the species of *Micrococca* which were not known to Hooker. In *C. Holstii* the male flowers are at the tips of minute bracteolate spikelets as in *C. oligandrum*, in *C. capense* they are in glomerules as in *Micrococca Mercurialis* itself. The two remaining species are interesting because they are intermediate; *C. Volkensii* has the flowers towards the upper part of the raceme glomerulate as in *C. Wightii*, those lower down on spikelets as in *C. oligandrum*, while *C. Humblotianum* has the male flowers glomerulate above, but towards the base of the rachis has them on short secondary branches with scattered and not imbricating bracteoles. Here again, then, in spite of its striking nature, the character is only of specific value.

There is, moreover, no definite association between the presence or absence of interstaminal glands and the development or suppression of these spikelets. Of the four Micrococcas which have interstaminal glands three (*Micrococca Mercurialis*, *C. Wightii*, and *C. hirsutum*) have glomerules, the fourth (*C. oligandrum*) has spikelets; of the five which have naked receptacles, one (*C. capense*) has glomerules only, two (*C. Beddomei* and *C. Holstii*) have spikelets, the remaining two (*C. Volkensii* and *C. Humblotianum*) have both glomerules and either spikelets or short secondary branches.

The conclusion that none of the species enumerated above can be included in the section *Euclaoxylon* is one that does not admit of doubt. Equally free from doubt is the conclusion, for which we are indebted to Hooker, that they belong to *Micrococca*. The same certainty, however, does not attend Hooker's further conclusion that the transfer of these species to *Micrococca* 'requires the suppression of the latter genus' (Flor. Brit. Ind., v. 410).[1] The necessity for this transfer has indeed shown that the characters on which Bentham relied in separating *Micrococca* from *Claoxylon* and on which Mueller depended in establishing his section of the same name are not diagnostic. But the fact that their significance was overlooked by Bentham and Mueller does not affect the existence of the important character derived from the nature of the hypogynous disc which we owe to

[1] It is a curious fact, and one that is not without a certain degree of interest, that, since 1880, no British botanist has accepted Bentham's opinion that *Micrococca* is, after all, a valid genus, while during the same period every Continental botanist has followed Bentham. It is, however, a fact that admits of simple explanation. In 1887 Hooker made the important discovery that *Micrococca* is not, as Bentham believed, a monotypic genus; whereas in 1900, when the account of the Euphorbiaceae in the Natürlichen Pflanzenfamilien appeared, Pax overlooked this discovery, and felt justified in accepting, without question, the conclusion at which Bentham had arrived ten years earlier. If Hooker has been followed by Trimen (Handb. Flor. Ceyl., iv. 63), by Hiern (Cat. Afr. Pl. Welw., 976), by Cooke (Flor. Bomb. Pres., ii. 609), and by the writer (Beng. Pl., 947), it must be admitted that none of us have added anything to what Hooker did. If Pax has been followed by Schweinfurth (Bull. Herb. Boiss., vii, App. 2, 306), by De Wildeman (Miss. Laurent., i. 129) and by Durand (Syll. Flor. Cong., 492), it must be equally admitted that none of these have added anything to what was already done by Pax. The acceptance or otherwise of Bentham's view has therefore been the result of accident, except in the case of Hooker to whom we are deeply indebted for having thrown a flood of light on an obscure and difficult question.

Thwaites, nor does it detract from the value of the still more important character afforded by the inflorescence for which we are indebted to Hooker. By means of these two characters Hooker has brought together, and has enabled others to augment, a compact and natural group of species which, when treated as Hooker has treated it, as a section of *Claoxylon*, proves to be much more distinct and far more easily separated from *Euclaoxylon* than Mueller believed it to be. So well characterized is this group and so clearly is it defined by the characters which Thwaites and Hooker have supplied, that the suppression of the genus *Micrococca*, so far from having been definitely effected, has become once more open to discussion.[1] The segregation of *Micrococca* has not been shown to be impossible; it has, instead, been made more simple. Since whatever tends to facilitate the separation of *Micrococca* makes for the acceptance of the view held by Bentham in preference to that adopted by Mueller, we are at liberty to inquire whether, in spite of the inadequacy of the criteria on which reliance was placed, the judgement of Bentham may not, after all, have been the sounder. Even if we concede that, in the light of the characters employed by Hooker, the evidence for and against the views held by Bentham and Mueller respectively is so evenly balanced as to justify either, one has only to look at the fruit in order to realize that Bentham's instinct was right. In *Claoxylon*, and in *Erythrococca* as well, the capsules have coriaceous walls which at first open loculicidally, the two valves of each coccus gaping to expose the seed. At a later stage the segments of the capsule break away septifragally from the relatively wide coriaceous columella. In *Micrococca*, however, the capsules, with thin crustaceous walls, open simultaneously both loculicidally and septicidally, and thus break up into 2-valved cocci, leaving behind them a slender woody columella. The nature of its fruit dispels all doubt as to the validity of the genus *Micrococca* and renders its differentiation from *Claoxylon* and *Erythrococca* a far simpler matter than the separation of these two genera from each other.

The Perulate Claoxyla.

The two Muellerian sections of *Claoxylon* which have still to be considered are *Adenoclaoxylon*, Muell. arg. (1864), and *Athroandra*, Hook. f. (1862), as modified by Mueller in 1866. These two sections agree with each other and differ from *Discoclaoxylon*, *Gymnoclaoxylon*, *Euclaoxylon*, and *Micrococca* in having perulate buds. It is convenient to deal with them

[1] It is largely because Thwaites was justified in referring *Claoxylon oligandrum* and *Micrococca Mercurialis* to one genus that there is a doubt as to the limits of that genus. This doubt is due to the uncertainty regarding the former species. Throughout its history the position of the latter has been much discussed, but its specific limits have been tolerably clearly understood. But, in the case of *C. oligandrum*, Mueller had to point out in 1866 that its specific identity had been misunderstood both by Baillon and by Thwaites, while in 1887 Hooker had to indicate that its position and its affinity had been equally misunderstood by Mueller.

more or less conjointly, but as *Athroandra*, in its original form, is the older of the two it is desirable to take it into consideration first. When, in 1862, Hooker proposed the section *Athroandra* (Journ. Linn. Soc. Bot., vi. 20) he pointed out that *C. Mannii*, Hook. f., the species on which the section was based, is 'probably generically distinct from *Claoxylon*, from all the species of which the perulate buds abundantly distinguish it'. Along with *C. Mannii* Hooker described another species, *C. Barteri*, Hook. f. (l. c., 21 *ad calc.*), which he referred to the same section. Hooker at the time was unaware, and indeed had no means of knowing, that specimens of the same species had, in 1860, been described by Baillon (Adansonia, i. 68) as *Trewia? africana*.[1] The suggestion of Baillon as to the generic position of this plant lends support, which is stronger for being indirect, to Hooker's belief that *Athroandra* is generically distinct from *Claoxylon*. As outlined by Hooker, the section *Athroandra* is based on species which agree with Mueller's section *Euclaoxylon* in having the male flowers with only interstaminal glands, but differ from *Euclaoxylon* in having perulate buds, and in having entire in place of plumosely laciniate stigmas. To the section *Athroandra* Mueller subsequently added five species which share the same characters:—*C. Welwitschianum*, Muell. arg. (Journ. Bot., ii. 333); *C. columnare*, Muell. arg. (Flora, xlvii. 437); *C. membranaceum*, Muell. arg. (Flora, xlvii. 437); *C. angolense*, Muell. arg. (Journ. Bot., ii. 333); and *C. rivulare*, Muell. arg. (Flora, xlvii. 518). The justice of Hooker's original view that *Athroandra* is probably generically distinct is further confirmed by the circumstance that Engler has based on specimens of *C. rivulare* his genus *Chloropatane* (Bot. Jahrb., xxvi. 383), and that Wright has referred specimens of *C. Welwitschianum* to the same genus (Flor. Trop. Afr., vi. 1, 169), under the name *Chloropatane Batesii*.

The section *Athroandra* as understood by Mueller is, however, somewhat wider in its limits than the *Athroandra* outlined by Hooker or the *Chloropatane* described by Engler and Wright. This is due to the fact that of the two characters, the perulate buds and the entire stigmas, which distinguish Hooker's section, Mueller has relied only upon the first and has treated the second as negligible. This has led him to include in *Athroandra*

[1] The oldest specimens of *Trewia? africana* were collected in Sierra Leone by Afzelius. These specimens bear a manuscript generic name, the existence of which perhaps indicates that Afzelius, too, had felt that this plant is not congeneric with the plant on which *Claoxylon*, A. Juss., was subsequently based: prior to 1824 the basis of *Claoxylon* was regarded as an *Acalypha*. The specimens on which Baillon based *Trewia? africana* were collected in 1859 by Perrottet in Senegambia, on the banks of the Casamance, long subsequent to the establishment of the genus *Claoxylon* by A. Jussieu. The specimens on which Hooker based *Claoxylon Barteri* were collected by Barter in Southern Nigeria—in Lagos Island, at Eppah and in the Yoruba forests. By a typographical error the name Yoruba appears in the Linnean Society's Journal as 'Gomba'. Mueller, when he detected the identity of *Claoxylon Barteri* and *Trewia? africana*, left this typographical error uncorrected and inadvertently introduced another by transferring the provenance of Perrottet's specimens of *Trewia? africana* from the banks of the Casamance to the neighbourhood of the Niger.

three species:—*C. pauciflorum*, Muell. arg.; *C. trichogyne*, Muell. arg.; and *C. triste*, Muell. arg. (Journ. Bot., ii. 333, 334); all of which agree with *Euclaoxylon*, but differ from Hooker's *Athroandra*, in having the stigmas plumosely laciniate and in having the hypogynous scales which alternate with their carpels discrete, whereas in *Athroandra* these scales, if free, are contiguous by their margins under the base of the ovary; more usually, however, they are connate in a disc. In the case of *C. trichogyne* and *C. triste*, moreover, the ovaries are densely strigose, whereas in every true *Athroandra* the ovary is glabrous; the stipules, too, are accrescent and harden into cartilaginous bosses or thornlets, whereas in every true *Athroandra* the stipules, although hyaline-scarious, are very minute and do not become altered or enlarged. But while it is, at least, inconvenient to include these species in *Athroandra*, their generic relationship is almost certain; the perulate buds, as Mueller remarks (DC. Prodr., xv. 2, 779), undoubtedly indicate their affinity.

The section *Adenoclaoxylon*, based on a solitary species, *C. Kirkii*, Muell. arg. (Flora, xlvii. 436), is as distinct and natural as the section *Athroandra* when taken in the sense outlined by Hooker. Now, however, that seven species belonging to this section are known, it is found that the arrangement of the receptacular glands, used by Mueller along with the character of perulate buds in its definition, is not constant. As originally described, the section differs from *Athroandra* and from *Euclaoxylon* by the presence of a ring of extrastaminal glands. But this extrastaminal ring may be imperfect or altogether absent, and the distinctive feature of the section, apart from the perulate buds, is the circumstance that the male flowers are in axillary glomerules, whereas in the rest of the genus these are in racemes. All the species in the section share with *C. trichogyne* and *C. triste* accrescent spinulous stipules, discrete hypogynous scales, and plumosely laciniate stigmas, and differ in these three characters from every true *Athroandra*. These three characters the section further shares with the genus *Erythrococca*, Benth. (Hook., Niger Flor., 506), to the consideration of which we may now give our attention.

Historical Review of Erythrococca.

The genus *Erythrococca* was founded by Bentham on specimens of a plant collected in Sierra Leone by Vogel and in Senegambia by Heudelot. But specimens of the same species, collected in Sierra Leone by Smeathman, had already been described by Poiret, in 1810, as *Adelia anomala*, Juss. (Encyc. Meth. Suppl., i. 132). Poiret only describes the female flowers; his general account contains the statement that the leaves are axillary to the spines, but omits to note the perulate buds. In 1824 A. de Jussieu dealt again with some of Smeathman's specimens (Tent. Gen. Euphorb., 32). He had male specimens before him, even if Poiret had not any; if he had a female

specimen it had no flowers. But de Jussieu's description of the male flower is not altogether clear, and his statement that the stamens are numerous (*plurima*) is not accurate. Poiret's account of the relative position of the leaves and spines is accepted by de Jussieu, who also omits to note that the buds are perulate. The animadversion on Poiret's account of the female flower is hardly called for: Poiret's description is correct so far as it goes, though it fails to state that the ovary is adpressed strigose and is subtended by hypogynous scales. Still Jussieu's explanation of Poiret's supposed error is interesting from its reference to the fact that in this species the male pedicels are jointed well above the base. Neither Poiret nor A. de Jussieu allude to the fruit or the seed.[1] But, as Bentham has indicated, to A. de Jussieu belongs the merit of pointing out that this species, owing to the nature of its anthers, is more nearly allied to *Claoxylon* than to *Adelia*.

The account of *Erythrococca* given by Bentham when, in 1849, he founded the genus, is somewhat brief. It points out that the spines are stipular and that the stamens are definite, thereby removing two previous misapprehensions. But there is no reference to the bud-scales, to the presence of receptacular glands in the male flower, to the fact that the ovary is strigose, or to the existence of hypogynous scales. The account of the stamens implies that they are 1-seriate and states that the filaments are connate in a ring. The fruit is said to be 'apparently' indehiscent, with a thin fleshy pericarp and a crustaceous endocarp.

The account of *Erythrococca* by Baillon (Étud. gén. Euphorb., 437) in 1858 deals at some length with the male receptacular glands, which are interpreted as a double disc. The stamens are described as more than 1-seriate, though the details given differ from those which are usual; the filaments are again interpreted as united at the base. Nothing is added to Bentham's account of the fruit, and Baillon does not note that the buds are perulate or that the ovary is strigose and subtended by hypogynous scales. This last omission was very soon rectified (Adansonia, i. 71); Baillon also soon discovered that the stamens are not always limited to a particular number (Adansonia, iii. 174).

A fuller account of *Erythrococca* was given by Mueller (DC. Prodr., xv. 2, 790) in 1866. The calyx is usually 3-partite, as Mueller says; occasionally, however, the male calyx may be 4-lobed. The stamens, though usually 6–7, as he states, may at times be more numerous; 11 is the highest

[1] Poiret explains that he saw specimens, collected by Smeathman, in the herbaria of A. L. de Jussieu and of Desfontaines. It is possible that A. de Jussieu only saw the specimens which had belonged to A. L. de Jussieu. It is also possible that neither of these herbaria had received fruiting specimens of Smeathman's plant, and it is further possible that A. L. de Jussieu never received a female specimen: Poiret's account of the female flower may have been based on a specimen which belonged to Desfontaines. But these questions happen to be of no practical consequence here, because the ample suite of specimens of this species collected in Sierra Leone by Smeathman, which is preserved in the British (Natural History) Museum, includes both sexes and also shows ripe fruits.

number so far observed. More important, however, is the account of the receptacular glands. Mueller describes those that constitute an extra-staminal ring as produced radially inwards between the filaments, and as confluent with the interstaminal glands so as to form the 'double disc' mentioned by Baillon, within which the stamens are enclosed. What Mueller describes is precisely what is seen when a carefully soaked flower is examined under a simple microscope. But when such a flower is kept under observation while it is parting with its extraneous moisture, the glands of the two series are seen to separate spontaneously, and to be in reality quite free. The confluence proves to be no more than an adhesion of these viscid bodies while they are wet; there is no organic union between the glands of the outer and the inner series. In *Erythrococca aculeata*, Benth., the receptacular glands are unusually large, about as long as the stamens; in *Claoxylon* (*Adenoclaoxylon*) *Kirkii* they are relatively small, shorter than the filaments. But in these two plants the receptacular glands do not otherwise differ; they are identical in character and are arranged in the same manner. Mueller does not remark that the buds of *Erythrococca* are perulate or that the ovary is adpressed strigose. He says nothing with regard to the seed, and his account of the fruit is taken from Bentham. He appears to accept as a fact what with Bentham was little more than a suggestion, and indicates (DC. Prodr., xv. 2, 791) his belief that it is only because its fruit is indehiscent that *Erythrococca* may be distinguished from *Claoxylon*.

In the revised definition of *Erythrococca*, published in 1880 by Bentham (Gen. Plant., iii. 308), the spines are described as infra-stipular. The number of stamens is now admitted to vary, but the filaments are still said to be connate in a ring. The presence of hypogynous scales in the female flower is accepted, but the existence of receptacular glands in the male flower, though in this species these glands are so large as almost to conceal the stamens, is not mentioned. The fruit is now said, without any reservation, to be indehiscent, with a thin fleshy exocarp and a crustaceous endocarp, but it is suggested that it may not always be monococcous. The spines are, however, as Bentham had stated in 1849, the stipules themselves. Under other circumstances the question as to whether the filaments be free as Mueller implies, or connate below as Bentham and Baillon suppose, might have been open to doubt. Had there been no interstaminal glands in the male flower, the structure which the attachment of these glands proves to be a receptacle might, without serious objection, have been interpreted as a short staminal 'column'. But even if this alternative explanation had been permissible, there is nothing in the appearance or the anatomy of this receptacle to suggest that it is 'annular'.

In 1890 Pax (Nat. Pflanzenf., iii. 5, 48) accepted the limitation of Bentham and Mueller. His brief diagnosis corrects the misapprehension

of 1880 as regards the spines, but omits any reference to the fruit. In the generic key which precedes his diagnosis, Pax has substituted for the character which in 1866 Mueller believed to be the only mark of distinction between *Erythrococca* and *Claoxylon*, alternative characters derived from the stamens and stigmas.[1]

Up to this point the history of *Erythrococca* has been that of a monotypic genus. In 1894 a new phase in this history was initiated by Pax, whose prolonged and fruitful study of the African Euphorbiaceae has been so rich in interesting results. In 1889 Schweinfurth collected in Arabia a plant previously obtained there by Deflers. This species, treated by Deflers as perhaps a *Mercurialis* (Voy. Yemen, 203), was distributed by Schweinfurth (Pl. Arab. Fel. exsicc. 933) as a *Claoxylon*. In 1892 Schweinfurth and Riva met with the same species in Abyssinia. On this occasion, as the sheets he distributed indicate, Schweinfurth concluded that the plant was not a *Claoxylon*, but must be either an *Erythrococca* or the type of a new genus. Deciding in the latter sense, he named the species *Deflersia erythrococca*, Schweinf. (Penzig *in* Atti Congr. Bot. 1892, Genova, 359). In 1894 Pax referred this plant to *Erythrococca*, as *E. abyssinica*, Pax (Engl. Bot. Jahrb., xix. 87); to this view Schweinfurth has since assented (Bull. Herb. Boiss., vii, app. 2, 306). If *Erythrococca* be indeed a valid genus, this action is fully justified; *Deflersia*, Schweinf., and *Erythrococca*, Benth., agree in having perulate buds, accrescent indurated stipules, both extrastaminal and interstaminal receptacular glands, discrete hypogynous scales, and free, linear, plumosely laciniate stigmas; they mainly differ in that the ovary in *Deflersia* is glabrous, but in *Erythrococca* is strigose, while in *Erythrococca* the stipular spines and the receptacular glands are larger than in *Deflersia*. Along with *E. abyssinica*, Pax added two other species to *Erythrococca* (Engl. Bot. Jahrb., xix. 88). One of these species, *E. Fischeri*, Pax, is interesting because it serves as a connecting link between *Deflersia*, Schweinf., and *Claoxylon* § *Adenoclaoxylon*, Muell. arg.; the other, *E. bongensis*, Pax, is a genuine *Adenoclaoxylon*. In thus widening the limits of *Erythrococca*, Pax has not stated what differential characters were relied on by him in distinguishing the genus from *Claoxylon*, though he has made it clear that he was not practically influenced by the criterion on which Mueller relied in 1866,[2] and that he was not guided by the criteria employed by himself in 1890.[3] The omission was, however, rectified in 1895 (Engl. Pflanzenw. Ost-Afrik., C, 238), when Pax supplied a new diagnosis; the only

[1] The characters adduced (l. c. 47) are: *Erythrococca*; stamens 3–6; styles short, plumosely laciniate, free from the base: *Claoxylon*; stamens usually numerous; styles linear, entire.

[2] Of the three species thus for the first time added to *Erythrococca*, the female flowers and fruits were unknown in *E. Fischeri* and *E. bongensis*, and although the female flowers of *E. abyssinica* are described there is no account of the fruit.

[3] *Claoxylon lasiococcum*, Pax (Engl. Bot. Jahrb., xix. 87), described at the same time as these three species of *Erythrococca*, has plumosely laciniate and not entire stigmas.

character included in this which is not also applicable to *Claoxylon*, is that *Erythrococca* has stipular thorns.[1]

When providing this new criterion for the genus Pax added a fifth species, *E. mitis*, which is certainly an *Adenoclaoxylon*, and is admittedly very nearly allied to *Claoxylon Kirkii* itself. More recently Rendle has added another *Adenoclaoxylon* to *Erythrococca*, *E. Paxii*, Rendle (Journ. Linn. Soc. Bot., xxxvii. 212), as nearly allied to *E. Fischeri* as *E. mitis* is to *Claoxylon Kirkii*; while Pax has described yet another, *E. rigidifolia*, Pax (Engl. Bot. Jahrb., xliii. 320), as nearly allied to *E. bongensis* as *E. Paxii* is to *E. Fischeri*. Every known *Adenoclaoxylon* has now been formally referred to *Erythrococca* except *Claoxylon Kirkii*, Muell. arg., the species on which the section was based.

Along with *E. rigidifolia*, Pax added to *Erythrococca* a species, *E. hirta*, Pax (Engl. Bot. Jahrb., xliii. 321), which is neither an *Adenoclaoxylon* nor a *Deflersia*, but is nearly allied to *Claoxylon trichogyne*, Muell. arg., one of the species added to *Athroandra* by Mueller which do not, on account of their laciniate stigmas, conform with that section as it was originally outlined. To this group belong, in addition to *E. hirta* and *C. trichogyne*, the following species described under *Claoxylon*:—*C. triste*, Muell. arg.; *C. lasiococcum*, Pax (Engl. Bot. Jahrb., xix. 87); *C. Menyharthii*, Pax (Bull. Herb. Boiss., sér. 2, i. 877); *C. Mildbraedii*, Pax (Engl. Bot. Jahrb., xliii. 80). All six agree in having stipular thorns, densely hirsute ovaries, and plumosely laciniate stigmas; where one of them is definitely placed the others must go. If *Erythrococca* be a valid genus, the reference thereto of *E. hirta* is as justifiable as was the reference to that genus of *E. abyssinica*; although the other five species named have not yet been formally included in *Erythrococca*, this does not lessen our indebtedness to Pax for having so enlarged our conception of the genus as to render it capable of accommodating any species, hitherto accounted for under *Claoxylon*, which combines the characters of perulate buds, stipular thorns, and plumosely laciniate stigmas.

The question that has first to be settled, therefore, is as to whether *Erythrococca* be really distinct from *Claoxylon*. The affinity between the two genera has been fully admitted since it was pointed out by A. Jussieu in 1824; the settlement of the question involves a scrutiny of the criteria which have from time to time been relied upon for their discrimination. These criteria have been three in number:—(1) that formulated by Mueller in 1866; *Erythrococca* has an indehiscent, while *Claoxylon* has a capsular fruit: (2) that employed by Pax in 1890; *Erythrococca* has 3–6 stamens and plumosely laciniate stigmas, while *Claoxylon* has usually many stamens and always entire stigmas: (3) that substituted by Pax in 1895; *Erythrococca* has stipular thorns, while, by implication, *Claoxylon* is unarmed.

[1] It is not clear that this was the criterion relied on in 1894, because *C. lasiococcum* is one of the species with stipular thorns.

Before Mueller's criterion can be considered it is desirable to provide, what so far has never been fully given, an account of the fruit and the seed of *Erythrococca aculeata*, Benth. The fruit of this species[1] is occasionally 3-coccous, all three carpels becoming fully developed; more often it is 2-coccous and didymous, not infrequently it is, by abortion, 1-coccous. The cocci are subspherical and have, when ripe, a dull green, sparingly setose, thinly coriaceous pericarp which opens loculicidally so as partially to expose the seed. The seed, which is almost globose, is completely enveloped in a bright scarlet arillus, organically quite free from the hard, crustaceous, nearly black, rugosely foveolate-reticulate testa. The albumen is fleshy, the conspicuous axile embryo has a conical radicle with two flat, expanded, suborbicular cotyledons. The coriaceous valves of the cocci remain long attached to the flat, subspathulate, flexible columella which is tipped by the marcescent stigmas; often the valves fall away before the seeds become detached. The accounts hitherto given of the fruit of *E. aculeata* have been incomplete descriptions of the seed; what has been termed a fleshy exocarp (Gen. Plant., iii. 308) or a thin sarcocarp (Engl. Bot. Jahrb., xix. 89) is the arillus, the crustaceous endocarp being the testa. We have seen that, although this is the only character on which Mueller relied in distinguishing *Erythrococca* from *Claoxylon*, it has not been used for this purpose by Pax. That author has, however, placed a certain amount of dependence upon the criterion in another connexion. An interesting plant collected in fruit by Pogge on the river Lulua, a tributary of the Congo, which has the perulate buds, the stipular thorns, and the plumosely laciniate stigmas of *Erythrococca*, has been treated by Pax as the type of a distinct genus *Poggeophyton* (Engl. Bot. Jahrb., xix. 88), partly because the glands which surround the base of the ovary differ from the hypogynous scales of *E. aculeata*, and partly because it has a dehiscent capsule. The fruit of *Poggeophyton aculeatum*, Pax, is, however, quite like that of *Erythrococca aculeata*, Benth., and the only difference in the seeds of the two is that in *Poggeophyton aculeatum* the arillus does not completely envelop the testa. The necessity for treating *Poggeophyton* as a genus apart from *Erythrococca* therefore rests on a difference in the appearance and disposition of the component parts of the hypogynous 'disc' in the two plants.

The two criteria employed by Pax in 1890 differ from the one relied upon by Mueller in 1866 in being real and not imaginary. As the staminal criterion is not looked upon by Pax as an absolute one, it is more convenient to consider the two separately. In considering them it is, moreover, necessary to take into account the circumstances under which they

[1] This account is based on an examination of (*a*) the original specimens of *Adelia anomala*, Juss., collected by Smeathman in Sierra Leone; (*b*) the original specimens of *Erythrococca aculeata*, Benth., collected by Vogel in Sierra Leone; (*c*) specimens of the same species collected by Scott Elliot in Sierra Leone.

were enunciated. Since these criteria were laid down, the limits of the genus *Erythrococca* have been extended, and this extension has involved the substitution of a new criterion. Our examination of the two characters must therefore have regard only to their applicability to a state of affairs in which *Erythrococca* was still looked upon as a monotypic genus, which had to be discriminated from a *Claoxylon* that, except for the removal therefrom of the genus *Micrococca*, Benth., was limited and subdivided in accordance with the system devised by Mueller in 1866. The stamens in *Erythrococca* are rarely, if ever, fewer than 6; they may be as many as 11.[1] In the section *Adenoclaoxylon*, as known to Mueller, the number of stamens is 7–8; in *Discoclaoxylon* the number is 6–12. In *C. trichogyne* and *C. triste*, the two species with stipular thorns and plumosely laciniate stigmas which Mueller added to *Athroandra*, but which do not belong to that section in its original sense, the male flowers are still unknown. But in *E. hirta*, Pax, which is so nearly allied to *C. trichogyne* that the two may be conspecific, there are only 10 stamens; in *C. Menyharthii*, Pax, which is equally nearly allied to *C. triste*, there are only 2–5 stamens. This staminal criterion is, on the whole, adequate so far as Mueller's sections *Gymnoclaoxylon* and *Euclaoxylon*[2] are concerned; it is also applicable to that portion of *Athroandra*, Muell. arg., which corresponds with the true *Athroandra*, Hook. f. But it is ineffective so far as Mueller's sections *Adenoclaoxylon* and *Discoclaoxylon* are concerned, and is probably equally so as regards that portion of *Athroandra*, Muell. arg., which does not belong to *Athroandra*, Hook. f.

The stigmatic criterion used by Pax in 1890 happens to be less effective as between *Erythrococca*, Benth., and *Claoxylon*, A. Juss., than the

[1] The experience of Bentham and Pax that the stamens may be fewer than 6 must be looked upon as, at least, somewhat exceptional. It is opposed to the experience of Mueller, who appears usually to have met with 6, occasionally with 7; 5 in an outer series, with always at least one quite central. In view of this conflict of experience, close attention has been given to this question, and no apology is needed for a statement of what has so far been actually observed. The usual arrangement is precisely that described by Mueller; no flower examined has been met with in which the outer series of 5 stamens was incomplete, nor has one been seen in which this outer series was unaccompanied by at least one central stamen. But very often there are 2–3 central stamens; occasionally there are 4–5 central stamens, and when this is the case these 4 or 5 are definitely disposed in a second (inner) series, accompanied by an additional ring of receptacular glands. Very rarely, when there are two complete series of stamens (5 + 5), an eleventh quite central stamen is present; this eleventh stamen, however, is hardly ever perfect; usually it has no anther; sometimes it has anther cells with no pollen. In flowers with 9–11 stamens the male calyx has always been found to be 4-partite; when there are 7–8 stamens the calyx is usually 4-partite. When the calyx is 3-partite there are almost always 6 stamens arranged as Mueller describes; occasionally, however, 3-partite calyxes may have 7 stamens, very rarely may have 8. A central imperfect stamen, exactly like that occasionally seen in *E. aculeata*, occurs frequently, though by no means invariably, in *Claoxylon Menyharthii*, and has been interpreted by Brown (Kew Bull. 1909, 141) as a rudimentary ovary (*C. virens*, N. E. Br.).

[2] It is, however, only because of Hooker's discovery that *C. oligandrum*, Muell. arg., is not an *Euclaoxylon* but a *Micrococca* that we are in a position to make this statement, because in *C. oligandrum* we sometimes find 5 stamens.

staminal one is. In every species where the female flowers are known the stigmas are plumosely laciniate throughout Mueller's sections *Discoclaoxylon*, *Adenoclaoxylon*, *Gymnoclaoxylon*, and *Euclaoxylon*. Even in the case of *Athroandra*, Muell. arg., the character is ineffective because Mueller has added to *Anthroandra*, Hook. f., three species, *C. trichogyne*, *triste*, and *pauciflorum*, which have plumosely laciniate stigmas.[1] The criterion, as between *Erythrococca* and *Claoxylon*, instead of being generally applicable, is only effective in separating from *Erythrococca*, Benth., the section *Athroandra* when that section is limited in accordance with Hooker's original intention.

The fact that a character may not prove effective in a particular direction does not, however, necessarily deprive it of value. In the present case this stigmatic character enables us to rectify the misapprehension entertained by Mueller with regard to the natural limits of the section *Athroandra*, Hook. f. But the character does more than this; it confirms from an independent source the opinion of Hooker that *Athroandra* is probably generically distinct from *Claoxylon*. Were this, indeed, the only character which had to be considered, the logical result of its application would be the simultaneous reduction of *Erythrococca* to *Claoxylon*.

We have, however, yet to consider another criterion which has been held by Pax to justify at once an extension of the limits of the genus *Erythrococca*, and the separation of that genus from *Claoxylon*. This criterion, suggested in 1895, is that *Erythrococca* may be recognized by the presence of stipular thorns. There is no doubt that the character is an important one. In the original *E. aculeata*, Benth., the thorns are so large as to compel attention, but in the three species of *Claoxylon* known to him in which the character is obvious (*C. Kirkii*, *trichogyne*, and *triste*) Mueller has treated it as negligible.[2] The criterion, as we have already seen, may be used to a greater extent than Pax has yet formally used it. It does not conflict with any other salient character; there is no species with stipular thorns which does not at the same time have perulate buds and plumosely laciniate stigmas. The only difficulty is that the criterion does not take us quite so far as these two other characters, used in conjunction, happen to carry us; there is one species, *C. pauciflorum*, which has perulate buds and plumosely laciniate stigmas, where the stipules remain unmodified. The perulate buds indicate that *C. pauciflorum* should be associated either with *Erythrococca* (including *Adenoclaoxylon*) or with *Athroandra*, yet the nature of the stipules excludes it from the former, the character of the stigmas prevents its reference to the latter.

[1] It is largely because these three species exhibit this character, whereas the stigmas of every true *Athroandra* are entire, that Mueller's treatment of the section is unsatisfactory.

[2] It is not stated why the character was considered unworthy of reference. But we know that it cannot have been overlooked, because Welwitsch, in his field-notes regarding *C. trichogyne*, expressly calls attention to the nature of the stipules (Cat. Afr. Pl. Welw., 975), and we also know that Mueller had access to these field-notes.

The chief objection to the exclusive employment of either the stigmatic or the stipular character is that both suggest a cleavage plane which intersects that indicated by the presence or absence of cartilaginous bud-scales, treated by Hooker as perhaps of generic, and by Mueller as certainly of sectional significance.[1] In their neglect of this character Bentham and Pax have only done what Mueller has done as regards both the stigmatic and the stipular ones. The action in both cases is quite legitimate; its explanation probably is a wish to avoid the simultaneous employment of characters which act at cross purposes. But the fact that they were not made use of by Mueller has not rendered the stigmatic and the stipular criteria less valuable; the fact that Bentham and Pax have not employed them leaves unaffected the importance of the bud-character pointed out by Hooker. When we find that, useful and valuable as they are, the stipular and stigmatic criteria alike fail to effect the complete and satisfactory differentiation of *Erythrococca* from *Claoxylon* which is desired, it is permissible to examine more closely the result of the application of the character afforded by the presence or absence of cartilaginous bud-scales.

When Hooker's character is treated as the primary one, we find that those species which have perulate buds, and at the same time have stipular thorns (*Erythrococca* as amplified by Pax), always agree with the non-perulate species (*Claoxylon* proper) as regards stigmas, while one half of them agree as regards male flowers. Those species with perulate buds, which at the same time have entire stigmas (*Athroandra* in the original Hookerian sense), always differ from the non-perulate species (*Claoxylon* proper) as regards female flowers, and with one exception[2] differ also as regards male flowers. It is clear, when this line of inquiry is taken, that *Athroandra*, Hook. f., is entitled to generic recognition as apart from *Claoxylon*. Nor in the case of *Erythrococca*, as amplified by Pax and as apart from *Athroandra*, is there room for serious doubt. It is permissible to argue that, where there is such substantial agreement as regards floral structure, no single character, even when so striking as that afforded by the presence of stipular thorns, can suffice to justify the recognition of a genus. But the argument is not really sound. The important character on which Pax has based his judgement does not stand alone; it is only ancillary to the still more important character of perulate buds. When this is realized all doubt as to the desirability of separating *Erythrococca* from *Claoxylon* disappears.

[1] According to the system of subdivision employed by Mueller in the case of *Claoxylon*, which is based conjointly on the presence or absence of bud-scales, and on the disposition of the receptacular glands in the male flower, the two sections *Athroandra* and *Euclaoxylon* agree as regards the latter, and are only distinguishable by the former character.

[2] *C. membranaceum*, Muell. arg., which, now that its male flowers are known, is found to have the filaments longer than the anthers, as is the case in *Claoxylon* proper and in all the species that have stipular thorns. In every other species which has entire stigmas the anthers are subsessile.

The question remains as to whether *Erythrococca*, as enlarged by Pax, and *Athroandra*, Hook. f., constitute two distinct genera, or if they may be treated as integral parts of one natural genus. As genera in Euphorbiaceae go, no great harm could accrue were they kept distinct; their discrimination would certainly present no difficulty. But while their separation would be a simple matter it does not appear to be necessary. In the first place the stipular character, which is perhaps unequivocal so far as the distinction between *Erythrococca* and *Claoxylon* is concerned, is not so definite as indicating a distinction between *Erythrococca* and *Athroandra*. The stipules in *Athroandra*, it is true, remain minute and unmodified. But these organs are not, in *Athroandra*, normal stipules; they are firm, persistent, hyaline or scarious bodies that, even in those species which have pubescent twigs and petioles, are polished and glabrous. They differ in degree rather than in kind from stipules of the *Erythrococca* type. In all those species which can be referred to *Erythrococca*, as understood by Pax, the stipules are accrescent, the filaments are longer than the anthers, the stigmas are plumosely laciniate, and the hypogynous scales which alternate with the carpels are free and discrete, or very rarely are accompanied by smaller additional scales. In all the species which can be referred to *Athroandra*, Hook. f., the stipules remain unmodified, the anthers are subsessile, the stigmas are entire, and the hypogynous scales which alternate with the carpels are rarely free, and if they be free are contiguous at their margins under the ovary; more usually they are connate in an urceolate disc. There is, however, one species with perulate buds, *C. pauciflorum*, Muell. arg., which has the stamens, the hypogynous scales, and the stigmas of *Erythrococca*, with the unmodified stipules of *Athroandra*. On the other hand, there is a perulate species, *C. membranaceum*, Muell. arg., with the unmodified stipules, the entire stigmas, and the contiguous hypogynous scales of *Athroandra*, which has anthers borne on long filaments, as in *Erythrococca*. These two species, one a somewhat aberrant *Erythrococca*, the other a somewhat aberrant *Athroandra*, serve as links between the groups to which they severally belong. The intimate relationship of these groups is further indicated by a third species with perulate buds, *C. polyandrum*, Pax and K. Hoffm. (Engl. Bot. Jahrb., xlv. 237). As regards the sum of its characters this species resembles *C. pauciflorum* so much that it may be looked on as the East African representative of that Angolan plant. It combines the anthers with long filaments and the discrete hypogynous scales of an *Erythrococca* with the unmodified stipules of an *Athroandra*. The stigmas, however, are curiously intermediate; they are ovate-lanceolate and divaricate, and are borne on a distinct, if short, style, exactly as they are in one-third of the species of *Athroandra*. But in place of being entire, as they always are in *Athroandra*, the stigmas of *C. polyandrum* have incised lobulate margins. They thus approach the plumosely laciniate

type of stigma characteristic of *Erythrococca*, to which group, in spite of its markedly aberrant features, *C. polyandrum* is most conveniently referred. The existence of these intermediate species justifies the treatment of *Athroandra* as a sub-genus of an *Erythrococca* somewhat wider even than Pax has indicated; the criterion which distinguishes this enlarged *Erythrococca* from *Claoxylon*, A. Juss., is that it has perulate buds, while *Claoxylon* has not.

Throughout this genus, in spite of the differences as regards hypogynous scales and stigmas which occur in the species of its two sub-genera, the fruit and the seed are remarkably uniform, and the account already given under *E. aculeata* is applicable to the fruit of all its congeners except that in the majority we find no hairs on the pericarp, and that in a few the arillus does not quite envelop the testa.

Mueller (DC. Prodr., xv. 2,775) terms the fruit in *Claoxylon*—which genus, as understood by him, included *Claoxylon* itself as here understood, *Micrococca* as here understood, and the whole of *Erythrococca* as here understood, except *E. aculeata*, Benth.—capsular, 2–3-coccous. This characterization is sufficiently general to admit of its employment for all three genera. The seed Mueller speaks of as ecarunculate and as covered by a coloured epidermis which soon becomes loose. This 'epidermis' is the arillus; it never *becomes* loose, because it shows no trace, at any stage, of organic union with the testa. Welwitsch, on the other hand, refers, in the case of *C. pauciflorum*, to a fleshy endocarp (Cat. Afr. Pl. Welw., 975); on examination Welwitsch's specimens show that this 'endocarp' is the arillus; there is no trace of organic union between it and the pericarp. The arillus is viscid, as Welwitsch remarks under *C. Welwitschianum*; it therefore sometimes adheres, now to the seed coat which it overlies, now to the inner wall of the pericarp which encloses it. The apparently incompatible interpretations of Mueller and Welwitsch are thus not only readily intelligible but easily reconciled. The seeds of *C. Welwitschianum* and of *C. triste* are said by Welwitsch to have an arillode, but his own specimens show that the structure is really an arillus.

Bentham (Gen. Plant., iii. 309) speaks of the seed of *Claoxylon*—the genus being understood by him as it was by Mueller, except that it does not include *Micrococca*—as 'estrophiolate'; he does not mention the arillus.[1] The fruit of *Claoxylon* is said by Bentham to break up into 2-valved cocci (*capsula in coccos 2-valves dissiliens*). This description applies only in a qualified sense to any species of the sections *Adenoclaoxylon* or *Athroandra*, which we have now transferred to *Erythrococca*. The same qualification is needed in the case of the section *Discoclaoxylon*, where the cocci, though

[1] The first author to allude definitely to the fact that the seed in *Claoxylon* is arillate was Kurz (For. Flor. Brit. Burma, ii. 395): Kurz had, however, the advantage of having collected the fruits and examined the seeds of certain species of *Claoxylon* in the field.

larger than in any *Erythrococca* and densely velvety outside, are again green and coriaceous when ripe, and open loculicidally without shedding their valves to expose the seeds, which in this case are bright yellow. In the case of the section *Euclaoxylon* (including *Gymnoclaoxylon*) the fruits and seeds are, at least as a rule, indistinguishable from those of *Erythrococca*, though it is stated by Hooker (Flor. Brit. Ind., v. 410) that in *Claoxylon* there are species with cocci which, besides being coriaceous, may be indehiscent, and that in some of the species the seed may have no arillus.[1] It is true that ultimately in *Claoxylon*, as in *Erythrococca*, the two half valves of the cocci fall away, but there is no splitting of the septa such as is met with in the species of the allied genus *Micrococca*, to the fruits of which the description given by Bentham is strictly applicable.

A character, more interesting than important, which at first sight appears almost distinctive of *E. aculeata*, on which the genus *Erythrococca* was based, deserves to be noted. The pedicels in the male flowers of *Erythrococca* are articulate. The articulation is usually opposite or below the tip of the corresponding bracteole; the portion of the pedicel under the joint is, as a rule, appreciably thicker than the portion above;[2] in cases where the rachis is pubescent while the pedicel is glabrous, the thicker pedicel base is often pubescent like the rachis, and not glabrous like the rest of the pedicel. In *E. aculeata* the joint is considerably above the level of the bracteole. But this feature is not peculiar to *E. aculeata*; it is well marked in *Claoxylon Menyharthii*, which differs considerably from *E. aculeata*, and in *E. natalensis* and *E. berberidea*, which differ considerably from both the foregoing. The meaning of this difference in the position of the articulation from that which usually obtains is not obvious; it is, however, so far as classification is concerned, only of specific value.

Another curious character met with in *E. aculeata* is that the male calyx is partite only to the middle, leaving below the teeth a distinct campanulate tube. In two other species, *E. berberidea* and *E. subspicata*, the calyx is two-thirds partite, but in the genus as a whole the calyx is partite almost to the base. This departure from the general rule is associated with a divergence from the usual conditions as regards the receptacular glands. In *E. aculeata* these are unusually large, as long as the stamens; in *E. berberidea* and *E. subspicata* they are also large, as long as the filaments; as a rule these glands are relatively small, generally minute. The horizontal plane to which division of the calyx extends in each case practically coincides with the level of the points of origin of the viscid apical

[1] The possible association of exarillate seeds and indehiscent cocci is one that will doubtless receive the attention it merits from whoever may prepare the revision of which the genus *Claoxylon* stands urgently in need.

[2] In the case of *C. columnare*, Muell. arg., the condition has been fully and correctly described by Mueller (DC. Prodr., xv. 2, 776); the character, however, is a generic and not, as Mueller's treatment seems to imply, a specific one.

hairs[1] so generally met with in the male flower; what at first seems a striking difference is therefore in reality only a case of conformity with a general rule.

In speaking of the bodies which bear these viscid hairs the term 'receptacular glands', used by Bentham, is preferable to the term 'disc' employed by Baillon and Mueller in the case of *E. aculeata*, and less definitely by Mueller as regards *Claoxylon* generally.[2] The word 'disc', owing to the wide morphological interpretation which it permits, is rather unsatisfactory, and its use, so far as the genus *Erythrococca* is concerned, is not altogether convenient. In some of the more nearly allied genera these glands are staminodes and show every transition from a perfect stamen to a simple, more or less amorphous gland. We have seen that, in *Claoxylon*, one species, *C. sandwicense*, affords equally direct evidence of the same transition. Direct testimony to this effect is not very often met with in *Erythrococca*, but indirect evidence as to the staminodial character of these glands is by no means wanting. Cases of this are encountered in the section *Adenoclaoxylon*. In *E. Kirkii*, where the extrastaminal glands are usually connate in an urceolum, we find no very definite indication of the staminodial nature of the glands, but in *E. mitis*, where these glands are free, they are frequently thickened and 2-lobed, and much resemble imperfect stamens. In *E. bongensis* and *E. rigidifolia*, where the extrastaminal ring met with in *E. Kirkii* and *E. mitis* does not occur, the stamens of the outermost series have each a pair of minute glands adnate to the base of the filaments. In *E. Paxii*, where there is usually an extrastaminal ring which is incomplete, some at least of the stamens exhibit the same association with a pair of receptacular glands.[3] The meaning of the arrangement becomes apparent in *E. olacifolia*, where there is an extrastaminal ring which is usually complete, but where, when the ring is incomplete, a missing gland may be replaced by a perfect stamen. The glands of the extrastaminal ring are usually considerably larger than the interstaminal glands of the same species, and may reasonably be interpreted as representing a fused pair of basal glands belonging to a stamen which is itself suppressed. This interpretation is more or less confirmed by the arrangement met with in *E. subspicata*, where the extrastaminal ring consists of twelve glands agreeing in shape and size with the interstaminal ones, but united at their bases as six 'pairs' of glands,

[1] The function of these viscid hairs, among which, in open flowers, insects are occasionally found entangled, calls for more attention in the field than it has so far received.

[2] In the cases of *Adenoclaoxylon* and *Athroandra*, which immediately concern us since both are now transferred to *Erythrococca*, and again in the case of *Discoclaoxylon*, Mueller avoids the use of the term 'disc'. But the 'urceolum' spoken of under *Adenoclaoxylon* and *Athroandra* and the 'urceolate ring of glands' described under *Discoclaoxylon* are definitely referred to under *Gymnoclaoxylon* as 'an extrastaminal disc' and under *Euclaoxylon* as a 'disc surrounding the receptacle'. Mueller, however, does not employ the term 'disc' in connexion with the interstaminal glands, which differ from the extrastaminal ones in situation, but do not differ from them in character.

[3] This is well shown by Rendle in Journ. Linn. Soc. Bot., xxxvii, t. 3, fig. 5.

the 'pairs', however, remaining perfectly distinct. The interstaminal glands throughout the genus are remarkably uniform; they are rhomboid and truncate or subtruncate at the apex where the viscid long hairs arise, and show no clear indication of their staminodial character. The chief deviation from this shape is in *E. zambesiaca* and *E. natalensis*, where the glands are flattened, suborbicular, and fringed with marginal short hairs; here, again, there is no indication that the glands are modified stamens. But there is another deviation from the rhomboid form which, though less marked, is more interesting. A few species in various sections, such as *E. rigidifolia* in *Adenoclaoxylon*, *E. Menyharthii* in *Trichogyne*, *E. polyandra* in *Pseudathroandra*, and *E. Molleri* in *Chloropatane*, have glands that are ovoid instead of rhomboid, and are then glabrous or nearly so. In the cases of *E. rigidifolia*, *E. polyandra*, and *E. Molleri*, where the glands are all interstaminal, they are not all sessile; some of the glands are distinctly stipitate and have all the appearance of being rudimentary stamens. In the case of *E. Menyharthii*, Brown has, indeed, interpreted the glands, which are all extrastaminal, as staminodes (Kew Bull., 1909, 141).

The word 'disc', in connexion with the female flower, has the sanction of general use and common consent; it is, however, doubtful if the term be more satisfactory there than it is in the case of the male. The hypogynous scales of which this 'disc' is composed in the sub-genus *Euerythrococca* are invariably discrete, but in *Athroandra* either are so large as to be contiguous under the base of the ovary or are connate in a lobed, rarely a subentire, shallow cup. The *Euerythrococca* arrangement is that which is met with in the genus *Micrococca*, and Hooker has entered a plea for caution in connexion with this group (Flor. Brit. Ind., v. 410), which deserves careful attention. Hooker has, indeed, tentatively suggested that the scales may perhaps be petals; their usual appearance and the circumstance that, as a rule, they alternate with the calyx-segments lend support to this view. But it has to be noted that they also alternate with the carpels,[1] and although, as a rule, the carpels are isomerous with and opposite to the calyx-segments, cases are by no means rare in which the carpels are fewer than the calyx-segments. When the number of carpels is thus reduced, there is a corresponding reduction in the number of hypogynous scales; this is not what might be anticipated, as an invariable occurrence, had the scales been modified petals. There is thus room for an alternative suggestion;

[1] An indication that this interpretation is not wholly satisfactory is afforded by the arrangement met with in *Claoxylon* § *Discoclaoxylon*, Muell. arg. In this case the female calyx, like the male calyx, is uniformly 4-partite. The ovary is always 2-locular; the disc consists of two very large, fleshy, reniform scales, alternate with the carpels and prolonged under the base of the ovary so that their edges are contiguous both in front and behind, as in the species of *Athroandra* § *Hemierythrococca*. In *Discoclaoxylon*, however, while the carpels are exactly opposite the anterior and the posterior calyx-lobes, the hypogynous scales, instead of alternating with a pair of calyx-lobes, are opposite the two calyx-lobes which constitute the lateral pair.

the scales may perhaps represent a modified androecium. That this suggestion is, at least sometimes, more satisfactory than the other, may be gathered from an examination of the arrangement which obtains in the plant on which the genus *Poggeophyton*, Pax (Engl. Bot. Jahrb., xix. 88), was based. This plant agrees with *Erythrococca* in having perulate buds, stipular thorns, and plumosely laciniate stigmas. It was believed, when the genus was proposed, that it differs from *Erythrococca* as regards its fruit. This, as we now know, is not the case; the difference between *Poggeophyton aculeatum* and an *Erythrococca*, such as *E. aculeata*, is confined to the hypogynous 'disc'. That difference is certainly striking. In *Erythrococca* the 'disc' is composed of small, flattened discrete scales, isomerous and alternate with the carpels. In *Poggeophyton* these hypogynous scales are replaced by large staminodes, isomerous and alternate with the carpels; what Pax treats as the 'disc' is a ring of smaller oblong glands hirsute at their tips with long viscid hairs. The glands which constitute this ring are sometimes free, sometimes slightly connate below, and belong to a whorl external to that in which the staminodes are situated. The staminodes are obviously only somewhat imperfect stamens with stout cylindric-clavate filaments longer than the anthers; the anther cells are two in number, erect, free except at the base, and without pollen. The 'glands', on the other hand, are identical in appearance and character with the glands that form the extrastaminal 'ring' or the extrastaminal 'urceolum' in the male flowers of an *Adenoclaoxylon*, such as *E. Kirkii* or *E. mitis*, or of a *Deflersia*, such as *E. abyssinica*, *E. aculeata*, or *E. subspicata*.

The difficulty presented by the conditions described is not, therefore, so formidable as it at first appears. The 'staminodes' of *Poggeophyton aculeatum* are identical in appearance as well as in position with the hypogynous scales of *Claoxylon triste* as described by Mueller (DC. Prodr., xv. 2, 779), and with those of *C. Menyharthii*, Pax, except that in *C. triste* and *C. Menyharthii* these scales are merely cylindric-clavate filaments which are not surmounted by empty anther cells. Moreover, in *C. Menyharthii*, apparently as a casual abnormality, one or two small, linear-oblong, glabrous glands may occasionally be met with in addition to the staminodial hypogynous scales, though these glands are less conspicuous than the corresponding ones in *Poggeophyton aculeatum*, and do not, as in that species, form a complete hypogynous ring.

Two other species, which also are confined to West Central Africa, agree in general facies with *Poggeophyton aculeatum*, and are evidently members of the same natural group; in both of these species, however, the staminodes characteristic of *P. aculeatum* are replaced by ovate, subacute, flattened hypogynous scales of the normal *Erythrococca* type. One of the species, *E. Laurentii*, is readily distinguished from the others by having leaves of a different shape with shorter and stouter petioles, but the second,

E. subspicata, resembles *Poggeophyton aculeatum* so closely that, apart from its 'disc', it is mainly separable because its leaves are denticulate in place of crenate. This fact induces, therefore, some doubt as to whether the presence of receptacular glands of a male type in the female flowers of *P. aculeatum* be more than an abnormality. That doubt is increased by the circumstance that one of Pogge's specimens exhibits an unusual irregularity; towards the base of some of its subspicate racemes, male flowers occupy the positions which higher up the spikes are occupied by ripe fruits.[1] These male flowers, except that they have 9 stamens instead of 12, and 5 pairs of extrastaminal glands in place of 6, are remarkably like the male flowers of *E. subspicata*. It may be argued that this monoecious condition affords an additional reason for treating *Poggeophyton aculeatum* as the type of a distinct genus. The argument would, however, be open to serious objection; the male flowers in question, in place of being glomerulate, are solitary as if they were female ones. The inference, therefore, rather is that the female flowers exhibit, in the androecial aspect which has been imparted to their 'disc', a complementary effect of the male influence in the plant. All that its characters justify us in concluding is that the particular plant from which Pogge's specimens were taken did not show that clearly defined diclinism which is characteristic of the genus to which it belongs.[2] The character of the disc in *Poggeophyton aculeatum*, in place of affording a criterion of generic import, supplies one that is doubtfully adequate for specific discrimination. What, in any case, seems clear, is that the claim of *Poggeophytum aculeatum* to a place, along with *E. subspicata*, in the genus *Erythrococca*, widened as Pax has widened it, is valid.

The fact that in *E. Poggeophyton*, *E. Menyharthii*, and *E. tristis*, the bodies which occupy the position of the flattened hypogynous scales met with in other nearly allied species are manifestly staminodes, while the scales which may accompany these staminodes in *E. Poggeophyton* and *E. Menyharthii* are comparable with male receptacular glands, themselves certainly staminodial, appears to warrant the belief that the hypogynous scales in the genus as a whole correspond morphologically with a modified androecium. Whether this interpretation be justified or not, the existence of the conditions that obtain in *Claoxylon Menyharthii* and in *Poggeophyton*

[1] The only instance in which a doubt has hitherto arisen as to whether a species of *Erythrococca* may be monoecious, has been in the case of *Claoxylon* (*Athroandra*) *Schweinfurthii*, Pax (Engl. Bot. Jahrb., xix. 86), a plant which in this paper is treated as a form of *C. flaccidum*, Pax. The circumstances have been fully explained by Pax (l. c.). In the case of *C. Schweinfurthii*, the two sexes are met with in specimens issued by Schweinfurth under the same field number; the presumption therefore is that they may have been found on one plant. But in this instance the male and female flowers are on distinct twigs, and we do not have the definite proof which *Poggeophyton aculeatum* affords as to the existence of a monoecious condition.

[2] Analogous instances of collateral manifestation of the male influence in a female flower and *vice versa* are not infrequent in *Cannabis sativa*; an account of some of these may be found in Sc. Mem. by Officers, Med. and Sanit. Dept. India, N.S., no. 12, 1904.

aculeatum assists us in interpreting the three types of 'disc' met with in the female flowers of *Erythrococca*. From a condition such as that occasionally seen in *Claoxylon Menyharthii*, in which the hypogynous bodies alternating with the carpels are staminodes which are accompanied by an incomplete ring of small free scales, to a condition in which these additional scales fuse with the adjacent staminodes to form as many discrete flattened hypogynous scales as there are carpels, is only a step. From a condition such as that characteristic of *Poggeophyton aculeatum*, in which the hypogynous bodies alternating with the carpels are staminodes which are accompanied by a complete ring of small free scales, to a condition in which these accessory scales fuse with the adjacent staminodes to form as many contiguous flattened hypogynous scales as there are carpels, is again only a step. From this latter condition to one where these contiguous glands are fused in a complete urceolate 'disc' is but another step in the same direction. That this may have been the history of the evolution of the 'disc' in *Erythrococca* is suggested by another consideration. In *E. africana* the disc at first is complete and entire; in fruit it is distinctly subequally 5-lobed; the number of lobes suggests the possibility of some relationship to the fact that in the male flowers there are 10 stamens in the outermost (marginal) series. Another species where there is the suggestion of a similar retrogression is *E. Molleri*; here the 'disc' in the female flower sometimes consists of two free scales with contiguous margins, sometimes is complete; when it is complete this disc at first has a uniform, even or 2-lobed margin, later on it splits along the edge into a number of crenulate lobules.

Summary.

The result of a detailed consideration of the characters exhibited by these various groups of species with anther cells that remain erect even when empty, is to confirm the conclusion to which Bentham came in 1849: *Erythrococca*, Benth., and *Micrococca*, Benth., though very closely allied to *Claoxylon*, A. Juss., are easily distinguishable and natural genera. But while Bentham's instinct was in each case right, neither genus is monotypic, as he believed them to be; to Hooker we are indebted both for one of the criteria which enable us to distinguish *Erythrococca* from its two allies, and for the demonstration of the fact that *Micrococca* is wider in its limits than Bentham supposed. For the proof of the necessity for an enlargement of the limits of *Erythrococca* we are indebted to Pax. The characters which enable us to discriminate the three genera may be summarized as follows:—

I. Erythrococca, Benth. ampl. Buds perulate. Racemes interrupted or uniformly floriferous. Capsule subglobose or didymous, coriaceous; cocci dehiscing loculicidally: subdivided into :—

1. **Euerythrococca.**[1] Stigmas plumosely laciniate or at least incised lobulate.

2. **Athroandra,** Hook. f. Stigmas entire.

II. CLAOXYLON, A. Juss. Buds not perulate. Racemes uniformly floriferous. Capsule subglobose, coriaceous; cocci dehiscing loculicidally: subdivided into:—

1. **Euclaoxylon,** Muell. arg.[2] Male flowers with only interstaminal glands.

2. **Discoclaoxylon,** Muell. arg. Male flowers with only an extra-staminal urceolum.

III. MICROCOCCA, Benth. ampl. Buds not perulate. Racemes interrupted. Capsule deeply 3-coccous, thinly crustaceous; cocci dehiscing both septicidally and loculicidally.

The necessity for some modification of view as regards the limitation of *Micrococca*, Benth., was impressed on Sir J. D. Hooker when engaged in the task of dealing with the Indian species of the genus *Claoxylon*. The necessity for a corresponding modification of view as regards the limits of *Erythrococca*, Benth., has been similarly impressed upon the writer while occupied in the preparation, at the request of Sir W. T. Thiselton-Dyer, of an account of the African species from time to time referred to *Claoxylon*, for the 'Flora of Tropical Africa'. The extension of the limits of *Micrococca*, which Hooker has shown to be essential, and the corresponding extension of *Erythrococca*, for which we are similarly indebted to Pax, to which effect is here given, departs from the treatment proposed by these authors only in that *Micrococca*, looked upon by Hooker as a section of *Claoxylon*, is here considered a distinct genus, while the genus *Erythrococca* is made to include, besides the section *Adenoclaoxylon*, also the section *Athroandra*.

The action here taken involves, therefore, some modification of the estimate hitherto formed with regard to the dimensions of the genus *Claoxylon*. The restriction of this latter genus is, however, to a certain extent compensated for by the fact that these transfers simplify materially our conception of its geographical distribution. *Micrococca*, which up to 1887 was believed to be monotypic, is now known to include eight distinct species and one recognizable variety, and to extend from South-East and East Africa and the Comoro Islands to South-West Peninsular India and Ceylon, with one of the species, which is a tropical field-weed, further spread throughout the greater part of Tropical Africa, Southern Arabia, the greater part of India, Western Indo-China, and the Malay Peninsula. *Erythrococca*, which up to 1894 was also believed to be monotypic, is now known to include over forty recognizable forms, all of them confined to the

[1] This sub-genus, as already explained, includes *Adenoclaoxylon*, Muell. arg.

[2] This sub-genus, as already explained, includes *Gymnoclaoxylon*, Muell. arg.

African continent or to the islands in the Gulf of Guinea, save one which extends from Abyssinia to Southern Arabia.

Claoxylon, on the other hand, which in 1866, when allowance has been made for the removal of *Adenoclaoxylon*, *Athroandra*, and *Micrococca*, was believed to contain thirty species, is now known to include about forty-five distinct forms, of which only three are African. The others are natives of the Mascarene Islands (fifteen), South-Eastern Asia (fourteen), Polynesia and New Caledonia (nine), and Australia (four). The three African species belong to an endemic section, *Discoclaoxylon*, so distinct that no harm could result were it treated as a different genus. A fourth African species, *C. sphaerocarpum*, Kuntze (Rev. Gen. Plant., iii. 2, 248), has indeed been described from South Africa. The material on which this species is based we have had no opportunity of seeing. Its author has indicated that this material is incomplete ; from the careful description which Kuntze has provided it is, however, almost certain that the plant in question belongs to another genus, and there is no evidence that any species belonging to *Claoxylon* proper, as originally defined by A. Jussieu, occurs on the African continent.

As the whole of the species included in *Micrococca* and *Erythrococca* have been described elsewhere, all that seems called for here is a revised definition of each of these genera, modified in accordance with existing knowledge and supplemented with a systematized enumeration of the forms that appear referable to each. In consequence of the fact that, of the two, *Erythrococca*—the inclusion of which in *Claoxylon* has never been proposed —is more closely related to *Claoxylon* than *Micrococca* is, it is desirable to reverse the order of their presentation. The enumerations themselves are based on an examination of the material of both genera preserved in the herbaria at Kew, the British Museum, and Berlin, with, in addition, in the case of *Erythrococca*, the material in the herbaria at Brussels and Paris ; in the case of Paris the African collections of Dr. Chevalier have also been available. The specimens of *Erythrococca* in the post-Prodromus collection of the de Candolle herbarium and in the herbarium of the Natal Botanic Garden have also been studied, as have the specimens, illustrating both genera, collected by Dr. Schweinfurth, which are preserved in the Boissier herbarium. To his friends Dr. Rendle, Professor Engler, Professor De Wildeman, Professor Lecomte, Mr. de Candolle, Mr. Medley Wood, and Mr. Barbey, the writer is deeply indebted for the kind help thus afforded. He is further greatly indebted to his friends Professor Schinz and Professor Briquet for permission to study the types of *Claoxylon Menyharthii*, which is preserved in the Zurich herbarium, and of *Trewia? africana*, which is preserved in the De Lessert herbarium.

ERYTHROCOCCA, Benth. ampl.

Erythrococca, Benth. *in* Hook., Niger Fl., 506 (1849) ; Baill., Étud. gén. Euphorb., 437, t. 21, f. 10 (1858) *et in* Adansonia, i. 71 (1860) ; Muell. arg. *in* DC. Prodr., xv. 2, 790 (1866) ; Benth. *in* Benth. et Hook. f. Gen. Plant., iii. 308 (1880); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48, fig. 29 A (1890) *necnon, sensu ampliore, in* Engl. Bot. Jahrb., xix. 87 (1894), *et in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895).

Adelia, Juss. *ex* Poir. *in* Encyc. Meth. Suppl., i. 132 (1810); A. Juss., Tent. Gen. Euphorb., 32, pro parte : *Claoxylo* quam *Adeliae* affinior (1824) : nec Linn.

Trewia, Baill., Adansonia, i. 68, pro parte et dubitanter (1860): nec Linn.

Mercurialis, Baill., Adansonia, iii. 176, *sensu ampliore* (1862) *et in* Hist. des Plantes, v. 210, *sensu ampliore* (1874) ; Deflers, Voy. Yemen, 203, dubitanter (1889) : nec Linn.

Claoxylon § *Athroandra*, Hook. f. *in* Journ. Linn. Soc. Bot., vi. 21 (1862) ; Muell. arg. *in* DC. Prodr., xv. 2, 776 (1866); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48, sphalm. *Arthroandra* (1890): nec *Claoxylon*, A. Juss.

Claoxylon § *Adenoclaoxylon*, Muell. arg. *in* Flora, xlvii. 436 (1864), *et in* DC. Prodr., xv. 2, 775 (1866); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48 (1890): nec *Claoxylon*, A. Juss.

Deflersia, Schweinf. *ex* Penzig *in* Atti Congr. Bot. 1892, Genova, 359 (1893), *et in* Abhandl. Akad. Berlin, xxxiii. 663, 667, 708: Abyssin. Pflanzennam., 17, 21, 62 (1893) : nomen tantum.

Poggeophyton, Pax *in* Engl. Bot. Jahrb., xix. 88 (1894).

Chloropatane, Engl. *in* Engl. Bot. Jahrb., xxvi. 383 (1899) ; Perkins & Gilg *in* Engl. Pflanzenr. : Monim. 24 (1901) ; Wright *in* Thiselton-Dyer, Flor. Trop. Afr., vi. 1, 168 (1909).

Rivinoides, Afzel. *in sched.* : nomen tantum.

Flores dioici vel rarissime forsan casu monoici, apetali. ♂ *Calyx* membranaceus in alabastro saepissime subglobosus apice minute apiculatus, raro conico-pyramidalis, primum clausus, sub anthesi valvatim 3–4-partitus, casu 5-partitus. *Stamina* definita vel indefinita (2–64), in receptaculo plus minusve elevato centralia, saepe pluriseriata, glandulis rhombeis subovoideisve vel rarissime complanatis saepissime inter se liberis et apice pilis viscidulis plerumque praelongis onustis consociata ; filamenta nunc antheris manifeste longiora, nunc perbrevia antherisque breviora; antherae 2-loculares; loculi obovoidei vel subglobosi, basifixi, erecti, praeter basin inter se liberi, extrorsim rimosi; glandulae plerumque totum receptaculum tegentes et

inter bases filamentorum dispersae vel per paria basibus ipsis affixae, rarissime circa bases in annulum vel urceolum extrastaminalem tantum dispositae, saepe tamen utroque modo simul in floribus evolutae. *Rudimentum ovarii* nullum. ♀ *Calyx* plerumque 2-partitus, nonnunquam 3-partitus, rarissime 4-partitus, lobis quam in mare saepius minoribus. *Ovarium* glabrum vel nonnunquam strigosum, 2–3-loculare; loculi laciniis calycis oppositi vel iis pauciores; ovula in quoque loculo solitaria; stigmata loculis ovarii isomera; nunc elongata, linearia, patula, a basi libera totaque saepissime plumoso-laciniata, raro laevia; nunc brevia vel brevissima, ovato-lanceolata vel suborbicularia, divaricata vel suberecta, subsessilia et basi connata vel in apice styli saepius brevis raro plane evoluti columnaris insidentia, laevia vel perraro papillosa, margine integra vel rarissime inciso-lobulata. *Discus hypogynus* e squamis complanatis vel rarissime cylindrico-clavatis staminodiiformibus et interdum glandulis minoribus eis maris extrastaminalibus conformibus in serie exteriore additis, majoribus semper cum carpidiis alternantibus eisque isomeris compositus; squamae nunc inter se liberae et saepissime plane discretae, rarius marginibus contiguae; nunc in urceolum plus minusve annularem margine lobatum vel rarissime subintegrum zygomorphon vel perraro eccentron connatae. *Fructus* capsularis; capsula plerumque 2-cocca, didyma, sed saepe 3-cocca, vel nonnunquam casu abortuque monococca; cocci subglobosi, coriacei, extra glabri vel nonnunquam setosi, loculicide 2-valvi; valvae subreflexae cum columella subspathulata coriacea stigmatibus marcescentibus coronata diu cohaerentes, nonnunquam tamen demum deciduae semina columellamque relinquentes. *Semina* fere sphaerica, arillo carnoso viscido laete colorato involuta: testa crustacea, foveolato-reticulata; albumen carnosum; embryo axilis; radicula conica vel subcylindrica; cotyledones lati, complanati, suborbiculares.— *Frutices* glabri vel plus minusve puberuli vel pubescentes; inermes vel armati; cortex lenticellatus. *Gemmae* perulatae; squamae subcoriaceo-scariosae, nitidae, saepissime diu persistentes. *Folia* decidua, alterna, breviter sed distincte petiolata, membranacea, margine glandulosa, crenata vel dentata; petioli supra canaliculati; stipulae coriaceae vel cartilagineae, glabrae, nitidae; nunc minimae, hyalino-scariosae, immutatae; nunc accrescentes et in mamillas vel umbones vel aculeolos conicos rectos hamatosve mutatae, nonnunquam spinulas basi dilatatas apice pungentes efficientes. *Flores* parvuli vel minuti, in glomerulos axillares sessiles pedunculatosve aggregati, vel secus rhachides racemorum laxorum vel congestorum nonnunquam spiciformium axillarium fasciculatim vel singillatim dispositi. *Racemi-glomerulive* fasciculati vel solitarii; pedunculi graciles; bracteae parvae; pedicelli capillares prope vel raro paulo ultra basin articulati; bracteolae minutae. *Bracteae* maris saepissime pluriflorae, raro 1-florae; feminei plerumque 1-florae, raro 2–3-florae.

CLAVIS SPECIERUM GREGATIM CONIECTARUM.

Stigmata plumoso-laciniata vel inciso-lobulata; squamae hypogynae liberae, discretae [I. EUERYTHROCOCCA]:—
- Flores glomeratim congesti; stipulae aculeolatae [§ 1. **Adenoclaoxylon**]:—
  - Rhachides pubescentes:—
    - Stamina 6–8 — ¶ 1. *Mites* (spp. 1, 2).
    - Stamina 9–15 — ¶ 2. *Rigidifoliae* (spp. 3–5).
  - Rhachides glabri — ¶ 3. '*Fischerianae*' (spp. 6, 7).

- Flores racemosi:—
  - Stipulae aculeolatae vel pungentes:—
    - Stamina glandulis juxtastaminalibus intermixta et simul glandulis extrastaminalibus circumcincta [§ 2. **Deflersia**]:—
      - Racemi foliis breviores:—
        - Ovarium glabrum:—
          - Stipulae aculeolatae; stamina 6–8 — ¶ 4. *Eudeflersieae* (spp. 8, 9).
          - Stipulae pungentes; stamina 15–30 — ¶ 5. *Berberideae* (spp. 10–12).
        - Ovarium strigosum — ¶ 6. '*Aculeatae*' (sp. 13).
      - Racemi foliis subaequilongi — ¶ 7. *Subspicatae* (spp. 14–16).
    - Stamina aut glandulis extrastaminalibus circumcincta aut glandulis juxtastaminalibus intermixta: glandulae nunquam in utroque modo simul in floribus evolutae; ovarium dense strigosum [§ 3. **Trichogyne**]:—
      - Glandulae receptaculares omnes extrastaminales; squamae hypogynae staminodiiformes — ¶ 8. *Tristes* (spp. 17, 18).
      - Glandulae receptaculares omnes juxtastaminales; squamae hypogynae complanatae — ¶ 9. *Lasiococcae* (spp. 19–23).
  - Stipulae immutatae [§ 4. **Pseudathroandra**] — ¶ 10. *Pauciflorae* (spp. 24, 25).

Stigmata integra; stipulae immutatae [II. ATHROANDRA]:—
- Stigmata a basi libera; squamae hypogynae liberae sed circa basin ovarii marginibus contiguae [§ 5. **Hemierythrococca**]:—
  - Filamenta antheris longiora — ¶ 11. *Membranaceae* (sp. 26).
  - Filamenta antheris breviora — ¶ 12. *Patulae* (spp. 27, 28).
- Stigmata basi connata vel in apice styli insidentia; squamae hypogynae fere semper in urceolum connatae [§ 6. **Chloropatane**]:—
  - Stylus subnullus:—
    - Stigmata suborbicularia, majuscula, suberecta — ¶ 13. '*Rivinoides*' (spp. 29–31).
    - Stigmata ovato-lanceolata, divaricata — ¶ 14. *Rivulares* (sp. 32).

Stylus distinctus:—
Stigmata suborbicularia, minima, suberecta,
stylo columnari multo breviora ¶ 15. *Columnares* (spp. 33–35).
Stigmata ovato-lanceolata, sub anthesi divaricata
demum recurva, stylo abbreviato longiora ¶ 16. *Oleraceae* (sp. 36–42).

Subgen. I. EUERYTHROCOCCA.

Erythrococca, Benth.; Baill.; Muell. arg.; Pax; ll. supra cit. *Adelia*, Juss.; A. Juss.; ll. supra cit. *Mercurialis*, Baill., pro parte; Deflers; ll. supra cit. *Claoxylon* § *Adenoclaoxylon*, Muell. arg.; loc. supra cit. *Deflersia*, Schweinf.; loc. supra cit. *Poggeophyton*, Pax; loc. supra cit. *Claoxylon* § *Athroandra*, Muell. arg., pro parte; loc. supra cit.: nequaquam § *Athroandra*, Hook. f.

Stipulae plerumque accrescentes, induratae, mammillas umbones aculeolos vel spinulas efficientes; rarissime immutatae. *Flores* plerumque minuti in glomerulos vel racemos dispositi. *Stamina* saepissime definita et nisi in *E. Menyharthii* glandulis juxtastaminalibus consociata, saepe simul glandulis extrastaminalibus circumcincta; filamenta antheris longiora; antherarum locelli subglobosi, rarius obovoidei. *Ovarium* glabrum vel strigosum; stigmata nisi in *E. polyandra* sessilia, libera, patula, a basi tota plumoso-laciniata—in *E. polyandra* stigmata in apice styli brevis insidentia, divaricata, margine inciso-lobulata. *Discus hypogynus* e squamis complanatis vel raro cylindrico-clavatis staminodiiformibus cum carpidiis alternantibus eisque isomeris rarissime alteris minoribus seriei extrastaminodialis additis compositus; squamae inter se liberae et minoribus neglectis plane discretae.

§ I. ADENOCLAOXYLON. *Flores* minuti in glomerulos axillares congesti. *Stamina* definita vel subdefinita glandulis juxtastaminalibus intermixta et saepe simul glandulis extrastaminalibus—his tamen nonnunquam plus minusve imperfectis—circumcincta; antherarum locelli subglobosi. *Ovarium* nisi in *E. olacifolia* glabrum vel raro pilis setosis paucis onustum —in *E. olacifolia* dense strigosum; squamae hypogynae complanatae, inter se discretae. *Perulae* persistentes. *Stipulae* mammillas umbones vel aculeolos efficientes.

¶ 1. **Mites.** Glomeruli *sessiles*; *rhachides pubescentes.* Stamina *saepissime* 8; *glandulae hirsutae, extrastaminales annulum vel urceolum perfectum efficientes.* Ovarium *glabrum.* Stipulae *in mammillas vel umbones tantum mutatae.*—Claoxylon § Adenoclaoxylon, *Muell. arg.* in *Flora*, xlvii. 436 (1864): *sensu stricto.*

1. **E. Kirkii.** *Folia* tenuia. *Pedicelli* masculi flaccidi, flexuosi. *Glandulae* extrastaminales saepissime in urceolum connatae.—*E. mitis*, Pax *in* Engl. Bot. Jahrb., xxiii. 524; partim et quoad spp. Stuhlmanniana apud Dar-es-Salam lecta (1897); vix Pax *in* Engl. Pflanzenw. Ost-Afrik., C.

Claoxylon Kirkii, Muell. arg. *in* Flora, xlvii. 436 (1864), *et in* DC. Prodr., xv. 2. 776 (1866); Pax *in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895); Sim *in* Flor. Port. E. Afr., 105 (1909).

[VIII a.] East Africa: **Mozambique.** Portuguese East Africa: coast near Beira, *Schlechter*! Coast near Mozambique, *Stuhlmann* 397!

[VIII b.] East Africa: **Zanzibaria.** German East Africa: Lindi; Rovuma Bay, *Kirk*! *Sim.* Dar-es-Salam; Kodenga, *Stuhlmann* 6369! Kinda, *Stuhlmann* 6511! Dar-es-Salam, *Stuhlmann* 7506! 7555! 7595! 7649! Bagamoyo; Bagamoyo, *Stuhlmann* 254! 7233! 7266! Tanga; Amboni, *Holst* 2709! British East Africa: Seyidieh; Mombasa, *Boivin*! *Hildebrandt* 2039!

This species, the basis of Mueller's section *Adenoclaoxylon*, has only been collected on or near the coast.

2. **E. mitis,** Pax *in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895). *Folia* firmula. *Pedicelli* masculi rigidiusculi, stricti. *Glandulae* extrastaminales saepissime liberae.—Pax *in* Engl. Bot. Jahrb., xxiii. 524 quoad spp. Volkensiana tantum (1897), *et in* Engl. Bot. Jahrb., xxx. 339 (1901).

[VIII b.] East Africa: **Zanzibaria.** German East Africa: Morogoro; Khutu Steppe, *Goetze* 105! Tanga; between Magila and Sega, *Volkens* 72!

Nearly allied to *E. Kirkii* and perhaps an inland form of that species, as Pax has suggested, but readily distinguishable by the firmer leaves, shorter stiffer male pedicels, and smaller thicker calyx-lobes.

¶ 2. **Rigidifoliae.** Glomeruli *sessiles vel pedunculati; rhachides pedunculisque pubescentes.* Stamina 9–15; *glandulae glabrae vel raro pilis perpaucis onustae; extrastaminales annulum perfectum vel incompletum efficientes, vel obsoletae.* Ovarium *glabrum vel strigosum.* Stipulae *manifeste in aculeolos conicos rectos hamatosve mutatae.* Folia *firmula.*

3. **E. bongensis,** Pax *in* Engl. Bot. Jahrb., xix. 88 (1894). *Glomeruli* sessiles. *Stamina* 9–12; annulus extrastaminalis incompletus. *Ovarium* ignotum. *Folia* plus minusve pubescentia.

[IX.] East Central Africa. German East Africa: Ruanda; Buganza, south of Lake Mohasi, *Mildbraed* 591! Congo State: Eastern Prov.; Rutschuru Steppe, *Mildbraed* 1874! Southern Sudan: Bahr-et-Ghazal; Bongo, Gurfala, *Schweinfurth* 2226 (Hb. Berlin)! 2296 (Hb. Kew)!

A very distinct species.

4. **E. rigidifolia,** Pax *in* Engl. Bot. Jahrb., xliii. 320 (1909). *Glomeruli* pedunculati. *Stamina* 9–15; annulus extrastaminalis obsoletus. *Ovarium* glabrum vel parce pilosum. *Folia* glabra vel parce pubescentia.

[IX.] East Central Africa. German East Africa: Bukoba; Karagwe, 4,000–6,000 ft., *Stuhlmann* 1760! *Scott Elliot* 8159! Ruanda; Mugarura Island in Lake Kiwa, *Mildbraed* 1112! Congo State: Eastern Province; north shore of Lake Albert Edward, 2,700 ft., *Mildbraed* 1935! Uganda: Western Prov.; Unyoro, Kaichura on east side of Lake Albert Nyanza, *Scott Elliot* 8033 (a stunted form with small leaves)! Uganda Prov.; marsh to the west of Lake Victoria Nyanza, *Scott Elliot* 7449! British East Africa: Naivasha; Nakuro, 6,000 ft., *Scott Elliot* 6793! Ukamba; Nairobi, *Linton* 159! Duruma, *Kassner* 301!

Very nearly allied to *E. bongensis*, from which it differs mainly in having the glomerules shortly peduncled, and in having usually larger leaves, though in the case of Scott Elliot 8033, from Unyoro, the leaves are no larger than in *E. bongensis*. The flowers only differ in that there is no trace of an extrastaminal ring of glands in *E. rigidifolia*.

5. **E. olacifolia,** Prain *in* Kew Bull. 1911, 89 (1911). *Glomeruli* pedunculati. *Stamina* 12; annulus extrastaminalis plerumque perfectus. *Ovarium* dense adpresse strigosum. *Folia* glabra.

[IX.] East Central Africa. Uganda: Western Prov.; Toro, Bukarungu, 2,900 ft., *Bagshawe* 1191!

Closely allied to *E. rigidifolia*, but readily distinguished by the longer petioles, the strigose ovary, and the sparingly setose capsule. The extrastaminal ring of glands is usually complete; sometimes a gland may be absent or replaced by a stamen. The hypogynous scales are large, prominent, and almost staminodial, their tips reaching the bases of the stigmas.

¶ 3. **'Fischerianae.'** Glomeruli *pedunculati; rhachides pedunculisque glabrae.* Stamina 10–21; *glandulae parce hirsutae, extrastaminales annulum incompletum vel perfectum efficientes.* Ovarium *glabrum.* Stipulae *manifeste in aculeolos conicos rectos hamatosve mutatae.* Folia *tenuia.*

6. **E. Paxii,** Rendle *in* Journ. Linn. Soc. Bot., xxxvii. 212, t. 3 (1905). *Pedunculi* glomerulis vix longiores. *Stamina* 15 vel 18 vel 21; annulus extrastaminalis saepissime incompletus.

[IX.] East Central Africa. German East Africa: Kilimanjaro; Marangu, 5,000 ft., *Volkens* 2354! Uganda: Western Prov.; Ankole, near the River Rufúa, *Bagshawe* 513!

Very nearly allied to *E. Fischeri*, from which it differs mainly in the shorter peduncles and the more numerous stamens, the latter surrounded by only an imperfect ring of glands.

7. **E. Fischeri,** Pax *in* Engl. Bot. Jahrb., xix. 88 (1894). *Pedunculi* glomerulis longiores; rhachis saepe elongata glomerulum alterum terminalem suffulciens. *Stamina* 10–12; annulus extrastaminalis perfectus glandulis

nonnunquam plus minusve connatis.—Pax *in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895).

[IX.] East Central Africa. German East Africa: without precise locality, *Fischer* 21!

A very distinct species; the elongated rachis with often a second somewhat smaller terminal glomerulus renders this species a connecting link between the sections *Adenoclaoxylon* and *Deflersia*.

§ II. Deflersia. *Flores* saepissime minuti in racemos axillares dispositi; racemi angustiores foliis breviores vel angustissimi spiciformes foliis subaequilongi. *Stamina* nisi in *E. natalensi* definita, semper glandulis juxtastaminalibus intermixta et simul glandulis extrastaminalibus circumcincta; antherarum locelli subglobosi. *Ovarium* nisi in *E. anomala* glabrum; squamae hypogynae nisi in *E. Poggeophyto* complanatae, inter se discretae—in *E. Poggeophyto* squamae cylindrico-clavatae, stamindiiformes, antheras cassas suffulcientes, glandulis minoribus eas floris masculi simulantibus additis. *Perulae* persistentes vel deciduae. *Stipulae* saepe spinulas basi dilatatas apice pungentes, raro umbones vel aculeolos tantum efficientes.

¶ 4. **Eudeflersieae.** Racemi *foliis breviores; pedicelli masculi prope basin articulati.* Flores *minuti; calyx masculus fere ad basin usque partitus.* Stamina 6–8; *glandulae rhombeae, hirsutae vel glabrae, filamentis breviores.* Ovarium *glabrum; squamae hypogynae complanatae, inter se discretae.* Stipulae *in mammillas vel aculeolos conicos mutatae.*—Deflersia, *Schweinf.* ex *Penzig* in *Atti. Congr. Bot.* 1892, *Genova*, 359 (1893): *sensu stricto.*

8. **E. abyssinica**, Pax *in* Engl. Bot. Jahrb., xix.* 87 (1894). *Folia* distincte petiolata. *Pedunculi* masculi glabri. *Calyx* masculus 3-partitus. *Glandulae* receptaculi hirsutae.—Schweinf. *in* Bull. Herb. Boiss., vii., app. 2, 306 (1899). *Mercurialis*? sp., Deflers, Voy. Yemen, 203 (1889). *Claoxylon Deflersii*, Schweinf. *in* Pl. Arab. Fel. exsicc. n. 933 (1891): nomen tantum. *Deflersia erythrococca*, Schweinf. ex Penzig *in* Atti Congr. Bot. 1892, Genova, 359 (1893), *et in* Abhandl. Akad. Berlin, xxxiii. 663, 667, 708: Abyssin. Pflanzennam. 17, 21, 62 (1893): nomen tantum.

[X.] North-East Africa. Abyssinia: Rora, 6,000 ft., *Hildebrandt* 509! Eritrea: Ghinda, 3,300 ft., *Schweinfurth* 327! near Felakhit, 3,000–3,500 ft., *Schweinfurth* 2239! near Acrour, 6,300 ft., *Schweinfurth & Riva* 1037! Mogod Valley, 4,650 ft., *Schweinfurth* 2059! Gheleb, 5,700 ft., *Schweinfurth & Riva* 1132! Arabia: Yemen; Mt. Masâr, near Attâra, 6,000 ft., *Deflers* 413; Wadi Madfar; near Hodjela, 2,700 ft., *Schweinfurth* 933! Wadi Chusiet; near 'Ussil, 4,000 ft., *Schweinfurth* 1176; Menacha, 8,000 ft., *Schweinfurth* 1690! Gebel Bura; above Hille, 3,000 ft., *Schweinfurth* 1813.

A very distinct species. The Mogod Valley specimens, cited by Schweinfurth as 2058, but actually numbered (in Herb. Boissier) 2059, have unusually large leaves, up to 10·5 cm. long by 6 cm. wide; so also have those of 2239 from near Felakhit, which reach a length of 13·5 cm. and are 5–6 cm. wide.

9. **E. usambarica,** Prain *in* Kew Bull. 1911, 90 (1911). *Folia* breve petiolata. *Pedunculi* masculi pubescentes. *Calyx* masculus 4-partitus, vel casu 5-partitus. *Glandulae* receptaculi glabrae.

[VIII b.] EAST AFRICA: **Zanzibaria.** German East Africa: Tanga; Derema, *Scheffler* 160! near Amani, 2,800–3,000 ft., *Engler* 708! Handai; Nquelo, 3,000 ft., *Heinsen* 74!

Nearly allied to *E. abyssinica*, of which it appears to be the East African representative.

¶ 5. **Berberideae.** Racemi *foliis breviores; pedicelli masculi nunc prope basin nunc plane ultra basin articulati.* Flores *parvi vel minuti; calyx masculus ultra medium vel fere ad basin usque partitus.* Stamina 15–30; *glandulae late ovatae vel oblongae, complanatae, hirsutae, filamentis breviores vel aequilongae.* Ovarium *glabrum; squamae hypogynae complanatae, inter se discretae.* Stipulae *in spinulas basi dilatatas apice pungentes mutatae.*

10. **E. zambesiaca,** Prain *in* Kew Bull. 1911, 90 (1911). *Folia* tenuia. *Pedunculi* masculi pubescentes. *Flores* masculi minuti, virides; pedicelli prope basin articulati. *Stamina* 15 vel 18; glandulae extrastaminales 6; juxtastaminales ovatae, filamentis breviores.

[VIII a.] EAST AFRICA: **Mozambique.** Nyasaland: Lower Shire; Chiromo, *Scott Elliot* 2795!

A very distinct species.

11. **E. natalensis,** Prain *in* Kew Bull. 1911, 91 (1911). *Folia* tenuia. *Pedunculi* masculi glabri. *Flores* masculi parvi nec tamen minuti, albi; pedicelli manifeste supra basin articulati. *Stamina* 30; glandulae extrastaminales 10; juxtastaminales ovatae, filamentis breviores.

[VII b.] SOUTH-EAST AFRICA: **Natal.** Inanda; Mt. Edgecumbe, *Wood* 1089! Mt. Moreland, 500 ft., *Wood* 1391! without precise locality, *Gerrard* 81!

A very distinct species.

12. **E. berberidea,** Prain *in* Kew Bull. 1911, 92 (1911). *Folia* firmula. *Pedunculi* masculi glabri. *Flores* masculi parvi nec tamen minuti, virides; pedicelli manifeste supra basin articulati. *Stamina* 15 vel 18; glandulae extrastaminales 5; juxtastaminales filamentis aequilongae.

[VII b.] South-East Africa: **Natal.** Near Durban, 200 ft., *Wood* 7582! 9439! 11810!

Very nearly allied to *E. natalensis*, but with thicker leaves, which are different in outline, and with fewer stamens, which are accompanied by different interstaminal glands.

¶ 6. '**Aculeatae.**' Racemi *foliis breviores; pedicelli masculi alte ultra basin articulati.* Flores *minuti; calyx masculus ad medium usque tantum fissus; tubus campanulatus.* Stamina 6–11; *glandulae rhombeae, crassae, hirsutae, filamentis longiores.* Ovarium *adpresse strigosum; squamae hypogynae complanatae, inter se discretae.* Stipulae *in spinulas basi dilatatas apice pungentes mutatae.*—Erythrococca, *Benth.* in *Hook., Niger Fl.*, 506 (1849), et in *Benth. et Hook. f. Gen. Plant.*, iii. 308 (1880): *sensu stricto.*

13. **E. anomala.** *Glandulae* receptaculares majusculae, staminibus antheris haud exceptis aequialtae.—*Adelia anomala*, Juss. *ex* Poir. *in* Encyc. Meth. Suppl., i. 132 (1810); A. Juss., Tent. Gen. Euphorb., 32 (1824). *Erythrococca aculeata*, Benth. *in* Hook., Niger Fl., 506 (1849); Baill., Étud. gén. Euphorb., 437, t. 21, f. 10 (1858); Muell. arg. *in* DC. Prodr., xv. 2, 791 (1866); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48, fig. 29 A (1890), *et in* Engl. Bot. Jahrb., xix. 87 (1894). *E. aculeata*, var. *acutissima*, N. E. Br. *ex* Stapf *in* Journ. Linn. Soc. Bot., xxxvii. 114 (1905), *et in* Johnst. Liberia, ii. 649 (1906). *Mercurialis aculeata*, Baill., Adansonia, iii. 173 (1862).

[IV a.] West Africa: **Upper Guinea.** Senegambia; neighbourhood of Boquet, *Heudelot* 856! Sierra Leone: without precise locality, *Smeathman*! *Afzelius*! *Vogel* 162! Bagroo River, *Mann* 879! Scarcies; near Wallia, *Scott Elliot* 4790! Limba; Makende, *Scott Eliott* 5694! Liberia: near Monrovia, *Whyte*! Ivory Coast: Bouroukrou, *Chevalier* 16579! Agnichy Valley; Copiekrou, *Chevalier* 17103! Sassandra; Soubré, *Chevalier* 17990! Southern Nigeria: Western Lagos, *Rowland*! Yorubaland; at Ibadan, *Schlechter* 13012! Olokemeji, *Foster* 285! Cameroons: Johann-Albrechtshohe, *Staudt* 466! Victoria, *Winkler* 115!

A very distinct species, the original basis of the genus: readily distinguished from every other species by the relatively large receptacular glands and the relatively shorter calyx-lobes. The form described as var. *acutissima* hardly differs sufficiently to deserve separate recognition. Heudelot notes that this plant is much in request owing to the high reputation in which it is held as a vermifuge.

¶ 7. **Subspicatae.** Racemi *arcte spiciformes foliis subaequilongi; pedicelli masculi prope basin articulati.* Flores *minuti; calyx masculus paulo ultra medium fissus; tubus late campanulatus.* Stamina 9–12; *glandulae oblongae, hirsutae, filamentis aequilongae; extrastaminales per paria inter se libera imo basi connatae.* Ovarium *glabrum*; *squamae hypogynae nunc complanatae, inter se discretae; nunc staminodiiformes cylindrico-*

clavatae, antheras locellis cassis suffulcientes annuloque glandularum minorum extrastaminodialium eis floris masculi similium circumcinctae. Stipulae *in spinulas basi dilatatas apice pungentes mutatae.*—Poggeophyton, *Pax* in *Engl. Bot. Jahrb.*, xix. 88 (1894).

14. **E. subspicata,** Prain *in* Kew Bull. 1911, 185 (1911). *Stamina* 12. *Squamae hypogynae* carpidiis alternantes ovato-lanceolatae, complanatae, in memoriam petala reducentes, inter se discretae. *Folia* distincte petiolata, ovato-oblonga, acuminata, margine denticulata subinde manifeste lobata.—*E. aculeata*, De Wild. & Dur., Ann. Mus. Congo, Bot., sér. 2, i. 50 (1899), *et* sér. 3, ii. 209 (1901); Dur., Syll. Flor. Cong., 491 (1909), pro parte : nec Benth.

[V.] West Central Africa. French Congo : Oubangi ; Krebedje, Fort Sibut, *Chevalier* 3617 ! Congo State : Equatorial Dist. ; Coquilhatville, *Dewevre* 692 !

A very distinct species.

15. **E. Laurentii,** Prain *in* Kew Bull. 1911, 186 (1911). *Stamina* ignota. *Squamae hypogynae* carpidiis alternantes ovato-lanceolatae, complanatae, in memoriam petala reducentes, inter se discretae. *Folia* breve petiolata, ovata, acuta, margine parum crenulata.—*E. aculeata*, De Wild., Miss. Laurent., 129 (1905); Dur., Syll. Flor. Cong., 491 (1909), pro parte : nec Benth.

[V.] West Central Africa. Congo State : Aruwimi ; Lie, *Laurent* !

Nearly allied to *E. spicata*, but readily distinguished by the differently shaped, smaller leaf-blades and the very much shorter, but at the same time considerably broader and stouter, petioles.

16. **E. Poggeophyton,** Prain *in* Kew Bull. 1911, 187 (1911). *Stamina* 9. *Squamae hypogynae* dimorphae, alterae carpidiis alternantes staminodiiformes subcylindrico-clavatae apice locellis antherarum cassis indutae, alterae minores glandulas maris receptaculares simillimae annulum exteriorem circa basin ovarii efficientes. *Folia* distincte petiolata, oblongo-lanceolata, acuta, margine parum crenulata.—*Poggeophyton aculeatum*, Pax *in* Engl. Jahrb., xix. 89 (1894); Dur. & Schinz, Étud. Flor. Cong., 245 (1896); Engl. *in* Sitzb. Preuss. Akad. Wiss., xxxviii. 829 (1908); Dur., Syll. Flor. Cong., 490, 656 (1909).

[V.] West Central Africa. Congo State : Kasai ; on the Lulua, *Pogge* 1370 !

Very near to *E. subspicata*, but distinguishable by the somewhat differently shaped leaves and by the dimorphic hypogynous glands, though it is doubtful whether this latter character may not be an individual instance of abnormality.

§ III. Trichogyne. *Flores* minuti, in racemos axillares dispositi. *Stamina* saepissime definita, aut glandulis extrastaminalibus circumcincta aut glandulis juxtastaminalibus intermixta ; glandulae nunquam in utroque

modo simul in floribus evolutae; antherarum locelli plerumque obovoidei. *Ovarium* semper dense strigosum; squamae hypogynae nunc cylindrico-clavatae staminodiiformes sed anantherae, aliquando glandulis minoribus eas floris masculi simulantibus additis; nunc complanatae, inter se discretae. *Perulae* persistentes. *Stipulae* umbones vel aculeolos efficientes.—*Claoxylon* § *Athroandra*, Muell. arg. *in* DC. Prodr., xv. 2, 776, pro parte (1866); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48, pro parte (1890): nec Hook. f.

¶ 8. **Tristes.** Pedicelli *alte ultra basin articulati.* Stamina 2–5, *glandulis extrastaminalibus glabris circumcincta; glandulae juxtastaminales* 0. Squamae hypogynae *carpidiis isomerae staminodiiformes subcylindrico-clavatae, apice integrae, aliquando glandulis minoribus eas floris masculi simulantibus additis.*

17. **E. Menyharthii.** *Pedunculi* feminei 2–4-flori.—*Claoxylon Menyharthii*, Pax *in* Bull. Herb. Boiss., sér. 2, i. 877 (1901). *Claoxylon* C. tristi *affine*, Pax *in* Baum, Kunene-Sambesi Geb., 283 (1903). *C. virens*, N. E. Br. *in* Kew Bull. 1909, 140 (1909).

[VI.] South-West Africa. Portuguese West Africa: Mossamedes; on the Kwito below its confluence with the Longa, *Baum* 549! German South-West Africa: Awas Mts., *Dinter* 808! Bechuanaland: ridge on the north side of Messeringa Vley, *Seiner* II. 277! Ngamiland: Kwebe Hills, 3,400 ft., *Mrs. Lugard* 51! *Lugard* 53! 94! Rhodesia: Mboruma on the Zambesi, *Menyharth* 889 *b*!

[IX.] East Central Africa. German East Africa: Magu Kagchi, *Fischer* 387!

Very nearly allied to *E. tristis*, of which the male flowers are still unknown. Female specimens of *E. Menyharthii* can only be distinguished from those of *E. tristis* by having several-flowered racemes instead of solitary flowers.

18. **E. tristis.** *Pedunculi* feminei 1-flori.—*Claoxylon triste*, Muell. arg. *in* Journ. Bot., ii. 334 (1864), *et in* DC. Prodr., xv. 2, 779 (1866); Hiern *in* Cat. Afr. Pl. Welw., 975 (1900).

[VI.] South-West Africa. Portuguese West Africa: Mossamedes; Huilla, near Humpata, in rocky places at 5,000 ft., *Welwitsch* 390! Morro de Lopollo, *Welwitsch* 391! Mounyino, 6,000 ft., *Antunes* 313!

A very distinct species; each female peduncle bears a solitary articulate pedicel.

¶ 9. **Lasiococcae.** Pedicelli *prope basin articulati.* Stamina *plerumque* 9–15, *in specie singula tamen* (E. Ledermanniana) 27, *glandulis juxtastaminalibus hirsutis intermixta*; *glandulae extrastaminales* 0. Squamae hypogynae *carpidiis isomerae, complanatae, inter se discretae.*

19. **E. lasiococca.** *Stamina* 15. *Calyx* femineus 2-lobus. *Folia* parva, pubescentia, 0·8–1·2 cm. longa; petiolus 3 mm. longus.—*Claoxylon lasio-*

coccum, Pax *in* Engl. Bot. Jahrb., xix. 87 (1894) *et in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895).

[IX.] East Central Africa. German East Africa: Eastern shore of Lake Tanganyika, *Scott Elliot* 8231! Uganda: without precise locality, *Stuhlmann* 1484!

A very distinct species, easily recognized within the group by its small leaves.

20. **E. Ledermanniana**, Prain *in* Kew Bull. 1911, 92 (1911). *Stamina* 27. *Calyx* femineus ignotus. *Folia* glabrescentia, 4–5 cm. longa; petiolus 6 mm. longus.

[IV a.] West Africa: **Upper Guinea.** Cameroons: Esole; on Mt. Basso, 6,100 ft., *Ledermann* 2032! Mfongu; Muti slopes, 5,600–6,300 ft., *Ledermann* 5877!

Most nearly allied to *E. Mildbraedii*, from which it differs in the shape and pubescence of the leaf, in the shorter petioles, and in the more numerous stamens with considerably longer filaments.

21. **E. Mildbraedii.** *Stamina* 9–12. *Calyx* femineus 2-lobus. *Folia* pubescentia, 5–7·5 cm. longa; petiolus 1·2 cm. longus.—*Claoxylon Mildbraedii*, Pax *in* Engl. Bot. Jahrb., xliii. 80 (1909).

[IX.] East Central Africa. German East Africa: Ruanda; Kissenge, 7,600 ft., *Mildbraed* 1452! Uganda: Ruwenzori; Kivata, 7,000–9,000 ft., *Scott Elliot* 7637!

A very distinct species.

22. **E. trichogyne.** *Stamina* ignota. *Calyx* femineus 2-lobus. *Folia* pubescentia, 4·5–6·5 cm. longa; petiolus 3 mm. longus.—*Claoxylon trichogyne*, Muell. arg. *in* Journ. Bot., ii. 334 (1864), *et in* DC. Prodr., xv. 2, 779 (1866); Hiern *in* Cat. Afr. Pl. Welw., 975 (1900).

[IV b.] West Africa: **Lower Guinea.** Portuguese West Africa: Angola; Golungo Alto, near Sange, *Welwitsch* 396!

A very distinct species.

23. **E. hirta**, Pax *in* Engl. Bot. Jahrb., xliii. 321 (1909). *Stamina* 10. *Calyx* femineus 4-lobus, raro 3-lobus. *Folia* pubescentia, 5–10 cm. longa; petiolus 5–6 mm. longus.

[IX.] East Central Africa. Uganda: Entebbe, 4,000 ft., *Bagshawe* 793! Ruwenzori; Butaga Forest, 7,000–8,000 ft., *Scott Elliot* 7998! Congo State: West Ruwenzori; Kalange, 3,600 ft., *Mildbraed* 2486!

Very closely allied to *E. trichogyne*, from which it mainly differs in having more numerous female calyx-lobes.

§ IV. Pseudathroandra. *Flores* parvi in racemos axillares dispositi. *Stamina* definita vel indefinita, glandulis juxtastaminalibus intermixta;

glandulae extrastaminales 0 ; antherarum locelli obovoidei. *Ovarium* glabrum vel fere glabrum ; squamae hypogynae complanatae, inter se discretae. *Perulae* persistentes. *Stipulae* minutae, scarioso-hyalinae, immutatae.—*Claoxylon* § *Athroandra*, Muell. arg. *in* DC. Prodr., xv. 2, 776, pro parte (1866) ; Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48, pro parte (1890) : nec Hook. f.

¶ 10. **Pauciflorae.** Folia *glabra, juvenilia purpurascentia.*

24. **E. pauciflora.** *Calyx* maris 4-lobus. *Stamina* 24, vel 27, vel 30 : saepius 27 ; glandulae rhombeae, hirsutae. *Stigmata* linearia, patula, a basi libera, tota plumoso-laciniata.—*Claoxylon pauciflorum*, Muell. arg. *in* Journ. Bot., ii. 333 (1864), *et in* DC. Prodr., xv. 2, 778 (1866) ; Hiern *in* Cat. Afr. Pl. Welw., 975 (1900).

[IV b.] West Africa : **Lower Guinea.** Portuguese West Africa : Cabinda ; Chinchoxo, *Soyaux* 29 ! Loanda ; near Musaque do Sul and above Loanda, *Welwitsch* 400 ! near Loanda, *Gossweiler* 195 ! *also without number* ! Malange, *Gossweiler* 452 ! Musaque, near the English Cemetery, *Gossweiler* 1519 !

A very distinct species, which combines the stigmas characteristic of an *Euerythrococca* with the stipules of an *Athroandra*.

25. **E. polyandra.** *Calyx* maris 3-lobus. *Stamina* 10–13 ; glandulae ovoideae, glabrae. *Stigmata* ovato-lanceolata, sub anthesi divaricata demum recurva, in apice styli brevis insidentia, margine inciso-lobulata.—*Claoxylon polyandrum*, Pax et K. Hoffm. *in* Engl. Bot. Jahrb., xlv. 327 (1910).

[VIII b.] East Africa : **Zanzibaria.** German East Africa : Wilhelmstal ; Kwai, *Albers* 36 ! Tanga ; Amani, *Warnecke* 219 ! *Kraenzlin* 2182 !

In general facies this species most resembles *E. pauciflora* and may perhaps be looked upon as its East African representative. But it approaches the sub-genus *Athroandra* more closely than *E. pauciflora* does ; besides having the unmodified stipules of an *Athroandra* it has stigmas that, but for being incised-lobulate along the margins, resemble those of the species in the group *Oleraceae*.

Subgen. II. Athroandra.

Mercurialis, Baill., pro parte ; loc. supra cit. *Claoxylon* § *Athroandra*, Hook. f. ; Muell. arg., pro parte ; ll. supra cit. *Trewia*, Baill. ; loc. supra cit. *Chloropatane*, Engl. ; Perkins & Gilg ; Wright ; ll. supra cit. *Rivinoides*, Afzel. MSS.

Stipulae immutatae, minutae, scarioso-hyalinae. *Perulae* semper persistentes. *Flores* parvi, vix tamen minuti, semper in racemos axillares dispositi. *Stamina* indefinita glandulis juxtastaminalibus consociata ; glandulae extrastaminales nunquam evolutae ; filamenta nisi in *E. membranacea* antheris manifeste breviora ; antherarum locelli obovoidei. *Ovarium*

semper glabrum ; stigmata laevia vel in specie singula (*E. africana*) papillosa, nunquam laciniata lobatave ; nunc sessilia, libera, elongata, linearia, patula vel recurva ; nunc basi connata vel in apice styli brevis raro distincti columnaris insidentia, brevia vel brevissima, ovato-lanceolata vel suborbicularia, divaricata vel suberecta. *Discus hypogynus* e squamis complanatis cum carpidiis alternantibus eisque isomeris inter se liberis sed circa basin ovarii contiguis vel plane in urceolum annularem lobatum vel raro subintegrum connatis compositus.

§ V. HEMIERYTHROCOCCA. *Stigmata* elongata, linearia, laevia, a basi libera. *Discus hypogynus* e squamis majusculis cum carpidiis alternantibus inter se liberis sed circa basin ovarii contiguis compositus. *Glandulae* juxtastaminales hirsutae.

¶ 11. **Membranaceae.** Calyx *maris in alabastro globosus apice minute apiculatus.* Filamenta *antheris longiora.* Stigmata *praelonga, arcuatim recurva.* Squamae *hypogynae apice 2-lobulatae.*

26. **E. membranacea.** *Ramuli* foliisque pilis patentibus molliter hispidi. *Flores* albi ; stamina 27.—*Claoxylon membranaceum*, Muell. arg. *in* Flora, xlvii. 437 (1864), *et in* DC. Prodr., xv. 778 (1866).

[IV a.] WEST AFRICA : **Upper Guinea.** Cameroons : Cameroon Mts., 4,000 ft., *Mann* 1197 ! Buea, 3,300 ft., *Lehmbach* 212 !

This species, in all other respects an *Athroandra*, has the long filaments characteristic of an *Euerythrococca*, and thus serves as a connecting link between the two sub-genera.

¶ 12. **Patulae.** Calyx *maris in alabastro conico-pyramidalis.* Filamenta *antheris multo breviora.* Stigmata *elongata, patula.* Squamae *hypogynae apice integrae.*

27. **E. patula.** *Folia* secus nervos subtus petiolisque pilis patentibus hispida, pallide viridia, basi rotundata, 20–25 cm. longa. *Stamina* 50–54.—*Claoxylon patulum*, Prain *in* Kew Bull. 1911, 93 (1911).

[IV a.] WEST AFRICA : **Upper Guinea.** Cameroons: Jabassi, 200–250 ft., *Ledermann* 1063 ! 1106 !

A very distinct species, but at the same time, owing to the absence of female flowers, one of somewhat doubtful affinity. The hispid hairs on the leaves suggest comparison with *E. hispida* on the one hand, and with *E. membranacea* on the other. From both of these it is, however, readily distinguished specifically by the differently shaped male flower-buds and the larger number of stamens ; both these species are, moreover, high-level plants. From *E. membranacea* it is, besides, at once distinguished by the short filaments. The similarity in shape of the male flower-buds suggests that the nearest affinity may be with the next species, but the question can only be definitely settled when the female flowers of *E. patula* are known.

28. **E. Mannii.** *Folia*, etiam juvenilia, glaberrima, saturate viridia, basi cuneata, 8–20 cm. longa. *Stamina* 24–30.—*Claoxylon Mannii*, Hook. f. *in* Journ. Linn. Soc. Bot., vi. 20 (1862); Muell. arg. *in* DC. Prodr., xv. 2, 778 (1866).

[IV a.] West Africa: **Upper Guinea.** Fernando Po: Clarence Peak, 5,000 ft., *Mann* 260! 633!

Mueller describes the stigmas of this species as papillose, but in Mann's original fruiting specimen they are smooth. This is the species on which *Athroandra*, Hook. f., was primarily based.

§ VI. Chloropatane. *Stigmata* brevia vel brevissima, ovato-lanceolata vel suborbicularia, laevia vel in specie singula (*E. africana*) papillosa, basi connata vel in apice styli brevis vel distincti insidentia. *Discus hypogynus* urceolaris, margine lobatus vel raro subinteger, vel casu e glandulis majusculis cum carpidiis alternantibus circa basin ovarii contiguis compositus. *Filamenta* antheris semper multo breviora.—*Chloropatane*, Engl. *in* Engl. Bot. Jahrb., xxvi. 383 (1899).

¶ 13. '**Rivinoides.**' Stylus *subnullus; stigmata suborbicularia, majuscula, basi connata, suberecta.* Calyx *maris in alabastro globosus minute apiculatus, vel breve conico-pyramidalis.*—Trewia, *Baill., Adansonia*, i. 68, *pro parte et dubitanter* (1860): *nec Linn.* Rivinoides, *Afzel. MSS.*

29. **E. africana.** *Stigmata* papillosa. *Calyx* maris in alabastro globosus minute apiculatus. *Stamina* 24; glandulae juxtastaminales hirsutae. *Discus hypogynus* sub anthesi subinteger, fructifer 5-lobus. *Folia* primum parce pubescentia, demum fere glabra.—*Trewia? africana*, Baill., Adansonia, i. 68 (1860). *Claoxylon Barteri*, Hook. f. *in* Journ. Linn. Soc. Bot., vi. 21 ad calc. (1862); Stapf *in* Johnst. Liberia, ii. 649 (1906). *C. africanum*, Muell. arg. *in* DC. Prodr., xv. 2, 777 (1866). *C. oleraceum*, Prain *in* Kew Bull. 1911, 93 (1911), pro parte et quoad Barter 2223 tantum.

[IV a.] West Africa: **Upper Guinea.** Senegambia: Casamance River, *Perrottet* 748! Sierra Leone: Crawford's Island, *Afzelius*! Bafodeya Hills, *Scott Elliot* 5505! 5647! Liberia: Sinöe Basin, *Whyte*! Ivory Coast: between Yaou and Ayame, *Chevalier* 17805 ter! Southern Nigeria: Lagos Island, *Barter* 2223! Western Lagos, *Millen* 163! *Rowland*! Eppah, *Barter* 3285! near Ipatu, *Foster* 184! Yoruba forests, *Barter* 3344!

This species, very distinct from every other member of the genus by its stigmas, was included, in a footnote, by Hooker, within his section *Athroandra*. It is also the species on which the genus *Rivinoides*, suggested by Afzelius, was intended to be based. But while very distinct as regards its female flowers, it is only distinguishable from *E. oleracea*, so far as its male flowers are concerned, by the smaller number of stamens. The specimen of Barter 2223 at Kew, which is in leaf only, is noted as having had 40 stamens, and on this account was referred, in the Kew Bulletin, l. c.,

to *E. oleracea*. Since the description of *E. oleracea* was published, an opportunity has occurred, through the kindness of Mr. Lecomte, of examining the specimen of Barter 2223 in the Paris herbarium. This specimen admits of its flowers being examined, and shows that its male flowers have 24 stamens only, and that it is identical with the type of *Trewia ? africana*, and with Barter 3285 and Barter 3344.

30. **E. Chevalieri.** *Stigmata* laevia. *Calyx* maris in alabastro globosus minute apiculatus. *Stamina* 30; glandulae juxtastaminales hirsutae. *Discus hypogynus* 2-lobus. *Folia* persistenter pubescentia.—*Claoxylon Chevalieri*, Beille *in* Bull. Soc. Bot. Fr., lv. Mém. 8, 75 (1908).

[IV a.] WEST AFRICA: **Upper Guinea.** French Guinea: Fouta Djalon; Labé plateau, 3,650 ft., *Chevalier* 12296! Diaguissa, 4,000 ft., *Chevalier* 12643! 12689!

A very distinct species, most nearly allied to *E. africana*, but readily recognized by its pubescent mature leaves and its smooth stigmas.

31. **E. Molleri.** *Stigmata* laevia. *Calyx* maris in alabastro breve conico-pyramidalis. *Stamina* 40; glandulae juxtastaminales glabrae. *Discus hypogynus* nunc completus aut late 2-lobus aut planus margine sub anthesi integer demum breviter crenulato-laciniatus; nunc e glandulis majusculis circa basin ovarii contiguis compositus. *Folia* glabra, juvenilia rubescentia vel purpurascentia.—*Claoxylon Molleri*, Pax *in* Bol. Broter., x. 160 (1892), *et in* Engl. Bot. Jahrb., xix. 84 (1894). *C. purpurascens*, Beille *in* Bull. Soc. Bot. Fr., lv. Mém. 8, 75 (1908).

[IV b.] WEST AFRICA: **Lower Guinea.** St. Thomas' Island: St. Nicholas, 3,000 ft., *Moller & Quintas* 13! *Moller* 136! *Henriques* 22! 24! St. Thomas' Peak, 4,650 ft., *Chevalier* 13652! 13656! 14527! 14582!

A very distinct species, and the only *Athroandra* so far known in which the receptacular glands are glabrous. Beille has expressed the opinion that it may be an *Euclaoxylon*; the character of the glands favour this view, but the fact that the buds are perulate shows that it cannot be sustained. The glabrous leaves suggest a comparison with *E. Mannii* on the one hand and with *E. rivularis* on the other, but the nature of the stigmas indicates that its most natural position is near *E. africana* and *E. Chevalieri*. The variable nature of the hypogynous disc is unusual; the fact that sometimes the glands which alternate with the carpels are not connate in front and behind makes it serve to some extent as a link between *Hemierythrococca* and *Chloropatane*.

¶ 14. **Rivulares.** Stylus *subnullus; stigmata laevia, ovato-lanceolata, basi connata, divaricata.* Calyx *maris in alabastro subtetragono-globosus minute apiculatus.* Glandulae *juxtastaminales hirsutae.*

32. **E. rivularis.** *Stamina* 40. *Discus hypogynus* breviter sed late 2-lobus. *Folia* glabra, 8–10-nervia, 10–20 cm. longa.—*Claoxylon rivulare*, Muell. arg. *in* Flora, xlvii. 518 (1864), *et in* DC. Prodr., xv. 2, 777 (1866). *Chloropatane africana*, Engl. *in* Engl. Bot. Jahrb, xxvi. 383 (1899); Perkins

& Gilg *in* Engl. Pflanzenr.: Monim., 24, f. 4 M–R (1901); Wright *in* Thiselton-Dyer, Flor. Trop. Afr., vi. 1, 169 (1909).

[IV b.] WEST AFRICA: **Lower Guinea.** Cameroons: Kribi; Great Batanga, *Dinklage* 1057! Yaunde; *Zenker* 494, 798 (ex Engler). Spanish Guinea: Mont John River; Kongui, *Mann* 1785!

A very distinct species, which forms the basis of the proposed genus *Chloropatane*. We have not seen specimens collected by Zenker at Yaunde, bearing the numbers cited.

¶ 15. **Columnares.** Stylus *columnaris*; *stigmata laevia*, *suborbicularia*, *minima*, *stylo multo breviora*, *suberecta*. Calyx *maris in alabastro globosus minute apiculatus*, *vel breve conico-pyramidalis*. Glandulae *juxtastaminales hirsutae*. Discus hypogynus *minute 2-lobus*.

33. **E. Welwitschiana.** *Bracteolae* ovatae, 1 mm. longae. *Calyx* maris in alabastro globosus, minute apiculatus. *Stamina* 30. *Folia* 10–15 cm. longa, demum fere glabra.—*Claoxylon Welwitschianum*, Muell. arg. *in* Journ. Bot., ii. 333 (1864), *et in* DC. Prodr., xv. 2, 776 (1866); Hiern *in* Cat. Afr. Pl. Welw., 975 (1900). *C. africanum*, De Wild. *in* Ann. Mus. Congo, Bot., sér. 5, ii. 279, in parte et quoad spp. Sapiniana apud Sankuru lecta tantum (1908); Dur., Syll. Flor. Cong., 491, in parte et quoad spp. Sapiniana (1909); De Wild., Comp. Kasai Miss. Scient., 330 (1910): nec Muell. arg. *Chloropatane Batesii*, Wright *in* Thiselton-Dyer, Flor. Trop. Afr., vi. 1, 169 (1909), *et in* Kew Bull. 1909, 214 (1909).

[IV b.] WEST AFRICA: **Lower Guinea.** Cameroons: Kribi; Bipindi, 500–600 ft., *Zenker* 1908! 2049! 2610! 2910! 2925! 3773! 3912! Ebolowa; Efulen, *Bates* 409! Spanish Guinea: Nkolentangan, *Tessmann* 296! Gaboon: without precise locality, *Klaine* 2976! Portuguese West Africa: Angola; Golungo Alto, on the Zuenza River, *Welwitsch* 397! *Gossweiler* 4412! Serra da Alta Queta, *Welwitsch* 398!

V. WEST CENTRAL AFRICA. Congo State: Kasai; Sankuru, *Sapin*!

A very distinct species. Sapin states that the leaves of this plant are edible and form an excellent vegetable.

34. **E. columnaris.** *Bracteolae* ovatae, 1 mm. longae. *Calyx* maris in alabastro conico-pyramidalis. *Stamina* 48–54. *Folia* 4–7 cm. longa, adpresse pubescentia.—*Claoxylon columnare*, Muell. arg. *in* Flora, xlvii. 437 (1864), *et in* DC. Prodr., xv. 2, 776 (1866).

[IV. b.] WEST AFRICA: **Lower Guinea.** Prince's Island: *Mann* 1139!

Very nearly allied to *E. Welwitschiana* and indistinguishable as regards female flowers; the male flowers differ considerably in shape and in having more numerous stamens. The two are, however, readily distinguished by their different leaves.

35. **E. Poggei.** *Bracteolae* subulatae, 2 mm. longae. *Calyx* maris in alabastro conico-pyramidalis. *Stamina* 60. *Folia* 4–7 cm. longa, patule pubescentia.—*Claoxylon columnare*, Engl. *in* Sitzb. Preuss. Akad. Wiss., xxxviii. 829 (1908); Dur., Syll. Flor. Cong., 656 (1909): nec Muell. arg. *C. Poggei*, Prain *in* Kew Bull. 1911, 93 (1911).

[IV. b] West Africa: **Lower Guinea.** Congo State: neighbourhood of Lazaret, *Vanderyst*!

[V.] West Central Africa. Congo State: Kasai; Mukenge, *Pogge* 1373! Kwango; without precise locality, *Butaye*!

The affinity of this species is somewhat doubtful, though the probability is that it may be most nearly allied to *E. columnaris*. The female plant is not yet known; should it prove to have a columnar style with minute stigmas it may be possible to adopt Engler's view and refer the plant to *E. columnaris*; even then, however, it will be necessary to treat it as a distinct variety, readily recognized by its somewhat hispidulous, spreading hairs, and its longer bracteoles.

¶ 16. **Oleraceae.** Stylus *brevis; stigmata laevia, ovato-lanceolata, sub anthesi divaricata dein recurva, stylo longiora.* Calyx *maris in alabastro globosus minute apiculatus.* Glandulae *juxtastaminales hirsutae.*

36. **E. atrovirens.** *Folia* adpresse pubescentia, basi cuneata vel rotundata, 8–10 cm. longa. *Stamina* 30–40. *Discus hypogynus* eccentros, integer. *Cocci* 6 mm. lati.—*Claoxylon atrovirens*, Pax *in* Engl. Bot. Jahrb., xix. 85 (1894); Dur. & Schinz *in* Étud. Flor. Cong., 245 (1896); Dur., Syll. Flor. Cong., 491 (1909). *Claoxylon* sp., Dawe, Bot. Miss. Ugand., 56 (1906). *C. inaequilaterum*, Pax *in* Engl. Bot. Jahrb., xliii. 320 (1909).

[IV a.] West Africa: **Upper Guinea.** Cameroons: Tchâpe Pass, 4,800 ft., *Ledermann* 2653! Babangi Tungo; Bamessing, 4,500 ft., *Ledermann* 5823!

[V.] West Central Africa. Congo State: Arumwimi; Yambuya, *Solheid* 32! Uelle; Bomokandi, at Kussumbo in the Monbuttu country, *Schweinfurth* 3186!

[IX.] East Central Africa. Congo State: Eastern Province; Kwa Muera, at Fort Beni on the Semliki, *Mildbraed* 2246! 2401! Muera, 3,300–3,600 ft., *Mildbraed* 2271! 2277! Uganda: Western Province; Unyoro, at Hoima, 3,500 ft., *Bagshawe* 1512! Uganda Province; Chagwe, 3,900 ft., *Dawe* 215! Entebbe, 3,900 ft., *Brown* 356! German East Africa: Bukoba; *Stuhlmann* 3923!

It has not been found possible to keep *Claoxylon inaequilaterum* apart from *E. atrovirens*; the flowers of the two are the same in both sexes. In some of the specimens of *C. inaequilaterum* the leaves agree in shape with those of some of the specimens of *E. oleracea*, but as regards indumentum they agree with those of

E. atrovirens, and the extreme forms, with cuneate and rounded bases respectively, are connected by intermediates.

37. **E. oleracea.** *Folia* secus nervos puberula mox glabrescentia, basi rotundata, 6–15 cm. longa. *Stamina* 30–40. *Discus hypogynus* eccentros, integer. *Cocci* 6 mm. lati.—*Claoxylon africanum*, De Wild. & Dur. *in* Bull. Herb. Boiss., sér. 2, i. 47 (1900), *et in* Ann. Mus. Congo, Bot., sér. 3, ii. 209 (1901); De Wild. *in* Miss. Laurent, i. 130 (1905); Rendle *in* Journ. Linn. Soc. Bot., xxxvii. 213 (1905); De Wild. *in* Ann. Mus. Congo, Bot., sér. 5, ii. 279, spp. Sapiniana tamen excludenda (1908); Dur., Syll. Flor. Cong., 491, spp. Sapiniana excludenda (1909): nec Muell. arg. *C. oleraceum*, Prain *in* Kew Bull. 1911, 94, syn. Hook. et Muell. arg. excludenda (1911).

[IV a.] West Africa: **Upper Guinea.** Cameroons: Tchâpe Pass, 5,000 ft., *Ledermann* 2845! Tibati, 3,000 ft., *Ledermann* 2423!

[IV b.] **Lower Guinea.** Cameroons: Yaunde, 2,700 ft., *Zenker & Staudt* 211! *Zenker* 184! 499! 712! Spanish Guinea: Bebao, *Tessmann* 555! Congo State (Lower Congo): Stanley Pool. Dist.; Kisantu, *Gillet* 37! 74! 1419! 1865! Kimuenza, *Gillet* 2144! Lukolela, *Dewevre* 748!

[V.] West Central Africa. Congo State: Lake Leopold II Dist.; near Lake Leopold II, *Body* 92! Equatorial Dist.; Eala, *Pynaert* 525! 920! Lulonga, *Pynaert* 767! Injolo, *Huyghe & Ledoux* 22! Bangala Dist.; Bumba, *Laurent*! Abumonbasi, *Thonner* 200! Uelle Dist.; Paku, *Seret*!

[IX.] East Central Africa. Uganda: Uganda Province; coast of Lake Victoria Nyanza, 4,000 ft., *Bagshawe* 588!

Very nearly allied to *E. atrovirens* and not distinguishable by floral characters, in either sex, from that well-marked species. As a rule, however, the two are quite unlike each other as regards shape of leaf, while they possess a different indumentum. So distinctive a facies is thus imparted to the two plants that hitherto no confusion has taken place between them. On the other hand, the general facies of *E. oleracea* is so like that of *E. Welwitschiana* that it bears as close a resemblance to *E. africana* as *E. Welwitschiana* does. Male specimens of these three species, on this account, call for care in their discrimination, and the writer has experienced exactly the difficulty which others have met with. Fortunately, however, the female flowers of these three plants are widely different, the stigmas of *E. africana* being subsessile and papillose, those of the other two smooth and at the apex of a distinct style. But these two are readily distinguishable because the stigmas of *E. Welwitschiana* are very small and suberect, much shorter than the style, while those of *E. oleracea* are at first divaricate and ultimately recurved and longer than the style. It is to be remarked that in the extreme west (N. Cameroons) of the wide area which the two allies occupy and again in the extreme east (Uganda proper) of this area, *E. atrovirens* and *E. oleracea* occur together. In the intervening region, however, *E. atrovirens* has so far only been found to the north of the Congo, while

E. oleracea appears to take its place to the south of that river, though it also occurs north of the Congo in Bangala and Uelle.

In Lake Leopold II district, according to Body, the leaves are used as a vegetable; according to Huyghe and Ledoux, both the leaves and the flowers are similarly employed in the Equatorial district.

38. **E. angolensis.** *Folia* adpresse pubescentia, basi late cuneata, 5–8 cm. longa. *Stamina* ignota. *Racemi* feminei 4-flori. *Discus hypogynus* zygomorphos, utrinque breviter 2-lobus. *Cocci* 6 mm. lati.—*Claoxylon angolense*, Muell. arg. *in* Journ. Bot., ii. 333 (1864), *et in* DC. Prodr., xv. 2, 777 (1866); Hiern *in* Cat. Afr. Pl. Welw., 975 (1900).

[IV b.] West Africa: **Lower Guinea.** Portuguese West Africa: Angola; Loanda, Pungo Andonga near Luxillo, *Welwitsch* 399!

A very distinct species.

39. **E. flaccida.** *Folia* adpresse pubescentia, basi late cuneata, 5–8 cm. longa. *Stamina* 34. *Racemi* feminei 12–16-flori. *Discus hypogynus* zygomorphos, utrinque altius 2-lobus. *Cocci* 6 mm. lati.—*Claoxylon flaccidum*, Pax *in* Engl. Bot. Jahrb., xix. 86 (1894); Dur. & Schinz *in* Étud. Flor. Cong., 245 (1896); Dur., Syll. Flor. Cong., 491 (1909). *C. Schweinfurthii*, Pax *in* Engl. Bot. Jahrb., xix. 86 (1894); Dur. & Schinz *in* Étud. Flor. Cong., 245 (1896).

[V.] West Central Africa. Niam-Niamland: Nabambisso, *Schweinfurth* 3056! Congo State: Uelle; Bomokandi, in the Monbuttu country, at Munsa's dorf, *Schweinfurth* 3351 in Herb. Berlin! Munsa, *Schweinfurth* 3355 in Herb. Kew!

Very nearly allied to *E. angolense*, and perhaps only a form of that species, from which it differs in having rather longer petioles, female racemes with more numerous flowers, and a more prominently 2-lobed hypogynous disc. No good character is available whereby to distinguish the Niam-Niam plant (*Claoxylon Schweinfurthii*, Pax) from the Monbuttu one (*C. flaccidum*, Pax).

40. **E. macrophylla.** *Folia* adpresse pubescentia, basi angustiore cuneata, 22–25 cm. longa. *Stamina* 24. *Discus hypogynus* ignotus. *Cocci* ignoti.—*Claoxylon macrophyllum*, Prain *in* Kew Bull. 1911, 95 (1911).

[IX.] East Central Africa. Congo State: Eastern Province; Fort Beni, Kwa Muera, *Mildbraed* 2197!

A very distinct species, the precise affinity of which cannot be determined in the absence of female flowers and fruits. It is perhaps most nearly allied to *E. Dewevrei* and *E. hispida*, but it is readily distinguished from both by the shape of its leaves and the absence of hispid hairs.

41. **E. hispida.** *Folia* secus nervos pilis patentibus hispida, subtus inter nervos glabra minute verrucosa, basi rotundata vel late cuneata, 12–20 cm. longa. *Stamina* 27. *Discus hypogynus* zygomorphos, utrinque

late 2-lobus. *Cocci* 9 mm. lati.—*Claoxylon hispidum*, Pax *in* Engl. Bot. Jahrb., xix. 85 (1894).

[IV a.] West Africa: **Upper Guinea.** Cameroons: Buea, 5,300 ft., *Preuss* 888! 908! *Deistel*! *Lehmbach* 179! *Reder* 638! Great Cameroon, 5,300 ft., *Mildbraed* 3459!

A very distinct species, readily distinguished from all the others except *E. Dewevrei* by its relatively large fruit.

42. **E. Dewevrei.** *Folia* secus nervos pilis patentibus hispida, subtus inter nervos densius tomentosa, basi rotundata, 10–12 cm. longa. *Stamina* 24. *Discus hypogynus* zygomorphos, utrinque late 2-lobus. *Cocci* 9 mm. lati.—*Claoxylon Dewevrei*, Pax *in* De Wild. & Dur., Ann. Mus. Congo, Bot., sér. 3, ii. 209 (1901), *et in* Engl. Bot. Jahrb., xxxii. 283 (1903); Dur., Syll. Flor. Cong., 491 (1909).

[IV a.] West Africa: **Upper Guinea.** Cameroons: Babadji, 5,200 ft., *Ledermann* 5988!

[V.] West Central Africa. Congo State: Eastern Province; Maniema, Nyangwe, *Dewevre* 947!

Very nearly allied to *E. hispida*, from which it differs in having the leaves rather densely tomentose beneath between the hispid nerves. The plant from Babadji, Cameroons, with male flowers, agrees well in this respect with *Dewevre* 947 from Nyangwe, Congo State, which is female. With *Dewevre* 947 in the original citation is associated another specimen, *Dewevre* 964 a, also from Nyangwe. This latter plant agrees as regards pubescence with *E. hispida* and not with *E. Dewevrei*, but it differs so markedly from both in the size and form of its leaves that it may be necessary, where fuller material is available, to treat it as a distinct species. Besides differing as regards foliage, it has a larger number of stamens than *E. hispida* has, or than the Cameroon plant which agrees as regards foliage with the female plant on which *E. Dewevrei* was based. In the meantime, it may be left in *E. Dewevrei*, where De Wildeman and Durand have placed it, but must be treated as a distinct variety, characterized as follows:—

E. Dewevrei, var. **inopinata.** *Folia* secus nervos pilis patentibus hispida, subtus inter nervos glabra minute verrucosa, basi cuneata, 5 cm. longa. *Stamina* 40. *Discus* ignotus. *Cocci* ignoti.—*Claoxylon Dewevrei*, De Wild. & Dur. *in* Ann. Mus. Congo, Bot., sér. 3, ii. 209, partim (1901); Dur., Syll. Flor. Cong., 491, partim et quoad Dewevre 964 a tantum (1909): vix Pax. An species distincta?

[V.] West Central Africa. Congo State; Eastern Province; Maniema, Nyangwe, *Dewevre* 964 a!

According to Dewevre the leaves of this variety are edible.

An examination of this conspectus shows that in a certain number of instances species may be barely distinguishable as regards foliage, and may even closely resemble each other as regards male flowers, but may

nevertheless be readily separable by their female flowers. This is particularly true of *E. subspicata* and *E. Poggeophyton*, which agree closely in other respects, yet differ so much as regards the female flowers that the latter species has been looked on as the type of a distinct genus. Another case in which this difficulty is marked is as regards *E. Welwitschiana* and *E. oleracea*, which can hardly be distinguished by their foliage, and again as regards *E. oleracea* and *E. africana*, which have been somewhat consistently mistaken for each other. Yet in the case of these three species the female flowers differ so greatly that each belongs to a distinct natural group.

But the conspectus reveals a converse fact. In a considerable number of cases the forms which it is necessary, for the moment, to recognize, are associated in pairs which practically agree as regards their flowers, and are only to be distinguished by differences in tomentum, in shape of leaf, or in the number of flowers in their respective inflorescences. Doubts therefore arise as to whether the differences in question do not indicate varieties rather than species; it is conceivable that in some cases further material may justify the union of these pairs. Aggregation has, however, been carried as far as the available material will justify; all that can be done is to provide, for the use of workers in the field, a brief synopsis of the situation, in the hope that the material which is required to confirm or dispel these doubts may be obtained.

In *Adenoclaoxylon*, *E. mitis*, Pax, may be only an inland form of the littoral *E. Kirkii* (*Claoxylon Kirkii*, Muell. arg.); *E. rigidifolia*, Pax, may be merely a state of *E. bongensis*, Pax; *E. Paxii*, Rendle, may be no more than a variety of *E. Fischeri*, Pax. In *Deflersia*, *E. berberidea*, Prain, may be only a very distinct variety of *E. natalensis*, Prain; *E. subspicata*, Prain, may prove to be the normal condition of a species whereof *Poggeophyton aculeatum*, Pax, is a teratological condition. In *Trichogyne*, *E. Menyharthii* (*Claoxylon Menyharthii*, Pax) may be the usual state of a species whereof *E. tristis* (*Claoxylon triste*, Muell. arg.) is a local and uncommon condition; *E. hirta*, Pax, may be only a variety of *E. trichogyne* (*Claoxylon trichogyne*, Muell. arg.). Finally, in *Chloropatane*, *E. Poggei*, Prain, may, as Engler has already stated, be referable to *E. columnaris* (*Claoxylon columnare*, Muell. arg.); *E. oleracea*, Prain, may prove to be only a distinct variety of *E. atrovirens* (*Claoxylon atrovirens*, Pax); *E. flaccida* (*Claoxylon flaccidum*, Pax) is almost certainly only a form of *E. angolensis* (*Claoxylon angolense*, Muell. arg.); *E. Dewevrei* (*Claoxylon Dewevrei*, Pax) is perhaps only the normal condition of a species whereof *E. hispida* (*Claoxylon hispidum*, Pax) is a striking local form.

However, even if, in each of these cases, the suggested reduction should ultimately prove to be necessary, the genus *Erythrococca*, as here recognized and limited, will still include over thirty well-characterized species.

MICROCOCCA, Benth. ampl.

Micrococca, Benth. *in* Hook., Niger. Fl., 503 (1849); Baill., Etud. gén. Euphorb., 436 (1858), pro parte; Benth. *in* Benth. et Hook. f. Gen. Plant., iii. 309 (1880); Pax *in* Engl. & Prantl, Nat. Pflanzenf., iii. 5, 48 (1890).

Mercurialis, Pluk., Phytogr., t. 205, f. 4 (1692), *et* Almag., 248 (1696); Lamk., Encyc. Meth., iv. 120 (1796); Baill., Étud. gén. Euphorb., 490 (1858), *in* Adansonia, i. 76 (1860), iii. 175 (1862), *et in* Hist. des Plantes, v. 210 (1874), pro parte: nec Linn.

Tragia, Linn. Sp. Pl., 980 (1753); Willd., Sp. Pl. iv. 324 (1805); Roxb., Flor. Ind., iii. 576 (1832); Grah., Cat. Bomb. Pl., 186 (1839), pro parte.

Microstachys, Dalz. & Gibs., Bomb. Flor., 227 (1861): nec A. Juss.

Claoxylon, Thw., Enum. Pl. Zeyl., 271 (1861); Muell. arg. *in* Linnaea, xxxiv. 166 (1865), *et in* DC. Prodr., xv. 2, 789 (1866), pro parte: Hook. f., Flor. Brit. Ind., v. 412 (1887), pro parte; Trimen, Handb. Flor. Ceyl., iv. 63 (1898); Hiern, Cat. Afr. Pl. Welw., 976 (1900), pro parte; Prain, Beng. Pl., 947 (1903); Cooke, Flor. Pres. Bomb., ii. 609 (1906): nec A. Juss.

Flores dioici vel monoici, apetali. ♂ *Calyx* membranaceus in alabastro globosus apice minute apiculatus, primum clausus, sub anthesi valvatim 3-partitus. *Stamina* definita vel indefinita (3–30), in receptaculo plus minusve elevato centralia, saepe pluriseriata, nunc glandulis rhombeis inter se liberis et apice pilis viscidulis onustis consociata, nunc receptaculo nudo inserta; filamenta antheras aequantia vel iis breviora; antherae 2-loculares; loculi obovoidei vel sublineares, basifixi, erecti, praeter basin inter se liberi, extrorsim rimosi. *Rudimentum ovarii* nullum. ♀ *Calyx* 3–4-partitus, lobis quam in mare majoribus, imbricatis. *Ovarium* plus minusve pubescens vel substrigosum, 3-loculare vel raro 4-loculare, profunde sulcatum; loculi laciniis calycis oppositi vel iis pauciores; ovula in quoque loculo solitaria; stigmata loculis ovarii isomera, elongata, linearia, patula, a basi libera totaque plumoso-laciniata. *Discus* hypogynus e squamis linearibus complanatisve cum carpidiis alternantibus eisque isomeris compositus. *Fructus* capsularis; capsula 3-cocca, raro 4-cocca, interdum abortu 2-cocca, didyma; cocci subglobosi, tenuiter crustacei, extra parce pubescentes vel subglabri, fere a basi distincti, loculicide septicideque simul dehiscentes et 2-valvatim dissilientes. *Semina* fere sphaerica, arillo pertenui involuta; testa crustacea, foveolato-reticulata; albumen carnosum; embryo axilis; radicula conica vel subcylindrica; cotyledones subangusti vel lati, complanati, oblongi vel suborbiculares.—*Frutices* vel *herbae*, inermes; gemmae haud perulatae. *Folia* alterna, petiolata, saepe primum purpurascentia, membranacea, margine plus minusve dentata; stipulae minutae. *Flores* minuti, secus rhachides racemorum axillarium in glomerulos vel in spiculas bracteolis imbricatis

obtectas dispositi ; glomeruli vel spiculae inter se distantes. *Racemi* fasciculati vel solitarii, saepius unisexuales, nonnunquam androgyni ; pedunculi rhachidibusque gracillimi ; bracteae parvae ; pedicelli articulati, masculi breves, feminei longiores ; bracteolae minutae. *Racemi* 1-*sexuales* nunc masculi glomerulis plurifloris, nunc feminei glomerulis 1–2-floris ; *racemi androgyni* nunc glomerulis masculis plurifloris cum singulo femineo terminali, nunc glomerulis paucifloris floribus masculis brevissime pedicellatis 1–2 cum unico centrali femineo longius pedicellato in quoque fasciculo.

CLAVIS SPECIERUM.

Racemi 1–sexuales ; frutices perennantes :—	
Receptaculum maris glandulis interstaminalibus instructum ; folia longe petiolata :—	
Racemi foliis longiores, spiculigeri	1. *M. oligandra.*
Racemi foliis breviores, glomeruligeri :—	
Folia parce pubescentia	2. *M. Wightii.*
Folia dense hirsuta	2 b. *M. Wightii*, var. *hirsuta.*
Receptaculum maris eglandulosum :—	
Folia basin versus longe gradatim attenuata, brevissime petiolata	3. *M. Humblotiana.*
Folia longe petiolata :—	
Racemi masculi glomeruligeri	4. *M. capensis.*
Racemi masculi spiculigeri :—	
Folia basi acuta	5. *M. Beddomei.*
Folia basi late cuneata vel obtusa	6. *M. Holstii.*
Racemi androgyni ; folia longe petiolata :—	
Frutex perennans ; flos terminalis in quoque racemo femineus ; receptaculum maris eglandulosum	7. *M. Volkensii.*
Herba annua ; flos centralis in quoque glomerulo femineus ; receptaculum maris glandulis interstaminalibus instructum	8. *M. Mercurialis.*

1. **M. oligandra.** *Racemi* 1-sexuales foliis longiores. *Flores* masculi ad apices spicularum remotarum bracteolis dense imbricatis obtectarum dispositi ; stamina glandulis interstaminalibus consociata. *Frutex* perennans ; folia ovato-lanceolata, longe petiolata, basi cuneata vel raro obtusa.—*Claoxylon longifolium*, Baill., Étud. gén. Euphorb., 493 (1858) in parte, syn. *Erythrochilus longifolius*, Bl. exclud. ; Thw., Enum. Pl. Zeyl., 271 (1859). *C. oligandrum*, Muell. arg. *in* Linnaea, xxxiv. 164 (1865), *et in* DC. Prodr., xv. 2, 784 (1866) ; Hook. f., Flor. Brit. Ind., v. 412 (1887) ; Trimen, Handb. Flor. Ceyl., iv. 64 (1898).

ASIA : **Ceylon.** Moist regions in forests at from 2,000 to 5,000 ft. ; Matale ; Deltota ; Maskeliya ; Maturata ; *Gardner* 6 ! 165 ! 780 ! *Walker* 37 ! *Thwaites* 2102 ! 2499 ! *Wight* 2641 !

2. **M. Wightii.** *Racemi* 1-sexuales foliis breviores. *Flores* masculi in glomerulos inter se remotos aggregati ; stamina glandulis interstaminalibus consociata. *Frutex* perennans ; folia oblongo-lanceolata, parce hispida, longe petiolata, basi cuneata.—*Claoxylon Wightii*, Hook. f., Flor. Brit. Ind., v. 413 (1887).

Asia : **India.** Malabaria : Travancore ; Courtallam, *Wight* 2676 ! Tinivelli Hills, *Beddome* !

var. **hirsuta.** *Folia* dense hispida.—*Claoxylon hirsutum*, Hook. f., Flor. Brit. Ind., v. 413 (1887).

Asia : **India.** Malabaria : Travancore ; Tinivelli Hills, *Beddome* !

3. **M. Humblotiana.** *Racemi* 1-sexuales foliis breviores. *Flores* masculi in glomerulos inter se remotos aggregati vel interdum inferiores in racemulos perbreves inter se remotos dispositi ; receptaculum maris eglandulosum. *Frutex* perennans ; folia oblanceolata basi sensim in petiolum abbreviatum attenuata.—*Claoxylon Humblotianum*, Baill., Bull. Soc. Linn. Par., 996 (1892).

Mascarene Islands : **Comoros.** Johanna ; 2,000 ft., *Humblot* 222 ! *Kirk* !

4. **M. capensis.** *Racemi* 1-sexuales foliis breviores vel aequilongi, vel masculi interdum foliis longiores. *Flores* masculi in glomerulos inter se remotos aggregati ; receptaculum maris eglandulosum. *Frutex* perennans ; folia elliptico-lanceolata, longe petiolata, basi cuneata.—*Claoxylon capense*, Baill., Étud. gén. Euphorb., 493 (1858), *et in* Adansonia, iii. 161 (1862) ; Muell. arg. *in* DC. Prodr., xv. 2, 786 (1866) ; Sim *in* Flor. Port. E. Afr., 105 (1909).

Africa : **South-East Africa.** Mozambique : Lourenço Marques, *Sim*. Zululand : Entumeni, *Wood* 3979 ! Ngoya, 1,000–2,000 ft., *Wylie* (*Herb. Wood* 7905) ! *Wood* 11570 ! Natal : without precise locality, *Gerrard* 1179 ! Pondoland : near the Umsikaba River, *Drège* 4636 ! without precise locality, *Bachmann* 805 !

5. **M. Beddomei.** *Racemi* 1-sexuales foliis aequilongi vel breviores. *Flores* masculi ad apices spicularum remotarum bracteolis dense imbricatis obtectarum dispositi ; receptaculum maris eglandulosum. *Frutex* perennans ; folia elliptico-lanceolata, longe petiolata, basi acuta.—*Claoxylon Beddomei*, Hook. f., Flor. Brit. Ind., v. 413 (1887).

Asia : **India.** Malabaria : Travancore ; Anamalai Hills, *Beddome* !

6. **M. Holstii.** *Racemi* 1-sexuales foliis aequilongi. *Flores* masculi ad apices spicularum remotarum bracteolis dense imbricatis obtectarum dispositi ; receptaculum maris eglandulosum. *Frutex* perennans ; folia ovata vel elliptica, longe petiolata, basi late cuneata.—*Claoxylon Holstii*, Pax *in* Engl. Bot. Jahrb., xxxiv. 372 (1904).

AFRICA: **East Africa.** German East Africa: East Usambara; near Amani, 2,800 ft., *Engler* 611! near Gonja, *Holst* 4261!

7. **M. Volkensii.** *Racemi* androgyni flore terminali femineo, foliis breviores. *Flores* masculi superne in glomerulos aggregati, basin versus ad apices spicularum bracteolis laxe imbricatis obtectarum dispositi; receptaculum maris eglandulosum. *Frutex* perennans; folia lanceolata, longe petiolata, basi acuta.—*Claoxylon Volkensii*, Pax *in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895), *et in* Engl. Bot. Jahrb., xxiii. 524 (1897).

AFRICA: **East Africa.** German East Africa: Kilimanjaro; Marangu, 7,000–7,500 ft., *Volkens* 1001! *Engler* 1766! *Uhlig* 131!

8. **M. Mercurialis,** Benth. *in* Hook., Niger Fl., 503 (1849). *Racemi* saepissime androgyni foliis aequilongi vel iis longiores. *Flores* masculi nunc pauci cum unico centrali femineo consociati nunc plures absque femineo in glomerulos inter se remotos aggregati; stamina glandulis interstaminalibus consociata. *Herba* agrestis annua; folia ovata, longe petiolata, basi cuneata vel obtusa.—Baill., Étud. gén. Euphorb., 436 (1858); Pax *in* Engl. Pflanzenw. Ost-Afrik., C, 238 (1895); Dur. & De Wild., Bull. Soc. Bot. Belg., xxxvii. 105 (1898); Schweinf., Bull. Herb. Boiss., vii. App. 2, 306 (1899); De Wild. & Dur., Ann. Mus. Congo, Bot., sér. 2, i. 50 (1899); sér. 2, ii. 57 (1900); sér. 3, ii. 209 (1901); De Wild., Miss. Laurent, i. 129 (1905), *et in* Ann. Mus. Congo, Bot., sér. 5, ii. 279 (1908); Dur., Syll. Flor. Cong., 492 (1909). *Mercurialis* maderaspatensis *tricoccos acetabulis destituta*, Pluk., Phytogr., t. 205, fig. 4 (1692), *et in* Almagest., 248 (1696). *Mercurialis alternifolia*, Lamk., Encyc. Meth., iv. 120 (1796); Baill., Étud. gén. Euphorb., 490 (1858), *et in* Adansonia, i. 76 (1860); iii. 175 (1862). *Tragia foliis ovatis*, Linn., Flor. Zeyl., 334 (1747). *T. Mercurialis*, Linn., Sp. Pl., 980 (1753); Willd., Sp. Pl., iv. 324 (1805); Roxb., Flor. Ind., iii. 576 (1832); Grah., Cat. Pl. Bomb., 186 (1839); syn. Rheede excl. *Microstachys Mercurialis*, Dalz. & Gibs., Bomb. Flor., 227 (1861). *Claoxylon Mercurialis*, Thw., Enum. Pl. Zeyl., 271 (1861); Hook. f., Flor. Brit. Ind., v. 412 (1887); Trimen, Handb. Flor. Ceyl., iv. 63 (1898); Woodr., Journ. Bomb. Nat. Hist. Soc., xii. 372 (1899); Hiern, Cat. Afr. Pl. Welw., 976 (1900); Prain, Beng. Pl., 947 (1903); Cooke, Flor. Bomb. Pres., ii. 609 (1906).

AFRICA: **West Africa (Upper Guinea).** Senegal: without precise locality, *Perrottet* 715! Sierra Leone: near Regent, *Scott Elliot* 4117! without precise locality, *Afzelius* 419! Liberia: Grand Bassa, at Fishtown and Monrovia, *Dinklage* 1659! Togo: Lome, *Warnecke* 187! Misahohe, *Baumann* 258! Bismarckburg, *Buettner* 321! Southern Nigeria: Lower Niger, *Vogel* 194! Nupe, *Barter* 1494! Cameroons: Kriegsschiffhafen, *Schlechter* 12394!

West Africa (Lower Guinea). Cameroons: Batanga, *Dinklage* 647! 648! *Bates* 122! Spanish Guinea: Nkolentangan, *Tessmann* 416! Congo

State (Lower Congo): Boma; Zambi, *Dupuis*. Mayumbe; Bingila, *Dupuis*. Matadi; near Matadi, *Laurent*. Stanley Pool Dist.; Kisantu, *Gillet*; near Dembo, *Gillet*; *Vanderyst*; near Yumbi, *Laurent* 421; near Kwamouth, *Bieler*. Angola: Golungo Alto; near Sobati, *Welwitsch* 394! Pungo Andongo; near Pedra Cabondo, *Welwitsch* 395!

West Central Africa. Congo State: Kwango; Eiolo, *Laurent*. Lake Leopold II Dist.; Kutu, *Laurent*; Kiri, *Laurent*. Aruwimi Dist.; near Bena-kamba, *Dewevre* 189; Limbutu, *Laurent*. Uelle; Niam-Niamland, at Mbomu, *Schweinfurth* 3997! Paku, *Seret*. Lualaba-Kasai Dist.; Mukenge, *Pogge* 1332!

East Africa (Mozambique). Nyasaland: Lower Shire; Elephants' Marsh, *Scott*!

East Africa (Zanzibaria). German East Africa: Usaramo, *Stuhlmann* 6380! Madossi, *Stuhlmann* 8119! Dar-es-Salam, *Stuhlmann* 8526! Island of Zanzibar: *Hildebrandt* 1038!

East Central Africa. Uganda: Entebbe, 3900 ft., *Brown* 25!

North-East Africa. Abyssinia: without precise locality, *Schimper*! *Ehrenberg*. Eritrea: Saati, 470 ft., *Schweinfurth* 22! *Schweinfurth & Riva* 487! Nubia: Samhar, near Massowah, *Hildebrandt* 736!

MASCARENE ISLANDS: **Madagascar.** Nossibé; *Boivin*!

ASIA: **Arabia.** Yemen: Agara near Hodjela, 1,750 ft., *Schweinfurth* 1973. Wadi Hille; near Wolledje, at the foot of Gebel Melhan, 2,000 ft., *Schweinfurth* 777.

India. Bombay: Porbundar; *Woodrow*. North Canara; Carwar, *Talbot*! Northern India: Behar; Monghir, *Wallich*! Southern India: Mysore; *Law*! base of Anamalai Hills, *Beddome*! Nilgiri Hills; Sigur, 3,000 ft., *Gamble*! Coromandel; near Madras, *Koenig*! *Wight*! *Shuter*! *G. Thomson*!

Ceylon. Throughout the low country, *Walker*! *Thwaites* 3310!

Indo-China. Burma: Pegu; Petroleum Wells on the Irrawadi, *Wallich*!

Malaya. Malay Peninsula: Pulo Uban; Granite quarries, *Ridley* 374!

DISTRIBUTION OF ERYTHROCOCCA AND MICROCOCCA.

In discussing the distribution of the African species of any genus we have at our disposal two somewhat different systems of subdivision of the continent; that adopted in the 'Flora of Tropical Africa', and that employed in 'Die Pflanzenwelt Afrikas'. Each of these systems serves very well the purpose it is intended to fulfil. Neither system, however, is wholly suitable to a sketch like the present; the former because of the respect it is compelled to pay to the accidental situation of existing political frontiers; the latter because of the detail to which the subdivision of the natural regions

recognizable on physiographical grounds is necessarily carried. These natural regions, ten in number, but in three instances conveniently subdivisible, are here treated as primary; to facilitate the use of a map, existing political areas are also indicated. To avoid confusion, the limits of these natural regions may be briefly stated:—

I. NORTH AFRICA. Mediterranean sea-board to the northern edge of the Sahara: Atlantic sea-board, between Tangier and Cape Nun, to the eastern fringe of the Libyan Waste.

II. THE SAHARA.

III. NORTH CENTRAL AFRICA. Southern edge of the Sahara to the northern boundary of Upper Guinea and the Congo-Chad divide; Atlantic sea-board, between Lake Taniahya and the Gambia, eastward to Dar Fertit and the Nile-Chad divide. This region includes the catchment areas of the Senegal to its mouth, of the Niger and the Benue to their confluence, and of the streams that empty into Lake Chad.

IV. WEST AFRICA. Belt roughly 200 miles wide inland from the Atlantic sea-board, the River Gambia on the north and Benguella on the south. This region includes the Lower Niger basin from Lokoja to the sea and the Lower Congo Basin below Stanley Pool; it admits of convenient subdivision into:—

(*a*) **Upper Guinea.** River Gambia to the River Sanaga; with Fernando Po.

(*b*) **Lower Guinea.** River Sanaga to Benguella; with Prince's Island and San Thomé.

V. WEST CENTRAL AFRICA. Catchment area of the Congo as far as Stanley Pool.

VI. SOUTH-WEST AFRICA. Congo-Kunene and Congo-Zambesi divides southward to the Roggeveld, Nieuweveld, and Zuurberg ranges: Atlantic sea-board, between Benguella and Olifant's River, eastward to Lake Nyasa, and the Kirk, Melsetter, Lebombo, and Drakensberg ranges. This region includes the catchment areas of the Cunene and the Orange-Vaal to their mouths, of the Zambesi to Tete, and of the Limpopo and other streams to the Mozambique frontier.

VII. SOUTH-EAST AFRICA. Coast and Central zones of Cape Colony and Natal, between the Roggeveld, Nieuweveld, Zuurberg, and Drakensberg ranges and the sea, from Olifant's River to Delagoa Bay. This region admits of convenient subdivision into:—

(*a*) **Cape.** Olifant's River to the Kei.

(*b*) **Natal.** Kei River to Delagoa Bay.

VIII. EAST AFRICA. Belt of varying width from the Indian Ocean

sea-board between Delagoa Bay and the River Juba, inland to the Lebombo, Melsetter, and Kirk ranges, Lake Nyasa, a line from the northern end of Nyasa to the eastern base of Kilimanjaro, thence to the eastern base of Kenia, thence to Lake Rudolf: limited on the north by a line joining the northern ends of Lakes Rudolf and Stefanie and projected eastward to the River Dawoe, thereafter by the Dawoe-Juba. This region admits of convenient subdivision into:—

(*a*) **Mozambique.** Delagoa Bay to the Rovuma.

(*b*) **Zanzibaria.** Rovuma River to the Juba; with Mafia, Zanzibar, and Pemba.

IX. East Central Africa. Limited on south and west by a line from the northern end of Nyasa to Tanganyika, by Lake Tanganyika, the Congo-Nile and the Nile-Chad divides as far as Dar Fertit; on the east by the western boundary of East Africa as far as the northern end of Lake Rudolf, thereafter by a line from Lake Rudolf to the River Sobat; on the north by the Sobat and the Bahr-el-Arab. This region includes the catchment area of the Upper Nile as far as Sobat with the *massifs* of Ruwenzori, Kilimanjaro, Elgon, and Kenia.

X. North-East Africa. Limited on the west by North Africa, the Sahara, and North Central Africa; on the south by East Central and East Africa. The region includes Egypt, Nubia, the Eastern Sudan, Abyssinia, and Somaliland, with Yemen and Socotra.

In the first three regions, North Africa, the Sahara, and North Central Africa, no species of *Erythrococca* has yet been met with; nor has any species been found in the western, or Cape, subdivision of South-East Africa. In all the other regions species of *Erythrococca* occur in numbers varying from nineteen in West Africa to one in North-East Africa.

Of the nineteen species which occur in West Africa eleven are found in Upper Guinea and nine are endemic there; nine are met with in Lower Guinea and six of these are endemic. Only one of the nineteen is found both in Upper and in Lower Guinea; this species extends across West Central Africa as far as Uganda in East Central Africa. One species is common to Upper Guinea and West Central Africa; two others are common to Lower Guinea and West Central Africa; yet another extends from Upper Guinea only across West Central to East Central Africa.

In West Central Africa there are nine species only; four of these are endemic. Of the remaining five, three extend to West Africa only; the other two extend both to West Africa and to East Central Africa.

In South-West Africa there are only two species, one of them being endemic and local; the other, which is very widely spread in the region, extends from German South-West Africa and Bechuanaland to Northern Rhodesia and German East Africa.

In South-East Africa there are, again, only two species; both are confined to the Eastern, or Natal, sub-region, and both are endemic.

In East Africa there are only five species; all of them, however, are endemic. One is confined to the southern or Mozambique sub-region; three are confined to the northern or 'Zanzibaria' sub-region; the fifth, which appears to be a purely littoral species, extends along the Indian Ocean seaboard from Beira in the south to Mombasa in the north.

In East Central Africa there are twelve species, nine of which are endemic; of the others, one is manifestly a South-West African overflow, the remaining two are clearly overflows from West Central Africa.

The solitary North-East African species is endemic, but occurs on both sides of the Red Sea, in Abyssinia and in Yemen.

The sub-genus *Euerythrococca*, with twenty-five species, is represented in every region where the genus occurs, but most fully so in East Central Africa, where there are nine species; eight of these are endemic, the ninth is an overflow from South-West Africa. In West Africa this subgenus is rather poorly represented, there being only four species, two in Upper and two in Lower Guinea; all four, however, are endemic. The subgenus *Athroandra*, on the other hand, though it extends through West Central to East Central Africa, is mainly West African, all but two of the seventeen species which it includes occurring there, and all but four of those that do occur there being endemic. Of the two which are not reported from West Africa, one is endemic in West Central Africa, the other has so far been met with just within the western border of East Central Africa, and, like the two remaining Athroandras in that region, may prove to be only an overflow into the portion of this area which has, as Engler remarks (Pflanzenw. Afr., i. t. 2), a flora of decidedly West African character.

The section *Adenoclaoxylon* is confined to East Africa, where there are two endemic species, and to East Central Africa, where there are five species, all endemic. The section *Deflersia* is more widely spread and extends from Yemen and Abyssinia to Natal, with a very distinctive outlying group of three nearly allied species in West Central Africa, and a solitary, but also very distinctive species in Upper Guinea. One species is endemic in North-East Africa, two are endemic in East Africa, and two in South-East Africa. The section is unrepresented in East Central Africa or in South-West Africa, and there is no species in Lower Guinea. The section *Trichogyne* may almost be said to occupy the area left vacant by *Deflersia*. There is no species in North-East Africa, East Africa, or South-East Africa, there is no species in West Central Africa, and the solitary species in Upper Guinea occurs just within the south-eastern margin of that sub-region. In East Central Africa *Trichogyne* is represented by four species, of which three are endemic, the fourth being an overflow from South-West Africa, where it is widely spread. In South-West Africa there is

a second species, local and endemic, near the southern border of Lower Guinea, in which area there is another endemic species. The section *Pseudathroandra* has only two species, one of them endemic in Lower Guinea, the other in the 'Zanzibaria' sub-region of East Africa.

The section *Hemierythrococca* is confined to West Africa; its three species are all endemic in Upper Guinea. The section *Chloropatane* has eleven species in West Africa; three of these are endemic in Upper and three in Lower Guinea. There is only one species common to both the Guineas, and this, which extends as far as East Central Africa, may be only an overflow from West Central Africa. Another species extends equally far to the East from Upper Guinea only; one more from Upper Guinea and two others from Lower Guinea extend into West Central Africa. Of the six species of *Chloropatane*, one of them with a very distinct variety, which occur in West Central Africa, one, and the variety in question, are endemic; the remaining five are distributed, two to Lower Guinea only, one to Upper Guinea only, the other two to East Central Africa on the one hand, to West Africa on the other. In East Central Africa there are but three species of *Chloropatane*, and only one of these, so far as is known, is endemic.

The distribution of the genus *Micrococca* is interesting, but presents no feature of difficulty. The only annual species, *M. Mercurialis*, is a widespread tropical weed in the Eastern Hemisphere. In Africa it is common in cultivated ground in West Africa, both in Upper and in Lower Guinea; it occurs both in West Central and in East Central Africa; it has been reported in East Africa, both from the Mozambique and the Zanzibaria subdivisions. It appears to be common also in North-East Africa, and extends thence to Southern Arabia. It has been met with in Madagascar. In Asia it is widely spread in Western, Northern, and Southern India, extends thence to Burma and the Malay Peninsula, and is common in Ceylon. The shrubby species have much more restricted ranges. One species, *M. capensis*, is confined to the eastern, or Natal, subdivision of South-East Africa, but is widely spread there from Pondoland to Delagoa Bay. Two species, *M. Volkensii* and *M. Holstii*, seem confined to the Zanzibaria subdivision of East Africa. One species, *M. Humblotiana*, is endemic in the Comoro Group; two, *M. Wightii*, with its distinct variety *hirsuta*, and *M. Beddomei*, are endemic in the Malabaria subdivision of India; the last species, *M. oligandra*, is endemic in Ceylon.

The subjoined tables show the details of this distribution more compactly.

I. Conspectus of the Distribution of Erythrococca.

Sub-genus.	Section.	Group.	Species.	I N. Afr.	II Sahara.	III N. C. Afr.	IVa W. Afr. (U. Guin.).	IVb W. Afr. (L. Guin.).	V W. C. Afr.	VI S. W. Afr.	VIIa S. E. Afr. (Cape).	VIIb S. E. Afr. (Natal).	VIIIa E. Afr. (Mozamb.).	VIIIb E. Afr. (Zanzib.).	IX E. C. Afr.	X N. E. Afr.
I.	§ 1.	¶ 1.	1. *E. Kirkii*	–	–	–	–	–	–	–	–	–	×	×	–	–
			2. *E. mitis*	–	–	–	–	–	–	–	–	–	–	×	–	–
		¶ 2.	3. *E. bongensis*	–	–	–	–	–	–	–	–	–	–	–	×	–
			4. *E. rigidifolia*	–	–	–	–	–	–	–	–	–	–	–	×	–
			5. *E. olacifolia*	–	–	–	–	–	–	–	–	–	–	–	×	–
		¶ 3.	6. *E. Paxii*	–	–	–	–	–	–	–	–	–	–	–	×	–
			7. *E. Fischeri*	–	–	–	–	–	–	–	–	–	–	–	×	–
	§ 2.	¶ 4.	8. *E. abyssinica*	–	–	–	–	–	–	–	–	–	–	–	–	×
			9. *E. usambarica*	–	–	–	–	–	–	–	–	–	–	×	–	–
		¶ 5.	10. *E. zambesiaca*	–	–	–	–	–	–	–	–	–	×	–	–	–
			11. *E. natalensis*	–	–	–	–	–	–	–	–	×	–	–	–	–
			12. *E. berberidea*	–	–	–	–	–	–	–	–	×	–	–	–	–
		¶ 6.	13. *E. anomala*	–	–	–	×	–	–	–	–	–	–	–	–	–
		¶ 7.	14. *E. subspicata*	–	–	–	–	–	×	–	–	–	–	–	–	–
			15. *E. Laurentii*	–	–	–	–	–	×	–	–	–	–	–	–	–
			16. *E. Poggeophyton*	–	–	–	–	–	×	–	–	–	–	–	–	–
	§ 3.	¶ 8.	17. *E. Menyharthii*	–	–	–	–	–	–	×	–	–	–	–	×	–
			18. *E. tristis*	–	–	–	–	–	–	×	–	–	–	–	–	–
		¶ 9.	19. *E. uasiococca*	–	–	–	–	–	–	–	–	–	–	–	×	–
			20. *E. Ledermanniana*	–	–	–	×	–	–	–	–	–	–	–	–	–
			21. *E. Mildbraedii*	–	–	–	–	–	–	–	–	–	–	–	×	–
			22. *E. trichogyne*	–	–	–	–	×	–	–	–	–	–	–	–	–
			23. *E. hirta*	–	–	–	–	–	–	–	–	–	–	–	×	–
	§ 4.	¶ 10.	24. *E. pauciflora*	–	–	–	–	×	–	–	–	–	–	–	–	–
			25. *E. polyandra*	–	–	–	–	–	–	–	–	–	–	×	–	–
II.	§ 5.	¶ 11.	26. *E. membranacea*	–	–	–	×	–	–	–	–	–	–	–	–	–
		¶ 12.	27. *E. patula*	–	–	–	×	–	–	–	–	–	–	–	–	–
			28. *E. Mannii*	–	–	–	×	–	–	–	–	–	–	–	–	–
	§ 6.	¶ 13.	29. *E. africana*	–	–	–	×	–	–	–	–	–	–	–	–	–
			30. *E. Chevalieri*	–	–	–	×	–	–	–	–	–	–	–	–	–
			31. *E. Molleri*	–	–	–	–	×	–	–	–	–	–	–	–	–
		¶ 14.	32. *E. rivularis*	–	–	–	–	×	–	–	–	–	–	–	–	–
		¶ 15.	33. *E. Welwitschiana*	–	–	–	–	×	×	–	–	–	–	–	–	–
			34. *E. columnaris*	–	–	–	–	×	–	–	–	–	–	–	–	–
			35. *E. Poggei*	–	–	–	–	×	×	–	–	–	–	–	–	–
		¶ 16.	36. *E. atrovirens*	–	–	–	×	–	×	–	–	–	–	–	×	–
			37. *E. oleracea*	–	–	–	×	×	×	–	–	–	–	–	×	–
			38. *E. angolensis*	–	–	–	–	×	–	–	–	–	–	–	–	–
			39. *E. flaccida*	–	–	–	–	–	×	–	–	–	–	–	–	–
			40. *E. macrophylla*	–	–	–	–	–	–	–	–	–	–	–	×	–
			41. *E. hispida*	–	–	–	×	–	–	–	–	–	–	–	–	–
			42. *E. Dewevrei*	–	–	–	×	–	×	–	–	–	–	–	–	–
			Totals	0	0	0	11	9	9	2	0	2	2	4	12	1

II. Conspectus of the Distribution of Micrococca.

Species.	Africa.							Asia.				
	W. Afr.	W. C. Afr.	S.E. Afr. (Natal).	E. Afr. (Mozamb.).	E. Afr. (Zanzib.).	Mascarenes.	N.E. Afr.	Arabia.	India (Malab.).	Ceylon.	India (Coroman.).	Indo-China (Burma).
1. *M. oligandrum*	—	—	—	—	—	—	—	—	—	×	—	—
2. *M. Wightii*	—	—	—	—	—	—	—	—	×	—	—	—
3. *M. Humblotiana*	—	—	—	—	—	×	—	—	—	—	—	—
4. *M. capensis*	—	—	×	—	—	—	—	—	—	—	—	—
5. *M. Beddomei*	—	—	—	—	—	—	—	—	×	—	—	—
6. *M. Holstii*	—	—	—	—	×	—	—	—	—	—	—	—
7. *M. Volkensii*	—	—	—	—	×	—	—	—	—	—	—	—
8. *M. Mercurialis*	×	×	—	×	×	×	×	×	×	×	×	×
Totals	1	1	1	1	3	2	1	1	3	2	1	1

Properties.

The species of *Erythrococca* and of *Micrococca* seem to be economically unimportant. Little has been recorded in the field as to the former, still less as to the latter. The only suggestion as to the possible presence of an active principle occurs in a note on *Erythrococca anomala* by Heudelot, who says: 'Les feuilles sont très recherchées par les habitants du Fouta Dhiallon comme anthelmintique; ils prétendent que c'est un spécifique contre le ver solitaire. Ils font souvent plus de 50 lieues pour se procurer des feuilles de cet arbuste, qui du reste est assez rare. Ils l'appellent *Sakadhöelly*.' The only other references come from the Congo State and suggest bland properties. Body notes that the leaves of *E. oleracea*, known as *Ejendje* in the Lake Leopold district, are eaten. Huyghe and Ledoux say that about Injolo in the Equatorial district the leaves and the flowers of this species are used as a vegetable. Dewevre remarks that in the Maniema district the leaves of *E. Dewevrei* var. *inopinata* are eaten, and Sapin records that, in the Kasai district, *E. Welwitschiana*, which is known in the Sankuru tongue as *Masoha* and in the Bangala dialect as *Ntenteke*, makes an excellent vegetable. These economic notes we owe to French and Belgian collectors; no German or English traveller has noted the utilization of any species either as a medicinal or as a food plant.

On the Gametophytes and Embryo of Pseudolarix.

BY

KIICHI MIYAKE

AND

KONO YASUI.

With Plate XLVIII.

ALTHOUGH the gametophytes, fertilization, and embryogeny of the Abietineae may, at present, be said to be the best known among the Coniferales, there remain still some genera of which the gametophytic structures are entirely untouched by investigators. Among the existing nine genera of the Abietineae, the genus *Pinus* has been most thoroughly investigated. We have also more or less complete records of the gametophytes of *Picea*, *Abies*, *Tsuga*, *Pseudotsuga*, and *Larix*.[1] But, in the remaining three genera—*Cedrus*, *Pseudolarix*, and *Keetleria*—this phase of life-history is entirely unknown. In light of the accumulating evidence in support of the view that the Abietineae is a very ancient group of the Coniferales,[2] it has been thought very desirable that the gametophytic phase of all the genera of the group should thoroughly be worked out. The present study has been undertaken with this object in view and to fill up one of the gaps in our knowledge of the life-history of the Abietineae.

Pseudolarix Kaempferi, the only species of the genus, is a native of China and seems to thrive quite well in Southern Europe. The material for the present study has been secured from Northern Italy, through the kindness of Dr. R. Rovelli in Pallanza, in whose garden a beautiful specimen of this tree is growing, and to whom we are very much indebted. During the years 1903 and 1904, it was sent from there to Bonn, Germany, where one of us (K. M.) was staying at the time. The living male or female cones with part of the attached branches were sent two or three times a month

[1] Chief contributions to our knowledge of the gametophytes and embryo of the Abietineae are those of Hofmeister ('51, '58), Strasburger ('69, '72, '78, '79, '84, '92), Goroschankin ('80, '83*a*, '83*b*), Sokolowa ('90), Mottier ('92), Belajeff ('93), Dixon ('94), Coulter ('97), Blackman ('98), Chamberlain ('99), Cavara ('00), Murrill ('00), Arnoldi ('00), Coulter and Chamberlain ('01, '10), Ferguson ('01*a*, '01*b*, '04), Miyake ('03*a*, '03*b*), Thomson ('05), Stopes and Fujii ('06), Kildahl ('07), and Lawson ('09).

[2] Concerning the anatomical and palaeontological evidences, see Jeffrey ('05, '08) and Hollick and Jeffrey ('09).

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during April to July. Part of the material was always fixed immediately after being received, and the remainder put in a moist chamber to be fixed later. The cone-bearing twigs were usually inserted into water under a bell-jar, and it was found that the cones showed normal development at least for several days.

The fixing was done usually with Flemming's chromosmo-acetic acid solution, chrom-acetic acid mixture being occasionally employed. After being embedded in paraffin in the usual way, the material was cut 5–10 μ in thickness. For staining, Flemming's triple combination or Heidenhain's iron-alum haematoxylin were used.

On account of the insufficiency of the material our results are not quite so complete as we had wished, but they show distinctly that the gametophytes and embryogeny of *Pseudolarix* closely follow the normal Abietinous type.

The Male Gametophyte.

The microspore seems to be quite mature at the end of March. In the male cones received early in April, the microspores were already fully formed, with well-developed wings, and many of them were found in the process of division. In fact, in cones of the same date we may find various stages of division which are described in the following paragraphs.

The nucleus of the mature microspore soon divides to form the first prothallial cell (Pl. XLVIII, Figs. 1–3). The first prothallial cell shows signs of disintegration soon after its formation and is at first found as a lenticular cell at the dorsal side of the microspore (Fig. 4). It, however, disorganizes quite rapidly and sooner or later becomes flattened against the spore wall (Figs. 5, 7). The second division follows immediately, and the second prothallial cell is organized, which soon shares the same fate as that of the first (Figs. 5, 7, 8–11). Fig. 6 shows a polar view of a spindle of one of these divisions and the number of chromosomes is seen to be distinctly twelve.

As the third division, resulting in the formation of the generative and tube cells, is completed, the two prothallial cells are seen as thin darkly staining bodies pressed against the dorsal wall of the pollen-grain (Fig. 8). The generative cell soon divides, and the so-called body and stalk cells are formed (Figs. 9–11). Now the pollen is ready for pollination. The number of cells in the mature pollen-grain of *Pseudolarix* is the same as in *Larix* (Strasburger '72, '92) and *Picea* (Strasburger '92, Miyake '03 *a*), while it differs from that of *Pinus*. In the latter the generative cell does not divide until some time after pollination (Strasburger '92, Ferguson '04).

Pollination seems to take place in Northern Italy about the middle or the latter part of April. It seems to occur somewhat later in Southern Germany; for, judging from the male cones received from Baden-Baden

on May 13, 1904, which were in the last stage of pollination, the latter had probably begun at the beginning of the same month.

The further development of the male gametophyte after pollination has not been investigated.

The Female Gametophyte.

The division in the formation of the megaspores seems to occur about the time of pollination, the megaspore mother-cell being as a rule solitary in each ovule. Stages of the division were not observed, nor has the number of potential megaspores been determined. The lowest of the megaspores soon begins to enlarge and develops into the female prothallium, while the upper sister cells gradually disintegrate and are crowded to the upper corner of the growing gametophyte. Fig. 12 shows a young female gametophyte with two free nuclei which are embedded in the parietal layer of cytoplasm surrounding a large central vacuole, and the disorganizing remains of the sterile megaspores are seen at the upper part of it. It is probable that the parietal layer of cytoplasm is organized at one-nuclear stage, as has already been reported in *Pinus* (Ferguson '04) and *Cunninghamia* (Miyake '10). The free nuclear division in the megasporic sac takes place hand in hand with the enlargement of the latter as in other Conifers.

Fig. 13 shows the condition of a young female prothallium about four weeks after that of Fig. 12. It is surrounded by several layers of well-developed tapetal cells. After much enlargement and more nuclear divisions, the walls are formed between the nuclei, and finally the entire megasporic sac is filled up with compact prothallial tissue. At this time the tapetum is much compressed by the growing prothallium, and shows signs of disintegration.

The so-called megaspore membrane enclosing the female prothallium is at first thin and delicate. During the growth of the prothallium the membrane becomes thicker and more conspicuous. Its structure and thickness are on the whole similar to those described for other Abietineae (Thomson '05, Lawson '09). Fig. 14 is drawn from the lateral basal region of a mature prothallium, showing the well-developed megaspore membrane, which measures about 4·5 μ in thickness. The exosporium shows characteristic radial striations, and is several times as thick as the homogeneous endosporium. Thomson ('05), who has studied the megaspore membrane in five genera of the Abietineae, states that in this group 'the coat is thick in the chalazal region, and thins out gradually towards the micropylar portion of the prothallium, being not more than one-third as thick at the apex as at the base of the macrospore'. He found, however, that in *Larix* 'there is scarcely a trace of the megaspore coat in the archegonial region'. Lawson ('09) also found a similar thing in *Pseudotsuga*, and says that 'at a plane

almost level with the base of the archegonia the membrane thins out rather abruptly, and from this region to the very apex of the prothallium no trace of the membrane could be detected'. In *Pseudolarix*, as in the majority of the Abietineae studied by Thomson, the megaspore membrane is present in the micropylar region of the prothallium, where it is much thinner than at the basal portion.

As soon as the female gametophyte is filled with solid prothallial tissue, archegonial initials appear at the micropylar end of the prothallium. This seems to take place early in June. One of the earliest stages of the young archegonia observed is shown in Pl. XLVIII, Fig. 15. This is figured from the material received on June 6. The archegonial initial has already divided once, and the primary neck-cell is cut off from the central cell.

The primary neck-cell is soon divided into two cells by an anticlinal wall (Fig. 16). The two cells then divide again by walls perpendicular to the first. The four cells thus formed usually divide once more by periclinal walls, and two tiers of four cells each are formed (Fig. 22). These constitute what may be called the normal neck of the mature archegonium. In some archegonia, however, the neck was found in a longitudinal section to consist of two tiers of four cells each, suggesting that there may be eight cells in each tier.

The central cell increases in size quite rapidly, and in about three weeks after the formation it reaches the full size. In the early stages of development it contains a large vacuole occupying the greater part of the cell (Figs. 15, 16). The presence of a similar vacuole has been observed in the young archegonia of *Tsuga* (Murrill '00), *Taxodium* (Coker '03), *Cunninghamia* (Miyake '10), *Dioon* (Chamberlain '06), and several Cupressineae (Strasburger '79, Land '02, Norén '07, Lawson '07), and may probably represent the normal condition in the young central cell of the Gymnosperms. The formation of the central vacuole may be considered as a result of the growth of a cell without a corresponding increase of its cytoplasm, as in the case of the young female gametophyte. Later, the vacuole gradually decreases in size, and the cell is gradually filled up with cytoplasm. The cytoplasm is at first vacuolate, but later it becomes more dense and granular. The nucleus is, from the first, always situated at the apex of the cell just beneath the neck (Figs. 15–17).

Enveloping the central cell is a layer of sheath or jacket cells, which are scarcely distinguishable from the adjacent cells of the prothallium in the early stage of development (Fig. 15). But later the cytoplasmic contents become more dense and the nuclei much larger, and in the mature archegonium they are well differentiated (Figs. 22, 23).

The number of archegonia in a single ovule varies from four to seven, the more usual number being five or six. As in other Abietineae the archegonia are arranged quite separate from one another, and they may

come in contact in the middle region where they are widest. Even there the egg-cells never come into direct contact as in the Cupressineae and some of the Taxodineae, but they are always separated by one or two layers of jacket cells (Fig. 23). Each archegonium has its own archegonial chamber, and this more or less funnel-shaped depression above the neck is not so deep as that in *Pinus* (Ferguson '04) and *Tsuga* (Murrill '00) (Figs. 22, 25, 30).

As the central cell of the archegonium reaches its full size, the nucleus prepares for division. Figs. 18 and 19 show the early prophase of the division, and we can see that the chromatic contents of the nucleus accumulate near the centre of the cavity in a condition suggesting the synapsis of the reduction division, as has already been observed in various other Abietineae (Murrill '00, Miyake '03 *a*, '03 *b*). The first indication of the spindle formation is the appearance of a fibrous cytoplasmic cap at the lower side of the nucleus (Fig. 19). The process of the division does not seem to differ from that of other Abietineae. Fig. 20 shows the metaphase of the division; the spindle is more or less pointed at the lower end and somewhat blunt on the upper side, as has often been observed in *Pinus* (Ferguson '04) and *Picea* (Miyake '03).

The result of the division is the formation of the ventral canal-cell. The cell shows, however, signs of disintegration as soon as it is formed (Fig. 21). In the mature archegonium ready for fertilization, the ventral canal-cell usually appears as a deeply-staining lenticular or crescent-shaped cap over the top of the egg (Figs. 22, 24).

The egg nucleus begins to enlarge soon after its formation, and moves down towards the centre of the egg. The mature egg nucleus situated near the centre of the archegonium is more or less spherical or elliptical and usually contains a nucleolus (Fig. 22). It has often been observed that the chromatic substance arranged in more or less irregular reticulum does not fill up the entire nuclear cavity, but is somewhat contracted on one side (Figs. 22, 24). This condition has been quite frequently found in apparently well-fixed eggs, but we have not been able to determine whether this is a normal structure or an artifact caused by reagents.

After the division of the central cell, the number and size of the vacuoles in the cytoplasm gradually decrease, and in the mature egg there are usually found a few smaller vacuoles. The number of so-called proteid vacuoles seem to be not so numerous as in some other Abietineae (Figs. 17–22).

In the mature archegonium previous to fertilization we have sometimes found an extra nucleus at the upper part of the egg (Fig. 24, *x*). A similar nucleus has been observed in the mature archegonium of *Tsuga* (Murrill '00) and *Abies* (Miyake '03 *b*). Murrill calls such a nucleus in *Tsuga* the nuclear vacuole. The origin and fate of the nucleus have not been followed.

The Embryo Formation.

Fertilization takes place about the end of June. The actual fusion of the sexual nuclei was not observed. But various stages of the embryo formation were followed. The process does not differ from the usual Abietineae type. The result of the division of the fusion nucleus is shown in Pl. XLVIII, Fig. 25. The two daughter nuclei soon divide, and the four free nuclei of the proembryo now move down towards the base of the archegonium (Fig. 26). Upon reaching the base of the egg, the four nuclei arrange themselves in a plane, as shown in Fig. 27. They then undergo further division, and the walls are formed between the eight daughter nuclei. Thus a tier of four completely walled cells is cut off below, and the upper four nuclei being separated from one another by walls are exposed above to the rest of the egg cytoplasm (Fig. 28).

The eight nuclei divide once more, and eventually there are formed four tiers of cells of four cells each, the upper tier being incompletely walled, since the nuclei are separated from one another by walls, but freely exposed above to the unsegmented part of the egg (Figs. 29, 30). The lowest tier forms the embryo proper, the tier above is elongated to form the suspensor, and the uppermost of the completely walled tiers constitutes the rosette (Fig. 31). The young embryo in Fig. 31 is apparently in eight-cell stage.

Summary.

The mature pollen-grain has wings and contains the large tube cell, smaller generative and stalk cells, besides the disintegrated remains of two prothallial cells. The stages of division in the formation of these cells were observed. The number of chromosomes was found to be twelve.

Pollination takes place in Northern Italy about the middle or the end of April, and it seems to be somewhat later in Southern Germany. The further development of the male gametophyte after pollination has not been investigated.

The formation of the megaspores seems to occur about the time of pollination, the megaspore mother-cell being usually solitary in each ovule. The lowest of a row of megaspores develops into the female gametophyte.

The large central vacuole of the young female prothallium seems to be formed already at one-cell stage. The enlargement of the functional megaspore goes hand in hand with the free nuclear division.

The young female gametophyte is surrounded by several layers of well-developed tapetal cells, which later show signs of disintegration.

The megaspore membrane is well developed in the mature prothallium and has about the same structure and thickness as that of other Abietineae. It is quite thick in the basal region and thins out gradually towards the micropylar region of the prothallium.

The female gametophyte is filled with solid tissue early in June, and soon the archegonial initials appear at the micropylar end of the prothallium. The archegonia reach the mature size in about three weeks.

The development and structure of the archegonium do not seem to differ much from those of other Abietineae. The number of archegonia in a single ovule varies from four to seven, the more usual number being five or six. The neck consists usually of eight cells, arranged in two tiers of four cells each.

In the division of the central cell of the archegonium, the chromatic contents of the nucleus at first accumulate near the centre of the cavity as in synapsis. The ventral canal-cell is formed as a result of the division, and it shows immediately signs of disorganization. The egg nucleus increases in size soon after its formation, and moves down towards the centre of the egg.

Fertilization takes place about the end of June. The actual fusion of the sexual nuclei was not observed. The first two divisions of the fertilized egg nucleus take place near the centre of the egg, and the four free nuclei of the proembryo move down to the base of the archegonium. The four nuclei now divide again, and the walls are then formed between eight free nuclei, which are arranged in two tiers.

The nuclei of both tiers divide once more, and eventually the proembryo consists of four tiers of four cells each, the uppermost of which is open at the top. The lowest of the tiers becomes the embryo proper, the next tier above elongates to form the suspensor, and the uppermost of the completely walled tiers constitutes the rosette.

The structure and development of the gametophytes and embryogeny of *Pseudolarix* follow the normal Abietinous type.

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EXPLANATION OF FIGURES IN PLATE XLVIII.

Illustrating Dr. Miyake and Miss Yasui's paper on *Pseudolarix*.

All figures were drawn with the aid of a camera lucida.

Figs. 1–3. Stages in the first division of the microspore. × 1,400. April 2.

Fig. 4. The first prothallial cell is formed. × 1,400.

Fig. 5. Division in the formation of the second prothallial cell. Disorganized first prothallial cell is seen as a thin deeply staining body. × 1,400.

Fig. 6. A polar view of a spindle of one of the first two divisions of germinating microspore, showing twelve chromosomes. × 1,400.

Fig. 7. The second prothallial cell is formed. × 1,400.

Fig. 8. The third division is completed. The generative cell, the tube cell, and the remnants of two degenerating prothallial cells are shown. × 1,400.

Figs. 9, 10. Stages in the division of the generative cell. × 1,400.

Fig. 11. Mature pollen-grain just before pollination, showing the tube cell, the body and stalk cells, besides two degenerating prothallial cells. × 1,400.

Fig. 12. The two-nucleated stage of the female gametophyte. One of the two nuclei, outline of which is shown here, appears in next section. × 480.

Fig. 13. Young female prothallium surrounded by several layers of tapetal cells. × 90. May 22.

Fig. 14. A lateral basal portion of the periphery of a mature female prothallium, showing the structure of the megaspore membrane. × 480.

Fig. 15. Young archegonium with a single primary neck-cell. × 160. June 6.

Fig. 16. A later stage with two neck-cells. × 160. June 13.

Fig. 17. Upper portion of a full-grown central cell shortly before division. × 310.

Figs. 18, 19. Prophase in the division of the central cell. × 310.

Fig. 20. Metaphase of the division. × 310.

Fig. 21. The division is almost completed. × 310.

Fig. 22. Mature archegonium. × 105. June 26.

Fig. 23. Cross-section of a mature prothallium showing seven archegonia. × 80.

Fig. 24. Upper half of a mature egg, showing an extra nucleus (*x*) just below the disintegrating ventral canal-cell (*v.c.c.*). × 160.

Fig. 25. The two daughter nuclei resulting from the first division after fertilization. × 105.

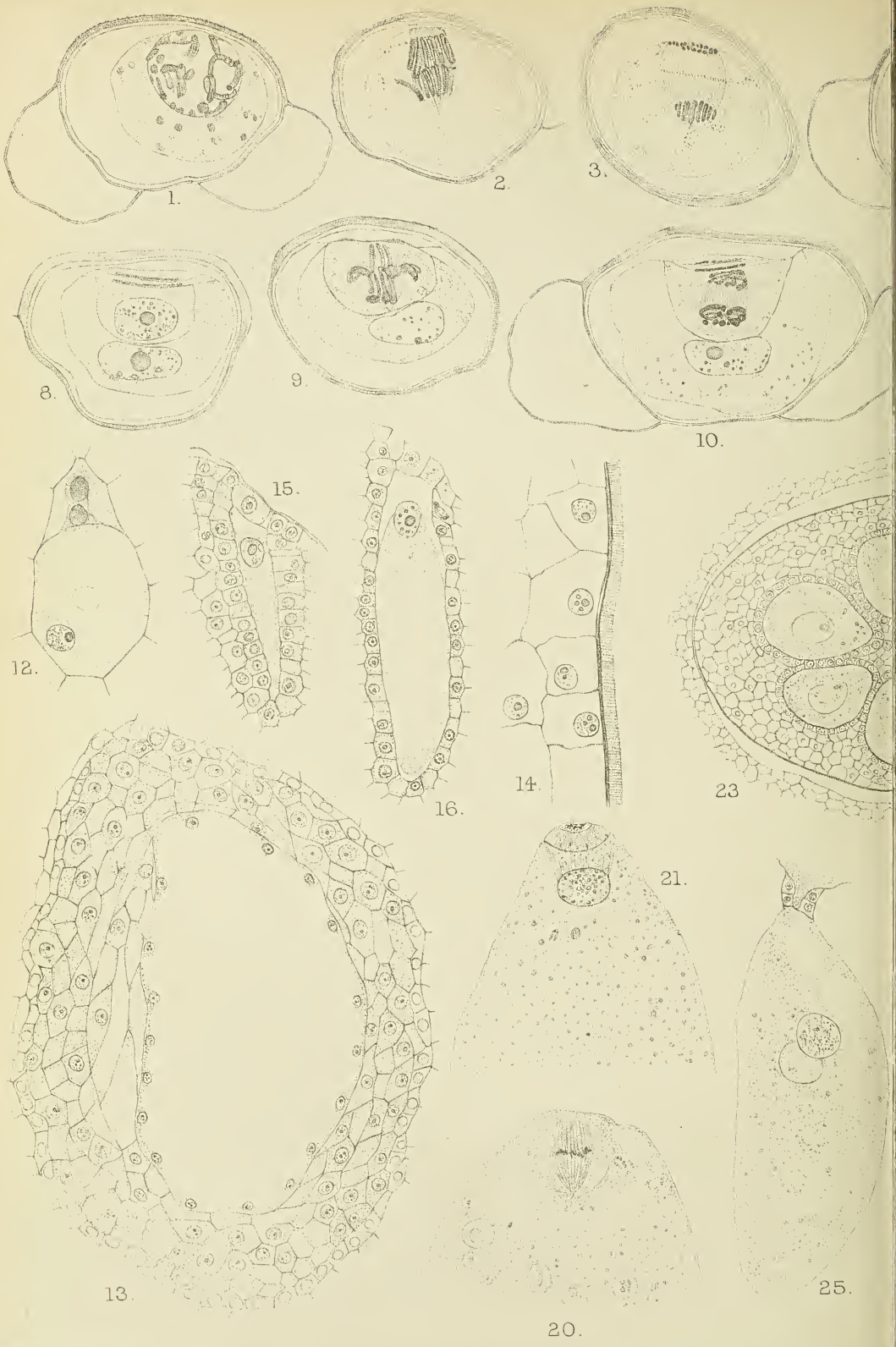
Fig. 26. A later stage, with the four free nuclei of the proembryo. × 105.

Fig. 27. The four nuclei at the base of the archegonium. Only two of them are shown. × 105.

Fig. 28. The eight-nuclear stage, showing the formation of walls between the nuclei. × 105.

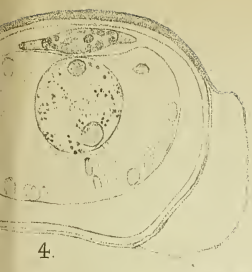
Figs. 29, 30. Formation of the proembryo is completed. × 105.

Fig. 31. A later stage, showing the elongating suspensor. × 105.



K. Miyake et K. Yasui, del.

MIYAKE & YASUI— PSEUDOLARIX.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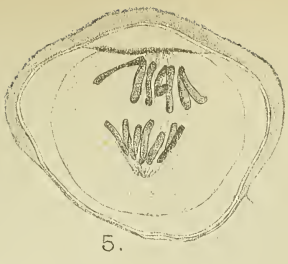
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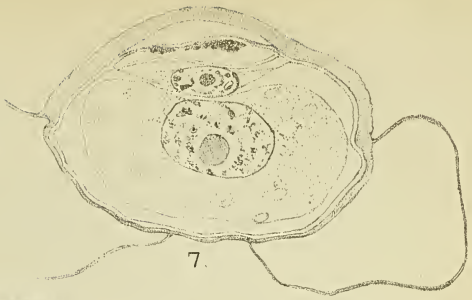
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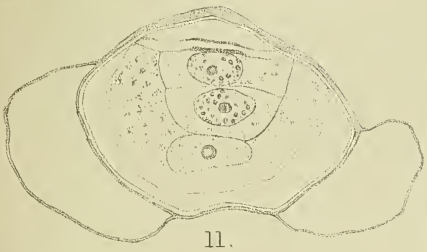
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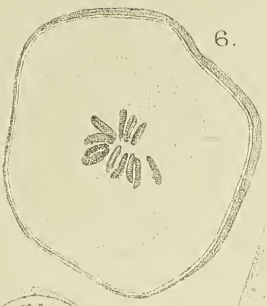
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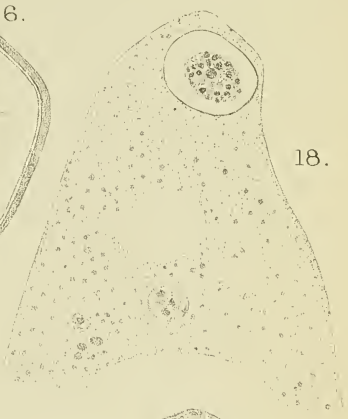
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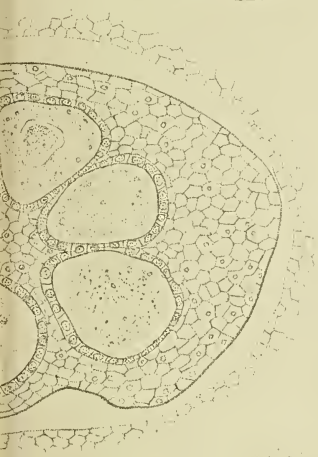


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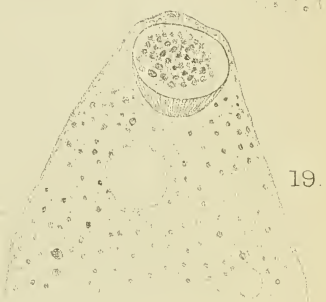
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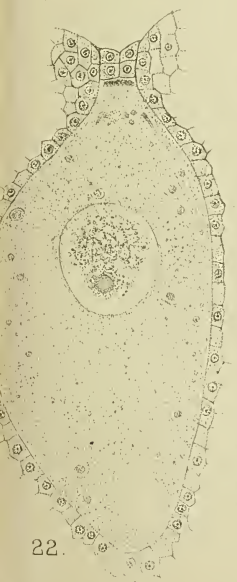
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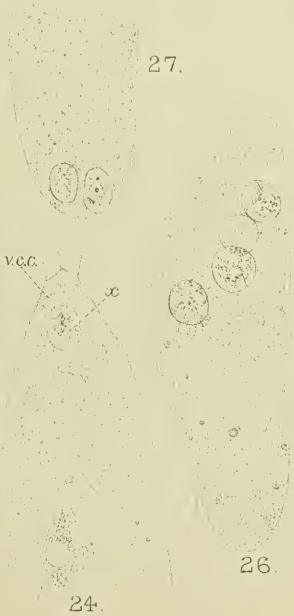
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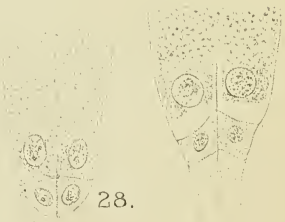
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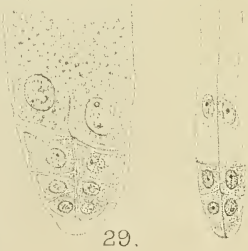
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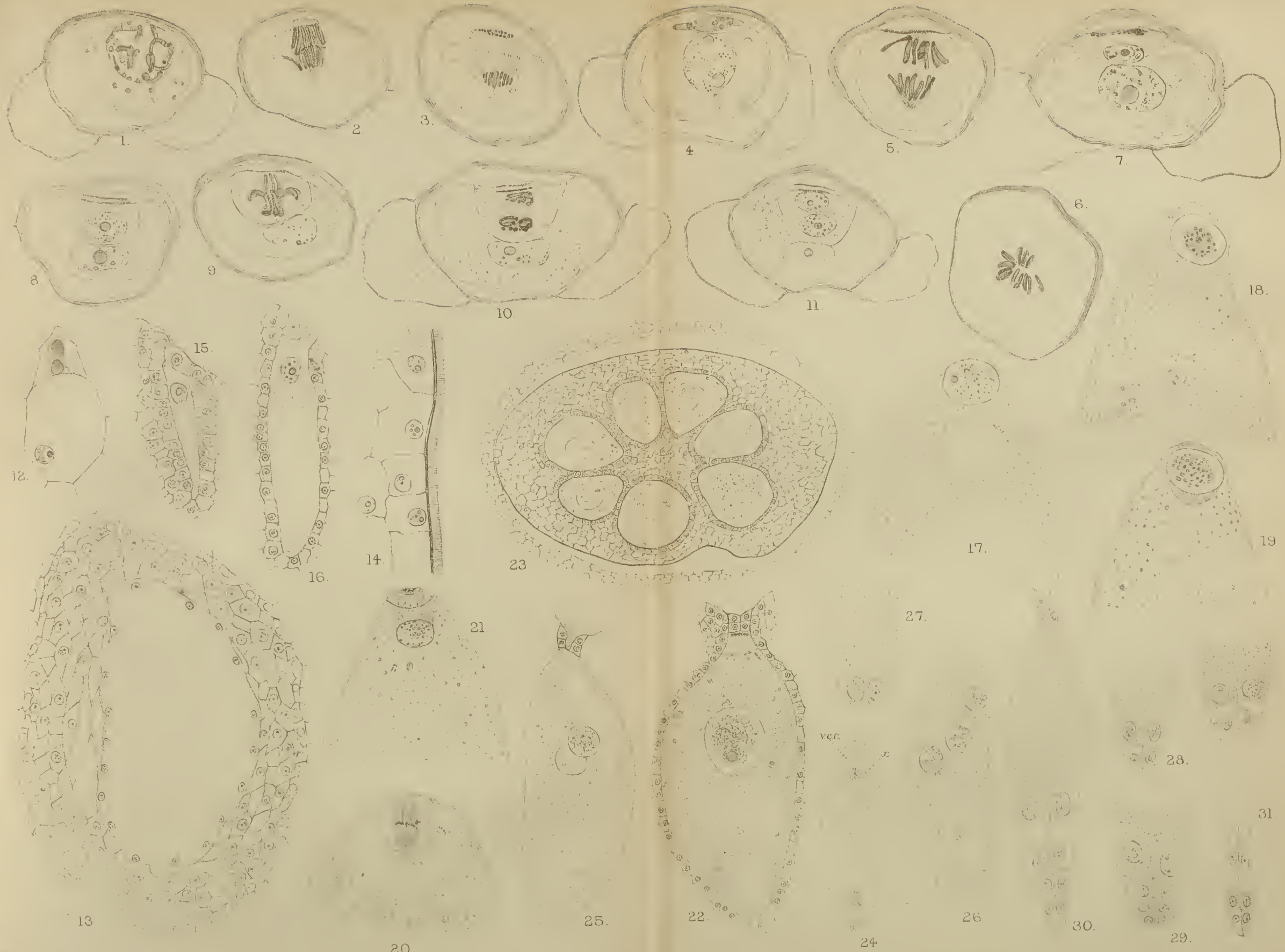
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The Cytology of the Laboulbeniales.

BY

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University of Toronto.

Introductory.

A PRELIMINARY note on the cytology of the ascus of the Laboulbeniaceae was published by the writer in 1906. Since then a considerable quantity of material has been accumulated and the earlier studies on this exceedingly interesting group have been extended to many additional forms and to the cytological phenomena of their entire life cycle. In the course of these studies the observations first recorded have been repeatedly verified, and many new facts have come to light. Several features are still under investigation. This introductory account of the researches so far accomplished will be followed by others dealing in greater detail with the topics presented below.

I wish here to express my obligations to Professor Roland Thaxter for his generous interest and kindnesses throughout, and to Professors Farlow and Thaxter for the facilities of the laboratories of Cryptogamic Botany of Harvard University so kindly placed at my disposal during several months of last year.

Spores.

The spores are uninucleate in their earliest stages. The nucleus divides once before the spore is mature, but a septum immediately effects the separation of the spore contents into two cells. In *Amorphomyces* alone of all the genera examined does this septum not form, in which case the lower of the two daughter nuclei (with reference to the orientation of the spores in the ascus) at once degenerates, so that when the spores are shed they are non-septate and contain but a single nucleus.

Thallus.

The cells of the thallus are characteristically monoenergid. After the thallus has completed its normal growth, the nucleus in the larger cells of the receptacle of certain genera may undergo one or several divisions. This

phenomenon has been noted frequently in *Ceratomyces* and *Laboulbenia*. The same thing is true of the nuclei of the richly stored inner cells of the perithecium.

The nucleus divides mitotically, not only in the young spores and thalli, but also in the older cells. An ordinary spindle is formed at each division, on which are arranged the chromosomes, and at each end of the spindle there is a central body.

The entire plant body is enclosed by a thin chitinous membrane that is exceedingly resistant to the penetration of fixing reagents. The walls of the cells are thick and laminated. Those that separate cells of common origin contain a single deep pit, interrupted only by the middle lamella. There are protoplasmic bridges, but sometimes, as in the receptacle and perithecium of *Laboulbenia chaetophora*, they are very tenuous.

The cytoplasm is finely granular or spongy-reticulate, and often contains abundant granular inclusions, or globules of oil. Very often, especially in *Laboulbenia*, one finds a more or less clearly defined organization of cytoplasmic strands bearing a definite relation to the pits or to the nuclei.

The Antheridia.

The antheridia, as is well known, are of two general types—*exogenous*, as in *Zodiomyces*, and *endogenous*, as in *Laboulbenia*. The endogenous are again of two sub-types—simple, as in *Stigmatomyces*, and compound, as in *Dichomyces*. The antheridia are usually borne along with the female organs on the same plant, but in *Amorphomyces* and a few others the sexes are separate.

Exogenous antheridia were studied in *Rhyncophoromyces* and *Zodiomyces*. They were found to be uninucleate. The spermatia are formed as short lateral branches at the upper end of the antheridia, and they, too, are uninucleate. There are indications, in my preparations of *Rhyncophoromyces*, that the spermatial rods, as soon as they drop off, are replaced by the outgrowth of others from the same base, in which case the antheridial nucleus must undergo repeated divisions in order to supply each spermatium in turn.

Simple endogenous antheridia were studied in *Stigmatomyces*, *Dioichomyces*, *Amorphomyces*, and *Laboulbenia*. The antheridium is invariably occupied by a single nucleus. Prior to the formation of a spermatium the nucleus divides mitotically, and the chromatin destined for the upper daughter nucleus, the nucleus of the male element, is literally pushed during the telophase stage of division up to the mouth of the efferent tube by the remarkably orientated and elongated spindle fibres. This phenomenon is regularly repeated at the birth of each spermatium. The spermatia contain a relatively large nucleus and a small amount of cytoplasm, and appear to be covered by nothing more than a thin protoplasmic membrane.

The compound antheridia of three genera were examined—*Dichomyces*, *Dimorphomyces*, and *Enarthromyces*. The nucleation observed was seen to differ in no way from that of the simple antheridia. The nature of the common chamber into which the efferent passages of the individual antheridia open was not determined. But an examination of specimens of *Enarthromyces* kindly loaned me by Professor Thaxter seems to show that they are, in this form at least, modified cells. It is possible, too, that the compound antheridium has originated quite independently more than once in the course of the evolution of the forms bearing simple antheridia, and in different ways, that is, on the presumption that the latter is the more primitive type.

The Procarp.

The procarp of several genera has been examined, but that of *Dioichomyces*, *Amorphomyces*, *Stigmatomyces*, and *Laboulbenia* has received most careful attention. There are many difficulties attendant on the elucidation of the nuclear phenomena, so that except in the case of *Laboulbenia chaetophora* I am not yet in a position to state with sufficient positiveness the origin of the pair of nuclei found eventually in the carpogenic cell.

The procarp has its beginnings as a single uninucleate cell, an outgrowth from the receptacle. It develops into a structure that consists of three parts—a uninucleate carpogenic cell, a uninucleate trichophoric cell, and a trichogyne. The trichogyne, as in *Stigmatomyces*, is unicellular and uninucleate, or, as in several species of *Laboulbenia*, is a more or less highly branched and septated organ. About the time the carpogonium becomes binucleate the trichogyne withers, and then the trichophoric cell begins to degenerate. I have seen spermatia attached to trichogynes, but, as yet, have not been able to demonstrate their entrance into or fusion with them, possibly on account of the dense stain taken by the receptive cells of the trichogyne at this stage. Nor have I been able to detect a nucleus migrating down through the trichophoric cell.

The carpogenic cell does acquire a second nucleus, and the ascogenic cells, too, in all the genera that have come under observation, are binucleate. In some forms the intermediate stages have been followed more or less completely, showing that the binucleate condition is maintained. I have seen no evidence of a nuclear fusion in the carpogenic cell or ascogonium, though the possibility of the occurrence of such a fusion is not precluded.

As for *Laboulbenia chaetophora*, it is possible to give a practically complete account of its developmental sequence. This species, like its near relative *L. Gyrinidarum*, is interesting because of the lack of antheridia, or of any organs that might function as antheridia. The trichophoric and carpogenic cells are uninucleate at the outset. The nucleus of the latter very soon divides, and one daughter passes to the lower end of the cell,

which is subsequently cut off as the inferior supporting cell, the other to the upper end. The nucleus of the trichophoric cell now moves down next to the carpogenic cell, and divides mitotically. The membrane separating the two cells at this time disappears. One daughter of the trichophoric nucleus next moves back to the earlier position occupied by the mother nucleus, and is later cut off by a septum ; the other remains with the upper nucleus of the carpogenic cell. This pair now undergo division. A transverse partition then cuts the carpogonium into a binucleate superior supporting cell and a binucleate ascogonium. Presumably the nuclei in the ascogonium again divide, to supply a binucleate secondary inferior supporting cell which is cut off from the lower end of the ascogonium. I cannot speak with certainty of the nucleation of the supporting cells in all instances. There may be variations such as have been found to be the case in the cells adjoining the ascogenic cells in many other Ascomycetes. The binucleate ascogonium now usually divides by a nearly vertical wall into a pair of binucleate ascogenic cells, but may at once, without dividing, begin to bud off asci.

The Ascus.

The two nuclei of an ascogenic cell divide simultaneously and mitotically at the advent of each ascus. A daughter of each passes into the young ascus, where they fuse. The definitive or fusion nucleus of the ascus now enters on a long period of growth, and finally attains a large size. Eventually it undergoes three successive mitoses. The first exhibits clearly the phenomena said to be characteristic of meiosis, except that neither here nor in the two subsequent divisions is there any change in the number of chromosomes. The central body is present and lies outside the nuclear membrane. In the prophases of mitosis its relation to the chromosomes is very evident. The second division follows rapidly on the first, but the third takes place more leisurely.

Directly after the third division spore formation begins, which differs in no respect from the method characteristic of sac fungi. The astral rays are thin, long, and quite conspicuous, and are to be seen within the spore for some time after its delimitation. They do not unite to form the protoplasmic membrane bounding the young spore, and, apparently unmodified, have been observed in some cases twisted about the long neck of the nucleus while the central body of the latter was still in connexion with the spore membrane. The cavities in which the spores lie are also lined by what appears to be a protoplasmic membrane. The spores of all the forms examined, with the exception of *Amorphomyces*, as already noted, become two-celled.

Four spores only are formed, four nuclei of the last division passing to the apex of the ascus, where they function no further, and finally disintegrate. The functional nuclei, in *Laboulbenia chaetophora* at least, are the

lower ones on the spindles of the last mitosis. There are some reasons for believing that the same is true of *Amorphomyces* and *Dioichomyces*, in which case sexual differentiation of the spores might be determined in the second division.

Relation to Host, and other Factors.

The foot of such forms as *Laboulbenia Gyrinidarum* and *L. chaetophora* lies closely appressed on the surface of the host, but does not penetrate its chitinous covering or produce any visible changes in its underlying tissues. This is not true of all, however, as, for example, of *Stigmatomyces*, *Dimeromyces*, and *Dioichomyces*. In the last there is a distinct hypertrophy of the tissues in the neighbourhood of the point of attachment of the fungus, and in the two former there is, especially under the older and larger tufts, often a disorganization of a more or less extensive patch of cells, which sometimes extends through the entire body wall. I have not had the opportunity of examining any species possessing rhizoids.

Whatever part the appendages play in the life of the fungus, that is to say, as to whether or not they act to a certain extent as absorbing organs, it may be stated here that their cells are well nourished throughout, exhibit some power of regeneration, and do not show signs of senescence often seen in the larger cells of the receptacle.

The members of several species—for example, of *Stigmatomyces* and *Dioichomyces*—lie flat against the hosts in their earlier stages of development. At maturity, however, the stalk cell of the receptacle bends so as to bring the plant into a position approximately at right angles to its former one. The young plants thus appear to enjoy the advantages of greater security against desiccation, and in the later stage spore dissemination is facilitated.

The moisture relation also exhibits itself in the relative thickness and permeability of the chitinous general envelope. In *Zodiomyces* and other aquatics this membrane is usually much thinner and more permeable than in terrestrial forms. The same may be said of species that grow on the under side of the body in comparison with those that grow in more exposed situations. This fact may be readily confirmed by reference to species of *Dioichomyces*, two or three of which are often to be found on the same insect, or to individuals of a species not restricted to a limited position on the host.

Theoretical.

One of the most obvious conclusions to be drawn from a cytological study of the Laboulbeniales is the fact of their evident Ascomycetous nature. The spore sac has quite the same right to be designated an *ascus* as has the spore sac of the most typical Ascomycetes. As to their place

among the Ascomycetes, if the possession of a perithecium is the one distinctive character of the Pyrenomycetes, then there is no good reason why the Laboulbeniales should not constitute a well-marked subdivision of this order.

The resemblance of the procarp to that of the Florideae, on the one hand, and to that of certain Fungi on the other, has long been recognized. Cytologically, it stands nearer the latter. The phenomena of conjugate nuclear division, and in *L. chaetophora* of a reduced type of sexuality, suggest similar phenomena in the rusts and certain Ascomycetes.

The nuclear phenomena in the antheridia appear to be of far-reaching import. The gap between the exogenous and the endogenous types is bridged by such a form as *Coreomyces*. Such features as a uninucleate antheridium, the possibility of proliferation of spermatia from the same antheridium, and the exogenous type of spermatium organization, suggest similar phenomena in the rusts, many Ascomycetes, and the Florideae. But further investigations are needed on the antheridia of all of these groups.

On the Histological Relations between Cuscuta and its Host.

BY

MARY G. THODAY (SYKES),

Girton College, Fellow of Newnham College, Cambridge.

With Plates XLIX–LI.

Introductory.

THE questions concerning the origin of protoplasmic connecting threads in plant tissues have long excited much interest. At present we are confronted with two main conflicting theories, namely, that of Strasburger,[1] who has hitherto maintained that connecting threads owe their origin to the boring out of the cell-walls by the protoplasm (the formation of 'plasmodesmic pseudopodia'), and that held by Gardiner[2] and previously suggested independently by Russow[2] and Kienitz-Gerloff[2], who regard connecting threads as derived primarily from spindle fibres, and as therefore only present when cell-wall formation follows on nuclear division. It is obvious that if we knew whether connecting threads are ever formed between cells or tissues which are not genetically continuous, we should be a step further towards a decision between these theories. On this point a large amount of evidence will no doubt be necessary, but it occurred to me that a good beginning might be made by a careful histological investigation of the haustoria of parasites, in order to determine whether sieve plates are ever formed in compound walls separating the cells of the host from those of the parasite. The work of previous authors[3] on parasite haustoria tends to support the former of the two theories, but recent improvement in histological methods now makes more minute examination possible.

Material of *Lathraea squamaria* and a species of *Orobanche* was preserved and examined, but both were found to be eminently unsuited to this kind of histological work. Various species of *Cuscuta* growing on

[1] Strasburger, 1901 and 1902.
[2] Gardiner, 1900 and 1907; Russow, 1883; Kienitz-Gerloff, 1891.
[3] Peirce, 1893 and 1894; Strasburger, 1901.

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various hosts were also studied; of these *Cuscuta europaea* on the Hop, *Cuscuta reflexa* on *Begonia* and *Ficus*, and an unnamed variety growing on *Solidago* were not found to be very useful; the best material was obtained from *Cuscuta europaea* growing on *Vitis*, and *Cuscuta reflexa* growing on *Salvia* sp. The former, growing out of doors in the Cambridge University Botanic Garden, was collected in July and October, the latter in June and July from a plant growing in the greenhouse.

Most of the more important results were obtained from *Cuscuta reflexa*, a perennial species growing on *Salvia*, and as the histology of the phloem in the latter genus had not been studied, it became necessary in the course of the work to make some investigations on its sieve tubes; these are recorded in the first part of the paper, while the second part deals with the histology of the parasite *Cuscuta*, particularly with the development of its sieve tubes. The investigation of the phloem of these two plants is of some interest in itself, since it is the first time that the phloem of so small a plant as *Salvia* or that of a parasite has been studied by recent methods. In the third part, which is of more interest to the general reader, an attempt is made to give some account of the manner in which the haustorium penetrates the host, and deals especially with the origin of the sieve tubes in the haustorium and the development of connexions with the sieve tubes of the host. The facts brought to light afford important evidence both on the value of sieve tubes for conduction, and on the efficiency of sieve plates; the paper closes with a short discussion of these matters, and of the conflicting theories concerning the nature and origin of connecting threads.

I am indebted to Mr. Lynch for kindly aiding me in obtaining material. The work for this paper was begun in the laboratory of the Royal Holloway College, but was chiefly carried out at the Botany School, Cambridge, while holding a Fellowship at Newnham College.

Methods.

The methods used for preservation and staining were based on those used by Gardiner,[1] Hill,[2] and the author[3] in previous work. The material found best for the minute histological work was preserved in a solution of 0·5 gramme iodine in 0·75 gramme potassium iodide in 100 c.c. of distilled water. The results obtained by the safranin method without a wall stain were confirmed by a study of paraffin microtomed sections stained in ruthenium red, chlor-zinc-iodine, or Delafield's haematoxylin; in some cases the actual sections used for the examination of sieve plates, &c. (and stained in safranin, aniline blue,[4] and London blue), were afterwards placed in Delafield's haematoxylin, so that the relations of the walls might be

[1] Gardiner, 1898 *a* and *b*. [2] Hill, 1901 and 1908. [3] Sykes, 1908.
[4] Sykes, p. 298, l. c. [Water blue (Hofmann's blue) made up with aniline.]

more clearly understood. The sieve plates could still be located, and the callus could still be stained; it was thus possible to obtain reciprocal preparations of the same element, in the first place with protoplasmic connexions and slime strings well shown, in the second with the walls stained (see Figs. 76–8, Pl. LI).

I. Notes on the Histology of Salvia (sp.?) (perennial).

(*a*) The sieve tubes of *Salvia* are found to resemble closely those of *Vitis*[1] in the general outlines of their development. The small size of the narrow terminal sieve plates makes them difficult to investigate, but it has been possible to make out sufficient of their structure for the present purpose, and it is interesting to find so close a resemblance between the sieve tubes of the Vine and other large plants heretofore examined, and those of a small plant like *Salvia*.

Fig. 1, Pl. XLIX, represents a young *terminal sieve plate* in section; it is seen to be unpitted and traversed by a number of fine protoplasmic threads, on some of which a minute swelling is visible at the middle lamella. On the upper surface of the plate faint specks of callus have already appeared at the ends of the threads. The extension of the callus change is seen in the terminal sieve plate in Fig. 2, where each thread is enclosed in a callus rod. Figs. 3, 4, 5, represent other sieve plates in the same and later stages.[2] Surface views at these stages showed each string to be in the centre of a hole traversing the plate and surrounded by a ring of callus. Shallow pits are usually formed in the young sieve plates, but are not universally present even in the quite mature plate. When present, each pit is always occupied by a single thread or slime string, the method of development being that of Hill's second type.[3]

All the older sieve tubes in this material were in the stage shown in Fig. 6, where a large mass of callus is seen on either side of the plate.

Lateral sieve plates were rare; they appeared to resemble the terminal sieve plates in all particulars (Figs. 4 and 8). Fig. 7 represents a lateral plate in surface view; the ends of the callus rods are arranged in groups, and the groups are distributed over the plate, collected together in well-defined areas. In one lateral sieve plate (Fig. 8) median nodes were clearly seen separating the two halves of the callus rod; these were also present in the terminal sieve plates, but are not shown in any of the figures.

The *sieve fields* are rather more abundant in the tangential than in the radial walls.[4] In surface view numerous elliptical depressions are seen in the wall; each depression is a shallow pit, the membrane of which is traversed by numerous fine threads (Figs. 10 and 11). Each thread is bored out and transformed into a slime string enclosed in a separate callus

[1] Hill, 1908.
[2] See description of plates.
[3] Hill, 1908, p. 274, Text-figs. 10, 11, 12.
[4] Cf. ibid., p. 268.

rod, and a median node is often visible at the middle lamella (Fig. 9, Pl. XLIX). In many cases each depression becomes a compound pit, and the group of callus rods is separated into smaller groups, each group in a small pit, or even each separate rod in a pit of its own (Fig. 9). Callus is deposited later over the heads of the groups (Figs. 10 and 12) and may occur in considerable quantity in the old tubes (Fig. 13).

The sieve fields of *Salvia* are of especial interest in connexion with the current research, since it is found that it is with these sieve fields that the parasitic cells form a junction in order to obtain food materials from their host. No cases of connexion by means of the terminal, and few by lateral, sieve plates have been demonstrated.

(*b*) My preparations contained numerous well-stained examples of the developing pits in the walls of both the pericyclic fibres and the lignified elements of the xylem. Three such pits in a young pitted vessel are shown in section in Fig. 14; groups of threads are seen to traverse the pit-closing membrane, connecting the pit fillings. In Fig. 15 the pit-closing membranes are seen in surface view, each traversed by a small number of threads.

II. On the Histology of Cuscuta.

(*a*) The walls of the *cortical cells* of all the species of *Cuscuta* examined were traversed by numerous connecting threads, generally arranged in small groups, each group being confined to a pit, and containing some 6–20 threads, or fewer in the case of the transverse walls. In many cases the pits were again divided into smaller pits (Figs. 16, 17, 18).

(*b*) The *sieve tubes* of several species of *Cuscuta* have been to some extent described by Mirande,[1] and my investigations have confirmed his account of their gross mature structure as he saw it when they were isolated by maceration. The sieve tubes of *Cuscuta* are much larger than those of *Salvia*.

As in *Salvia*, however, the development of the sieve plates, in the two species which I studied carefully, agrees in all essentials with Hill's description of the development of the sieve plates in *Vitis*,[2] and is also closely comparable with that of the sieves in the sieve tubes of *Laminaria*.[3] Except when otherwise stated the following account, and also the greater number of the figures, are obtained from *Cuscuta reflexa*.[4]

The terminal sieve plates. In the two species examined the terminal sieve plates are simple, each pit containing a single thread or callus rod; groups of threads or callus rods in each pit are, however, occasionally found in the terminal plates of the sieve tubes in the haustorium of *Cuscuta europaea* on the Vine.

[1] Mirande, 1898. [2] Hill, 1908. [3] Sykes, 1908.

[4] According to Solereder, 1908, pp. 1003–5, there are sieve plates in the longitudinal walls of the sieve tubes of the Monostyleae only. But I have found no difference in this respect between *C. reflexa* (Monostyleae) and *C. europaea* (Distyleae).

In the youngest stage the transverse wall of the young sieve tube is traversed by a number of faintly staining threads, on which a median thickening is sometimes visible.[1] The wall is commonly unpitted. In Fig. 19 the pitting is more marked than is usual in so young a transverse wall, and the threads are exceptionally crowded. The threads soon stain more darkly, and at the same time specks of callus appear at their ends, very speedily spreading along them (Fig. 20). Owing to the small dimensions of the plates, it is difficult to follow the details of this process, but it can be seen that at the next stage each thread stains very darkly with protoplasmic stains, and is enclosed in a tube of callus. In a few cases a median nodule[2] has been distinguished on the callus rod, which is thus seen to be separated into two plugs. (Cf. Fig. 20 B.) Even at this stage the sieve plate is only very slightly pitted; as a rule a single thread or slime string is found in each pit. Fig. 21 shows groups of strings occurring together in small depressions, and is taken from a sieve tube of *C. europaea.*

The formation of callus next spreads over the region in between the pits, and the sieve plate is now fully mature (Figs. 22 and 23). In surface view the perforations are easily demonstrated, each pore being lined with callus and traversed by a single slime string (Figs. 25 and 26). In the material of *C. europaea*, preserved out of doors in October, some accumulation of callus had taken place over the sieve plates, but very little was present in greenhouse material of *C. reflexa* in October (Figs. 24 and 27). In *C. reflexa* growing on *Ficus* in the greenhouse in November, large masses of callus had accumulated. Mirande[3] has also figured still larger masses of callus in other species.

Lateral walls of the sieve tubes. Lateral sieve plates were only rarely found; in them all the phases in the development of slime strings and callus rods appear to be similar to those in the terminal sieve plate. Fig. 27 represents a lateral sieve plate, against which a large accumulation of callus has taken place.[4] Short strands of cells with sieve plates on their transverse walls are occasionally found connecting two strands of sieve tubes; such a connecting element is shown in Fig. 28.

Groups of threads appear to be equally numerous in the tangential and radial walls of the young sieve tubes.[5] Sometimes each group contains very few threads, usually in a small pit (Fig. 30, *C. europaeus*); sometimes a large group stretches across the sieve-tube wall, forming a wider 'sieve field' (Fig. 29, *C. reflexa*).

The development of the threads in the lateral walls, each into a slime

[1] Cf. Hill, *a* and *b*, 1901; 1908. Sykes, 1908.

[2] Cf. Hill, 1901 *b*, Figs. 20 and 21, Pl. XXXIII; 1908, Figs. 34, &c., Pl. XVIII.

[3] Mirande, p. 88 and Fig. 7.

[4] From *C. europaeus* on *Vitis*, October; cf. Peirce, 1893, p. 294.

[5] For a more detailed description of the distribution of pores in the lateral walls, see Mirande, pp. 85–7, and Figs. 6 and 7.

string enclosed in its callus rod, is similar to the development of the threads in the terminal sieve plate; the only difference is that the perforations are here much finer.

Each fully formed slime string thus becomes enclosed in a very minute callus rod which is only very slightly bored out (Figs. 30 A and 37 A, Pl. XLIX).

The median thickenings are found to be peculiarly conspicuous on the young threads in the lateral walls (cf. Figs. 19 and 31); a median node is also often clearly visible on the mature callus rod (Figs. 32 and 39). In Fig. 31 A a very early stage of callus formation is seen, and in one group the change has proceeded as far as the middle lamella from one side, but has not taken place at all on the other side of the middle lamella.[1]

In the walls of the sieve tubes which abut on a companion cell, numerous small pits usually occur, and in these pits groups of threads are found. In many cases that half of the thread which is on the sieve-tube side of the wall has undergone development into a short slime string enclosed in a callus rod, while the half on the side of the wall which belongs to the companion cell has remained unchanged[2] (Figs. 19, 24, 41, and 42).

In the summer material very small accumulations of callus were sometimes formed over the pits in the lateral walls, but in the material preserved in October much larger masses were present, which often extended over a considerable portion of the intervening wall. Figs. 35 and 39 represent large groups of slime strings in the lateral walls of two sieve tubes, over which small pads of callus have been deposited; Fig. 38 shows in surface view a wall in which many small groups occur, and a small pad of callus has been deposited over each group; Fig. 40 represents a wall inside which the callus forms an almost continuous layer. The pencilled lines in the blue callus indicate the 'paths' above the slime strings, through which continuity with the pores is for a long time maintained.[3]

Sieve tubes of the haustorium. The sieve tubes of the haustorium are composed of very short elements, and sieves are numerous in all their walls. It appears that any parenchymatous cell may become organized as a sieve tube, but companion cells are by no means universal.

The histology of the haustorial sieve tubes corresponds in nearly all respects with that of the sieve tubes of the main stem. It has already been mentioned that the threads or slime strings in the transverse plates are commonly arranged in groups. As a rule, the callus rods traverse the entire thickness of the wall which separates the phloem elements. Callus appears to be formed earlier in the haustorium than in the main stem; in *Cuscuta reflexa* large masses of callus were already

[1] Hill, 1901 *b*, Fig. 18, Pl. XXXIII, and p. 589; Hill, 1908, Figs. 23, 24, &c., Pl. XVII, and pp. 281, 367; Sykes, 1908, Figs. 14 and 15, Pl. XIX, and pp. 307, 316.

[2] Hill, 1901, pp. 600–1; 1908, pp. 279–80; Sykes, 1908, p. 317.

[3] Hill, 1901; 1908.

present in the haustorium in October, but were not formed in the sieve tubes of the main stem till November (Figs. 44 and 45). On the transverse plates the callus is sometimes nearly all collected on the parasite side of the plate (Fig. 45) ; in other cases, a continuous layer of callus lines all the walls of the elements of a sieve tube of the haustorium. Here, as in other cases, the callus is apparently deposited by the protoplasm.[1]

It is interesting to find that the haustorial sieve tubes do not terminate at the level opposite the phloem of the host, but are continued down the axis of the haustorium into the host pith (*C. reflexa* on *Begonia*, *C.* sp. on *Solidago*, &c.).

Some attempt was made to determine the number of threads present in the longitudinal walls of a short young sieve tube of the haustorium (Fig. 43). The two groups, A and B, were made up of 11 and 16 threads respectively. The two groups together measured 11·2 μ across, the whole cell being 24·4 μ across at this point and 64·4 μ in length. From these numbers some estimation may be made of the large number of threads present in the wall of such a cell.

III. On the Relations between Host and Parasite.

A. *Outlines of the Development of the Haustorium.*

The broad outlines of the development of the young haustorium in *Cuscuta* and its manner of penetrating the host have been described to some extent by Peirce.[2] A sucker-like organ first arises from the epidermis of the mother stem and adheres firmly to the host plant; its formation is then followed by the ingrowth of the true haustorium, which has originated endogenously, mainly from the cortical region just outside the pericycle, and seems very properly regarded as morphologically an adventitious root.[3] The cells of the sucker, or 'prehaustorium', dissolve their way into the host plant, partly by pressure, partly by the excretion of a ferment,[4] and into the space thus made the haustorium grows, enlarging the opening and becoming surrounded by a mass of compacted dead cell-walls.

The haustorium grows at its tip and finally becomes connected with the bundles of the host plant, its method of growth, either continuing in a straight line towards a single host bundle, or branching into two parts which diverge to join more than one host bundle, varying with the arrangement and size of the bundles of the host.[5] These variations in form do not concern us here.

The following more detailed account is derived in great part from

[1] Hill, 1901 *b*, pp. 597-600; 1908, pp. 277-8; Sykes, 1908, pp. 316-17.

[2] Peirce, 1893, pp. 295 ff., and 1894, pp. 99 ff.

[3] Ibid., 1893, p. 305.

[4] Ibid., 1894, pp. 99-106; the invading cells were found to have the power of corroding starch grains.

[5] See ibid., 1893.

C. reflexa parasitic upon *Salvia*, with corroborative evidence from *C. europaea* on *Vitis*. These hosts were found to be the most favourable for histological work; on the one hand their cell-walls are not so thin or their tissues so parenchymatous as, e. g., in *Begonia* or *Impatiens*, and on the other hand their cortex and pith are fairly free from the numerous crystals, fibres, stone cells, &c., which make the use of the freezing microtome so difficult with, e. g., the hop. In both cases, too, the compacted mass of dead cell-walls which at an early stage surrounds the haustorium is very soon absorbed; in some hosts attacked by *Cuscuta* they are hardened, and form an almost insuperable barrier to histological investigation.[1]

The ingrowth of the haustorium is mainly due to the superficial cells covering its tip, which undergo a remarkable series of changes. They become greatly elongated,[2] and each forms a hypha-like element of very large dimensions. They have all the features characteristic of secretory cells: their walls are at first very thin, especially at the tip; their protoplasmic contents are very dense, and their nuclei are exceedingly large. In many cases the diameter of the nucleus of an invading hypha was found to be greater than the diameter of the whole cell invaded.

Cell-division occurs at first chiefly in the cells immediately behind the hyphal cells,[3] but as the tips of the hyphae grow inwards their hinder portions become also subdivided, their elongated apices remaining, however, unicellular throughout all the young and growing stages. As they extend further into the host plant it is common to find, in the tip of a single hypha, from two to five of the large characteristic nuclei (Figs. 46 and 47, Pl. XLIX); these unicellular and multinucleate elements may branch at an early stage, but it is not until later that they become subdivided transversely and longitudinally almost up to the tip, each of the nuclei then being surrounded by a cell-wall.

From each hypha a strand of cells is thus developed; the further development of the different strands depends on their position with regard to the tissues of the host. The end of the invading haustorium in the cortex of the host may be compared with a brush of hairs, separated somewhat from one another in the early stages. In *C. reflexa* on *Salvia* and in *C. europaea* on *Vitis* the central hairs (hyphae) push straight through the vascular cylinder into the thick-walled pith of the host; they do not become subdivided for some distance from the tip, and generally remain small and undifferentiated. In a few cases, however, strands of sieve tubes were found to be developed from them even in *C. reflexa* in the empty, innutritious

[1] This is still more strikingly the case in plants attacked by *Lathraea squamaria*, where the mass of dead cell-walls stains with lignin stains.

[2] Cf. Peirce, 1893, esp. Figs. 15, 16, 18, Pl. XIV; Heinricher, 1894 (*Lathraea*), p. 325, Fig. 1, Pl. IX, and Fig. 1, Pl. X; Solms Laubach, 1868, Figs. 2, 5, 6, Taf. XXXV; Leclerc du Sablon, 1894 (*Melampyrum*), Figs. 3, 5, &c., Pl. I.

[3] See Peirce, p. 304, &c., and Fig. 5 G, Pl. XIII.

pith of *Salvia*,[1] and such strands were quite common in *C. reflexa* in the juicy pith of *Begonia*.

The hyphae immediately surrounding the central core become applied to the xylem elements of the host; the strands of cells to which these hyphae give rise become organized as strands of reticulately pitted tracheides and are connected with the xylem of the main stem of the parasite. Concerning the details of the development of the xylem connexions I have little to say; Peirce has given some descriptions of the mature state in his paper, and my observations, as far as they go, do not differ from his to any important extent. The hyphae apply themselves firmly to the xylem elements of the host; and the thin walls at their tips, which are at first undifferentiated, later become lignified, thick and thin areas being developed which correspond to the thick and thin areas of the host wall.

The peripheral hyphae of the haustorium (outer hairs of the brush) do not extend so far into the host plant, but many of them push their way straight through the older layers of the host phloem (Fig. 60, Pl. L, is an example of a parasite hypha seen boring through a host sieve tube), and, making for the functional and developing sieve tubes from which the most nutriment can be obtained, become applied to their walls and form intimate connexions with them. It is generally found that in the phloem the haustorium expands considerably (especially in *C. europaea* on *Vitis* and *C.* sp.? on *Solidago*). At the surface of the host the haustorium is circular in section; it becomes oval or lozenge shaped in the cortex of the host, the long axis of the oval being parallel to the length of the host stem. When it reaches the phloem it expands laterally and may again become roughly circular in section. Contact is thus made with a large number of sieve tubes outside the region originally pierced by the shaft of the haustorium. When a connexion has been made, the applied hypha becomes subdivided to form a strand of cells from which a string of short sieve tubes is developed; these are connected by other short sieve tubes in the shaft of the haustorium with the phloem in the parasite stem (Figs. 62–4). It is the development of the phloem connexions and of the strings of sieve tubes with which I am mainly concerned.

Some of the separate hyphae on the outer edge of the brush have got no further than the cortex, others have pierced a little deeper, but only as far as the old disused sieve tubes. It is not possible to determine how far these have still the power to work further into the functional phloem, but it seems most likely that at a certain stage their development stops, the total number of connexions being then adequate to the needs of the parasite. Occasionally a hypha after reaching the old phloem turns back, bending sharply on itself, and returns into the cortex. Such hyphae presumably are unable to find food in the old phloem, and seek it in the

[1] Cf. also Peirce, 1893, p. 309, for development in an innutritious pith.

more nutritious cortex, where they were occasionally found applied to a secretory cell. The hyphae in the cortex are smaller and shorter and have smaller nuclei.

Finally, in the fully developed haustorium, the brush of loose hairs has become a tissue of closely compacted cells, a few on the extreme periphery of the brush alone remaining separate from one another. It must be clearly realized, however, that the tissue owes its origin to the fusion of separate strands of cells, and that therefore all the cells composing it are not genetically connected with one another. Some of the longitudinal walls have originally formed the walls of separate invading hyphae, and have become fused together later; these walls afford an excellent opportunity for the investigation of the question which forms the main object of this paper, viz. whether connecting threads are formed between non-genetically connected cells.

B. *Histology.*

We are now in a position to consider in detail the manner in which the young invading hyphae make their way through the cortex and pericycle of the host, and through the old disused sieve tubes, form connexions with the functional sieve tubes of the host, and, finally, give rise each to a strand of sieve tubes which conveys the food products absorbed from the host to the main parasite stem.

Fusion of lateral walls of invading hyphae to form a tissue. The tips of the invading hyphae are for some time separated from one another, but later, when the more advanced of the hyphae have been joined by those which lagged behind, they become closely applied to one another. Their adjoining walls are then fused together so closely that all sign of their separate origin is lost. Fig. 51 A, Pl. XLIX, represents small portions of the longitudinal walls of two adjacent hyphae near their tips. The section was stained with London blue, and the blue colour in the figures indicates the distribution of the bluish-green colour so characteristic of callus and substances like it. The inner and outer superficial layers of the walls of both hyphae were stained blue. Apparently the blue colour is characteristic of various substances produced by the hydrolysis of ordinary cellulose;[1] these, being mucilaginous, facilitate the fusion of the walls. The softening is a temporary process and is lost when its object is accomplished. In Fig. 51 B the two adjacent walls have fused, and the wavy blue line marks the still visible softening along the line of fusion. In Fig. 51 C the line of fusion no longer stains blue, but can still be clearly seen (see also F, Figs. 76, 77, 78, Pl. LI). As in Fig. 51 A, Pl. XLIX, the inner layers of the walls stain blue; in some cases even larger portions of the wall may be temporarily softened. Since the hydrolysis of the outer layer is probably affected by the ferment

[1] See Sykes, 1908, p. 315.

secreted by the protoplasm, it is easy to understand that the ferment may change other parts of the wall on its way outwards.

Just behind the fusing tips, cell-divisions are now taking place in the hyphae. Fig. 49 represents a transverse section of a hypha which has been divided into three cells; the newly formed cell-walls are packed with connecting threads, but there are no connecting threads in the outer fusion walls. *No connecting threads have ever been seen in the fusion walls separating adjacent hyphae* or adjacent strands of cells formed from these hyphae. In quite mature haustoria it is often very difficult to make out the boundaries of the original strands, but whenever the preparation is sufficiently good it is clear that, whereas sieve tube areas are common in the walls subdividing the hyphae, they are not formed on their original longitudinal walls (see Figs. 62 and 64, Pl. L; w = original wall).

These facts are perhaps the most interesting outcome of this investigation, since it would appear that here is at least one incontrovertible case in which the distribution of connecting threads is clearly coincident with the distribution of genetically connected cell-walls, and does not extend to walls associated with one another by later growth.

Behaviour of the wall at the tip of an invading hypha. It has already been stated that the tip of an invading hypha is at first covered by a thin, very soft and plastic wall with which the protoplasm is closely in contact.[1] The plasticity of the wall in this region is illustrated by Figs. 46 A and 46 B, in which hyphae are shown on their way through the walls of cortical cells; the invading element makes a clean hole in the cortical wall, and squeezes its way through the hole. It is often much contracted in this process, expanding again when it reaches the cavity of the cortical cell; later the hole is enlarged and the hypha regains its original shape. At the edges of the hole, host and parasite are almost from the first so firmly cemented together that it is difficult to ascertain at exactly what point one wall begins and the other ends.[2] In preparations stained with Delafield's haematoxylin the common portion of the wall takes the stain more deeply, and is thus to a certain extent distinguishable.

As the hyphae approach the central cylinder their end walls become conspicuously swollen and mucilaginous, and at the same time still more plastic. Fig. 50, Pl. XLIX (less magnified than Fig. 46 B), represents two adjacent hyphae passing through the pericycle; the wall of the right-hand element is slightly swollen at the tip.

The thick-walled cells of the pericycle in *Salvia* must offer some considerable resistance to the passage of the hyphae. Cases have been met

[1] Peirce also suggested that the end walls are not quite solid; 'kept in partial solution by the enzyme they are secreting,' 1894, p. 114.

[2] Cf. Peirce, 1894, pp. 113–14, and Figs. 8 and 9, Pl. VIII. It is this firm union which even at an early stage prevents the haustorium from being pulled out of the host tissues.

with again and again in which the pressure of a hypha has bulged in a thick-walled cell to the extent of causing its two longitudinal walls to touch each other, obliterating the cavity between them. Sooner or later, however, the solvent action of the hypha dissolves even the thick walls of these elements. In Fig. 52, Pl. L, are shown two invading hyphae which have penetrated for some distance into the pericycle; a portion of the wall of the last cell penetrated (F, Fig. 52) is still visible in the space between their tips. The next pericycle cells are in their turn about to succumb, and the wall of the cell on the right is already considerably bulged in, and is dissolved as far as the middle lamella.

Occasionally when an invading hypha has made an entry into the cavity of a thick-walled cell it turns and for a short time pursues its course longitudinally in the cavity of the host cell, following the path of least resistance and widening the cavity by its pressure.

The wall at the tip of an invading hypha becomes more and more swollen and mucilaginous as it penetrates the pericycle and reaches the disused phloem; the inner layers of the wall now commonly stain with London blue or water blue, and are evidently softened in the same way as has been described in the lateral walls of adjacent hyphae which are about to join with one another.[1] Occasionally even in the inner layers of the pericycle (Fig. 53) the tip of the hyphal wall is sufficiently hydrolysed to stain blue; this is of some importance, since it makes it appear certain that the blue-staining substance is the product of some activity of the hyphal cell itself (probably of a hydrolysing ferment), and is not due to an interaction between the parasite and the old sieve tubes of the host, which contain much blue-staining callus. The same conclusion is suggested by the fact that it is the *inner* layers of the wall which first undergo the change. Fig. 55 shows a very early stage in which only two minute patches of blue-staining substance have yet been produced (see also Figs. 56, 58, &c.). Only a few cases were seen in which hydrolysis in the walls of the hyphal tips had gone far enough for them to stain with water blue,[2] but the less hydrolysed state which stains with London blue was common even in hyphae in the outer zones of the old host phloem.

Sometimes the blue staining substance occurs at first only in patches (Fig. 61); later it generally extends throughout the inner layers. A thin outer layer of unstained cellulose is visible until the invading cell reaches the functional phloem (Figs. 56, 58, &c.). Then, on the edge of the functional phloem, there is a short stage in which the whole wall at the tip of the hypha stains blue. Being transient, this stage is not very often seen. Figs. 59 and 60 represent hyphae just getting to this stage; in Fig. 59 two small blue patches are seen in the outer layers as well as the large swollen mass within. Fig. 60 represents a transverse section through

[1] *Ante*, p. 664. [2] Sykes, 1908, p. 315.

the tip of a hypha making its way straight through the wall of a host sieve tube; the inner and outer layers of its wall both stain blue.

In two of the elements shown in Fig. 57, and probably also in the left-hand element in Fig. 65, the blue stain is taken by the entire thickness of the much swollen wall at the tip of the hypha.

When the hyphae have penetrated well into the functional phloem these swollen blue-staining tips are very rarely met with, though occasionally irregular masses of blue mucilaginous-looking substance are found, apparently in the cavity of the hyphae (see Figs. 62 and 67).

Formation of junctions between the tips of the hyphae and the sieve areas of the host. It is by no means an easy matter to ascertain exactly what does occur when the tip of a hypha forms a junction with a sieve area of the host. The accumulated mass of evidence seems, however, to demonstrate conclusively that *the formation of a junction is effected by the application of the naked protoplasm of the hyphal tip to a functional sieve plate or sieve field of the host.* The swollen and mucilaginous parasite wall is entirely dissolved away in the region immediately over the sieve area.

Before proceeding to give the evidence on which this conclusion is based it may be well to state that it is opposed to that reached by Peirce and Strasburger. Peirce[1] demonstrated the fact that the haustorium forms junctions with the phloem as well as the xylem of the host; having described the sieve plates between host and parasite, and having seen callus on either side of their plates, he stated his view that half of the 'compound' sieve plate is derived from the host wall and half from that of the parasite. Strasburger[2] came to a similar conclusion. He could not demonstrate any simple protoplasmic connexions between other cells of the host and parasite, a fact which he attributes to absence of near relationship between the two; he states, however, that open communication is established in the pores of the compound sieve plates, and he enumerates the facts among other evidence possibly in favour of his view that protoplasmic communication may be established between non-genetically connected tissues.

When the junction sieve areas are carefully examined by suitable methods, the most obvious fact concerning them is that *they resemble exactly in appearance and dimensions, and in every detail, the lateral sieve plates or sieve fields already described in the respective hosts*, Salvia *and* Vitis. The junction sieve areas are certainly no thicker than those in the undisturbed host phloem, as they would almost necessarily be if they owed their origin to a fusion between the host sieve area and the thick swollen wall of the tip of the parasite hypha. The presumption is therefore that these are not

[1] Peirce, 1893, p. 301 and p. 314, Fig. 13, Pl. XIV. The account given by Peirce of his observations and the figures given by him are by no means conclusive. This is partly due to his researches having been conducted on alcoholic material. His figures by no means preclude the possibility that in the formation of the junction sieve plate, one or other of the host and parasite walls is absorbed.

[2] Strasburger, 1901, pp. 601–2.

compound sieve plates, but are derived from the host wall only, and are utilized in their original condition by the parasite for its own convenience.[1]

We are at once confronted with the question as to what is the ultimate fate of the portion of the wall of the parasite intervening between host sieve area and parasite protoplasm, since it does not form part of a 'compound sieve plate'. There are obviously two possibilities: the food material may pass through the soft and plastic terminal wall, or the wall may be dissolved and the naked protoplasm apply itself to the host sieve area.

It has already been stated that after the parasite hyphae have penetrated into the functional phloem, thick mucilaginous blue-staining tips are no longer to be found. Some change in their nature must therefore have taken place. When an invading cell is applied to a young or mature sieve plate, in nearly all cases an unstained area is found between the parasite protoplasm and the sieve plate. Since it is the essence of the methods employed in this investigation that they demonstrate protoplasm, slime strings, callus, &c., but leave the cell-wall unstained, it is not easy in such cases as Figs. 76 and 77, Pl. LI, to decide whether this unstained area (between the parasite protoplasm and the sieve area J) be wall or space. It was not found practicable to use a wall stain conjointly with safranin and London blue, &c., and in preparations in which wall stains were employed by themselves it was very difficult to locate the junction sieve areas. In a few cases, however, this method led to useful results. Fig. 72, Pl. L, is an example of a junction plate between parasite and host, stained in Delafield's haematoxylin. Between the parasite protoplasm and the sieve field there is a large unstained area (*sp*) which is here undoubtedly a space, and must have been caused by the solution of the parasite wall in this region. *x*, *x* mark the termination of the unaltered portion of parasite wall. In Figs. 76 and 77, Pl. LI, also similar knob-like endings are labelled *x*, *x* and suggested a similar interpretation even when examined without a wall stain. The same preparation was later stained in haematoxylin, and is drawn in Fig. 78, where the relations of the walls are clearly demonstrated.[2] It was then quite clear that the unstained area here also is a space, and *that the intervening parasite wall must have been absorbed.*

In Fig. 78, at R, R, are shown small remnants of the parasite wall which have apparently not been dissolved with the rest.

The formation of a space on the parasite side of the sieve area is no

[1] It appears to be always with the longitudinal wall between two sieve tubes that junctions are effected; at any rate in *Salvia* no satisfactory cases were observed in which a junction was made with the wall between a sieve tube and a companion cell; in *Vitis* there was some evidence that such junctions may occasionally be developed.

[2] In such preparations, stained with haematoxylin, after having been examined by the usual methods for demonstrating the structure of the sieve areas, the walls stain quite sharply, notwithstanding the processes employed to slightly swell them, and the termination of the parasite wall round the hole which is formed over the sieve area is perfectly clear.

doubt due to artificial shrinkage of the protoplasm. It was most pronounced in preparations which had at any stage been placed in alcohol (Fig. 72, Pl. L); it is probable that the protoplasm in this region is easily shrunk, having only recently come to occupy its position against the sieve area, consequent on the solution of its own wall. In some preparations there was hardly any sign of shrinkage, e. g. in Fig. 68.

There can be little doubt that the *mucilaginous change described at the tips of the parasite hyphae is preparatory to dissolution.* The actual disappearance of the blue mass is rapid and is difficult to detect. Fig. 71 probably represents one of the last stages in its solution, the blue line being a last remnant of the mucilaginous mass. Several other cases, none of them very clear, were also met with, in which irregular 'blobs' of callus separated the parasite protoplasm from the junction sieve area. Figs. 62 and 67 were drawn from two rather curious cases, in which a relatively large mass of blue mucilaginous substance probably represented the dissolving tip of the wall. Apparently the protoplasm had already crept past the jelly-like mass and applied itself to the junction sieve plate.

C. *Functional Efficiency of the Arrangement.*

It is probable that the orientation of the hyphae which make connexion with the host phloem is significant from the point of view of the efficiency of the junctions.

It has already been stated that the haustorium widens out when it reaches the phloem, its transverse section becoming much greater than before. As the hyphae enter the old phloem of the host they also change their direction slightly, and, instead of running in a straight line with the long axis of the haustorium, they twist a little obliquely and finally turn outwards at an angle with the long axis, thus coming into contact with sieve tubes which have not been interrupted by other ingrowing elements. In the functional and young phloem the invading hyphae turn still more, often twisting repeatedly so that their course is difficult to follow under the microscope, and they never remain long at the same focus. Finally, the tips of many of them run nearly at right angles to their original direction, that is, almost parallel with the host sieve tubes (see Figs. 59, 68, and 70, Pl. L, Figs. 77 and 78, Pl. LI, &c.). The advantage of this change of direction is clear when it is remembered that it is always with the *lateral* sieve fields (or occasionally with the lateral sieve plates) that junctions are effected. The hypha may be said to lay itself alongside the host sieve tube; it is thus able to bring as large an area as possible into contact with a sieve field. At the same time it runs the least risk of injuring the sieve-tube wall, since it avoids bringing to bear on it the terminal pressure, which we have already seen to be sufficiently great to bulge in even the thick wall of a pericycle cell.

It is not, of course, possible to be quite clear as to whether the lateral twisting of the end of the hypha always takes place before contact with a sieve area is actually made (as appears to be the case in Fig. 68, Pl. L), or whether in some cases it is after the formation of a junction has begun that the hypha continues its growth in a lateral direction in order to avoid terminal pressure and not to injure the junction plate.

However it is brought about, lateral contact is so common that it would seem probable that even the few cases in which the extreme tip appeared to be utilized, e. g. Fig. 76, Pl. LI, are probably to be accounted for by the plane of the section passing through the heel of the slipper-shaped end. Such an arrangement as is seen in Fig. 76 would be obtained by taking a section at right angles to that of the adjoining element in Fig. 77, passing through the left-hand end of the junction sieve area.

Even in the element drawn in Fig. 77 the extreme tip really extended further at another focus for some distance beyond the point shown in the diagram.

These facts naturally raise the question whether, after a junction has been formed with a sieve area in the wall of one sieve tube, the hypha ever goes on to form a junction with other sieve tubes. As my methods do not admit of cutting sections in series, it is not possible to deny absolutely that this ever happens. If it does occur, it must be only on the extreme periphery of the haustorium, where the invading elements are less closely packed together. There could, however, be no advantage to the parasite in forming junctions with a second series of sieve tubes if, in order to do so, it would be necessary to make holes in the sieve tubes already attacked, and thus probably to destroy their efficiency. It may well be that the further growth of the hyphae depends on the composition of the phloem in the host. In *Salvia* the phloem consists largely of sieve tubes, and it would thus be almost necessary after the formation of a junction to bore through a sieve tube in order to reach others. In other hosts, such as *Vitis*, where the phloem contains much parenchyma, the hypha might conceivably make its way from one sieve tube to another by way of intervening parenchymatous cells, thus to a less extent disturbing the sieve tubes. No sign of any such second junction plate has, however, been found even in *Vitis*, nor any sign of a re-formation of the dissolved portion of the wall of the hypha, nor any other adaptation to protect the naked area of parasite protoplasm, such as would probably be necessary in the neighbourhood of the disused sieve plate. Several hyphae may apply themselves laterally to the same sieve tube, but apparently each hypha only forms one such lateral connexion during its course. The arrangement would thus be similar to that prevalent among the hyphae which form junctions with the xylem. These, having made one junction, become lignified, and of course lose the power of further growth. We may conclude that the development of the invading hyphae

ceases when an adequate number of connexions has been established between host and parasite.

The formation of single lateral connexions has probably the additional advantage that it disturbs to the smallest possible extent the mechanics of the sieve tubes. It appears to be necessary that the connexion should be established with a host element whose wall is otherwise uninjured, though occasionally another element of the same tube may have been pierced by an invading hypha. No connexion has ever been found between a parasite hypha and a sieve area in the wall of an element which has been broken through by other hyphae on their way further into the host. That is to say, at least one transverse sieve plate always intervenes between a broken place in a sieve wall and a junction sieve area.

It is generally accepted that the substances translocating in the sieve tubes are under considerable pressure;[1] in consequence of this pressure their contents flow out laterally through the sieve fields as well as terminally through the sieve plates. In the special case, when the sieve tube has been interrupted by the growing shaft of the haustorium, the translocating substances will all the more attempt to find some lateral outlet, and, as in the normal case, will flow gently through the lateral sieve fields into the trap laid for them by the invading hyphae. The actual passage of food material would thus appear to be more a passive than an active process, due mainly to filtration from an enclosed space under pressure. Under these conditions a very close connexion between the parasite and host protoplasm in the pores of the sieve area would not be necessary. I have no conclusive observations to demonstrate how far the parasite protoplasm penetrates the pores of the sieve plate or establishes connexion with the slime strings of the host or with the protoplasm enclosing them. The ease, however, with which the protoplasm of the parasite shrinks away from the junction plate is very evident, and one may conclude from this fact, with some degree of probability, that the two are never very closely connected. Evidence that this connexion is, nevertheless, sufficient for all requirements has been obtained by Mr. Mangham,[2] who has seen in microchemical preparations indications of the actual passage of sugars from host to parasite.

IV. General Observations.

A. *The efficiency of sieve tubes.* The contrast between the behaviour of the parasite hyphae in the cortex and the phloem affords evidence of the efficiency of the sieve tubes and sieve areas for conduction.

[1] As shown by the work of Fischer, Sachs, &c. See Mangham, 1910, p. 265.

[2] I am indebted to Mr. Mangham for his kind permission to mention his observations. The methods employed were those indicated in his paper in Science Progress, 1911, and by means of which he obtained the results communicated to the Brit. Assoc. at Sheffield, 1910.

In the cortex the parasite hypha bores straight through the wall into the cavity of the cell, without apparently taking any account of the presence of connecting threads; in the phloem, on the other hand, it makes connexion with the unchanged sieve area. The junction is effected in such a way as to disturb as little as possible the mechanics of the sieve tube, and the hypha thus secures a long-continued supply of food.[1]

The adaptation of the unchanged sieve area to the purposes of the parasite points to the great efficiency of the multiperforate septum[2] of which the sieve area is composed. In the cortex of the host the connecting threads appear to be inadequate for bringing a supply of food to the hyphae. Even those hyphae which finally extend freely into the cortex of the host seem always to make a hole in the wall of the last cortical cell attacked. These facts may be regarded as affording further evidence in favour of the view that while connecting threads seem to be of value rather for the conduction of impulses (stimuli) than of food,[3] the slime strings of the sieve areas afford an excellent medium for the translocation of food substances.

It would be of interest to know what would occur if an invading hypha made contact with the wall between a sieve tube and a companion cell of the host, i. e. with a wall crossed by connecting threads bored out only on the sieve-tube side as far as the middle lamella. Unfortunately no satisfactory cases of this kind have been observed.[4]

B. *Callus*. Substances which stain with callus stains are not all identical. I have already shown that in the seaweeds[5] there are various states of hydration of the cell-wall, in which it stains with one or other of the callus stains; in the least hydrated condition it is affected by London blue alone. 'Callus' is thus a collective term which applies to several different states of hydration, if not actually to more than one chemical substance.[6]

In the haustorium of *Cuscuta* also, there are several substances which stain with callus stains and which play an important part in the history of the parasite. There is the ordinary callus in the sieve plates which stains with all the callus stains and which, as has been made clear by recent work, is due in the young sieve plate to the hydrolysis of the cellulose wall by a ferment, but is added to in the later stages by accumulations of callus directly deposited by the protoplasm.[7]

[1] It would be of interest to know what would occur if an invading hypha made contact with the wall between a sieve tube and a companion cell of the host, i.e. with a wall crossed by connecting threads bored out only on the sieve-tube side as far as the middle lamella. Unfortunately no satisfactory cases of this kind have been observed.

[2] See Hill, 1901 *b*, p. 604.

[3] Hill, 1908, pp. 281–3; on the other hand, Gardiner, 1898 *a*, p. 111 (food and impulses).

[4] See p. 668, footnote 1.

[5] Sykes, 1908, pp. 315–17.

[6] Cf. Mangin, 1892.

[7] Hill, 1901 *b*, pp. 597–600, and 1908; Sykes, 1908.

Again, there is the blue-staining mucilaginous substance which helps to fuse together the lateral walls of the separate invading hyphae during the process of the formation of a compact tissue.[1] This only stains with London blue, and does not show in preparations in which water blue only has been used. It is probably less hydrated than the sieve-tube callus.

Still more important is the substance formed from the tips of the walls of the invading hyphae, on their way to apply themselves to the host sieve areas. It is this blue-staining substance in the tips which dissolves at the junction sieve plate by a process which is perhaps best described as mucilaginous degeneration of a limited area of the cell-wall. Sometimes this substance stains with water blue, more often with London blue only, but it has none of the bright, refractive appearance of ordinary callus. Both here and in the lateral walls the mucilage is clearly derived from the already formed cell-wall, probably by the hydrolytic action of a ferment in the hyphae.

A substance staining with callus stains is found in still another form as granules, which often accumulate to form large blue-staining masses, very conspicuous, especially in the old haustoria. These granules are bright and refractive, staining both with water blue and London blue. Very few, if any, were found in the haustoria of *C. reflexa* or *Begonia*, but they were common in *C. europaea* on *Vitis*, and still more numerous in *C. reflexa* on *Salvia*. They never occurred in the main stem, but were found both in the sieve elements and in the parenchymatous cells of the haustorium, chiefly where it abutted on the pericycle and phloem of the host. They did not appear in the early stages of the growth of the haustorium, but increased in number later. In *C. reflexa* on *Salvia* they were particularly numerous in the part of the haustorium near the pericycle of the host and were found also in the haustorial cells which had invaded the cortex and pith. They even occur, though then generally small in size, in the tips of the invading hyphae (Figs. 68, 73, Pl. L). In the old decayed haustorium they were very numerous, often swelling during the processes of staining, &c., and making the preparation obscure. Occurring in the midst of the protoplasm without relation to the cell wall, they must be formed by direct deposition from the protoplasm itself[2] (Figs. 73, 74, 75).

They are probably of the same nature as the granules recently described by Dr. Benson in the 'phleotracheides' of *Exocarpus* and *Thesium*,[3] which also stain with water (aniline) blue. In these plants the granules apparently never attain the great size of those in *Cuscuta*. It has already been suggested that they are due to the deposition of the hydro-

[1] *Ante*, pp. 664, 665. Cf. the 'callus' which unites the separate members of a tetrad of pollen grains, by the solution of which the four grains are set free. Mangin, 1892.

[2] Cf. Hill, 1901 *b*, p. 599, and Fig. 26, Pl. XXXIII.

[3] Benson, 1910, pp. 673 ff.

lysed products of the cellulose which is continually being absorbed by the parasite as it burrows through the cell-walls of the host. In this connexion it is interesting to notice the absence of granules in *Cuscuta* growing on the thin-walled *Begonia*, and their great number in the same species of *Cuscuta* growing on *Salvia*, with its thick-walled pith and pericycle. Heinricher's [1] examination of the smaller and more compact granules in *Lathraea* [2] led him to describe them as 'amylo-dextrin'. These granules have no very close resemblance to the more irregular masses in *Cuscuta*, and it is not known whether they stain with callus stains. They are present both in tracheides and parenchyma; their reactions resemble to some extent those in *Cuscuta*. Both turn a darkish brown with iodine in potassium iodide, the colour fading later, and both lose their refractiveness on swelling. With sulphuric acid there is a great deal of swelling and degeneration into mucilaginous drops.[3]

It appears probable that the granules and masses thus occurring in so many parasite haustoria are at any rate of allied nature. In *Cuscuta* their absence at an early stage even when growing on *Salvia* suggests that at first all the cellulose dissolved by the ingrowing haustorium is needed by the parasite, and is converted into soluble form and translocated; later, as the hyphae reach the functional phloem, and richer supplies of food are at their service, the hydrolysed cellulose is deposited in the form of these blue-staining granules and masses. Their accumulation at a later stage must to some extent choke the old haustoria.

C. *The origin of connecting threads.* It has been suggested independently by Russow [4] and Kienitz-Gerloff,[5] and the view is strongly supported by Gardiner,[6] that the connecting threads found throughout plant tissues are present *ab initio* in the young cell-wall. Their origin is regarded as connected with the spindle fibres found between the dividing halves of the nucleus, which are so to speak 'imprisoned in the young wall'.[7]

On the other hand Strasburger [8] remains the chief exponent of the view that connecting threads, though often appearing in the young tissues, owe their origin to the formation of 'plasmodesmic pseudopodia', processes growing out from the protoplasm and boring through the young cell-wall. On this view the development of protoplasmic connexions need not be confined to genetically connected cells.

It will not be easy to obtain direct developmental evidence such as would be conclusively in favour of either of these two views. To find

[1] Heinricher, 1894, p. 349, and 1901, p. 725.
[2] Ibid., Fig. 7, Pl. IX.
[3] Cf. Heinricher, 1894, pp. 343 ff.
[4] Russow, 1883.
[5] Kienitz-Gerloff, 1891.
[6] Gardiner, 1898 *a*, 1900, 1907.
[7] Gardiner, 1898 *a*, p. 110; see also Hill, 1908, pp. 282–3.
[8] Strasburger, 1901, pp. 493 ff. and 1902, pp. 52–3.

such evidence research must go back to the developing cell plate, of which our knowledge is at present so meagre.

The presence or absence of connecting threads between non-genetically connected tissues is, however, at any rate a strong indirect argument for or against the view that their formation is intimately connected with the genesis of the cell. Strasburger apparently expects to find such threads [1] at least between cells of the same or of sufficiently closely allied plants.

When working on the histology of *Macrocystis pyrifera* and *Laminaria saccharina* [2] I tried to find out whether connecting threads were developed in the septa formed between the tips of the invading cortical hyphae and the longitudinal walls of the primary pith filaments. It was, however, not only very difficult to distinguish these septa, but I had no young material adequate for their study.

We are also still ignorant as to what happens in the case of sliding growth and grafts.[3]

But the present investigation has, I believe, made it clear that in two cases in which cells not genetically connected are brought into contact later, no connecting threads are formed. In the one case, that of the approximation of the separate invading hyphae to form a tissue, the adjacent longitudinal walls fuse together, but no connecting threads are ever developed in them.

Now this is a case of fusion between the young thin cell-walls of cells which belong, not to different plants, however closely allied, but to the same plant. The fusing cells are, moreover, in an identical stage of development, so that if ever the formation of connecting threads were to be expected between cells not genetically connected it would be here.

The other case, that of fusion of part of the tip of a hypha with a host sieve area, has formerly been described as resulting in a fusion sieve plate, the slime strings of which would, one supposes, be derived from both host and parasite. But I have been unable to find any evidence that this is so. The junction sieve area is merely the intact sieve area of the host sieve tube; the parasite wall immediately overlying it first becomes swollen and mucilaginous and then is completely dissolved away.

It would seem that the application of naked protoplasm to a bored-out wall would be especially favourable to the formation of 'plasmodesmic pseudopodia' which should project into the wall and fuse with the host protoplasm. Yet not only is there no sign of the development of such processes, but the parasite protoplasm is probably not even very closely applied to the junction sieve area. The junction between the sieve tubes of *Cuscuta* and its host therefore afford no argument in favour of Strasburger's hypothesis. Indeed, the two cases, taken together, may be regarded as a strong argument against such a view.

[1] Strasburger, 1901. [2] Sykes, M. G., 1908. [3] Gardiner, 1898 *a*, p. 310.

V. Summary.

1. The development of the sieve plates and sieve fields in the phloem of *Salvia* and *Cuscuta* is found to agree in all essentials with *Vitis* (Hill) and *Laminaria* (Sykes). It is interesting to find the same type of development in the comparatively large sieve tubes of the climber *Vitis*, in the minute sieve tubes of a small plant like *Salvia*, in a non-chlorophyllaceous parasite, and in a seaweed. In every case the connecting threads in the young transverse wall of the sieve tubes are each bored out to form a single slime string enclosed in a tube of callus.

2. The outlines of the development of the young haustorium in *Cuscuta* are described. The invading haustorium is compared with a brush of hyphae; the central hyphae push into the pith or fuse with the xylem of the host; the ones next surrounding these fuse with the sieve tubes; the peripheral ones remain in the cortex. The main mass of the originally separate hyphae form in the mature haustorium, by lateral fusion with one another, a compact tissue.

3. Each hypha composing the tissue becomes subdivided into a number of cells forming a strand. When the tip of the hypha is connected with a sieve tube of the host, this strand of cells is developed into a strand of short sieve tubes. Connecting threads and sieves are numerous in the subdivision walls, but are never formed in the fusion walls between the originally separated hyphae—that is, they are only formed in those cell-walls which are genetically connected.

4. The wall at the tip of the invading hyphae becomes more and more mucilaginous as it approaches the functional phloem. In the inner layers of the pericycle it is sometimes sufficiently hydrolysed for some of its inner layers to stain with callus stains; this hydrolysis spreads till it affects the entire thickness of the wall at the tip.

5. Preparatory to the formation of a junction with a host sieve tube, an invading hypha lays itself more or less alongside the sieve-tube wall, so that the two come into lateral contact. The mucilaginous wall of the parasite where it touches a sieve area is then absorbed, and the naked protoplasm of the hypha applies itself to the sieve area of the host. It is nearly always with a sieve field, occasionally with a lateral plate, that the junction is made; the junction sieve plates and sieve fields in all cases exactly resemble those of the host under normal conditions.

6. The protoplasm of the parasite is easily shrunk away from the sieve area, and is probably never closely fused with the host protoplasm. The translocation of food substances from host to parasite would appear to be of the nature of passive filtration, the contents of the sieve tubes, forced by internal pressure, escaping through the lateral sieve fields into the parasite. This arrangement probably disturbs as little as possible the

normal mechanics of the sieve tubes of the host and ensures for the parasite a long-continued supply of nutriment.

7. The facts accumulated afford evidence on three interesting theoretical questions:—

A. That the parasite takes so much trouble to make use of the host sieve fields as they are, and not to disturb the mechanics of the sieve tubes, is important testimony in favour of the functional efficiency of sieve tubes in general and sieve fields and sieve plates in particular.

B. Various cases of hydrolysis of the cell-wall have been demonstrated; not only are substances staining with callus stains formed in the ordinary way in the sieve tubes, but they occur in the fusing longitudinal walls of adjacent hyphae, in the dissolving walls at the tips of the hyphae, before junctions with the phloem are developed, and in the form of granules or masses of varying size in many of the cells of the old haustorium. Hydrolysis has not proceeded to the same extent in all these cases. In the first three places the hydrolysed substance is produced from already formed cell-wall; in the last place it is laid down directly by the protoplasm, and probably owes its origin to the superabundance of cellulose absorbed by the parasite.

The haustorium of *Cuscuta* thus provides us with unmistakable cases of the formation of callus, both by direct deposition and by changes in already formed wall.

C. Connecting threads are not found in the fusion walls between adjacent hyphae. Also the wall at the tip of a hypha is not burrowed through by connecting threads or slime strings when a junction with a sieve area is to be formed; but instead the whole area of the parasite wall abutting on the sieve fields is absorbed. These facts militate against Strasburger's theory of the origin of connecting threads through boring out by the protoplasm, and may be regarded as indirect evidence in favour of Gardiner's view that connecting threads only occur between genetically connected cells, their origin being associated with the processes of cell-division.

Botany School, Cambridge,
February, 1911.

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DESCRIPTION OF PLATES XLIX–LI.

Illustrating Mrs. Thoday's paper on *Cuscuta* and its Host.

The lenses used were Swift's 3 mm. objective of 1·4 N.A., Zeiss 2 mm. objective of 1·4 N.A.; Zeiss oculars × 6, 8, 12, and 18. Unless otherwise specified the preparations were stained by the safranin method followed by treatment with London blue. The blue colour in the figures represents substances staining with London blue.

PLATE XLIX.

I. *Salvia* sp.

Fig. 1. Young terminal sieve plate in longitudinal section, traversed by fine connecting threads. At the upper end of some of them a faint callus change is already discernible. × 500.

Fig. 2. Terminal sieve plate in which each thread is enclosed in a callus rod, and is situated in a minute depression. A number of fairly deep pits are visible in the radial wall; in each pit a group of fine callus rods are visible. × 800.

Fig. 3. Terminal sieve plate at about the same stage, but more deeply pitted, and with slime strings more bored out. × 1,000.

Fig. 4. A lateral plate, similar to the terminal plate in character. × 500.

Fig. 5. A terminal plate in which the formation of callus has extended over the part of the wall in between the pits. × 500.

Fig. 6. An old terminal sieve plate, with a large deposit of callus on either side. The majority of the sieve tubes in the material were at this stage. × 500.

Fig. 7. Lateral sieve plate in surface view. × 1,000.

Fig. 8. Lateral sieve plate in section; in each pit is a single slime string surrounded by a callus rod, which is interrupted at the middle lamella by a median node. × 1,000.

Fig. 9. Sieve field in section. The wall is profusely pitted; each slime string has its own callus rod; the median nodes are clearly shown. × 1,500.

Fig. 10. Ditto. Shows more uniform pitting; each pit is occupied by a group of callus rods. Most of the plates to which the haustorial cells apply themselves resemble this figure. (London blue only.) × 750.

Fig. 11. Surface view of sieve fields. The small figure shows a few of the slime strings, each traversing a hole in the wall, and surrounded by a tube of callus. × 800 and 1,500.

Fig. 12. Sieve field; in which the formation of callus has extended over the areas of wall between the threads. × 800.

Fig. 13. Sieve field over which large masses of callus have been deposited. × 800.

Fig. 14. Longitudinal section of wall of developing xylem element, showing the pit-closing membrane in section, traversed by a group of threads. × 1,500.

Fig. 15. Wall of the same. M = pit-closing membrane with ends of threads in surface view; P = pit in section showing pit filling. × 1,000.

II. *Cuscuta.*

(Preserved June or July unless otherwise mentioned).

Fig. 16. Radial wall of cortical cell of *C. reflexa*, showing the connecting threads traversing the pit-closing membranes. × 1,500.

Fig. 17. Longitudinal wall of cortical cell of *C. europaea*. × 1,000.

Fig. 18. Cortical cell of *C. reflexa* in section, showing the groups of threads traversing the pits in the longitudinal walls seen both in surface view and in section, and also the much smaller groups in the transverse walls. × 1,000.

Fig. 19. Longitudinal section of sieve tube and companion cell from the main stem of *C. reflexa*. The threads in the transverse wall are arranged in groups and are still unaltered. In the right-hand longitudinal wall (between two sieve tubes) the wall surrounding them has become changed into callus, in the left-hand one (between sieve tube and companion cell) the change has only occurred in the sieve-tube side of the wall. *cc.* = companion cell. × 1,000.

Fig. 20. Terminal sieve plate of sieve tube of main stem of *C. reflexa*; each thread has given rise to a slime string enclosed in a callus rod. Fig. 20 A illustrates the stages in the development of the slime strings as seen in longitudinal section. × 1,000. Fig. 20 B is part of an old transverse plate in longitudinal section, showing the fully developed callus rods interrupted at the middle lamella by a well-marked median node. × 1,500.

Fig. 21. A sieve tube from the main stem of *C. europaea*, showing a case in which the strings are arranged in groups and not distributed uniformly. × 600.

Fig. 22. Sieve tube and companion cell from main stem of *C. reflexa*. The surface of the wall in between the individual callus rods is now coated with a thin layer of callus. In the wall between sieve tube and companion cell the callus change appears only to occur in the sieve-tube side of the wall. *cc.* = companion wall. × 1,000.

Fig. 23. Fully developed sieve plate as Fig. 22; slime strings more bored out. × 1,000.

Fig. 24. Old sieve tube from *C. europaea* (preserved in October), showing accumulation of callus on one side of sieve plate and over the sieve-tube end of the groups of strings in the longitudinal wall between sieve tube and companion cell. *cc.* = companion cell. × 600.

Fig. 25. Surface view of a transverse sieve-plate from the haustorium of *C. reflexa*; the callus rods are evenly distributed and each contains a single slime string. × 1,500.

Fig. 26. Part of an old transverse sieve plate from the main stem of same, showing the callus change extending to the wall between the rods. × 1,500.

Fig. 27. Lateral plate from sieve tube of *C. europaea* (preserved in October), showing accumulation of callus. × 750.

Fig. 28. *C. reflexa.* A and B = sieve tubes of two separate groups, separated by parenchyma and connected by transverse connexion D. The longitudinal wall of A is traversed by numerous groups of minute callus rods. × 500.

Fig. 29. Surface view of longitudinal wall of sieve plate of *C. reflexa*, showing groups of connecting threads, some of which are beginning to be bored out. × 600.

Fig. 30. Surface view of sieve field on longitudinal wall of sieve tube of *C. europaea.* The slime strings are arranged in little groups, and each string is enclosed in a minute callus rod (shown in Fig. 30 A). × 600.

Figs. 31 A and B. Stages in development of the lateral sieve fields (*C. reflexa*) seen in section. × 1,000.

Fig. 32. Longitudinal wall of a mature sieve tube of *C. reflexa*, showing well-marked pits, each traversed by a group of slime strings. Median nodules well defined. × 1,500.

Fig. 33. Ditto, showing a larger sieve field. × 1,000.

Fig. 34. Single pit more highly magnified.

Fig. 35. Longitudinal wall from sieve tube of *C. europaea* (preserved in October), showing accumulation of callus over each pit. × 500.

Fig. 36. Surface view of lateral sieve fields in *C. reflexa*, showing spread of callus change. × 500.

Fig. 37. Ditto, smaller fields and callus change further advanced. × 500.

Fig. 38. Ditto, showing large accumulation of callus over the ends of the small sieve fields. × 500.

Fig. 39. Longitudinal section of sieve field from a sieve tube of *C. europaea* (preserved in October), showing remarkably large median nodules and considerable accumulation of callus. × 1,000.

Fig. 40. Longitudinal wall of sieve tube of *C. reflexa* in section, showing large accumulation of callus on the sieve-tube side of the wall. 'Paths' through callus. × 1,000.

Fig. 41. Wall between sieve tube and companion cell (*C. reflexa*), showing callus change on sieve-tube side of wall only. × 1,000.

Fig. 42. Ditto, *C. europaea* (October). × 800.

Fig. 43. Short element of sieve tube from haustorium of *C. reflexa.* Each thread has its own callus rod; at *c* a small accumulation of callus has taken place. (Greatest length of cell = 64·4 μ; A–B = 11·2 μ; the group A consists of eleven threads; the group B of sixteen.) × 500.

Fig. 44. Short element from haustorium of *C. reflexa*, showing accumulations of callus. × 1,000.

Fig. 45. An exceptionally large mass of callus in old haustorium, nearly filling cell. × 1,000.

Relation of Host and Parasite.

Fig. 46 A. Tip of invading hyphal cell from haustorium of *C. europaea*, making its way through the cortex of *Vitis.* It has already two large nuclei. × 800.

Fig. 46 B. A similar cell of *C. reflexa*, passing through the longitudinal wall of a cortical cell of *Salvia* and boring a clean hole in it. At the edges of the hole the two walls are fused together. × 500.

Fig. 47. (Figs. 47, 48, 49, 51 from *C. europaea* on *Vitis.*) An invading hyphal cell which has nearly reached the host phloem and is four-nucleate. × 800.

Fig. 48. An invading hypha which has reached the phloem of the host. The large size of the nucleus is apparent from a comparison with the dimensions of the transverse section of a host sieve tube. × 800.

Fig. 49. Transverse section of an invading element, which has reached the phloem, taken at some distance from the tip. Longitudinal divisions have taken place to form a strand, and the thin longitudinal walls (*l*) are seen to be packed with connecting threads. × 1,000.

Fig. 50. Two invading cells of *C. reflexa*, near host phloem. The right-hand element has just bored through a host element; its compressed shape may be taken as an indication of the softness

and plasticity of its walls. No connecting threads are present between the two adjoining elements, but they are always found in the transverse walls. A transverse wall is shown in which they have been converted into fine slime strings enclosed in callus rods. × 500.

Fig. 51 A. Parts of the adjoining walls of two invading hyphae, near their tips. The walls are not yet fused together, but their surfaces appear to be becoming mucilaginous and stain with London blue. × 800.

Fig. 51 B. A later stage, in which the adjoining walls are fused; the wavy blue line indicates a line of mucilaginous substance along which fusion has occurred. × 800.

Fig. 51 C. A similar case, in which, however, the fusion line no longer stains with London blue. Faint indications of a blue stain are seen also on the inner edges of the two walls. × 800. (Figs. 51 A, B, C, from *C. europaea* on *Vitis*.)

PLATE L.

(In Plates L and LI the drawings are obtained, unless otherwise specified, from *C. reflexa* on *Salvia*.)

(Here, as also in Pl. LI : H = host element; P = parasite element; HS = host sieve tube; HC = host companion cell; *sp.* = space; *x* = broken or partly dissolved parasite wall; J = sieve plate at junction between host and parasite.)

Fig. 52. Two invading hyphae which have just reached the pericycle of the host (*Salvia*). On the left the host element is merely slightly bulged inwards; on the right the bulging has been carried further, and the wall of the pericycle cell (f_1) has been absorbed as far as the middle lamella. *f* = the remains of the wall of the last fibre, the rest of which has been absorbed. (Stained Delafield's haematoxylin.) × 750.

Fig. 53. A similar invading hypha which has got through the greater part of the pericycle and has nearly penetrated into the phloem. Part of the wall over the tip of the hypha stained with London blue. × 500.

Fig. 54. An invading hypha which has reached the old phloem; the wall at its tip, where it is applied to the host sieve-tube wall, is still unaltered. It appeared at another focus that a continuation of this element went on further into the phloem. (Stained first in safranin and London blue and afterwards in Delafield's haematoxylin.) × 600.

Fig. 55. A somewhat similar case; two adjoining hyphae in the host phloem. In the tip of the left-hand element the inner layers of the thick cell-wall are apparently beginning to be softened and stain in patches with London blue. (Stained as Fig. 54.) × 600.

Fig. 56. Two invading hyphae in which the process of softening the wall appears to have proceeded further, and the inner layer of the wall is swollen up and stains with London blue. × 600.

Fig. 57. Three similar elements which have penetrated still further into the host phloem; in two of them the whole thickness of the wall at the tip now takes the blue stain with London blue. × 450.

Fig. 58. The tip of another such element, a thin outer layer of the wall still unstained. × 600.

Fig. 59. A similar element near a lateral sieve plate (*s*) of the functional host phloem, to which it is very probable that it was about to apply itself. The relations of the walls were almost impossible to make out; it appeared that even at the tip a layer of unaltered parasite wall was still present, but was just being softened so as to stain with blue on the outer surface also. × 600.

Fig. 60. A parasite hypha seen in transverse section making its way at right angles to the old host sieve tubes and going straight through a hole made in one of them. The wall of the hypha stains blue in parts, the section being probably taken near its tip. × 500.

Fig. 61. The surface view of an invading hypha; the wall as yet only stains blue in patches. × 600.

Fig. 62. A strand of sieve tubes derived from an invading hypha. A connexion has been established with a host sieve area and most of the walls subdividing the hypha have become bored out and developed as sieve areas. W = original wall of hypha in which no connecting threads or slime strings are found. The relations of the walls of the host and parasite in the region of junction could not be clearly made out; possibly the mass of blue-staining substance may represent the dissolving wall of the tip of the parasite hypha in a last stage of degeneration. (Ditto in Fig. 67.) × 600.

Fig. 63. Part of a strand of sieve tubes from an old and possibly degenerating haustorium. The

arrows pointing to P and H indicate the direction of the ends of the haustorium which belong respectively to parasite and host; the element cut in transverse section belongs to the phloem of the main parasite stem. Callus changes have taken place to an unusually large extent in the longitudinal walls. × 600.

Fig. 64. Strand of sieve tubes from the haustorium of *C. europaea* on *Vitis* (preserved in October). Accumulation of callus had taken place over the sieve areas on the subdivision walls, but none was found on the original walls of the hypha. W = wall of the original hyphal element which has now become subdivided. C = crushed cells on the periphery of the invading strand. It was not found possible to make out the exact relations of parasite and host. *x* = a thin ridge or broken wall. (Cf. Figs. 68, 76, &c.) × 500.

Fig. 65. Ends of three invading hyphae, closely appressed to one another; the left-hand element resembles Figs. 58, 59, &c.; the right-hand one is applied to a lateral sieve field of the host. X is probably the partially dissolved remains of the parasite wall. × 600.

Fig. 66. An invading hypha in contact with a host sieve tube, of which a lateral sieve field is seen in surface view. × 600.

Fig. 67. Compare Fig. 62. The end wall of the parasite cell beyond X has probably been dissolved, and the blue mass may represent part of its degenerating remains. The naked protoplasm of the parasite is seen extending beyond the blue mass, and would appear to have applied itself closely to the host sieve area. × 600.

Fig. 68. Two invading elements which have turned more or less at right angles to their original course and are now running in almost the same direction as the host sieve tubes. The lower one is as yet unattached; two small blue-staining granules are seen embedded in its protoplasm. (Cf. Figs. 73–5.) The upper element has applied itself to a lateral sieve plate. No cell-wall could be distinguished between the sieve plate and the parasite protoplasm at X. (The preparation was stained first with safranin and London blue; afterwards light green and Delafield's haematoxylin were used successively as wall stains.) × 500.

Fig. 69. From a transverse section of *C.* sp.? on *Solidago* sp.? The tip of an invading parasite hypha is applied to a lateral sieve plate, and no cell-wall appears to be present between the sieve plate and the parasite protoplasm. × 600.

Fig. 70. An empty parasite element whose wall appears to terminate in a ridge at X. J = the host sieve field to which the protoplasm of the parasite element was probably applied. × 500.

Fig. 71. An empty parasite element applied to a sieve field, J. The remains of the wall (stained blue) at the tip X are apparently dissolving. × 600.

Fig 72. From a paraffin preparation stained only with Delafield haematoxylin. The invading element with its enormous nucleus is applied to the sieve field J. In this case it was fairly clear that the parasite wall terminated on either side of the sieve plate at X × 750.

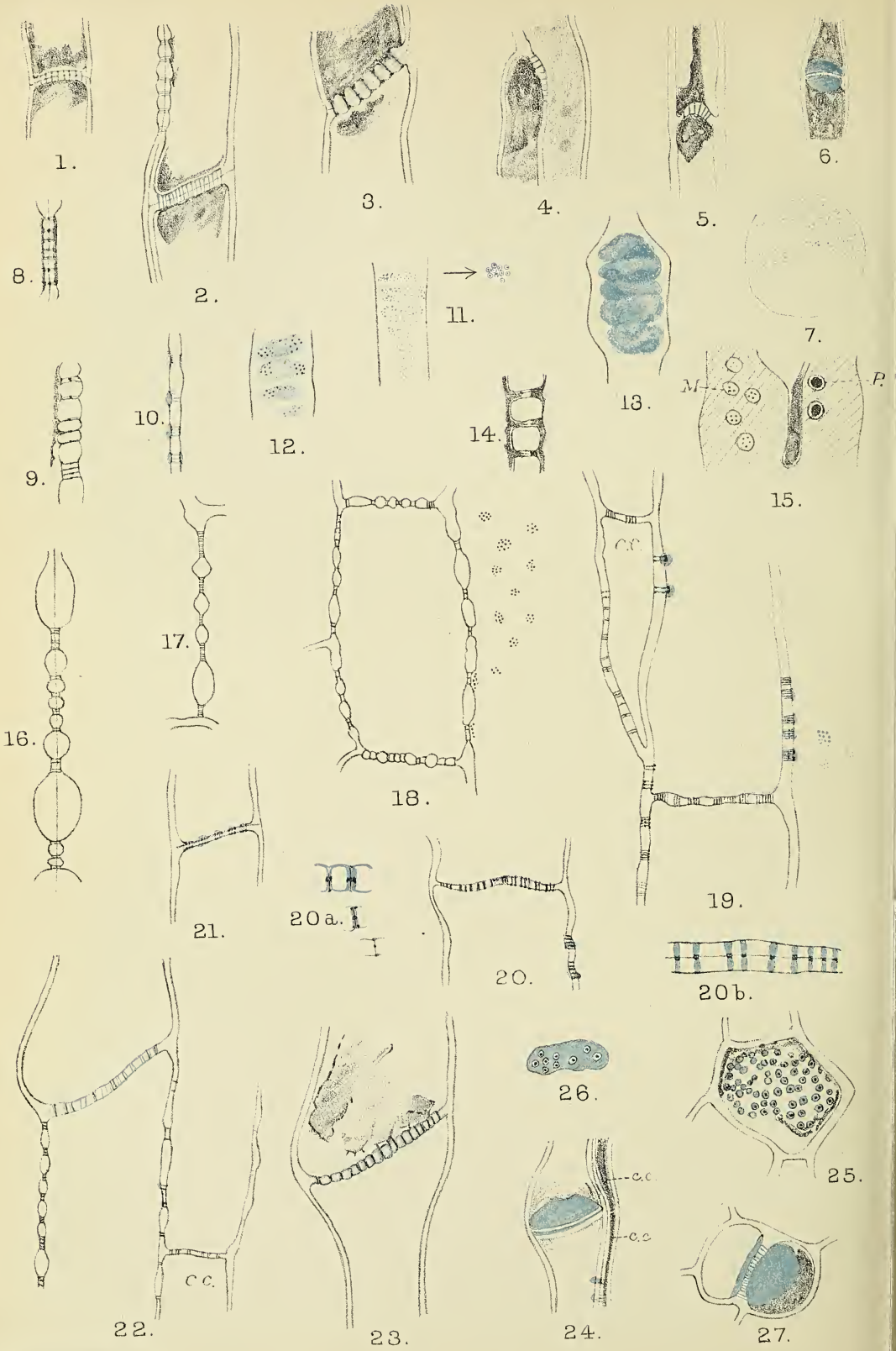
Figs. 73, 74, 75. Haustorial cells showing blue-staining masses in various stages of development embedded in the protoplasm. All from old haustoria. × 750.

PLATE LI.

Figs. 76, 77, 78.[1] The ends of three invading hyphae which have formed connexions with sieve areas in the host phloem. Figs. 76 and 77 were obtained from a preparation stained with safranin, aniline blue, and London blue. The preparation was afterwards stained in Delafield's haematoxylin, and Fig. 78 was drawn from the same three elements. The difference in proportion between the same element stained in the two ways is partly due to different foci, and the difference in thickness of the walls to the difficulty in focusing sharply walls which are unstained.

The protoplasm of the parasite cells is only shrunk very slightly from the sieve plate, and it is fairly clear even from Figs. 76 and 77 that no wall intervenes between it and the sieve plate. This is made unquestionable by Fig. 78, the small space remaining quite unstained. In Figs. 76 and 77, *x* represents what was regarded as being probably the termination of the parasite wall. In Fig. 78 these points are more sharply identified. H = host sieve tube. P = parasite element. J = sieve field of host. W = wall seen obliquely. F = line of fusion between walls of adjoining parasite elements. R = remains of almost dissolved parasite wall. In Fig. 78 the host walls are shaded, the parasite walls left unshaded. Fig. 78 magnified 1,000, Figs. 76 and 77 slightly less.

[1] Fig. 78; D. Thoday *del.*



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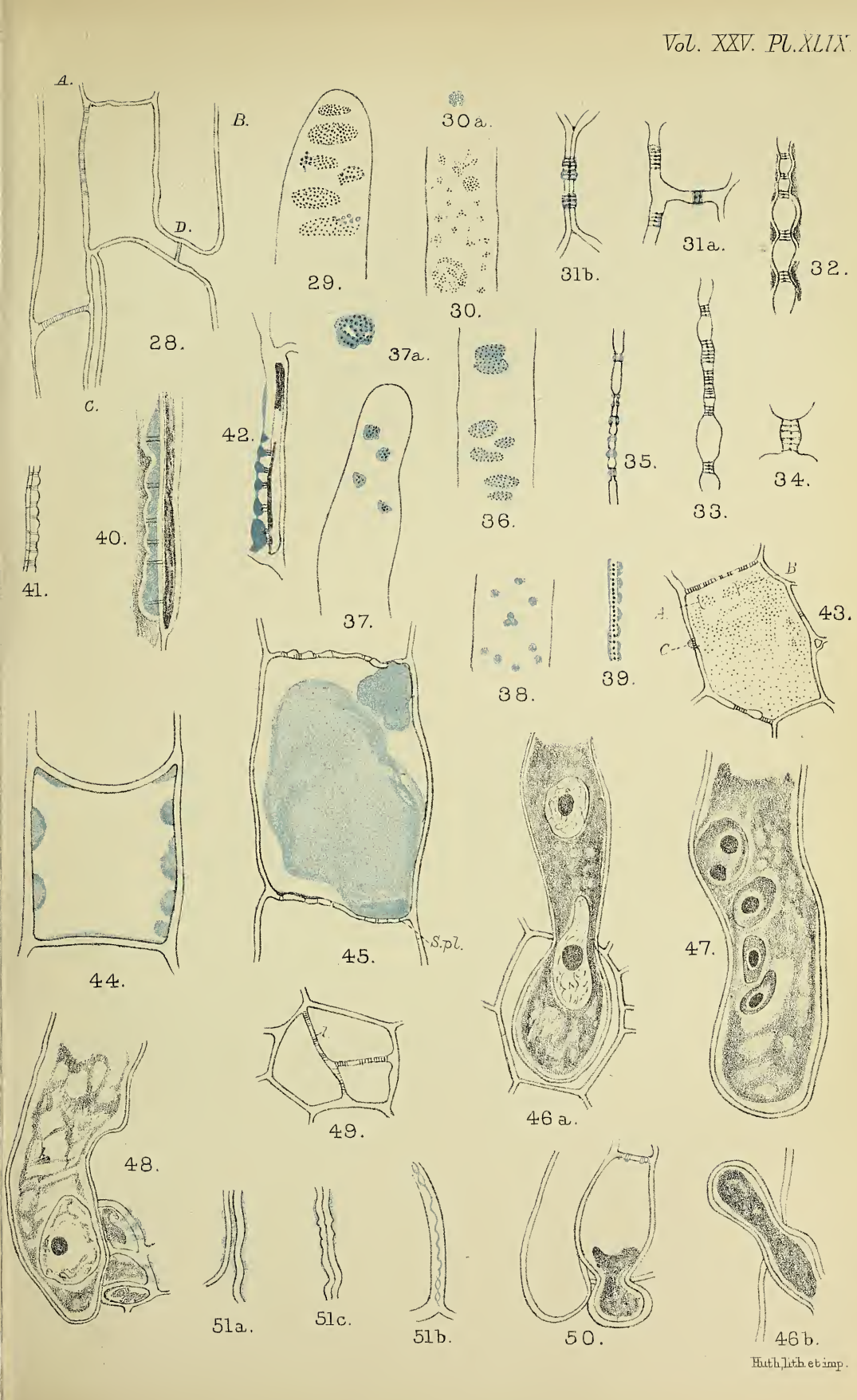
THODAY — SALVIA AND CUSCUTA, HISTOLOGY.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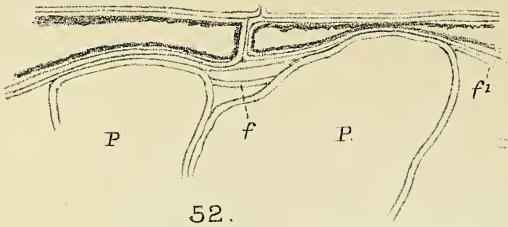

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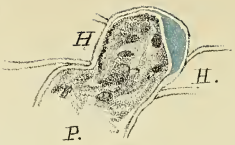

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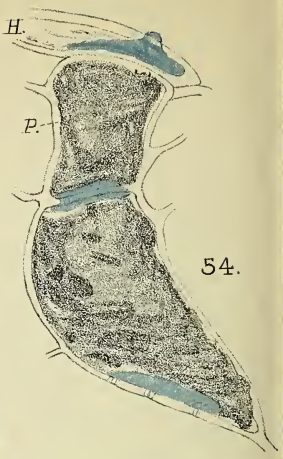

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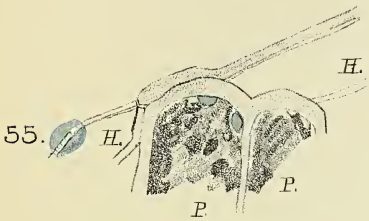

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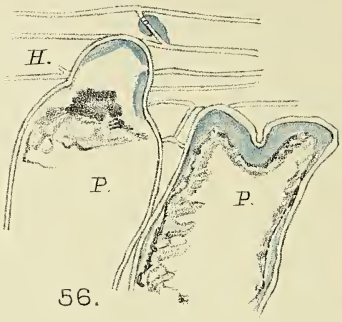

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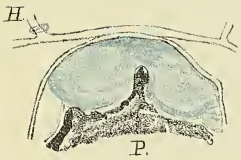

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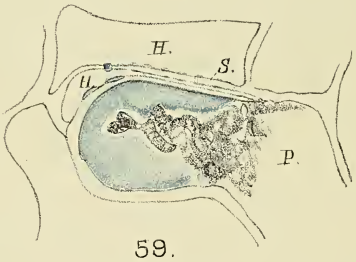

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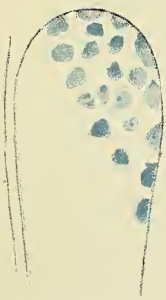

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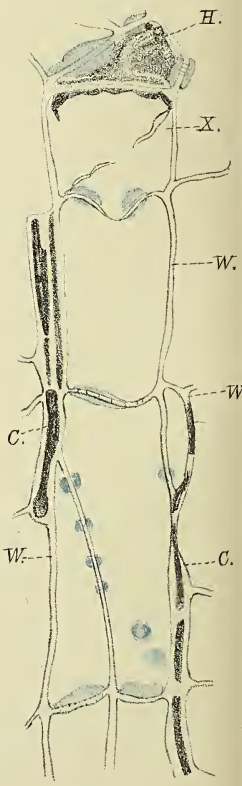

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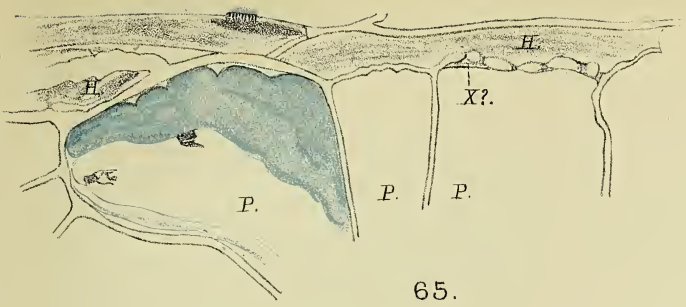
M. G. Thoday (Sykes) del.

THODAY — CUSCUTA, HISTOLOGY.



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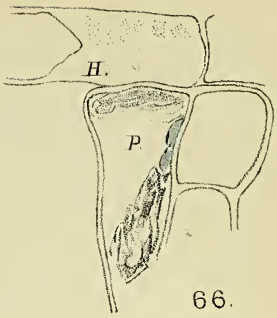

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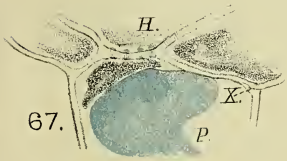

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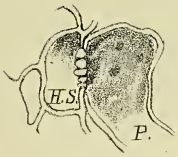

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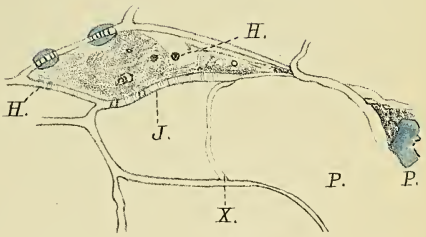

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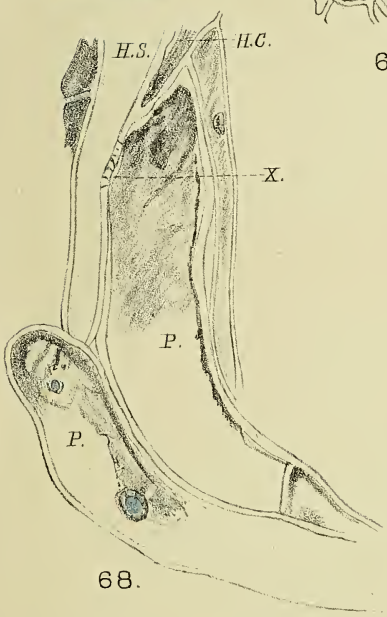

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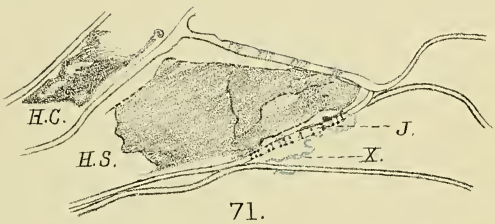

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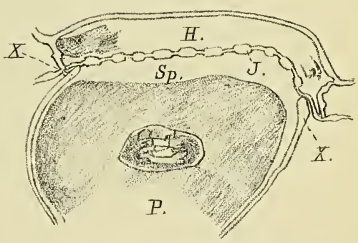

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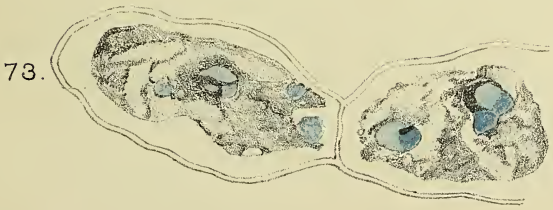

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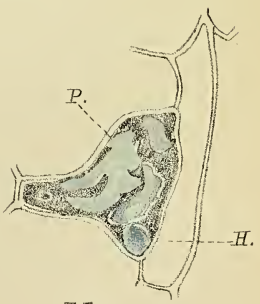

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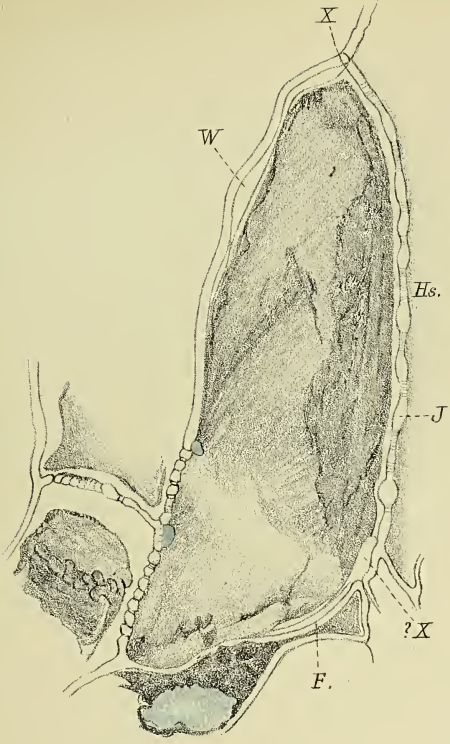

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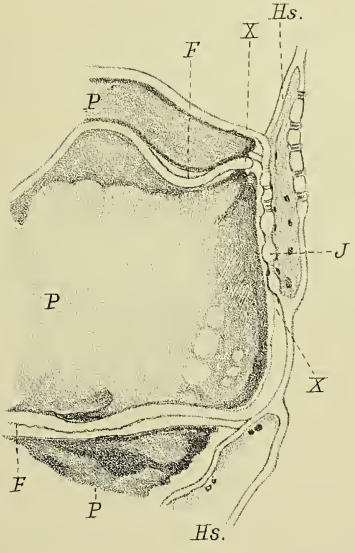

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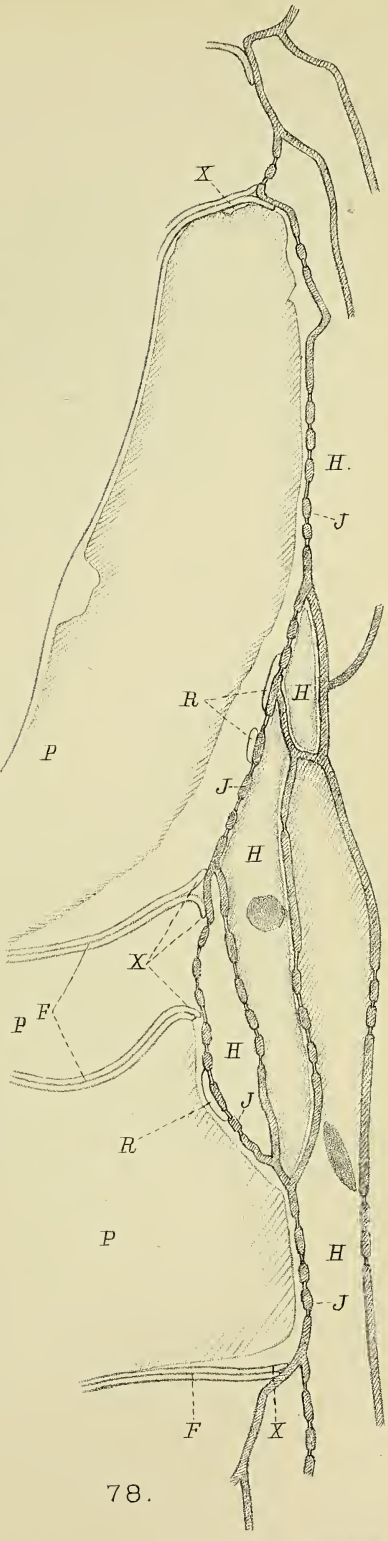

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M.G. Thoday (Sykes) del. Huth lith et imp.

THODAY — CUSCUTA, HISTOLOGY.

Notes on the Development of the Carpophore of some Agaricaceae.

BY

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

With Plate LII.

THE exact order and manner in which the different parts of the carpophore develop is still uncertain in a large number of Agaricaceae. In his earlier work De Bary (1) attributed an internal origin to the hymenium of *Agaricus* (*Psalliota*) *campestris* and some other Agarics which he had studied. In 1874, however, Robert Hartig (3) described the development of the carpophore of *Agaricus* (*Armillaria*) *melleus*, and he found that the pileus arises through a superficial annular furrow which in the beginning is completely open to the outside, and that later, through growth of the marginal hyphae of the pileus and of the stem, the annular furrow becomes covered over with a hyphal layer, the veil.

In his 'Comparative Morphology and Biology of the Fungi, &c.,' published in 1884, De Bary (2) renounces his former opinion and accepts that of R. Hartig. He now believes that in *Agaricus campestris*, as in *A. melleus*, the primordium of the hymenium is first exposed in the open annular furrow which marks off the rudimentary pileus from the stem, and that only subsequently it becomes enclosed by hyphae, which grow towards one another from the edges of the furrow and form the marginal veil.

Fayod (4) studied a very large number of Agaricaceae, and he concluded that the primordium of the pileus was always the first to be differentiated *within* the rudiment of the carpophore. It arises as a definite layer, the 'couche piléogène,' which has the form of a shallow inverted bowl, convex above and concave below. This is surrounded externally by a thin layer which he calls the 'cuticule primordiale'. Fayod divides the Agaricaceae into angiocarpous, subangiocarpous, gymnocarpous, and endocarpous forms, and he attempts to trace his 'cuticule primordiale' in all of them. He very obviously, however, brings together several very different structures under the one name. For seventeen years after the publication of Fayod's work

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no additions were made to our knowledge of the development of the fruit-bodies of these Fungi. In 1906, however, the long silence was broken, and Professor G. F. Atkinson (5) published an admirable account of the development of the carpophore of the mushroom.

He found that the young homogeneous fruit-body, in its earliest stage, shows no differentiation into parts (except the rudimentary universal veil), and is to be considered as the primordium of the carpophore. The first differentiation consists in the appearance of a ring of deeply staining hyphae near the upper end of the young fruit-body and some distance below the surface. This is the primordium of the hymenium, and it marks the differentiation of the primordium of the fruit-body into the primordium of the pileus and that of the stem and marginal veil, the latter being the tissue of the young fruit-body external to the hymenial primordium and continuous with what is to be the margin of the pileus above and with the undifferentiated stem surface below.

Soon after the hymenial primordium has been formed the tissue of the pileus primordium becomes definitely organized, and appears as a deeply staining area lying some depth below the surface.

The three salient facts which Professor Atkinson demonstrated in *Agaricus campestris* were :—

1. The hymenial primordium was the first structure to become differentiated in the homogeneous fruit-body.

2. This hymenial primordium arises endogenously and the marginal veil is not an aftergrowth.

3. The tissue of the pileus (the 'couche piléogène' of Fayod) arises in this plant after the appearance of the hymenial ring.

A few years later C. C. E. Fischer (6) published an account of his study of the development of the carpophore of *Armillaria mucida*. He finds that this does not at all agree with the description given by Robert Hartig in the case of *A. mellea*.

No open furrow is formed round the young fruit-body which subsequently becomes closed by the aftergrowth of a marginal veil. On the contrary, Fischer finds the development of this plant to resemble that of the mushroom, as given by Atkinson, in so far as, in both species, the hymenium originates endogenously and is separated from the exterior from the first by a layer of neutral tissue which constitutes the marginal veil.

If I read his account correctly, however, *Armillaria mucida* differs from *Agaricus campestris* in that in the former plant the pileus ('couche piléogène' of Fayod) precedes the appearance of the hymenial rudiment.

Fischer states that the hyphae near the apex of the young fruit-body show a tendency to radiate towards the periphery and form a subcuticular palisade layer of tissue. This palisade layer defines the rudiment of the

pileus. Later the palisade tissue spreads inwards to form the primordium of the hymenium.

Some time ago I collected material of a number of Agarics in order to determine their mode of development. A few of these have now been sectioned, and in the present note I have brought together the photographs of these sections and have added a few words of description.

The great difficulty in studying the development of the carpophore of these Fungi, when growing in the open, usually lies in obtaining sufficiently young stages. In the case of *Hypholoma fasciculare* (Huds.), however, no trouble is experienced in finding all stages of development from the youngest rudiment to the mature fruit-body.

In the earliest stage the primordium of the carpophore consists of a mass of narrow, closely packed, much interwoven hyphae, which for the most part take a clearly marked longitudinal course. Over the surface the hyphae are broader in diameter and more loosely arranged. This layer of superficial hyphae constitutes the universal veil. In Fig. 1, Pl. LII, three young fruit-bodies at this stage are seen developing side by side.

Fig. 2 represents a slightly older carpophore rudiment. It will be noticed that the body has elongated considerably and that it now shows the first signs of a differentiation of its parts. Near the apex a cup-shaped layer of hyphae has become conspicuous in consequence of the deeper stain which it takes. This layer constitutes the primordium of the pileus, the 'couche piléogène' of Fayod. The hyphae which compose it appear to be richer in protoplasm than their neighbours; they do not assume, in this fungus, a palisade arrangement such as Fischer described in *Armillaria mucida*, but on the contrary they tend rather to run transversely, so that their course is parallel with the surface of the 'cup' which they form.

The layer of deeply staining hyphae increases in thickness and then spreads inwards, so that the edges of the 'cup' become incurved towards the centre of the young carpophore. This incurved and somewhat thickened area of the deeply staining layer of hyphae forms the rudiment of the hymenium (Fig. 3). In the next figure (Fig. 4) a slightly older stage is represented.

In the section of the fruit-body here represented there are to be seen the first signs of the loosening of the tissue just below the primordium of the hymenium. The hyphae in this locality cease to keep pace with the growth of the rest of the carpophore. In consequence there is first a loosening of the tissue below the hymenial rudiment, and a little later an actual cavity is formed there. This cavity is the gill cavity. An early stage in its formation is shown in Fig. 5. In this photograph we can distinguish the different parts of the carpophore: the universal veil, the pileus, the stipe, the hymenium, the gill cavity, and the marginal veil. In slightly older fruit-bodies we find that the whole structure, and more particularly the pileus, is broadening

out laterally. This can be seen by comparing Fig. 6 with Fig. 5, Pl. LII. At this period of the development the hymenial layer is composed of a large number of narrow hyphae, densely filled with deeply staining protoplasm, and arranged very regularly and closely together side by side over the whole hymenial surface. The marginal veil can now be very clearly distinguished, and it will be seen that it is composed of the neutral tissue lying just outside the gill cavity and within the universal veil. About this time or slightly later the stipe shows a differentiation into medullary and cortical regions. The former is composed of loosely arranged hyphae which stain very lightly, whilst in the cortical area the hyphae are more closely arranged and stain more deeply. The gill cavity continues to enlarge as the carpophore grows and the hymenial surface increases in area (Fig. 7). The gills are next developed as a series of down-growths from the hymenial surface (Fig. 8).

The further history of the fruit-body is largely that of the expansion of its parts. The increased growth of the lower surface of the pileus and of the gills at length leads to the rupture of the marginal veil, which is left in the older carpophore as a fringe to the edge of the pileus and a darkened area upon the stipe. The stipe has in the meanwhile become hollow.

Another Agaric which I have examined is *Clitocybe laccatus* (Scop.).

Here also the first differentiation of the carpophore primordium consists in the demarcation of the pileus (Fig. 9). Just below the surface in the upper part of the elongated carpophore primordium a cup-shaped aggregate of closely interwoven hyphae appears and marks the rudiment of the pileus. Soon the rim or edge of the 'cup' grows inwards, and this internal extension of the closely arranged hyphae forms the rudiment of the hymenial layer. It will be noticed in Fig. 10, which represents this stage, that both pileus and hymenium have originated below the surface of the fruit-body, and that this is completely surrounded by a rather poorly developed, but still quite distinct, universal veil. The pileus now grows laterally, and in doing so soon ruptures the feebly developed universal veil (Fig. 11). The rest of the development of the hymenium takes place whilst this is exposed to the air and uncovered by any universal or marginal veil (Fig. 12).

I have also cut a series of sections of the young fruit-bodies of *Armillaria mellea* (Vahl). These preparations do not bear out the account given of the development of this carpophore by R. Hartig; they are, on the contrary, in general agreement with the description given by Atkinson of the mushroom and that given by Fischer of *Armillaria mucida*.

The first differentiation of the previously homogeneous fruit-body consists in the appearance (in longitudinal sections) of two darkly stained patches lying a little way below the surface and near the upper end of the carpophore (Fig. 13). These darkly coloured patches in the longitudinal

sections naturally correspond to a ring-shaped area in the entire carpophore, and they represent the primordium of the hymenium. Very soon afterwards the differentiation of a cup-shaped layer of deeply staining hyphae takes place, which extends upwards from the primordium of the hymenium over the summit of the carpophore, but always some distance below its surface (Fig. 14). This cup-shaped layer forms the primordium of the pileus. A little later a gill cavity is formed just below the hymenial primordium by the cessation in growth of the hyphae at that spot (Fig. 15). The gill cavity increases in size and the differentiation of the various parts of the fruit-body becomes more distinct (Fig. 16). The marginal veil, formed of neutral tissue *present from the first*, covers in the gill cavity. The universal veil, which is well developed over the summit of the young pileus, can only with difficulty be traced in the neighbourhood of the marginal veil as a distinct structure from this. The whole development of the hymenium and the formation of the gills takes place within the cavity which is curtained off from the exterior by the marginal veil (Fig. 17).

Not until a late stage does the growth of the pileus rupture the marginal veil along its margin and leave this structure as the 'annulus' upon the stipe. The chief facts which result from my observations upon *Armillaria mellea* are that the primordium of the hymenium is the first part of the carpophore to be differentiated in this plant, and that this differentiation takes place endogenously. Fischer found that a palisade layer, marking off the pileus, was the first area to differentiate in *Armillaria mucida*. It is not unlikely that there may be some variation in the exact spot in which the differentiation commences; in any case, my preparations of *A. mellea* show that the differentiation of the pileus follows rapidly upon that of the hymenium, and it is quite possible that under certain circumstances the differentiation of pileus and hymenium may be practically simultaneous, or that the 'couche piléogène' may even be distinguished before the hymenium.

On the other hand, however, the order of differentiation may be constantly distinct in the fruit-bodies of *Armillaria mellea* and *A. mucida*. Further observations can alone decide this. However this may be, the particular set of plants which I have examined correspond in this respect more closely with Atkinson's observations upon *Agaricus campestris*, in which he found the primordium of the hymenium to be the first part to differentiate in the young carpophore. I am in complete agreement with both Atkinson and Fischer with regard to the endogenous origin of the hymenial primordium, and in finding the marginal veil to be present from the first and not formed as an aftergrowth as Hartig believed.

In conclusion, I should like to express my thanks to the staff of the department of Cryptogamic Botany at the British Museum (Natural History) for their kind assistance in identifying the Fungi described above.

Summary.

A. *Hypholoma fasciculare* (Huds.).

1. The very young carpophore consists of a mass of densely interwoven hyphae enveloped in a layer of looser hyphae.

2. The first differentiation of parts in this carpophore consists in the appearance of a cup-shaped layer of deeply staining hyphae a little way below the surface at the upper end of the young fruit-body. This marks the primordium of the pileus, which is thus the first part to differentiate in *Hypholoma.*

3. The inward extension of the edge of this cup defines the hymenial layer.

4. Below this primordium of the hymenium an air-space—the gill cavity—is formed. A marginal veil can now be clearly distinguished. It is derived from the neutral tissue just outside and below the hymenial layer; it is present from the first and is not an aftergrowth.

5. The stipe differentiates into a cortical and a medullary region. It later becomes hollow.

B. *Clitocybe laccatus* (Scop.).

6. In this plant also the first differentiation of the carpophore consists in the demarcation of the pileus. It appears as a cup-shaped layer of hyphae lying a little way below the surface at the upper end of the elongated carpophore primordium.

7. The rim or edge of the 'cup' grows inward to form the rudiment of the hymenial layer.

8. A poorly-developed universal veil surrounds the whole carpophore at first. At an early stage, however, the lateral growth of the pileus ruptures this universal veil, and the whole of the rest of the development of the hymenium takes place whilst this is exposed to the air and unprotected by either a marginal or universal veil.

C. *Armillaria mellea* (Vahl.).

9. The primordium of the hymenium is the first part to become differentiated in the plants which I have examined. It has an endogenous origin.

10. The pileus becomes differentiated soon after the hymenium has been marked off.

11. The hymenium is never exposed in an open furrow. On the contrary, the marginal veil is present from the first, and is never an aftergrowth as Hartig supposed.

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6. Fischer, C. C. E.: On the Development of the Fructification of *Armillaria mucida* (Schrad.). Ann. Bot., vol. xxiii, July 1909.

EXPLANATION OF FIGURES IN PLATE LII.

Illustrating Mr. Beer's paper on the Carpophore of some Agaricaceae.

All the figures are from untouched photographs taken by the author.

Figs. 1–8 represent *Hypholoma fasciculare* (Huds.).

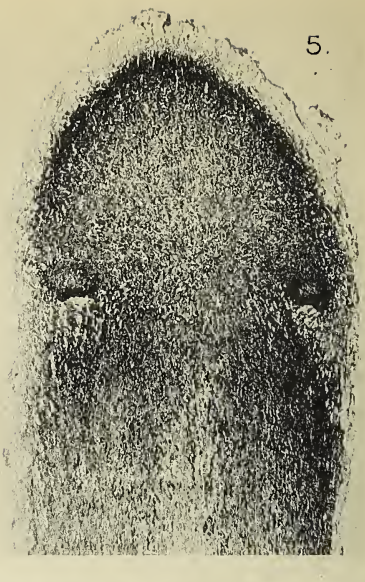
Fig. 1. Three very young carpophores with tissues still undifferentiated. × 41.
Fig. 2. Differentiation of rudiment of pileus. × 41.
Fig. 3. Further differentiation of pileus and commencement of differentiation of hymenial rudiment. × 46.
Fig. 4. Loosening of hyphae below the hymenial rudiment to form gill cavity. × 44.
Fig. 5. Early stage in development of gill cavity. × 45.
Fig. 6. Broadening of pileus and further development of gill cavity. × 45.
Fig. 7. Gill cavity further enlarged. Universal and marginal veils shown clearly. × 42.
Fig. 8. Later stage of development. Formation of gills. × 44.

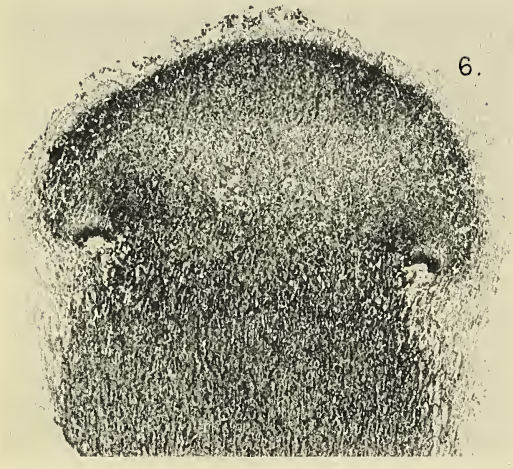
Figs. 9–12 represent *Clitocybe laccatus* (Scop.).

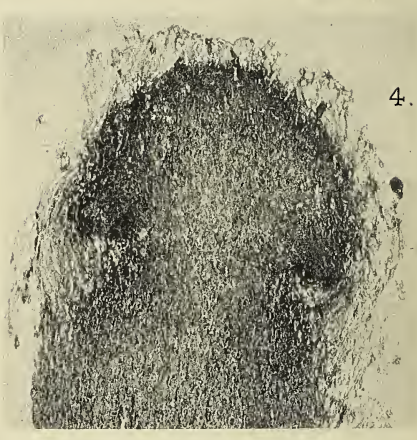
Fig. 9. Differentiation of pileus. × 45.
Fig 10. Differentiation of hymenial rudiment. × 45.
Fig. 11. Hymenial layer exposed by lateral expansion of the pileus. × 43.
Fig. 12. Older stage. × 43.

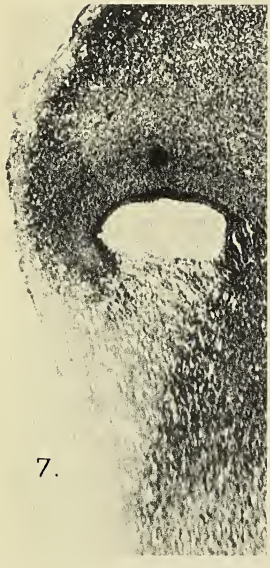
Figs. 13–17 represent *Armillaria mellea* (Vahl.).

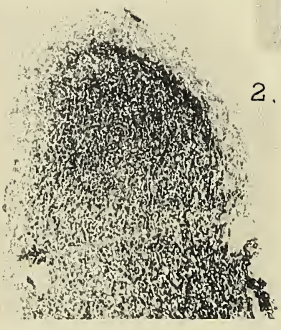
Fig. 13. Differentiation of hymenial rudiment. × 41.
Fig. 14. Differentiation of pileus. × 41.
Fig. 15. First indication of formation of gill cavity. × 41.
Fig. 16. Older stage. × 41.
Fig. 17. Development of gills. × 43.

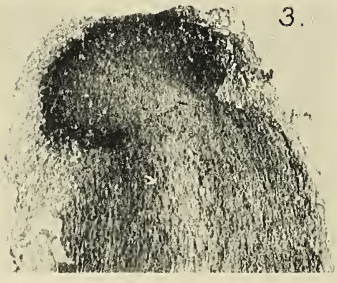


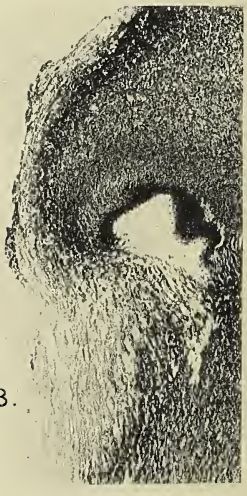












BEER—AGARICACEAE.

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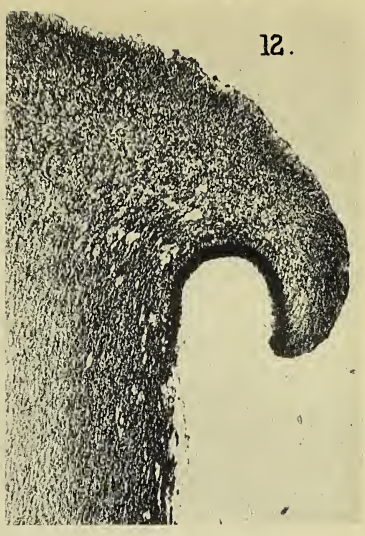

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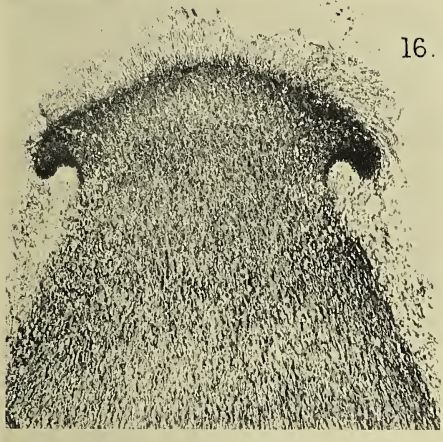

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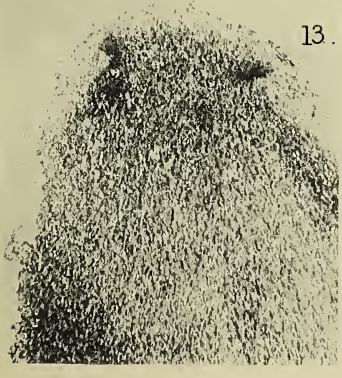

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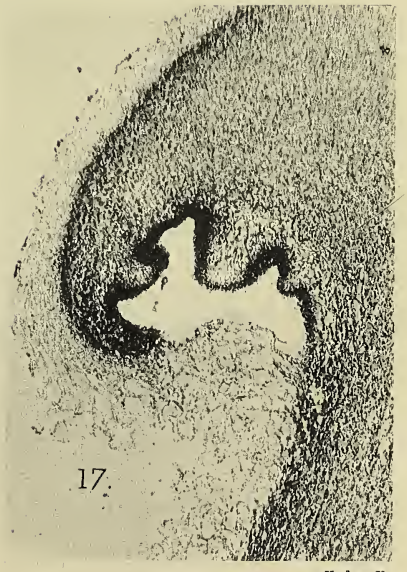

Huth, coll.

The Development of Costaria, Undaria, and Laminaria.

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With Plates LIII-LV.

QUITE recently, Drew[1] has discussed the behaviour of the spores of *Laminaria*, and concluded that they are gametes and not zoospores. This diverges somewhat widely from the view hitherto held by eminent botanists for many years. Yet I hope his observation has revealed the truth, as there seem to occur in nature other cases which may be more easily explained by granting the fusion of two spores of Laminariaceae. For instance, *Hirome undarioides*, Yendo, stands as an intermediate form between *Undaria pinnatifida*, Sur., and *Laminaria radicosa*, Kjellm., in its habit, texture, and propagating organs. An explanation of the genetic relationships between them may be facilitated if hybrids of Laminariaceous species have been proved possible.

Whatever the spores in the sori of Laminariaceae may be, and whatever sort of evolution takes place during germination, 'the full development from spore to adult has not yet been studied for any member of the Laminariaceae.'[2] Thuret[3] briefly described and beautifully figured some embryonal stages of *Saccorhiza bulbosa* some sixty years ago; and this is the only direct evidence we have with reference to the sporelings of the Laminariaceae. Barber, Setchell, Reinke, Foslie, McMillan, Griggs, and others who have traced the stages of development in various members of the Laminariaceae have started from much later stages than Thuret described. Among these authors Setchell gives the most important account as regards the details concerning the development of the internal structure. As the result of the studies of these botanists we are led to believe, though without exact evidence on many particular points, that most members of the Laminariaceae are simply rows of cells at the beginning; that they then become monostromatic, and add the new layers of cells at the lower middle part of the lamina; that the simple

[1] Drew: Reproduction and Early Development of *Laminaria digitata* and *L. saccharina*. Ann. of Bot., xxiv, No. 93, 1910.

[2] Setchell: Post-embryonal Stages of the Laminariaceae. Univ. of Cal. Public., vol. ii, No. 4, 1905.

[3] Thuret: Recherches sur les Zoospores des Algues. Ann. des Sci. Nat., Bot., 1850.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

Laminaria-like frond increases in its length by intercalary growth, or, as Thuret calls it, by stipo-frondal growth, and adds to its thickness by the formation of cortex and medulla.

The present writer has had opportunities for years of collecting and examining the early stages of development of various species of Laminariaceae on the coasts of Japan. In most cases the mode of development of these members coincided with what we had reason to expect. Among them *Costaria Turneri* has supplied especially favourable material for investigation, and the results of the study proved interesting. The present paper is prepared to elucidate the development of this species in detail, as well as of *Undaria pinnatifida* and *Laminaria* sp., referring to some parallel accounts of other genera previously studied.

I must not miss this opportunity of expressing my hearty thanks to Dr. D. H. Scott for so kindly taking the trouble of looking over the manuscript and proofs. It is due to this that the present writer has been able to publish his paper on the opposite side of the globe.

I. Costaria Turneri.

Costaria Turneri is an inhabitant of the North Pacific at low-water mark, descending to several fathoms of depth. It is found abundantly on the coasts of northern Japan, especially that of the Hokkaido Island (Yesso). In the vicinity of the Otaru Harbour, on the west side of the island, the plant begins to appear late in December. Numerous young fronds are found on rocks as well as on *Sargassum Thunbergii*, *Rhodomela Larix*, and some others which abound about there. These epiphytic specimens usually lose their anchorage as the fronds grow larger, and only those on rocks can survive to attain to their maturity. For the purpose of searching for the youngest individuals of the species, *Rhodomela Larix* serves as an especially suitable host. The latter is, at this season of the year, covered with *Sphacelaria*, *Ulothrix*, *Bangia*, &c., and the spores of *Costaria* seem to seek their resting-places among the filamentous Algae. Several young shoots of *Costaria* are usually found growing together at a point. An examination of such points under a dissecting microscope generally reveals a number of minute fronds of much earlier stages. I shall first treat of the embryonal stages and then trace the more advanced forms.

Embryonal Stages.

It is a rather puzzling matter to give an exact definition of the embryonal stage in the life-history of Algae. The term can by no means be applied with clear limitations as in animal embryology. For Laminariaceous plants Setchell [1] includes in the embryonal stages the development

[1] Setchell: Post-embryonal Stages of the Laminariaceae. Univ. of Calif. Public., Botany, vol. ii, 1905, p. 1.

from the germinating spore till the simple Laminarioid frond is completed, with its various medullary, cortical, and epidermal tissues. Reinke [1] insists upon the term 'embryonal stages' for all those before sorus-formation. In the present paper the term is taken as by Setchell, but a little extended; viz. from the sporeling up to the time when all the organs (the sorus excepted) found in matured plants have been indicated, though in a primitive state.

The youngest stage of *Costaria Turneri* that I could find was a confervoid body measuring about 70 μ in length (Pl. LIII, Fig. 1). It was a simple filament of seven cells disposed in a row. The cells were cylindrical without any marked constrictions at the septal points, and the length was nearly equal to the diameter. The apical cell was more or less elongated compared with the rest, and was slightly swollen, with a round head. Chromoplasts were present in each cell, filling up nearly the whole room in the terminal cell, but rather poor in the others. The basal part of the confervoid body could not be examined in a satisfactory manner, as this portion was entirely hidden in the interwoven filaments of *Sphacelaria*, *Ulothrix*, &c. Judging, however, from observations on the later stages, it is very likely that the lowermost cell of the confervoid body is elongated into a long siphonal tube penetrating the felty substratum.

The growth of the confervoid body in length results from successive divisions of the apical cell in transversal planes perpendicular to the axis of the filament.

Areschoug [2] described the sporelings of *Chorda tomentosa* as becoming simple or branched monosiphonous filaments. Similar sporelings have been observed by Williams [3] in certain members of the Laminariaceae, and by Okamura [4] in *Leathesia*. Kützing [5] delineated three very young stages of *Laminaria saccharina* growing in association. In his figure he indicated the confervoid, branching bodies at the base of a young plant. He does not mention them as young stages of the plant, remarking simply: 'Conferva-artige Fäden, welche den protonematischen Bildungen der Moose ähnlich sind.' It is very probable, however, that he regarded these bodies as the primary stage of the plant jointly illustrated with them. These facts might suffice to convince us that various brown Algae of three dimensions are at the beginning confervoid. In *Saccorhiza bulbosa*, according to observations by Thuret,[6] the monosiphonous filament does not branch at all, and ceases to add to its own length after there are half a dozen cells in a row.

The zoospores of *Costaria Turneri* are freed from the mother frond late

[1] Reinke: Studien zur vergleichenden Entwicklungsgeschichte der Laminariaceen, 1903, p. 11.
[2] Areschoug: Observationes Phycol., iii, p. 15, Tab. I, Fig. 1.
[3] Williams: Nature, vol. lxii, p. 613.
[4] Okamura: Icon. of Japan. Algae, vol. i, No. 4, Pl. XVIII, Figs. 10–12.
[5] Kützing: Phycol. Generalis, p. 345, Pl. XXIV, I, Fig. 5.
[6] Thuret: l. c., Pl. XXX, Fig. 10.

in autumn or early in winter. If they germinate within a short time after liberation, the confervoid body observed must itself have passed through a resting stage of several weeks. Or, if the confervoid body had only just been formed as the result of germination, the spore must have had a certain period of rest. I am rather inclined to believe that the zoospores must pass through a resting period without germinating. The actual occurrence of this has been verified by Williams.[1] Okamura[2] reported on *Undaria pinnatifida* that its spores germinated in July, as he observed at Omori, near Tokyo, shortly after the liberation of the zoospores. In Tokyo Bay, the young blades of that species are first seen late in autumn or early in winter. If so, the filamentous sporelings of *Undaria* must have spent a few months in developing into a minute frond. Drew's recent observation that the gametospores produce several sporophytes after a complicated process adds a new and quite different case to those already recorded. His view, however, important as it is, requires further proof before universal acceptance.

In the next stage, the apical cell of the confervoid body no longer divides in a transverse direction, but by a longitudinal septum through the middle point. The longitudinal division extends downwards, leaving a few basal cells in the initial state. The result is a linear-spathulate lamina constructed with two rows of cells in a single layer, with the monosiphonous stipe attached to the lower end. An exactly similar stage has been illustrated by Thuret[3] in *Saccorhiza bulbosa.*

Another longitudinal division takes place in the cells of the lamina by two planes parallel to the first division-plane. The apical two cells, resulting from the primary division, do not divide in this case. As a consequence, each terminal cell is now seated upon the two newly formed cells. Thus the lamina becomes practically composed of four rows of cells disposed in a single layer.

The second division above described begins its action in the cells next to the apical pair. The process goes on downwards, segment after segment, till it reaches the basal one upon the stipe.

Successive divisions proceed, after the secondary division has been completed. They take place in two directions, the one parallel to the sagittal plane and the other passing through the middle of each segment, and perpendicular to the other. In any case, the two contiguous cells which are seated beneath the apical pair and embrace the axis of the lamina divide one step further than the rest. The lamina in these stages eventually becomes obovate in outline, the broadest part being at about the level of the two cells just mentioned.

Figs. 1–5, Pl. LIII, illustrate the various stages of development referred to above, all carefully drawn by the aid of Abbe's reflecting camera. The

[1] Williams: l. c. [2] Okamura: Botanical Magazine, Tokyo, vol. xv, No. 163, 1900, p. 230.
[3] Thuret: l. c.

geometrical figure (Fig. 18) is diagrammatized from this series. From the diagrammatic figure it may be easily understood that the growth of the embryonal lamina of *Costaria Turneri* results from the division of the two initial cells situated side by side beneath the apical cap. This mode of cellular arrangement is rather disturbed in *Saccorhiza bulbosa*, judging from the figure given by Thuret,[1] and in *Undaria* and *Laminaria* as described below.

The cells which constitute the monosiphonous stipe elongate as the lamina extends, to a length much exceeding the diameter. At the same time they divide longitudinally and transversely, quite irregularly, to form a cylindrical stipe. The resulting cells are poor in chromatophores, and those in the lower part are entirely devoid of them. From the basal cells of the stipe, a few rhizoidal filaments are given off, in a manner reminding us of the root-hairs of a land plant (Fig. 5). Two forms answering to such a stage have been illustrated by Kützing[2] in *Laminaria saccharina*; in them, the terminal cells are shown in regular transverse zones, but their longitudinal arrangement is not in any apparent order.

When a plant has attained about 2 mm. in length, the monostromatic lamina becomes distromatic. The process is first carried on at the point which corresponds to the trasitional region in an adult frond. The division of the cells is by a simple plane passing through the middle points between the two surfaces of the lamina. Hence, in the cross-section of a lamina at this stage, the sister cells of the two layers are exactly facing one another.

At this stage the cells at the transitional region undoubtedly play the part of a meristem, although the apical cells multiply as well. The activity of the latter, however, is gradually retarded as the blade is extended by the so-called stipo-frondal growth. Most former writers, if not all, seem to have omitted to recognize the apical growth of the sporelings of Laminariaceae, and hence the crucial stage in the mode of growth has not been hitherto considered. So far as I am aware, Reinke[3] is the only one who, arguing from the figures given by Thuret, has remarked that the embryonal blades of Laminariaceae extend at first by cell-division in all parts of their area, and that afterwards the intercalary growth is localized in the transitional region.

While the lamina becomes two-layered after the monostromatic stage, the stipe becomes gradually thicker. Cross-sections show many polygonal cells compactly but irregularly arranged without any remarkable distinction in their size. The boundary between the distromatic lamina and the polysiphonous stipe lies, of course, at the transitional point.

When the stipe has become cylindrical the rhizoidal filaments are copiously given off from its basal point (Fig. 14). Each filament is simply

[1] Thuret : l. c. [2] Kützing : l. c., p. 345, Tab. XXV, I, Fig. 5.

[3] Reinke : Studien zur vergleichenden Entwicklungsgeschichte der Laminariaceen, 1903, p. 8.

an elongation of an epidermal cell without any septum throughout the whole length. This is somewhat different from the case which Kützing[1] has illustrated in *Laminaria saccharina*. Griggs[2] found tufts of filamentous strands at the base of *Lessoniopsis littoralis* which measured about 1·1 mm. in length. He has not remarked on the finer structure of the strands. The filaments, as the plant grows on, add to their number, resulting finally in a cluster of conical shape in general outline—the primary haptere. This process agrees in a striking manner with what has been observed in *Chorda* and *Punctaria*. Strömpfelt's[3] description of the formation of the primary haptere of *Laminaria* is in some points inapplicable to our case.

Soon after the blade has extended its area by the stipo-frondal growth and its greater part has become distromatic, another layer of large parenchymatous cells makes its appearance. This layer originates from the transitional point between the blade and the stipe. So far as my researches extend, it has no direct genetic relation with the already existing two layers. In other words, the cells which constitute the new layer are generated at the transitional point from the internal cells of the polysiphonous stipe. As a striking analogy, I cannot help mentioning, superfluous as it may seem, the mesoderm formation in the primary stage of an animal embryo.

Fig. 21, Pl. LIV, shows a cross-section through the upper part of the four-layered area of a frond about 1·5 cm. in total length. About the point *a* the axis passes; the right half and the left monostromatic marginal part are not shown in the figure. About *b* we clearly find the new layer interposed between the older two. The cells composing the latter still correspond to one another in their position, though somewhat more disturbed than before. Those of the new layer are quite different in size from those of the two older layers, and the septa are not at all related. At the point *c* we find a distromatic portion without any layer interposed, and about *d* the lamina in the initial state. In the middle of the frond, the new layer is again split into two; each cell of one layer has its double in the other, showing that the two facing layers are sisters. These two new layers initiate both the cortex and the medulla in more advanced stages, as will be explained hereafter. Hence I prefer to assign the name 'precortical layers' to them for the sake of convenience.

Fig. 20 shows the cross-section of the same frond through the apex of the tristromatic area. Here we see the terminal cell of the new layer penetrating the apical portion of the distromatic area.

[1] Kützing: l. c., Tab. XXV, I, Fig. 5.

[2] Griggs: Juvenile Kelps and the Recapitulation Theory. American Naturalist, vol. xviii, No. 506, 1909, p. 10.

[3] Strömpfelt: Untersuchungen über die Haftorgane der Algen. Bot. Centralbl., Bd. xxxiii, 1888, p. 398.

Longitudinal sections of fronds at a similar stage of development have revealed that the constituent cells of the precortical layer are isodiametrical, but much flattened. The length and the breadth are 3–4 times as large as those of the pre-existing cells, but the height is nearly alike. The relative sizes of the cells of the three layers may also be recognized by observing such fronds in surface view after being stained and clarified *in toto*.

When the lamina has attained to 5–7 mm. in length, its general outline becomes lanceolate or elliptical-lanceolate with tapering base and acute apex. The length of stipe varies according to the place where the plant is growing. Those found among the caespitose filaments of *Sphacelaria* or such-like Algae naturally have the stipe longer (Figs. 6–10, Pl. LIII).

Kuckuck [1] has delineated a cross-section of a frond of *Laminaria* apparently corresponding to the above-mentioned stage. He has not given an explanation in full of what he has delineated. But his illustration suggests a conception diverging too widely from what I have observed on my material. I reproduce here the figure given by him (Fig. 22, Pl. LIV). Others besides myself might suppose from this figure that the middle layer is the primary one, and that the peripheral layers have been formed on both surfaces of the initial layer.

The process of gradual increase of the cell-layers is essentially the same in the blades of *Costaria*, *Undaria*, and *Laminaria* treated in the present paper. They agree in the formation of the peripheral or epidermal layers, as they may be termed, previously to that of the precortical layer. And so far as I have observed in Dictyotae, Zonariae, Punctariae, &c., which have one or more layers of parenchymatous cells between the two single epidermal layers, the formation of the latter from the former is a view hardly to be accepted.

The multiplication or the addition of layers has its centre practically at the transitional region. The process extends more rapidly in the longitudinal than in the transverse direction. The boundaries between the successive layers may be readily traced by observing such a lamina under a low power of the microscope after the specimen has been stained and clarified by a suitable method. Fig. 13, Pl. LIII, shows diagrammatically a frond which measures about 1·5 cm. in total length. In *Costaria* the formation and interpolation of the tertiary layer follows directly after the formation of the distromatic layers. The genuine distromatic structure is therefore in most cases able to be traced along the outer margin of the tristromatic area. Generally speaking, the boundaries of the different numbers of layers form concentric lines parallel to the periphery of the lamina, converging towards the transitional region.

Comparing the blades at these various stages of development, the

[1] Kuckuck: Bemerkungen zur marinen Algenvegetation von Helgoland. Wiss. Meeresuntersuch., N. F., Helgoland, 1906, p. 250, Fig. 19.

monostromatic part first formed seems to have never decreased in its area, nor does the distromatic, &c. In other words, the areas of simpler structure do not add any complexity to the tissue by the later development except for the extension in area. The additions of new elements always commence in the transitional region.

Close examination under a higher power of the microscope shows, however, that the boundaries are not in straight lines, but very irregularly serrated. Setchell[1] mentions a similar condition in the youngest fronds of *Saccorhiza dermatodea*. Besides, there are many small areas detached from the principal part, but with a similar addition of layers. This is especially the case for the distromatic layer. Such oases must have resulted from an abnormal meristematic activity retained in the cells of the monostromatic area. Fig. 16, Pl. LIII, illustrates a part of the lamina under such a condition, the shaded part signifying a di- or tri-stromatic area ; Fig. 17 shows one of the oases still more magnified. The irregularity of the border line is due to the fact that certain cells in a group—areoles of cells as it were—which have been derived shortly before from the same mother-cell are simultaneously divided into two layers. Fig. 19 shows a part of Fig. 16 under a high power.

Kützing[2] seems to have observed a similar structure in an embryonal blade of *Laminaria saccharina*. He remarks: 'Man bemerkt, dass an einigen Stellen die Randzellen sich in kleine Kügelchen aufgelöst haben, die je 4 und 4 zusammenstehen (Exanthem ?).' He seems to have regarded the areolar cells as having a pathological origin. I am rather inclined to suppose, if I may be allowed the suggestion, that his specimens had the epidermal cells plasmolysed.

In *Costaria Turneri* the apical portion of the blade remains in its monostromatic state for a considerable time. I found several specimens measuring 3–4 cm. in length, with the three costae already formed, which still had the apical region in a single layer of cells.

Similar stages of development—polystromatic lamina with monostromatic apex—have been recorded by Kützing, Kuckuck, Reinke, Setchell, Griggs, &c., in *Laminaria saccharina*, *L. ephemera*, *Saccorhiza dermatodea*, *Alaria esculenta*, &c. The present writer has collected many specimens of *Undaria* and *Laminaria* with a similar structure. Hence, it may be safely stated that most members of the Laminariaceae have once passed through such a stage during their life-history.

In *Costaria Turneri*, some species of *Laminaria*, and certain others, the apical growth seems to continue, however, in a slighter degree than before, even after the greater part of the blade has become polystromatic. It

[1] Setchell: Concerning the Life-history of *Saccorhiza dermatodea*. Proc. of Amer. Acad. of Art and Sc., 1891, p. 182, Figs. 1 and 2.

[2] Kützing: l. c., p. 345.

is some time after the formation of all the ribs that we find the lamina of *Costaria Turneri* with an eroded apex. Griggs [1] states that *Hedophyllum sessile* measuring 2·3 mm. in length had already 'several' layers of cells in the middle region and towards the base, but with a monostromatic edge. In *Lessoniopsis littoralis* measuring about 1·1 mm. in length he also found its lamina already 'several' layers of cells thick, even at the edge. Of the latter specimen he remarks: 'It hardly seems possible that there could have been any remnant of the one-layered lamina which had not been transformed into the many-layered adult blade.' In this respect he mentions *Cymathere* as the opposite case, 'in which a large portion of the embryonic lamina is not changed, but continues to grow and persists until the plant is more than 20 cm. long.' As regards the 1·1 mm. specimen of *Lessoniopsis* he does not state whether the apex had been eroded or not. Judging, however, from his figure, the apical portion is clearly worn away. Still, his remark above quoted remains open to question unless the apex of the lamina had been proved to be persistent. Putting aside this questionable case as exceptional, there is ample reason to conclude that in Laminariaceae the monostromatic area of the blade is sooner or later atrophied without developing into complex tissue.

Setchell [2] and McMillan [3] give the name 'midrib' to the thickened area which runs longitudinally in the blades of *Saccorhiza* and *Pterygophora* respectively. In the present paper the area is mentioned under the name of meridional region in order to distinguish it from the true midrib. McMillan remarks that this area makes its first appearance in *Pterygophora*, when the plant has grown to several centimetres in height. So also in the case of *Laminaria* sp., as is described below. But in *Costaria Turneri*, the primary midrib is first seen as a longitudinal elevation in the polystromatic transitional region when a frond has attained 1 cm. in the total height (Fig. 6). To the naked eye, however, it is not yet sharply defined from the adjacent region. Transverse sections through such points reveal the first step in the formation of the midrib as a few parenchymatous cells aggregated beneath the precortical layer of the ventral side. I mean by 'ventral' the surface with the midrib elevated. The dorsiventrality of the lamina of *Costaria* is thus begun at an early stage of development.

After the primary midrib has been formed and the frond has attained about 1·5 cm. in length, the lateral ribs begin to come out, one on each half of the lamina. The new ribs are shorter than the primary one already in existence and are elevated on the dorsal surface. The three are parallel to one another for the greater part of their length, but gradually converge

[1] Griggs, l. c. [2] Setchell: Concerning the Life-history, &c., p. 182.

[3] McMillan: Observations on *Pterygophora*. Minnesota Botanical Studies, 2nd series, Part VI, 1902, pp. 725-6.

in the transitional region and finally unite together at the base of the blade.

Fig. 24, Pl. LIV, is drawn with the aid of Abbe's reflecting camera from a cross-section at the transitional region of a frond about 12 mm. in total length. The three ribs are clearly shown. The essential part of the ribs is built up of the subcortical cells, which are much more increased in number than in the other parts. The epidermal layer is a single sheet of cells, now anticlinally elongated.

The figure shows at the same time how the medulla is generated. The two layers of the precortical cells are separated one from the other by the intervening space filled with a gelatinous matrix. The latter substance is undoubtedly secreted from the precortical cells. As a consequence, the cell-ends facing the gelatinous matrix become convex by their own turgescence. The hyphal cells are now generated from some of the precortical cells on the side towards the space. At the time when the hyphae are just formed, they contain, as in the cortical cells, a small number of chromoplasts. It is, however, in the later stages that the hyphae fill up the medulla as a compactly interwoven filamentous tissue.

When a blade with the hyphae just formed is observed from the surface under a low power, they are seen as delicate veinlets traversing longitudinally the polystromatic area (Fig. 15, Pl. LIII). Their course has been very difficult to trace, owing, no doubt, to the fact that they run freely through the gelatinous matrix. This was also the case in *Undaria*. In *Laminaria* the two precortical layers remained in close contact for a considerable time after the hyphal cells had been well developed (Figs. 44–6, Pl. LV). More precise accounts of the formation of hyphae will be therefore given in a later chapter. It is noteworthy, however, that in some specimens of *Costaria* measuring 10 cm. or more, the upper part of the four-layered area remained without the hyphal cells.

Soon after the formation of the three ribs, a row of tiny bullations begin to attract our attention on both sides of the midrib (Fig. 10, Pl. LIII). The lamina continues its stipo-frondal growth, adding new bullae successively below. The bullation of *Costaria Turneri* is not a simple over-extension of the lamina at certain points. From the beginning it is an elevation and depression occurring regularly and alternately, and parallel to one another. They are slightly transversely stretched at right angles to the adjacent ribs. At a somewhat more advanced stage an end of the elevation—depression on the other surface—bends at right angles and parallel to the rib. The depression adjacent to the elevation also bends in like manner, but at the opposite end and in the opposite direction to fit the former. The process will be more easily understood by a glance at the illustration, than by imitating an enunciation by Euclid (Figs. 11, 12). Briefly speaking, the bullation reminds us of a sort of carved fretwork. These bullations

are undoubtedly due to the quicker growth of the area adjacent to the ribs, while the latter elongate at a less speed. Yet the characteristic regularity of the bullating process deserves special attention.

A similar bullation is to be seen in the young blades of *Laminaria cichorioides* and several others, and still more strikingly in the whole life of *Kjellmaniella* (*Laminaria*) *gyrata*. The bullation found in the blades of *Laminaria bullata* and its forms comes out at a later period of life and is rather of a secondary nature.

When a frond has reached a total length of 3–4 cm. another rib begins to appear in each marginal area. It is at first indicated by the formation of bullae along the external side of the lateral ribs (Fig. 11). The newly formed external ribs are elevated towards the ventral surface, i.e. similar to the midrib ; they converge downwards with the already existing ones to meet together at the base of the lamina. After the new ribs have been completed, one more row of bullations makes its appearance along the external side of each rib (Fig. 12).

When the five ribs have been completed we generally find the monostromatic apex of the lamina more or less worn away. But the distromatic area may be still seen remaining in the upper portion of the lamina even after that.

The first appearance of cryptostomata takes place in the area between the first three ribs, after the bullation has begun to attract our notice. The seats of the hairs in such an area are more or less depressed below the surrounding epidermis, as shown in Fig. 26, Pl. LIV, but never so deeply as to be called pits even in an adult frond. The depression appears to be due to the fact that the cells in this area neither increase in diameter nor divide tangentially, while the surrounding epidermal cells are multiplying actively to extend the lamina.

In examining the earlier stages of the cryptostomata we find a group of epidermal cells almost simultaneously elongated in height and each finally septated (Fig. 25). I could never detect a special cell which corresponds to the so-called initial cell commonly occurring in the cryptostomata of Fucaceae.[1]

The slight depression and the lack of the initial cell in the cryptostomata of *Costaria* are characters parallel to those of *Alaria*.[2] Exact similarity is found in *Undaria*, as will be seen below (p. 705). Comparing these members of the family with *Adenocystis* [3] and *Saccorhiza* [4] we might with safety come to the conclusion that the cryptostomata in Laminariaceous species are different from those of Fucaceous Algae in the mode of formation.

[1] Simons : A Morphological Study of *Sargassum filipendula* Bot. Gaz., vol. 49.
[2] Murray : On the cryptostomata of *Adenocystis*, *Alaria*, *and Saccorhiza*. Phyc. Mem., x, 1893.
[3] Ibid. [4] Ibid.

Setchell[1] has found small clusters of hairs in *Saccorhiza dermatodea*, not only in the area of complex structure but also in the monostromatic part of the blade of some young specimens. In *Costaria*, the simplest structure provided with them, so far as I could ascertain, was not less than four layers of cells thick (Fig. 27, Pl. LIV). Before the three ribs have been marked out, no indication of cryptostomata could be found either in the four-layered area or in the transitional region of more complex structure. I am inclined to think from this fact that the cryptostomata are formed in all parts of the blade, though there may be differences in structure, after a plant has attained to a certain stage of development.

The primary haptere, after being formed in the manner before described, remains simple until the first three ribs have appeared. When the bullations begin to attract our notice, new rhizines are given off in a radiating manner above the primary one. These rhizines follow the growth of the frond to a certain extent, but mostly remain in the simple state. The new whorls of rhizines above the old ones grow and ramify to play the important part of the adult holdfast.

Post-embryonal Stages.

The appearance of the last pair of ribs may be taken as an indication that the embryonal stage of the plant has ended. At this stage, indeed, all the characters of the species that might be found in an adult frond are manifested, though in a primitive state.

The further development of the fronds after this stage is practically in the internal structure. Every element which makes up the blade, stipe, and holdfast gradually adds to its thickness and becomes more complicated. The external characters undergo but little change except that the size of the frond gains in an enormous degree. The fronds which measure 10–15 cm. at the end of December will attain to a total length of as much as 1·5 metres by the beginning of April. Among the minor changes in the external characters, the appearance of the longitudinal furrows on the stipe and the gradual change in the shape of the base of the lamina are to be mentioned.

As I remarked several years ago,[2] the general outline of the blade of a Laminariaceous plant undergoes gradual change by age. The important change is in the transitional region. Those species with a round or even cordate base of the lamina in the adult have, in a majority of cases, a cuneate or acute base while yet young. So also in the case of *Costaria*, as shown in Figs. 11, 12, Pl. LIII, and Fig. 29, Pl. LV. After a blade has been furnished with the five ribs, it begins to add to its length at a

[1] Setchell: Concerning the Life-History of *Saccorhiza*, p. 182.

[2] Yendo: Three New Marine Algae from Japan. Bot. Mag., Tokyo, 1903, vol. xvii, p. 101.

considerable speed, the breadth not following the growth in the same ratio. Late in spring we find many narrowly lanceolate blades, 30–50 cm. in length and 3–4 cm. in breadth, with the base gradually attenuating towards the stipe. At the beginning of summer the growth in length seems practically to have ceased in the blades and the increase in breadth becomes remarkable. The wearing away of the terminal portion of the blade proceeds downwards. As in other costated species the membranous part decays off one step further than the ribs. Finally the general outline of the blades become ovate with a broad cordate base (Fig. 29, Pl. LV).

At the stage when the growth in length of the blade becomes somewhat slow, a number of longitudinal ridges and furrows make their appearance on the stipe. They run longitudinally from the transitional region and disappear before reaching the holdfast. In dried specimens they are liable to be mistaken for the wrinkles caused by shrinking of the stipe on drying. The ridges have undoubtedly a certain connexion with the ribs in their position, but there seems to be no apparent rule in their relation. The intercostal area, however, becomes narrow at the transitional region and is always continued into one of the furrows. The ribs themselves have a furrow on each at the base of the blade. Some of the furrows are continuous for the whole way, but others are discontinuous (Fig. 29).

The cross-sections of the furrowed stipe show the arrangement of the subcortical cells in a peculiar manner. In general, the parenchymatous cells are arranged radially, as in other members of the Laminariaceae. Towards the depressions, however, the cell-rows gradually converge and in the elevations they diverge (Fig. 31). In the latter case the cell-rows do not radially split to increase their number fastigiately, but each cell below the anticlinal point is much stretched tangentially, as shown in Fig. 31. What makes it necessary to have such a peculiar construction in the stipe is at present beyond our comprehension. For the purpose of mechanical strength the structure of the ridges seems to be hardly expedient. Barber[1] has called attention to the fact that the ridge peculiar to the stipe of *Saccorhiza bulbosa* does not result from a rapid multiplication of the cortical cells, but from their increase in size. I mention this here as a curious coincidence in such widely different cases.

Harvey[2] described a perforated specimen of *Costaria Turneri* as a special form, calling it *forma pertusa*. It is, however, a rather aberrant case to find a frond of *Costaria Turneri* at an adult stage without any perforation. Generally speaking, a few holes are completed when a frond has attained some 20 cm. or so, and gradually increase in number as the blade extends. In the area above the transitional region, the holes are especially numerous, but in the upper portions of the blade new holes may also make

[1] Barber: Development of *Laminaria bulbosa*. Ann. of Bot., vol. iii, No. 9, p. 52.
[2] Harvey: Characters of New Algae, &c. Proc. of Amer. Academy, vol. iv, 1859, p. 329.

their appearance. The number of holes varies very much according to the habitat of the plant; in some cases there is a considerable number and in others only a few.

The mode of formation of the holes is essentially similar to that in *Agarum Turneri*, which has been studied by Humphrey.[1] In *Costaria*, however, the points of formation are not elevated on one side so remarkably as in *Agarum*. In a majority of cases one surface is slightly depressed and the other much more deeply (Fig. 28, Pl. LIV). The tissue at this point, with both surfaces thus drawn together, is gradually atrophied. The epidermal layer of the less depressed surface lasts longer than the other, so that a round, milky-white remnant of membrane may be often still found stretching over a small hole. The healthy borders of the two epidermal layers come together as the atrophied tissue melts away, and finally the scar is entirely healed. The youngest holes visible to the naked eye measure about 2 mm. in diameter. They are widened as the blade extends and may reach often as much as 1 cm. in diameter.

At the beginning of a post-embryonal stage the five ribs are evenly distributed in the lamina, dividing the entire breadth into six longitudinal strips. But in more advanced stages the marginal regions are far wider than the other intercostal areas and the lateral two ribs are more approximated to one another than to the midrib (Fig. 29, Pl. LV).

II. Undaria.

Embryonal Stages.

The early stages of development of *Undaria*, from the filamentous sporelings until about 2 mm. in total height, are in the essential points similar to those of *Costaria*. The cellular arrangement, however, so far as the specimens in my hands have shown, is not so regular as in the latter case. Even in the blades comparable with the stage shown in Fig. 4, Pl. LIII, the whole arrangement of cells could hardly be schematized in a simple geometrical figure. A frond measuring 0·9 mm. in total height, with a very short stipe and roundish blade—aberrant from the normal shape—had the cells in an especially disturbed condition.

The precortical layer makes its appearance when a plant has attained 1·7 mm. in total height. The mode of formation of this layer is quite similar to that in the case of *Costaria*. The tristromatic area, so far as any difference can be made out, is extended longitudinally, while attaining comparatively less width.

The young fronds a few centimetres high of *Costaria* and *Undaria* are hardly separable one from the other by the naked eye. But in *Undaria* as

[1] Humphrey: On the Anatomy and Development of *Agarum Turneri*. Proc. of Amer. Academy, 1886.

soon as the blade has attained to the length of 7·5 mm. or more, a conspicuous number of mucilage glands may be clearly seen under a hand lens. As an exceptional case I found a frond with the blade about 2 cm. in length and the midrib running half of its whole length—without the slightest doubt of its being *Undaria*—in which no gland could be detected.

The glands are far more numerous in the embryonal blades than they are in the youngest ligules of a more developed stage. They are denser in the marginal region than in the middle of the blade and the transitional region. Along the margin of a blade about 2·5 cm. in length I counted 120 glands in a square millimetre, taking both surfaces together.

The formation of the glands takes place after the hyphal structure has appeared in the meridional area above the transitional region. But when it has commenced its work, the glands are generated in the two-layered as well as in the more complex area (Fig. 34, Pl. LV). Hence, the formation of the glands is related to the age of a plant and not to the structure. In other words, the glands are formed after the epidermal layer has attained to a certain stage of development. An analogous case has been pointed out in the formation of cryptostomata in *Costaria*.

In my former paper[1] I have remarked that each glandular cell, as a rule, originates from a single cortical cell which is in contact with the epidermal layer. In the majority of Laminariaceae, the peripheral layers of the blade have distinct features from those of the underlying tissue in the cellular arrangement and in the cell-contents. In my use of the term 'cortical cells' I follow the practice of many algologists and mean the cells which constitute the layers interposed between the epidermal layers and medulla. It is a well known fact, however, that in the stipes the peripheral cells are hard to distinguish from the underlying layer, as the former give rise to the latter by the formation of tangential walls. German botanists include the whole portion of such tissue, in the stipes as well as in the blades, which has nothing to do with the hyphal cells, under the name 'Rinde'. Setchell[2] applies the term 'limiting layer' to the peripheral portion of the 'Rinde'. This layer is characterized by having its cells much smaller than those of the underlying layer. In a young blade which has a single layer of large parenchymatous cells between the well defined epidermis and medulla, he calls the interposed layer 'cortex'. And, in a more complex structure, he applies the term 'outer cortex' to the layers of small cells beneath the epidermis, and 'inner cortex' to those of large parenchymatous cells near the medulla. The outer cortex in his sense is nothing but the 'limiting layer' minus the epidermis. Barber[3] also noticed in the stipes of *Saccorhiza bulbosa* that the peripheral two layers are constructed of much smaller

[1] Yendo: Mucilage Glands of *Undaria*. Ann. of Bot., vol. xxiii, No. xcii, 1909, p. 621.
[2] Setchell: Concerning the Life-history of *Saccorhiza*, p. 200.
[3] Barber: l. c., p. 53, Fig. 11.

cells than the deeper-seated layers. He seems to believe all the layers to have originated from the same meristematic one, and he regards the above-mentioned differences as indicating that the growth of the stipe by formation of new cells is exceedingly slow.

In the young blade of *Undaria* I have noticed the transverse division of the well-defined epidermal cells in a plane parallel to the surface. The daughter-cells form a discontinuous stratum of cells under the epidermis; they are nearly equal in size to their mother-cells and much smaller than the pre-existing cortical cells. The latter are precortical cells or their derivatives. As the epidermis of the blade is a layer well defined from the beginning, the name 'limiting layer' cannot be applied to the resulting layers of *Undaria* without modifying Setchell's conception. The name 'Rinde', again, when applied to the tissue of the blade, comprises two distinct parts, one originating from the precortical and the other from the epidermal layers. Similarly the 'cortex' comprises the cells interposed between the epidermis and the medulla whatever may be the difference in their origin. The distinction of the cortex into outer and inner layers as proposed by Setchell in the case of the blades of *Saccorhiza* comes near to separating the tissues according to their origin.

Considering the origin of the glandular cells with reference to these conceptions, it may be advisable to modify my former statement concerning this point, as follows:—Each glandular cell, as a rule, originates from a subepidermal cell which has been generated from the epidermal cell above it.

The cryptostomata are produced from the epidermal cells after the glands have appeared. Their number is far less than that of the glands. On examining the mode of development of the cryptostomata, I cannot find any essential difference from what has been seen in *Costaria*, *Alaria*,[1] and *Saccorhiza*.[2] At the start, the position of the hairs is at the depth of one epidermal cell and is slightly depressed below the surrounding level. There is no special initiating cell comparable with that characterizing the Fucaceous cryptostomata (Fig. 35, Pl. LV).

The young blade of *Undaria* increases in area, adding the glands and cryptostomata proportionately. The appearance of the rachis takes place after the blades have attained at least 4 cm. in length. The primary rachis is constructed by cell-aggregation along the median line of blade. The origin of the cells is similar to that in the case of *Costaria*, but on both sides instead of one. The rachis is, of course, more sharply defined near the transitional region and gradually evanescent above. Such a stage of the plant is hardly distinguishable from the young lamina of *Alaria*, unless the presence of glands is taken into account.

[1] Murray: l. c., Plate XVI, Fig. 5–7.

[2] Murray: l. c.

Post-embryonal Stages.

The appearance of the four organs, rhizines, glands, cryptostomata and midrib, may be taken as marking the end of the embryonal stages of *Undaria*. The formation of pinnules and sporophylls remains to be accomplished before the individual is adult.

The indications of the pinnules are seen as minute lateral ligules on both sides of the base of the blade (Fig. 32). These ligules are shifted upwards, increasing more or less in size, as the blade grows at the transitional region. The result is the dentation of the blade margins near the base. New ligules are successively given off below the older ones as the plant grows larger and larger (Fig. 33). Full-grown adult plants have the blades 1·5–2·0 feet long and 6–9 inches broad. The ligules follow the growth of the blade, extending more in the transverse than in the longitudinal direction of the frond, to form the pinnules characteristic of the species. The sinuses between the adjacent pinnules are round, and in some forms they are very deep, approaching near the midrib, while in others they are comparatively shallow. It is clear from these facts that the extension of the ligules takes place in the entire area from the very tip down to the midrib. If the meristematic tissue were limited to the area in the transitional region near the midrib, the ligules should remain as dentations along the margin of the blade without adding to their size; and if it were situated in the ligule proper, the sinuses must reach down to the rib.

At the time when the primary ligules begin to appear, the cylindrical stipe becomes gradually compressed. In a middle-aged frond it is ancipitous, with prominent margins. The cross-sections of such a stipe show the medullary layer in a narrow strip bridging the space between the marginal ridges. The greater part of the section consists of thick-walled quadrangular cells, disposed in rows radiating from the medullary slit.

The sporophylls are practically over-extensions of the marginal ridges. As a plant approaches maturity, the marginal ridges extend in width as well as in length. The rate of growth in length of the sporophyll is far quicker than that of the stipe, which is often negligible at this stage. The undulation of the sporophyll naturally results. The matured *Undaria* has its stipe almost covered by the ruff-like foldings from both sides. As a rule, the extension is gradually propagated upwards from the point of its commencement. The point of commencement of the extension varies according to the locality where the plant grows. In the northern form, which has an enormous length of stipe, it is localized at the basal part of the stipe; in the short-stiped southern form the upper portions of the sporophylls are confluent with the base of the lamina. Moreover, in the southern form, sterile ligules from the margins of the sporophylls are generally found,

which is never the case in the northern. From these variations we may distinguish three forms of *Undaria pinnatifida*, Sur., thus:—

f. *typica*.
= *Undaria pinnatifida*, Sur. Illustr. Alg. du Japon, p. 77, Pl. VI–VII.
Ditto; Alg. Jap., Pl. X.
f. *distans*, Miy. et Okam.; in Okamura, Nippon Sōrui Mei, i, p. 128.
= *Alaria pinnatifida*, Harv. Charact. of New Algae (Proc. Amer. Acad., vol. iv, 1859), p. 329.
= *Ulopterix pinnatifida*, Kjellm.; in Kjellm. et Peters, Om Japans Lam., p. 275, Pl. XI.
= *Undaria distans*, Miy. et Okam. Report on Fisheries of Hokkaido, vol. iii, p. 57, Pl. XXVI.

Stipite longitudinem laminae profunde pinnatifidae subaequante, sporophyllis ad basin stipitis limitatis, majoribus, nudis.

Notice.—The present form has once been mentioned with specific rank by Miyabe, but afterwards as a form by himself and Okamura jointly. In both cases the diagnosis has been incompletely given, in Japanese only. I hope these authors will publish their discoveries in full detail in a form universally accessible. The Japanese characters are, unfortunately for us, unfamiliar to scientific circles. The present writer feels obliged to mention the manuscript names.

f. *narutensis*.
Stipite brevissimo, sporophyllis pauci-undulatis lamina confluentibus e margine ligulas steriles proliferentibus.

III. Laminaria.

According to Miyabe,[1] there are three species of *Laminaria* in the vicinity of Otaru Harbour, viz. *L. ochotensis*, Miy., *L. religiosa*, Miy., and *L. cichorioides*, Miy. The former two are so nearly related that we very often meet with specimens to which either description may be applied equally well. In the sterile, and even more so in the young fronds, the specific distinction is almost impossible.

In April, 1910, I collected numerous sporelings of a *Laminaria* at the Marine Station of Oshoro, near Otaru. The specimens may be either *L. religiosa* or *L. ochotensis*, but I cannot tell to which species they belong, for the reason above stated.

An examination of the sporelings showed close agreement in various points with the results obtained by many former observers on various species of Laminariaceae, as well as with the above statements on *Costaria* and *Undaria*. *Laminaria*, however, is separated from these two essentially by the presence of mucilage canals and the absence of cryptostomata

[1] Miyabe: Laminaria Industry. Report on Fisheries of Hokkaido, vol. iii, 1899, in Japanese.

and midrib. As it may not be uninteresting to compare these three members of the order, and as some points seem worth mentioning, a brief account of the early stages of development is given below.

Embryonal Stages.

In my collection the youngest specimen measured 0·45 mm. in total length, with a lanceolate monostromatic blade and short stipe. The transitional region was already three-layered, as seen under the microscope by varying the focus. Although I was not able to find the confervoid stage of the present species, I do not doubt that it may be similar to what has been observed in *Saccorhiza* by Thuret and in *Costaria* by myself.

A specimen measuring 1·62 mm. in total length is shown in Fig. 36, Pl. LV. The greater part of the blade, except a small area at the transitional region, is monostromatic. In the apical point we see the coaxial hyperbolic arrangement of cells still beautifully regular (Fig. 37). In more advanced stages when the blade has attained 4 mm. or more, the complex structure in the transitional region is highly developed. The primary hyphal cells traverse it longitudinally, having a vein-like appearance, anastomosing with the adjacent ones by short lateral branches. The minute structure of these cells, together with their relation to the precortical cells, is vividly seen in this species, by observing the blade *in toto* under a microscope. In *Costaria* and *Undaria* we could not trace the veinlets completely (Fig. 15, Pl. LIII). Fig. 45, Pl. LV, is drawn from a specimen with the frond about 2 cm. in total length. It is from about the middle part of the blade. In the lower portions towards the transitional region, the hyphae gradually become hard to trace, as the complexity of the structure makes the preparations less translucent. It will be recognized from this figure that room for the passage of the hyphae is sought in the intercellular spaces of the precortical cells. But this fact is more easily understood when observed in cross-sections of the blade at earlier stages.

Figs. 43 and 44 are cross-sections through the middle part and the transitional region respectively of a frond about 5 mm. in total length. In the former we see two hyphal cells in section at the meeting points of four rectangular precortical cells. An exactly similar figure has been drawn by Kützing[1] from a four-layered stage of *Laminaria saccharina*, though he does not give a full explanation of it. In the transitional region, which shows a more advanced state of the tissue, the two layers of the precortical cells are separated by the gelatinous matrix interposed between them. The hyphal cells are no longer compressed in the intercellular spaces, but have a free medium around them. They terminate at the external limit of the four-layered area, finally joining on to a comparatively small precortical cell.

[1] Kützing: Phyc. Gen., Pl. XXIV, I, Fig. 3.

The latter, as in the case of *Costaria* above alluded to, result from the division of the pre-existing cells. The lower limit of the hyphal strands runs into the complex tissue of the transitional region. Judging from these facts, the hyphal cells seem to be added to the distal ones at the transitional region. They elongate to follow the extension of the surrounding area, keeping their original diameter. Hence, it may be understood that the primary septa of the hyphal filaments, at least in the embryonal stages of the blades, do not result from the division of the already completed hyphal cells. At a later period new hyphal cells may grow out as branches of the inner cortical cells, or the older cells may multiply by formation of septa. It is very interesting to note that the junctions of the hyphae are mostly at the intercellular spaces of the precortical cells; and that the expansion of the terminal portion of the cell, to form the so-called trumpet cell, is already to be seen at such an early period of development.

Taking the results obtained in *Costaria* and *Undaria* into account, it will be clearly seen that the sporelings of the Laminariaceous Algae start at first from a simple cell-row, then become monostromatic, distromatic, and so on, and finally proceed to the complex structure with hyphal tissue in the medulla. This accords satisfactorily with the conclusions arrived at by former investigators. One thing, however, remains to be remarked about the sclerenchymatous cells of *Saccorhiza dermatodea* which were termed 'tubular cells' by Setchell.[1]

Setchell [2] describes, in a very young blade of *Saccorhiza dermatodea*, a layer of the tubular cells interposed between the two epidermal, or, as he calls them, limiting layers. These tubular cells are, according to him, an important element of the medulla in the blade and stipe. He further describes a plant which has the first set of hapteres well developed. In it, at the tip of the blade, he finds 'a well-defined limiting layer on each surface, and within this, on both sides, a cortical layer of a single series of cells, and in the centre the medulla, represented by a few scattered tubes'.[3] He has nowhere given any decisive information relating to the origin of either the tubular cells or the cortical cells. But it is possible to understand from what he describes in various parts of his paper, that he regarded them both as having originated from the epidermal layers. It naturally follows from his statements that the medullary elements at the beginning, and the cortical at the next stage, are generated from the primary epidermal tissue.

The same writer attributes the origin of the hyphal cells to the ramifications of the inner cells of the two- or three-layered cortex. This leads us to suppose that in *Saccorhiza dermatodea* the hyphal cells are developed at a somewhat later stage than in our plants.

As in the case of *Costaria* and *Undaria*, the blades of the present

[1] Setchell: Concerning the Life-history of *Saccorhiza*, p. 195.
[2] Ibid. [3] Setchell: l. c., p. 204.

Laminaria, even after they have attained to several centimetres in length, still keep their primitive structure. In a blade of 5–6 cm. in length we usually have the apical portion in a monostromatic state, and the greater part of the area in the four-layered condition. The diagrammatic figure showing the boundaries of the different numbers of strata is represented in Fig. 39, Pl. LV. Compare this with Fig. 13, Pl. LIII, which is of *Costaria*. Only in the transitional region do the precortical layers leave some spaces between them filled with a gelatinous matrix; they are in close contact in the remaining area. The meridional area can only be distinguished from the marginal when a blade has attained 10–12 cm. in length. In the younger stages the marginal parts of course have a more simple structure when compared with the middle. Still, we can by no means regard the latter as the indication of the meridional area.

In most Japanese forms of *Laminaria*, the meridional area is much thicker than the marginal regions. It has, in some species, a different structure when compared with the latter. For example, the meridional area of some species is provided with double layers of lacunae while there is a single layer in the marginal regions. Still more remarkably, the meridional area is depressed on one surface and elevated on the other. Along each side of the area, in *Laminaria ochotensis*, there is a narrow canaliculation which dies out near the transitional area. In some species the meridional area is comparatively narrow, and in others much broader. The cross-sections of a blade, therefore, give a bilaterally symmetrical undulating figure, characteristic of the species (Fig. 46, Pl. LV). Actual observation of the growing plants reveals that the depressed meridional area is always turned towards the source of light unless disturbed by an external mechanical force. The appearance of sori mostly begins on the shaded side, and is often limited to it. Thus in the blade of *Laminaria*, at least in the Japanese forms, there is a dorsiventral differentiation.

The dorsiventrality of the blade in the species under consideration is indicated in the transitional region when a plant has attained 2 cm. in total length. The one side, probably the future under-side, is elevated, while the other remains flat (Fig. 38). The characteristic canaliculation, however, becomes recognizable in much more advanced stages, viz. after a blade has attained 50–70 cm. in length. In some individuals two rows of dimples with regular intervals appear on both sides of the meridional area, no doubt owing to the difference in the speed of growth of the two areas. The canaliculations are not really distinct until the dimples have disappeared.

The differentiation in the internal structure of the stipe is first to be noticed when a plant has attained 4–5 cm. in total length. The medulla is not sharply delimited from the inner cortex, but may be more or less distinguished by the cellular arrangement. The loose anastomosing structure of the hyphal cells is limited to the base of the transitional region (Fig. 38).

In a primary stipe which is a few millimetres in length, the medulla is quite wanting. As the meristematic point lies in the transitional region, and as the hyphal cells are generated after the precortical layers have been formed, the lack of the medullary tissue in the primary stipe is to be expected. Barber,[1] speaking of the stipe of *Saccorhiza bulbosa*, states that in the portion below the ridge and in the hapteres there is no formation of the hyphal tissue. Setchell[2] noticed also in a young frond of *S. dermatodea* the presence of the hyphal cells at the transitional point only.

The primary root of a plant measuring about 2 cm. in total length is round and disc-shaped with a diameter of about 1 mm. (Fig. 40, Pl. LV). The periphery is still felty at this stage. When a frond has attained the height of 7 cm. or so, the disc-shaped root extends horizontally, forming a haptere or scutellum with an uneven surface and a few shallow notches on the margin (Fig. 41). The periphery is now thick and rounded, and is undoubtedly constructed of a compact tissue of minute cells. Indications of the future rhizines may be seen as insignificant outgrowths around the point just above the primary haptere. These rhizines quickly elongate, the primary haptere extending as well, first horizontally, but soon bending downwards to form the primary holdfast (Fig. 42). The further development consists in the addition of new whorls of rhizines above the older ones, the diameter of the stipe and rhizines increasing at the same time.

The part of the primary stem in which the medulla was absent is practically lowered down as the stipe elongates, and becomes buried within a part of the axis of the holdfast. And this part decays away before long with the primary haptere. Hence, in an adult form, no part of the stipe is devoid of medulla.

The two species of *Laminaria* above mentioned are characterized by the constant presence of lacunae in the stipe as well as in the lamina. In these, the lacunae are situated close along the periphery and are regularly arranged, with narrow intervals between one another.

A search for the lacunae in young specimens gave a negative result. In a specimen with the total height 54 mm., maximum breadth of blade 21 mm., and diameter of stipe 2 mm., no indication of the lacunae could be seen in the whole length of the plant. The younger specimens of course were devoid of them. These searches have been carried out by making sections at every millimetre of the frond, and also by staining, *in toto*, in aqueous solution of aniline blue. The blade of *Laminaria*, suitably fixed beforehand, and preserved in alcohol, is dipped in aniline blue (after Ranvier) for half an hour; then washed thoroughly in 70 % alcohol. The lacunae only are then stained a deep blue. Thus the presence and the distribution of the lacunae may be satisfactorily demonstrated.

The specimens collected in July and August, which had the stipes

[1] Barber: l. c., p. 54. [2] Setchell: l. c., p. 197.

3·5–4·5 mm. in diameter, are provided with well-developed lacunae. But in a specimen collected late in June the lacunae are generally narrow and slit-like, although their number may be quite as great as in the August specimens. Unfortunately, I have no set of specimens from April to June, and so am unable to trace the exact stage of the plant when the lacunae begin to appear. It is a fact, however, that the lacunae in our species are not formed until the frond has attained a considerable height. The period when the first-year plant has ceased its quick growth in length and begun to add to its width may probably be the time of lacunae-formation.

General Conclusions.

1. The earliest stage of development of the sporelings of the Laminariaceae investigated is a confervoid body growing by a single apical cell. The confervoid body becomes monostromatic in the next stage, with a monosiphonous stipe. The growth of the monostromatic blade is initiated by the two cells situated side by side at the same level beneath the apical cell, the axis of the blade passing between the two cells.

2. The monostromatic blade becomes distromatic at its base; the monosiphonous stipe becomes polysiphonous at the same time. A new meristematic tissue begins to appear at the transitional region between the blade and the stipe.

3. The growth in length as well as in breadth is due, at a certain period, to both the apical and the stipo-frondal growth. The apical growth is gradually retarded, and finally ceases. Erosion of the apex of the blade follows next.

4. A single precortical layer of large parenchymatous cells is generated at the transitional region between the already-existing two layers. The former soon becomes two-layered, and adds to the number of its layers later on. Additions of layers of cells are, as a rule, limited to, and begin at, the transitional region.

5. The hyphal cells are generated as the precortical layer becomes doubled, and the expansion of their distal ends into a trumpet shape takes place at the intercellular spaces.

6. The rib and the meridional region are formed by special thickening of the cortical layers. The dorsiventrality of the lamina, if it exists, is indicated simultaneously with the formation of such parts.

7. In *Undaria* the mucilage glands are developed at an early stage, but in *Laminaria* the appearance of the lacunae does not take place before the blade has attained to a considerable length.

8. The cryptostomata in the Laminariaceae are not generated from a single initial cell. Each hair has its origin in an epidermal cell of equal value, except that those in the middle develop earlier than the peripheral cells.

EXPLANATION OF PLATES LIII–LV.

Illustrating Professor Yendo's paper on *Costaria*, *Undaria*, and *Laminaria*.

Figs. 1–31. *Costaria Turneri.*

PLATE LIII.

Fig. 1. Filamentous stage of a sporeling; the basal portion unknown, as it was hidden in a tuft of *Elachista*. × 450. From a fresh specimen.

Figs. 2–4. Further advanced stages. × 450. From fresh specimens.

Fig. 5. More advanced stage with root-strands just formed; the stipe has become poly-stromatic. × 150.

Figs. 6–10. Stages from the beginning of the appearance of the midrib up to the formation of the three ribs. The shaded area shows the di- and poly-stromatic portion, and the non-shaded area the monostromatic. All in nat. size.

Figs. 11 and 12. Five ribs formed; the characteristic bullation is especially conspicuous along the midrib. Nat. size.

Fig. 13. A diagrammatic figure showing the boundaries of the variously layered areas; *a*, *b*, *c*, *d*, *e*, mono-, di-, tri-, tetra-, poly-stromatic respectively.

Fig. 14. Primary root of a frond measuring about 5 mm. in total length. × 150.

Fig. 15. A frond before formation of the midrib; the outer area without shading is monostromatic, in which, however, oases of distromatic spots are scattered. Observe the irregularly toothed boundary of the polystromatic area. The hyphae are also seen as anastomosing, longitudinally running veinlets in the polystromatic area. × 15.

Fig. 16. The upper half of the blade shown in the preceding figure more highly magnified.

Fig. 17. One of the small distromatic oases, showing the irregular cell-division; the underlying cells are shaded, with finer outlines. × 220.

Fig. 18. Schematic figure reduced from Fig. 4, showing the hyperbolic arrangement of cells; *a*, *a*, the two initiating cells.

PLATE LIV.

Fig. 19. A part of Fig. 17 still more highly magnified; areoles of the epidermal cells, which have lately divided from a common mother-cell, are defined with thicker walls. The shaded area signifies the distromatic part; in this, the irregularity of the boundaries between the monostromatic and the distromatic is seen to be related to the areoles. × 220.

Fig. 20. Cross-section of blade of a young plant about 1·5 mm. in total length, through the apex of tristromatic area; in the middle a primary cortical cell is seen. × 240.

Fig. 21. Cross-section of the same lamina through the upper part of the four-layered area; about *a* the axis of the lamina passes. The areas with different numbers of cell-layers are seen with remarkable regularity. × 240.

Fig. 22. Reproduction of a figure delineated by Kuckuck. The figure is said to show a cross-section of a blade of a young *Laminaria*. × 150.

Fig. 23. Diagrammatic figure showing longitudinal section of a blade with the primary medulla formed.

Fig. 24. Cross-section of the transitional region of a plant with the blade about 12 mm. in length. × 100.

Fig. 25. Cr ss-section of a blade with the three ribs just formed, to show the origin of the hairs. × 450.

Figs. 26 and 27. Cross-sections of blade of a plant about 10 cm. in total length, through points about 1 cm. from the base and 2 cm. from the apex of the blade respectively. From a fresh specimen. × 450.

Fig. 28. Cross-section of a blade through a hole in course of formation. × 25.

PLATE LV.

Fig. 29. Lower portion of an adult frond. *p*, the perforations. Nat. size.

Fig. 30. Cross-section of the stipe at a point about 1 cm. from the base of the blade. × 2.

Fig. 31. A part of the above, to show the structure of the ridges and furrows. × 54.

Figs. 32–5. *Undaria pinnatifida* f. *distans*.

Figs. 32 and 33. Young blades with the midrib and the ligules formed. Cryptostomata are frosted over the blade except at the transitional region and on the midrib. Nat. size.

Fig. 34. Cross-section of blade of a plant about 6 mm. in total length, to show the 'Anlage' of three mucilage glands in the distromatic area. × 450.

Fig. 35. Cross-section of blade through a young cryptostoma. × 450.

Figs. 36–46. *Laminaria* sp.

Fig. 36. An embryonal frond with polystromatic transitional region. × 36.

Fig. 37. The apex of the same. × 240.

Fig. 38. Cross-section of the basal portion of blade of a plant about 2 cm. in total length. About *a* is the axis of the blade. × 240.

Fig. 39. A diagrammatic figure to show the boundaries of variously layered areas; *a*, *b*, *c*, *d*, *e*, mono-, di-, tri-, tetra-, poly-stromatic area respectively.

Fig. 40. The simple primary root from a plant about 2 cm. in total height. × 10.

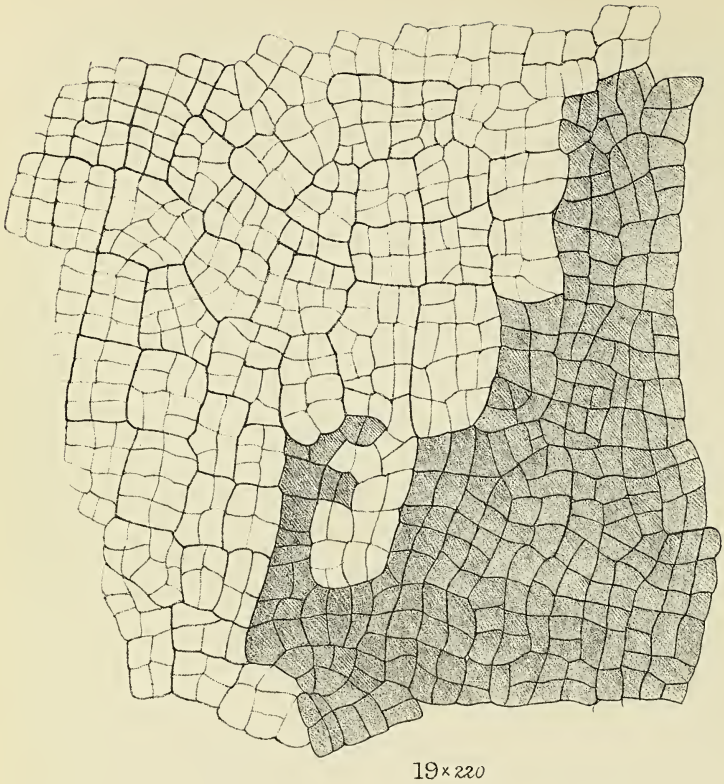
Fig. 41. The same, from a plant about 7 cm. in total height; the 'Anlagen' of the rhizines are seen as small wart-like protuberances near the scutellate root. × 10.

Fig. 42. A more advanced stage of the same, from a frond about 20 cm. in total height. × 5.

Figs. 43 and 44. Cross-sections of middle and transitional region respectively of a blade measuring about 4 mm. in length. × 450.

Fig. 45. A portion of four-layered area with hyphal cells seen from the surface by varying the focus. The small cells at the right hand show the epidermis; large cells, the precortical layer; the shaded cells, the hyphal filaments. The left upper side is the limit of the four-layered area. × 240.

Fig. 46. Cross-section of blade of *Laminaria ochotensis* at middle part of its whole length. Nat. size.



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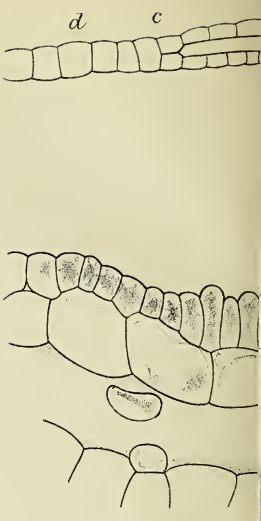

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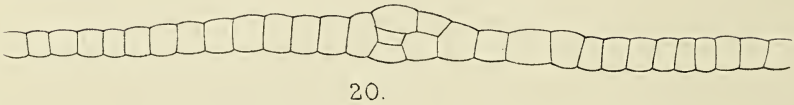

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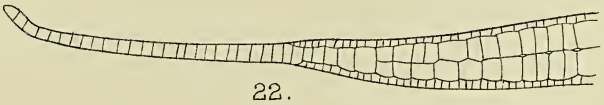

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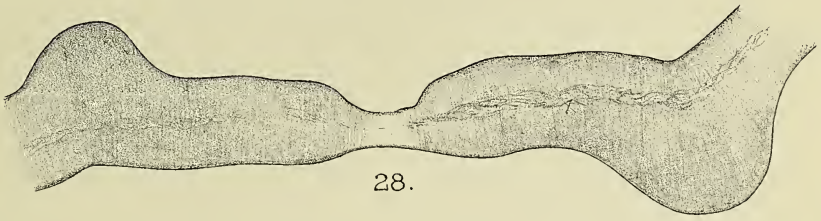

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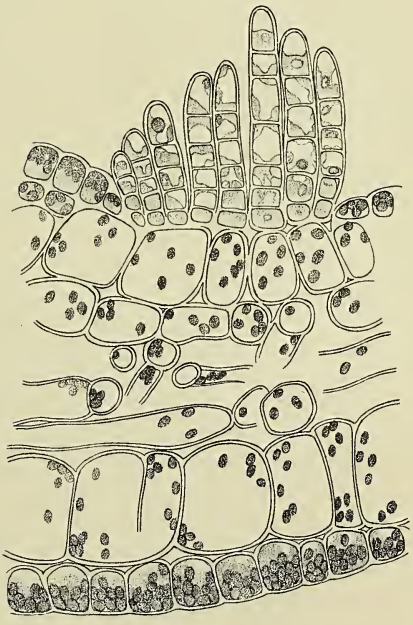

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Huth, lith. et imp.

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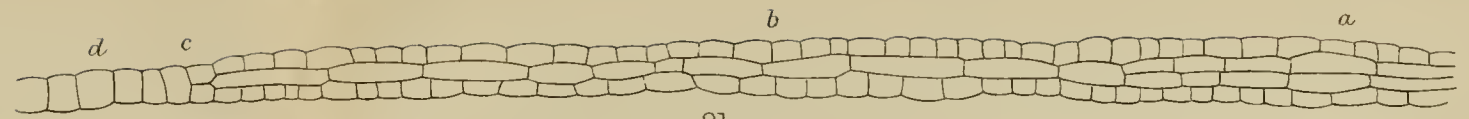

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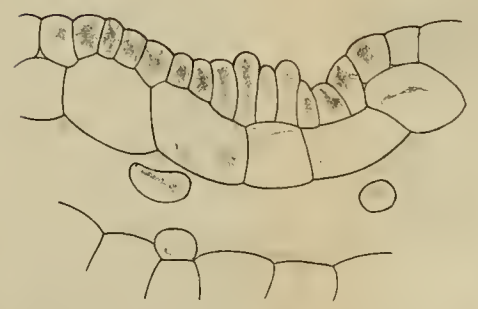

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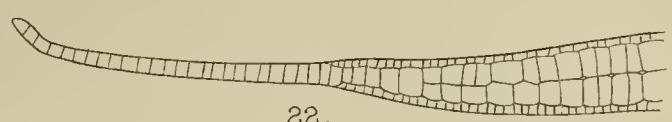

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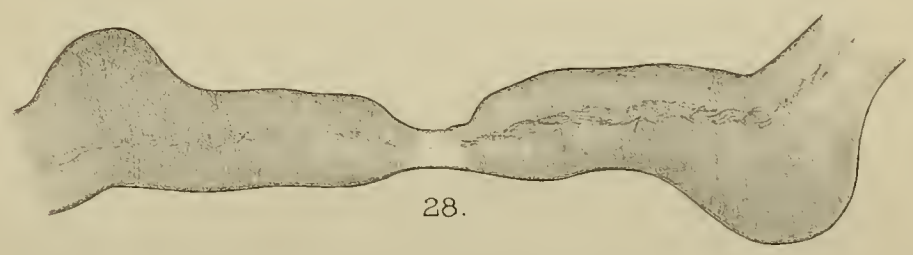

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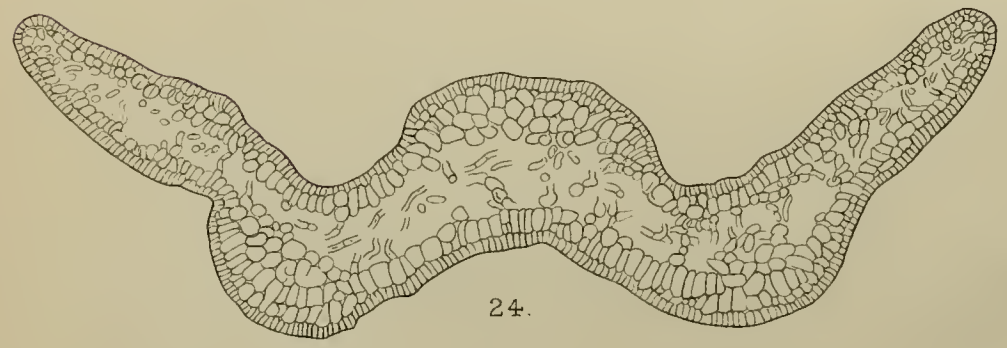

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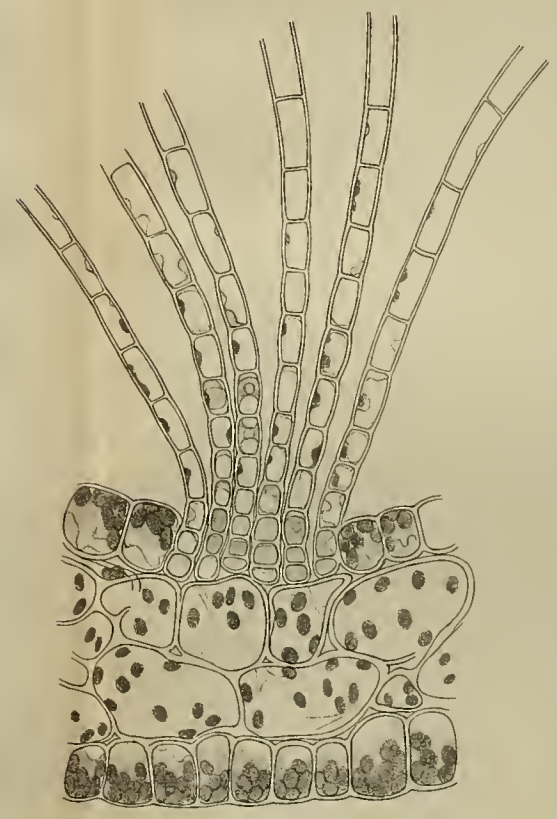

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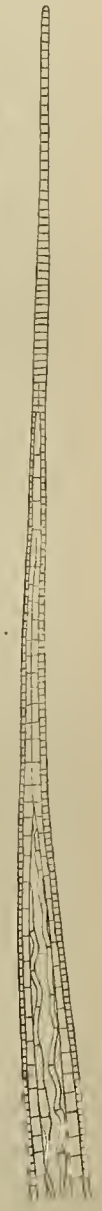

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YENDO——LAMINARIACEAE.

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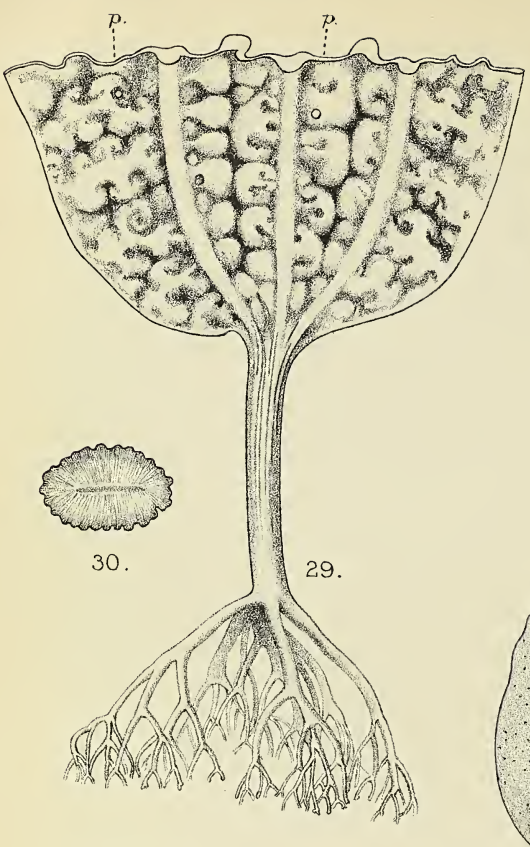

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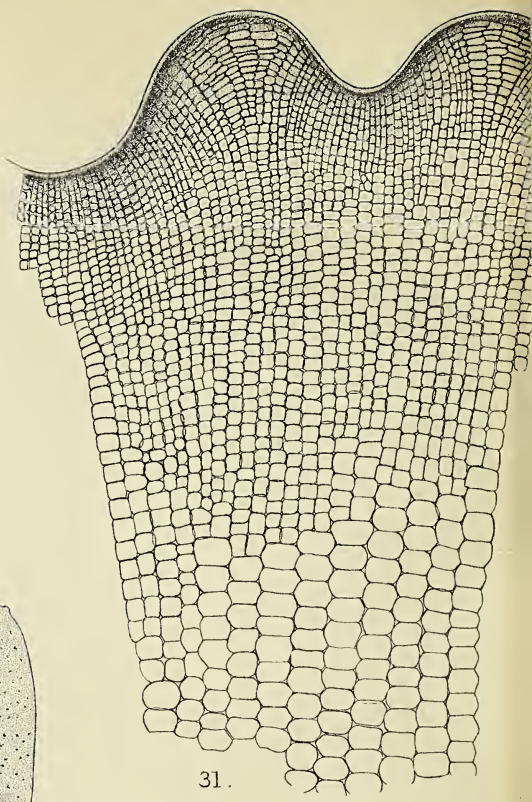

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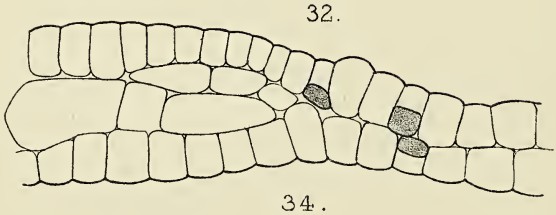

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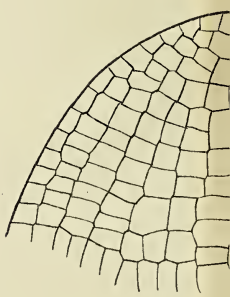

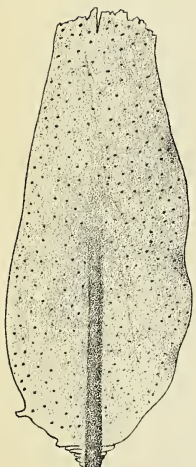

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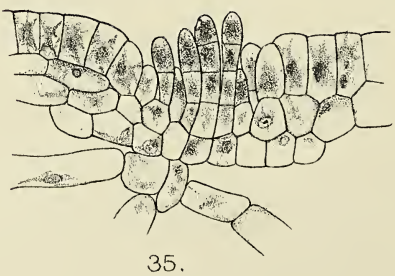

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YENDO —— LAMINARIACEAE.

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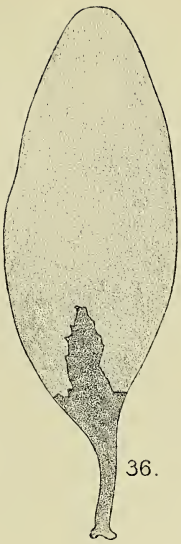

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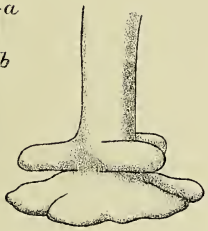

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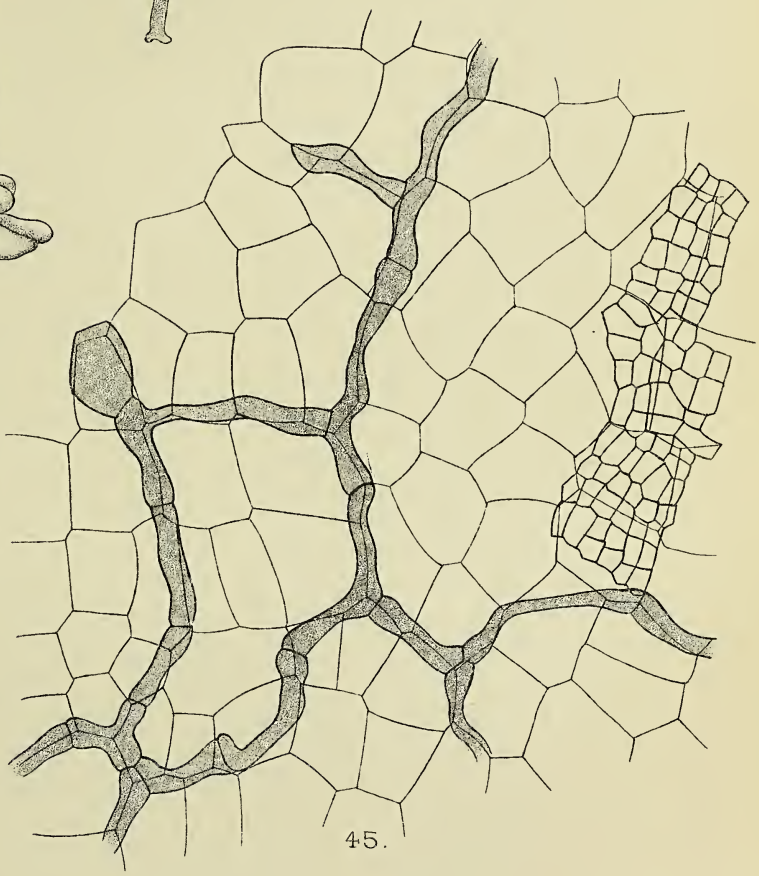

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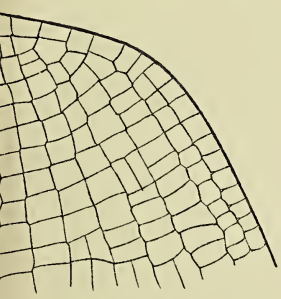

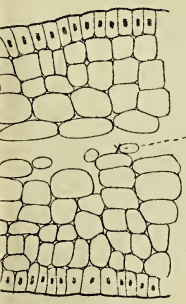

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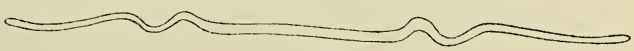

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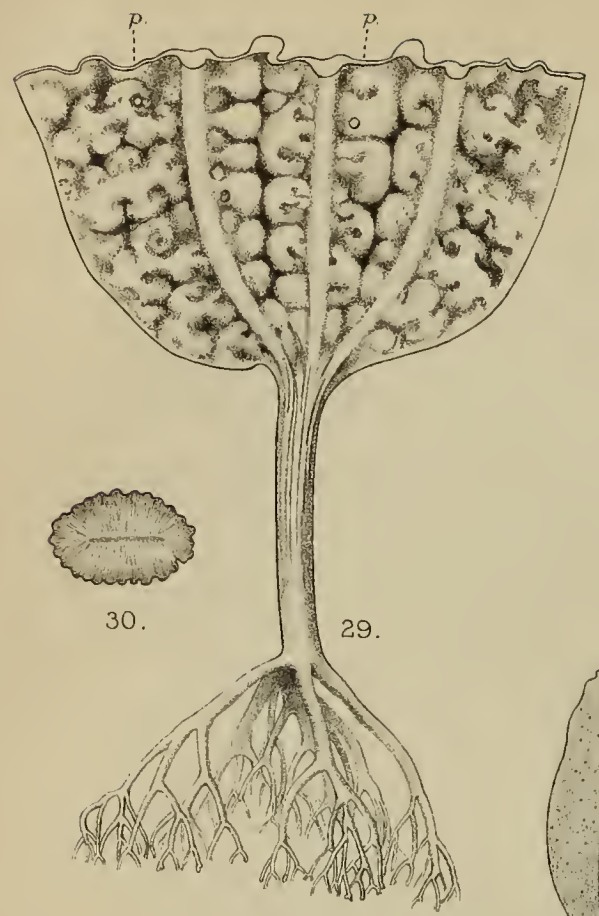

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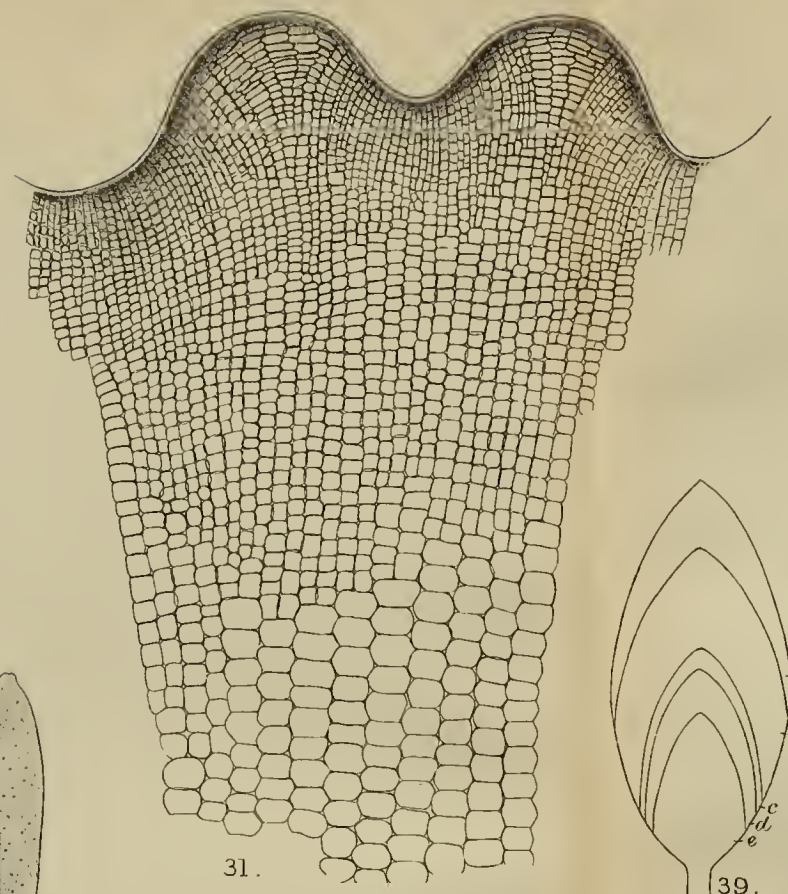

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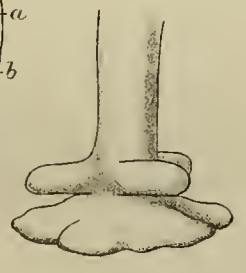

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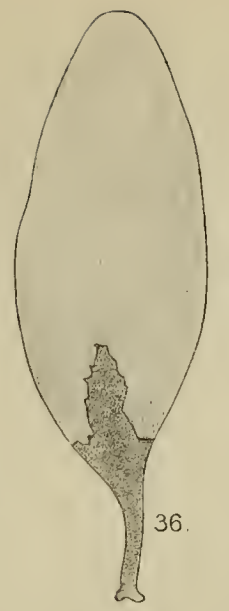

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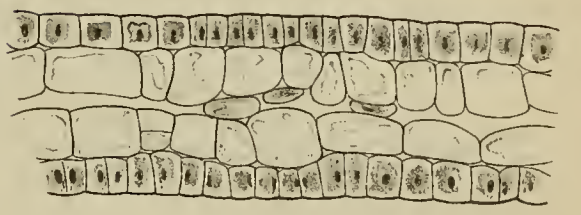

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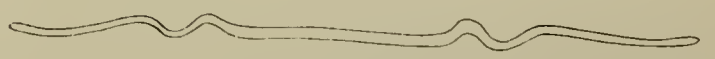

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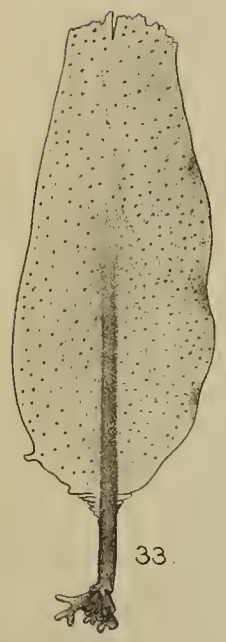

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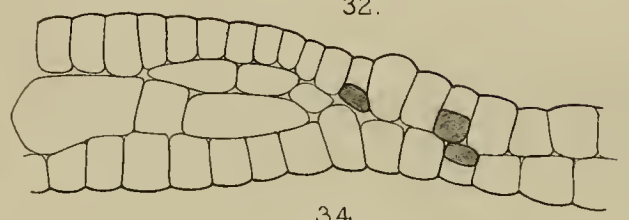

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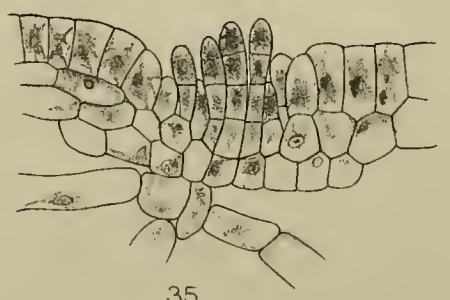

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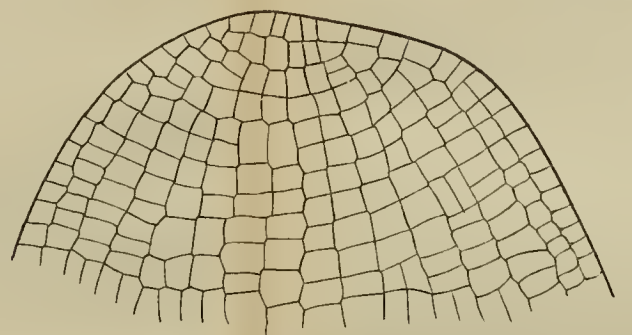

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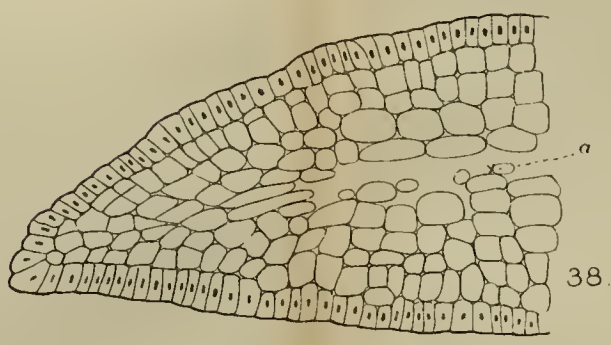

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YENDO —— LAMINARIACEAE.

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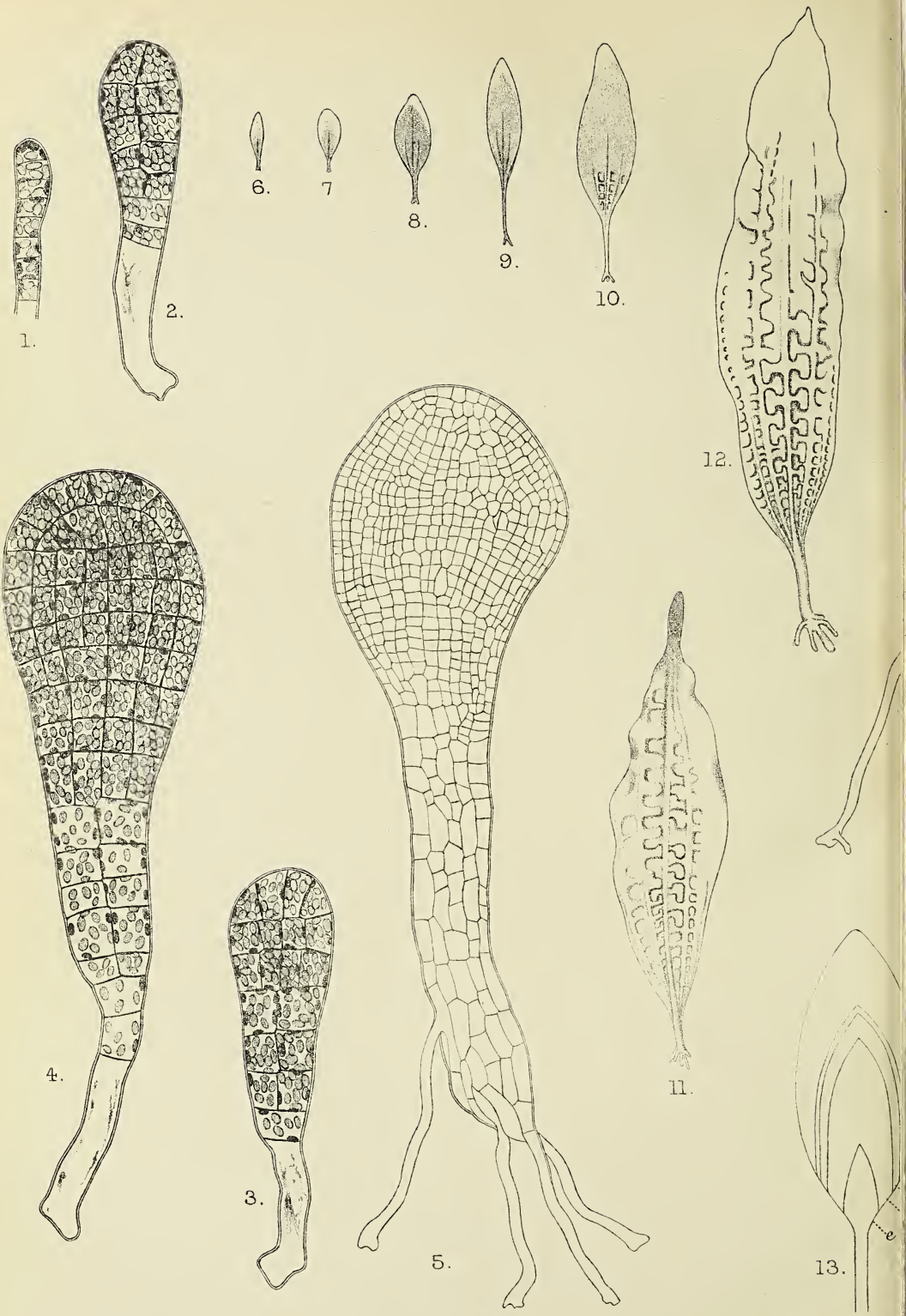

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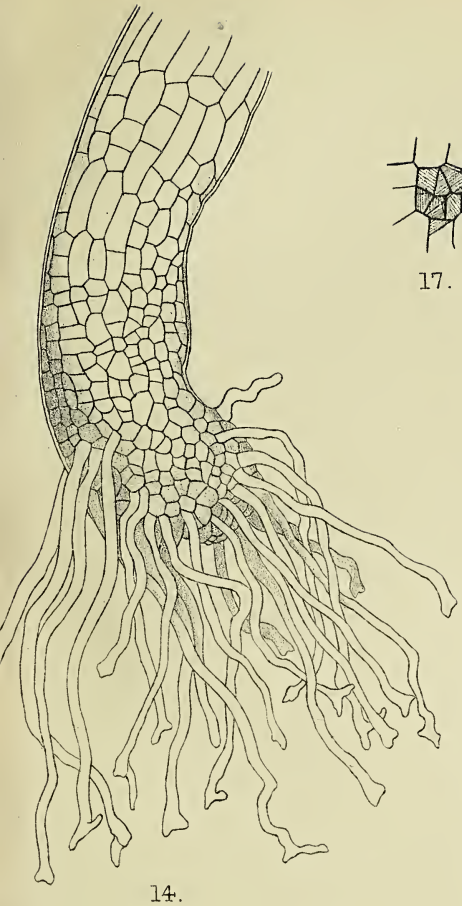

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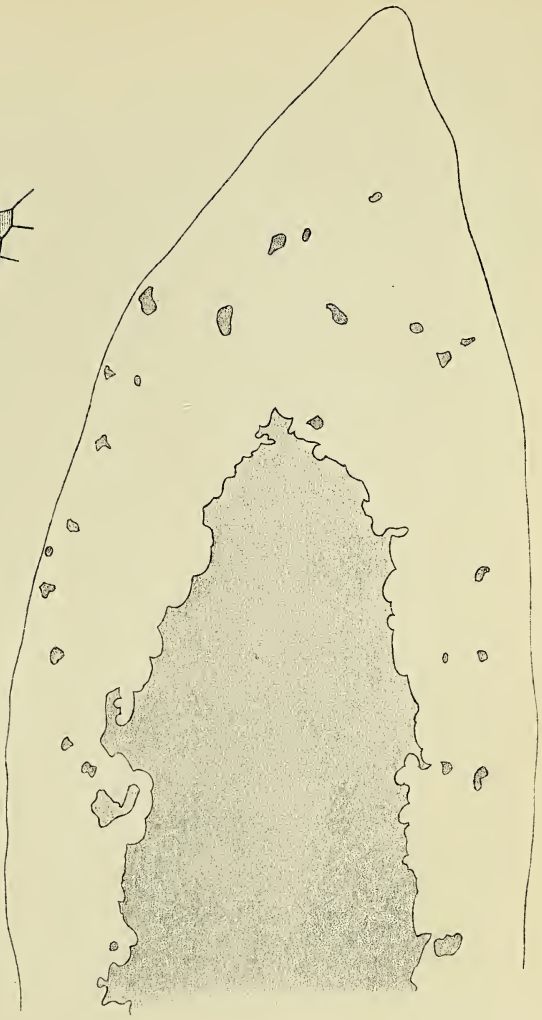

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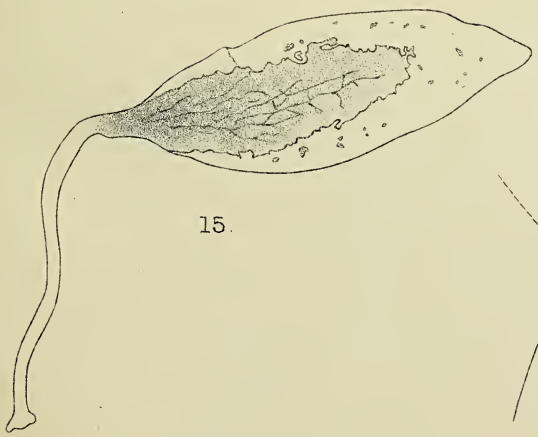

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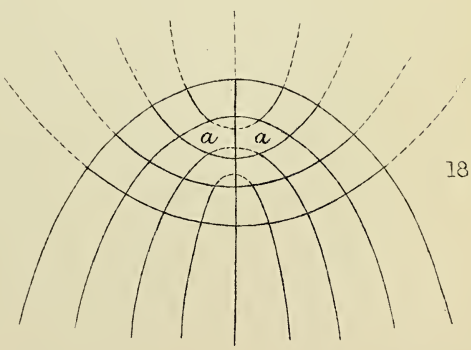

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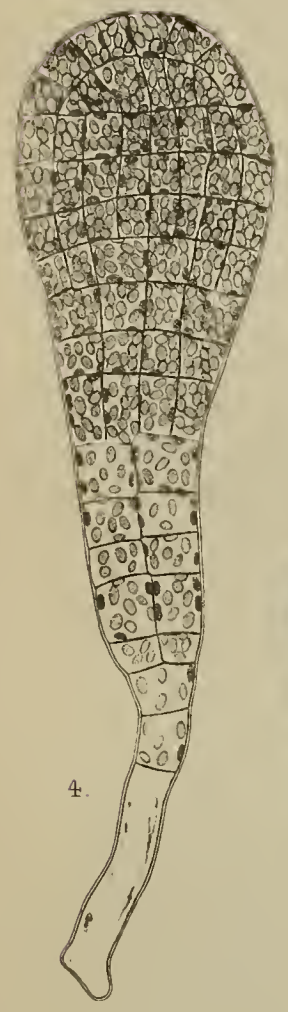
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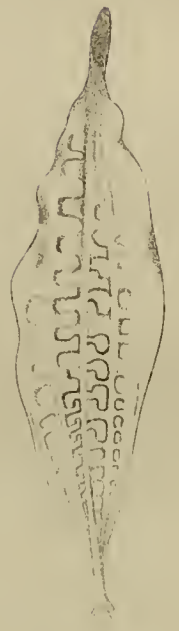
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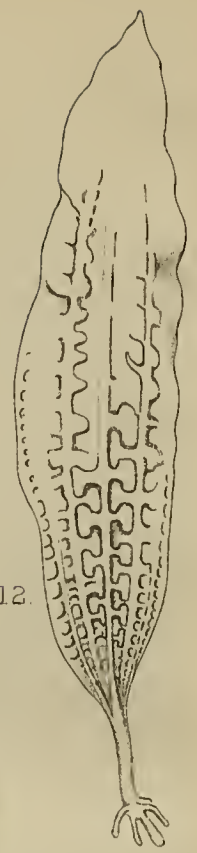
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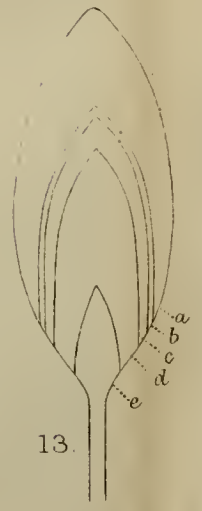

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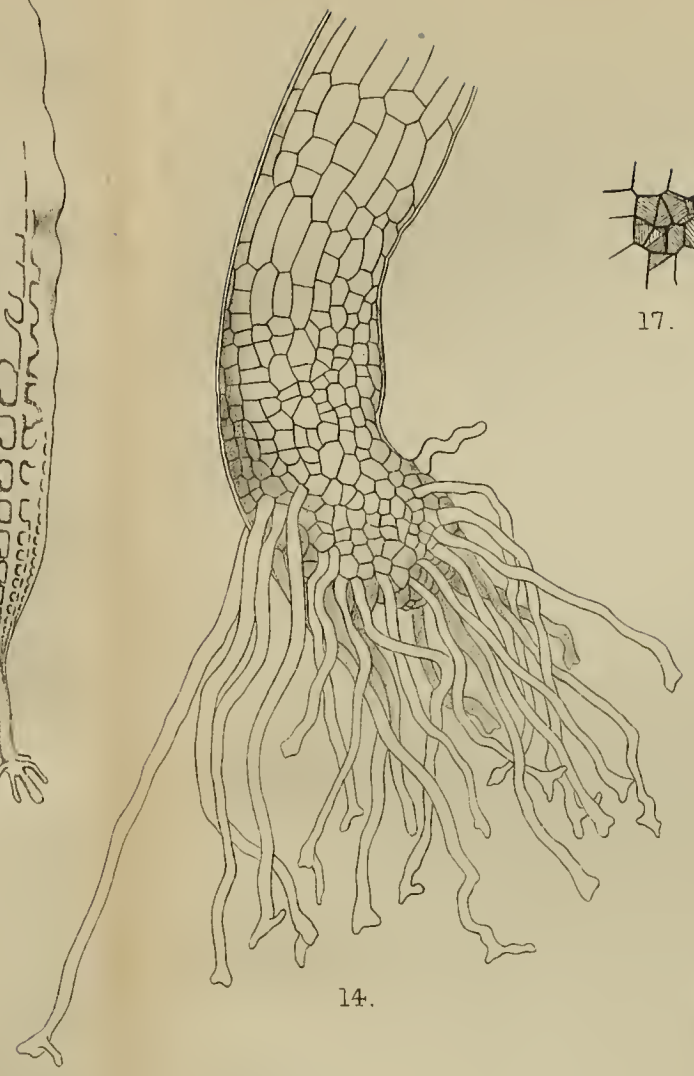
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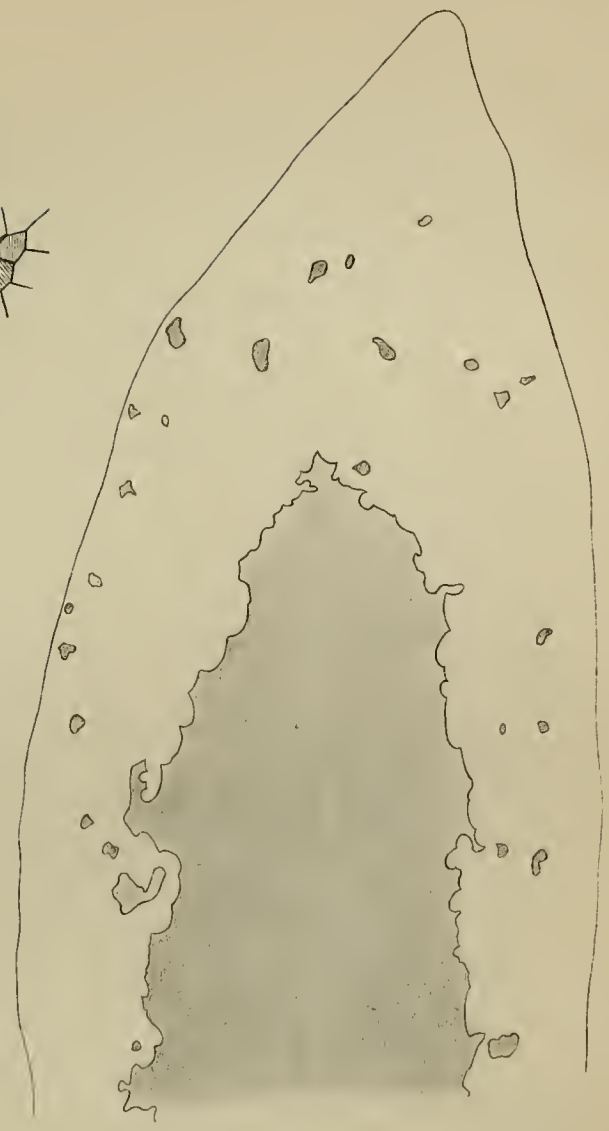
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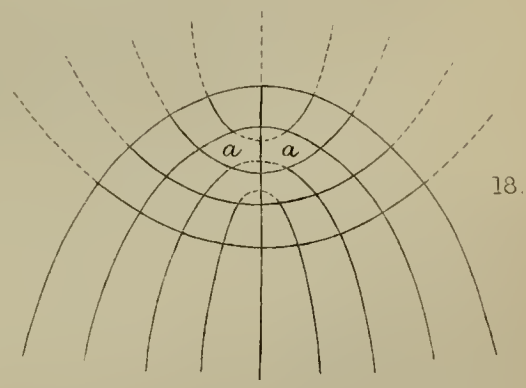

18.

YENDO——LAMINARIACEAE.

Huth. lith. et imp.

The Origin of Monocotyledons from Dicotyledons, through Self-adaptation to a Moist or Aquatic Habit.[1]

BY

G. HENSLOW, M.A., F.L.S., F.G.S., F.R.H.S., V.M.H.

CONTENTS.

1. INTRODUCTION.

NEARLY twenty years ago (1892), I pointed out that there is a large number of coincidences between the morphological and anatomical characters of Monocotyledons and Aquatic Dicotyledons, sufficient, in fact, to justify the conception that the former class had been evolved from the latter. Beyond a limited extent in experiments upon adaptation, the

[1] Supplementary Observations and Experiments to 'A Theoretical Origin of Endogens from Exogens, through Self-adaptation to an Aquatic Habit'. Journ. Lin. Soc. Bot., xxix, 1892, p 485.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

'theory', as I then called it, was not experimentally verified. Several corroborative experiments, however, have been made since; which prove incontestably that the special and hereditary, acquired characters residing in the roots, stems, and leaves of Monocotyledons are precisely the same as those which arise in Dicotyledonous plants, when responding to an aquatic environment, whether seen in Nature or in artificial experiments at the present day. Therefore, one need not entirely rest satisfied with Induction, or the accumulation of a very large number of coincidences, all of which conspire, more or less independently, to establish the conclusion herein maintained.

Since my former paper was published, numerous other coincidences have been noticed, so that my present object is to supplement that paper, and thereby to strengthen the argument with *addenda*, fortified by experiments.

2. Evidences from Geology.[1]

During the last twenty years much new knowledge has been acquired about fossil plants. The researches of Prof. Seward, Dr. Scott, and others have been ably put before us. For instance, Dr. Scott thus writes with regard to the relationship between Monocotyledons and Dicotyledons: 'If the Angiosperms were derived from the Cycadophyta, it would appear to follow that the Dicotyledons were first evolved, for their structure has clearly much more in common with the Cycad type than that of Monocotyledons. The latter would thus be regarded as a branch line of descent, diverging, no doubt at a very early stage, from the main Dicotyledonous stock. This view has been maintained, on other grounds, by various modern botanists. So far, however, as the palaeontological record shows, the two classes are of almost equal antiquity, both appearing for the first time in Lower Cretaceous rocks.'[2]

The reason of our great absence of knowledge of plant life during the Secondary Epoch is because the Liassic and Oolitic series of strata are almost entirely marine; so that countless ages passed, during which we know comparatively little of the land floras.

As exceptions to this statement, the 'Dirt-bed' of the Purbecks, which I investigated many years ago, is very rich in plant remains; and Dr. Scott reminds me of the American deposits which are extraordinarily rich, being contemporary with our own Oolites. Still, taking all the plant beds known of the Secondary Epoch, they do not amount to very much in

[1] See loc. cit., p. 486. Similar references are always to my former paper.

[2] Studies in Fossil Botany, Pt. II, p. 660.

comparison with what must have been the whole land surface of the globe during that prolonged period.

That Monocotyledons should have arisen nearly contemporaneously with Dicotyledons is not surprising if my contention be true; because rivers and lakes as well as morasses, we may be sure, abounded in all ages.

It has been suggested that the most primitive plants were aquatic, and that some took to land and became terrestrial. Unfortunately, Geology has revealed nothing to support this theory or the reverse; but that all Dicotyledons now existing have been evolved from xerophytic Gymnosperms is generally admitted, and that Monocotyledons, as now limited, were derived from the former is becoming generally accepted. There are about a dozen genera of the order Naiadaceae, all of which are aquatic plants, most of them living in the sea; but that they are *degradations* and not primitive is obvious. No existing order of either class can be said to be primitive, because of their simple structure; for this, in all cases, is the result of degradations. The same must be said of fresh-water plants.

3. Distribution and Percentages of the Natural Orders of Monocotyledons as compared with those of Dicotyledons.[1]

If it be asked where Monocotyledons were evolved, the present distribution of this class may perhaps indicate the region. Of the thirty-two orders described by Bentham and Hooker in the 'Genera Plantarum', twenty-one are chiefly found in the Tropics and warmer regions of the Temperate Zones; whereas only six have species reaching the Arctic and Antarctic regions.

Sir J. D. Hooker, in his Introductory Essay to the 'Flora of Tasmania', tells us that there are the following proportions between Monocotyledons and Dicotyledons:—

	Temperate Floras.	
Europe.	Monocotyledons : Dicotyledons	1 : 5·2
Russian Empire	. . . . .	1 : 5·1
British N. America	. . . . .	1 : 3·8
South Africa	. . . . .	1 : 4·2
Australia	. . . . .	1 : 5·0
	Tropical Floras.	
W. Tropical Africa	. . . . .	1 : 3·6
Ceylon	. . . . .	1 : 3·1
India	. . . . .	1 : 3·8
Tropics generally	. . . . .	1 : 3·0
Australia	. . . . .	1 : 3·5

This table, therefore, illustrates the same phenomenon.

[1] loc. cit., p. 487.

Now if the order Cycadaceae, represented by the Bennettiteae, perhaps a very characteristic group of the Secondary Epoch, gives us any clue as to climate, then it might be inferred from the present distribution of the Cycadaceae that Monocotyledons arose in the warmer regions of the world.

4. Degeneracy of Monocotyledons.

Professor Sacco draws the conclusion from Palms being almost entirely restricted to the tropics, that they cannot adapt themselves to cooler temperate climates, either morphologically or physiologically.[1]

This would seem to apply, however, to the great majority of Monocotyledons in different degrees, and the explanation of the fact, I would suggest, is, that having become degenerate in structure by having lived originally in water, they cannot now, as land plants, recover to the full the power necessary to construct such tissues as are found in Dicotyledons capable of resisting the injurious effects of a cold climate. Moreover, having lost the power of making massive timber of concentric cylinders of wood—really girders in combination—they cannot support large trunks.

The degraded condition of all Monocotyledons is also insisted upon by Prof. Hugo de Vries. He says:[2] 'Monocotyledons are obviously a reduced branch of the primitive Dicotyledons. In Orchids and Aroids, in Grasses and Sedges, reduction plays a most important part, leaving its traces on the flowers as well as on the embryo of the seed.

'The whole evolution of Monocotyledons from the lowest orders [?] of Dicotyledons implies the seeming loss of cambial growth and many other qualities.'

'Retrogression is everywhere so active, that it can almost be said to be the prevailing movement. Reduction in the Vegetative and Generative organs, in the Anatomical structure and growth of the stems, and in sundry other ways, is the method by which the Monocotyledons, as a group, have originated from their supposed ancestors among the lower [?] dicotyledonous families. Retrogression is the leading idea in the larger families of the group, as for instance in the Aroids [especially their allies Lemnaceae] and Grasses. Retrograde evolution is also typical in the highest and most highly differentiated family, the Orchids, which have but one or two stamens.' Prof. de Vries might have added the rudimentary condition of their ovules and seeds, and consequently the difficulty in securing the germination of Orchids.

Prof. de Vries, like other writers, fails to see that water has been the *cause* of degeneration in Monocotyledons; though all the *now* ter-

[1] L'Évolution biologique et humaine, p. 201.

[2] Species and Varieties, their Origin by Mutation, p. 15.

restrial species have acquired sufficient adaptations to maintain an aerial existence.

The degeneracy of Monocotyledons is seen in every organ, as e. g. in the exhausting process of flowering and seeding. Thus bulbs, so characteristic of certain orders, which throw up a central flowering stem, perish with the completion of the reproductive process. Larger plants, as *Agave* and *Aloë*, die in the same manner. Lastly, Palms sometimes follow suit. Thus is it with *Corypha umbraculifera*, the Talipot Palm of Ceylon and Southern India. It has enormous leaves, a single blade, it is said, being large enough to cover a whole family. In about seventy years it is then able to flower; but the entire tree perishes immediately after the effort has been made.[1]

Miss Sargant observes that: 'The reduction in structure so characteristic of aquatic species is very strongly marked in their young seedlings. . . . Ancestral features have commonly disappeared or become obscure in the general loss of differentiation. Thus, the fact that terminal leaves seem characteristic of aquatic embryos suggests very strongly that their peculiar position is due rather to *suppression of the axis* than to any reminiscence of a stemless period in the history of the race.'[2]

'Considerable reduction of structure commonly occurs in *the embryo* and *seedling* of aquatic and semi-aquatic plants'[3] [my itals.].

The above quotations apply as well to Monocotyledons generally.

Miss Sargant elsewhere notices how 'the petiolar tube of cohering cotyledons is accompanied by a thickening, or at least much shortened hypocotyl', and adds that it is 'commonly very short in Monocotyledons', the thickening leading to bulbs, corms, &c. This is what might be anticipated from the subsequent tufted condition of the leaves of so many of that class, as well as in perennial dicotyledonous hygrophytes and hydrophytes, in which not only the primary internode or hypocotyl, but the subsequent ones are suppressed.

Miss Sargant calls attention to the prevalence of tufted or 'squat' plants on the Alps, and attributes it to the short hot summer following the melting of the snows. Insolation, however, may assist in arresting the axis. In South Africa, she adds, 'parallel conditions are seen in the dry season being followed by the heavy rains of the wet one.'[4]

The following British plants will remind the reader of this correlation between tufted foliage, arising from perennial axial structures, and an aquatic habit. Many Grasses, Rushes, and Sedges, *Iris*, *Eriocaulon*, *Isoëtes*, *Narthecium*, *Toffieldia*, *Stratiotes*, *Alisma*, *Sparganium*, *Hydrocharis*, *Lit-*

[1] Gardeners' Chronicle, vol. xlvii, p. 426, and Supplementary Illustration.
[2] Reconstruction of a Race of Primitive Angiosperms. Ann. of Bot., xxii, p. 151.
[3] Ibid., p. 152.
Theory of Origin of Monocotyledons, &c. Ann. of Bot., vol. xvii, p. 79.

torella, *Drosera*, *Pinguicula*, *Menyanthes*, *Samolus*, *Primula*, and most bulbous plants, &c.

It must not be forgotten that starvation and strong light can produce the same arrest of the internodes, as seen in plants growing on walls, &c., as *Draba*, *Sedum*, *Arabis Thaliana*, and many others. So that this peculiarity of Monocotyledons is not *caused* by the arrested habit, but this itself may be the result of varying degrees of water, or else in consequence of other inducements.

Degeneracy is very obvious where the aquatic form is contrasted with the land form of amphibious plants, such as *Bidens cernua*, *Ranunculus Lingua*, *Polygonum amphibium*, &c. As these plants grow equally well in either environment, they retain the capacity for adapting themselves to both conditions; hence neither form is hereditary, so as to develop the characters of any one form when grown in a different environment.

It is only, as a rule, after a prolonged residence of many generations (in water or in great drought, for example) that the acquired characters of adaptation are developed when the seeds are grown in totally different circumstances. Thus *Ranunculus heterophyllus*, the Water Crowfoot, retains the dissected and floating types of foliage on land; and the fleshy Cactaceous plants 'come true' to seed in England.

Miss Sargant observes that 'Monocotyledons may be shown to be on the whole a decadent race, of which some branches have been driven to an aquatic habit to escape the severer competition on land'.[1] Unfortunately, they do not escape it. Rivers, ponds, &c., soon get choked with a mixture of aquatic plants; e. g. Water Crowfoots or *Elodea*. If by 'decadent' she means *prior* to their entry into water, the question arises—What caused the decadence on land? The author does not appear to see that it is *water* itself which has caused the degeneracy in all aquatic plants.

'We may look on living Monocotyledons as a race which has been on the whole unsuccessful in the struggle for existence; and in consequence maintains itself chiefly in situations where the local conditions are exceptionally favourable to its peculiar characters.'[2] But grasses with 3,200 species in all kinds of soils and climates are ubiquitous; Palms have 1,100; Orchids, 4,000 to 5,000 species, &c. Such do not seem to warrant Miss Sargant's hypothesis.

Degeneracy is well seen in the structure of the stems. The cylinders of wood of a Dicotyledon form a combination of girders, and whether hollow or not, afford ample strength to support the weight of the tree or herb; but the dislocation of the strands in Monocotyledons brings about a mechanical weakness. Hence, palm stems made into walking-sticks are sometimes very brittle. The petiole of rhubarb leaves, naturally a hygro-

[1] Reconstruction, &c., p. 175.

[2] Reconstruction, &c., p. 176.

phyte, in which the strands are scattered, is equally weak,[1] and snaps across with the greatest ease.

Herr Ph. Eberhardt has shown how even a moist air has a similar effect to water upon woody stems, by greatly reducing the mechanical tissues of support.[2] Further illustrations will be mentioned later.

5. Possible Aquatic Origin of Palms.

Certain special resemblances are well illustrated by Palms. Though solid, silicified stems have been found fossil in Cretaceous rocks of America and in the Miocene of Antigua, some were remarkable for having a portion of the ground tissue in the condition of aerenchyma, a truly aquatic character. MM. G. Bonnier and Leclerc du Sablon illustrate this fact by a section of the stem of *Palmoxylon lacunosum*.[3] Mr. Drabble has also shown that many living Palms have roots full of lacunae, and names *Kentia*, *Metroxylon*, *Phoenix*, *Caryota*, *Areca*, &c., as well as *Phytelephas*.[4]

Another peculiarity about Palm-roots is the presence of what Jost calls 'pneumathodes'. They are described as *plaques farineuses*, probably from some resemblance to lenticels; they surround the bases of roots. M. C. L. Gatin, who gives an account of Jost's paper, would refer them to something more analogous to what is found on Ferns of the orders Marattiaceae and Cyatheaceae (two of the most ancient kinds of Ferns), consisting of fine trees with noble fronds growing in moist ravines. He concludes his article as follows:—'Il ne me paraît pas sans intérêt au moment où les travaux de l'école anglaise ont remis à l'ordre du jour les importantes questions de phylogénie des grands groupes végétaux, de constater une grande analogie de structure entre les organes respiratoires des Palmiers et ceux des Cyathœacées et des Marattiacées.'[5]

Though most Palms have tall stems *now*, as terrestrial trees, it does not necessarily follow that primitive Palms were not small shrubs.

Of course, pneumatophores are well known as occurring on the roots of hygrophytic trees and herbs, as *Taxodium distichum*, &c.; but they often fail to be produced if the tree grows in dry ground, as is the case with this deciduous Cypress.

[1] The rhizome, used as a drug, is known by the absence of a compact cylinder of xylem, which is replaced by stellate clusters.

[2] Ann. des Sci. Nat., tome xviii, 1903, p. 61.

[3] Cours de Botanique, p. 1214, Fig. 2193.

[4] The Anatomy of Roots of Palms. Trans. Lin. Soc., 2nd Ser., Bot., vi, p. 427.

[5] Observations sur l'appareil respiratoire des organes souterrains des Palmiers, par M. C. L. Gatin. Rev. Gén. de Bot., xix, p. 193.

6. Leaves of Large Size characteristic of many Aquatic Plants.

With regard to the foliage, the enormous size to which the leaves can attain is a characteristic feature of certain hygrophytes and hydrophytes. Of the former are the Ferns just referred to, *Gunnera*, *Rheum* (from Rha, the old name of the Volga), *Petasites* (as contrasted with *Tussilago*), &c.

Of the latter may be mentioned the *Victoria regia*. Of Monocotyledons, Aroids, Bananas, and Palms illustrate the same fact, whether they be hygrophytes or not at the present time. On the other hand, the largest trees among Dicotyledons or Gymnosperms, such as *Eucalyptus* and *Sequoia*, have no such leaves.

7. Water-storage Organs.

If plants had been more or less aquatic, and subsequently became xerophytes, it is presumable that they would only be able to sustain life during great and prolonged droughts, by adopting methods of storing water. Such is not infrequent in both classes. Species of *Erodium* and many others utilize various internal structures for storing water in the deserts near Cairo. *Oxalis cernua* does the same in South Africa and Malta, &c. *Poa bulbosa* has swollen nodes in dry ground, but not in moist soil.

Hence, tuberous roots, swollen internodes, bulbs, and corms are often acquired in adaptation to arid conditions on land. The prevalence of them among Monocotyledons, as seen in the orders Orchideae, Iridaceae, Amaryllidaceae, and Liliaceae, would seem to indicate an ancestral aquatic life. I would, therefore, regard the object of providing plants with corms, bulbs, and rhizomes to be threefold, viz. to enable them to be perennials, to be reservoirs of food, and if they be hydrophytes or hygrophytes which became xerophytes, then their third use is for water storage.

8. The Requirement of much Water by many Terrestrial Monocotyledons.

In support of this contention, not only are there still living aquatic members of the above-mentioned families, but under cultivation they all require a fair amount of water during growth, while some, such as Lilies, *Aspidistra*, &c., revel in it.

'*Watsonia*, for example, succeeds best when the saucer is kept full of water.' This genus in cultivation 'requires to be dried off gradually and then to be submitted to a baking in the sun', evidently in adaptation to the South African droughts.

'Liliaceae, on the other hand, requires an abundance of water, and the genera never like being very dry; so their bulbs should not be taken up.

The finest specimens of *Lilium cordifolium* growing wild are on the shores of lakes, in eighteen inches of water, thus being true aquatics. Hyacinths cannot be grown in this country as in Holland; without doubt this is a question of water. There they get an abundance of it. Tulips require less water than Hyacinths.'[1]

Mr. N. S. Pillans, the well-known Cape botanist, writes me from Cape Town: 'One question you ask I can reply to, that is whether Cape bulbous plants flourish best when their roots get an abundance of moisture. *They certainly do*, and most species flourish best under those conditions. Some become so luxuriant as to change their appearance, just as some succulents do.'

The leek is a bulbous plant when wild, as in the dry fields of Malta; but under a rich cultivation it has reverted to its, presumably, ancestral bulbless form.

I would suggest that the trunks of Palms, like the Baobab and 'bottle' trees, store water in the ground tissue; for some species resemble the latter at their base. Indeed, a palm may be compared with the bulb without internodes, but with the axis or stem drawn out; just as some Lilies pass gradually from bulbs to rhizomes.

Cycad stems may be compared to Palms, for both living and extinct plants of Cycadaceae and Bennettiteae are palm-like. The large pith of the latter, as seen in *Bennettites Saxbyanus*, was probably adapted for water storage.[2] Similarly the figure given of Cordaites[3] shows a strong zone of xylem with a large and discoidal pith, admirably adapted for water storage. These and other features seem to prove that such Cycadaceous plants were *not* hygrophytes or hydrophytes but entirely xerophytes.[4] It is notorious how the Date-palm craves for water.

9. Cycads and Monocotyledons.

It is a remarkable fact that Robert Brown, from the study of the embryo, thought he saw an affinity to Monocotyledons in Cycads; so he placed Cycadeae at the end of that class, immediately preceding Dicotyledons. 'In this order,' he says, 'we have at least, in respect of the external structure of the embryo, a *transition* from Monocotyledons to Dicotyledons. The development of the plumule agrees better with that of the former class; for in all these (Grasses and Aroids excepted) the primary

[1] The above facts have been kindly communicated to me by Mr. Rudolf Barr, the experienced cultivator of bulbs.

[2] Scott, op. cit., ii, p. 563, Fig. 201.

[3] Ibid., p. 528, Fig. 190.

[4] See my paper on the Xerophytic Characters of Coal-Plants. Quart. Journal Geol. Soc., lxiii, 1907, p. 282.

leaves are always scale-like or are mere sheaths ; while in all Dicotyledons perfect leaves are developed.' [1]

The primary leaves of the seedlings of *Victoria regia*, however, are similarly more or less arrested in development, and are comparable with those of *Sagittaria*.[2]

It might be suggested that *Cycas*, like Palms with a similar pseudo-pectinate or compound leaves, was derived from an aquatic ancestor, but now become a strong xerophyte, like *Agave* and *Aloë*, which are comparable in form with *Stratiotes Aloides*. But, on the other hand, intense drought may bring about an imitative result, as in *Hakea* (Proteaceae). This has more probably been the case with *Cycas*. These 'pectinate' leaves of xerophytes closely resemble in *form* the submerged leaves of *Proserpinaca palustris*.

Ceratozamia is the only known Cycad with only one cotyledon, though the second can be, and has been developed. The ovules are shed shortly after fertilization, so that the cotyledon is not developed until the seed lies on the ground. It arises at one, the lower, side, and gradually enlarging, the margins touch each other with the sheathing base. It thus agrees with hygrophytic and hydrophytic plants of both classes, as well as terrestrial Monocotyledons.

A longitudinal section shows a 'scale' opposite to the cotyledon, just as in the Oat. An inequality in size appears to be not unusual in other Cycads, as it is also in *Nymphaea*.

The reason why the lower cotyledon, i.e. as the seed lay on the soil, was only developed was clearly not in consequence of gravity, which would act equally on the development of both, but lies in the presence of water, whereby the lower one gets more saturated. When arrangements are made so as to equalize the moisture all round the seed, and it is made to germinate on the clinostat, both cotyledons are developed. Rotation is found to retard the growth. According to the figures given the primary root is very slender, and adventitious roots are represented as issuing from the hypocotyl close to the cotyledon.[3]

[1] 'In utrinque longitudinalis, plus minus obvius, cotyledonum duorum accretionem indicat, earumque separatio interna in regione Plumulae ante germinationem certe conspicuae manifesta est.

'In hoc ordine igitur habemus, saltim respectu structurae externae Embryonis, transitum a Monocotyledonibus ad Dicotyledones. Plumulae autem evolutio cum priori classe magis convenit; in his omnibus enim, Gramineis Aroideisque exceptis, foliola primaria hujus organi semper abortiva et squamiformia, vel merae vaginae sunt; dum in Dicotyledonibus omnibus folia perfecta evadunt. Aliud discrimen forsan obtinet inter Plumulas duarum classium; in Dicotyledonibus ejusdem foliola primaria cum cotyledonibus alternant [as in *Tamus* and *Asparagus*, which have rudimentary second cotyledons]. In Gramineis et Aroideis contra in quibus solummodo e Monocotyledonum Classi hanc determinare possumus, cotyledoni opponuntur. Cycadeae quantum e re tam minuta judicare liceat, hac ratione cum Dicotyledonibus quadrant' (Prodromus Florae Novae Hollandiae, p. 347).

It will be noticed that Brown wrongly uses the word *cum*. It only means 'with' in the sense of 'accompaniment'.

[2] loc. cit., p. 515.

[3] Morphology of Gymnosperms, by Coulter and Chamberlain, pp. 99 ff., Figs. 75-9 ; Figs. 187-

The development of the lower cotyledon only is probably analogous to the well-known fact that of two opposite leaves growing on a horizontal branch the lower will be larger than the upper. This is said to be due to gravity; but as the larger leaf requires the most water, it must be upon *that* which gravity acts. If the bough be kept reversed, then the leaves which would have been naturally uppermost, becoming the lower, now grow to be the larger.[1]

10. Monocotyledonous Dicotyledons.

In addition to the plants mentioned in my previous paper, *Corydalis solida* may be considered, for although Sir J. D. Hooker, in his Student's Flora, regards it as having connate cotyledons, M. Velenovsky considers it to have one; and he discovered an additional coincidence with Monocotyledons. It consisted in a peculiar adaptation of the hypocotyl and the petiole of the cotyledon, in that these are covered with absorbing hairs and can give rise to endogenous adventitious roots, so that the petiole acts as a true root.

This is not an isolated fact, for M. Gatin had previously observed that among certain Monocotyledons the petiole and cotyledonary sheath are sometimes covered with absorbing hairs, as e.g. in *Trachycarpus Martiana* and *Strelitzia*.[2]

It may also be noted that *Corydalis solida* has a corm and glabrous dissected foliage; the former may perhaps primitively have been for storage of water and the leaf submerged. In *Ranunculus Ficaria* and *Carum Bulbocastanum* the embryo is greatly arrested in the ripe seed; so that germination in both alike is very slow, features due to degeneracy which water can account for.

Miss Sargant, quoting from Hegelmaier's observations, shows that the development of the cotyledon is in a crescent-shaped ridge which thus agrees with the Water-lily, thereby acquiring a sheathing base. 'Hegelmaier and Schmid consider one possibility only—the formation of a single cotyledon from the original pair by suppression of the second.'[3] Miss Sargant also refers to *Corydalis cava*, as having an 'embryo monocotyledonous from the first. . . . From the researches of Dr. Schmid, we know that no traces of the

194 (p. 155); Fig. 462 (p. 429); quoted from The Embryo of *Ceratozamia* by H. A. Dorety. Bot. Gaz. xlv, 1908, pp. 412–16, Figs. 1–7.

[1] Influence des agents extérieurs sur l'organisation polaire et dorsiventrale des plantes, par M. L. Kolderup Rosenvinge. Rev. Gén. de Bot., vol. i, Figs. 12, 13, p. 131. See Author's experiments, Origin of Plant Structures, p. 205.

[2] La morphologie de la germination et ses rapports avec l'anatomie, par M. C. L. Gatin. Rev. Gén. de Bot., xx, p. 273.

[3] The Reconstruction of a Race of Primitive Angiosperms. Ann. Bot., vol. xxii, p. 157.

original bicotyledonary structures are to be found in the early history of the embryo.'[1]

Miss Sargant gives a list of Pseudo-monocotyledons, under five orders —Fumariaceae, 3; Umbelliferae, 3; Primulaceae, 1; Lentibularineae, 2; Nyctagineae, 3—and observes they are 'also characterized by the early formation of tubers [or corms], or at least by the development of a much shortened squat axis'.[2]

The following is Darwin's observation on *Abronia* (Nyctagineae) :—'In *A. umbellata* and *A. arenaria* one of the cotyledons is quite rudimentary and destitute of a petiole. At first it stood opposite to the larger cotyledon; but as the petiole of the latter grew in the *same line with the hypocotyl* [i.e. making it to be falsely terminal] the rudiment appeared in older seedlings as if seated some way down the hypocotyl. . . . In both these species the hypocotyl is so much enlarged, especially at a very early age, that it might almost be called a corm. . . . In *Cyclamen persicum*, the hypocotyl, even while still within the seed, is enlarged into a regular corm. . . . With several Cacteae, the hypocotyl is from the first much enlarged, and both cotyledons are almost or quite rudimentary, as in *Cereus Landbeckei*, *Rhipsalis cassytha*, &c. So, too, is it in *Stapelia sarpedon*.'[3] Darwin reasons as to the cause of the enlargement of the hypocotyl, in its being correlated with the arrest of one or both the cotyledons, and suggests the necessity of storage of *food*. At the early stage it would seem to be more probably *water*, whether in plants descended from aquatics or habitually living in *drought*.

The *Cyclamen* is another genus which has usually only one cotyledon, though a second has been seen. From the late Dr. M. T. Masters' investigations the genus agrees with Monocotyledons in the following details :—

1. The early disappearance of the primary root.

2. One cotyledon is normally absent.

3. The cotyledon acts as an absorbing organ for consuming the endosperm, just as in *Trapa* and Monocotyledons.

4. The first leaf is over the site of the missing cotyledon, and the second nearly over the existing one. Hence the phyllotaxis begins as $\frac{1}{2}$, as in nearly all Monocotyledons.

5. If four leaves occur there is an attempt to return to the $\frac{2}{5}$ divergence with the persistent quinary arrangement of the flower.

6. The development of the large corm is analogous to those of Iridaceae.

7. The glabrous, cordate leaf is paralleled by that of *Ranunculus Ficaria*, *Nymphaea*, *Limnanthemum*, and *Hydrocharis*.

[1] Theory of the Origin of Monocotyledons, &c. Ann. of Bot., vol. xvii, p. 69.

[2] Ibid., pp. 76–8.

[3] Movements of Plants, p. 95 ff. (abridged; my itals.).

Miss Sargant, in her paper 'On the Origin of the Seed-leaf in Monocotyledons',[1] takes the present prevailing view that this class was not precedent to, but descended from Dicotyledons, and says, with regard to *Allium*, 'the second half of the root-stele might originally have been supplied by the trace of a second cotyledon opposite the first.'[2] She also suggests that the two bundles of the cotyledon and hypocotyl of *Anemarrhena* may be traces representing the two distinct cotyledons of some ancestor. If this view is justified the 'cotyledon' of *Anemarrhena*, and probably that of all Monocotyledons, must be equivalent to both the seed-leaves of Dicotyledons.'[3] Miss Sargant would seem thus to consider that the single 'cotyledon' of Monocotyledons results from a fusion of the two, characteristic of Dicotyledons; but there is some ambiguity in her use of the term 'cotyledon'. Thus, in this sentence: 'Three bundles from each cotyledon [meaning the *blade*] enter the petiolar cylinder.'[4] But elsewhere, speaking of *Eranthis hiemalis*, she says: 'The partial union of two cotyledons [meaning *the petioles only*] is undisputed.'[5] Again, 'The two cotyledons of Primitive Angiosperms have united to form the single member in Monocotyledons.'[6] This change in the use of the term is a little confusing. *R. Ficaria* has been thought to indicate a fusion because its single cotyledon has often an emarginate blade, supposed to consist of two united; but it sometimes has none, or again two sinus. Miss Sargant adds that each of the two strands in the petiolar cylinder of *Eranthis* gives rise to three just before entering the blade. But those of *Anemarrhena* supply none. This countenances the view I have expressed elsewhere, and maintained by certain botanists in 1875, that the ordinary so-called 'leaf' of Monocotyledons with parallel venation is really homologous with the petiole only.[7] Others, as Sachs, regard the *blade* as arising directly from the sheath; but no mention is made of the parallel venation, which, to my mind, decides the question.

I would summarize my position as follows:—

What is suppressed in the embryo of Monocotyledons consists of—

1. The *blade* of the existing so-called single cotyledon, this being homologous with the petiole.

2. Both the *blade* and the *petiole* of the missing cotyledon.

What is retained consists of—

1. The petiole only of the existing cotyledon.

2. The single bundle of the petiole of the missing cotyledon (if present).

[1] New Phytologist, i, p. 107.

[2] op. cit., p. 109.

[3] op. cit., p. 110.

[4] Origin of the Seed-leaf in Monocotyledons. New Phytologist, vol. i, p. 111.

[5] op. cit., p. 113.

[6] Reconstruction, &c. Ann. Bot., xxii, p. 183.

[7] M. D. Clos mentions de Candolle and Treviranus, in Des éléments morphologiques de la feuille chez les Monocotylés. Mém. de l'Acad. des Sci. Toulouse, 1875.

This is then 'caught' within the broad or sheathing base of the single petiolate cotyledon.

This retention of the bundle from a lost organ may be paralleled by those of the five lost stamens being retained within the perianth segments of the flowers of Orchids.

This does not alter the fact that the organs, the cotyledon, and the stamens are suppressed.

Moreover, one cotyledon may be suppressed, but it does not *necessarily* follow that its representative strand should pass into the sheathing base of the one developed.

The rudiments of abortive cotyledons remain in *Ceratozamia* and *Trapa*, and also in the Oat; but no cord belonging to the lost one enters the developed cotyledon.

In *Ranunculus Ficaria*, Irmisch figures the petiole of the single cotyledon, showing the sections of the midrib and two marginal strands characteristic of most dicotyledonous leaves.[1] *Bunium* is simpler in having only one central strand.[2] *Anemone apennina* has the two blades of the cotyledons *sometimes* coherent half-way up. Then the two strands are included, but diverge as midribs.[3]

With regard to *Ranunculus Ficaria*, I have noticed some other features than those mentioned in my former paper as indicative of an ancestral aquatic habit.[4]

Both the upper (as on floating leaves) and lower epidermis are provided with stomata; while the cells of the lower have a wavy or indented outline, each has a cluster of chlorophyll granules in it. The cells on the upper have much less irregular walls, and are devoid of chlorophyll. The guard cells of the stomata are provided with chlorophyll.

The leaves have been described as 'opposite' (Hooker), but while the first two leaves on the flowering stem appear to be a pair of leaves, i. e. one opposite to the other, the sheathing base of one *overlaps* that of the other. Other leaves on the same stem are single.

The phyllotaxis, therefore, is $\frac{1}{2}$. This arrest of an internode between the first two leaves on a vigorously growing, erect stem is perhaps, the result of a habit not yet quite abandoned, when the stem was enabled to grow, on the plant becoming terrestrial. The leaves were doubtless originally crowded together, as is more generally the case now, arising from a very short thick 'stock', giving the 'squat' habit to the Lesser Celandine, growing, as it usually does, in damp soils.

[1] Beiträge zur vergleichenden Morphologie der Pflanzen, Parts I and II (1854).

[2] Irmisch, op. cit.

[3] Miss Sargant mentions *Ranunculus chius, R. repens, Phlomis, Urtica*, &c., as having been found with the two blades of the cotyledon more or *less* united, but it is in all cases exceptional or 'monstrous'.

[4] loc. cit., p. 495.

Professor Hugo de Vries[1] has shown by experiment that there are three causes which give rise to annuals; that is, they hasten the production of the central stem, viz. freedom of growth, by the absence of crowding, insolation, and a rich soil. Conversely, we may assume that crowding, shade, and a poor soil, besides water, may be expected to produce 'tufting' rosettes and a 'squat' habit.

The phyllotaxis of the Lesser Celandine suggests the origin of the three sepals and eight or nine petals, as the normal numbers. The absence of fives being accounted for by the single cotyledon and the $\frac{1}{2}$ arrangement of the leaves, these alone *cannot* give rise to the $\frac{2}{5}$ arrangement.

It has also been noticed that the fibrovascular bundles or strands are 'closed' as in Monocotyledons. This will be referred to again, as recorded by Professor Vines.

In allusion to *Ranunculus Ficaria*, *Corydalis cava*, and *Carum Bulbocastanum*, Miss Sargant observes that 'the embryo of all these species is very little developed in the ripe seed. A long period of maturation is necessary before germination can take place. In each, the single cotyledon was certainly derived from two; the ancestral genera certainly had two.'

'The actual process in all three forms bears a strong resemblance to the development of the embryo in *Tamus*. The cotyledon appears on the flattened apex of the pro-embryo as a peripheral ridge [this also occurs in *Castalia*, &c.]. At first it is circular, but it soon becomes crescent shaped by the rapid growth of one side. The stem apex is often late in appearing. It is always formed in the central depression outlined by the circular ring. Very soon the stem-bud is completely dominated by the lateral member, which embraces, and at last arches over it. This lateral member is, of course, the single cotyledon which becomes apparently terminal in the mature embryo, for it manages to push the stem apex to one side. In the interpretation of their results, Hegelmaier and Schmid consider one possibility only—the formation of a single cotyledon from the original pair by suppression of the second.'[2]

11. The Effect of Water upon Roots.

An ordinary dicotyledonous seedling growing in moist soil develops its primary root normally, and frequently to a great length, as under cultivation, the presence of water acting as a stimulus. Thus a turnip root, finding its way into a field drain-pipe, elongated to an extent of upwards of six feet.

If, however, seeds of Dicotyledons be grown in a net over water, as soon as the radicles penetrate it, they perish. Adventitious roots now appear from the hypocotyl. This arrest of the axial root is characteristic

[1] Alternating Annual and Biennial Habit: The Mutation Theory, vol. ii, p. 291, P. 25.

[2] Reconstruction, &c., pp. 156–7. See refs. in Miss Sargant's References to Literature, op. cit., p. 183.

of all aquatic Dicotyledons and of all Monocotyledons, whether aquatic or terrestrial. This was known to Richard and Poiteaux in 1808.

This difference, as well as others, is well seen in dicotyledonous amphibians, such as *Bidens cernua*.[1]

It has long been recognized that a characteristic feature of Monocotyledons, in addition to the arrest of the primary root, is to have the root-cap together with its formative tissue distinct from that of the root behind it.[2]

Numerous experiments with seedling Dicotyledons germinating in water, not only invariably showed the arrest of the primary root, with the evolution of secondary or adventitious ones; but as one of the latter grows from a lateral papilla, with a common meristem, this latter contracts, as it were, often appearing as pointed, with the calyptra of about four layers, more or less perfectly distinct from the root. If the complete separation is not always fully secured at first, the tendency to divide the merismatic tissue is always present. If sufficient time be allowed before examination, the calyptra becomes distinct.

Miss Sargant calls attention to the '*Absence of a true epidermis in the root above the root-sheath in Monocotyledons*'. This character of the root is so far as we know universal among Monocotyledons, but not confined to them. It is found in the Nymphaeaceae.[3] Since the degradation of the epidermis to the epithelioid state is characteristic of all submerged plants, the above loss is probably a further instance of the original effect of water.

12. Origin of the formerly called 'Endogenous' Arrangement of the Cauline Bundles of Monocotyledons.

Professor Vines observes[4]: 'In Schizostely the single primitive stele breaks up into as many distinct strands as it possesses vascular bundles . . . each schizostele being invested by its own segment of the endodermis. This obtains among Phanerogams in the Nymphaeaceae, some species of *Ranunculus*, as *R. aquatilis* and *R. Lingua*, &c., and in the Monocotyledons *Hydrocleis* and *Limnocharis* (Alismaceae).

'Stems with cauline bundles may be monostelic or polystelic; monostelic stems with cauline bundles are generally gamodesmic, having a solid vascular cylinder, e. g. *Lycopodium*, some aquatic Dicotyledons, e. g. *Utricularia*, *Aldrovanda*, *Callitriche*, *Myriophyllum*, *Ceratophyllum*; some aquatic Monocotyledons, e. g. *Elodea*, *Hydrilla*, &c.'[5]

[1] I have described and figured the two kinds of roots in this plant, the Bur-Marigold, in my Heredity of Acquired Characters in Plants (Murray).

[2] loc. cit., p. 507.

[3] Theory of the Origin of Monocotyledons, etc. Ann. Bot., xxvii, p. 83.

[4] Students' Text-Book of Botany, i. 152.

[5] Ibid., p. 173. It will be noted that the above quotations only prove that water affects Dicotyledons and Monocotyledons alike. But the object of this paper is to show that precisely the same results are now to be seen in *terrestrial* Monocotyledons, whose aquatic ancestors have returned to land.

Professor Vines adds: 'Some few Dicotyledons have closed bundles, i. e. without cambium in the stem, e. g. *Adoxa*, *Ranunculus Ficaria*, Nymphaeaceae, *Myriophyllum*, *Utricularia*, &c.' Such, of course, agree with *all* Monocotyledons.

With regard to the similar and the usual condition of monocotyledonous stem-bundles, MM. G. Bonnier and Leclerc du Sablon observe: 'Chaque faisceau est entouré d'une gaine continue de fibres à parois épaissies et lignifiées *qui ont la même origine que le parenchyme qui les entoure*. . . . Les faisceaux offrent une disposition analogue dans certaines Dicotylédones, telles que les Ranoncules, *Thalictrum*, beaucoup d'autres plantes de la famille des Ranonculacées et quelques autres encore.' [1]

Podophyllum peltatum has been described by Mr. Holm,[2] who notes the following facts. The petioles of the cotyledons are united into a long tube. Such is characteristic of many geophilous plants with a moist soil, as species of *Anemone* and other genera of Ranunculaceae. The vascular system of the stem recalls that of a Monocotyledon. The radical leaves are net-veined, but their petioles are expanded at the base into a sheath, from which many traces enter the subterranean stem.[3] This gives rise to the dispersed strands in the stem. The traces in the erect stem have lost their cambium and are 'closed' like those of a Monocotyledon. Cambium, however, is found in the bundles of the rhizome; these are arranged in a single circle, but without interfascular cambium.

This species of *Podophyllum* grows in swampy woods in the Southern States of North America. It has a 6-leaved perianth.

The origin of the coherent petioles may perhaps be traced to the ring found on the axis, whence the cotyledons arise, the two crescents of which cohere by their edges. This crescent or ring, as stated, is seen in Nymphaeaceae.

Though the rhizome of *Nymphaea* first suggested the similarity to the scattered bundles of Monocotyledons, it is by no means confined to submerged rhizomes. Mr. Worsdell, for example, figures the flowering stems of *Anemone rivularis, Caltha palustris, Podophyllum peltatum*, and *Hydrastis canadensis*.[4] To these petioles may be added those of *Thalictrum flavum* and *Rheum*.

As the 'closed' bundles of Monocotyledons and of certain Dicotyle-

[1] Cours de Botanique, pp. 182–4 ff.; Figs. 258, 259 (Monocotyledons), Fig. 260 (B) *Thalictrum*. A slight discrepancy seems to be noticeable between Vines's and Bonnier's descriptions in the words I have italicized in the account of the latter botanist; for while Vines regards the bundle sheath as due to the *endoderm*, which it certainly is in many cases, Bonnier attributes it to the medulla or *ground tissue* of the root. I would venture to suggest that both cases may occur in different plants.

[2] Bot. Gaz., xxvii, 1899, p. 419.

[3] See my paper, On the Origin of the Sheathing Petiole, p. 499.

[4] A Study of the Vascular System in certain Orders of Ranales. Ann. of Bot., xxii, Pl. 32, 33.

dons, chiefly aquatic plants, are mostly *without* cambium, so, on the other hand, *traces of cambium* are to be found in Grasses and Sedges.

Mr. A. Chrysler thus writes:[1] 'A well-marked, though generally short-lived cambium occurs in the bundles just above the node and near the base of the leaf-sheath in certain Grasses.' This fact is considered by him to lend support to the view that 'Monocotyledons have been derived from some group possessing a cambium, probably the Dicotyledons'.

'A cambium[2] is present also in the bundles at the nodes of *Scirpus cyperinus* and other species. These features indicate that the Cyperaceae is one of the most primitive groups of Monocotyledons. The view which derives this class from an essentially dicotyledonous ancestry receives further support.'

In my former paper[3] I drew attention to the series of concentric circles in *Nelumbium*, apparently indicating the first stage of their dislocation from a primitive and more compact zone of fibro-vascular bundles. Miss Sargant observes that the 'regular orientation—xylem inwards, phloem outwards—suggests that the leaf-traces were formerly linked together by a cambium.'

Miss Sargant also observes:[4] 'Traces of a cambium in the vascular bundles of monocotyledonous seedlings have been recorded by several observers, as in *Zea*, *Typha*, *Lilium*, *Dracaena*, and in the hypocotyl of *Yucca arborescens*, &c.'

She quotes Professor Quéva as saying: 'The persistence of a cambium zone in the bundles of certain Monocotyledons shows that we may logically consider them as derived from the more primitive Dicotyledons, by means of the early disappearance of the cambium, and an increase in the number of traces from each leaf.'[5]

In addition to the above, Mr. Chrysler observes that 'something similar occurs in the development of the central cylinder of Araceae and Liliaceae.[6] The simple siphonostelic stage persists in *Acorus* for several internodes, and the stem looks much like that of a Dicotyledon; higher up some segments of the stele become amphiasol (*sic.* amphivasal?), and this may be regarded as the first appearance of a monocotyledonous character.'

Again, in speaking of the central cylinder, he adds: '*Medeola* and *Lilium* show the effect of long internodes combined with extended gaps in breaking up the central cylinder into several strands arranged in the circumference of the circle.'[7] 'The plan of the young stele,' continues Mr. Chrysler, 'as, e.g., in *Smilacina*, bears a close resemblance to that of Dicotyledons, and differs from the older state of the latter only in the absence of cambium. These considerations lead to the conclusion that the

[1] Bot. Gaz., xli, p. 11 ff. (Jan. 1906).
[2] Rev. by A. B. Plowman, Ann. of Bot., xx, pp. 1–33, Pl. I, II, 1906.
[3] loc. cit., p. 512. [4] Reconstruction, &c., p. 144. [5] op. cit., p. 145.
[6] Bot. Gaz., xxxviii, pp. 161–4. [7] ibid., p. 177.

Monocotyledons are *not* an ancient group, but that they have branched off from the Dicotyledons, or that both groups have sprung from a stock which resembled the modern Dicotyledons more closely than it did the Monocotyledons.'

Mr. Chrysler, like the other writers quoted, does not appear to see that this close connexion is really due to a common cause, viz. degradation through an aquatic habit of life.

13. The Forms and Structure of Aquatic Leaves are the Result of the Direct Action of Water.

In my former paper[1] I suggested that the 'gashes' of some Aroids, as well as 'fenestrations' (e.g. *Anadendrum Monstera*, *Caladium*, *Pothos*, &c.)—recalling those of the lattice-leaved plant (*Ouvirandra fenestralis*), such also occasionally occurring in our pond-weeds—were formerly due to water, and that they are now hereditary in terrestrial species.

The pinnate or palmate forms of the leaves of some Aroids and, perhaps we may add, Palms, the marginal incisions being quite unlike those of a Dicotyledon, may have been acquired in their days of antiquity when submerged.

In support of this contention I can now add that the dissected type of foliage when submerged has been proved experimentally to be due to the degenerating effect of water upon the protoplasm of the stem. Mr. MacCallum succeeded in showing this by setting up osmosis artificially. He dissolved certain nutritive salts in the water in which he grew plants of *Proserpinaca palustris*. The result was just what he anticipated, namely, that the superfluous water, with which the protoplasm of the stem was saturated, was withdrawn, so that the plant was now enabled to bear, and produced subsequently fully developed leaves of the aeriform, pinnately nerved, lanceolate type.[2]

In the case of *Ranunculus heterophyllus* the dissected type of leaf has been proved, both by Nature and my own experiments, to grow equally well in air after being sown in a border, and is thus hereditary, though it is a character entirely acquired by the *soma*. Sowing the seeds of *Ranunculus heterophyllus* in a garden border, they all came up and developed dissected leaves, but perfectly in adaptation to air. Subsequently the floating type appeared, as if the stem had reached to the surface of imaginary water, and finally the flowers were borne. This heredity of the acquired characters of the foliage lends countenance to the view that the pinnate and palmate characters of Aroids and Palms are due to the same cause, as well as all their other permanent aquatic types of structure.

[1] loc. cit., p. 522.

[2] Bot. Gaz., xxxiv, p. 106. I have described and figured this more fully in my Heredity of Acquired Characters in Plants, pp. 34 ff. (Murray).

Another common feature of nearly all Monocotyledons and aquatic Dicotyledons is the total absence of hair, 'glabrous' being the characteristic feature of nearly all alike.

14. The Reticulated Venation of some Monocotyledons is only imitative of that of Dicotyledons.

An important fact must be noticed. The reticulated leaves of certain Monocotyledons are *not identical* with, but only *imitate*, by approximating the structure of, those of Dicotyledons. The typical monocotyledonous leaf being really homologous with a petiole only, and therefore phyllodinous, has parallel strands. If such be an aquatic plant, it may be so only in comparatively deep water, as *Sagittaria*. If it bear floating leaves, the usual change is to widen the intervals at the upper end by curving the strands so as to make a curvinerved or elliptical blade. This now introduces transverse cross-bars, thereby strengthening the whole. If the blade becomes aerial considerable changes take place; the first is for the two outermost strands to curve downwards, so that the blade assumes a spear-head or hastate shape. By the prolongation of the downward points it becomes like an arrow-head or sagittate. These points may be rounded off and so a cordate form is acquired, being specially adapted, according to Mr. Hiern,[1] for resisting the strain of running water when floating.

The last change or advance is to form tissue between the lower points or fill in the cordate gap. The blade then becomes peltate, as in *Alocasia* and *Nelumbium*, as well as our own *Hydrocotyle*.[2]

The minuter venation changes with these forms; the first appearance of an irregular network is seen between the small divisions of the first described. The veins, primarily at right angles to the longitudinal strands, become oblique; the number of main strands may be reduced even to three, so that plenty of space is allowed for more uniform reticulations. The blade then closely resembles an ordinary one of a Dicotyledon, but a prominent midrib, like that of a Dicotyledon, is absent in Monocotyledons; for when the leaf is large, as in *Caladium*, &c., *a cluster of strands become crowded together*, but as their ends successively diverge obliquely across the blade, becoming reticulated, the median cluster is reduced to nothing towards the apex of the blade.

We thus discover that aerial reticulated leaf-blades of Monocotyledons are not *identical*, but only *imitative* of the fibro-vascular system of an ordinary dicotyledonous leaf. The change is very well seen by comparing the small elliptical blades which first arise from the tuber with the subsequent sagittate ones of *Arum maculatum*.

Indeed, one is tempted to speculate upon this fact. For no Monocotyledon would seem to possess a decidedly aquatic form resembling an aerial

[1] Proc. of the Cam. Phil. Soc., Oct. 1872.

[2] loc. cit., pp. 515 ff.

leaf-blade of a Dicotyledon. Conversely, a submerged leaf of any aquatic plant of this class rarely, if ever, agrees with a Monocotyledon. Thus the ribbon-like form of *Lobelia Dortmanna*[1] and others has really a midrib with lateral ribs branching from it at the base. They subsequently run parallel, *imitating*, but are not *identical* with, say, those of a grass blade or *Sagittaria*. *Hippuris* has a single midrib without lateral ones, and thus is degraded to a very simple condition.

Could, therefore, Monocotyledons have arisen so early as to have *preceded* the reticulated venation of Dicotyledons? The earliest kind of branching venation appears to have been dichotomous, as seen in Ferns (*Adiantum*) and the *Ginkgo* of to-day; but in *Cordaites laevis*, as figured by Dr. Scott[2] after Renault, the leaf is represented of the simplest kind with parallel venation.

Something of that sort, perhaps, supplied the original type of leaf of Monocotyledons, now represented by the petiole only. This, however, professes to be no more than guess-work and a suggestion only. At all events, as Miss Sargant notes, the cotyledons of Monocotyledons would seem to carry the two strands, one being the relic of a lost cotyledon, with no *third* strand in the place of a true midrib as in Dicotyledons. In some cases, as *Eucomis nana*, according to her description, the two cotyledonary strands approach so as to make a pseudo-midrib 'exactly opposite to the first leaf'.[3] It also occurs, she adds, in *Lilium* and *Allium*.

15. Reproductive Organs.

In the section with this heading[4] I omitted to allude to the degraded flowers of some aquatic Dicotyledons. Thus the Halorageae, apparently derived from Onagraceae, exhibit several instances of aquatic genera having much reduced flowers, both in size and number of parts, such as in wanting a corolla and being unisexual. Elatineae, again, affords another instance. *Hydrocotyle* has no vittae, in accordance with the well-known fact that oils and resins are much reduced or absent from plants growing in moist regions.

Similarly the degradations of the flowers of Grasses, Sedges, *Lemna*, *Zostera*, and many others are very obvious. On the other hand, as long as the flowers of aquatic plants are visited by insects, there is no reason why they should not resist degeneration in size and attractiveness or number of parts, as is the case in Nymphaeaceae, &c.; though in the Water-crowfoots the flowers are, mostly, considerably degraded.[5]

[1] Bentham spells this *Dortmanni*; Hooker, *Dortmanna*. L. is the authority for both.
[2] Studies in Fossil Botany, p. 522.
[3] Theory of the Origin of Monocots., etc. Ann. of Bot., vol. xvii, p. 19; cp. Pl. I, Fig. 6.
[4] loc. cit., p. 524.
[5] See my paper on the Self-fertilisation of Plants, Trans. Lin. Soc., Sec. Ser.—Bot., i, p. 347.

16. Cytological and Embryological Investigations.

Some important researches have been made in the more minute details of embryology, which bear upon this subject.

Messrs. W. Lubimenko and A. Maige have published an article entitled—*Recherches cytologiques sur le développement des cellules-mères du pollen chez les Nymphéacées.*

'Indépendamment des caractères morphologiques et anatomiques bien connus, certaines particularités cytologiques, comme la grosseur des noyaux et la simplicité de la membrane des grains de pollen, rapprochent cette famille des Monocotylédones. . . . Les études cytologiques faites jusqu'à présent sur le développement du pollen . . . ont permis d'établir des caractères distinctifs assez constants entre les Monocotylédones et les Dicotylédones, et elles peuvent fournir, de cette manière, des documents suffisants pour la comparaison avec les Nymphéacées.'[1]

In conclusion the authors add:—

'Les deux espèces (*Nymphaea lutea* and *Nuphar luteum*) se rapprochent des Monocotylédones par la nature simple du réseau au stade de prosynapsis et par la dissociation précoce des cellules-mères du pollen.'[2]

Mr. Melville T. Cook has written upon the Embryogony of some Cuban Nymphaeaceae.[3] The following is a brief analysis of his paper bearing on the present subject:—

Mr. Lyon, describing *Nelumbo*, considers it should be classed as a Monocotyledon. Cook strengthened his view with *Castalia odorata* and *Nuphar advena*; Schaffner does so by comparing Nymphaeaceae with Helobiae, observing that the number and arrangement of ovules connect the order with Monocotyledons.

The formation of the tube-sac in the embryo-sac is like that of *Saururus* (Piperaceae) (aquatic).

The mention of *Saururus* at once suggests *Peperomia.* Species of this genus have been described by Mr. Hill[4] and others. Miss Sargant quotes details as follows:—'There are two cotyledons present in the seed. One is hypogeal and acts as a sucker (comparable with *Trapa*, *Cyclamen*, *Elettaria*, Palms, &c.), the other has assumed the appearance and functions of a foliage leaf. It is geophilous in structure. . . . Its seedlings seem to germinate in the spongy herbage of the locality.'[5]

Mr. Cook calls the 'tube-sac' a 'nucellar tube'. It extends from the antipodal to the chalazal end of the ovule. It originates as a great mass

[1] Rev. Gén. de Bot., xix, 1907, p. 402–3. [2] Idem, p. 499.

[3] Bot. Gaz., xlii, 1906, p. 376, Pl. 16–18; with Bibliography.

[4] Morphology and Seedling Structure of the geophilous species of *Peperomia*, together with some views on the Origin of Monocotyledons. Ann. of Bot., xx, 1906, p. 395.

[5] The Seedling Structure of some *Piperales*. Ann. of Bot., xx, 1906, p. 161.

Miss Sargant discusses the embryology of *P. pellucida* in her Reconstruction, etc. (op. cit., p. 128).

of cells elongated in its direction and rich in protoplasm. These dissolve by disintegration and the tube is formed. The behaviour of the endosperm and nucleus is similar to that of *Sagittaria variabilis*, but this has no nucellar tube.

Mr. Cook refers to Hall as finding resemblances in *Limnocharis*; Strasburger in *Ceratophyllum*; Campbell in *Naias* and *Zannichellia*; all being aquatic plants.

The author calls attention to a probable physiological connexion between the tube and the antipodals in Ranunculaceae, *Sparganium*, and *Vaillantia*, as furnishing nourishment for the embryo. The large lower nucleus formed by a division of the endosperm-nucleus (equivalent to the nucellar-tube nucleus) in *Sagittaria* and Nymphaeaceae showed a resemblance which appeared to indicate a similar function, viz. for supplying food to the embryo through the agency of the endosperm. In *Castalia* (*Nymphaea*) this is done by the 'core' of cells, subsequently by the tube.

With regard to the embryo, there are the following stages of development:—

1. A globular pro-embryo with or without a suspensor, resembling that of *Sparganium simplex* [and *Ranunculus Ficaria*].

2. A cotyledonary 'ridge', nearly circular, enclosing the plumule in the middle. N.B.—This ridge is characteristic of Monocotyledons and is also present in the pro-embryo of *Ranunculus Ficaria*, *Corydalis cava*, *Carum Bulbocastanum*, as also on *Tamus* and *Commelyna*. This ridge represents the commencement of the usual sheathing base of the cotyledon.

3. Two cotyledonary lobes are developed from the ridge, giving the dicotyledonous character.

It may be noted that considerable variation occurs. In *one*, it was so great as to give the appearance of *two equal* cotyledons. [This variety appears to indicate the true dicotyledonous origin, but the ridge being *not quite circular* points to partial arrest of one cotyledon.]

Brassenia and *Cabomba* differ (*a*) in having no cell-walls at first in the endosperm, (*b*) the dicotyledonous character appears very rarely. [Hence these two genera may indicate the more primitive conditions.]

The young embryo of *Nymphaea advena* resembles that of *Lysichiton Kamtschatense* (Aroideae) and of *Ceratophyllum*. Those of other genera are like *Sparganium simplex*, *Naias flexilis* and *Zannichellia*, *Potamogeton* and *Lemnocharis*, *Alisma*, &c.

Mr. Cook writes elsewhere upon Nymphaeaceae and *Sagittaria*.[1]

In certain Nymphaeaceae the endosperm-nucleus divides into an upper and a lower nucleus, which become separated by a wall formed across the embryo-sac. The upper nucleus forms the endosperm. From the lower

[1] Development of the Embryo-sac in *Castalia* and *Nymphaea*. Bull. Torr. Bot., ch. xxix, 1902.

cell is formed a long tube which grows towards the chalazal end of the ovule, forming a passage by the absorption of the nucellus. It thus plays a nutritive part comparable to that of the antipodal cells in many genera. A similar formation of endosperm has been described in *Sagittaria* by Rendle.[1]

The details should satisfy the reader that they are all the result of an aquatic habit of life, thus bringing all Monocotyledons into close connexion with aquatic Dicotyledons.

17. Speculations on the Arrest of one Cotyledon.

Perhaps the most difficult question to answer is: What were the exact conditions under which one cotyledon was arrested? Such arrest occurs in plants associated more or less with water now, as *Ranunculus Ficaria* and *Pinguicula*, hygrophytes; or which were probably ancestrally aquatic, as all Monocotyledons. But the reduction of the embryo may take place also under other conditions of life, as in parasitism, by drought, &c.

We have seen above that it was clearly proved that the arrest of the intercostal tissue in a submerged leaf is actually due to the weakening effect of water by saturating the protoplasm, so that it is unable to develop a complete blade. From this we may confidently generalize upon the effect of an excess of water upon all parts of aquatic plants.

When we try to connect water with a reduced size of the two cotyledons in the seed or with only one, or again with their total arrest, we are confronted with the fact that existing aquatic Dicotyledons have, as a rule, two cotyledons; but as such aquatic genera or species mostly belong to present terrestrial orders, they are of comparatively modern origin, and we can only assume that heredity has overcome any deteriorating effect sufficient to totally arrest one cotyledon. Still, the size of the cotyledons is not infrequently greatly reduced when in the seed; indeed, they may be arrested in the pro-embryonic condition, so that we seem to see degrees of degradation. Thus:—

1. The embryo may be relatively minute as in Ranunculaceae, in *Dionaea*, *Drosera*, *Gunnera*, and *Eriocaulon*, more or less aquatic plants. On the other hand the xerophytic Holly has a very small embryo embedded in a mass of albumen.

2. In some seeds the cotyledons are small and arrested as compared with the size of the radicle, e.g. *Pinguicula*, genera of Halorageae, *Parnassia*.

3. The embryo may remain in the seed arrested at the pro-embryonic condition of an undifferentiated globular mass of cells, as in *Ranunculus Ficaria*, several species of *Anemone*, *Utricularia*, &c.

4. In the next place there may be an inequality in the size of the embryo, as in *Nymphaea*, &c.

[1] See also Class. of Flowering Plants, i, 163 (Rendle); and Bot. Gaz., xxiii, 6, 260.

In *Ceratozamia* it has been shown above that as a seed lies flat on moist ground, the cotyledon on the *lower* side only is developed; but if the seed be kept uniformly moist all round, then both of the cotyledons become developed.

Now, seeds (provided with a fitting temperature) will not germinate unless water be present, either within the seed or acquired from without; so that whatever may be the differential causes acting on the two cells or groups of cells of the pro-embryo, whence originate the two cotyledons, if *one* receive a trifle *more* moisture than the other, it may, perhaps, determine the development of the former in xerophytes, and the latter in hydrophytes.

Miss Sargant has also shown that the two cotyledonary strands, corresponding to the two cotyledons, one of which is present, but carries the strand of the other, are probably never exactly alike, as in *Eranthis* and *Anemarrhena.* If her figures were drawn under the camera, the *size* of the xylem vessels, expressly concerned in carrying water, are not precisely alike,—and such may, perhaps, make all the difference in deciding which blade shall be arrested, as in all Monocotyledons.

However it may have been brought about in the first Monocotyledons evolved, there are too many collateral coincidences, speaking broadly, between water-effects on Dicotyledons and existing Monocotyledons, in all their organs, to gainsay the probabilities that water was, in some unknown way, the primary cause of the monocotyledonous condition.

Monocotyledons were probably first evolved in the early part of the Secondary Epoch as soon as Angiosperms were established from Gymnosperms; and as we do not even know what the first dicotyledonous Angiosperms were like, one cannot avoid speculating as to the process; for no existing order of Monocotyledons can be pronounced primitive with any degree of safety; because degradations are misleading, if any be selected as such, from being of a more simple type.

18. Non-inheritance, Imperfect and Complete Inheritance, of Acquired Characters.

Since external conditions may graduate one into another, so that mesophytes are intermediate between xerophytes and hygrophytes, so, too, the same species may find it necessary to adapt itself to soils, &c., of more than one kind. To do this the plant retains the power of response to either condition, and the characters acquired in one are *not* necessarily hereditary in the other, as in the case mentioned of normally amphibious plants.

To become hereditary under *any* change of environment, it is necessary, as a rule, for the species to have lived for many generations under the same conditions as those which produced its special characteristics.

A partial inheritance may last for one or two years; just enough to

prove that acquired characters *can* be reproduced under diametrically opposite conditions of life.

I have shown this to be the case with *Ononis spinosa*, &c.[1] If seeds be sown in a constantly wet soil and atmosphere, the spines are at first present, but reduced, in the seedlings. They grow into leafy branches in the second year. Similarly, if there be a warm, moist spring, the spines of the Barberry will grow out into large leaves. So, too, the Beet, naturally a perennial, under cultivation has become a biennial, this character being now hereditary; but it has not prevented its 'bolting' and so *passing* into an annual. Lettuces, on the other hand, are naturally annuals, but when bolting *return* to this condition.

But, in nature, innumerable instances exist which prove incontestably that acquired characters of plants can be maintained quite irrespective of the environment. This is seen in strong xerophytes with massy fleshy stems, such as of the *Aloë* and *Agave* type, as well as the Cactaceous, and in aquatics; of which *Ranunculus heterophyllus*, described above, is a familiar example. Of course, this also applies to *all* terrestrial Monocotyledons.

According to Weismann, acquired characters, derived by the *soma* from changes in the conditions of life, can only be hereditary, provided they influence the germ cells, which are supposed to exist contemporaneously with the direct actions of the environment. Plants, however, are unlike animals, in passing through a more or less extended period of vegetation from germination, during which those activities are at work upon the plant's *soma*, i. e. *before* any trace of reproductive cells exist. It is not until the vegetative activities decline that certain *vegetative cells* pass over into or become *reproductive cells*. The continuity of protoplasm extending from one daughter nucleus to the other *through* the 'equatorial' cell-plate, may be the means of communicating the effects of external irritations, &c., to the very cells which become reproductive germ cells and sperm cells.

It may be suggested that the movements of the chromosomes to the 'north' and 'south' poles from the 'equator' are the means required for making these protoplasmic strands of continuity, whereby the communications are kept up between the surface and the interior.

Dr. Hugo de Vries' experiments on Mutations clearly prove that marked differences in seedlings (greater than the ordinary 'individual differences') may be permanent from their origin, at least under circumstances in which they appeared; for he does not seem to have tested them in markedly different soils. I notice Professor J. S. Henslow found, in 1827, the same thing to occur with Primulas; varieties came up in his sowings, of which he observed that some were permanent, others reverted, &c.[2]

[1] Heredity of Acquired Characters in Plants (Murray). See also Origin of Plant Structures, 1895, p. 54.

[2] Loudon's Magazine of Natural History, iii. 406 and ix. 153.

19. Isolation and Natural Selection.

It may be observed that I have said nothing about 'Isolation' and 'Natural Selection'. Instead of the former, I prefer to say a *new environment*, however the seeds may have happened to get into it. With regard to the latter phrase, Darwin described it as being only metaphorical.[1]

I follow his later ecological explanation of evolution; for I find that in all cases, if a quantity of seeds germinate and grow under new conditions sufficient to cause them to vary, they *all vary alike*, i.e. what he called 'definitely', just as Darwin said: 'There can be little doubt that the tendency [i.e. response] to vary in the same manner has often been so strong, that all the individuals of the same species have been similarly modified without the aid of any form of selection.'[2]

20. Conclusion.

Miss Sargant concludes her long and interesting paper on the 'Reconstruction of a Race of Primitive Angiosperms' as follows:—'The hypotheses which have been considered . . . have led to two phylogenetic schemes only, which attempt to explain the evolution of Monocotyledons from Dicotyledons from a common ancestor. The first is that of Professor G. Henslow. The second is the fusion (of the two cotyledons) hypothesis.'

If the *blades* only be thought to be 'fused' then there is no proof of such being the case, as shown by *Ranunculus Ficaria*. But I understand Miss Sargant to mean by 'cotyledons' solely the *strand* or the vascular bundle of the lost cotyledon to be retained in the sheath and petiole of the one present; as she has shown to be the case in *Anemarrhena*.

All I would maintain is that the strand is the last relic of the cotyledon which has been otherwise totally arrested by water.

The reader will now probably have read enough to perceive, *first*, that there is an immense number of *coincidences* which occur among *aquatic* plants of both classes. *Secondly*, that all *terrestrial* Monocotyledons exhibit the same coincidences, but coupled with re-adaptations for living in air. These coincidences afford an abundance of *inductive evidence* of their having had a common origin in ancestral hygrophytes or hydrophytes among Dicotyledons.

Thirdly, experimental verification now covers the peculiarities of the roots, stems, and leaves of Monocotyledons; proving that water *was* the actual source of what Darwin called 'the direct action of the conditions of life', to which the plant responds. Such acquired, aquatic, structural

[1] Origin, &c., 6th ed., p. 65.

[2] Ibid., p. 72. The paragraph containing this quotation is *not* in the first edition.

characters have become hereditary, and are still retained in *all* Monocotyledons, whether they be aquatic or terrestrial, now.

In the title of my first paper in 1892, I used the word 'Theory', but judging from the very large amount of *inductive evidence* derived from Morphology and Microscopical Histology, coupled with the experimental verifications now recorded, I feel justified in abandoning the term; for I would maintain that the conclusion has passed the stage of hypothesis and probability only, to that of a *demonstrated fact.*

As a corollary it may be added that all the facts herein stated prove incontestably that the morphological and other characters, which constitute the classificatory distinctions between Dicotyledons and Monocotyledons, have all been acquired by the response of the 'soma'—often long before any reproductive organs exist—to the aquatic conditions of life. Such are now permanent and hereditary.

On the Xylem Elements of the Pteridophyta.

BY

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With Plate LVI and three Figures in the Text.

A. Historical.

THE question regarding the nature of the xylem elements of Pteridophyta—whether they are vessels of ordinary type or of a special type, or whether they are tracheides—is one which has given rise to much discussion. The differences of opinion on this point are due to the difficulties attending the detection of the primary portions of the cell-wall in much altered tissues; for upon the recognition of the presence of a pit-membrane, itself a part of the middle lamella of the primary cell-wall, depends the solution of the whole question. Dr. Fr. Halft (3) has recently given an excellent *résumé* of the views of the earlier investigators of this subject, pointing out that it was Sanio (11) who, in 1873, caused general opinion to turn in favour of the presence of the pit-closing membrane, at least in Phanerogams.

With regard to the Vascular Cryptogams, it was at first maintained that their xylem elements were true vessels, the presence of a pit-membrane being denied. De Bary (1), however, after the appearance of Sanio's work on the Scots Pine was led to investigate other forms, including Vascular Cryptogams. He, too, noted the pit-membrane, mentioning especially *Pteris Aquilina*, where it occurs in the side walls of the elements; and in 1891 Strasburger (16) demonstrated that the Vascular Cryptogams in general possess pointed tracheides, dovetailing into one another and presenting no distinction between their end and side walls. At present only two exceptions to this general rule are known—the root of *Nephrodium Filix-mas* and *Pteris Aquilina* having true vessels, since the end walls are perforated (Russow, 10).

In 1908 Prof. Gwynne-Vaughan (2) returned to the former theory of the vascular nature of the xylem elements of Ferns. His conclusions are based upon investigations suggested by the appearance of transverse sections of certain fossil forms referred to the Osmundaceae (Kidston and

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Gwynne-Vaughan, 5 and 6). A transverse section of *Osmundites skidegatensis*, for example, shows, between the lignified parts of two adjacent elements, distinct splits not containing any trace of a 'middle substance'; and Gwynne-Vaughan compares with such a section one of the recent species, *Osmunda cinnamomea*, in which also he finds splits or spaces between the transverse bars of lignified substance.

Gwynne-Vaughan distinguishes two main types of 'vessel', represented by the elements of *Pteris Aquilina*, and by those of *Osmunda cinnamomea* and *Nephrodium Filix-mas* respectively.

In *Pteris Aquilina*, as typical of the one group, he found that, although 'those parts of the primary wall that connect the opposite transverse bars of secondary thickening in the middle of the wall are here maintained intact even at maturity' (p. 521), the pits on both end and side walls of the elements are true perforations. There is here, then, a free passage vertically and horizontally from element to element.

The second main type of 'vessel' includes some in which the pits occur in more than one series on each facet, and others in which there is only one series.

Osmunda cinnamomea—representative of the Osmundaceae as a whole—has typical multiseriate pitted elements. In these, according to Gwynne-Vaughan, the whole of the primary wall, including the middle lamella, disappears entirely, except at the angles of the elements and between the series of pits, where the wall remains solid. If this be the case, it follows that there must be a free passage vertically and horizontally as in *Pteris*, with an additional vertical passage in the spaces left by the disappearance of the primary wall.

Nephrodium Filix-mas possesses uniseriately pitted xylem elements, and in these the primary pectose layers and middle lamella are held by Gwynne-Vaughan to disappear entirely, except at the angles. Here, therefore, there would seem to be still greater freedom for the movement of water, for not only must the elements be in communication vertically and horizontally, but the entire disappearance of the primary wall from between the transverse lignified bars must leave considerable spaces for the passage of water vertically between the elements, outside their actual cavities.

Gwynne-Vaughan thus concludes that the xylem elements of the Pteridophyta are mostly vessels, these being in certain cases, as typified by *Osmunda* and *Nephrodium*, of a special kind.

In 1910 Halft's dissertation 'Die Schliesshaut der Hoftüpfel im Xylem der Gefässkryptogamen' appeared, but it seems to have excited little attention in this country. The conclusions of this investigator entirely refute those of Gwynne-Vaughan, and support Strasburger's views as to the tracheidal nature of the xylem of Vascular Cryptogams (Halft, 3); for, by experiment and by examination of both longitudinal and transverse

sections, he has been able to demonstrate that the pit-membranes are typically present in the end, as well as in the lateral walls of the elements. (In *Pteris Aquilina* the pit-membranes disappear from the cross walls, these being much less inclined to the lateral walls than is usual.) Halft further demonstrates that the primary wall remains between the bars of secondary thickening. The difference in the amount of cohesion of the bars gives rise to the presence or absence of the 'split' between them, as seen in transverse section.

The results obtained by the writer lend strong support to the views put forward by Halft with regard to the tracheidal nature of the xylem elements of the majority, at least, of the Pteridophyta.

B. Methods and Material.

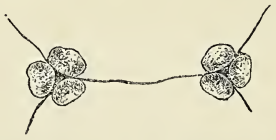
Unstained transverse sections of certain types, e. g. *Gleichenia* spp., *Lygodium* spp., and others, gave the impression that true empty spaces occurred between contiguous elements of the xylem, as Gwynne-Vaughan maintains. These sections when stained, however, failed to confirm the idea, the spaces never appearing clear, even under a magnification of 325 diameters.[1] The appearance of Halft's paper suggested the reason for this and led to the following investigations.

I. *Microchemical Tests for Pectic Substances of the Primary Walls.*

Several chemical methods were employed in order to prove the presence of primary pectic substances separating the secondary lignified layers of the xylem elements. The first of these methods was that mentioned by Halft (3). This investigator treated transverse sections of *Pteris Aquilina* and *Osmunda regalis* with Schulze's maceration mixture (concentrated nitric acid and potassium chlorate), until the lignin of the secondary layers of thickening had disappeared, but apparently before the stronger lignification of the primary walls of the elements had been destroyed. It is a well-known fact that lignified walls, after treatment with this maceration fluid, give the cellulose reaction. Halft therefore removed the residue of cellulose from his sections by means of strong sulphuric acid, and found that in the cases mentioned the primary walls and pit-membranes remained as a delicate network on the slide (cf. Strasburger, 17). In his description of the method he remarks that if the maceration fluid had not acted for a sufficient time the middle substances were much swollen, and that after treatment with H_2SO_4 they showed as a deep brown line, the secondary layers of thickening not disappearing entirely. If, on the other hand, the macerating medium had acted for too long a time the primary wall vanished along with the secondary layers on the addition of H_2SO_4.

[1] Swift's 1/6 objective. No. 3, ocular.

Halft's method was followed in the treatment of sections of the petioles of *Marattia elegans* and *M. laxa*, but the results seemed to indicate that the mature tissues used were too strongly lignified for the maceration fluid to remove the lignin completely before the dissolution of the lamella took place. Other experiments, however, were decidedly satisfactory, sections of *Selaginella Wildenovii*, treated for a very short time with the maceration fluid and then transferred to strong H_2SO_4, giving the desired result. The primary membranes remained after the removal of the secondary layers, but at the corners of the cells small patches of lignified substance also remained, demonstrating that these were the most strongly lignified areas, and therefore the most resistant to the macerating fluid (cf. Text-fig. 1).



TEXT-FIG. 1. Part of a transverse section of *Selaginella Wildenovii* after treatment with Schulze's maceration fluid and H_2SO_4, showing the primary wall and the patches of lignified substance.

A further microchemical test giving uniformly good results was used. Thin sections of *Marattia* spp. and *Gleichenia circinata* were stained with methylene blue, the lignified walls of the xylem elements taking on a bright blue colour. Between the lignified bars was a substance which stained violet-blue, this indicating its pectic character (Stevens, 15). On mounting in glycerine the violet-blue of the middle substance faded rapidly, while the bright blue of the lignified parts remained unchanged. The decolorization in glycerine is a further characteristic of pectic substances when stained with methylene blue (Mangin, 7–9).

Following Mangin's method, thin sections of *Selaginella Wildenovii* were treated for twenty-four hours with acid-alcohol (one part of acid to five of alcohol), then thoroughly washed with distilled water, and stained with methylene blue. In this case also the primary parts were differentiated (Mangin, 7–9).

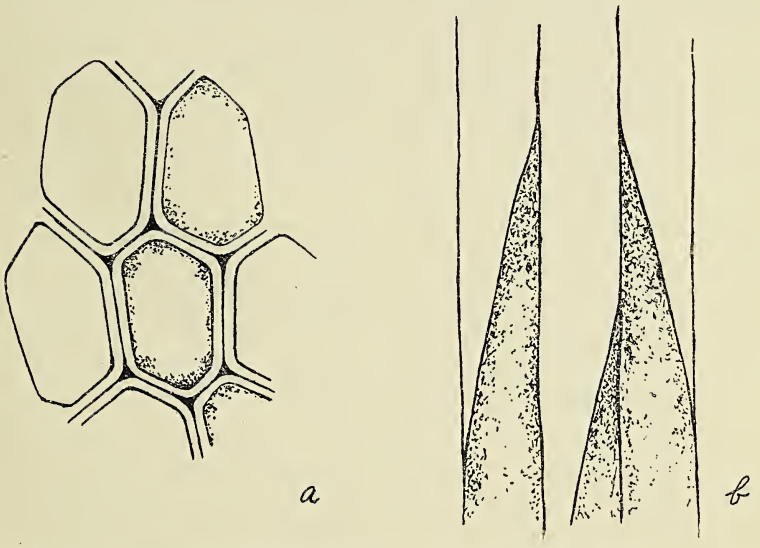
It will thus be seen that the microchemical evidence for the presence of a primary membrane is decidedly convincing.

II. *Physical Methods of Testing the Nature of the Xylem Elements.*

The methods used by Halft were not repeated, but one of his experiments was utilized in a somewhat modified form.

Portions of the petioles of *Marattia* spp., *Angiopteris evecta*, and several polypodiaceous types, and also pieces of the stems of *Selaginella Wildenovii* and *Psilotum triquetrum*, were injected (by means of an air-pump) with water containing in suspension a considerable amount of finely ground

vermilion or Indian ink. Longitudinal sections of the injected stems showed that the granules of colouring matter rose to different heights throughout the xylem, and that they were crowded together in the ends of the pointed elements. It was also noted, both in transverse and longitudinal section, that one element might show granules, while its neighbours were quite free from them (cf. Text-fig. 2 (*a*) and (*b*)).



TEXT-FIG. 2. (*a*) Diagrammatic transverse section of the xylem elements of *Marattia laxa*, illustrating the presence or absence, at the same level, of injected matter adhering to the inner surface of the cell-wall. (*b*) Diagrammatic longitudinal section of *M. laxa*, showing the accumulation of granules in the pointed ends of the elements.

If the xylem had been a communicating system, as Gwynne-Vaughan states, the granules of cinnabar or Indian ink would have been drawn to the same height throughout, not being impeded by either end or side walls. Again, if the xylem had consisted of true vessels, there would have been no great amount of accumulation of colouring matter in the pointed ends of the elements, for the perforations would have allowed of their passage. But the fact of the presence of injected matter in some, and of its absence in others, of neighbouring elements at the same level, points to the presence of a membrane closing the pits of the lateral walls. Hence it would appear that the xylem of Pteridophyta cannot be a communicating system of the type described by Gwynne-Vaughan ; neither can it consist of vessels (at least in the majority of known cases), for the accumulation of granules at the pointed ends of elements shows that the end walls must have acted as filters, not permitting the passage of suspended matter. This points to the presence of a pit-closing membrane here, as well as in the truly lateral portions of the walls ; so that the above-described physical tests strongly

support Halft's views as to the tracheidal nature of the xylem elements of the Pteridophyta in general.

III. *Microscopical Examination of Material.*

1. Material was chosen with a view to extending observations to the lower Filicales, Marattiales, Psilotales, and to other members of those groups representatives of which have already been investigated by Halft.

The types examined include:—

Equisetum limosum	aerial stem
Lycopodium alpinum	stem
L. Phlegmaria	„
L. selago	„
Selaginella Kraussiana	„
S. Wildenovii	„
S. Martensii	„
Psilotum triquetrum	rhizome to aerial stem.
Tmesipteris tannensis	„ „ „ „
Angiopteris evecta	petiole
Marattia laxa	„
M. elegans	„
M. fraxinea	root and petiole
Osmunda regalis	petiole
O. Claytoniana	rhizome and petiole
O. cinnamomea	rhizome
Todea hymenophylloides	rhizome and petiole
Lygodium scandens	petiole
L. dichotomum	„
Aneimia fraxinifolia	„
Mohria caffrorum	„
Gleichenia circinata	„
Matonia pectinata	rhizome
Trichomanes radicans	rhizome and petiole
Polystichum angulare	petiole
Blechnum brasiliense	„
Allosorus crispus	„
Asplenium bulbiferum	rhizome and petiole
Phlebodium glaucum	rhizome
Marsilia Drummondii	„

2. Fixing.—The different solutions used for fixing the material were:—

Alcohol (93 % or 95 %).
Farmer's fluid (acid-alcohol).
Jeffrey's solution.

3. Embedding.—For microtoming, small pieces of material from which all hard cortical layers had been removed were dehydrated, cleared in cedar-wood oil, and embedded in paraffin (M. P. 49°–51°).

4. Sectioning.—The microtome-sections used were mostly 6 or 8 μ in thickness. In the majority of cases both longitudinal and transverse sections were cut. It was recognized that a comparison of the two was necessary for the satisfactory demonstration of the middle lamella, and also for the explanation of the presence or absence of spaces between contiguous xylem elements. As Halft has pointed out, it is difficult to obtain good longitudinal sections with the microtome, owing to the hardness and structure of the material, and to the nature of the embedding medium. Free-hand sections were found, in the case of the larger and harder specimens, to give more satisfactory results.

5. Staining.—A combination of ruthenium red and methylene blue was most generally employed. For microtome-sections, the ruthenium red solution was of the strength mentioned by Gwynne-Vaughan (2, p. 519). For hand-sections, the stain was used very much stronger, only small quantities of solution being made at a time, since the fluid stain soon loses its power. In this way much better results were obtained, the pectic 'middle substance' staining a bright red.

Halft recommends Haidenhain's iron-haematoxylin and haematoxylin-alum, used singly. These stain the middle lamella, but do not affect, or at least only slightly, the lignified bars. In the present investigations both these stains were used with good results.[1]

Other stains found to be effective were Bismarck brown and methyl green; aniline blue and carthamine; Delafield's haematoxylin and safranin; the first named in each case differentiating the primary substances.

A microscopical examination of sections, prepared as described, affords conclusive evidence as to the accuracy of the inferences drawn from micro-chemical and physical tests respectively, and demonstrates that the xylem of Pteridophyta is typically composed of tracheides, the middle lamella and pit-membranes remaining in both end and side walls.

C. Discussion.

Comparing the views of the two most recent writers on the subject of the Pteridophyte xylem elements, it will be seen how entirely conflicting they are :—

Halft holds that the pit-membranes remain; Gwynne-Vaughan, that they disappear, the pits being actual perforations.

[1] Zimmermann (Botanical Microtechnique, 1893, p. 142) notes that haematoxylin is most satisfactory for the recognition of the closing membrane of bordered pits. He used principally Böhmer's solution.

Halft finds true tracheides; Gwynne-Vaughan finds true vessels, often of a peculiar type.

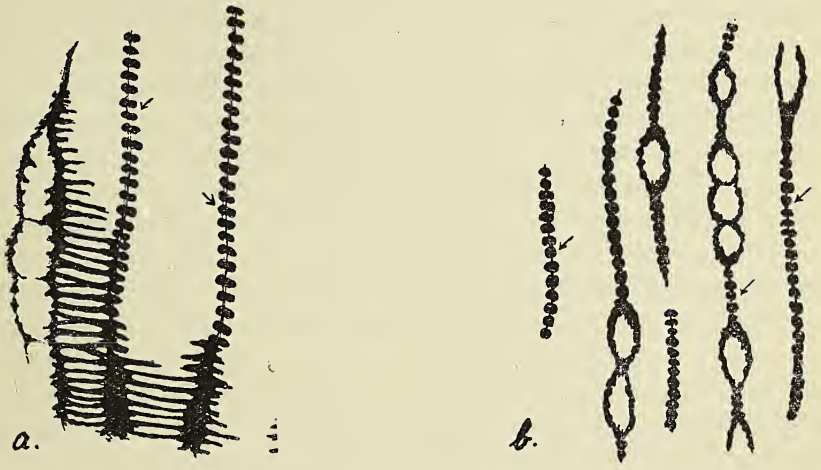
Halft's observations point to the retention of the primary wall between the transverse bars of lignified substance—those of Gwynne-Vaughan to its retention in elements of the type presented by *Pteris Aquilina*; to its partial disappearance in multiseriately pitted types of element such as those of *Osmunda cinnamomea*; and to its complete disappearance (except, of course, at the angles of the cells) in uniseriately pitted forms, as found in *Nephrodium Filix-mas.*

As mentioned in the Introduction, Gwynne-Vaughan was led to his conclusions by observations made in connexion with the fossil Osmundaceae. Transverse sections of *Osmundites skidegatensis* showed apparently clear splits between contiguous xylem elements (cf. 2, Pl. XXVIII, Fig. 2). In the present investigations it was found that sections must be examined under a high magnification (550–1500 diameters), and that they must be fairly thin, if the middle lamella and its continuation as pit-membrane were to be seen distinctly. For example, in a transverse section of *Matonia pectinata* stained lightly with ruthenium red and methylene blue, the middle lamella was at first overlooked, under a magnification of 325 diameters, though a higher power revealed its presence. But the ordinary fossil section is not prepared for examination under a very high power, and extremely minute histological points may easily be overlooked. Miss Kershaw mentions and figures the occurrence of spaces between the xylem elements in *Solenostelopteris* (4, p. 686, Pl. LVIII, Fig. 5); and an examination of transverse sections of the fossil plants *Botryopteris* sp., *Psaronius* sp., *Lepidodendron Harcourtii*, *L. vasculare*, *Lepidophloios fuliginosus*, *Sphenophyllum plurifoliatum*, and others, proved the frequent occurrence of these apparently clear spaces. It seems, however, unlikely that this appearance is due to the fact that the lamella has disappeared (at least normally—it may be broken down by the attacks of Fungi (cf. Seward, 12)). It is more probable that the means of preparation and examination are, as a rule, not perfect enough to demonstrate its presence, in the case of fossil specimens, especially since its occurrence has been definitely proved in allied types among recent plants, e.g. in members of the Ophioglossaceae, Osmundaceae, and Hymenophyllaceae (cf. Seward, 13; Kidston and Gwynne-Vaughan, 5 and 6); the Marattiaceae; the Lycopodiales; and the Psilotales.

In the xylem of the Lepidodendreae, fine connexions are often present between adjacent bars of secondary lignified substance, as, for example, in the short elements in the central part of the protostele of *Lepidodendron vasculare* (Seward, 13). Professor Seward, in describing these delicate connexions, refers to Gwynne-Vaughan's view and remarks that ' in the *Lepidodendron* tracheae we seem to have a stage in which the intervening membrane is in process of absorption. It is, however, possible that the threads

may be the result of contraction and splitting of the membrane during drying or decay.' In view of later work it would seem that Professor Seward's second suggestion is the more probable explanation of the apparent connexions.

An examination of a fairly representative collection of fossil slides resulted in the discovery of two excellent longitudinal sections—one of *Stigmaria ficoides* and one of *Sphenophyllum plurifoliatum*—showing the middle lamella distinctly (Text-fig. 3, *a* and *b*).



TEXT-FIG. 3. (*a*) *Stigmaria ficoides*. Longitudinal section of xylem. (From a photograph.) (*b*) *Sphenophyllum plurifoliatum*. Longitudinal section of xylem. (From a photograph.) (The arrows in both cases point to the middle lamella.)

In *S. ficoides* the lamella appears as a double line. This is probably due to the fact that the two edges of a thick section are seen, the section being also somewhat oblique, as shown by the bars, and the fact that one line, representing one edge of the arc of lamella, is at a lower focus than the other.[1]

To return to the recent forms, in a fair number of cases longitudinal hand-sections were obtained in which it was clear that a series of scalariform bars must have been cut through twice. But the sections of the bars did not fall away from one another, as would have been the case if Gwynne-Vaughan's view of the absence of a connecting membrane were correct. In fact, it would have been impossible to obtain such a section by hand as that shown in Pl. LVI, Fig. 4.

[1] Since the above was sent to press, the writer finds that Williamson and Scott figured the middle lamella in *Calamites* in their paper 'Further Observations on the Structure of the Fossil Plants of the Coal Measures,' Part I, p. 883, Pl. 78, Fig. 9 (Phil. Trans. B., vol. clxxxv, 1894).

It would therefore appear that there must be some connexion between the bars, holding them in position, and a high magnification proved this to be the case.

Gwynne-Vaughan considered that where he saw the connecting middle substance between the bars in longitudinal section, he was looking down at the corner of an element where he recognized that the primary parts were retained. This being the case, it is probable that his general reliance upon transverse sections was the cause of his overlooking the presence of a pit-closing membrane.

In the case of *Pteris Aquilina*, in which Gwynne-Vaughan recognizes the presence of the primary wall at least between the thickened areas, he says 'these persistent parts . . . do not become lignified, but remain pectic in character and stain readily with ruthenium red. When a tracheal wall treated with this reagent is regarded in surface view, the pectic middle substance will appear as a red area shining through the lignified and unstained secondary layers and outlining the inner limits of the pit cavities' (cf. 2, Pl. XXVIII, Fig. 10). A surface view of a tracheide of *Lycopodium selago*, stained with ruthenium red and methylene blue, showed in certain foci the appearance mentioned by Gwynne-Vaughan, except that the lignified layers, unstained in his sections, were here stained blue. At a lower focus, however, the red of the pit-membrane itself was readily seen. It is suggested that this effect was produced by the separation and raising of the lignified layers away from the membrane at the pits (as in a bordered pit), so that they occupied a different focus from that of the membrane, this being, in the actual pit-opening, at too low a focus to be seen. In the areas immediately surrounding the pit-space the primary red-stained pectic layers did not shine through, as here they were not backed and covered closely by the lightly blue-stained parts.

A simple illustration will make this last suggestion more clear. Suppose a piece of red paper to represent the middle primary substance stained with ruthenium red, and two pieces of thin blue paper placed closely, one behind and one in front of it, to represent the layers of secondary thickening stained with methylene blue. If the light is allowed to pass through these three layers, when in contact with one another, the red will be seen through the blue. But if the three layers are separated, as is the case in a pit-area, the red will not shine through the blue so readily.

In his description of the growth and formation of the xylem elements in *Osmunda cinnamomea* (as typical of the Osmundaceae and of multiseriately pitted forms in general), Gwynne-Vaughan remarks that the pectose primary wall is present in immature elements, and that, as maturity is attained and lignification of the secondary layers becomes fairly strong, it gradually disintegrates, except at the angles and (in this particular type) between the series of pits. This is clearly shown in his Fig. 8. Eventually,

according to his view, disintegration is complete and an empty space is left between the thickening bars of contiguous elements.

Halft's researches prove, however, that in *Osmunda regalis*, as well as in other forms, the spaces between the lignified bars are not entirely empty —the middle lamella stretches across from end to end. In this connexion Mr. Sinnott's excellent photographs, illustrating 'Foliar Gaps in the Osmundaceae', may be mentioned. His Fig. 8 (*Todea hymenophylloides*) shows the presence of the lamella perfectly (Sinnott, 14). The writer has also observed the lamella in *Osmunda Claytoniana*, *O. cinnamomea*, and *Todea hymenophylloides* amongst the Osmundaceae, as well as in representatives of other families of the Filicales and of other groups of the Pteridophyta.

In *Gleichenia dicarpa* good evidence of the presence of the lamella was afforded by a torn section, in which adjacent bars of secondary thickening were broken and displaced, while the lamella projected from between the bars, having given way at one of the angles (Pl. LVI, Fig. 6).

Halft used principally only mature material in order to prove the persistence of the middle substance, even in the later stages; but he appears to have sectioned young xylem of *Osmunda* also, for he mentions that it does not show splits, the 'springing apart' of the lignified bars not having taken place at this stage.

Not having sectioned xylem of different ages, he has failed to see that a certain amount of disintegration actually does occur. An examination of *Todea hymenophylloides* and *Tmesipteris tannensis* has shown that the splits between the bars of thickening of adjacent elements are formed by the partial disappearance of the primary wall, leaving the middle membrane traversing the space from end to end. Its extreme delicacy renders it difficult of observation, especially if the staining is light and the focus not extremely exact.

Halft appears to think that the presence of a split is due to the mere 'springing apart' of the bars of thickening, and that the primary wall remains between, no dissolution taking place. But the pit-membrane is middle lamella only, and as the primary wall consists of middle lamella and layers of pectic substances on either side, it would seem that some disintegration of the primary wall must occur where the middle lamella is exposed and separated from the secondary bars. Where the bars actually join, it may be supposed that the primary layers remain, as well as the middle lamella. The longitudinal section of *Tmesipteris tannensis* (Fig. 15) in particular supports this view—the primary layers being distinctly thicker where the bars join than where they separate, thus indicating what the transverse sections showed to be the case—that some disintegration has taken place.

It may be that a certain amount of 'springing apart' helps the widening

of the splits in some cases, when the bars are no longer held together by the primary wall; but it seems reasonable to maintain that some disintegration precedes the separation, which begins before lignification is very far advanced.

It is further suggested that upon the actual amount of breaking down of the primary wall between corresponding pairs of bars depends the presence or absence of a 'split' as seen in transverse section.

If the disintegration is great, the bars will be connected only for a very small fraction of their whole width, as in *Matonia pectinata* (Pl. LVI, Fig. 2). The corresponding transverse section (Fig. 1) shows the splits wide and clear, and it is obvious that in transverse sections it will be comparatively rarely that the junction of the bars is cut through—hence the general appearance of splits in such a type. If the amount of disintegration is slight, as shown in the longitudinal section of *Marattia fraxinea* (Fig. 4), the connexion between the bars extends nearly their whole width, and the prevalence of the 'non-split' appearance as seen in transverse section (Fig. 3) is clearly due to the plane of section cutting (in the majority of cases) the regions where the bars are joined. The variation in the width of the splits in different types may be the result of the different amounts of the raising or springing apart of the bars in the pit areas.

It seems unwise to attempt to classify types under the head of 'split' or 'non-split', for between the two extremes illustrated by *Matonia* and *Marattia* there may be any number of cases showing intermediate degrees of separation of the thickening bars, representing not only different amounts of disintegration of the primary wall, but also different degrees of springing apart of the secondary layers, the splits being sometimes wide, as in *Matonia pectinata* (Figs. 1 and 2) and *Lygodium scandens* (Fig. 11), and sometimes narrow, as in *Selaginella Wildenovii* (Figs. 7 and 8) and *Lygodium dichotomum* (Figs. 12 and 13). A transverse section of *Aneimiodictyon* sp. (petiole) showed a type nearest that of *Marattia*, though not so extreme, while allies of this species, *Aneimia fraxinifolia* (Figs. 16 and 17) and *Mohria caffrorum*, presented a type almost as extreme as *Marattia*. *Psilotum triquetrum* (Figs. 9 and 10) and *Tmesipteris tannensis* (Figs. 14 and 15) showed fairly wide splits on the whole, though the relative width of these seemed to vary in different parts of the plant. In the species of *Osmunda* examined, the xylem of the rhizome was found to have large splits, while that of the petioles exhibited the opposite extreme. This being the case, it is hardly surprising that there is little constancy of type among species of the same genus or family, though *Marattia laxa*, *M. fraxinea*, *M. elegans*, *Angiopteris evecta*, and *Danaea* sp. (cf. Figs. 3, 4, and 5) were found to approximate to the same type.

Where the connexion between successive pairs of thickening bars is narrow it is clear that the mechanical efficiency of the xylem elements will

be much less than where the connexion of the bars is wider. It is tentatively suggested that there may be a mechanical explanation of the variation in type. In the case of *Osmunda*, for example, where there is variation within the organs of the same plant, the mechanical needs are clearly greater in the petiole than in the rhizome, and the efficiency of the xylem as a strengthening tissue is here raised by the wider connexions of the bars.

Again, where the connexion of the thickening bars is slight, there will be a larger area through which transfusion of water and solutions may take place, in a lateral direction as well as vertically.

The different parts of the conducting system in this case will be in more facile communication than where the exposed areas of lamella are smaller and transfusion is consequently somewhat retarded.

With regard to the detection of true vessels,[1] such as were found by Halft in *Pteris Aquilina*, longitudinal sections are necessary, for it seems that the perforation of the end walls of the xylem elements depends, in a great measure at least, upon the amount of their inclination, and that where end and side walls grade into one another and are not properly differentiated, as is the case in the pointed elements of *Osmunda regalis*, pit-membranes remain throughout; but where end walls are well defined and very little inclined to the lateral walls, the need for true perforations may arise. In all the cases where longitudinal sections were used as controls to the transverse (as in the majority of the types mentioned), true pointed tracheides were found, confirming the opinion of Halft that these are the rule in Vascular Cryptogams.

Summary.

1. The xylem elements of Pteridophyta are typically pointed tracheides, the pits on both end and side walls being closed by a membrane formed by the persistent middle lamella.

2. The middle lamella is believed to be exposed in the pit areas by the disintegration of the rest of the primary wall.

3. The varying amounts of disintegration give rise to the prevalence of the 'split' or 'non-split' appearance between the secondary lignified layers, as seen in transverse section.

4. The width of the split depends largely upon the degree of separation of the secondary layers in the areas surrounding the pit openings.

The grateful acknowledgements of the writer are due to Dr. H. C. I. Fraser for drawing attention to this problem; to Professor Carr for material and opportunities for research; and to Professor Gwynne-Vaughan and Mr. H. S. Holden for valuable criticism and advice.

[1] Halft employs the term 'trachea' in its limited sense meaning 'vessel', whilst Gwynne-Vaughan apparently follows De Bary's usage, giving it a comprehensive application. In the present instance also, 'trachea' is understood to include both 'vessel' and 'tracheide'.

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(Further references may be found in the works quoted.)

EXPLANATION OF PLATE LVI.

Illustrating Miss Bancroft's paper on the Xylem Elements of the Pteridophyta.

(The figures are semi-diagrammatic, the magnification being 1,500 diams. in each case).

Fig. 1. *Matonia pectinata.* Transverse section of xylem elements of rhizome, showing the middle lamella traversing wide spaces between them.

Fig. 2. *Matonia pectinata.* Longitudinal section, showing narrow connexions of the thickening bars.

Fig. 3. *Marattia fraxinea.* Transverse section of xylem of petiole. The thickening bars are closely connected; no spaces show between them.

Fig. 4. *Marattia fraxinea.* Longitudinal section through a series of lignified bars. Note the wide connexions between successive pairs. A pointed end of an element is also shown (*a*).

Fig. 5. *Angiopteris evecta.* Longitudinal sections. Cf. Fig. 4, showing the same type.

Fig. 6. *Gleichenia dicarpa.* Transverse section of xylem of rhizome, showing the torn lignified bars and the projecting lamella.

Fig. 7. *Selaginella Wildenovii.* Transverse section of xylem of stem. The spaces between the bars are relatively narrow.

Fig. 8. *Selaginella Wildenovii.* Longitudinal section corresponding to the transverse section shown in the previous figure.

Fig. 9. *Psilotum triquetrum.* Transverse section of xylem of aerial stem—the middle lamella traverses spaces between the elements.

Fig. 10. *Psilotum triquetrum.* Longitudinal sections, showing the narrow connexions between the lignified layers.

Fig. 11. *Lygodium scandens.* Transverse section of xylem of twining petiole. Compare the wide splits exhibited by this part of the xylem with those in

Fig. 12. *L. dichotomum* (petiole), where they are relatively much narrower.

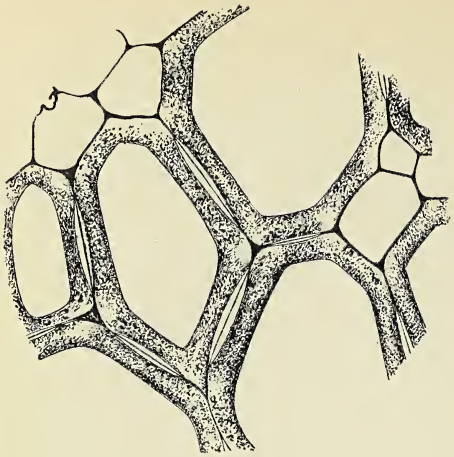
Fig. 13. *L. dichotomum.* Longitudinal section, corresponding to Fig. 12.

Fig. 14. *Tmesipteris tannensis.* Transverse section of xylem of aerial stem. The lamella is seen in the spaces between the tracheides.

Fig. 15. *Tmesipteris tannensis.* Longitudinal section, showing the connexions between the thickening bars.

Fig. 16. *Aneimia fraxinifolia.* Transverse section of xylem of petiole. No spaces are shown. Cf. *Marattia fraxinea.*

Fig. 17. *Aneimia fraxinifolia.* Longitudinal section, corresponding to Fig. 16.



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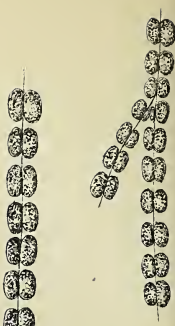

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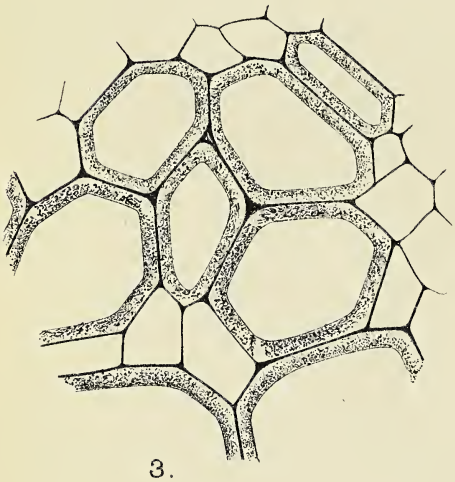

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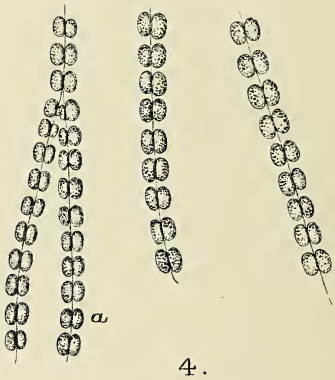

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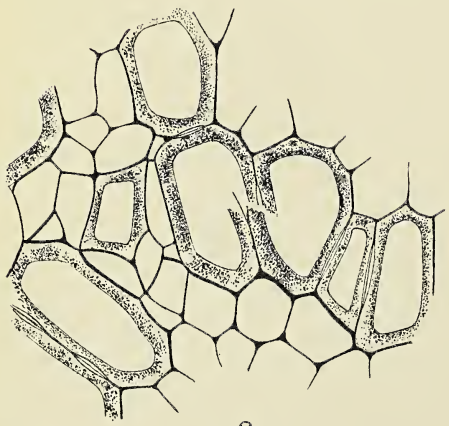

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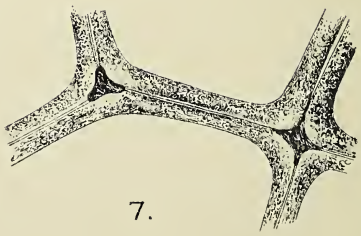

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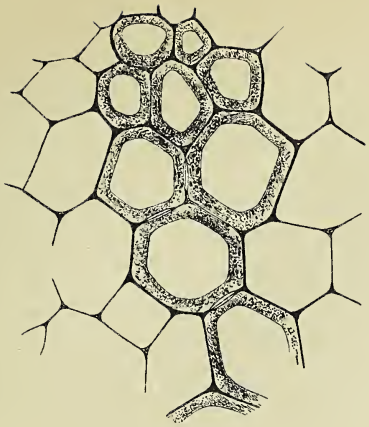
BANCROFT — XYLEM ELEMENTS OF PTERIDOPHYTA.



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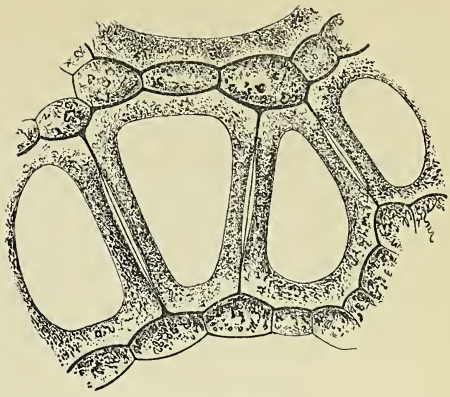

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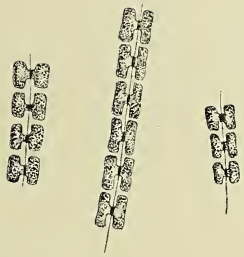

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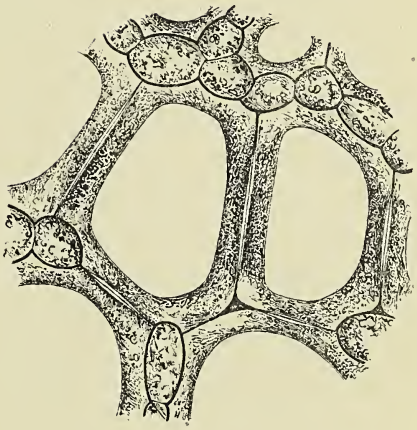

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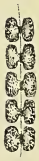

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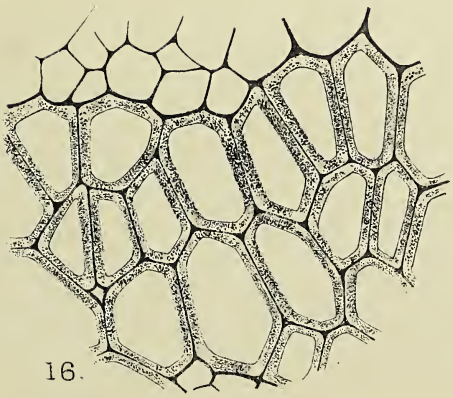

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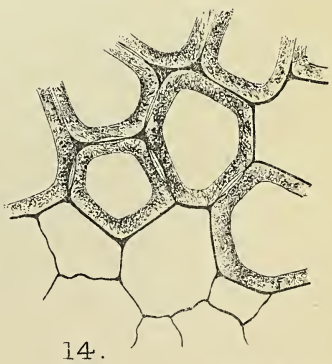

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The Ontogenetic Development of the Stele in Two Species of Dipteris.

BY

HELENA DE BRUYN,

Newnham College, Cambridge.

With Plates LVII and LVIII.

THE material investigated was gathered by Mr. Tansley in 1901 on Mount Ophir in the Malay Peninsula. The amount of material available was not very large, and often the series of sections were not quite complete, so that not all the stages wanted were obtained; enough facts, however, were gathered to form some conclusions on the development of the stelar structure.

The plants investigated were *Dipteris conjugata*, Reinwardt, and *D. Lobbiana*, Moore. Both have a creeping rhizome with leaves going off in alternate rows. Fig. 3, Pl. LVII, shows a young plant of *D. Lobbiana*; the first leaves are divided once, in the latest formed a second dichotomy has taken place. The veins in the leaves branch dichotomously and anastomose, even in young leaves (*D. conjugata*, Fig. 5; *D. Lobbiana*, Fig. 6, Pl. LVII). In the adult stems of both species a solenostele is present. The anatomy of the stele of the mature plant of *D. conjugata* is fully described by Seward and Dale.[1]

Dipteris conjugata.

Endodermis. Surrounding the steles of the young plants we find, in the first place, a layer of endodermal cells easily to be recognized by the dark contents of its cells. An endodermis consisting of cells with dark brown contents is a very widely spread phenomenon among Ferns, and endodermal cells with similar contents are even found in the fossil solenostelic Fern described by Miss Kershaw.[2] In the regions near the growing point, however, and sometimes also in older parts, the dark secretion is either absent or, owing to bad preservation or other causes, is not apparent, and

[1] Seward and Dale: On the Structure and Affinities of *Dipteris*. Phil. Trans. Roy. Soc., London, B. 204, vol. cxciv, 1901.

[2] Kershaw: A Fossil Solenostelic Fern. Ann. of Bot., vol. xxiv, 1910, p. 686.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

this makes the endodermis, especially in the young plants, often difficult to recognize.

Pericycle. The pericycle consists of one or two rows of cells, and these cells may have grey contents. The pericycle of the mature plant, as stated by Seward and Dale,[1] consists of two or three rows of cells.

Xylem. In the young plants the xylem is formed of scalariform tracheides, between which occasionally parenchymatous cells are found; in older plants the parenchymatous cells are more numerous.

Phloem. The phloem consists of small elements, many of which often possess nuclei. In the older plants a pith is present, the cells of which have sclerotic walls; they are very much like the cells of the inner cortex next to the stele.

Cortex. The cortex in the youngest stems is formed by uniform cells. In the older plants the outer layers of the cortex have cells with thicker walls than the inner ones, and in quite young plants this difference can already be noticed.

The Ontogeny of the Stele. The extreme base of the stem of *D. conjugata* was not present at all; it is therefore impossible to be certain that a protostele is present, although it is highly probable. The stele of the young stem is often of the *Lindsaya* type[2] (amphiphloic protostele), and the ventral side of the xylem-ring is frequently much thicker than the dorsal, as in the adult stem of *Lindsaya* itself. This is not, however, a constant feature, as we may find young stages with a more equally developed xylem-ring. Near the base of the stem the tissue surrounded by the xylem has a parenchymatous structure. It is very difficult to tell in the early stages whether any of the cells of the central parenchyma are sieve tubes. Later on undoubted phloem is found, and only then can we say with certainty that the whole stele is of the *Lindsaya* type. A striking phenomenon is the appearance of cells with dark contents in the central tissue. Only a very few of these dark cells are formed at first. They do not form any regular layer, neither have they a constant position. They generally closely resemble the endodermal or pericyclic cells. The contents of the pericyclic cells are mostly not so dark as those of the endodermal ones. It is quite possible that the dark cells formed in the centre of the young stele should really be regarded as the common mother-cells of pericycle and endodermis.

The behaviour of these dark internal cells during the first stages is quite interesting. As stated, a few of these cells appear in the centre of the stele, but in the next sections their number decreases or they disappear completely. That they are really absent and not merely unrecognizable owing to the absence of their dark contents, is shown by the fact that when they disappear the whole central tissue is formed by a smaller number of

[1] Seward and Dale, loc. cit., p. 494.

[2] Tansley and Lulham: On a New Type of Fern Stele, &c. Ann. of Bot., vol. xvi, 1902.

cells. Some sections later the central tissue has again increased and dark cells are formed, but afterwards they disappear again. The position in which these cells appear varies a good deal. Sometimes they are formed when a leaf-trace is about to depart, sometimes just before the xylem-gap is closed, but they may be absent from both these positions. Fig. 13, Pl. LVIII, shows the stele of a young plant. The rhizome of this plant was 4 mm. in length and had formed eleven leaves. In this stage a leaf-trace has left the stele some little distance below, and the xylem is not yet quite closed. Two dark cells are seen in the centre surrounded by phloem. Fig. 14 is a section of the same rhizome after four more leaf-traces have gone off. No dark cells are present here. The xylem is much more developed on the ventral side. At the departure of the first leaf-traces there is no connexion whatever between the dark inner cells and the outer endodermis, but after some more leaf-traces have gone off, the dark inner cells come into connexion with the outer endodermis, when the leaf-trace separates from the stem-stele, and a real leaf-gap is formed for the first time. It may happen, however, that on the departure of the next leaf-trace there is again no connexion of the outer endodermis and the inner dark cells. The inner endodermis can now often be distinguished from the inner pericycle as a definite tissue, but it does not yet form a regular layer. The number of its cells constantly changes, and it may even disappear completely.

The presence of internal endodermal cells in the stele, before a real leaf-gap is formed and before any connexion has taken place between the outer endodermis and the inner tissue, shows that the inner endodermis is formed independently in the centre of the stele, and that the inner endodermal cells are not in any sense formed by 'intrusions' of the outer ones through the leaf-gaps.

In a series from an older stem we find a true solenostele, but the xylem-ring is still quite obviously thicker on the ventral than on the dorsal side. Protoxylems are apparently absent, as is generally the case in the first-formed stems of Ferns. The pith consists in the first section only of one cell (Fig. 1, Pl. LVII), and a few sections later no pith cell can be recognized. At the end of the series, however, the pith is quite a distinct tissue. Through the whole series the cells forming the pith and the internal endodermis are constantly decreasing and increasing in number; on the whole, of course, they increase. In the first appearance of the internal endodermis this fluctuation in development is already observed. It is seen in all the different tissues of the stele in the youngest stages, and here in the older stem it is again quite obvious, especially in the pith. The leaf-traces succeed each other in this stem very rapidly, and are rather small in comparison with the stele. They mostly go off with two xylem-strands.

In a still older stem the structure is much more like that of the mature

plant. The xylem cylinder is mostly uniform laterally and ventrally, but in some places the ventral side is still thicker than the dorsal one. The xylem consists of tracheides with rows of parenchymatous cells. Some of these have brown contents; probably this secretion is tannin, since in the mature plant the presence of tannin sacs is a constant feature. Mesarch protoxylems are perhaps present, but they are not very distinct. In the series described here the leaf-traces follow each other very quickly, and before a gap is closed the next leaf-trace is already prepared for by the part of the xylem-ring destined for the next trace becoming thinner and projecting outwards. The result is that a complete uniform xylem-ring is never present. The gap is, however, always closed before the projecting arc of xylem separates from the rest, so that the stele is still a true solenostele. In older stems the distance between two leaves is much greater, and there are long internodal stretches with an equally developed xylem-ring. An interesting fact is that the xylem at the margins of both sides of the gaps of this plant is distinctly thickened. The endodermis does not closely follow this thickening. In the mature plant, as described by Seward and Dale,[1] this thickening of the margins always occurs and is still more developed. Here all the other tissues have the same shape. In an old plant examined by me the closing of the leaf-gap was effected by these thickened gap-margins approaching each other on both sides till they fused. The stele had then the form of a ring whose xylem is much thicker on the dorsal than on the ventral side. Gradually this dorsal part becomes thinner till the tissue of the whole xylem-ring has the same thickness. This behaviour does not agree with the statements of Gwynne-Vaughan and Miss Kershaw. Thus Gwynne-Vaughan[2] writes of *D. conjugata*: 'The free xylem-strand has almost separated off from the solenostele altogether, being connected with it only at two points between which a tongue of ground tissue has inserted itself.' This statement may perhaps refer to the parenchymatous tissue which is formed in the abaxial part of the thickening, but this part belongs to the arc which goes off as the leaf-trace. The parenchyma separates the xylem of the limb of the horseshoe from its curved end. There is no sign whatever of a free xylem-strand nor of any thickening in the internodes. The figure of Seward and Dale[3] of the mature stem shows an equally developed solenostele. Miss Kershaw[4] writes: 'In *D. conjugata* 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.'

[1] Seward and Dale, loc. cit., p. 499.

[2] Gwynne-Vaughan: Observations on the Anatomy of Solenostelic Ferns, Part II. Ann. of Bot., vol. xvii, 1903, p. 700.

[3] Seward and Dale, loc. cit., Fig. 8.

[4] Kershaw, loc. cit., p. 687.

Nothing of the kind is figured by Seward and Dale, nor has it been noticed in the material examined by me.

As a result of the investigation of the different stages of the stem in regard to the development of the stele, we may say in general that probably first a protostele is formed, then the *Lindsaya* type occurs, and this then passes into a solenostele, which at first has thicker xylem on the ventral side, but afterwards comes to possess a xylem-ring equally developed on all sides. The xylem of the margin of the leaf-gaps becomes thickened, and higher up the stem this thickening is still more developed. There is, however, a great fluctuation in development in all the tissues. They may for some time go back again to the condition of a less developed stage. In one series a certain stage may be less developed than in another, or may last for a shorter period.

Roots. The roots of old plants in both species have triarch structure; but the first-formed roots of both species are probably diarch. In the mature plants roots are given off from any part of the surface of the stem (Seward and Dale [1]) and independently of the leaf-traces. The same is the case in the young plants. In one case a root was even given off from the thinner and outwardly projecting part of the xylem-ring destined to form a leaf-trace.

Petiole. The first-formed petioles have a simple structure. The xylem forms a flattish arch and is surrounded by phloem. In petioles which are a little older the xylem is horseshoe-shaped. The xylem-arch is interrupted by parenchyma, as seen in Fig. 2, Pl. LVII, which shows the leaf-trace of a rather small plant while still included in the main axis. As already mentioned, the leaf-traces at this stage often depart with two xylem-strands. In petioles formed by an older plant the xylem is broken up into many more pieces. In the petioles which have already horseshoe-shaped xylem, the phloem surrounds the xylem, running in a straight line across the concavity from limb to limb of the horseshoe, but not lining the concavity. In Fig. 2 there is no phloem present at the adaxial side of the xylem. The part of the xylem forming the leaf-trace but still attached to the stele of the stem had here probably no lining of phloem on the inner side. In petioles formed later still we get the same characters as in the mature plants. The xylem is horseshoe-shaped, with the ends curved inwards, and the row of tracheides is interrupted by parenchyma. The phloem and also the endodermis follows the xylem-arch. Secretory sacs, which are absent in the young petioles, are present, and between them protoxylems are found. The investigation of the petioles of the different stages shows very distinctly that there is a gradual transition from the simple structure of the petiolar stele to the more complicated type of the mature plants. Thus we find some petioles which have at their base a horse-

[1] Seward and Dale, loc. cit., p. 495.

shoe-shaped stele, with the endodermis invaginated, while higher up the endodermis and pericycle form a straight line on the adaxial side. In some of the petioles of this stage a tissue of sclerenchymatous cells, surrounded by an endodermis, is found in the centre of the petiolar stele. These steles show a very close resemblance to those in the base of the petioles of *Matonia sarmentosa* (Compton [1]), and of some species of *Gleichenia* (Poirault [2]). This structure is not found through the whole petiole but only in parts of it, and is not always the same in different leaves. In one petiole, e. g., the whole stele has horseshoe-shaped structure at the base. Then the ends of the limbs of the horseshoe approach each other till they meet. The endodermis of both ends joins together, and attached to it is a loop of endodermal cells, including a strand of ground tissue. The loop becomes detached from the outer endodermis, and thus a patch of sclerenchyma surrounded by a ring of endodermal cells is formed inside the stele. These endodermal cells are at first separated by only one layer of pericyclic cells from the endodermis surrounding the stele, but soon the patch of sclerenchyma with its endodermis is found more in the centre of the stele. This structure is reached at 0·5 cm. from the base. The resemblance to *Matonia sarmentosa* and some species of *Gleichenia* is now very striking. At a higher level more changes take place. The outer endodermis projects somewhat inwards, and the internal sclerenchyma changes its position more towards the adaxial side of the petiolar stele. Finally the inner and outer endodermis come into connexion with each other, and a normal horseshoe-shaped stele is again present at 1·5 cm. from the base. For a distance of 1·25 cm. this structure remains; then the ends of the limbs of the horseshoe approach each other again, and at 3 cm. from the base there is again internal sclerenchyma; but now only one cell, surrounded by endodermis, is present inside the stele. At 3·5 cm. the number of sclerenchymatous cells has increased to three. At a higher level it was impossible to tell if internal sclerenchyma or endodermis were still present, but the outer endodermis still formed a straight line at the adaxial side. Unfortunately the petiole was broken off at this point. At the highest level starch was still present in the stelar tissue.

At the base of another petiole, 5 cm. long, the endodermis and pericycle formed a straight line on the adaxial side. Inside the stele one or two dark cells were present which might be internal endodermal cells. Soon a whole group of these internal endodermal cells was formed, and in the midst of these one or two sclerenchymatous cells appeared. The sclerenchymatous cells surrounded by the endodermis come nearer to the outer endodermis and come into connexion with it. After some time they are separated again from the outer endodermis by a layer of pericyclic cells, and

[1] R. H. Compton: The Anatomy of *Matonia sarmentosa*, Baker. New Phyt., vol. viii, 1909, p. 302.

[2] Poirault: Recherches anatomiques sur les Cryptogames vasculaires. Ann. des Sciences nat. (Bot.), série vii, tome 18, 1893, p. 181.

then gradually the internal endodermal and the sclerenchymatous cells disappear.

Thus the behaviour of the petiolar stele was in this case quite different from the one described before. In the first one the internal sclerenchyma was at first in connexion with the cortex, and the internal endodermis was continuous with the endodermis surrounding the stele ; in the second petiole the internal sclerenchyma and endodermis are formed in the stele itself, and have no connexion with cortical tissue and outer endodermis. In the last-mentioned petiole the stele was never horseshoe-shaped, for the endodermis is never invaginated on the adaxial side.

In a third petiole the different tissues behaved more like those in the petiole described first, but here the loop of endodermal cells surrounding the sclerenchymatous cells is never separated from the external endodermis. The two limbs of the horseshoe-shaped stele approach each other, separate, and approach each other again. The inner sclerenchymatous cells, or the ones in the curve or in the loop, have often thicker walls and are smaller than the cells of the cortex near the stele.

In many petioles of approximately the same age as the ones described, no internal sclerenchyma or endodermis was found. The changes described take place rather suddenly ; it may therefore be that in some the structure was present in a small part, but not found. Seward and Dale [1] have found similar internal fibres in the stele of the main ribs of the leaf of mature plants.

As already mentioned, *Matonia sarmentosa* has in its mature petioles internal sclerenchyma at the base, while on a higher level a horseshoe-shaped stele is present. Perhaps some may think that the resemblance of the structure in some young plants of *D. conjugata* with the mature *M. sarmentosa*, and with some species of *Gleichenia*, must be regarded as an ancestral character, and that it adds support to the view that *Dipteris*, *Matonia*, and *Gleichenia* are related forms. But the irregularity of the behaviour of the internal tissues, the different ways in which they are formed, their rare occurrence and their presence in old leaves, are all facts which suggest to the writer that too much stress should not be laid on this character.

It seems to me possible to explain the facts as follows. When the petiole is getting larger and the limbs of the horseshoe become more divergent, the central tissue gets a tendency to form sclerenchyma. In connexion with this, endodermal cells are formed separating the sclerenchymatous cells from the other tissues. According as different parts of the stele are affected the different structures occur: i.e. the internal sclerenchyma is surrounded by an endodermis in the centre of the stele or is attached to the outer endodermis or directly to the horseshoe-shaped strand. In older petioles, in which the limbs of the horseshoe are more divergent, the stele has always the horseshoe shape. We find a similar case in different species of *Gleichenia*,

[1] Seward and Dale, loc. cit., p. 498, Fig. 38.

in which the centre of the petiole is often for part of its course sclerotic. In *Gleichenia dicarpa*, belonging to the sub-genus *Eugleichenia*, the central region, which Boodle [1] regards as being pericycle, consists partly of parenchyma, partly of sclerotic elements. No internal endodermis is present here, but it is found in other species of *Gleichenia*. The petiole of the sub-genus *Mertensia* has an invaginated endodermis. Boodle writes of it (p. 716): 'The central region is occupied by sclerotic tissue continuous with the cortex and apparently belonging to it instead of to the pericycle as in *G. dicarpa*.' In *G. pedalis* the endodermis is only slightly incurved, and here the pericycle is again partly sclerotic as in some Eugleichenias. We see, therefore, that in *Gleichenia* there seems likewise to be a tendency to form sclerenchyma in the central part of the stele, and this is brought about in the different species in various ways resembling the different structures of *D. conjugata*.

Often tannin sacs are not present at the base of the petiole, but appear at a higher level; this is also the case with the protoxylems. Division of the stele into two by constriction in the median vertical plain takes place only just before the lamina is reached and is soon followed by a second division. The veins of the leaf are collateral.

Dipteris Lobbiana.

Stem. *Dipteris Lobbiana* has in many respects the same characters as *D. conjugata*. Fortunately the very first stages were present, but the series were more incomplete and broken than those of *D. conjugata*.

The diarch primary root passes into a protostele (Fig. 4, Pl. LVII). A part of the xylem projects and passes out as the first leaf-trace. The further stages often show the same types as are found in *D. conjugata*. The *Lindsaya* stage is passed through and a few dark cells are formed in the centre, and these disappear again afterwards. A xylem-ring is formed with unequally developed ventral and dorsal side, but the difference in thickness of the two sides is not so striking as in *D. conjugata*.

In one of the series we find the stage represented by Fig. 15, Pl. LVIII. The xylem has the appearance of not being fully differentiated yet, and the section looks as if it were near the meristematic region. Some sections nearer the stem apex, however, the xylem-elements increase very much, in connexion with the formation of a root (Fig. 16), and some sections further on still a complete xylem-ring is formed again. This ring is only one cell thick at the dorsal side and the xylem is much more developed at the point of insertion of the root. This series is again an example of the very irregular development which is so striking a character in *D. conjugata*. The increase of xylem-elements in connexion with root-formation is a very constant feature. When the meristematic region is reached, the xylem is

[1] Boodle: On the Anatomy of the Gleicheniaceae. Ann. of Bot., vol. xv, 1901, p. 714.

represented by a few scattered elements and a group of connected tracheides at one side. These connected tracheides form a fully differentiated leaf-trace (Fig. 8, Pl. LVII). This departure of a leaf-trace while the rest of the xylem is in the meristematic condition is found in all cases, and also in *D. conjugata.* It is a common phenomenon at the apices of Fern stems. But the local failure to develop xylem further back in the stem does not seem to have been described in other Ferns.

In an older stem a normal solenostele is found with an equally developed xylem-ring. The pericycle is formed by a row of 1–2 cells. The cells of the phloem have very thick walls. No protoxylems are apparently present in the xylem, which consists of scalariform tracheides with parenchymatous cells in between. A leaf-trace goes off with two separate xylem-groups. Still within the main axis it turns, ceasing to be opposite the leaf-gap. Fig. 7 is a section of the stele at this stage. The xylem-ring is still open and the internal endodermis is represented by a single cell only. The stele gradually increases in size and a pith is formed. The cells of the pith have thicker walls than those of the cortex near the stele. Near the growing point, when the xylem and phloem are already fully differentiated, the pith and cortex cells have still thin walls, and the endodermal cells have no contents and are not easily recognized. This shows that the xylem and phloem are sooner differentiated than the other tissues.

A still older stem is represented by Fig. 17, Pl. LVIII. On the whole it has the same characters as the one just described. The xylem consists of tracheides with dark brown cells in between. This brown substance may be a secretion of tannin. These tannin cells are represented in the younger plants by the colourless parenchymatous cells. No older plants of *D. Lobbiana* were available to enable one to form definite conclusions, but the structure does not differ very much from the one found in *D. conjugata* of approximately the same age.

A marked difference between the two species is that the leaf-trace in *D. Lobbiana* is given off as two separate strands, one after the other on the same side of the xylem-gap, as shown by the diagrams (Figs. 9, 10, 11, 12, Pl. LVII). No thickening takes place at the margin of the xylem leaf-gap in this species. On the whole, however, there is a great resemblance in the development of the stelar structure of the two species.

Petiole. The first-formed petioles have here also a simple structure. In a petiole 1 cm. long, the lamina of which divides twice dichotomously, the vascular strand is composed of a few xylem-elements forming a slightly curved arch and surrounded by phloem. Probably protoxylem is present. The pericycle consists of one layer of cells. The stele divides into two at the base of the lamina.

A petiole 3·5 cm. long had a lamina which showed three successive dichotomous branchings. At the base of the petiole the stele is formed by

two xylem-groups (Fig. 18, Pl. LVIII). Protoxylem is probably present. The phloem of the ventral side has joined that of the dorsal side and the endodermis extends somewhat inwards. The whole strand has the appearance of the beginning of a branching, but 1 cm. higher the strand has again single structure (Fig. 19). The tracheides form here an uninterrupted arch surrounded by phloem which does not line the concavity. Protoxylem is again present. At the distance of 1·5 cm. from the lamina the strand divides into two, and near the lamina a second dichotomy occurs. Four strands are thus formed, as seen in Fig. 20. Protoxylem remains visible. The veins in the leaves are eventually collateral; they are often surrounded by sclerotic tissue.

In the oldest plant the petiole has two separate strands. Only a very small part of the petiole was available, and in this part (3 cm. long) no change takes place, the two separate bundles maintaining the same distance apart. Each bundle is formed by a xylem arch surrounded by phloem. Endarch protoxylem is probably present. In the first section no tannin cells are found, but somewhat higher characteristic tannin sacs occur. They are not, however, always formed in the same tissue, for they may be next to the xylem in the conjunctive parenchyma, or they may occur in the phloem.

The petiole described first has a single stelar structure, and branching takes place only quite near the lamina; in the oldest plant the leaf-traces go off with a double strand from the stem stele. The transition stage between these two types is quite distinctly formed by the second plant with its double xylem-strand at the base, and again single structure afterwards.

In conclusion I want to express my thanks to Mr. Tansley for supplying me with the material and for all his kind help and advice throughout my work. The photographs were taken by Mr. Mangham, to whom I should also like to express my gratitude.

Summary.

1. The evolution of the stele in the young plants of *Dipteris conjugata* and *D. Lobbiana* appears to be essentially similar. In *D. conjugata* the earliest stages were not available, but there is little doubt that a protostele would be found at the extreme base of the young stem, as it is in *D. Lobbiana*.

2. The central tissue formed in the middle of the xylem appears at first to consist largely, perhaps sometimes exclusively, of parenchyma. Later, sieve tubes appear and a typical *Lindsaya* stage is reached. In *D. conjugata* the ventral side of the xylem-ring is distinctly thicker than the dorsal.

3. Cells with dark contents, probably representing the beginning of an internal endodermis, appear isolated or in irregular masses in the midst of the central tissue (phloem or parenchyma). These cells may appear and disappear again several times as the stele is traced upwards, but make no connexion with the external endodermis at the leaf-gaps or elsewhere till after the departure of several traces.

4. Regular connexion of the dark cell-strand with the outer endodermis at the succeeding leaf-gaps occurs after some time; eventually pith cells appear in the centre of the group of dark cells and a normal solenostele is formed. The solenostele of *D. conjugata* frequently retains for some time the inequality of thickness of its xylem on the dorsal and ventral sides noticed in the *Lindsaya* stage, but the xylem-ring eventually becomes of uniform thickness all round.

5. The thickening of the xylem on the edges of the leaf-gap described by Seward and Dale in mature plants of *D. conjugata* occurs in the solenostele of quite young plants.

6. A feature of the steles of the young plants in both the species of *Dipteris* investigated is the irregularity of development in several of the stelar tissues. Thus the external endodermis is often irregularly arranged and is frequently double for part of its course. The pericycle sometimes contains similar dark contents. The dark cells which appear in the midst of the internal phloem or parenchyma in the young plants are generally regarded as being endodermal in nature, but they may be partly of pericyclic nature, or they may represent the common mother-cells of endodermis and pericycle. The development of the xylem is frequently irregular, the tracheides failing to develop in parts of the xylem-ring, which again becomes completely differentiated nearer the apex.

Local development of xylem in advance of the general development is often found in the neighbourhood of the apex of the rhizome at the point where a root or a leaf-trace is inserted.

7. In *Dipteris conjugata* the petiolar stele is always single, in the earlier leaf-traces a flattish arch of xylem surrounded by phloem, in the later a more strongly curved arch. Sometimes sclerenchyma surrounded by an endodermis is found in the centre of the stele of young petioles. These internal tissues differ very much in structure and are not formed in the same way in different petioles. Sometimes the internal sclerenchyma and internal endodermis are in connexion with the cortex and external endodermis; in other cases they arise and disappear quite independently. Dichotomy of the leaf-trace to form the main veins of the leaf takes place only at the upper end of the petiole.

In *D. Lobbiana* the petiolar steles of the first-formed leaves resemble those of *D. conjugata*, but in later-formed leaf-traces a double structure may be evident in the lower part of the petiole; this becomes single again

nearer the lamina and ultimately branches dichotomously before the lamina is reached. The latest formed leaf-traces investigated depart from the edge of the leaf-gap as two successively separated cylindrical strands. This phenomenon may be connected with the more thorough dichotomous division of the leaves of *D. Lobbiana*.

Botany School, Cambridge.
March, 1911.

EXPLANATION OF FIGURES IN PLATES LVII AND LVIII.

Illustrating Miss de Bruyn's paper on *Dipteris*.

PLATE LVII.

Fig. 1. *D. conjugata* rhizome. Fairly young plant. Transverse section of the stele. Pith consisting of only one cell. × 340.

Fig. 2. *D. conjugata* rhizome. Fairly young plant. Transverse section of the leaf-trace still in the main axis. × 340.

Fig. 3. *D. Lobbiana*. Young plant. × 2.

Fig. 4. *D. Lobbiana* rhizome, just above primary root. Transverse section of the stele, showing protostelic structure. × 290.

Fig. 5. *D. conjugata*. One of the first-formed leaves, showing reticulate venation. × 8.

Fig. 6. *D. Lobbiana*. One of the first-formed leaves. × 8.

Fig. 7. *D. Lobbiana* rhizome. Fairly young plant. Transverse section of the stele. Only one internal endodermal cell. × 260.

Fig. 8. *D. Lobbiana* rhizome. Young plant. Transverse section of the stele near the apex. Stele in meristematic condition, except one part of it, which forms a leaf-trace. × 260.

Figs. 9, 10, 11, 12. *D. Lobbiana* rhizome. Older plant. Diagram showing the formation of the double leaf-trace.

PLATE LVIII.

Fig. 13. *D. conjugata* rhizome. Young plant. Transverse section of the stele, showing two dark internal cells. × 156.

Fig. 14. *D. conjugata* rhizome. Same plant as Fig. 13. Section nearer to the apex. Transverse section of the stele, showing *Lindsaya* type and unequally developed xylem-ring. × 156.

Fig. 15. *D. Lobbiana* rhizome. Young plant. Transverse section of the stele. Xylem in scattered groups. × 156.

Fig. 16. *D. Lobbiana* rhizome. Same plant as Fig. 15. Section nearer to the apex. Transverse section of the stele. Xylem very much developed in connexion with root formation. × 156.

Fig. 17. *D. Lobbiana* rhizome. Older plant. Transverse section of the stele. × 100.

Fig. 18. *D. Lobbiana* petiole, 3·5 cm. in length. Transverse section of the stele near the base. × 156.

Fig. 19. *D. Lobbiana* petiole. Same petiole as Fig. 18. Section at 1 cm. from the base. Transverse section of the stele. × 156.

Fig. 20. *D. Lobbiana* petiole. Same petiole as Figs. 18 and 19. Section near the lamina. Transverse section of the bundles. × 156.

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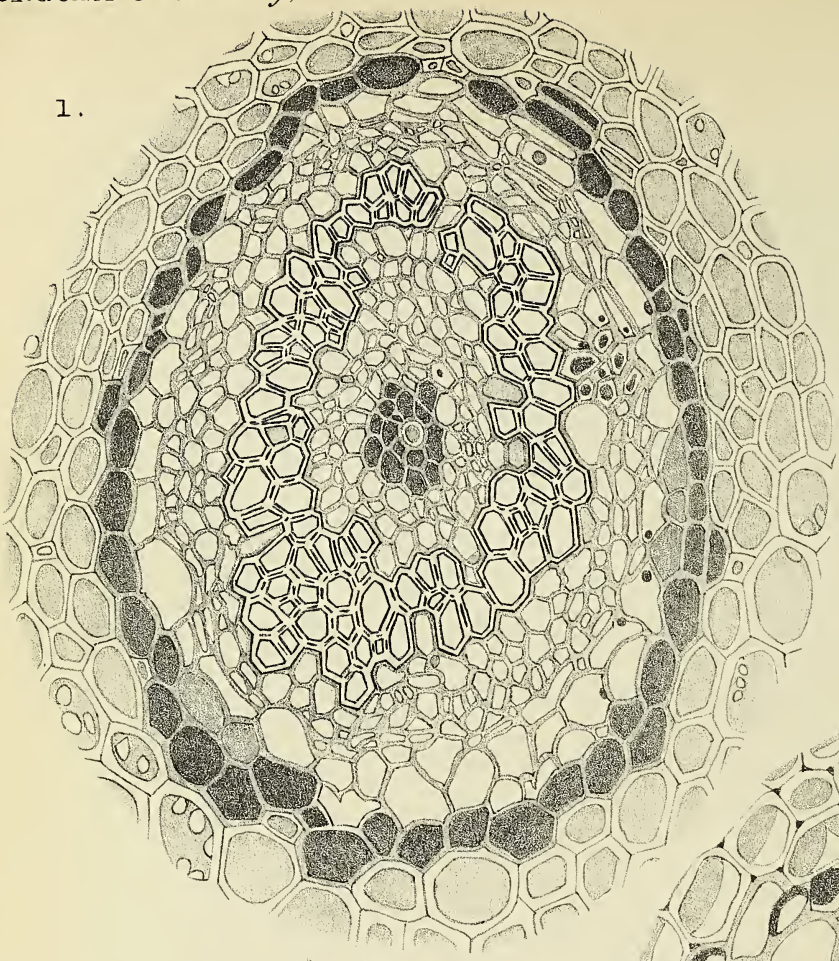

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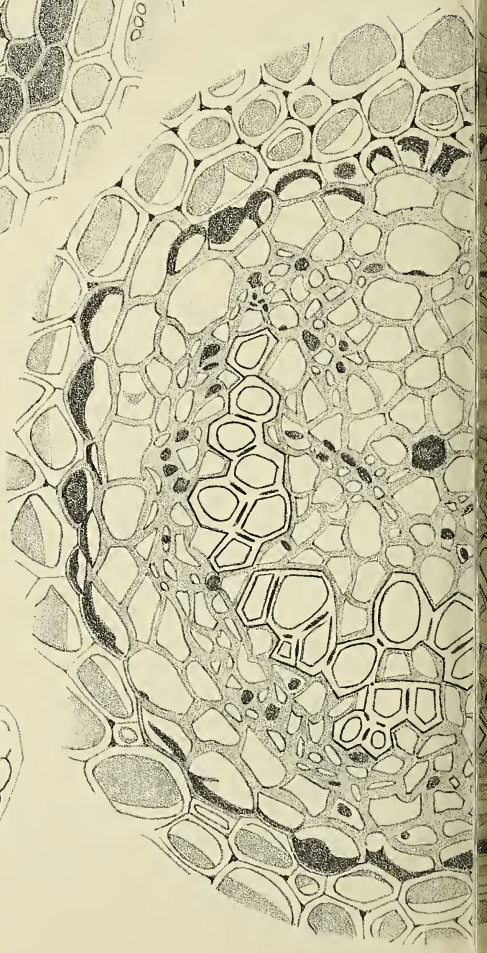

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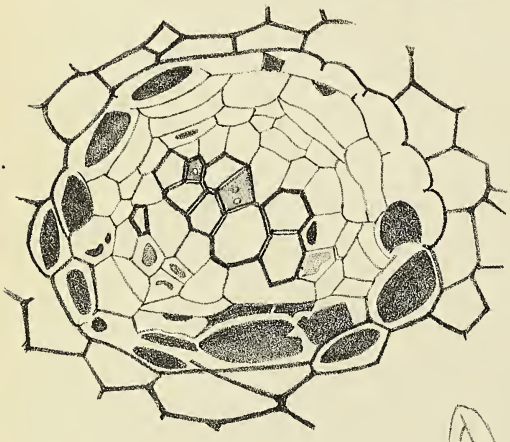

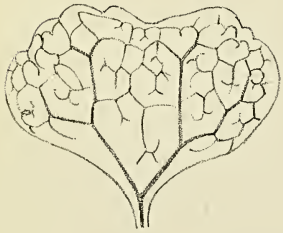

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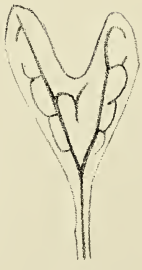

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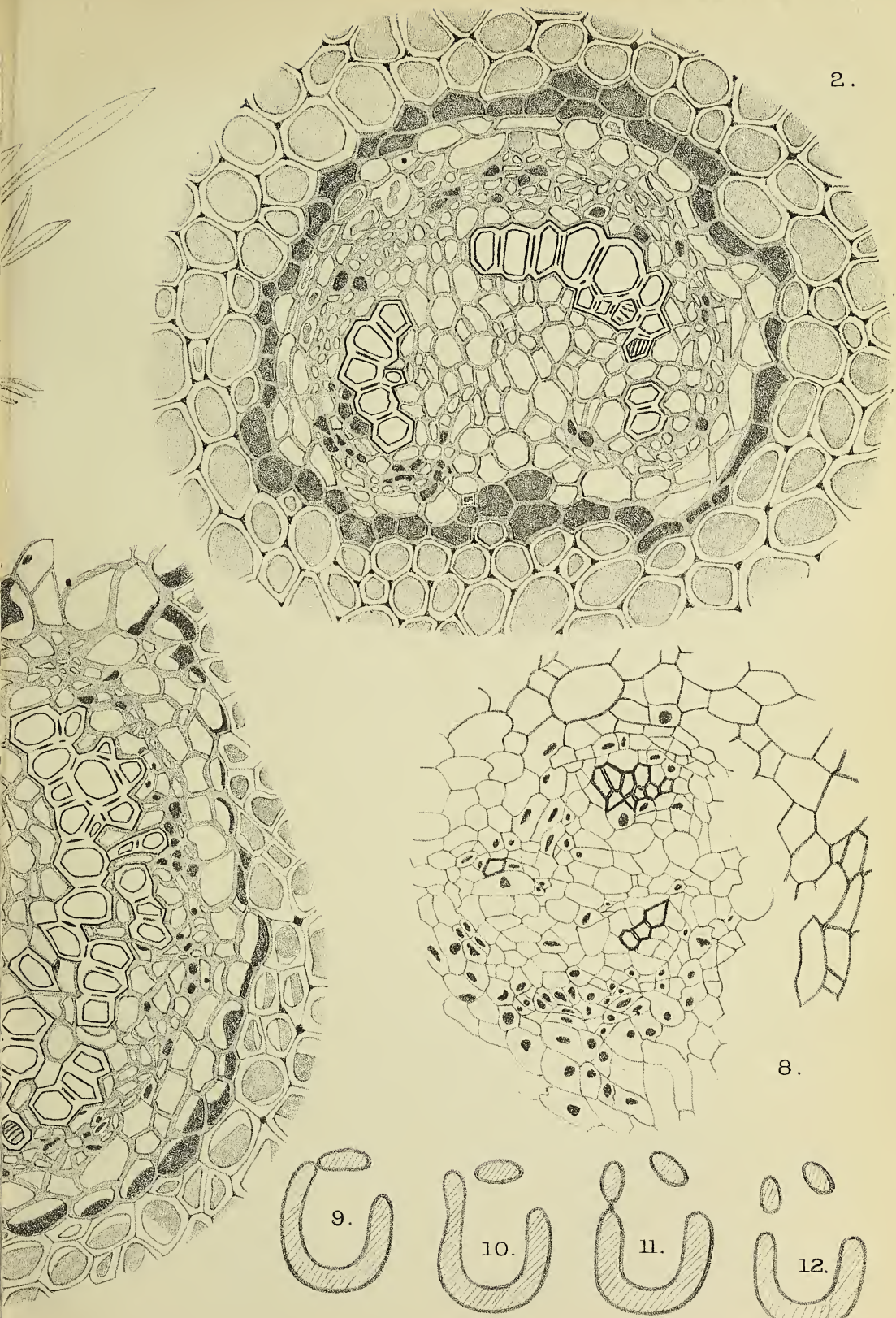

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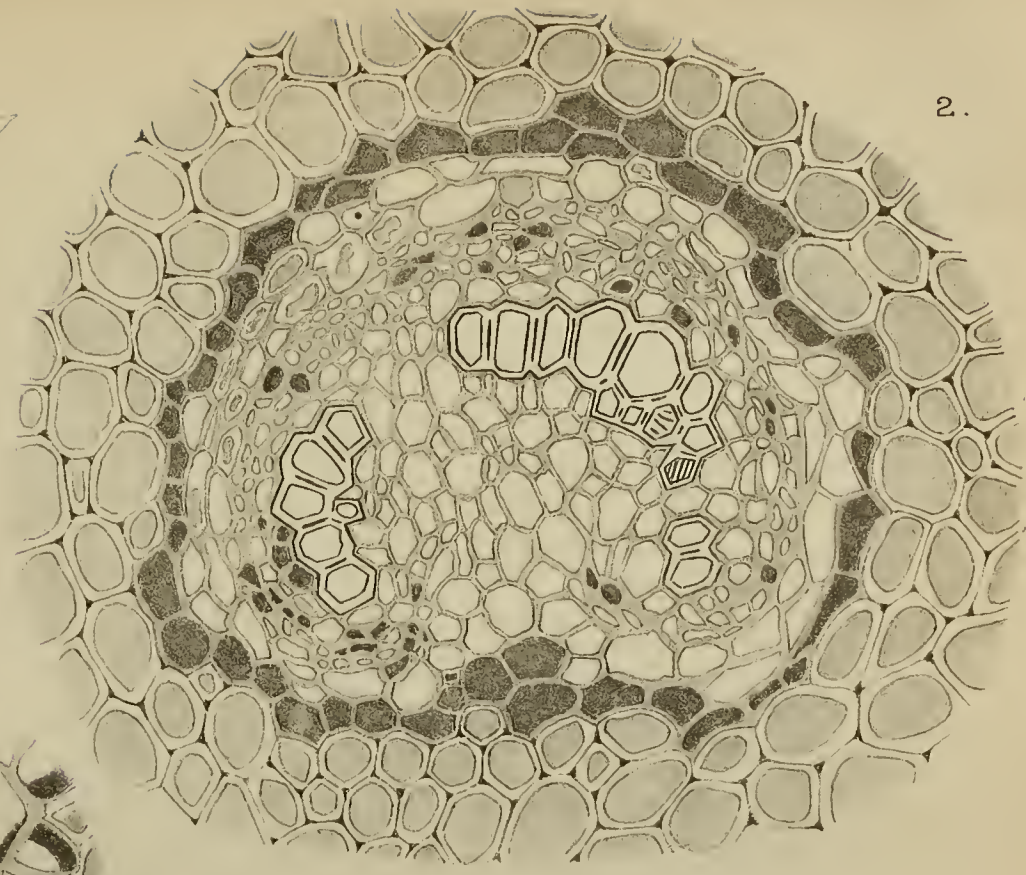

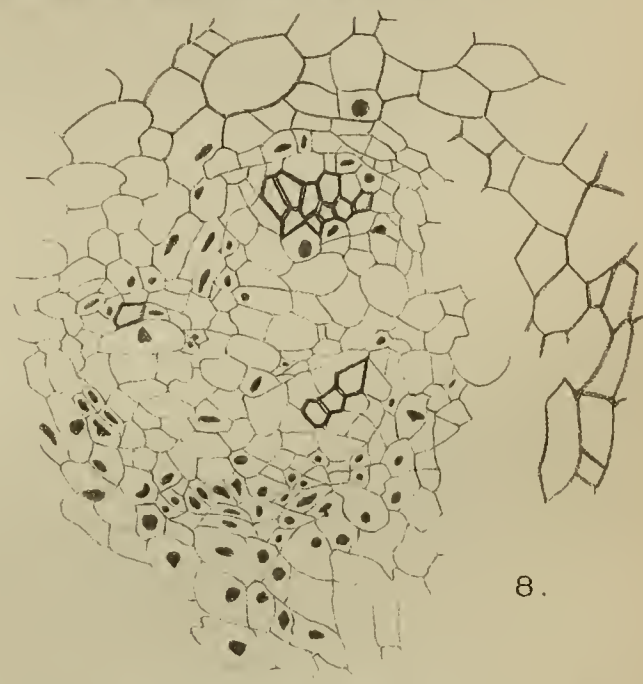

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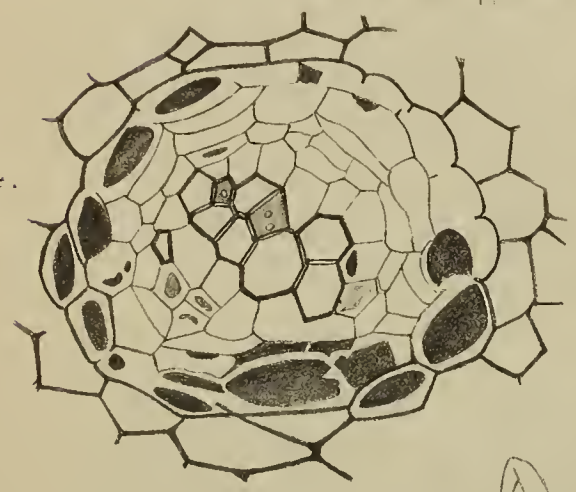

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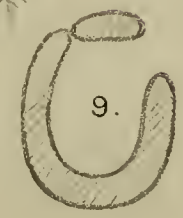

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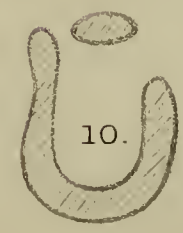

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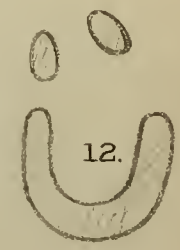

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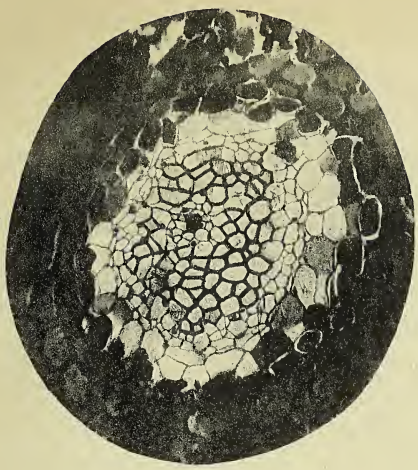

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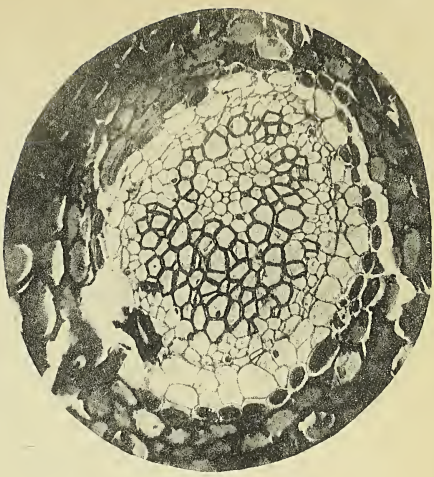

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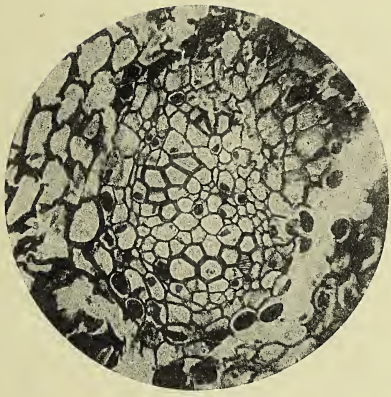

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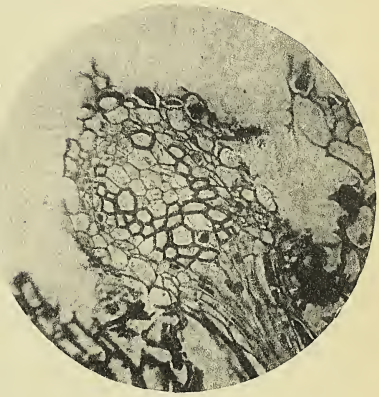

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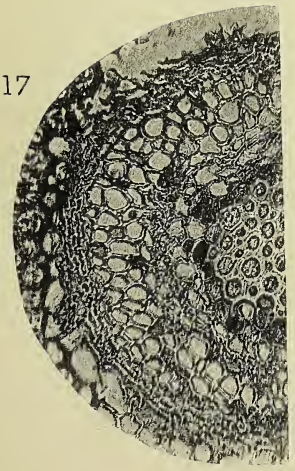

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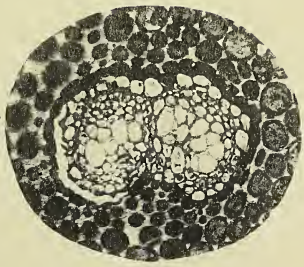

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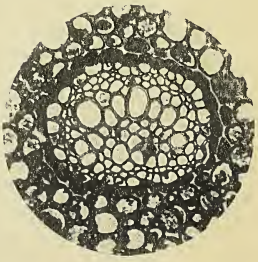

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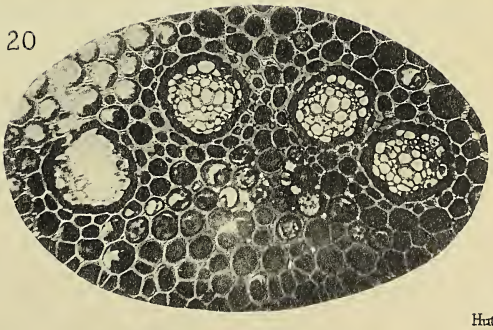

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The Embryo-sac of Pandanus.

BY

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With Plates LIX and LX and two Figures in the Text.

IT is evident that the number of Angiosperms in which the embryo-sac shows more or less marked departures from the usual eight-nucleate type is larger than has been supposed. The results of recent investigations are sufficiently striking to warrant the hope that forms may yet be discovered which will throw more light upon the homologies of the structures in the embryo-sac of the Angiosperms, and their relation to the corresponding structures in the Gymnosperms and heterosporous Pteridophytes.

In selecting forms for investigation one naturally chooses those genera which for other reasons are supposed to be primitive types. Among the Monocotyledons the Pandanaceae have been placed low down in the series,[1] and it seemed not unlikely that *Pandanus* might be a satisfactory subject for study; and such has proved to be the case, as it shows the least reduced type of female gametophyte that has yet been discovered among the Angiosperms.

The Pandanaceae include the genera *Pandanus* and *Freycinetia*. They are confined to the Old World, being especially abundant in the Malayan region, where they constitute a conspicuous feature of the flora. One species of each occurs in Hawaii.

During a visit to the East Indies in 1906 a large amount of material was collected, mostly in Java. There is a very extensive collection of Pandanaceae at Buitenzorg, and during my stay there from March to June repeated collections were made. When this material was examined, however, it was found to be too young to show the completed structures of the embryo-sac. An account of the development of the embryo-sac, as far as it could be followed in this material, has already been published,[2] but the study of the further details had to be postponed until older material could be procured.

The oldest stages secured from the Javanese material showed fourteen or sixteen nuclei in the embryo-sac, instead of the eight typical of most

[1] Pandanaceae. Engleru Prantl, Natürliche Pflanzenfamilien, Part II.

[2] The Embryo-sac of *Pandanus*. Bulletin of the Torrey Botanical Club, xxxvi, 1909, pp. 205–20.

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

Angiosperms. Of these nuclei two were at the upper end of the sac, the others at the antipodal end. It was impossible to tell whether this stage represented the condition at the time of fertilization, and an effort was made to obtain material which would settle this question.

Through the kindness of Dr. W. R. Shaw, of Manila, formerly instructor at Stanford University, a supply of carefully preserved material was secured, which furnished the later stages of development. Dr. Shaw writes that the Philippine species is probably *P. coronatus*, Martelli, a name supposed to be synonymous with *P. tectorius*, Soland.

A study of the more advanced stages of *P. coronatus* showed that the older stages found in the Javanese species were very far from mature. A brief account of the embryo-sac of *P. coronatus* was published, but the details were reserved for further study.[1]

A summary of the results published in the earlier papers is given here, and in addition the history of the embryo-sac from the latest stages secured in the Javanese species to the condition of the mature embryo-sac shown in *P. coronatus*. The post-fertilization stages have also been carefully followed, including the endosperm formation and the early history of the embryo.

The species collected in Java included two small ones, *P. Artocarpus*, Griff., and *P. affinis*, Kurz, and the larger widespread species, *P. odoratissimus*, L. f., which reaches to Hawaii. Of these *P. Artocarpus* furnished the greater part of the material that was studied.

All of the Pandanaceae are dioecious. The pistillate flowers in *Pandanus* are in dense heads, which in the smaller species are borne several together at the end of a short branch, each head subtended by a conspicuous bract. In the larger species the inflorescence is usually solitary and enveloped in a large number of bracts. The head of fruit may occasionally be nearly or quite a foot in diameter.

The Pandanaceae have been placed close to the Sparganiaceae, the flowers as well as the spiky heads of fruit, especially in the smaller species, closely resembling those of *Sparganium*. In the smaller ones like *P. Artocarpus* and *P. affinis*, the individual flower consists of a single carpel, which closely resembles that of *Sparganium simplex*, except for the absence of the conspicuous scale-like bracts that are found in the latter. In the larger species several carpels are more or less completely coherent and may be compared to the compound pistils of *Sparganium eurycarpum*. The carpels in *P. Artocarpus* and *P. affinis* (Pl. LIX, Fig. 1) are slender and much crowded together into nearly globular heads. The sessile stigma is elongated and sharply pointed, extending down the inner face of the carpel for about one-third of its length. In *P. coronatus* and the other species with large syncarpous fruits, the stigmas are discoid.

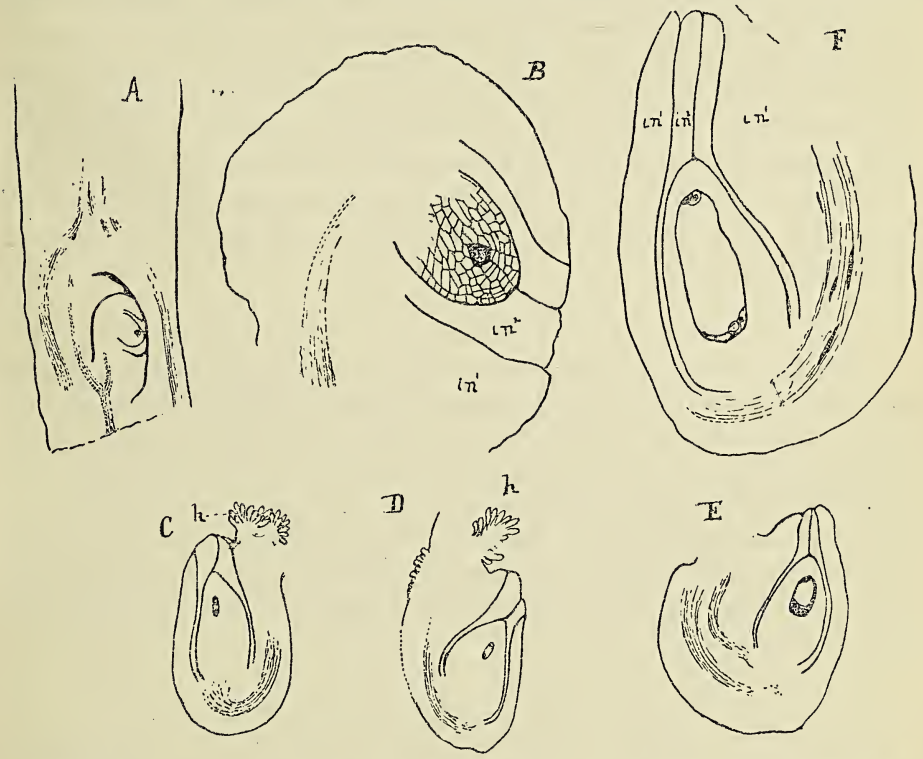
Each carpel contains a solitary anatropous ovule attached to one side

[1] Bulletin of the Torrey Botanical Club, xxxvii, 1910, 293–5.

of the ovarian cavity near its base. The placenta and the base of the funiculus bear closely placed glandular hairs (Text-fig. 1, C, D). These hairs are probably concerned with the conduction of the pollen-tube to the micropyle. On the inner side of the ovule the outer integument is completely fused with the funiculus.

The Ovule.

The youngest ovules that were secured were from *P. affinis*. Text-fig. 1, A, shows a median section through the base of a young carpel of this



Text-fig. 1. A. Section through the base of a young carpel of *Pandanus affinis*, Kurz, showing the single anatropous ovule. × about 35. B. The ovule more highly magnified. The sporogenous cell is shaded. × 235. in^1, in^2, the integuments. C–E. Older ovules of *P. Artocarpus*, Griff., showing the young embryo-sac. The embryo-sac shown in E contained fourteen nuclei. × about 35; *h*, secretory hairs upon the funiculus. F. An ovule of *P. coronatus* about ready for fertilization. × about 35. There were two 'polar' nuclei in process of fusion.

species. The ovule almost completely fills the ovarian cavity, and at this stage is a good deal flattened and the integuments do not extend much beyond the apex of the nucellus. The ovule is shown more enlarged at B.

Text-figs. 1, C, D, represent similar sections of older ovules of *P. Artocarpus*. In these older stages the nucellus becomes more elongated and the terminal portion of the integuments also becomes lengthened.

As the ovule develops there is relatively little increase in the size of the nucellus, which becomes replaced by the enlarging embryo-sac, but the funiculus and the part of the outer integument adjoining it become much enlarged, so that the ovule has quite a different form from the earlier stages. This recalls the ovules of many Araceae, where there is a similar enlargement of the basal region of the ovule. Traversing the funiculus there is a single fibro-vascular bundle.

P. affinis differs slightly from the other species examined in the form of the embryo-sac mother-cell, as well as in the greater thickness of the apical tissue of the nucellus. As the older stages of this species were not available it is impossible to say how these would compare with corresponding stages in the other species that were studied. The embryo-sac mother-cell (Fig. 3, Pl. LIX) was undivided, but it could be easily recognized by its dense contents and its very conspicuous nucleus. In section it appears almost triangular in form, with pointed base and broad apex. Above it there lies a group of cells, probably tapetal or parietal cells, which to judge from their position are the product of the repeated division of a primary tapetal cell.

The Pollen.

Only a very casual study of the pollen was made. The apparently ripe pollen-spores from an undetermined species (Fig. 2) were found to have a small cell (*m*) cut off from the body of the spore. The nucleus of this small cell was much smaller than that of the body of the spore. It is probable that the small cell is the antheridial or generative cell, and that its nucleus later divides into two. There is a possibility, however, that it represents the small sterile or prothallial cell which occurs in *Sparganium simplex*, and that the large nucleus divides again to form the generative and tube nuclei.

The Embryo-sac of Pandanus Artocarpus.

The earlier stages of the embryo-sac found in *P. Artocarpus* (Figs. 4–6) were somewhat more advanced than that described in *P. affinis*. The mother-cell is more nearly cylindrical and the base is truncate, and sometimes almost as broad as the apex, and in such cases it is the lowermost of a single series of cells, or at least such is the appearance in longitudinal section (Fig. 6). More commonly the upper end of the mother-cell is rather broader, and there are two series of parietal cells to be seen in longitudinal section (Fig. 5). There are two other layers of these parietal cells between the young embryo-sac and the epidermis at the apex of the nucellus. The sporogenous cell divides transversely into two, one of which, the lower, is the larger and becomes the embryo-sac (*e. s.*). The upper cell (*x*) divides into two by a vertical wall, and these two small cells persist with

little change for some time, but they finally disintegrate and are visible only as two small, darkly stained, shrunken bodies lying above the apex of the embryo-sac. In these early stages *Pandanus* closely resembles the ordinary Angiosperms, except for the vertical division in the upper sporogenous cell. It differs from *Peperomia* and *Gunnera*, which in some respects resemble it more nearly than any other Angiosperms, in the formation of three cells which may be interpreted as megaspores, instead of having the primary sporogenous cell develop directly into the embryo-sac.

At what stage the reduction divisions in the nuclei occur could not be determined, since for some reason, perhaps owing to the time of day at which the material was collected, no nuclear divisions could be found.

The young embryo-sac (*e. s.*) is easily recognizable, the cytoplasm being noticeably more densely granular than the adjoining cells of the nucellus, and the nucleus is somewhat larger. The position of the nucleolus lying in the cytoplasm next to the nucleus, as shown in the figure, is no doubt the result of an accident.

Fig. 4, Pl. LIX, shows a slightly more advanced stage. In this case the upper sporogenous cell is smaller and the nucleus less conspicuous than usual. The cytoplasm of the young embryo-sac contains many vacuoles and the conspicuous nucleus occupies the centre of the cell. A slightly older stage is shown in Fig. 5. The sister cell of the young embryo-sac divides longitudinally, instead of transversely, as is the case in most Angiosperms. This has, however, been noted in a number of other forms, both Monocotyledons and Dicotyledons.[1]

The young embryo-sac increases rapidly in size and the nucleus divides, one of the daughter nuclei moving to the upper end of the sac, the other to the chalazal end. The two nuclei are quite similar in appearance (Fig. 7), and the cytoplasm still fills the whole cavity of the sac, although there are several large vacuoles. These vacuoles finally unite, and by the time the second mitosis is complete, a single large vacuole occupies the greater part of the sac, the cytoplasm being mainly confined to the ends, while there is only a thin layer lining the lateral walls (Figs. 8 and 9).

Up to this point *Pandanus* agrees exactly with the typical Angiosperms, but the later history of the embryo-sac is decidedly different. The two nuclei at the micropylar end of the sac remain for a long time undivided, and the next nuclear divisions are confined to the chalazal region. Stages with four, six, eight, and twelve nuclei at the chalazal end were met with, but in all of these there were but two micropylar nuclei. The cytoplasm in the basal region of the embryo-sac increases very much in amount as the nuclear divisions proceed. None of the nuclei at this stage were found in division, so that the sequence of nuclear divisions in

[1] Coulter, J. M., and Chamberlain, C. J.: Morphology of Angiosperms, 1903, pp. 75, 76.

the chalazal region could not be determined. Several cases, one of which is shown in Fig. 12, Pl. LIX, had six chalazal nuclei, but it could not be determined which of the four nuclei of the preceding stage gave rise to the two extra nuclei. There is some uncertainty as to the subsequent divisions by which the number of chalazal nuclei increase to eight, and finally to twelve. Usually, at least, there are finally twelve of these chalazal nuclei, although it is possible sometimes there may be only ten. There is some variation in the form of the lower end of the embryo-sac, which may be either somewhat pointed, or broad and rounded (see Figs. 14 and 15).

In the oldest stages that were secured in *P. Artocarpus*, the embryo-sac, which had increased materially in size, showed at the somewhat narrower micropylar end two nuclei, while at the chalazal end there were twelve large nuclei surrounded by a large mass of granular cytoplasm containing several conspicuous vacuoles (Figs. 14 and 15). A similar, but smaller, mass of cytoplasm surrounds the nuclei at the upper end of the sac, and the large central vacuole is bounded laterally by a rather thick layer of cytoplasm which, however, contains no nuclei. In most cases there was no apparent differentiation of the cytoplasm at the upper end of the sac, although in a few instances there was a slight indication of what looked like the separation of an egg-cell and synergid; but this was very vague.

Corresponding to the enlargement of the embryo-sac there is a marked increase in the size of the nuclei, which at the same time show a distinct reticulum, while in the younger sac the contents of the nucleus appear more uniform. The nucleolus is very conspicuous. In *P. Artocarpus* the increase in size is more marked in the chalazal nuclei than in the micropylar ones, which are noticeably smaller. In the most advanced stages found in *P. Artocarpus* the arrangement of the nucleus is very similar to that described by Johnson for *Peperomia hispidula*,[1] except that there are fourteen instead of twelve chalazal nuclei in the latter, and these ultimately fuse into one enormous endosperm nucleus.

Owing to the similarity of the nuclei in the nucellus cells adjacent to the young embryo-sac, and those of the sac itself, the former may be mistaken sometimes for nuclei belonging to the embryo-sac, but in the later stages the greater size of the embryo-sac nuclei usually makes it easy to distinguish them. There is sometimes found a small cell (Fig. 13, *y*), apparently cut from the side of the embryo-sac, as occurs in the embryo-sac in *Peperomia*. The contents of these cells are densely granular like the cytoplasm of the embryo-sac, but the nuclei are small, and it is quite likely that these small cells really belong to the nucellus. The occasional occurrence of small nuclei apparently free in the cytoplasm of the embryo-

[1] Johnson, D. S.: A New Type of Embryo-sac in *Peperomia*. Johns Hopkins University Circular, 1907, No. 3, pp. 19–21.

sac, and differing in appearance from the other nuclei, suggests that perhaps the wall of an adjacent cell of the nucellus may have broken down and discharged the nucleus into the embryo-sac. These points, however, can only be settled when the history of the nuclear division is known.

All of the cells surrounding the embryo-sac differ more or less from the outer tissue of the nucellus, having more watery contents and sometimes rather larger nuclei; they are probably concerned to some extent with the nutrition of the embryo-sac, and sometimes this central mass of tissue suggests a mass of sporogenous cells; and it is not impossible that it really may represent a mass of sporogenous tissue of which one cell only is functional. In Fig. 18, Pl. LX, part of this tissue is shown with two conspicuous cells that very much resemble young embryo-sacs.

The Embryo-sac of Pandanus odoratissimus.

Pandanus odoratissimus was examined for comparison with *P. Artocarpus*, from which it was found to differ only in some minor particulars. This species has large fruits with the carpels united into groups, forming more or less complete compound pistils, but the union of the carpels is a very loose one. The upper part of the carpel soon becomes very hard and woody, but the base remains succulent for some time, and is easily sectioned. The inflorescences from which the preparations were made were about six centimetres in diameter, and it was supposed that fertilization had already taken place. It was therefore hoped that the later stages of the embryo-sac could be obtained, but on examination it was found that in spite of the large size of the carpels, which were nearly two centimetres in length, the ovules were little further advanced, and not noticeably larger than those in the apparently much younger and smaller flowers of *P. Artocarpus*. Pollen-spores were found on the stigma, and some of these had sent out their pollen-tubes. It was found, however, that the pollen-tubes had not yet reached the ovules. In *P. odoratissimus* the two micropylar nuclei are quite as large as those of the chalazal region, and the largest sacs found in this species were slightly larger than those of apparently the same age in *P. Artocarpus*. In some cases fourteen chalazal nuclei were counted, and occasionally a nucleus was found containing two nucleoli, looking in some cases as if there might have been fusion of two nuclei (see Fig. 16, Pl. LIX, and Fig. 17, Pl. LX).

The Embryo-sac of Pandanus coronatus.

The further study of the embryo-sac was confined to *P. coronatus*, as this was the only species secured in which the older embryo-sacs were found.

Fig. 21, Pl. LX, shows an embryo-sac of this species corresponding to the oldest stages found in *P. Artocarpus* and *P. odoratissimus*, which it closely resembles, except for its decidedly larger size and somewhat more

elongated form. A somewhat younger stage, with eight chalazal nuclei, is shown in Fig. 19, Pl. LX.

Fig. 20 shows the base of an embryo-sac, differing somewhat from the usual type. It was apparently somewhat older than that figured in 21, being decidedly larger. There were two micropylar nuclei and ten large nuclei in the antipodal region. There were also present two or three small nuclei, but it was a question whether these properly belonged to the embryo-sac. The most remarkable feature of this embryo-sac was a very large free nucleus, closely resembling the primary endosperm nucleus formed in the older embryo-sac as the result of the fusion of the 'polar' nuclei. This nucleus (Fig. 20, *b*) was embedded in a mass of cytoplasm, and contained two very conspicuous nucleoli, which gave it very much the appearance of having resulted from the fusion of two of the twelve original antipodal nuclei. As a rule no such fusion nuclei were found until a much later stage of development.

In the next stages met with, the two micropylar nuclei had divided, so that there were four nuclei at the upper end of the sac, and the eight or twelve chalazal nuclei had increased in number to 32–6. Although many preparations were made, none of them showed the division stages, and it is impossible to say whether the divisions of all the chalazal nuclei take place simultaneously, or whether the nuclei divide at the same time as the two micropylar nuclei.

In all these later stages the nuclei, which in the earlier condition were free, are separated by evident cell-walls, so that the base of the embryo-sac is occupied by a broad mass of antipodal cells very much like those that occur in *Sparganium* after fertilization has taken place.[1] There is, however, a marked difference to be noted. In *Sparganium*, at the time of fertilization, the embryo-sac has the usual form with three distinct antipodal cells. The very greatly increased number of antipodals found later results from a subsequent division of these three original antipodal cells. In *Pandanus*, however, up to the time that twelve or sometimes fourteen nuclei are present in the antipodal region, there is no trace of cell-division. The formation of this mass of antipodal cells in *Pandanus* resembles, therefore, very closely the formation of the endosperm after fertilization has taken place, and it is quite likely, although this is not easy to demonstrate, that more than one nucleus may sometimes be enclosed within the irregular cells composing the antipodal tissue..

In the micropylar region three of the four nuclei form a fairly typical egg apparatus (Figs. 22–4). Two of these, probably sister nuclei, give rise to the synergidae. The latter are separated by what seems to be a definite cell-wall, and are rounded off below, not presenting any peculiar character-

[1] See Campbell, Studies on the Flower and Embryo of *Sparganium*. Proc. Calif. Acad. of Sciences, 3rd Ser., Bot., vol. i, No. 9, 1899.

istics different from those in the ordinary embryo-sac. The nuclei are about half the size of the primary micropylar nuclei, and the nucleolus is relatively larger and more conspicuous. The second micropylar nucleus presumably gives rise to the nucleus of the egg and a free nucleus, the upper polar nucleus (*p. n.*). This upper polar nucleus later moves towards the base of the sac and fuses with the lower polar nuclei to form the primary endosperm nucleus.

The cytoplasm of the egg-cell, which is not always very clearly differentiated, is less dense than that of the synergidae, and the nucleus, sometimes at least (see Fig. 22, *c*), is smaller, with a less conspicuous nucleolus.

The polar nucleus is at first in close contact with the egg, and in size and structure resembles its sister nucleus. As it moves towards the lower part of the embryo-sac it becomes very much larger, and at the time it fuses with the polar nuclei from the antipodal end it is impossible to distinguish it from them.

The Antipodal Cells.

The exact number of antipodal cells is not easy to determine, as sometimes the nuclei of the adjacent nuclear tissue become enlarged and strongly resemble the nuclei of the antipodal cells, with the outermost of which they may readily be confused. Moreover, there is undoubtedly much variation in the number of antipodal cells. In several cases thirty-two of these could be counted, while in others there were as many as sixty-four, and possibly the number may be even greater than this. In some of the preparations a number of the antipodal nuclei were in process of division, but no rule could be discovered governing the time of division. It is probable that the number of antipodal cells present at the time fertilization takes place depends, at least in part, upon the number of free nuclei in the chalazal region at the time cell-formation begins. So far as could be determined the number of these free nuclei may range from eight to fourteen, and it seems likely, although this could not be proved, that the next division in these nuclei is accompanied by the formation of cell-walls. The antipodal cells (Figs. 22–4) are of very irregular form and contain a good deal of granular cytoplasm which is more or less vacuolated, especially in the upper cells. These cells in the earlier stages are strongly turgescent, and the upper ones project into the cavity of the embryo-sac. In the later stages these upper cells appear more or less flattened or even collapsed (Fig. 25), but how far this is the normal appearance, and how far it is due to shrinkage in the preparation of material, is impossible to say.

From one or more of the upper cells the large nuclei are discharged into the cavity of the embryo-sac and constitute the lower polar nucleus

(or nuclei). Figs. 23, 24, *p. n.*, Pl. LX, show the antipodal cell containing the polar nucleus projecting into the cavity of the embryo-sac. The intermediate stages between this condition and that where the nuclei lie free within the cavity of the embryo-sac were not seen, but as there were in no cases any free basal nuclei in such stages as those shown in Figs. 23 and 24, it seems pretty certain that the nuclei of these protruding antipodal cells are ultimately discharged into the cavity of the sac. The number of the basal polar nuclei varies from a single one to six or more. Most commonly there seem to be two or three, and the number is probably to some extent governed by the number of antipodal cells. There was no evidence that the increased number of polar nuclei was due to a division of the primary ones, although, of course, there is a possibility that such is the case.

At the time of fertilization the much enlarged embryo-sac shows a pretty well marked egg apparatus at the upper end, while at the chalazal end (Fig. 25) there may be seen a somewhat flattened mass of antipodal cells, in this case about sixty-four. The lowest antipodal cells are still turgescent and contain a good deal of granular cytoplasm, but the other ones appear collapsed and have very little contents, looking as if they were disintegrating. Some of these cells may perhaps be those from which the lower polar nuclei have been discharged.

Above the antipodal cells at the time of fertilization there may be seen a group of large nuclei closely crowded together, and beginning to fuse. As we have already seen, the number is usually from two to six, and it is quite impossible to tell which of these is the polar nucleus derived from the upper end of the sac, and which nuclei originated from the antipodal region. These nuclei subsequently fuse into a single large endosperm nucleus.

Fertilization.

The entrance of the pollen-tube into the embryo-sac was seen in a number of cases, but no satisfactory study of the details of fertilization could be made out. The pollen-tube is small, and could in some cases be seen forcing its way between the cells at the apex of the nucellus, but in no case were the nuclei satisfactorily shown, and although it is probable that there are, as usual, two generative nuclei, this cannot be positively asserted. Where the end of the tube could be seen within the sac, one of the synergidae, as usual, was destroyed. Presumably one of the generative nuclei is discharged into the sac, and small nuclei were sometimes seen in the neighbourhood of the recently fertilized egg which might possibly have been the generative nuclei, but it was quite as likely that these small nuclei were really derived from some of the nucellus cells which had been cut in sectioning the ovule. Nor were any satisfactory

demonstrations made of the presence of a male generative nucleus within the egg, or its fusion with the egg nucleus.

The fusion of the polar nuclei takes place about the same time as the fertilization of the egg, but there is probably some variation in this respect. The fusing polar nuclei (Figs. 25, 26, Pl. LX) are extremely conspicuous. Each one contains as a rule a single very large nucleolus and shows an evident reticulum, which does not stain at all strongly and is apparently rather deficient in chromatin. The nuclei become closely appressed and their cavities gradually fuse until there results a single very large nucleus, the primary endosperm nucleus, within which can still be clearly seen the separate nucleoli of the component nuclei. In the nucleus figured there were six large nucleoli, only two of which show in the figure. It is probable that the division of the primary endosperm nucleus follows quickly, as only one resting stage was found, while a number of cases were seen in which the nucleus was in process of division. In the resting nucleus which is figured the nuclear reticulum was more conspicuous than it is in the unfused polar nuclei, and there is much more chromatin, which shows in the form of distinct strongly staining granules.

In Figs. 30–2 are shown the details from a very peculiar embryo-sac. At the upper end of the sac there was an imperfectly differentiated egg apparatus which showed four instead of three nuclei, and it was impossible to tell which of the four represented the egg. What looked like the end of the pollen-tube (*p.t.*) could be seen at the apex, but if this structure really was the pollen-tube, it had not yet destroyed either of the synergidae. Near the egg apparatus there was a single cell (*y*) which looked very much like an egg-cell of the normal type. Near the middle of the sac there was a large mass of cytoplasm surrounding a group of about twelve large nuclei which were in process of fusion. Fig. 32 shows five of these. Near this group of nuclei there was a second cytoplasmic mass (*x*) attached to the wall of the embryo-sac and containing what looked like two nuclei, but these were not very distinct. What would have been the further history of this abnormal embryo-sac is impossible to conjecture.

In other cases apparently otherwise normal it looked as if there were two primary endosperm nuclei. Thus in the embryo-sac from which the endosperm nucleus shown in Fig. 27 was taken, there was a second group of seven nearly completely fused nuclei.

The few preparations that were secured showing the division of the primary endosperm nucleus were all rather advanced metaphases of mitosis, no examples of prophases being found. Fig. 28, *a*, shows the nuclear spindle of the dividing primary endosperm nucleus. The chromosomes form an undivided equatorial plate. The number of the chromosomes, as might be expected from a nucleus made up of the fusion of several, is extremely large, and their small size and crowded position made it prac-

tically impossible to determine their number. The two nuclei resulting from this first division were not seen, and the next stage met with showed four large endosperm nuclei, one of which is shown in section in Fig. 29, *a*, Pl. LX. These nuclei were of about the same size as the primary endosperm nucleus and showed much the same structure, containing several large nucleoli and a conspicuous reticulum.

With the successive divisions of the endosperm nuclei there is a marked reduction in their size, and this reduction in size is accompanied by a reduced number of chromosomes, as may be seen by comparing Fig. 28, *b*, with Fig. 28, *a*. The former nuclear spindle was from a nucleus of about the same size as that shown in Fig. 29. The free endosperm nuclei are evenly distributed through the cytoplasm lining the embryo-sac, and this cytoplasmic layer increases a good deal in thickness before the formation of cell-walls in the endosperm begins. At the time the first walls are formed in the endosperm, the nuclei are still further diminished in size (Fig. 29, *a*), and presumably the number of chromosomes is correspondingly less than in the earlier and larger free endosperm nuclei.

The Embryo.

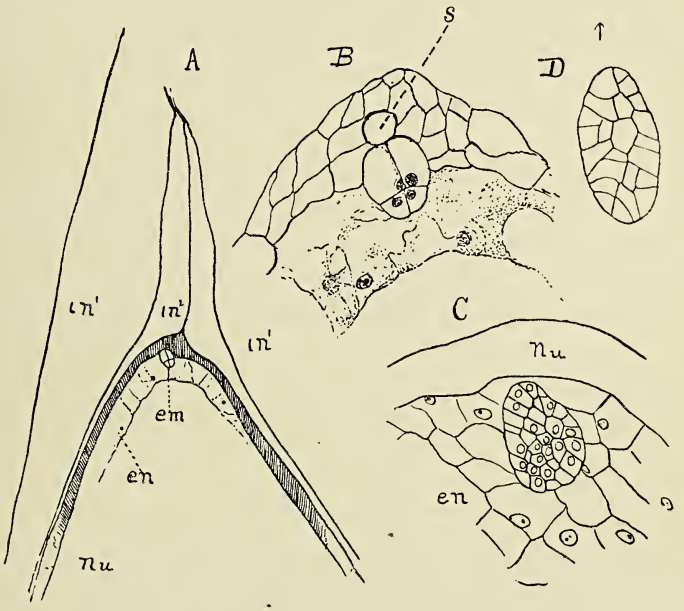
The embryo in *Pandanus* remains very small even in the ripe seed, and in the largest ovules that were sectioned, which had attained a length of more than a centimetre, the embryo was still quite undifferentiated. The early divisions take place slowly and the embryo remains unicellular for a long time after fertilization (Fig. 33). The first division, in some cases at least, is transverse and separates a short suspensor cell from a larger terminal cell (Fig. 34), but the number of young embryos examined was too small to make it certain whether the first divisions are always the same. Text-fig. 2, A, B, shows a four-celled embryo with probably a one-celled suspensor (*s*), although it is possible that this basal cell may belong to the nucellus.

The oldest embryos seen are shown in Text-fig. 2, C, D. These were nearly oval bodies without any definite suspensor. They showed no signs of the permanent organs of the young plant, and it is therefore impossible to say what is the method of origin of the stem apex and the other organs of the seedling. In order to follow out the further history of the embryo it would probably be necessary to germinate the seeds, as even in the ripe seed the embryo is quite rudimentary.

In the development of the embryo *Pandanus* differs very much from *Sparganium*, where the embryo in the ripe seed is very large and all the organs perfectly developed. The earlier stages in *Pandanus* are not very unlike the corresponding ones in *Sparganium* (compare for example Fig. 37 of my paper on *Sparganium* with Text-fig. 2, C).

The development of the endosperm begins at the apex of the embryo-sac, and the first division walls are formed while the embryo is still very small (Text-fig. 2, A, B). The development of the endosperm tissue does not seem to differ in any way from the ordinary method, and no particular study was made of this point. Between the nuclei there arise simultaneously the division walls, which form an irregular tissue increasing by division and growth until the whole embryo-sac is filled with a continuous mass of endosperm cells.

The hard testa of the seed is derived mainly from the outer integument, the inner integument being almost obliterated during the ripening of the seed.



TEXT-FIG. 2. A. Upper part of the ovule and embryo-sac of *Pandanus coronatus*, Martelli, showing a young embryo embedded in the endosperm, *en*. The shaded portion represents the tissue of the nucellus. × about 40. B. The embryo shown in A, more highly magnified. The round cell at the base of the four-celled embryo may be the suspensor, but this is not certain. C. An older embryo, embedded in the endosperm, *en*. × 225. D. A still older embryo. × 225. The arrow points towards the micropyle.

SUMMARY.

1. The primary sporogenous cell is separated from the epidermis of the nucellus by several layers of parietal cells presumably derived from the division of a single primary tapetal cell. The cells of the nucellus adjacent to the embryo-sac sometimes show an appearance suggesting that they may be abortive sporogenous cells.

2. The sporogenous cell divides into a large lower cell and a smaller

upper one, the latter dividing again by an anticlinal wall. The lower cell is the young embryo-sac.

3. The early divisions of the embryo-sac follow the usual course up to the stage with four nuclei, two being at each pole of the embryo-sac.

4. The next divisions are confined to the antipodal region where the two original nuclei, by subsequent divisions, give rise to a group of large free nuclei, usually twelve in number.

5. The two micropylar nuclei divide once, and there is formed a typical egg apparatus and an upper polar nucleus.

6. The antipodal nuclei divide further, the later divisions being accompanied by the formation of cell-walls much as in the formation of the endosperm. The number of antipodal cells may finally exceed sixty-four.

7. A varying number of the antipodal nuclei become free and assume the rôle of polar nuclei, fusing with the upper polar nucleus into a single large endosperm nucleus. Sometimes the fusion results in two primary endosperm nuclei.

8. The secondary endosperm nuclei diminish in size as division proceeds, and this diminution in size is accompanied by a corresponding reduction in the number of chromosomes.

9. The endosperm formation is of the usual type. After a large number of free nuclei have been formed, cell-walls arise between them, and cell-formation proceeds centripetally until the whole embryo-sac is filled with solid endosperm.

10. The embryo is very small and shows no external differentiation.

Conclusions.

In *Pandanus* the embryo-sac reaches the highest development before fertilization that has yet been recorded for the Angiosperms. Instead of the eight nuclei of the ordinary embryo-sac there are at least thirty-six, and sometimes twice that number, at the time fertilization occurs.

The early history of the embryo-sac follows closely the usual course up to the time that four nuclei have been formed, and then a difference is to be noted. The condition with two micropylar nuclei and eight to fourteen free nuclei at the base of the embryo-sac may be compared to that described by Johnson for *Peperomia hispidula*,[1] where there is a similar arrangement of the nuclei. The eight-nucleate stage found in *Gunnera macrophylla*[2] also resembles this stage of *Pandanus*, but the final nuclear division in *Gunnera*, resulting in sixteen nuclei, is accompanied by

[1] Johnson, D. S.: A New Type of Embryo-sac in *Peperomia*. Johns Hopkins University Circular, 1907, No. 3, pp. 19–21.

[2] Ernst, A.: Zur Phylogenie des Embryosackes der Angiospermen. Ber. der deutsch. bot. Gesellsch., xxvi, 1908, pp. 419–38.

the formation of division cells, while in *Pandanus* cell-formation does not take place until a later period.

It is in the subsequent history of the embryo-sac that *Pandanus* shows the greatest departure from the usual angiospermous type. The extraordinary development of the antipodal cells at the time of fertilization exceeds that found in any other form. In *Peperomia pellucida* and in *Gunnera*, where there are sixteen nuclei in the adult embryo-sac, there are six antipodal cells, and in some of the Grasses[1] there may be as many as thirty-six, and the number is often increased in many Compositae. But in all of these except *Peperomia* and *Gunnera* there is first developed a typical embryo-sac with three antipodal nuclei. These later undergo secondary divisions resulting in the increased number of antipodal cells. The increased number of antipodal cells, subsequent to fertilization, has been observed in a considerable number of forms, both Monocotyledons and Dicotyledons.[2]

In *Pandanus*, unlike these other forms, the mass of antipodal tissue is preceded by the formation of a considerable number of free nuclei, and the solid mass of antipodal tissue is formed by the subsequent division by cell-walls between these free nuclei exactly as is the case in the formation of the endosperm. This emphasizes the homology which undoubtedly exists between the two types of gametophytic tissue developed before and after fertilization.

The formation of the endosperm nucleus from the fusion of several nuclei is not peculiar to *Pandanus*. The same thing occurs regularly in *Peperomia* and *Gunnera*, and there are numerous other instances that have been observed in other plants. It is pretty clear from a study of these forms that the theory of the fusion of the polar nuclei being a sort of sexual process is quite untenable. Just what it means is hard to say.

While there are marked differences between *Pandanus* and *Sparganium*, especially in the condition of the embryo-sac at the time of fertilization, and the much better developed embryo in *Sparganium*, still the general morphology, and particularly the great development of antipodal tissue which finally is formed in *Sparganium*, confirm the view that the Pandanaceae and Sparganiaceae do really belong near together in the system.

There seems no valid reason for supposing that the condition of the embryo-sac found in *Pandanus* is not really primitive. The genus on other grounds has been placed near the bottom of the series of Monocotyledons, and the structure of the embryo-sac certainly confirms this view. The arguments that have been brought against assuming that the

[1] Cannon, W. A.: A Morphological Study of the Flower and Embryo of the Wild Oat, *Avena fatua*, L. Proc. Calif. Acad., 3rd Ser., Bot., vol. i, 1900, pp. 329–64.

[2] For a full account of the occurrence of more than three antipodal cells, see Coulter and Chamberlain, Morphology of Angiosperms, 1903, pp. 97–102.

sixteen-nucleate embryo-sac of *Peperomia* is primitive (which we do not think are very convincing)[1] cannot be applied to *Pandanus*. Since there are three megaspores produced in *Pandanus*, the embryo-sac can at most represent two of these, and as it may contain more than sixty-four nuclei, to speak of a reduction is out of the question. We entirely agree with Ernst[2] in believing that the time has come to recognize that the embryo-sacs with an increased number of nuclei are not abnormalities, but are rather older types of embryo-sacs which have survived. It is more than likely that the number of these types will be increased as a further investigation of the lower members of both the monocotyledonous and dicotyledonous series is made.

Whether the indefinite type of structure shown by *Peperomia*, with its poorly organized egg apparatus, is an older type than that of *Pandanus* or *Gunnera*, where a typical egg apparatus is present, is not easy to answer, although we are inclined to believe that it is.

There is little question that the type of *Pandanus* is more primitive than that of *Sparganium*, where the large development of antipodal tissue is secondary. The latter may very well be derived from the former, but the reverse is hardly conceivable.

EXPLANATION OF PLATES LIX AND LX.

Illustrating Prof. Campbell's paper on the Embryo-sac of *Pandanus*.

PLATE LIX.

Fig. 1. *a*, two young pistillate flowers of *Pandanus affinis*, Kurz. × 3. *b*, a somewhat older flower of *P. Artocarpus*, Griff. × 3.

Fig. 2. Two pollen-spores of *Pandanus* sp., showing the small prothallial (?) cell (m_1). × 600.

Fig. 3. Median section of the nucellus of a young ovule of *P. affinis*. The sporogenous cell is still undivided. × 600.

Fig. 4. The young embryo-sac of *P. Artocarpus*, with the sister cell, *x*. × 600.

Fig. 5. The upper part of the young nucellus of *P. Artocarpus*, showing the embryo-sac and the two cells, *x*, derived from its sister cell : *t*, the tapetal or parietal cells. × 600.

Fig. 6. The young embryo-sac of *P. Artocarpus*, with a single row of tapetal cells above it. The cell *x* is probably a sister cell of the embryo-sac. × 600.

Fig. 7. Young embryo-sac of *P. Artocarpus*, in which the primary nucleus has divided.

Fig. 8. A somewhat older embryo-sac of the same species; a large vacuole has developed in the embryo-sac. × 600.

Figs. 9–11. Young embryo-sac of *P. Artocarpus* with four nuclei. In Figs. 9 and 10, *a* is the micropylar region ; *b*, the chalazal. × 600.

Fig. 12. *a*, nearly median section of an embryo-sac of *P. Artocarpus* with six chalazal nuclei, of which three show in this section; one of the two micropylar nuclei can be seen; *b*, another section of the same, showing the second micropylar nucleus and the two sister cells of the embryo-sac.

[1] See Bulletin of the Torrey Botanical Club, xxxvi, 1909, pp. 205–20.

[2] Ernst, A.: Zur Phylogenie des Embryosackes der Angiospermen. Ber. der deutsch. bot. Gesellsch., xxvi, 1908, pp. 419–38.

Fig. 13. Two sections of an embryo-sac of *P. Artocarpus* with twelve (?) chalazal nuclei ; the small cell, *y*, probably belongs to the nucellus. × 300.

Fig. 14. Embryo-sac of *P. Artocarpus*, with six (?) chalazal nuclei ; *b*, the micropylar part of the same, showing the two sister cells (*x*) of the embryo-sac.

Fig. 15. Three sections of an embryo-sac of *P. Artocarpus* with twelve chalazal nuclei. The two micropylar nuclei are shown in *a* and *b*. × 300.

Fig. 16. Two sections of an embryo-sac of *P. odoratissimus*, L. f., with fourteen chalazal nuclei ; two of the nuclei may have belonged to the adjoining nucellar tissue. × 300.

PLATE LX.

All figures except 17 and 18 refer to *Pandanus coronatus*.

Fig. 17. *a*, cross-section through the apex of the embryo-sac of *Pandanus odoratissimus*, showing the two micropylar nuclei ; *b*, section through the base of the embryo-sac, showing six of the fourteen chalazal nuclei. × 300.

Fig. 18. Tissue of the nucellus near the embryo-sac, showing cells that resemble young secondary sacs. × 600.

Fig. 19. Longitudinal section of an embryo-sac, showing one of the two micropylar nuclei and three of the eight chalazal nuclei. × 300.

Fig. 20. *a*, base of an embryo-sac with ten nuclei in addition to the large nucleus shown at *b*. × 300.

Fig. 21. Embryo-sac with two micropylar and twelve chalazal nuclei. × 300.

Fig. 22. *a*, embryo-sac, in which there were thirty-two antipodal cells and four micropylar nuclei, forming an egg apparatus and polar nucleus, *p. n.* ; *b*, *c*, two sections of the egg apparatus from the same sac ; *a*, egg ; *sy*, synergidae. × 300.

Fig. 23. *a*, *b*, sections of the egg apparatus from a nearly mature embryo-sac ; *c*, the basal part of the same sac, showing the lower polar nucleus, *p. n.*, just separating from the mass of antipodal cells. × 300.

Fig. 24. *a*, egg apparatus and polar nucleus, *p. n.*, from a nearly mature embryo-sac ; *b*, basal region of the same. There were about thirty-six antipodals. A single polar nucleus, *p. n.*, was separating from the mass of antipodal tissue. × 300.

Fig. 25. Basal region of an embryo-sac, about the time of fertilization. There were about sixty-four antipodal cells, and three polar nuclei, the latter in process of fusion. × 400.

Fig. 26. Four fusing polar nuclei from a sac that has just been fertilized. × 400.

Fig. 27. Endosperm nucleus formed by the complete fusion of probably six polar nuclei. A second group of fusing nuclei, seven in number, was present in this same sac. × 600.

Fig. 28. *a*, the primary endosperm nucleus in process of division. × 600. *b*, dividing endosperm nucleus of about the same age as that shown in Fig. 29, *b*.

Fig. 29. *a*, one of four nuclei, resulting from the division of the primary endosperm nucleus ; *b*, *c*, endosperm nuclei from a more advanced embryo-sac, but before the development of any cell-walls in the endosperm ; *d*, nucleus at the time of the formation of the first cell-walls in the endosperm. × 600.

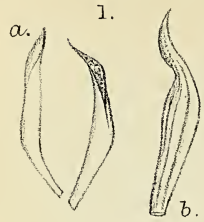
Fig. 30. *a*, an embryo-sac in which there was a group of twelve fusion nuclei, *e. n.*, and a second double nucleus, *x*, as well as a cell (*y*) resembling an egg-cell, in addition to an egg apparatus of four cells. × 95. *b*, *c*, *x* and *y* of *a*—more enlarged.

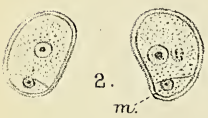
Fig. 31. Two sections of the egg apparatus of the embryo-sac shown in Fig. 30. There were four nuclei, but the synergidae and egg were not clearly defined. A structure (*p. t.*) resembling a pollen-tube could also be seen, but the nature of this was doubtful. × 600.

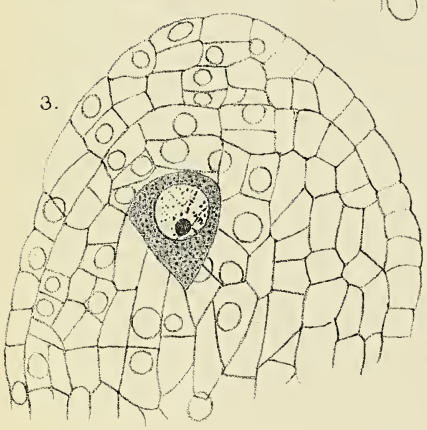
Fig. 32. Five of the twelve polar nuclei of the sac shown in Fig. 30. × 600.

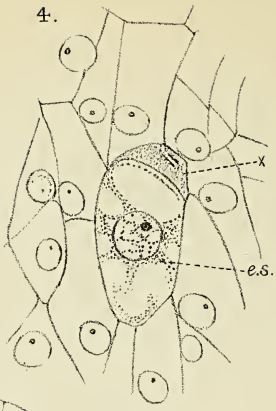
Fig. 33. *a*, upper part of the nucellus and embryo-sac, showing a one-celled embryo : *sy*, remains of a synergid. × 235. *b*, the embryo. × 500.

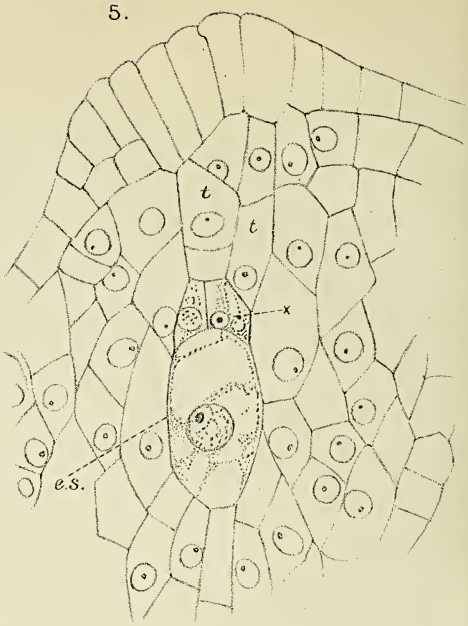
Fig. 34. Two sections of a two-celled embryo. × 500. *sy*, remains of the synergidae. × 500.

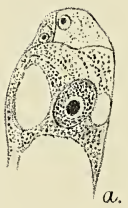


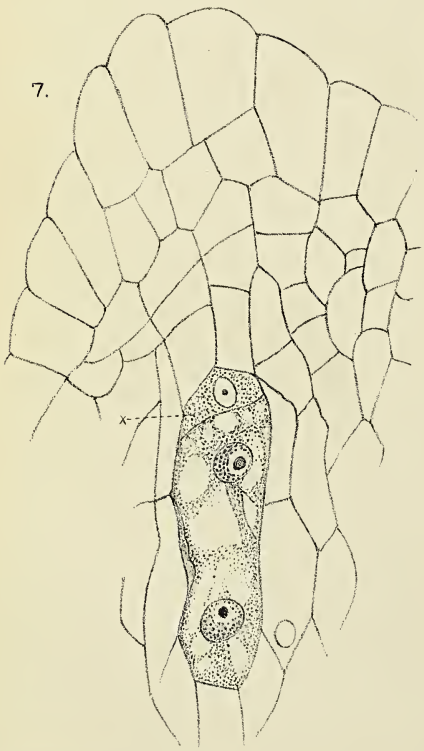


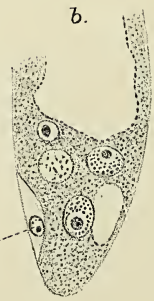












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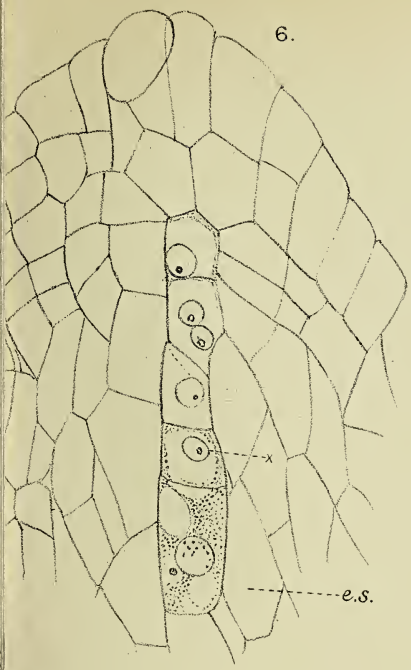

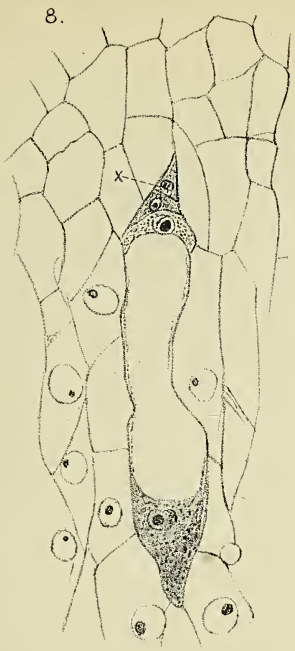

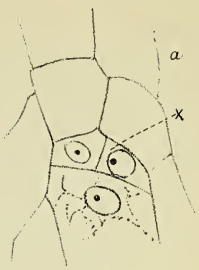

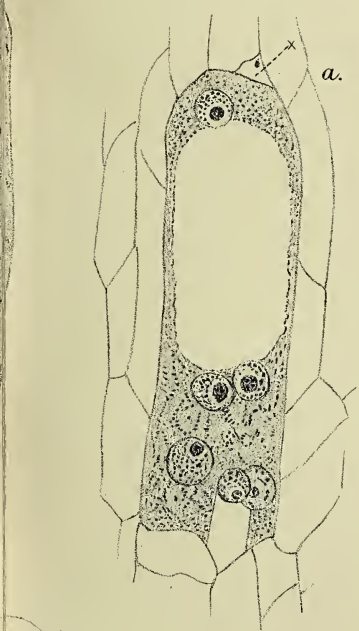

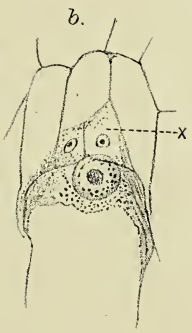

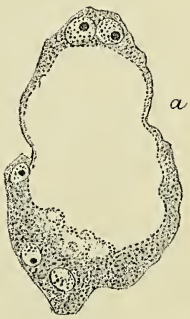

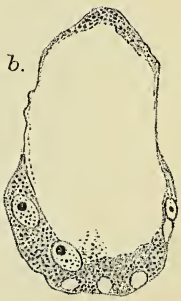

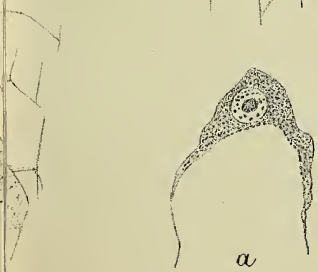

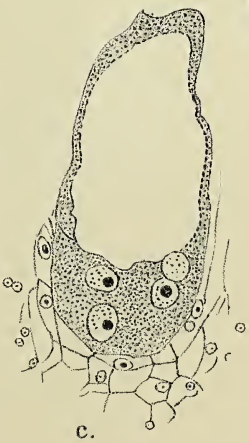

Huth, lith et imp.

1. a. b.

2. m.

3.

4. x e.s.

5. t t x e.s.

6. x e.s.

7. x

8. x

9. a. b.

10. a x b.

11.

12. a. b.

13. a. b. y.

14. a. b. x

15. a. b.

16. a b.

c.

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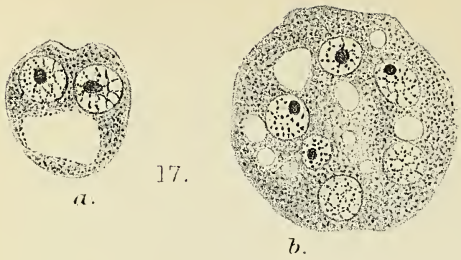

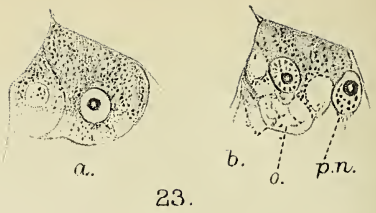

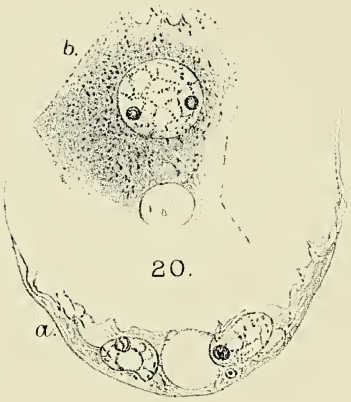

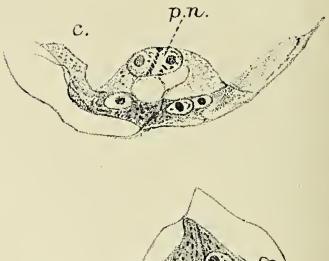

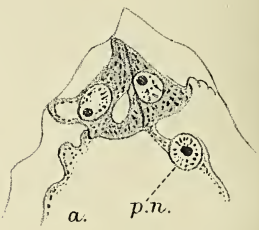

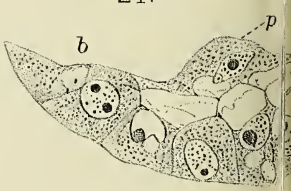

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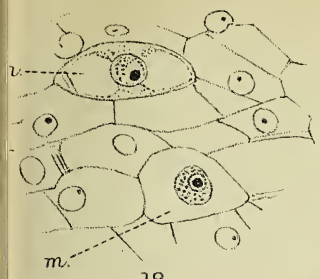

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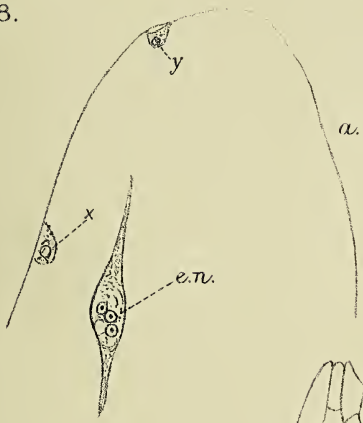

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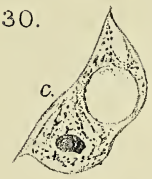

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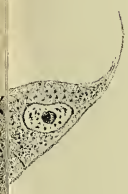

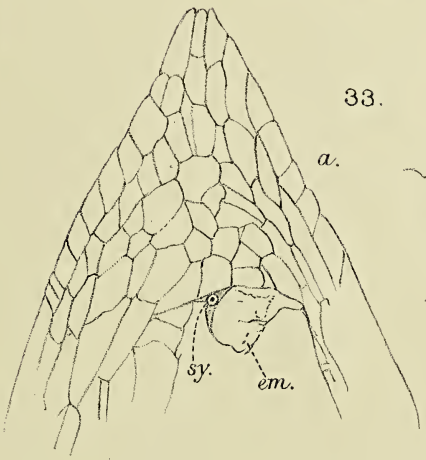

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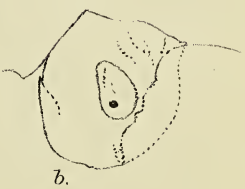

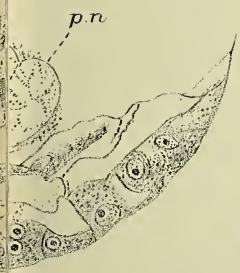

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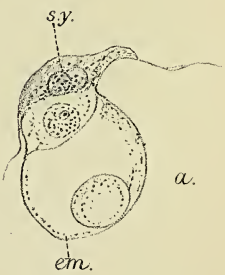

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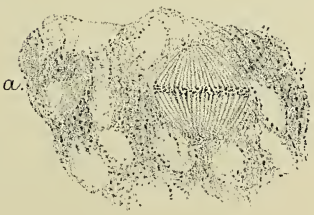

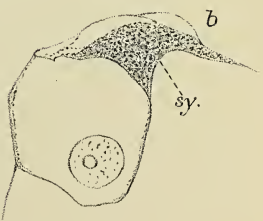

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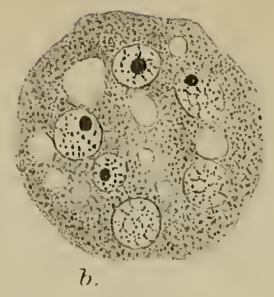

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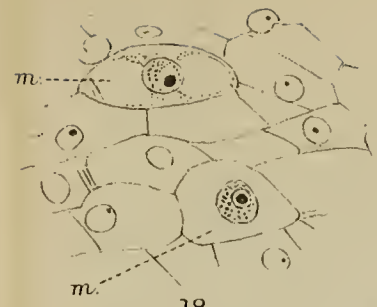

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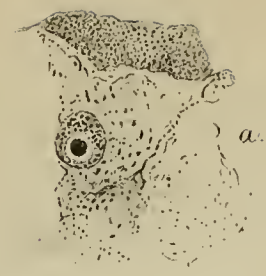

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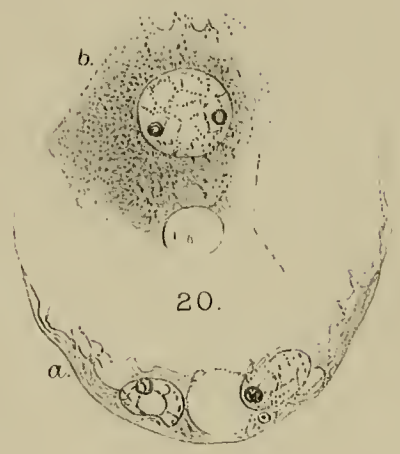

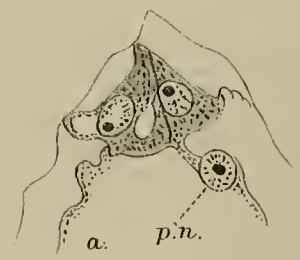

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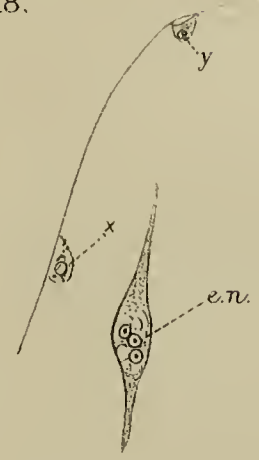

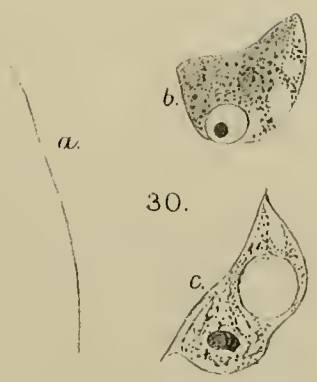

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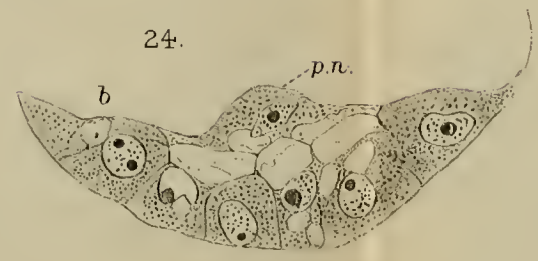

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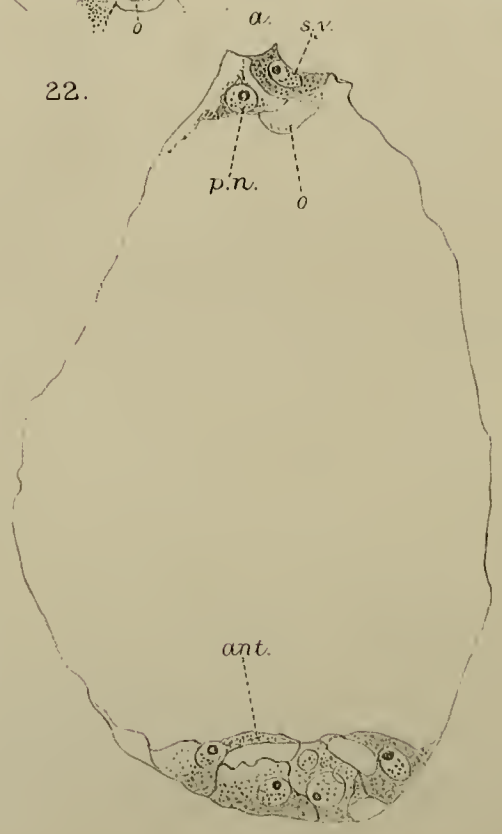

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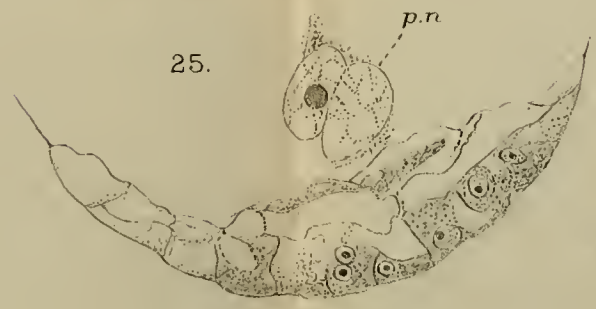

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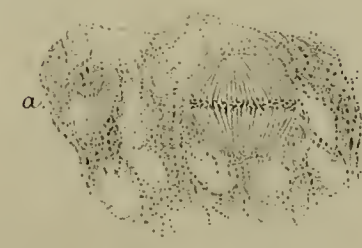

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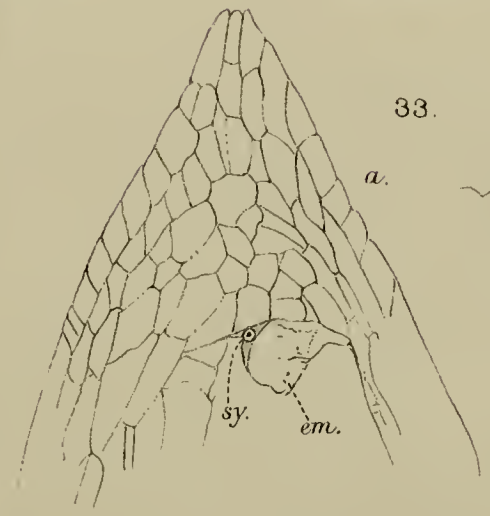

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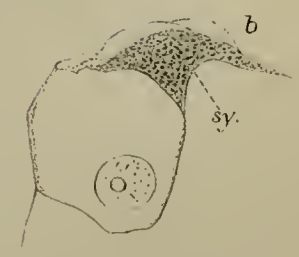

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CAMPBELL—— PANDANUS.

Huth, lith et imp

The Life-history and Cytology of Sorosphaera Graminis.

BY

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

With Plate LXI.

IN the course of some field work in the spring of 1910 I found, near Sevenoaks Railway Station, some grasses, the roots of which were in parts considerably swollen so as to give the appearance of small nodules or tumours. A cursory microscopical examination of these swellings revealed the presence of 'eel-worms', which apparently were the cause of the formation of the tumours. Being engaged at the time on work on *Sorosphaera Junci*, the small amount of material was laid by until the close of the summer of the same year, when I re-examined it with the object of ascertaining whether the 'eel-worms' were the sole parasites or whether, as I thought probable, and as is so often the case, they were accompanied by parasitic Fungi whose presence I had perhaps overlooked during my earlier examination. I was pleased to find that my surmise was a correct one, and that the grass roots harboured a second parasite, which proved to be an amoeboid organism allied closely to the *S. Junci* mentioned above. This fungoid organism, which forms a new member of the Plasmodiophoraceae, I propose to call '*Sorosphaera Graminis*', it being a *Sorosphaera* parasitic on the roots of various grasses. The result of the study of the life-history and cytology of this new *Sorosphaera* is embodied in the present communication. The genus *Sorosphaera*, containing originally the single species, *S. Veronicae*, was described by Schröter in 1878 in Engler and Prantl's 'Die natürlichen Pflanzenfamilien'; in this account the genus is distinguished from the other genera of the Plasmodiophoraceae by its hollow spherical collections of spores, which are enclosed by a common membrane. This diagnosis was subsequently modified by the present writer so as to include *S. Junci*, in which, although typical sorospheres are to be found, yet the more usual form of spore collections is of ellipsoidal or irregular shape.

The cytology and life-history of *S. Veronicae* has been described by Maire and Tison (1) in the 'Annales Mycologici' in 1909, and almost simul-

[Annals of Botany, Vol. XXV. No. XCIX. July, 1911.]

taneously by Blomfield and the present writer (2) early in 1910. These accounts in their main features agree closely with the life-history of *Plasmodiophora Brassicae* as described by Nawaschin (4). In July, 1910, I published an account of the life-history and cytology of a new species, *S. Junci*, parasitic on the roots of the various species of *Juncus*, which showed a close agreement with that of its nearly related *S. Veronicae*.

In January, 1911, in the 'Annals of Botany', Osborn, in a short note on *Spongospora Solani*, describes the fusion in pairs of the nuclei of the plasmodia prior to the nuclear divisions preceding the formation of the spores; in the same number another short account of the same species is given by Horne.

It is interesting to add that *Plasmodiophora Brassicae* has been found by Marchand (3) as a parasite in the roots of the Melon, Celery, and Sorrel, in which it caused malformations similar to those on the Cabbage.

The material used in the course of this investigation was collected by myself in the neighbourhood of Sevenoaks; some of it was fixed immediately after uprooting, the fixative used being usually Bouin's picro-formol solution and occasionally Flemming's solution. With a view to increasing the number of nuclear divisions, a number of the uprooted plants were kept from one to one and a half hours with their roots immersed in warm water at about 22° C., a temperature considerably above that in the open, which was about 10° C.; on their removal from the water they were at once placed in Bouin's solution. In this latter material the nuclear divisions were somewhat more common, although they were still few in number, and it needed considerable search to find any. Benda's iron haematoxylin was almost entirely used as a nuclear stain.

Occurrence of Sorosphaera Graminis. As previously stated, the first diseased plants I found were growing in the vicinity of Sevenoaks Station; from this spot, however, I obtained only a few plants. Later on in the summer I was fortunate in obtaining a good supply from a locality close at hand, the diseased grasses being found growing as weeds along the border of a gravelled path in the garden of my home. The spot was shady and the plants were growing directly in the gravel (Kentish ragstone) of the path; they were mostly gathered in the late summer when the ground was damp. Where soil had infiltrated into the gravel the grass plants were free from disease and grew somewhat more luxuriantly. The situation was on the slope of a hill with a northern aspect, and in summer it was a dry one; it was distant about a mile from the spot where I had found the diseased *Junci*. Several other likely places in the district were searched for diseased plants, but with no success, except in the case of a shady roadside close to the garden path above mentioned, and evidently forming part of the same diseased area, so that, as far as my experience warrants the expression of an opinion, the disease is not a common one. The grasses

attacked by the *Sorosphaera* were not so well developed as other healthy grasses in the same area, and did not so commonly flower. It was not always possible to determine with certainty the species of grass, but frequently the parasite was found in the roots of *Poa Annua*, a common grass weed on gravelled paths and a short-lived annual. Roots and stems of *Veronica Chamaedrys*, growing close to some of the diseased grasses, were microscopically examined, but found to be free from disease.

Structure and appearance of diseased roots. Almost without exception, the roots of diseased plants were in parts hypertrophied, although, as a general rule, the swollen portion was not invaded by the *Sorosphaera*, but was found to be the seat of 'eel-worm' infection. Probably the eel-worms form one means by which infection with the *Sorosphaera* is effected or conveyed, but as nearly all the grasses in the vicinity of my diseased area had their roots attacked by eel-worm, this might account for the fact of all the grasses infected by the *Sorosphaera* showing also the presence of eel-worm. However, I found the *Sorosphaera* much more commonly on the actual roots bearing the tubercles with eel-worm than on the non-tuberculate roots of the same or close neighbouring plants; this points to the eel-worm as being concerned in producing infection, at any rate the eel-worms would enlarge the area of disease by helping in the dispersal of spores. *Protomyces Rhizobius*, Trail, has been stated to be the cause of the formation of small tubercles on the roots of *Poa Annua*. I have but very rarely observed this fungus in the roots, and never actually in the tubercles themselves; these latter, in my opinion, are caused by the presence of the worm, and both the *Protomyces* and the *Sorosphaera* are secondary infections, and mostly confined to parts of the roots which show no signs of hypertrophy.

The swollen portion of the root may be terminal; not so commonly, however, the root may continue its growth, leaving the swelling behind; in this latter case the root usually becomes twisted. The swellings, which are often of crescent shape and of small size, are shown in Pl. LXI, Figs. 1 and 2; in transverse section they show a central stele surrounded by an enlarged parenchymatous cortex, the individual cells of which are of normal size. They contain cavities caused by the eel-worms which may be seen in them. Rarely some of the cells show the presence of the amoebae of the *Sorosphaera*; the latter are, however, usually to be found in the root bearing the tubercle, though frequently the grass plant infected by the nematode is quite free from the *Sorosphaera*. Both the tubercles and the roots themselves are free from reserve starch grains. In roots in which the disease is in an early stage we find the amoebae of the *Sorosphaera* in the outer cortical cells, some of which may be completely filled by the parasite, whilst others may contain but a single mononucleate amoeba or a few small-sized independent amoebae. The root-cells themselves are not hypertrophied to any great extent, although many of them are considerably elon-

gated, either through failure in the formation of transverse walls or through the absorption of such walls by the parasite. The nuclei of the root-cells, when visible, show some hypertrophy and signs of degeneration. In this stage, which is shown in Pl. LXI, Fig. 15, the appearance of the root is very similar to that of a diseased *Juncus* root.

In roots in which the disease has reached a more advanced stage, many of the cortical cells are filled with the spore collections of the *Sorosphaera*; the true spherical sorosphere is, however, but rarely to be seen, the most usual form being oblong, with slightly rounded ends as shown in Figs. 14 and 20. Other cortical cells may contain a crowded mass of small mononucleate amoebae in an incipient stage of spore formation (as seen in Fig. 10). The general appearance of such roots is considerably different from those of diseased *Junci*; the diseased cells, however, are frequently to be seen in longitudinal rows as they were in the *Juncus* root.

In addition to the probability of infection being effected through the agency of eel-worms, the amoebae may obtain an entrance into the root by the penetration of a mononucleate amoeba into a root-hair. I have observed root-hairs containing amoebae of various sizes, one of which is shown in Fig. 7, in which the nuclei are to be seen in the dumb-bell stage of division. This mode of infection is similar to what I have observed with *Sorosphaera Junci*, and doubtless it is the usual method by which the members of the Plasmodiophoraceae enter their host plant. I have also found root-hairs of *Zannichellia Palustris* serving in similar fashion for the entrance of *Tetramyxa parasitica*. The root apices probably serve also as points of entrance, as they are frequently diseased; this mode of infection would probably give rise to the longitudinal rows of diseased cells at times to be seen in the roots. Young branch roots often show the first signs of the presence of disease at or near to their apices.

Cytology of Sorosphaera Graminis. The amoeboid organism varies considerably in size from a quite small mononucleated mass of protoplasm, as shown in Fig. 5, *d*, to one of considerable size, as shown in Fig. 15. The young amoebae are often pear shaped, and in response to some attraction make their way to the nuclei of the plant cells; two such amoebae are shown in Fig. 16 with the plant nucleus between them. At times these small amoebae fuse together to form a plasmodium, which, after repeated nuclear division, will increase in size and finally occupy the whole of the root-cell. The smaller amoebae, as shown in Figs. 5 and 15, are very irregular in shape, and put out pseudopodia or protoplasmic threads, which appear at times to pass through the walls of the cells. I have never observed, however, any migration of a nucleus from one cell into a neighbouring one.

The life-history of the parasite may, as in the case of other Plasmodiophoraceae, be divided into two stages, viz. the vegetative and the reproduc-

tive, each stage being characterized by its nuclei. In *Sorosphaera Graminis*, which I have found considerably better for the observation of the nuclear changes than *S. Junci*, these two stages are well marked. The nucleus in the vegetative stage usually consists of a central mass of chromatin, the nucleolus or karyosome, situated at the centre of a small, spherical, clear space, the whole being surrounded by a delicate nuclear membrane. At times, more especially prior to nuclear division, granules of chromatin are to be seen on the inner side of this membrane. The nucleus stains well with Benda's iron haematoxylin. The first stage in the division of the vegetative nucleus consists in its becoming somewhat elliptical in shape, the karyosome elongates, and the chromatin granules collect together to form an equatorial plate, the whole giving the appearance of a cruciform structure, as shown in Fig. 8, and exactly similar to the corresponding phase that has been observed in other Plasmodiophoraceae. The next step consists in the splitting of this plate and the passage of the halved portions, one to each end of the elongated karyosome to form the 'dumb-bell' or 'double-anchor' stage shown in Fig. 7, which is similar to that of *S. Veronicae*. I have been fortunate in finding a nuclear division showing the actual splitting of the equatorial plate, which is drawn in Fig. 6; this phase I have never actually observed either in *S. Veronicae* or *S. Junci*. It appears, however, to be somewhat abnormal, as the karyosome is larger and more compact and not so elongated as I have usually observed it to be in the 'cruciform' stage prior to the actual splitting taking place; it seems probable that the equatorial plate has split just before the complete elongation of the karyosome. The completion of the nuclear division after the formation of the 'dumb-bell' is precisely like that in other Sorosphaerae, the elongated karyosome becoming more slender and at length disappearing, whilst the nuclear membrane of the two daughter nuclei is completed by the formation of a new equatorial portion. Figs. 7, 8, 9, and 19 show these various stages.

In the vegetative phase the organism increases in size by the division of its nuclei as described above, and may completely fill the root-cell in which it is found. On the other hand, many of the cells contain one or more irregular amoeboid bodies which at times give rise to daughter amoebae by a process of constriction—the so-called schizogony. As is the case in the other members of the Plasmodiophoraceae, all the nuclei of a given amoebae divide simultaneously, so that they are all to be seen in the same phase of the division, as is shown in Fig. 8.

At the close of the vegetative stage the karyosome gradually decreases in size, its chromatin being apparently passed out into the surrounding cytoplasm, so that we get a mass of protoplasm with a number of more or less spherical vacuoles in the place of the previous nuclei. In these vacuoles apparently fresh nuclei are formed, and the organism enters into its repro-

ductive stage. This intervening stage, in which we get the disappearance and reformation of the nuclei, is known as the akaryote or chromidial stage, and is to be seen in Pl. LXI, Figs. 11 and 12. The nuclei of the reproductive stage next undergo typical karyokinetic mitoses, as shown in Fig. 13. I have not been successful in finding any reduction nuclear division, there not being any noticeable difference in the size of the spindles. In all probability, however, meiosis takes place, as has been observed by myself and others in various members of the Plasmodiophoraceae. At the close of these nuclear divisions the nuclei are considerably reduced in size; unlike the vegetative nucleus, the chromatin is not collected into a central karyosome, but is found round the periphery of the nucleus, the centre of which is often clear. Cleavage occurs in the protoplasm, and a number of small independent uninucleate amoebae are formed. These amoebulae are crowded together, sometimes in spherical or ellipsoidal masses. After the secretion of a cell-wall by each amoebula a spore is formed, and the masses of amoebulae are converted into the collections of spores or sorospheres. These spore masses may completely fill the cell or they may be comparatively small; they are usually hollow and enclosed by a common membrane. The akaryote stage is shown in Figs. 11 and 12, and various forms of spore masses are to be seen in Figs. 14 and 20. I have observed no conjugation of nuclei such as has been described by Prowazek in *Plasmodiophora* and Osborn in *Spongospora*, although I have carefully searched for it. The reduction in size of the spindles in the two reproductive mitoses prior to spore formation has been observed by myself in both *S. Veronicae* and *S. Junci*, and doubtless points to a corresponding karyogamy such as has been described by Osborn. The spores vary considerably in size, and have an average diameter of about 6 μ.

Affinities of S. Graminis. It is obvious that *S. Graminis* is very closely allied to *S. Junci*, the main points of difference being the greater rarity of the true sorosphere (spherical in shape) and the more amoeboid form of the organism. The affinities of the Plasmodiophoraceae with other Fungi I have discussed in my paper on *S. Junci*; they differ from the non-parasitic Mycetozoa by their distinctive form of vegetative nuclear division, which has not been observed in the latter, in which also the akaryote stage is absent.

Attempts were made to infect plants of *Poa Annua* by planting them in pots of soil infected with *S. Junci*, in which also some diseased *Junci* were planted. The *Poa* plants, however, kept quite healthy, thus showing the two organisms, *S. Junci* and *S. Graminis*, to be distinct.

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

Summary.

1. *Sorosphaera Graminis* is parasitic on the roots of various Grasses, though not the actual cause of the swellings sometimes found on them.

2. *S. Graminis* is closely allied to *S. Junci* and *S. Veronicae,* its life-history being similar in character.

3. No hypertrophy takes place in the diseased roots, the disease being only detected after microscopical examination of the root.

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2. Blomfield and Schwartz ('10): *Sorosphaera Veronicae.* Annals of Botany, Jan., 1910.
3. Marchand ('10): *Plasmodiophora Brassicae.* Comptes rendus, tome cl, No. 21, p. 1348.
4. Nawaschin ('99): *Plasmodiophora Brassicae.* Flora, Band lxxxvi.
5. Schwartz ('10): *Sorosphaera Junci.* Annals of Botany, July, 1910.

EXPLANATION OF PLATE LXI.

Illustrating the paper by Mr. Schwartz on the Life-history and Cytology of *Sorosphaera Graminis.*

The drawings were made with the aid of the camera lucida.

Fig. 1. Diseased plant of *Poa Annua*. Natural size.
Fig. 2. Swellings on the roots. About twice natural size.
Fig. 3. Transverse section of diseased root. × 512.
Fig. 4. Transverse section of young diseased root. × 512.
Fig. 5 (*a*, *b*, *c*, *d*). Various forms of amoebae. × 730.
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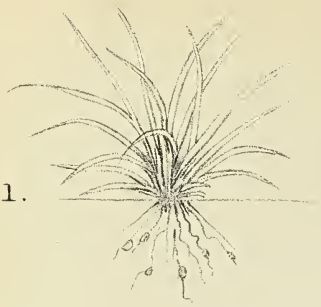

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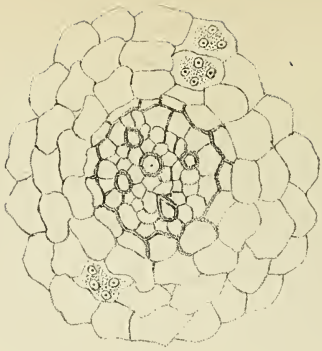

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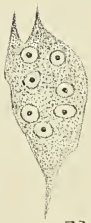

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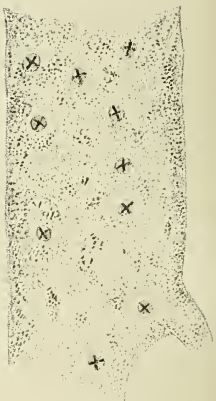

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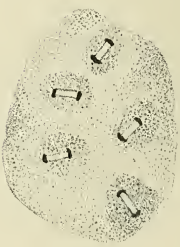

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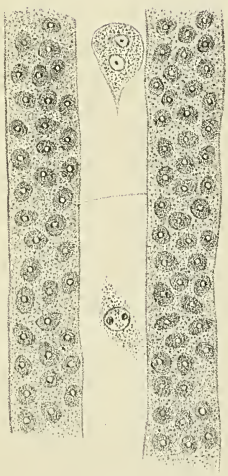

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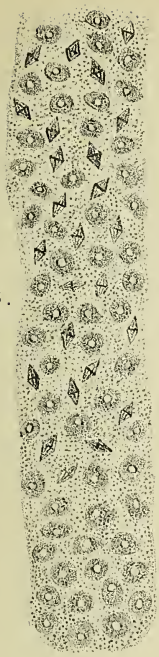
SCHWARTZ — SOROSPHAERA.



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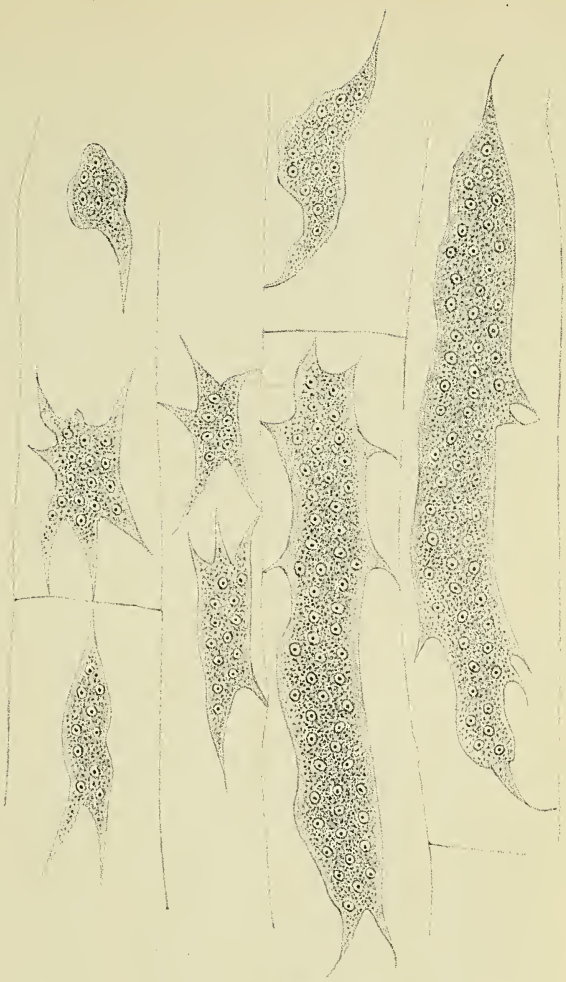

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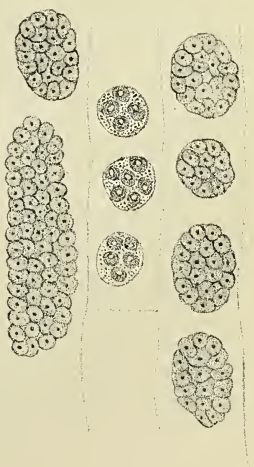

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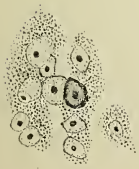

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Huth, lith et imp.

A Research into the Amyloclastic Secretory Capacities of the Embryo and Aleurone Layer of Hordeum with Special Reference to the Question of the Vitality and Auto-depletion of the Endosperm.

PART I.

BY

F. STOWARD.

Contents of Part I.

I. Introduction.

THE statement that the digestion and the ultimate depletion of the storage-reserve materials of the endosperms of cereal seeds, during the progress of the germinative process, is induced by enzymes, meets with universal acceptance.

Considerable divergence of opinion, however, exists as to whether the digestive phenomena observed are to be ascribed solely to the action of enzymes which arise as secretions either of the embryo or aleurone layer, or to both, or whether digestion and ultimate depletion of the reserve contents of the inner endosperm [1] are induced solely by enzymes which originate in or are generated by this tissue itself. That is to say, whether the inner

[1] The term 'inner endosperm', employed throughout in this paper, signifies that portion of the endosperm which remains when the endosperm is completely divested of its aleurone layer.

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endosperm, independently of the secretions of the embryo and aleurone layer, is capable of digesting its food reserves.

The subject has been attacked from various standpoints by different investigators. A general survey of the observations and results arising from their investigation and their interpretation bears witness to the divergence of the conclusions arrived at.

The earlier attempts to elucidate the interesting but complex phenomena presented by the physiology of germination as exemplified by cereal and other seeds are due to Gris[1] and Van Tieghem.[2]

Subsequently, more penetrative examination of certain of the aspects of this many-sided phenomenon was undertaken by Brown and Morris,[3] Hansteen,[4] Puriewitsch,[5] Linz,[6] Grüss,[7] Haberlandt,[8] Brown and Escombe,[9] Bruschi,[10] and quite recently by Ford and Guthrie.[11]

Examination of the literature dealing with these different researches shows that the greatest divergence in the conclusions arrived at by the different investigators mentioned centres itself in the question *whether* or *not* the inner endosperm possesses an auto-depletive capacity.

The adherents of the rival hypotheses advanced to elucidate the observed experimental phenomena range themselves into two distinct groups. On the one hand, Brown and Morris and Brown and Escombe claim that the inner endosperm (barley) possesses neither vitality (or at most only the veriest trace) nor a capacity for self-digestion. On the other hand, Hansteen, Puriewitsch, Grüss (barley), and Linz (maize) suggest that the inner endosperm consists of living cells which are capable of digesting their storage reserves.

Bruschi asserts that the inner endosperm (barley) may possess some residual vitality, that part of the tissue to which this attribute is ascribed being the more peripherally situated cells immediately subjacent to the triple-celled aleurone layer.

The twofold claim made by Hansteen and Puriewitsch that the endosperm possesses vitality and also a self-depletive capacity requires closer examination.

This claim may be at once disposed of by stating that the criterion employed by these authors, viz. the demonstration of enzymatic activity in

[1] Gris: Ann. Sci. Nat., Bot., 5e sér., ii, 1864, p. 90.
[2] Van Tieghem: Compt. rend., lxxxiv, 1877, p. 582.
[3] Brown and Morris: Journ. Chem. Soc., lvii, 1890, p. 458.
[4] Hansteen: Flora, lxix, 1894, p. 419.
[5] Puriewitsch: Jahrb. wiss. Bot., xxxi, 1897, p. 13.
[6] Linz: Jahrb. wiss. Bot., xxix, 1896, p. 267.
[7] Grüss: Ber. d. D. Bot. Ges., xi, 1893, p. 286; Jahrb. wiss. Bot., xxx, 1897, p. 644.
[8] Haberlandt: Ber. d. D. Bot. Ges., viii, 1890, p. 441.
[9] Brown and Escombe: Proc. Roy. Soc. Lond., lxiii, 1898, p. 3.
[10] Bruschi: Ann. Bot., July, 1908.
[11] Ford and Guthrie: Journ. Inst. of Brewing, xiv, 1908, p. 61.

a given tissue is no longer regarded as of value for the determination of the possession or non-possession of vitality by that tissue.

The unqualified statement that the endosperm (barley) is auto-depletive is supported by experimental demonstration of the phenomenon, but the conclusion that the demonstration of an auto-digestive capacity for the endosperm necessarily proves the claim that the inner endosperm is capable of inducing the complete self-depletion of its storage reserves independently of the aleurone layer is (as will be shown) probably inadmissible. Again, demonstration of the capacity of a tissue to augment its enzyme-content (such a capacity the inner endosperm possesses) can hardly be claimed to afford adequate evidence of the possession of a capacity for auto-digestion unless it be shown that digestive changes in the storage materials of sufficient magnitude either accompany or immediately succeed this augmentative capacity.

Apart from the work of Brown and Morris (embryo of barley) the methods of determining relative capacities of isolated endosperm and inner endosperm have been either qualitative or of such kind as to afford little or no means of evaluating the relative capacities for doing enzyme-work possessed by the different anatomical parts of the endosperm.

The study of the phenomenon of endospermic depletion as investigated by the method of experiment of Hansteen and Puriewitsch fails to indicate in what relative degree the aleurone layer and inner endosperm participate in or are contributory to the processes involved and take insufficient cognizance of possible differences in the properties and potentialities of the amyloclastic enzymes which originate in these tissues.

In particular the methods employed by Linz and Grüss in experiments with intact isolated endosperms of maize (although separate estimations of the amylase content of the aleurone layer were made at the termination of the germination experiment) are open to the objection that they fail to take account of the amount of enzyme *secreted* by the aleuronic tissue during the period of cultivation. They amount really to demonstrations of the capacity this or that structural part of the seed possesses of augmenting its amylase-content.

Similarly Grüss's demonstration of the presence of copper-reducing substances in thin sections taken from isolated endosperms under germination conditions, in the absence of the removal of the aleurone layer, fails to show that the apparent hydrolysis of starch implied by these experiments was due solely to an amyloclastic enzyme generated *in situ*.

Material. Two barleys have served as experimental material throughout the course of investigation, the one a North African and the other a brewing Chilian.

Both were mixtures of *H. vulgare* and *H. hexastichum* of the year, possessing the customary characteristics of barleys from these sources

of supply, huskiness and thick skin. Their germinative capacities were satisfactory and were subjected to frequent trials at intervals throughout the course of the inquiry.

Aims and methods of investigation. The principal aims of the investigation have been to endeavour to (1) show whence the amyloclastic enzymes are derived which are operative in influencing digestion of the amyliferous storage reserves of the inner endosperm and to (2) demonstrate in an approximate manner the contributory shares taken by these enzymes in the work of endospermic depletion.

Three possible sources of amylolytic enzyme apparently exist, the scutellar epithelium, aleurone layer, and inner endosperm.

The enzymes furnished by the two former are elaborated by cells which possess a secretory mechanism, and the continuance of their secretory activity depends upon their vitality. The amylases from both these sources are characterized by the possession of a marked power of attack on the *mature starch grains of the inner endosperm* under conditions which are approximately identical with those which obtain during the progress of normal germination.

The increase of enzyme pre-existent in the inner endosperm (augmentation of which occurs under certain experimental conditions to be described) does not with certainty depend upon the possession of *an undoubted secretory capacity* by this tissue; on the contrary, this enzyme possesses but feeble powers of attack on the fully formed starch grains which form by far the greater bulk of the storage reserves of the mature grain.

The augmentation of enzyme and the comparatively restricted amount of amylolytic change it is capable of inducing are therefore preferably referred to as being due to the generative or auto-digestive capacity of this tissue.

At the outset the fact must be clearly emphasized that the amylase which originates solely in the inner endosperm is sharply differentiated from those which are elaborated by the scutellar tissue and aleurone layer by its feebler power of attacking, under comparable experimental conditions, the fully formed starch grains of the mature inner endosperm, and the adequate recognition of the differential powers of attack on the mature starch grain possessed by these amylolytic enzymes is, as we shall see later, of prime importance in any attempt to elucidate and explain the phenomenon of starch digestion of the endospermic starch reserves, and we shall further see that the mere capacity of a given tissue to augment its amylase-content is of subordinate importance unless this augmentation of enzyme is accompanied by evidence of a commensurate increase in the amount of starch digestion.

The interest attached to the work of Ford and Guthrie (l. c.) relates to amylase of the resting grain and the possible rôle it may play in inner

endospermic depletion. Fuller reference to this phase of the question may, however, be deferred until experimental evidence has been adduced which bears on the nature of the amylase existent in the inner endosperm and the behaviour of the isolated tissue under the variable experimental conditions selected.

The chief result of their interesting inquiry is to demonstrate that under certain conditions of extraction the amylase of the resting seed undergoes a tangible *increase in quantity*.

That the embryo secretes an active amyloclastic enzyme, the secretory function being localized in the scutellar epithelium, has been convincingly demonstrated by the investigations of Brown and Morris (l. c.).

The glandular nature and secretory capacity of the aleurone layer has been rendered highly probable by the work of Brown and Escombe (barley) and Haberlandt (rye), and suggested by the researches of Linz (maize) and Grüss (barley), but opposed by Hansteen and Puriewitsch, who contend that its enzyme-secretory or generative capacity is not superior to that possessed by the amyliferous cells, in fact that it (the aleurone layer) represents physiologically an undifferentiated portion of the reserve system of the seed.

Throughout, the capacity to secrete or generate amylase possessed by the different anatomical parts of the grain (embryo, aleurone layer, endosperm, and inner endosperm) has been investigated as far as possible *quantitatively*. The methods devised and adopted comprised the preparation of sterile material, the establishment of cultures of isolated parts of the seed on nutrient or experimental substrata and their maintenance under *aseptic* conditions, and the subsequent *quantitative* investigation of both objects and media either for amylolytic enzyme or the products or evidence of amylolytic activity.

Sterilization of material. Sterilization of grains was necessarily resorted to to eliminate the possibility of the disturbing influence of micro-organisms, and because of the inadmissibility of the employment in the media of the experiments to be described of antiseptic reagents even in minute concentrations.

Further, in certain instances, notably in the case of experiments with inner endosperms, it was necessary to carry out cultural experiments of lengthy duration in order to afford these objects full opportunity of displaying their presumed powers of auto-depletion.

Intact air-dried seeds were subjected to a preliminary steeping in either[1] 10 % $CuSO_4$ or [2] absolute alcohol for periods of time varying from 24 to 72 hours.

[1] Latterly, in order to provide for the more efficient aeration of the material, the closely fitting stopper was removed and the bottle covered with a sterilized funnel.

[2] Wherever departure from the method described above has been made reference is made to the fact in the text.

Immediately after removal of the objects from the copper sulphate solution, they were divested of their paleae, and either washed as thoroughly as possible with sterilized tap-water only or washed and re-steeped in tap-water for 24 to 48 hours.

When absolute alcohol served as the antiseptic steeping reagent this re-steeping in water was invariably resorted to, and subsequent experience demonstrated the value of employing two to three changes of water or of placing the seeds under aeration conditions for 24 hours after removal from the water-steep.

For special purposes endosperms and inner endosperms prepared from air-dried seeds were successively steeped in absolute alcohol and water. In every instance the volume of steeping liquid used was just sufficient to cover the material. Small well-stoppered weighing bottles were employed, and each phase of the operation was carried out under strictly aseptic conditions.

The efficiency of the reagents employed and the method of manipulation followed was amply foreshadowed by the results furnished by several series of preliminary experiments.

Seeds treated as described were placed in tubes containing various nutrient media, and were then incubated at 30° C. for 7 to 14 days. In the majority of instances the contents of the tubes remained absolutely sterile :— in a small percentage only, moulds, but not bacterial forms developed, thus demonstrating the fact that the spores of the former organisms alone survived the toxic action of the reagents employed.

Preparation of sterilized objects for cultural purposes and investigation of their relative amylolytic secretory or generative capacities. The dissection of the steeped sterilized material was carried out in a Hansen inoculation chamber under the following aseptic conditions :—

A flat glass plate was placed on the floor of the chamber (the interior of which, just prior to use, was thoroughly swabbed with 1 % $HgCl_2$ solution) and covered with an inverted glass funnel. The depaled, steeped, thoroughly washed grains were then introduced *en masse* under the funnel.

Each grain was then successively withdrawn, dissected as required, and returned under the funnel until a sufficient number of objects for a culture experiment were prepared.

Dissections of grains into embryos and endosperms were accomplished by cutting through the integuments in the neighbourhood of the scutellar margin and gently lifting out the embryo with a scalpel: the further dissection of the endosperm into inner endosperm and aleurone layer was carried out by carefully cutting off the latter with a sharp razor in such a manner as to include the merest traces only of the subjacent amyliferous tissue.[1]

[1] This was subsequently controlled by filing off the aleurone layer of a number of air-dried grains and comparing the dry weights of this material after desiccation for 24 hours at 30° C., with

These somewhat tedious operations, affording by far the chief opportunity for reinfection of the material, were carried out with special care and precautions, the apparatus, dissecting instruments, and the operator's hands being, just prior to the commencement of the operation, thoroughly sterilized by passage through the flame of a Bunsen burner.

It is of interest to note that the successful sterilization of the grains turns on the discovery by A. J. Brown [1] of the existence of a semipermeable or selective property possessed by one of the integuments of the seed. The steeping of intact uninjured grains in the reagents mentioned is thereby rendered possible, as neither the copper sulphate nor absolute alcohol penetrates into the interior of the grain.

Method of establishing and conducting cultures. The sterilized material was quickly transferred to the liquid or semi-solid culture medium contained in small Petri dishes; either a definite number (5 or some multiple of 5) of objects (embryos and aleurone layer fragments) were placed on the surface of the medium, scutellar or inner surface respectively downwards, or a definite number of inner endosperms and endosperms were embedded in a thin stratum of semi-solid culture medium of just sufficient depth to cover them completely.

Other methods of establishment of the culture having reference to special experiments are described in the sections dealing with these experiments.

The cultures thus established were conducted *under absolutely sterile conditions*, at the ordinary temperature of the laboratory (15° to 18° C.), for periods of time varying in duration from 3 to 68 days.

In the case of cultures on liquid media the sterility of the culture medium was, at the termination of the culture experiment, controlled by removing a small quantity (0·5 c.c.) and introducing it into a series of tubes containing various nutrient media—dextrose-wort, dextrose-maltose yeast water, &c.)—and subsequently incubating these tubes at 30° C. for 7 to 21 days.

Such inoculations invariably furnished negative results, affording in these instances ample confirmation of sterility of the culture already indicated by macroscopic inspection.

The subsequent employment of semi-solid gelatine and agar medium rendered this system of control unnecessary, and, moreover, provided more favourable conditions for the display of the vital activity of certain of the objects under culture.

Numerous series of petty cultures were also instituted with embryo and aleurone layer fragments for the purpose of ascertaining whether or not the scutellar epithelium and aleurone layer, as stated by Brown and Morris and

that of an equal number of aleurone layers removed by cutting in the manner described above. The difference in no case amounted to more than 4 to 5 %, the former value being the nearer.

[1] Ann. Bot., xxi, p. 79 (1907).

Brown and Escombe respectively, secrete a cytoclastic enzyme, and also to study the mode of attack on starch grains of the amyloclastic enzymes which these tissues secrete.

The data derived from these variously conducted experiments and their discussion are embodied in the separate sections which follow.

II. Culture Media and Substrata.

Variously constituted culture media and cultural conditions have, during the course of the inquiry, been employed.

Reference need only be made at this juncture to the composition of the medium which has served as the *basis* of those used in the majority of the culture experiments to be described. Wherever modification of its composition, either by the inclusion of other substances or change of concentration of a particular component, has been made, attention is directed to the fact.

The composition of the medium shortly referred to in this paper as the ' mineral salt solution ', or more briefly, as ' M.S. solution', is the following:—

Per 100 c.c. of solution.[1]	
Calcium sulphate	0·100 grm.
Potassium chloride	0·25
Magnesium sulphate	0·25
Potassium dihydrogen phosphate	0·025

2 drops of N/5 Fe_2Cl_6 per litre of solution.

Method of investigating material and culture media for amylase. At the termination of the culture experiment the objects and corresponding culture medium from a given plate culture were separately investigated for amylase by digestion with soluble starch under definite and comparable conditions of time, temperature, and concentration of starch solution.

These digestions were carried out in the following uniform manner:— Into each of a series of 50 c.c. conical Jena flasks, previously cleaned by steaming them out, 25–30 c.c. of freshly prepared soluble starch containing 3 grm. starch solids per 100 c.c. of solution were pipetted. The flasks and their contents with the addition of a drop of antiseptic (nitrobenzene or toluene) were at once transferred to a thermostat regulated at 30° C.[2]

Those required for the *control* starch digestions, before addition of the enzyme-containing material, were rendered distinctly, but not excessively, alkaline by the addition of a few drops of 30 %–40 % NaOH solution.

The objects, previously desiccated for some hours (5–20) at 30° C., were divided into two portions, each consisting of an equal number and representing one-half the total number on the plate, were separately and uniformly

[1] Trans. of Guinness Research Lab., vol. i, Part II, p. 290, Brown, Millar, McMullen, and Escombe.

[2] Unless otherwise stated, it is to be understood that all starch digestions referred to in the text were carried out at this temperature; the temperature of the thermostat never varied throughout by more than + or −0·5° C.

ground up with chemically purified sand in a porcelain mortar. When the flasks and their contents had acquired the temperature of the thermostat (usually after an interval of 10–15 minutes) the finely ground material, placed on a small paper chute, was brushed little by little into the digestion flask, thoroughly distributed, and by constant agitation mixed with the starch solution.[1]

One half of the material serves as the *experimental* digestion ; the other, added to the alkalinized starch solution, as the corresponding *control* digestion. The latter, invariably carried out in all these digestion experiments, affords the means of correcting for the reducing substances pre-existent in the starch solution and added material.[2]

The culture liquid from a given plate was transferred to a calibrated tube and its volume brought to 20 c.c. with distilled water.[3] The tube and its contents were then transferred to the thermostat, and having attained the desired temperature, one half of this volume (experimental digest, 10 c.c.), representing the amount of amylase derived from one-half the total number of objects under culture, was added and thoroughly mixed with the unalkalinized starch solution, the other half (control digest) being added to the alkalinized starch solution. The flasks were then securely corked and the digestion continued for definite but variable periods of time (varying necessarily according to the amylolytic capacities of the material under investigation), at the conclusion of which the experimental digests were arrested by one or other of the methods already described. Finally, the digests were cooled, diluted to 100 c.c., filtered, and the copper-reducing power determined on an aliquot portion of the *clear* filtrate.

The amyloclastic capacity deduced from the experimental data thus determined is expressed throughout in this paper in terms of the weight of copper found, in milligrammes, and by calculation referred to the generative or secretory capacity possessed or displayed by twenty objects, and further reduced to a comparable basis by calculating the output (amylase in the culture medium) or augmentation (amylase in the tissue of enzyme) for a uniform period of digestion of one hour.

Throughout it is to be noted that the evaluation of this capacity refers

[1] Unless this method of transferring the material is adhered to (i.e. if the finely ground material is added to the starch solution *en masse*) 'balling' invariably ensues, and this may be so pronounced as to prevent a portion of the enzyme from gaining access to the starch solution during the earlier phase of the digestion, and may constitute a source of quite considerable error.

[2] For the purpose of checking this method of arresting amylolytic action, and further to unmask any possible action of the alkali on reducing sugars present, in many instances the controls were also checked by *boiling* the mixture of starch solution and material. No objection can be raised to the former method provided the use of large excess of alkali is avoided. The foregoing statement applies also to the arrests of the experimental digests by the alkali method.

[3] The distilled water employed in these experiments in the preparation of starch and nutrient solutions was prepared by means of a copper still modelled specially on the design given by Ford (Journ. Inst. of Brewing, ix, 1907 (5), p. 206).

to a definite number of organisms (embryos) or structural parts (aleurone layers, endosperms, inner endosperms) of the seed. The system adopted appeared to be preferable to that of referring it to *definite weights* of the various objects. If weight of material is taken as the basis of reference, several well-founded objections present themselves. Determination of definite weights of material necessitates desiccation under fixed conditions of time and temperature, and even if these be well below that calculated to be dangerous to the activity of the enzyme present, it does not follow that the enzyme may not undergo, as the result of influence of heat, inactivation or actual destruction. Moreover, the possibility of the desiccation of material of such different origin and constitution in such a manner as to render the weights comparable is excluded by the fact that loss of moisture (if the loss is wholly to be attributed to this) by such diverse material is by no means uniform, and consequently the residual weights of the various structural parts are more or less inadmissible as a basis for calculating their enzymatic capacities.

Were such comparisons confined to objects of the same kind, as, for example, isolated endosperms under cultural conditions to be described, the objection still would remain. The individuality possessed by the objects, the varying degree to which digestive changes progress, leading at every stage to variable proportions of unchanged and partly changed starch, the outward diffusion of products of change, all conspire to render the expression of result on any other than the numerical basis alluded to inadmissible and impracticable.

The manner of expressing results may perhaps be rendered clearer by taking an illustrative example:—a plate culture consisting of ten aleurone layers is established, continued for several days and finally terminated by removal of the cultural objects. Objects and medium are separately digested with soluble starch solution for a period of *half an hour* and the amount of reducing sugars determined and corrected in the manner described. In the experimental and control digestions five objects each are used; in the similarly conducted digestions with the diluted culture medium one half (10 c.c.) serves for the experimental, the other half (10 c.c.) for the control starch digestion, each half-volume of the total diluted culture medium obviously containing an amount of amylase derived from one-half (five objects) the total number of objects on the plate.

Let us suppose that the copper reduction values of the objects and medium after applying the control corrections are x and y milligrammes of copper respectively, then we shall have:—

Amylase per 20 objects per hour
in objects equivalent to $(x \times \frac{20}{5} \times \frac{1}{2} \times 2) = 4x$ milligrammes Cu.

Amylase per 20 objects per hour
in culture medium equivalent to $(y \times \frac{20}{5} \times \frac{1}{2} \times 2) = 4y$ milligrammes Cu.

The above example will serve to explain the mode of expression used in the tabular statements in the different sections descriptive of experimental work.

It is further assumed that, at least in the majority of instances, the augmentations of amyloclastic enzyme unmasked under the conditions of experiment are actually due to an increase in amount of the enzyme itself and not to activation of a non-increasing mass of enzyme. That is to say, production of enzyme as opposed to augmentation of enzymatic activity is implied, although it is conceded that the latter phenomenon may in the ordinary course of events obtrude itself, but purely in a subordinate measure.

It is conceivable that during the various progressive phases of culture, e. g. of isolated endosperms, the chemical and physical changes in residual endospermic substance may at each phase influence its amyloclastic activity, some idea of the extent to which this occurs being rendered evident by starch digestion experiments. The chief factors comprised here are actual augmentation of amylase together with modification of its activity by concomitant changes in the reaction medium induced by changes in the chemical and physical properties of the material under examination.

Inactivation of an enzyme can readily be induced, the agent and its approximate amount being readily enough determined; it is otherwise, however, when we turn to the question of the activation of an enzyme. The view held here is that a given enzyme possesses a finite capacity for work which does not exceed certain limits, and the display of this maximal capacity depends upon the provision of optimal working conditions. Wherever apparent activation occurs it is because in the previous conditions of experiment a retarding factor or factors were included.

In dealing with material possessing such variable amyloclastic capacities as structural parts of seed, the adoption of the foregoing method of expressing results is permissible. In order to meet the changes in magnitude of this capacity modifications in the method of starch digestion are unavoidable. Moreover, for the purpose of rendering the results of one series of experiments comparable with those of others, the conditions must be selected in such manner that the R of the mixed starch digestion products does not exceed the limiting value indicated by Kjeldahl's 'proportionality law', and this condition has been strictly adhered to throughout the course of this inquiry.

Experiments such as those briefly outlined afford striking evidence of the individuality of the objects which have been subjected to examination, and this factor alone probably overshadows those which may intervene during starch digestion, conditioned by alteration in number of objects, time, and concentration of starch employed.

The selection of what may appear to be comparatively small numbers

of objects for plate cultures turns on the fact, fully recognized at the very commencement of the inquiry, that the risks of reinfection increase greatly with increase in the number of objects handled. This refers more especially to objects like aleurone layers and inner endosperms, which have to be prepared by careful dissection of the aleurone layer. The successful preparation of sterile cultures of endosperms and embryos is a far easier task by reason of the more rapid handling of these objects.

Method of determining copper-reducing power. Many of the earlier determinations were made by the standard gravimetric method, the cuprous oxide being weighed as such, and the Cu calculated by applying the appropriate factor or the oxide reduced in a current of dry H and weighed directly.

The greater number of estimations have, however, been made by the extremely convenient and rapid method of *Bertrand*.[1]

In every instance these reductions were carried out in duplicate. The following comparative results show how closely estimations by the two methods approximate each other:—

1·1875 grm. of cane sugar were hydrolysed with HCl, neutralized with Na_2CO_3, cooled and diluted to 250 c.c.; duplicate determinations by the two methods made on 10 c.c. of the dilution gave—

Gravimetric method.	*Bertrand's method.*
97·2 mg. Cu, 49·5 mg. invert sugar	93·58 mg. Cu, 49 mg. invert sugar
95·6 ,, Cu, 48·5 ,, ,, ,,	92·59 ,, Cu, 48·5 ,, ,, ,,
Theoretically 10 c.c. of dilution contains	49·97 ,, ,, ,,

Soluble starches employed. The soluble starches used in the digestions represent three separate specimens prepared by precisely the same method from one and the same stock of potato starch. They were prior to use and during the progress of the inquiry subjected to critical control, either with a standard malt of known amylolytic power kept for the purpose or by means of a standardized enzyme solution.

The enzyme solutions employed in the following digestions were taken from a single embryo-plate culture.

COMPARISON OF SOLUBLE STARCHES.

Soluble starch.	*Starch solids per 100 c.c. of solution.*	*Amylase per 20 objects per hour equivalent to mg. of Cu.*
A_1	2·62	90·8
A_2	2·67	85·2
A_3	2·63	94·7

These values not only show how little these starch preparations differ

[1] Bertrand: Bull. Soc. Chim., t. xxxv, 1906, p. 1285.

from, but also how closely the amyloclastic secretory powers of the objects parallel each other; an approximation of sufficient nearness for ordinary biological investigation.

III. Amyloclastic Secretory Capacity of the Embryo of Barley.

The salient features of the general anatomy and histology of the barley embryo have been so frequently described that this portion of the subject calls for limited reference only.

The organ of special interest, the scutellum, is a shield-shaped expansion which over its greater area is in close apposition, but not organically united to the proximal end of the inner endosperm, the limiting layer forming the boundary between the scutellum and endosperm consisting of a single layer of columnar cells.

The functions of the scutellum are twofold—absorptive and secretory. It is regarded from the standpoint of morphological botany as a cotyledonary organ, and from the physiological standpoint as a glandular tissue, the secretory function being localized, at least principally, in the columnar or epidermal layer; the enzymes arising as a result of activity of secretory mechanism, amylase, cytase, and probably others being largely destined for extra-cellular work.

While it is broadly accepted that the scutellum possesses and exercises both absorptive and secretory functions it is perhaps not so generally recognized how important is the former function in the regulation of the complex processes which have their seat of action in the inner endosperm during the progress of normal germination, not only in regard to the removal of simplified products of enzymatic action, but to the accumulation of enzymes and the continuance of their specific activity.

It has been, and still is, a question of controversy whether the various degradation changes of the complex storage substances in inner endosperms can be or are induced solely by the action of enzymes which are elaborated by the embryo, or by enzymes which arise in or are generated by the inner endosperm independently of the embryo.

Demonstration of the possession by the embryo of a distinct amyloclastic secretory function and its localization in the columnar or secretory epithelium is due to Brown and Morris (l. c.), but this particular aspect of the subject, as far as can be ascertained, does not appear to have been the object of any subsequent independent reinvestigation.

The secretory function ascribed to the embryos by Brown and Morris, although generally accepted, has been more recently challenged by Ling.[1]

The investigation of the amylolytic secretory power of the embryo in this inquiry embraces two lines of attack: (1) cytological, (2) biochemical,

[1] Journ. Inst. Brewing, xv, p. 651.

and to the consideration of evidence afforded by application of these methods of investigation we must now turn.

1. *Cytological.* Embryos, removed from seeds after adequate steeping, were germinated for varying periods of time in Coldewe germinators at laboratory temperatures, 15° C.–20° C.; the material, after fixation, was embedded, microtome sections prepared, and the stained preparations examined and studied.

Briefly stated, cytological study of the scutellum, and particularly of the columnar epithelial cells during progressive phases of germination, reveals the fact that these cells are, as far as cytological evidence permits this statement to be made, distinctly secretory in function.

During the earlier phases of germination, 0–4 days, their cytoplasmic contents undergo marked changes—solubilization of specific granular inclusions which are probably destined to furnish enzymatic as well as nutritive material,—changes which are accompanied by significant and comparatively well-defined changes in the nucleus and its structures.

The succeeding interval, 4–8 days, presents features which, if unsupplemented by collateral biochemical evidence, are not so easy to follow or interpret. There is during this interval a gradual but complete disappearance of those granular contents (which various authors in other fields of cytological research have regarded as either enzymatic or pre-enzymatic in constitution) which are never renewed. Nevertheless, nuclear and cytoplasmic activity persists, as shown by the more or less continued production of chromatin and nucleolar substance, and by the vacuolate condition of the cytoplasm.

The final interval, 8–11 days, is marked by the gradual disorganization of cytoplasm and degeneration of the nucleus.

Judged solely by the cytological results of study, the most active phase of secretory activity would be ascribed to the earlier phase comprised within the 0–4 days' interval.

As we shall see, the biochemical evidence to be adduced not only supports the original contention of Brown and Morris that the columnar epithelium consists of cells which are secretory in function, but helps to elucidate the cytological evidence in a significant manner.

2. *Biochemical.* Embryos from sterilized steeped seeds were placed on various kinds of media, and after the lapse of definite time periods, either the culture medium or both culture medium and objects were investigated under uniform conditions for amylase.

Preliminary experiments in which embryos were cultivated on (1) water (starvation conditions), (2) cane sugar plus mineral salt solution, (3) 0·55 % asparagin solution, evidenced that the latter solution yielded the most favourable results. The amounts of amylase were exceedingly small, however, and as the seed from which the embryos were derived had been steri-

lized in $CuSO_4$ solution, it was surmised that this reagent, manifesting its influence more especially during the digestion with soluble starch, constituted a probable disturbing factor. This suggestion was, however, negatived by similarly established cultures with embryos derived from seeds which had been steeped successively in absolute alcohol and water yielding equally low results.

Asparagin and KH_2PO_4 are amphoteric substances, and, as shown by Ford [1] and Ford and Guthrie,[2] such substances may function in a protective manner towards barley amylase by maintaining the (1) extraction or (2) digestion medium in a state of comparative neutrality.

Whether this holds during the different phases of culture on nutrient substrata in the experiments to be described presently, or whether they serve mainly as nutritive substances and partly by stimulating secretory activity, lies beyond the actual scope of this inquiry.

The fact that in general enhanced secretion of amylase by the embryo follows on their use warrants their employment.

The results given in the following table may be taken as typical examples of possession by the embryo of this secretory capacity, of its variation with different concentrations of asparagin, and of its progressive increase with prolongation of the culture period.

TABLE I.

Cultures of Embryos on Asparagin-Mineral Salt Medium.

Concentration of asparagin per 100 *c.c. of solution.*	*Duration of culture.*	*Digestion period.*	*Amylase in culture medium per* 20 *embryos (equivalent to mg. of Cu .*
		(African barley.)	
Experiment 1.	Seeds 48 hours absolute alcohol, 48 hours water.		
1·65	4 days	2½ hours	20·1 mg.
1·10	,,	,,	16·1
0·55	,,	,,	30·5
0·055	,,	,,	32·2
		(African barley.)	
Experiment 2.	Seeds 48 hours $CuSO_4$.		
1·65	8 days	2½ hours	50·9
1·10	,,	,,	31·9
0·55	,,	,,	75·7
0·055	,,	,,	95·9
		(African barley.)	
Experiment 3.	Seeds 48 hours $CuSO_4$, 24 hours water.		
0·55 (asparagin only)	8 days	20 hours	5·7

The results show clearly, as already demonstrated by the work of Brown and Morris, that the barley embryo undoubtedly possesses the power of secreting amylase.

[1] Journ. Soc. Chem. Ind., 1904, xxiii. 414; Journ. Chem. Soc., 1906, lxxxix. 76.
[2] Journ. Inst. Brewing, 1908, xiv, i. 61–87.

An obvious increase in the amount of enzyme secreted with prolongation of the culture periods is evident, and the optimal output of amylase evidently does not coincide with the higher concentrations of asparagin, but lies between the limits comprised in the 0·55 and 0·055 concentrations, if not beyond this latter.

When asparagin only is used, as shown by the results of Experiment 3, the secretory activity of the embryo falls enormously, and in comparison with Experiments 1 and 2 the result points to the probable influence exercised by the mineral salts or by certain of them under the particular conditions selected.

The results of experiments with embryos on mineral salt medium with and without asparagin, comprised in the following table, lend support to this suggestion.

TABLE II.

Contemporaneous Cultures of Embryos on Mineral Salt Medium, with and without Asparagin.

Composition of culture medium.	*Duration of culture.*	*Digestion period.*	*Amylase per 20 embryos in medium, equivalent to mg. of Cu.*
Experiment 1. Seeds 48 hours 10 % copper sulphate.			
0·55 % asparagin-mineral salts.	4 days	2½ hours	61·7 mg.
Mineral salts only.	,,	,,	16·0
Experiment 2. Seeds 48 hours 10 % copper sulphate.			
5 % gelatine, 0·55 % asparagin-mineral salts	7 days	2½ hours	172·7 mg.
5 % gelatine, mineral salts only.	,,	,,	114·7

These experimental results indicate that the mixture of asparagin and mineral salts influences the secretory function more favourably than either alone.

This influence is, however, only apparently shown in Experiment 1. In order to prevent misapprehension it must be stated that the gelatine medium employed in Experiment 2, by affording mechanical support to the plantlets, eliminates a disturbing effect which is liable to ensue in Experiment 1, namely, partial submersion of the embryo, which at once interferes with the exercise of its normal functional activities as a whole.

The general conclusion, therefore, to be derived from these experiments is that the secretion of amylase by the embryo is principally influenced in some way by the presence of mineral salts, and that that induced by the inclusion of asparagin in the culture substratum is adjunctive.

The following experiment throws some light on the question as to whether mineral salts or certain of them stimulate the elaboration and excretion of amylase by the embryo; that is to say, whether their influence is to be regarded as physiological or otherwise.

We have seen that the secretory activity of the embryo cultivated upon a 0·55 % solution of asparagin is minimal.

If we take such culture solutions upon which embryos have been growing for some days and digest them separately with (1) soluble starch alone, or with (2) soluble starch containing mineral salts in the concentrations in which they occur initially in the small quantity (10 c.c.) of culture medium employed for each plate culture, the results should indicate whether the influence of these salts takes place externally, i. e. merely protects the amylase secreted during the culture period, or whether they influence the general metabolism of the plant and, consequently, its secretory activity.

The following experimental results, taken in conjunction with those of Tables I and II, favour the latter alternative, namely, that the influence they exercise is mainly physiological.[1]

TABLE III.

Digestions of Culture Medium with Soluble Starch in the Presence and Absence of Mineral Salts.

	Digestion period.	*Composition of digestion mixture.*	*Amylase per 20 objects in culture medium (equivalent to mg. of Cu).*
1.	20 hours	Starch only	6·0
2.	,,	Starch and mineral salts	4·0

The culture liquids investigated were derived from two cultures of 40 embryos each, cultivated on 0·055 % asparagin for seven days.

It is obvious from the above results, by comparison with Experiment 3, Table I, that the mineral salts used in the experiments previously described enter in some way into the general metabolism of the embryo, and thereby influence its secretory functions, thus negativing the suggestion that under the conditions of culture employed they—or certain of them, notably the phosphate—function as protective agents to the enzyme excreted into culture medium. It is not denied that they do not function in the manner indicated during certain phases of the culture experiment, but it is suggested that they principally influence secretion by entering into or influencing the general metabolic activities of the plantlet.

In order to demonstrate further the possession of an amylo-secretory function by the embryo, cultures of these objects were established on a medium consisting of 5 % gelatine and finely ground barley endosperm

[1] H. M. Richards: Ann. Bot., xxi, 1907, p. 501.

substance; the latter being effectively sterilized and the pre-existent amylase completely annihilated (as controlled by inoculation and digestion experiments respectively) by heating in a Koch's steam sterilizer for two hours at 100° C.

In order to avoid gelatinization of the starch grains, the finely ground sterilized endospermic substance was thoroughly mixed with the cooled, still mobile gelatine prior to the transference of the mixture to the Petri culture dish.

The results are embodied in the following table:—

TABLE IV.

Cultures of Embryos on 5 % Gelatine-Barley Endosperm Substance.

Seeds (African barley) 48 hours $CuSO_4$, 48 hours water.

Exp.	*Duration of culture.*	*Digestion period.*	*Amylase per 20 objects in medium (equivalent to mg. of Cu).*
1.	6 days	1 hour	80·5
2.	8 ,,	,,	96·7

As already evidenced (Table I), the secretory capacity of the embryo augments as duration of culture period is extended.

The following typical experiments afford further evidence of this feature:—

TABLE V.

Cultures of Embryos on 0·55 % Asparagin-Mineral Salt Medium.

Seeds 48 hours 10 % $CuSO_4$, 1 day under aseptic germination conditions.

Exp.	*Objects.*	*Duration of culture.*	*Digestion period.*	*Amylase per 20 objects in medium (equivalent to mg. of Cu).*	*Relative augmentation of secretory capacity, 24 hours.*
1.	40 embryos	2 days	2 hours	11	
2.	,,	3 ,,	1 hour	10	
3.	,,	4 ,,	,,	69	60 mg.
4.	,,	6 ,,	,,	119	25 ,,
5.	,,	8 ,,	,,	150	14 ,,

There is an obvious progressive augmentation of the secretory capacity of the embryo accompanying extension of the period of culture.

From the results afforded by these experiments under the conditions selected, it may reasonably be assumed (in the absence of direct experimental investigation) that they are really the net result of two series of factors which are opposed to each other. On the one hand, production of enzyme by the embryo; on the other, destruction or inactivation of enzyme by the operation of factors in and external to the medium, such as changes

in composition of the medium and oxidation by atmospheric oxygen. The relative extent to which these two series of opposed factors operate varies with the particular phase of the culture experiment considered. Presumably it is most marked during the period 0–3 days; from this period onward the relative augmentation of the secretory capacity is well maintained.

Although in the majority of instances, especially in gelatine media, the embryo exhibits good growth accompanied by considerable increase in its root and plumular development, there is reason to believe that under these conditions development is by no means strictly normal. In particular is this reflected on its amyloclastic secretory capacity; the results given in the foregoing tables distinctly under-estimate the potentiality of its secretory functions. The values of the secretory capacity of the embryo are of practically the same order as in the preceding experiments on purely artificial media.

The preliminary treatment to which the barley endosperm substance was subjected probably induces changes in it of both a physical and a chemical nature which render it less readily attackable by the embryo.

The experiment furnishes an example of the difficulty of preparing a culture substratum which shall serve as an adequate substitute for the natural inner endosperm.

The positive nature of the evidence adduced furnishes direct proof that the barley embryo secretes an amyloclastic enzyme. Initially the embryo, as stated by Brown and Morris (loc. cit., p. 492) and confirmed by direct examination by myself, contains little (not more than 1–2 mg. per 20 objects) or no pre-existent amylase at the moment of removal from the seed at the termination of the steeping operation carried out under the conditions described. The amounts of enzyme found in the medium at the termination of the cultural experiment therefore represent the magnitude of its varying secretory capacity under the conditions of experiment selected.

IV. Amyloclastic Secretory Capacity of the Aleurone Layer of Barley.

The earlier researches of Brown and Morris (l. c.) indicate that these authors regarded the aleurone layer not as a glandular tissue, but as functioning in some serviceable way in the general economy of the seed during the later stages of the germinative process.

It was shown by Haberlandt (l. c.) that the aleurone layer of rye possesses amyloclastic secretory powers, and it was suggested by him that the possession of this capacity was probably a common attribute of the aleurone layer in other members of the Grasses.

Hansteen and Puriewitsch (l. c.) contend that the aleurone layer does not constitute a differentiated glandular tissue, and that the secretory capacity of the aleurone layer cells is not, unit for unit, superior to that possessed by those of the subjacent amyliferous tissue.

In other words, they regard the aleurone layer as forming an undifferentiated portion of the reserve storage system of the seed.

Re-investigation of the work of Brown and Morris by Brown and Escombe led the latter authors to consider that the aleurone layer of barley represents a secretory tissue.

The assertion that the aleurone layer consists of units which are distinctly living receives general acceptance. In the barley grain it consists of a triple layer of cubical cells with highly cuticularized cell-walls (probably the thickest and most resistant in the entire seed) which form an almost complete envelope to the inner endosperm.

Each cell possesses a conspicuous nucleus, centrally disposed, and a cytoplasmic network which, prior to the inauguration of the germinative process, is densely crowded with, for the most part, large and conspicuous protein grains—the so-called aleurone grains.

Cytological investigation shows that the aleurone layer cells closely parallel the columnar epithelial cells in many important respects. Fixed, embedded, stained microtomed material from seeds in various stages of germination presents features which bear close similarity to those described for the columnar cells of the scutellum.

In particular, the nuclear and cytoplasmic changes and those undergone by the granular contents of the cytoplasm not only closely resemble but synchronize with these same general phenomena in the epithelial cells.

Were judgement by analogy strictly permissible, the deduction might at once be made that, although morphologically distinct, these cells are similar in function to those of the columnar epithelium.

It is noteworthy that hitherto experiments destined to demonstrate the possible possession of amylolytic secretory powers by the isolated aleurone layer have been entirely qualitative.

Investigation of the enzymatic secretory capacity of a given tissue which confines itself to the determination of the capacity which that tissue (such as the aleurone layer) may possess of augmenting its enzyme content intracellularly is necessarily incomplete because it takes no account of the quantity of enzyme excreted, and is therefore inadequate as a criterion of its total secretory powers.

Throughout this inquiry the treatment of the subject has been as far as possible distinctly quantitative, and the apparent non-recognition of the desirability of quantitative investigation has led to misapprehension regarding (1) the secretion of amylase, (2) its sources of origin in the germinating seed, and (3) the general trend of endospermic depletion.

As in the case of the embryo, before it is possible to evaluate its secretory capacity it is necessary to determine the initial amylase content of the aleurone layer directly after its removal from the steeped seed. The results of such a determination are given in the following table:—

TABLE VI.

Initial Amylase Content of Aleurone Layer of Steeped Seed.

Chilian barley. Seeds 48 hours absolute alcohol, 48 hours water.

Experiment.	*Amylase per 20 aleurone layers (equivalent to mg. of Cu).*
1. 2·5 aleurone layers	506
2. ,, ,,	675
3. ,, ,,	675

Typical examples of the secretory capacity of the aleurone layer are given in the following tabulations:—

TABLE VII.

Secretion of Amylase by Aleurone Layer.

Cultures of dorsal aleurone layers (African barley) on liquid and on solid substrata. Seeds 24 hours 10 % $CuSO_4$.

Exp.	*No. of objects on culture medium.*	*Duration of culture period.*	*Medium.*	*Amylase per 20 objects in culture medium (equivalent to mg. of Cu).*
1.	20 dorsal aleurone layers	2 days	0·55 % asparagin solution	365
2.	,, ,, ,,	3 ,,	,, ,, ,,	362
3.	,, ,, ,,	4 ,,	,, ,, ,,	414
4.	,, ,, ,,	6 ,,	,, ,, ,,	269
5.	,, ,, ,,	7 ,,	10 % gel., 0·55 % asparagin-M.S.	852
6.	,, ,, ,,	7 ,,	10 % gel.-M.S.	552

The above experiments, it is to be noted, were carried out with material prepared from seeds sterilized by steeping in copper sulphate solution, as will be shown later; there are certain objections to the use of this reagent in the case of aleurone layers, endosperms, and inner endosperms.

The superior results in Exp. 5 and 6 are not wholly due to prolongation of the culture period, but to substitution of a solid for a liquid substratum. In the latter circumstances the aleurone layer fragments are apt to be drowned, and this seriously interferes with their secretory functions.

The results comprised in the following table (VIII) may be taken as typical examples of the amyloclastic secretory powers of isolated aleurone layers prepared from alcohol-water steeped seeds.

TABLE VIII.

Secretion of Amylase by the Aleurone Layer (Chilian Barley).

Cultures of isolated aleurone layer on 5 % gelatine subs. Seeds 48 hours absolute alcohol, 48 hours water (aerated).

Exp.	*No. of objects on culture medium.*	*Culture period.*	*Amylase per 20 objects (equivalent to mg. of Cu) in:—* *Medium.*	*Objects.*	*Total.*	*Augmentation of amylase.*
1.	5 aleurone layers	4 days	2222	396	2618	1943
2.	,, ,,	6 ,,	2172	198	2370	1675
3.	,, ,,	7 ,,	1428	634	2062	1387
	Amylase initially present in 20 aleurone layers . .				675	

The augmentation of amylase observed in these and similarly conducted experiments depends principally on the substitution of absolute alcohol for $CuSO_4$ in the sterilization of the seeds, and also on the employment of a semi-solid medium which affords the fragments adequate mechanical support and provides for their aeration.

The examples given afford sufficient justification for regarding the aleurone layer as a glandular tissue, one of the functions of which is the secretion of an amylolytic enzyme, and they further furnish evidence that the amylolytic capacity of the aleurone layer, *as suggested by Brown and Escombe* (l. c.), is greatly superior to that of the embryo.

In order to show definitely that passage of amylolytic enzyme from the objects cultivated (embryos and aleurone layers) under the conditions described actually represents a secretory act, and not a mere passive straining out of enzyme pre-existent in the tissues of the embryo and aleurone layer, the following experiments were instituted :—

Embryos and dorsal aleurone layers were removed from steeped seeds and anaesthetized by immersion for forty-eight hours, at laboratory temperature, in a saturated aqueous solution of either chloroform or nitrobenzene contained in closed vessels. They were then cultivated on 0·55 % asparagin-mineral salt solution for seven days under the usual conditions. Examination of the culture media at the termination of the experiments afforded the following data :—

TABLE IX.

Culture of Anaesthetized Embryos and Aleurone Layers.

Seeds (African barley) 24 hours 10 % $CuSO_4$, 24 hours water.

Exp.	*Objects.*	*Anaesthetic reagent.*	*Digestion period.*	*Amylase in culture medium per 20 objects.*
1.	40 embryos	Chloroform	20 hours	2 mg.
2.	,,	Nitrobenzene	,,	4 ,,
3.	20 dorsal aleurone layers	Chloroform	1 hour	42 ,,
4.	,, ,, ,,	Nitrobenzene	,,	130 ,,

The embryos exhibited no signs of growth. These results, on the one hand, show that the treatment described is fatal alike to the vitality and secretory functions of the embryo, and inferentially, and by comparison with results of experiments (Tables I, II, &c.), the same deduction may be drawn from the results furnished by the aleurone layer experiments.

The anaesthetic reagents employed at first suspend and then completely annihilate the secretory mechanism of both the embryo and aleurone layer.

One probable reason for the comparatively small reduction of the secretory capacity of the aleurone layer is the difficulty offered to rapid penetration of the anaesthetic reagents through or across the highly cuticularized walls of the cells of this tissue.[1]

That this is probable is shown by the fact that when immersion of aleurone layer fragments in aqueous solutions of these reagents was restricted to twenty-four hours the reduction in the amount of amylase found in the *culture medium* was still *less* than in the foregoing experiments.[2]

It is equally probable that these agents in small quantity may stimulate secretion during the earlier stages of anaesthesia.

The results are also of interest because they serve to explain certain anomalies in the work of Brown and Morris,[3] dealing with the alleged non-glandular nature of the aleurone layer.

The statement is made that, in contrast to the embryo, fragments of the aleurone layer, after treatment with chloroform vapour, retain their diastatic powers quite unimpaired. Such fragments placed upon starch grains attacked these quite as quickly and completely as non-chloroformed fragments; that is to say, treatment which was found to be fatal to the embryo and completely arrested its amyloclastic functions, failed to do so in the case of fragments of the aleurone layer. Both tissues are regarded by the authors as possessing vitality, yet in one case (embryos) arrest of vitality means arrest of the secretory mechanism, in the other (aleurone layers) arrest of vitality signifies apparent non-arrest of the secretory function. The anomaly is evident, and the qualitative method of investigation used by the authors is largely responsible for the interpretation placed upon the phenomenon observed, as the foregoing experiments and those which follow show that treatment with chloroform is fatal to the life of both the embryo and aleurone layer. The embryo excised from the steeped seed contains little, if any, preformed amylase, and consequently when it or sections of the scutellum are placed on moistened starch grains no action occurs. Fragments of aleurone layer taken from the steeped or germinating seed invariably contain a considerable quantity of preformed enzyme, and

[1] Stoward, Ann. Bot., xxii, 1908, pp. 87, 442.

[2] The probable meaning of this is that with shorter steeping *less* amylase diffuses from the fragments of killed tissue into the steeping medium, and hence *more* enzyme is found subsequently in the culture medium.

[3] Journ. Chem. Soc., lvii, 1890, p. 525.

this diffusing from the chloroformed and killed tissue attacks the starch grains placed at its disposal.

The evidence adduced by Brown and Morris therefore is valueless as a proof of the non-possession of an amyloclastic secretory capacity by the aleurone layer.

The experimental data given in the following table furnish additional evidence on this important point.

TABLE X.

Cultures of Aleurone Layers on 5 % Gelatine-Mineral Salts in the Presence of (1) Nitrobenzene, (2) Chloroform.

Chilian barley: seeds steeped successively in absolute alcohol and water; 48 hours in each reagent. Culture period, 7 days.

Exp.	*Objects.*	*Anaesthetic reagent.*	*Amylase per 20 objects per hour (equivalent to mg. of Cu) in:—*		
			Medium.	*Objects.*	*Total.*
1.	10 aleurone layers	Chloroform[1]	1160	105	1265
2.	,, ,,	Nitrobenzene	1202	85	1287
	Amylase initially present in 20 aleurone layers . . .				675

When fragments of aleurone layer are removed from steeped seeds (loss of amylase during steeping is thereby eliminated), and at once placed on the surface of gelatine media with which are mingled copious amounts of chloroform or nitrobenzene, there are found, just as in experiments with similarly prepared and similar fragments of aleurone layers, considerable quantities of amylase in both medium and objects. Apparently, then, the fragments of aleurone layer are either non-living, or if living, these antiseptics behave in an anomalous manner.

Neither alternative, however, is correct. Under the conditions selected, although secretion of amylase apparently takes place, it occurs prior to the complete annihilation of the secretory mechanism. The amylase found *quantitatively in medium and objects on comparison with that found in experiments* on gelatine media without the addition of an antiseptic (compare experiments, Table VIII) is observed to have *undergone marked reduction in amount.* As a result of the influence of the antiseptic the augmentative capacity of the tissue falls. The amounts of amylase found represent the amounts of amylase pre-existent in and secreted by the tissue prior to the complete cessation of the activities of the secretory mechanism. The comparatively large amounts of enzyme are probably due to the fact that the action of the antiseptic is not abrupt because of the resistance to its rapid entry offered by the highly cuticularized cell-walls of this tissue.

[1] Chloroform renewed daily; both cultures remained absolutely sterile throughout the course of the experiment.

It is to be noted in these experiments that *at least two* different anaesthetic agents have been employed.

In short, the action of these agents on the embryo is signalized by the complete and rapid arrest of its secretory functions, minimal amounts only of enzyme being pre-existent in its scutellar tissues, and consequently only minimal amounts of enzyme are found in the culture medium. On the other hand, the inhibitory and fatal action of the same agents on the aleurone layer is marked by comparative slowness, the secretory function being more tardily impaired. Although considerable amounts of amylase are thus accumulated by the anaesthetized aleurone layer, its augmentative capacity, *as compared with similar objects not subjected to the influence of such agents, suffers marked reduction.*

This reduction of amyloclastic enzyme, as the result of the interference with and arrest of the secretory mechanism of a *living* tissue, is the kind of behaviour we should anticipate in the case of the aleurone layer. Complete and prompt arrest of its secretory powers (as in the case of the embryo as observed by Brown and Morris) could not occur; the *apparent* secretion of enzyme by the aleurone layer under complete anaesthesia, described in this section, really represents for the most part the passive diffusion of the accumulation of pre-existent enzyme[1] in its cells—a fact to be elicited only by the employment of quantitative methods of inquiry.

The augmentative capacity of the aleurone layer under anaesthetic conditions of culture suffers marked reduction, and this is taken to signify that the aleurone layer is a living tissue—a fact fully supported by other considerations.

Had its augmentative capacity been equal to or greater than that exhibited by similar aleurone layer fragments cultivated on similar media without the addition of antiseptics recognized as agents fatal to living protoplasm, the claim that this tissue possesses vitality would be rendered extremely difficult to maintain. The inner endosperm, as we shall see (Section IV A, pp. 824–37), does actually exercise its augmentative capacity as rapidly and to the same extent in the presence or absence of antiseptics.

The argument may be advanced that the method of treatment to which the isolated aleurone layers were subjected does not preclude the possibility of loss of amylase by the enzyme diffusing from the fragments of tissue into the steeping medium. The fact remains, however, that neither the aleurone layer nor the embryo after such preliminary treatment any longer possesses the power of exercising its secretory powers to the same extent as non-anaesthetized objects.

The following experiments will serve to show that in the foregoing experiments with anaesthetized aleurone layers (Tables IX and X) the

[1] The chloroformed embryos examined by Brown and Morris were probably taken from seeds immediately after steeping; at this stage they would contain little or no amylase. If taken at later stages from germinating seeds, the accumulated amylase would probably diffuse out and action upon barley starch would have been demonstrable, and unless quantitative examination were resorted to confusion similar to that instanced in the case of the aleurone layer would have arisen.

amylase found in the culture medium really represents that present initially in the tissue at the moment of the removal of objects from the anaesthetic steep solution.

Three series of ten dorsal aleurone layers each, removed from seeds steeped for forty-eight hours in water, were prepared. Of them, one was dried at 30° for forty-eight hours, and the other two were steeped for the same period in a saturated aqueous solution of either chloroform or nitrobenzene contained in closed vessels. The amounts of amylase were then determined in the steep media and also in the steeped and unsteeped objects after short desiccation at 30° C.

The results are given in the following table:—

TABLE XI.

Loss of Amylase by Isolated Dorsal Aleurone Layer during Steeping in Anaesthetic Solutions.

Exp.	*Objects.*	*Anaesthetic steep solution.*	*Duration of steep.*	*Amylase per 20 objects (equivalent to mg. of Cu) in:—* *Steep medium.*	*Objects.*
1.	10 dorsal layers	5 c.c. sat. aq. chloroform	48 hours	258·5	126·6
2.	,, ,,	5 c.c. sat. aq. nitrobenzene	,,	237·0	85·0
3.	,, ,,	Desiccated at 30° C.	,,	—	211·0

The general conclusions to be derived from these experiments with the embryo and aleurone layer may be briefly summarized:—

Both the embryo and aleurone layer possess an amyloclastic secretory capacity.

The magnitude of the aleurone layer capacity is considerably greater than that of the similar capacity of the embryo.

Prolonged action of saturated aqueous solutions of chloroform or nitrobenzene annihilates both the vitality and secretory functions of the embryo and aleurone layer. The amylase found in the culture liquids upon which anaesthetized objects have been placed represents merely the enzyme pre-existent in these objects at the commencement of the culture experiments.

The secretion of amylase by the embryo and aleurone layer is notably influenced (1) by the inclusion in the culture substratum of certain mineral salts, the action of which appears to be chiefly physiological; and (2) by physical conditions, notably by substitution of a semi-solid for a liquid medium.

IV A. The Relative Amyloclastic Secretory and Self-depletive Capacities of the Endosperm and the Inner Endosperm of Barley.

The endosperm of barley constitutes the great mass of the resting mature seed and consists of an inner portion—the inner endosperm—composed of large irregularly isodiametric cells, in each of which there

is a much attenuated network of cytoplasm, the meshes of which are densely crowded, principally with variously sized starch grains, and among the other far less abundant inclusions are those of a protein nature.

Immediately external to the amyliferous cells is the aleurone layer, forming as already stated, with the exception of that portion of the inner endosperm which abuts on the scutellum, an investing envelope to the entire inner endosperm. External to the aleurone layer is the spermoderm comprising the different external integuments of the seed.

The principal point of interest in the present inquiry refers to the question as to whether or not the inner endosperm, independently of the amyloclastic secretions of the embryo and aleurone layer, possesses a capacity for self-depletion, and if so, in what degree transformation of the starch storage reserves is induced solely by the pre-existent amylase of the resting seed.

The conclusions of the different investigators who have attacked this aspect of the question are marked by their extreme divergency.

Brown and Morris (loc. cit. p. 482) state that the inner endosperm consists of a mass of non-living cellular units which are unable to induce self-digestion of their starch contents.

Hansteen (loc. cit.) and also Puriewitsch (loc. cit.) contend that the endosperm of barley is self-digesting and that each cell of the inner endosperm is a living functionary unit.

The claim of vitality for the inner endosperm rests almost entirely on the demonstration of enzyme action in this tissue, this constituting a criterion which, as already stated, is no longer regarded as valid in determining whether a tissue possesses vitality or otherwise. Although Puriewitsch's view is opposed to Haberlandt's conclusion that the aleurone layer possesses a secretory function, yet many of his experimental results really suggest and support the correctness of Haberlandt's contention.

Brown and Escombe,[1] as a result of a reinvestigation of the work of Brown and Morris (loc. cit.), modify the views of the latter authors materially.

The statement by Brown and Morris that the accumulation of amylase which occurs in the inner endosperm during the germinative process is largely due to the secretory activity of the embryo they amend by conceding that the contributory share in the sum total of the amylase found in the endosperm furnished by the embryo was, no doubt, considerably over-estimated. On the other hand, they substantiate Haberlandt's suggestion that the aleurone layer possesses glandular functions and claim that it plays a much greater rôle in endospermic depletion than had hitherto been assigned to it.

Experiments devised to ascertain if the isolated inner endosperm

[1] Proc. Roy. Soc., lxiii, 1898, p. 3.

was capable of inducing self-digestion of its starch contents afforded negative results only, and the authors adhere to the opinion of Brown and Morris that this tissue possesses neither vitality nor a self-digestive capacity.

Brown and Morris recognize the existence of two amyloclastic enzymes, the one, the more active amylase designated 'secretion' amylase, the other, a feebler type which they regard as being similar to, if not identical with, the 'translocation' variety generally met with in plant tissues.

The former readily erodes starch-grains and liquefies and saccharifies starch-paste; the latter dissolves, but not by erosion, solid starch-grains and liquefies gelatinized starch much less readily than does the 'secretion' variety, which the authors contend is principally responsible for the dissolution and digestion of the *solid* starch reserves of the endosperm.

The amylase of ungerminated barley, according to Brown and Morris, is an unused residue of the 'translocation' variety and probably owes its origin to the action of an acid produced by the developing embryo upon a zymogen in the endosperm cells, and in their opinion plays a subordinate rôle only in endospermic depletion.

It is to this enzyme and its augmentation that the work of Ford and Guthrie refers specially; extracts of finely ground ungerminated barley prepared under certain conditions, i. e. the auto- and papain pre-digestion methods of these authors, exhibit higher amylolytic activity than extracts prepared by the customary short method of extraction. The general conclusion derived from their investigation appears to be that the results obtained are due, not to an increase of amylolytic activity, but to an augmentation of the enzyme itself, and the whole question implied, though not specifically raised, is whether the pre-existent enzyme of the resting grain does not contribute, in greater measure than was supposed by Brown and Morris, to the dissolution and depletion of the starch reserves of the inner endosperm.

The experimental work described in this section, therefore, represents an attempt to ascertain whether the amylase, which is pre-existent in the inner endosperm (and which augments under the varying conditions of experiment selected), is capable of inducing the depletion of its starch storage materials.

Perhaps no portion of the subject presents such difficulties of attack as the investigation of the relative amyloclastic secretory and self-depletive capacities of the endosperm and inner endosperm of the barley seed.

First, the difficulty encountered in the preparation of sterile material is greatly enhanced by the fact that the employment of copper sulphate as an antiseptic steep-reagent is inadmissible because of its tendency to adhere in small traces to these objects even after subsequent steeping in water for some time, and therefore the use of absolute alcohol, which is admittedly a less powerful agent, has to be resorted to.

Secondly, complexity is introduced into any attempt at solution of the question by the fact that the isolated endosperm (just as occurs when similarly prepared objects are investigated by the auto-digestion method) is capable of autonomously augmenting its amylase content. In comparable experiments with isolated endosperms, therefore, the augmentation of enzyme which invariably takes place cannot be wholly assigned to the secretory and excretory activity of the aleurone layer.

Thirdly, the induction and maintenance of endospermic depletion processes, either in the *absence* or *presence* of the aleurone layer, demand the provision of conditions which must at least parallel in some measure those provided by the embryo, namely, ready means for the outward diffusion of the mobilized products of amyloclastic activity.[1]

Culture Experiments with Endosperms and Inner Endosperms in 5% Gelatine with and without Mineral Salts.

In the various experiments described in this section the sterilization, preparation of material, establishment and maintenance and subsequent examination of the culture medium and objects, &c., were carried out in the manner previously described.

In the following table are shown typical examples of the relative augmentation capacities of endosperms and inner endosperms :—

TABLE XII.

Cultures of Endosperms and Inner Endosperms on 5% Gelatine with and without Mineral Salts.

Chilian barley. Culture period, 11 days. Seeds 48 hours absolute alcohol, 48 hours water.

Exp.	*No. of objects on plate.*	*Medium.*	*Amylase per 20 objects (equivalent to mg. of Cu.) in :—*		
			Medium.	*Objects.*	*Total.*
1.	10 endosperms (whole)	Gelatine	2474	1190	3664
2.	„ „	Gelatine-M.S.	2634	1349	3980
3.	10 endosperms (halved)[2]	Gelatine	2914	1349	4236
4.	10 inner endosperms (halved)[2]	Gelatine-M.S.	1508	396	1904
5.	„ „ „ [2]	„	1239	396	1635
	Amylase present initially in 20 endosperms				1181
	„ „ „ 20 inner endosperms				844

We may for the present confine our attention solely to the relative capacities these two classes of objects, differing only by removal of the

[1] The important influence which the embryo has on endospermic depletion during the progress of the germinative processes can be more readily realized by the morphological changes these cells undergo. Their elongation lengthwise and partial separation laterally increase their absorptive capacity very considerably.

Similar changes occur in the secretory epithelial cells of tea, as shown by Reed (Ann. Bot., xviii, 1904, p. 267). In addition deep glands are conspicuous features in the scutellum of tea, described by Misses Sargant and Robertson (Ann. Bot., xix, 1905, p. 115) and wholly confirmed by the writer's observations.

[2] Objects halved longitudinally to facilitate diffusion processes.

aleurone layer, possess of augmenting their amylase content. The salient point of difference to be noted is that under precisely parallel external conditions and quite apart from any possible differences in the attributes of the amylase from the two available sources (aleurone layer and inner endosperm), the amounts of enzyme which have *diffused* from the objects into the culture medium and naturally the total amounts of enzyme in objects and medium in Experiments 1, 2, 3 (endosperms) are decidedly superior to those in Experiments 4, 5 (inner endosperms).[1]

If certain provisional assumptions are made, certain tentative evaluations of the relative augmentative capacities of the endosperm and inner endosperm under the selected conditions of experiment may be formed. These are that (1) destruction of enzyme falls equally on the enzyme from either source ; (2) both enzymes have similar properties or attributes ; (3) in both series of experiments the inner endosperm comports itself in precisely the same manner as regards its enzyme augmentative capacity, i.e. the presence of the aleurone layer does not influence the augmentative capacity of the inner endosperm ; in short, the inner endosperm retains its individuality. Then, from the data given in Table XII, an approximate calculation may be hazarded of the relative augmentative capacities of the endosperm, inner endosperm, and of the aleurone layer.

The total augmentation of enzyme in each experiment is readily arrived at by deducting the initial amylase content from the total amylase content. Thus in Experiments 1 and 4 we have:—

Exp. 1. $3664 - 1181 = 2483$ mg.

Exp. 4. $1904 - 844 = 1060$ mg.

The relative contributory shares of the aleurone layer and inner endosperm may be computed by deducting the augmentation of enzyme in the inner endosperm from the augmentation in the endosperm. Thus in Experiments 1 and 4 we have :—

$$2483 - 1060 = 1423 \text{ mg.}$$

For the convenience of survey these results are given in tabulated form.

[1] These experiments afford clear evidence of the diffusibility of one colloidal substance into another (see Brown and Morris, Chemistry and Physiology of Foliage Leaves, Journ. Chem. Soc., lxiii, 1893, p. 657). Diffusion of enzyme across the aleurone layer cell-walls and spermoderm probably only takes place to a very limited extent. In Experiments 1 and 2 (endosperms), therefore, the enzyme must pass principally if not wholly via the 'germ bed' or exposed proximal end of the amyliferous tissue. Although the conditions in Experiments 4 and 5 (inner endosperms) for outward diffusion of enzyme apparently are greatly improved by removal of the aleurone layer, yet the amount of enzyme found in the medium is (as compared with Experiments 1, 2, 3) considerably less. The result is hardly that which anticipation would lead one to expect, if the units of the inner endosperm and aleurone layer possessed equal capacities for augmenting their content of amyloclastic enzyme.

Apart from the provision of external conditions favourable to outward diffusion, however, as we shall see later, the presence of a cytoclastic enzyme in the endosperm modifies certain internal conditions which probably favour diffusion. Removal of the aleurone layer means elimination of one of the principal sources of this cytoclastic enzyme.

TABLE XIII.

Relative Amylase Augmentative Capacities of Endosperms and Inner Endosperms.

Exp.		*Total augmentation of amylase.* (*equivalent to mg. of Cu.*)	*Relative augmentative capacities of:—* *Aleurone layer.*	*Inner endosperms.*
1.	Endosperms	2483	1423	1060
2.	,,	2802	1742	1060
3.	,,	3082	2022	1060
4.	Inner endosperms	1060	—	1060
5.	,, ,,	791	—	791

The tentative values given in the foregoing table show that the contribution of amylolytic enzyme derived from the secretory activity of the aleurone layer forms no mean proportion of the total augmentation of amylase found in the isolated endosperm under the conditions of experiment described.

In order further to investigate the augmentative capacity possessed by the inner endosperm, and for the purpose of comparison with similar objects under other experimental conditions, a further series of experiments was instituted with inner endosperms.

In this series of experiments the copper-reducing powers of the control digestions of the culture medium and object were specially noted, and careful macroscopical and microscopical examination was made of the condition of the starch contents taken from various parts of the objects under culture at the termination of their sojourn on the plates. The object was to ascertain whether any marked evidence suggestive of enzymatic action, the presence of reducing sugars, evidence of the corrosion or dissolution of starch grains, or the friability of the objects was demonstrable in the inner endosperm contents.

In the following table are grouped the results of the separate digestions of the culture medium and objects with soluble starch:—

TABLE XIV.

Cultures of Inner Endosperms on 5 % Gelatine-Mineral Salts.

Chilian barley. Culture period, 13 days. Seeds absolute alcohol 48 hours, water 48 hours.

Exp.	*Objects on plate.*	*Amylase per 20 objects* (*equivalent to mg. of Cu*):— *Medium.*	*Objects.*	*Total.*	*Augmentation of amylase.*
1.	5 inner endosperms (halved)	1587	634	2221	1371
2.	,, ,, ,,	1031	396	1427	583
3.	,, ,, ,,	1507	634	2141	1297
4.	,, ,, ,,	2304	238	2542	1698
	Amylase present initially in 20 inner endosperms .			844	

In the objects themselves there were no reducing sugars in any of the experiments, showing that although augmentation of amylase had taken place it was unaccompanied by any demonstrable evidence of hydrolysis of the starch reserves.

Reducing sugars equivalent to 35 mg. maltose were found in Exp. 4 only, representing at best a very feeble amount of amylolytic action.

Macroscopically the objects were devoid of any outward indications of change; they had retained their intact form and mass, and microscopical examination of specimens of starch taken from different parts of an object failed to reveal any indication of action on the starch grains.

Thin sections of objects taken in various planes and freed from their starch contents by digestion at 30° C. with filtered, diluted saliva, and then examined microscopically, showed clearly that their cell-walls and cytoplasmic contents were quite intact.

After desiccation at 30° C. for twenty-four hours the objects were subjected to physical examination, with the result that *friability* of the inner endospermic substance, one of the usual and invariable accompaniments of recognizable change in the cell-walls of the amyliferous cells, was found to be entirely absent.

The results so far adduced, while they show that the inner endosperm is capable of generating and accumulating amylolytic enzyme in its tissue, negative the view that this enzyme very readily or vigorously acts upon the semi-solid barley starch at its disposal.

In the following table the data derived from similarly established comparative experiments with inner endosperms and endosperms are summarized, the only difference in the experimental conditions between these and the preceding experiments being the doubling of the concentration (indicated by the symbol P_2) of KH_2Po_4.

The objects were not macroscopically and microscopically examined, but for present purposes it is only necessary to direct attention to the quantities of reducing sugars in the culture media in the endosperm experiments of this series, since this criterion affords a far more accurate and critical means of forming an opinion of the relative amylolytic activities possessed by the amylases (whatever may be their other specific attributes) which originate in the inner endosperm and aleurone layer respectively, than do measurements of the capacities possessed by these tissues to increase their amyloclastic enzyme content.

TABLE XV.

Cultures of Endosperms and Inner Endosperms on 5 % Gelatine-Mineral Salts (P_2).[1]

Chilian barley. Culture period, 7 days. Seeds 72 hours absolute alcohol, 48 hours water.

Exp.	*Objects.*	*Amylase per 20 objects (equivalent to mg. of Cu) in:—*			*Reducing sugars per 20 in medium.*
		Medium.	*Objects.*	*Total.*	
1.	5 inner endosperms	1111	317	1428	0.00
2.	5 endosperms	2222	974	3196	159
3.	,,	2761	1349	4111	238

The amounts of reducing sugars found in the culture media in the case of the endosperm experiments, 2 and 3, afford convincing proof that when the aleurone layer is left intact, not only is there proof of augmentation of amylolytic enzyme by the endosperms, but there is also evidence of marked amylolytic action.

In Experiment 1, just as in the preceding experiments with similarly prepared objects, there is undoubtedly an increase of amylase, but there is an *entire absence of reducing sugars in demonstrable amount in the culture medium, over and beyond that initially present in the objects.*

The suggestion may be advanced that in the experiment under consideration the period of time to which the experiment was restricted may have been insufficient for the normal display of the presumed amylolytic function possessed by the inner endosperm.

The experimental data summarized in the following table fail to lend support to such a suggestion, as shown by the results of Experiment 3, with inner endosperms, given in the table below.

TABLE XVI.

Cultures of Endosperms and Inner Endosperms on 5 % Gelatine-Mineral Salts (P_2).

Chilian barley. Culture periods, 3 and 17 days. Seeds 48 hours absolute alcohol, 48 hours water.

Exp.	*Objects.*	*Duration of culture.*	*Amylase per 20 objects in:—*			*Reducing sugars in medium (equivalent to mg. of Cu).*
			Medium.	*Objects.*	*Total.*	
1.	5 endosperms (halved)	3 days	1070	1130	2200	0.00
2.	,, ,,	17 ,,	2202	634	2836	313
3.	5 inner endosperms (halved)	17 ,,	1309	317	1626	0.00

It may be noticed in passing that as a general *result of increased amylolytic activity during prolongation of the culture period, in these culture experiments a diminution of enzyme occurs.* That is, enzyme activity in

[1] KH_2PO_4 concentration doubled.

these conditions entails enzyme destruction; only by prolongation of the experiment can this feature be rendered apparent, because during earlier phases of the culture experiment enzyme elaboration probably overshadows the opposed processes of enzyme inactivation and destruction. During normal germination within the limits of the malting period the accumulation of amyloclastic and other enzymes must necessarily be greatly in excess of the demand in order to ensure finally a substantial amount of residual enzyme.

These experimental results afforded by cultures of inner endosperms and endosperms on gelatine mineral salt medium may be briefly summarized.

Both types of experimental objects possess the capacity of augmenting their amylase-content, *the capacity of the endosperm, by virtue of exercise of the secretory functions of the aleurone layer, being distinctly superior to that of the inner endosperm.*

These experiments therefore furnish indirect collateral confirmation of the results already afforded by independent investigation of the isolated aleurone layer.

The secretion of amylase in the endosperm culture experiments is, if the experiment is of sufficient duration, invariably accompanied by the appearance of reducing sugars in the culture medium in easily demonstrable amounts.

In the inner endosperm experiments on this medium, this phenomenon is either absent or barely demonstrable, the amount of amylolytic action being *minimal.*

Macroscopically and microscopically the two series of objects, i. e. endosperms and inner endosperms, at the termination of the culture experiment, present features which are in marked contrast to each other.

There is invariably, in the case of the endosperm, reduction in the mass of culture objects, marked erosion of starch grains, and considerable alteration in the cell-walls of the amyliferous cells, features which are entirely absent in the inner endosperm cultivated and examined under identical conditions. The mass of the inner endosperm in these latter experiments remains practically unchanged, and there is an entire absence of any visible or readily recognizable change in either the starch contents or the cell-walls of the storage tissue.

Since in the one case the aleurone layer remains, and in the other it is removed, and further since direct experiment demonstrates that the aleurone layer possesses the capacity of secreting an active variety of amylase, it is difficult to avoid the conclusion that the observed phenomena are primarily due to the amylase which arises as a product of the secretory activity of the aleurone layer cells.

Cultures of Endosperms and Inner Endosperms on Agar Media.

It appeared most desirable not to confine this important division of the inquiry to *one particular type of experimental substratum.*

Experiments were therefore instituted with endosperms and inner endosperms upon a 0·8 % agar medium, this percentage of agar being chosen as the result of several preliminary trials, because of the additional facilities such a medium might offer in the promotion of the complex diffusion processes which present themselves in the type of experiments under consideration.

In the following table are summarized the results of a typical experimental series :—

TABLE XVII.

Cultures of Endosperms and Inner Endosperms on 0·8 % Agar with and without Mineral Salts (P_2).

Chilian barley. Culture period, 19 days. Seeds absolute alcohol 72 hours, water 48 hours (2 changes).

Exp.	*Objects.*	*Medium.*	*Amylase per 20 in :— Medium.*	*Object.*	*Total.* (*equivalent to mg. of Cu.*)	*Reducing sugars per 20 in :— Medium.*	*Objects.*
1.	10 endosperms (halved)	Agar-M.S.	1358	1707	3065	465	0·00
2.	,, ,,	Agar only	776	1552	2328	465	0·00
3.	10 inner endosperms (halved)	,, ,,	1435	232	1667	174	0·00

Again the fact is evident that the augmentative capacity of the endosperm distinctly surpasses that of the inner endosperm. The results further bear evidence that the enzyme generated by the inner endosperm does possess a certain capacity for attacking its *amyliferous storage reserves*,[1] but this capacity is decidedly inferior to that exhibited by the endosperm, as the comparison of Experiments 1 and 2 with Experiment 3 very clearly indicates.

In these experiments examination of the two types of objects at the termination of the culture period reveals the same differential features as those presented by the gelatine cultures, and their significance does not require further detailed reference.

[1] If specimens of barley starch taken from various parts of the inner endosperm are examined microscopically, it is invariably found that intermingled with the large fully formed starch grains are a considerable number of very minute grains which stain blue on treatment with iodine, and therefore consist of starch. It is highly probable that these minute grains are more readily attacked than are the larger ones by the amylase of the inner endosperm. These minute grains are probably either of the nature of or very similar to 'transitory' starch.

Often repeated and searching microscopical examination of specimens of the starch contents from inner endosperms after removal from the culture medium in which reducing sugars were demonstrable, as in Experiment 3 above, fail to reveal any evidence of visible dissolution of the larger fully formed mature starch grains.

In the following series of experiments, Experiment 4 calls for special notice because it was designed to show, under conditions which are strictly comparable as regards external circumstances, the very different behaviour of the two halves of the endosperm under the following conditions :—

The dorsal portion of the aleurone layer was completely removed from each endosperm, and the latter was divided into two longitudinal halves by a plane traversing it midway between its dorsal and ventral halves ; on each plate, therefore, in Experiments 3 and 4 there are ten dorsal halves of endosperms deprived of their aleurone layers, and ten corresponding ventral halves with their aleurone layers still adherent.

The numerical data furnished by these experiments are embodied in the following table :—

TABLE XVIII.

Cultures of Endosperms on 0·8 % Agar-Mineral Salts (P_4).[1]

Chilian barley. Culture period, 19 days. Seeds 48 hours absolute alcohol, 48 hours water.

Exp.	*Objects.*	*Amylase per 20 in :—* *Medium.* (*equivalent to mg. of Cu.*)	*Objects.*	*Reducing sugars in medium* (*equivalent to mg. of Cu*).
1.	10 endosperms (whole)	0·00	620	378
2.	10 endosperms (halved)	0·00	698	543
3.	10 endosperms deprived of dorsal aleurone layers	0·00	124	494
4.	10 endosperms, halved, and dorsal halves deprived of their aleurone layers	0·00	232	378

Just as in the experiments of Table XVII, there is a considerable amount of reducing sugar present in the culture medium in each experiment. The absence of amylase in the culture medium in this series apparently turns on the presence of the higher concentration of KH_2PO_4 employed. Whether this reagent is operative in the culture medium or during digestion with soluble starch is undecided and remains a question for future investigation.

The results show on the one hand (Exps. 3 and 4) that even when the area of the aleurone layer is reduced to practically one-half its original extent, and the reduction in the area of the amylase-secretory tissue is very considerable, the amylolytic activity of the remaining secretory tissue is still able to effect a considerable amount of transformation in the starch storage materials, as shown by the quantities of reducing sugars in the medium of these experiments.

It is, however, the *macroscopical and microscopical features presented by the ventral and dorsal halves in Exps. 3 and 4 which require mention.*

The former show the usual phenomena, already enumerated in the case of endosperm cultures : reduction in the mass of and rounding off

[1] Concentration of KH_2PO_4 fourfold.

of the contours of the inner endosperm tissue, erosion of starch granules, and evidence of marked change in the cell-walls of the amyliferous tissue.

The latter objects, on the contrary, exhibit little if any evidence of visible change either in mass or condition. In one or two instances, however, where a minute fragment of the aleurone layer was inadvertently left attached, the amyliferous tissue immediately underneath and on its margins when viewed by transmitted light was distinctly less opaque, and the fragment had literally sunk into the subjacent tissue as dissolution of the starch had progressed.

Invariably, in the endosperm of the intact seed under normal germination conditions, in the isolated endosperm under the cultural conditions here employed, action on the starch contents proceeds most rapidly, in point of time, in *the tissue subjacent to the dorsal aleurone layer*. The experiments just referred to demonstrate that the ventral portion of the aleurone layer is equally efficient in inducing transformation of the starch reserves placed at its disposal. The important and crucial point is that marked depletive change takes place, under the conditions selected, only in those halved objects which were not deprived of their aleurone layers. Where a minute fragment was all that remained, there was striking evidence of its influence in determining dissolution of the subjacent starch, an observation rendered possible only by the lengthy duration of these culture experiments.

The demonstration of the amylolytic capacity of the inner endosperm in Experiment 3, Table XVII, p. 833, was confined to the examination of cultures of these objects on agar alone. An additional series of experiments was therefore undertaken to ascertain whether the inclusion of mineral salts with variation of the concentration of the phosphate would influence the process of self-digestion exhibited by the inner endosperms.

These experiments are summarized in the table below, and additional data of interest are also included, namely, direct and indirect estimations of the amounts of reducing sugars in both objects and medium, and the weights of the objects after removal from the culture medium and subsequent desiccation for twenty-four hours at 30° C.

Two methods of preparing the objects were adopted :—

(1) The aleurone layers were filed off the air-dried endosperms, and the inner endosperms thus prepared were then steeped successively in absolute alcohol and water, forty-eight hours in each reagent (Experiments 1, 2, 3, and 4).

(2) *Endosperms* were steeped as in (1), and the aleurone layer removed with a razor (Experiments 5 and 6).

Method (1) excludes the possibility of enzyme passing by diffusion from the aleurone layer and embryo to the subjacent tissue during steeping.

Method (2) eliminates the possibility of the embryo secretion diffusing into the endosperm.

TABLE XIX.

Cultures of Inner Endosperms on 0·8 % Agar-Mineral Salts (P_2 and P_4).[1]

Chilian barley. Culture periods, 14 and 20 days.

Exp.	*Objects.*	*Concentration of KH_2PO_4 in culture medium.*	*Duration of culture experiment.*	*Amylase per 20 objects per hour in:—*		*Reducing[2] sugars per 20 objects in:—*		*Weight per 5 objects at termination of culture experiment.*[3]
				Medium.	*Objects.*	*Medium.*	*Objects.*	
				(equivalent to mg. of Cu.)				
1.	5 inner endosperms	P_2	14 days	0·00	379[4]	124	—	—
2.	,, ,,	P_4	14 ,,	0·00	221[4]	103	—	—
3.	,, ,,	P_2	20 ,,	—	—	26	0·00	167 mg.
4.	,, ,,	P_4	20 ,,	—	—	26	0·00	142 ,,
5.	,, ,,	P_2	20 ,,	85	2532[5]	113	—	139 ,,
6.	,, ,,	P_4	20 ,,	0·00	3798[5]	29	—	125 ,,

Average initial weight of 5 inner endosperms, 175.

Possible objections to these methods of preparation will be discussed under the next subsection (IV B). It may, however, be noted here that very little, if any, diffusion of enzyme appears to take place when intact seeds are steeped as previously described.

The general character of these results, minimal depletive action, absence of obvious evidence of action on the cell-walls and contents, so closely parallels in every respect those yielded by similar objects prepared from intact steeped seeds that further reference to them is unnecessary.

In Experiments 3 and 4 the objects were directly investigated for reducing sugars, to test whether, in the absence of demonstrable cytohydrolysis of the cell-walls and the consequent presumed hindrance to the ready outward diffusion, the products of amylohydrolytic action had accumulated in the objects themselves. The foregoing experimental evidence, however, negatives this presupposition, and it is evident that the inferior autodepletive capacity of the isolated inner endosperm cannot be attributed to this factor.

To confirm the statement frequently employed in previous sections, that

[1] The 0·8 % agar solution *per se* was invariably faint alkaline to alizarin. The phosphate employed in the concentration P_2 was apparently just sufficient to render the 0·8 % agar-M.S. neutral.

[2] The value given for the reducing sugars here and elsewhere is corrected for the small amount of reducing sugars present initially.

[3] The reactions of the culture medium towards alizarin at the termination of the culture experiment were—Experiments 1, 3, and 5, neutral, and Experiments 2, 4, and 6, acid.

[4] Determined by direct-digestion method.

[5] Determined by papain-digestion method.

no appreciable reduction in the mass of inner endosperms takes place even when the culture period covers 14 to 20 days, the actual weights of the objects in Experiments 3 to 6 after 24 hours' desiccation at 30° C. were determined, and also for comparison the initial average weight of 5 inner endosperms similarly prepared and steeped only.

The influence of the phosphate in concentrations ranging from P_2 to P_4 appears to be in some way detrimental to the amylase which passes by diffusion into the medium.

Inner endosperms (Table XVII, p. 833) on agar *alone* apparently, as judged by the amount of reducing sugar in the medium, exhibit greater depletive power than on the same medium with the addition of mineral salts.

The evidence so far accumulated again tends to show that, while the inner endosperm does exhibit an auto-depletive capacity, that capacity is, as compared with that possessed by the endosperm, relatively limited.

IV B. Cultures of Endosperms and Inner Endosperms on Moist Calcium Sulphate.

Under the experimental conditions described in the preceding sections the quantitative method employed elicited two important facts, viz. that the isolated inner endosperm possessed a capacity for augmenting its amylase content, and an auto-depletive capacity, both of which, however, are inferior to the similar capacities possessed by the endosperm. Since the endosperm for our purpose consists of inner endosperm and aleurone layer, and since evidence has been adduced showing that the latter tissue is to be regarded as a secretory tissue, the marked signs of depletion which the endosperm exhibits may fairly be attributed to the action of an active amylolytic enzyme elaborated by the cells of the aleurone layer.

General considerations derived from a survey of the foregoing experimental results indicated that, as in all biological problems, the relation of the object [1] to its substrate is an important consideration.

The employment of gelatine or agar media involves the use of substrata whose chemical *reaction* may vary from time to time within quite small limits, yet slight changes in this respect may introduce factors whose influence it is at once difficult to detect and control. It was therefore necessary to choose a single and preferably neutral inorganic substance in order to ensure the uniformity of the substrate.

[1] As will be pointed out later, we are probably dealing, in the case of inner endosperms, with the relation of an enzyme to its substrate.

Calcium sulphate appeared to offer many advantages: it was used successfully by Hansteen, by Puriewitsch, and latterly by Miss Bruschi, in their studies of endospermic depletion, and Ford and Guthrie have shown it to be one of those salts which solubilize the 'latent' amylase of the resting grain.

Experiments with endosperms and inner endosperms were therefore carried out on this substrate in the following manner:—

In each of a series of Petri dishes was placed a layer of calcium sulphate 15 mm. in depth; these were sterilized and, after cooling, sufficient sterilized tap-water was added to form a semi-solid stratum. The objects (endosperms and inner endosperms) were then inserted by their proximal ends, for a distance of a few millimetres, into the substratum, and finally water added in such quantity that, on tilting the dish slightly, water was visible at the lower margin of the semi-solid stratum. Under these conditions the objects received sufficient mechanical support to retain their vertical positions throughout the course of the experiment, and were virtually partially immersed by their proximal ends in a saturated solution of calcium sulphate. The conditions selected probably afford better conditions for the diffusion processes, both inward and outward, than the use of the *solid* gypsum columns adopted by Hansteen and others.

Typical examples of the results furnished by experiments established on these lines are given in the table which follows. They are not illustrative of the results furnished by one series of experiments merely, but of many similarly conducted essays.

After transferring to a beaker, the calcium sulphate substrate was treated with almost boiling water to arrest further amylolytic action, the mass well stirred and thrown on a filter, washed, cooled, and the filtrate and washings diluted to 100 c.c., and duplicate copper reductions carried out by Bertrand's method.[1]

The sterility of the medium was controlled by inoculating small quantities of liquid and solid portions of the substratum into yeast-water-dextrose-maltose medium and incubating for 14 days at 30°.

[1] The residues from these filtrates were each evaporated to small bulk, refluxed with 90% alcohol, the alcoholic extract filtered and after expulsion of the alcohol examined polarimetrically; each was found to be dextro-rotatory. On treatment with phenylhydrazine each preparation yielded apparently a mixture of two osazones. These facts leave no doubt as to the nature of the reducing substances found in the various media after termination of the culture experiment.

TABLE XX.

Culture of Endosperms and Inner Endosperms on Moist Calcium Sulphate (Kahlbaum).

Chilian barley. Culture period, 10 days.

Exp.	*Objects.*	*Total reducing sugars in medium equivalent to milligrams of Cu.*
1.	20 endosperms	601
2.	20 inner endosperms	133
3.	,, ,,	123

Experiment 1. Endosperms of air-dried seed steeped successively in (1) absolute alcohol; (2) water; 48 hours in each reagent.

Experiment 2. Aleurone layers filed off endosperms of air-dried seed; inner endosperms, thus prepared, then steeped as in Experiment 1.

Experiment 3. Intact seeds steeped successively in (1) absolute alcohol; (2) water; 48 hours in each reagent; embryos excised and aleurone layers removed with razor.

The contrast in the results afforded by Experiment 1 and Experiments 2 and 3 is most marked. In the space of 10 days 75 % *of the endosperms in Experiment 1 were almost completely emptied of their inner endosperm contents*; in the remaining 25 % the contents were in a semi-solid condition, and gentle pressure only was necessary to cause free ejection of their contents.

The inner endosperms (Experiments 2 and 3), on the contrary, had suffered little or no reduction in mass, and had the semi-solid consistency characteristic of similar objects which had been simply steeped and subsequently maintained in a moist condition.

These experimental results afford most convincing evidence, on the one hand, of the capacity of the endosperm, and, on the other, of the relative incapacity of the inner endosperm to induce complete and veritable depletion of the inner endosperm contents. The former result is conditioned by the presence of the aleurone layer, the latter by its absence.

The relative order of magnitude of the two capacities is also well shown by the relative amounts of reducing sugars in the substrata given in the above table.

The points of difference are still further emphasized by the contrast revealed by microscopical examination of starch granules from the two types of objects. These, in Experiment 1, exhibited extensive erosion, which is so closely similar to that hitherto attributed exclusively to the amylase secreted by the embryo ('amylase of secretion') that there is full justification for the statement that the enzyme secreted by the aleurone layer is *identical* with that elaborated by the embryo.

Microscopical examination of starch grains taken from various parts of the inner endosperms (Experiments 2 and 3) fails to reveal either any signs of erosion or any visible evidence of dissolution of the larger mature starch granules; they retain their characteristic lenticular shape, and the opinion is reiterated that in all probability the attack of the amylase on the inner

endosperm is restricted to the immature starch granules of minute size, which are always present in all parts of this tissue.

Invariably, in endosperm experiments such as Experiment 1, not only are the starch reserves degraded, but there is overwhelming evidence of advanced cytoclastic action. The cell-walls of the amyliferous cells exhibit every gradation of disintegrative change, from the initial swelling up and splitting into laminae to their complete dissolution. Careful, often repeated, examination of the cell-walls of objects 'cultivated' as in Experiments 2 and 3 fails to afford any conclusive evidence of cell-wall disintegration. Thin sections of such objects, treated with diluted saliva at 30° C. for several hours in order to dissolve out their starch contents, do not exhibit any recognizable sign of having undergone even partial cytohydrolysis.

Physical examination of endosperms and inner endosperms removed after periods (varying from 7 to 20 days) of cultivation on calcium sulphate and subsequent desiccation for 24 hours at 30° C. shows that, while the former objects are distinctly friable, the latter do not exhibit this attribute, and the almost unavoidable inference to be derived from these observations is that cytohydrolysis of the cell-walls, even where it does not result in their complete disintegration, is correlated in some way with acquisition of friability by the endospermic contents.

Finally, examination of the action, either of these two types of objects directly or of aqueous extracts prepared from them, on gelatinized starch affords a further means of emphasizing points of difference in the attributes of the amyloclastic enzymes, which take their origin in the aleurone layer and inner endosperm respectively.

The objection may be raised that the method of preparing inner endosperms in Experiment 2 does not preclude, on the one hand, possible coagulation of amyloclastic enzyme by absolute alcohol; on the other, considerable loss of enzyme by aqueous extraction during the steeping in water.

This objection is partly met by the results of Experiment 3, in which, by the method of steeping adopted, the inner endosperm was not exposed to these influences. In order to dispel this objection, inner endosperms were prepared as described in Experiment 2, and determinations were made of the amount of amylase which had diffused into the steep-water during steeping, and of the amylase present in the objects immediately after removal from the steep and subsequent desiccation at 30° for 24 hours.

The results were:—

	Amylase per 20 per hour (equivalent to mg. of Cu).
Amylase in steep water	261
Amylase in inner endosperms (direct digestion) . .	1055
Amylase in inner endosperms (0·5 % papain digestion)	2966

These data show that loss of amylase does occur under the circumstances, as one would have surmised; but as direct digestion, and particularly papain digestion, demonstrate, very considerable amounts of amylase, both pre-existent or 'free' and 'latent', are still present in the tissue, prior to the objects being placed in the experimental substrate.

The general conclusion to be deduced from the experimental results comprised in this section, in which the relative powers of the endosperm and inner endosperm to induce auto-depletive change have been further examined, is that the endosperm, by virtue principally of the secretory functions of the aleurone layer, is capable of veritable auto-depletion. The inner endosperm, on the contrary, possesses this capacity in a restricted sense only, the process it is capable of inducing being one of limited auto-digestion, which never attains or approaches that of complete depletion.

NOTE.

NOTE ON THE BIOLOGY OF THE GENUS SEPTOBASIDIUM.—The genus *Septobasidium*, Pat., was instituted for the reception of a fairly well-defined group of Basidiomycetous Fungi which had been included by previous authors under *Thelephora*, *Lachnocladium*, *Corticium*, &c. They are practically confined to tropical countries, and therefore very little has been recorded concerning their biology. As a rule they are found, encrusting living stems or leaves, up to a height of 10 feet or more from the ground. In Ceylon, several species, or forms, are quite common, though it is difficult to determine how many species occur, because the majority of the specimens collected are sterile, and in that condition they are all very much alike. One species (? *Corticium murinum*, B. and Br.) frequently causes alarm by clothing the stems of tea bushes from top to bottom; another (*Thelephora lichenicola*, B. and Br.) forms brown sheets which extend for a length of several feet along the stems of mango trees; while two others (*Thelephora suffulta*, B. and Br., and *Lachnocladium rameale*, B. and Br.) similarly encrust the twigs of shrubs in up-country jungles. But as the stems, twigs, or leaves are in no case killed or noticeably injured by the fungi, one is immediately led to question their supposed parasitism.

From an examination of a long series of specimens, it has been determined that these Fungi are parasitic on colonies of scale insects, which they overgrow and destroy completely. One purple-black species which is fairly common on tea always grows over the insect *Chionaspis biclavis*, as was pointed out to me by Mr. E. E. Green. An examination of the specimens in the Kew herbarium demonstrates that this habit is not confined to Ceylon species, for a sterile specimen there from North America, included under *Thelephora lichenicola*, also shows a colony of scale insects beneath the subiculum.

These Fungi live, not on the secretions of the insects, as in the case of *Meliola*, but upon the insects themselves: biologically, therefore, they afford a parallel to the genus *Hypocrella* among the *Pyrenomycetae*.

T. PETCH.

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The Vegetative Divisions in Vicia Faba.

BY

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AND

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With Plates LXII and LXIII.

THE nuclear divisions in *Vicia Faba* have long been a common object of laboratory demonstration, and preparations made early in 1910 for this purpose proved so suggestive that a detailed study was undertaken, first of the somatic and later of the meiotic stages.

The discussion of meiosis is reserved for a later paper, and we propose at present to deal only with the vegetative divisions. These were studied chiefly in the root apex, but also in the young petals, stamens, and ovary, and finally in the pollen-grain.

Plants of Carter's 'Monarch' were grown in the spring and summer of 1910 partly in the greenhouse of this Department and partly in a garden at Penge under the care of one of us.

The tissues were fixed in various media, of which Flemming's strong fluid diluted with an equal quantity of water proved the most successful. Sections were cut from 4μ to 5μ in thickness, and were stained sometimes with Flemming's triple stain or with Heidenhain's iron haematoxylin, but most frequently with the combination of Brynal, which, when successfully used, far surpassed either of the others for this purpose.[1]

Mitosis in the Sporophyte.

It is convenient to begin the account of the mitoses in the Broad Bean at the stage when the chromosomes are reaching the poles of the spindle, up the outer fibres of which they travel so that they form a hollow truncated cone. The individual chromosomes at this stage are

[1] I am indebted to the Government Grant Committee of the Royal Society for various lenses and certain other apparatus used in this work. H. C. I. F.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

straight or hooked rods (Pl. LXII, Fig. 1). Some of them are sharply contracted near the middle, and all of them show a slight irregularity of outline.

As they reach the narrow end of the spindle they shorten and thicken (Fig. 2), become more closely massed, and come into contact with their neighbours on either side.

The daughter nuclei thus newly formed become rounder and more definite in outline, and a faint differentiation of the cytoplasm suggests that the nuclear membrane has been formed. At the same time the close aggregation of the chromosomes loosens, but each remains attached to its neighbours by threads of stainable material. While this is taking place slits are observed here and there in the middle line of each chromosome and seem by their position to be due to the pull of the lateral attachments (Fig. 3).

With the increase of the young nuclei in size both the slits and the cross attachments become more marked (Figs. 4, 5); after a time the nucleus reaches an approximately spherical shape, and the chromosome complex is spread over the surface of the sphere just inside the now clearly defined nuclear membrane (Fig. 6). Now also the nucleolus becomes visible, appearing first as a drop or drops of stainable material (Fig. 7), and usually in relation to one or more of the chromosomes.

The chromosomes themselves become much less regular in outline as their stainable substance is aggregated here and there in irregular masses, leaving a relatively thin thread between (Figs. 7, 9).

At this stage the centre of the nucleus in median section appears empty—except perhaps for the presence of one or two nucleoli—and is surrounded by a ring of chromatin threads. But above or below the middle line a network may be focused, and in it the split and laterally attached chromosomes are clearly recognized (Fig. 8). Their ends are by this time no longer to be found, and have presumably become joined either to those of their lateral neighbours or to those meeting them across the poles of the nucleus; and the longitudinal slits have been pulled out to form diamond-shaped areas (Fig. 9). As time goes on, the limits of the individual chromosome become less obvious, but the extent to which they may be recognized depends partly also on the angle from which the nucleus is viewed. Thus the so-called resting stage is reached, and the nuclei, which may differ one from another considerably in size (cf. Figs. 9, 10), either pass into the 'permanent state', or after a varying period once more prepare for division.

The first indication that division is about to begin is found in the breaking down of certain threads of the reticulum which, in favourable cases, may be recognized as the cross attachments between the chromosomes of the preceding telophase.

At the same time, perhaps we may say in consequence of this change, other meshes approximate so that a double thread is formed (Fig. 11). It shows an appearance similar to that of the split chromosomes when passing into the resting stage (cf. Figs. 5, 11), and may safely be identified with them. The spireme thus produced is from its formation a double structure.

As development proceeds there becomes evident a definite relation between the nuclear thread and the nucleolus; the spireme is arranged in coils and loops radiating somewhat irregularly from the nucleolus in a way that recalls the 'second contraction' of meiosis (Figs. 12, 13), and may perhaps be compared to the polarization observed by Farmer and Shove (3) at a similar stage in *Tradescantia*. The nucleolus becomes vacuolate and decreases in size, doubtless giving up material to the chromatin thread, and as it disappears the spireme straightens (Fig. 14) and becomes coiled with more or less regularity either around or up and down the nuclear area (Fig. 15). The remains of the cross attachments have by this time disappeared, and the stainable substance has come to be pretty uniformly distributed along the thread, which is double throughout its length.

At about this stage the segmentation of the spireme into chromosomes occurs (Figs. 16, 17); this takes place gradually, the spireme breaking across first at one point and then at another, and for some time the separated chromosomes may adhere one to another by fine threads (Fig. 18). Throughout the subsequent stages certain chromosomes are again and again observed, as in the telophase, to be made up of two, or in one or two cases more, distinct segments (Figs. 1, 19, 23), and when separation of the daughter chromosomes has taken place it is possible to recognize this in each member of a pair.

While the development of the chromosomes is proceeding, spindle formation has taken place. A thickening of the cytoplasm around the nucleus occurs at an early stage (Fig. 16), and later, as the nuclear membrane breaks down, delicate fibres are visible (Fig. 18), running from end to end or from corner to corner of the cell.

It would appear that the chromosomes take up their position on the spindle very soon after it is formed (Fig. 18) and remain for some time lying against it; we observed, at any rate, numerous stages such as that shown in Fig. 19, while Fig. 18 represents a stage comparatively rare.

Throughout the development of the spireme the longitudinal fission of the thread is very clear, but after segmentation it becomes inconspicuous, and may sometimes seem to be completely obliterated (Pl. LXIII, Fig. 27). Even at this stage, however, it can be identified in favourable preparations (Pl. LXII, Fig. 18), and by the time that the chromosomes have passed on to the spindle it is once more evident.

The double chromosome is attached to the spindle by one end, and at first lies more or less parallel to or across the spindle fibres (Fig. 19).

Gradually it becomes erected till it stands out at right angles to the fibres (Pl. LXII, Figs. 19, 20), and the ends of the two daughter chromosomes, which at first lay close together, begin to slide away from one another along the spindle (Fig. 20) till the double rod becomes transformed into a wide V or U, at the apex of which the ends remote from the spindle lie pressed together or just crossing one another (Figs. 21, 22). The ends upon the spindle travel further and further apart till the daughter chromosomes are no longer in contact and lie flat or almost flat along the fibres (Fig. 23). They have the form of straight or somewhat sinuous rods; frequently the end towards the pole of the spindle is bent over, forming a hook; whether this takes place depends on the position of the chromosome during the early stages of its attachment to the spindle. Indications that such hooks will be produced may be recognized in Figs. 20 and 21.

The daughter chromosomes have now reached the stage represented in Fig. 1, and they show no recognizable indication of the longitudinal split which is about to occur; indeed, we may perhaps suppose that, for the short period between the metaphase and the telophase, the chromosome is a single structure.

Mitosis in the Gametophyte.

Various authors have called attention to the double nature of the chromatin elements in somatic cells, and it has been interpreted by some of them (Overton (12), Sykes (14), Takara (15)) as due to the lateral approximation of structures derived respectively from the male and female gametes, while to other investigators (Hof (7), Digby (1)) it has appeared rather that the duplication is brought about by an early longitudinal fission, which in the telophase of one division already prepares for the next.

In the Broad Bean a study of these stages in the cells of the sporophyte seemed to us to point pretty clearly to the latter interpretation; in order to test this conclusion we proceeded to a study of the vegetative divisions of the gametophyte, and examined the nuclei of the pollen-grains in various stages of development.

Here the chromosome number is seven instead of fourteen as in the diploid cells, and there is no question of the association of paternal and maternal structures since only a single set of chromosomes is present. But in the pollen-grain, exactly as in the cells of the root or flower, a double reticulum was observed (Pl. LXII, Fig. 24; Pl. LXIII, Figs. 31, 32), and here also the first evidence of duplication appears in the late telophase (Figs. 30, 31).

The formation of the first 'resting' gametophyte nucleus is preceded by the homotype division. It is proposed to discuss the meiotic stages in a forthcoming paper, and it suffices here to say that in the homotype telophase, as in all others studied, longitudinal fission can be seen.

After the conclusion of the homotype division, while the pollen-grains are still grouped in tetrads, the nuclei are small and very dense, with a fine reticulum in which the details of the arrangement of the thread are not readily made out.

As the nucleus increases in size the reticulum loosens, and its double nature is very evident (Pl. LXII, Fig. 24). The nucleus remains in the resting stage for some little time, while the wall of the pollen-grain becomes thickened, and then mitosis begins and hardly differs except in chromosome number from the corresponding process in diploid nuclei. The longitudinal fission is clear in all stages (Pl. LXIII, Figs. 25, 26, &c.), except that here, as in the somatic cells, it may be more or less obliterated when the chromosomes are passing on to the spindle (Fig. 27). The metaphases and anaphases differ from those of the sporophyte in the narrower spindle (Fig. 28); the individual chromosome presents the same appearance and shows the same somewhat irregular outline (Fig. 29). Longitudinal fission of the daughter chromosomes usually appears in the tube nucleus (Fig. 30) a little before it is seen in the generative nucleus (Fig. 31). The reticulum (Pl. LXII, Fig. 24; Pl. LXIII, Fig. 31) is quite like that of the sporophyte, though it gives the effect of being made up of fewer threads. As in the sporophyte the cross attachments break down so that the spireme is double from its formation. The nucleolus, sometimes at any rate, persists longer, and may be seen (Fig. 25) after the spireme has divided into its constituent chromosomes.

Discussion.

The longitudinal fission of the Chromosomes.

In view of the evidence derived both from the diploid and haploid nuclei there seems to us little doubt that in the Bean the separation of the chromosomes into two equivalent portions on the spindle is already foreshadowed by their fission in the preceding telophase.

That such a state of affairs is of common occurrence is suggested by the observations of Grégoire and Wygaerts (6) on *Trilium*, of Grégoire (7) and of Merriman (10) on *Allium*, and of Digby (1), who not only studied the sporophyte, but also observed parallelisms in the nucleus of the young pollen-grain on *Galtonia*.

In *Vicia Faba* the chromosomes do not appear to break up as in *Galtonia* (Digby (1)), but become joined to one another both end to end [1] and laterally. In the region of the lateral attachments the sides of the split chromosomes are pulled apart, and in this way the network of the

[1] Grégoire lays special stress on his observation that in *Allium* the ends of the chromosomes remain independent; it seems to us very improbable that such is the case in *Vicia*, for no free ends can be identified either in the reticulum or in the spireme stage, except such as inevitably occur in nuclei cut by the microtome knife.

resting stages is produced. Later the cross attachments break down so that the spireme consists of the split daughter chromosomes of the last division arranged presumably in the same order in which their ends became united in the telophase.

The double arrangement persists till the two halves separate on the new spindle, and it is only at one stage even temporarily obscured, namely, after the segmentation of the spireme when the chromosomes are passing on to the spindle. This is in part no doubt due to a fresh supply of stainable substance which has been taken up from the nucleolus, but we have also found it useful in this connexion to recall the behaviour of two parallel pieces of elastic band: if these are held just touching one another and then pulled out by their ends, the sides separate to come together once more when the strain is relaxed. But if the now slack pieces are bent or twisted their independence is again obvious. So, it appears to us, the taut spireme shows a duplication which may disappear when it segments, but which is once more obvious when the separate pieces come to lie variously curved upon the spindle. It must further be borne in mind that the chromosome is split only in one plane, and that from certain points of view the fission is therefore invisible. Moreover, in favourable preparations the duplication can even now be recognized, and it seems to us very doubtful whether complete closure of the split ever occurs.

The recognition of the longitudinal fission in the chromosome is thus carried back from the prophase to the preceding telophase, but the mechanism by which it is accomplished is still to seek. Grégoire (4, 5) describes the fission in the telophase as due to the development of alveoli within the chromosome, though he does not connect this process with the subsequent duplication of the spireme; and a corresponding explanation has been put forward by Stomps (13), who described a process of vacuolization in *Spinacia*, which divides the chromosomes of the telophase into series of parallel lamellae. We have seen no indication of more than one line of fission in the Bean, and we are inclined to suggest that the pull of the lateral attachments may play an important part in bringing this fission about.

One is driven throughout the study of mitosis in the Bean to visualize the chromosomes as somewhat elastic, viscous bands, easily adhering to one another, easily splitting in their more fluid interior, but retaining their form by reason of the greater density of their surface layer, which, as Livingston (9) points out for the ectoplasm, may be differentiated by mere contact with the external solution. From such a point of view it will follow that the narrow cross attachments, consisting as they must almost entirely of the transformed outer layer, may cohere sufficiently to pull asunder, as the nucleus expands, the unaltered centres of the chromosomes to which they are laterally attached.

It remains to be questioned why the cross attachments formed at this stage are stronger, as the above implies, than the attachments formed between sister chromosomes (Pl. LXII, Fig. 20), or indeed any near neighbours in the prophase and early metaphase. It is probably a relevant consideration that the chromosomes, crowded together at the narrow end of the spindle, seem to be in contact under pressure, whereas they lie loosely side by side in the earlier stages.

As far as we can see, though fission is begun in the telophase, it is not complete till after the spireme is formed, for, even in the early spireme stages (Fig. 11), the thread in *Vicia* is made up of alternate double and apparently single portions, and in Figs. 5 and 8 a clear relation seems to exist between the cross attachments and the points of fission. It must here again be borne in mind that the thread in the regions of cross attachment is comparatively tense, and that it is comparatively slack between these points, so that there the sides of a split thread might readily fall together. The possibility thus remains that the cross attachments do not cause the split, but only make evident a fission already accomplished.

Mechanism of Mitosis.

It is difficult again to obtain useful evidence as to the mechanism which brings about the separation of the daughter chromosomes, but the stages by which this is accomplished in *Vicia* seem fairly simple. The chromosomes become attached by one end to the spindle, they lie lax for a time along it, they are swung out at right angles to the spindle axis, and the separation of the two halves of the attached end begins. The halves remain in contact at the free end for a considerable time, so that the daughter chromosomes form first an acute and later an increasingly obtuse angle, till they come to lie almost along the same straight line. By this time they are quite free one of another; they show no decrease in length till they approach the poles, when they are shortened and thickened, forming the dense mass described. The contraction of the chromosomes at this stage is no doubt due to the continued action on their lagging ends of whatever force is responsible for their movement along the spindle.

It is well known that after good fixation the spindle fibres are often less conspicuous than when inferior fixatives are used. This fact is evidence against the recognition of the fibres as definite cell entities: Farmer and Moore (2) have regarded them as protoplasm modified by the forces at work in the cell, and we are led to suggest that an important part may be played by currents of altered cytoplasm in Angiosperms, much as these have been suggested (Fraser and Welsford (4)) to be responsible for the changes taking place in the cytoplasm of the ascus among Fungi.

If it be credible that such an alteration is in the direction of greater osmotic activity, then we should have a mass of osmotically active substance

(the end of the central spindle) lying within each group of chromosomes at the pole. When the nuclear membrane is formed water would pass into this mass from the outer cytoplasm, which is *ex hypothesi* less osmotically active, and the nuclear area would enlarge. If with the nuclear membrane the chromosome complex is carried outwards and therefore distended, the necessary conditions are obtained for the fission of the chromosomes in their more fluid interior under the pull of the lateral attachments.

Abnormal Conditions.

Some study has been made in recent years of the effect of abnormal conditions on nuclei, and *Vicia* has often been employed for this purpose. Němec (11) in an investigation of the effect of chloral hydrate and other reagents lately described a stage similar to that shown in Fig. 19 as representing the separation of the gemini in a heterotype division consequent on the nuclear fusions he records. This stage is a very common one in our material, both in the roots and in other vegetative organs grown under natural conditions, and we are constrained to regard it as a normal phase of karyokinesis. That this is the case is perhaps worth recording in view of Kemp's failure to find meiotic stages even in material subjected to abnormal conditions (8).

We noticed, also, considerable variation in the size of the nuclei even in neighbouring cells (cf. Pl. LXX, Figs. 9 and 10), but nuclei of irregular form, tripolar spindles, and other evidences of abnormality were entirely absent, as was indeed to be expected.

Segmented Chromosomes.

In a considerable number of cases some of the chromosomes on the spindle were seen to be made up of two or occasionally more distinct segments. It is perhaps possible to imagine that the segment rather than the chromosome or chromomere represents a discrete (hereditary) unit, and it might be suggested that the arrangement of these units in the chromosomes is indifferent and may vary. Such a speculation may throw light upon the fact that the number of independent Mendelian characters is in certain organisms greater than the haploid number of chromosomes, and moreover, if it were of at all general application, it would account for the often described variation in the chromosome number. Thus, if the average number of segments in certain chromosomes be two, their occasional independence or the union of three or four together would increase or diminish the apparent number of chromosomes. Again, the more or less permanent association of two segments would produce an appropriate physical basis for the Mendelian phenomenon of coupling.

Summary.

1. There are fourteen chromosomes in the sporophyte and seven in the gametophyte of *Vicia Faba.*

2. On reaching the pole of the spindle the chromosomes become massed together and come into contact one with another.

When this aggregation loosens, the chromosomes remain laterally attached to their neighbours and show longitudinal fission.

The fission persists and is pulled out in the regions of the cross attachments to form the diamond-shaped meshes of the reticulum. The ends of the chromosomes also unite, and one or two nucleoli appear. Thus the 'resting stage' is produced.

3. On the initiation of a new division the cross attachments break down, the sides of the diamond-shaped areas approximate, and a double spireme is formed; this ultimately breaks transversely into longitudinally split chromosomes.

4. The line of separation of the daughter chromosomes on the spindle is therefore marked out in the preceding telophase, and persists throughout the intervening stages.

5. The chromosomes are frequently constricted into segments, and it seems probable that the way in which the segments are grouped to form chromosomes may vary. An explanation here suggests itself of the often recorded variation in the chromosome number, and possibly also of the Mendelian phenomenon of coupling.

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EXPLANATION OF PLATES LXII AND LXIII.

Illustrating Dr. Helen Fraser's and Mr. Snell's paper on Vegetative Divisions in *Vicia Faba*.

All figures were drawn with a camera lucida under a 2 mm. apoch. hom. imm. Zeiss N.A. 1.40 comp., oc. 12. Magnification × 2,000 throughout.

PLATE LXII.

Fig. 1. Root; late anaphase, showing segmented chromosomes.

Fig. 2. Ovary; telophase, chromosomes contracted.

Fig. 3. Stamen; loosening of aggregation of chromosomes; cross attachments formed; longitudinal fission begun.

Fig. 4. Root; later stage of same.

Fig. 5. Root; same, still later.

Fig. 6. Root; early reticulum.

Fig. 7. Root; early reticulum; nucleolus present.

Fig. 8. Root; reticulum in upper nucleus; in lower (sister) nucleus spireme formation has already begun.

Figs. 9 and 10. Root; 'resting' stages.

Fig. 11. Root; breaking down of cross connexions in formation of spireme.

Fig. 12. Root; early spireme, showing relation to nucleolus.

Fig. 13. Root; same, rather later.

Figs. 14 and 15. Root; later stages of spireme; disappearance of nucleolus.

Fig. 16. Root; beginning of segmentation of spireme.

Fig. 17. Root; tangential section of nucleus, showing newly formed chromosomes.

Fig. 18. Root; chromosomes passing on to spindle.

Fig. 19. Root; chromosomes attached to spindle by one end, and for the most part lying parallel to it.

Fig. 20. Root; individual chromosomes on spindle, (*a*) cross attachment between members of a pair of daughter chromosomes; (*b*) and (*c*) early stages of separation of daughter chromosomes.

Fig. 21. Root; metaphase.

Fig. 22. Root; later stage of same.

Fig. 23. Root; early anaphase, showing segmented chromosomes.

Fig. 24. Young pollen-grain in transverse section; 'resting' nucleus, longitudinal fission visible in threads of reticulum.

PLATE LXIII.

Fig. 25. Pollen-grain in oblique section; longitudinally split chromosomes; remains of vacuolate nucleolus.

Fig. 26. Pollen-grain in transverse section; chromosomes after disappearance of nuclear membrane.

Fig. 27. Pollen-grain in longitudinal section; chromosomes passing on to spindle; split, not recognizable.

Fig. 28. Pollen-grain in longitudinal section; metaphase.

Fig. 29. Pollen-grain in longitudinal section; anaphase.

Fig. 30. Pollen-grain in longitudinal section; late telophase; in the larger (tube) nucleus the chromosomes have already undergone fission.

Fig. 31. Pollen-grain in longitudinal section; tube nucleus showing reticulum; chromosomes, in which fission has taken place, still recognizable in generative nucleus.

Fig. 32. Pollen-grain in transverse section; reticulum in 'resting' nucleus.

Fig. 33. Pollen-grain in transverse section; nucleus with spireme beginning to form.

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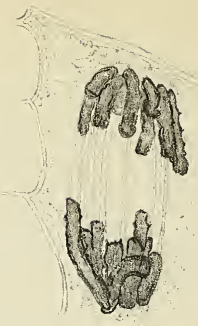

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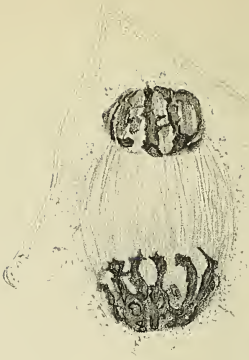

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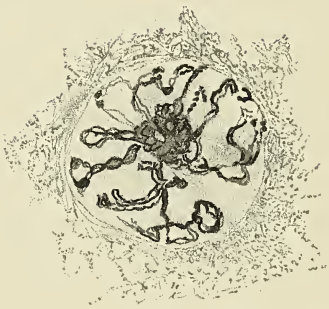

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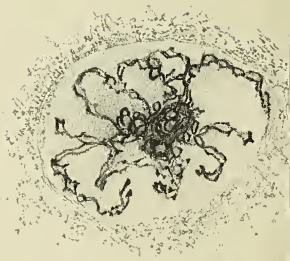

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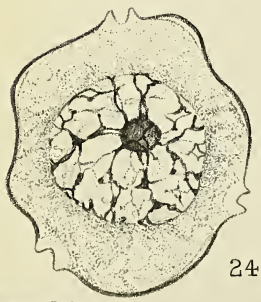

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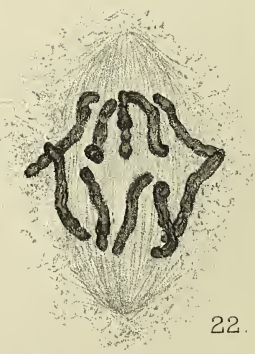

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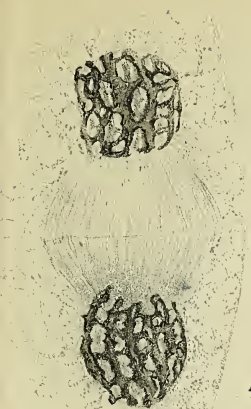

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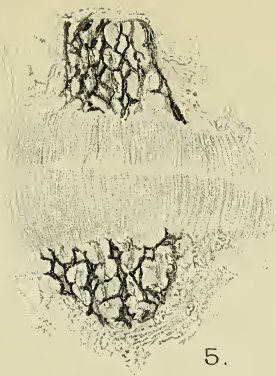

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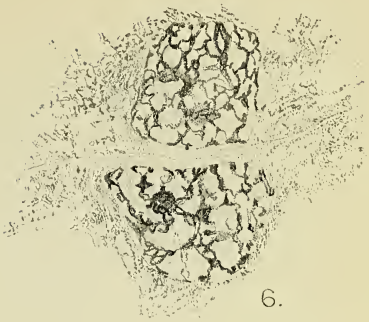

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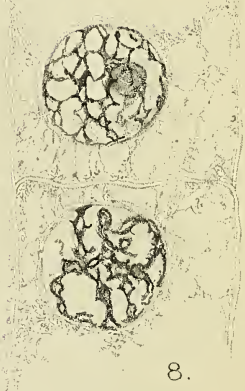

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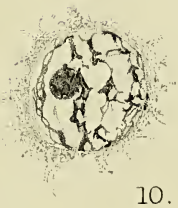

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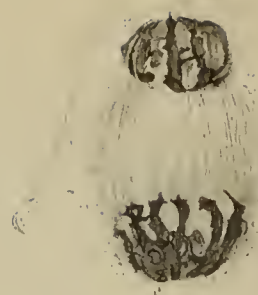

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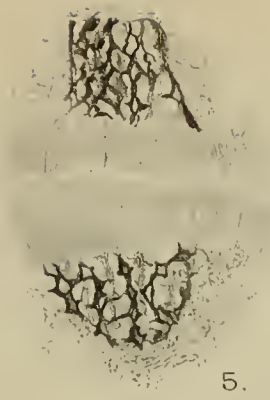

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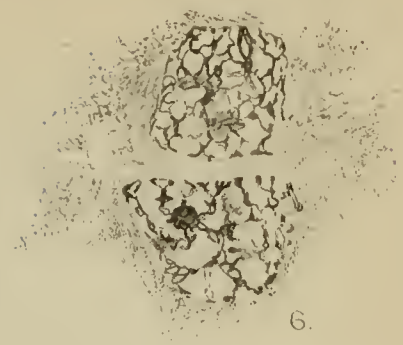

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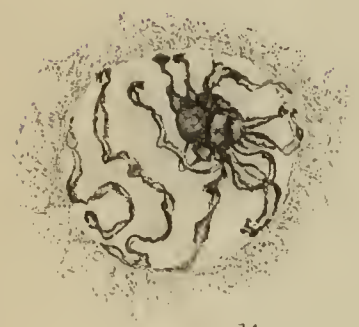

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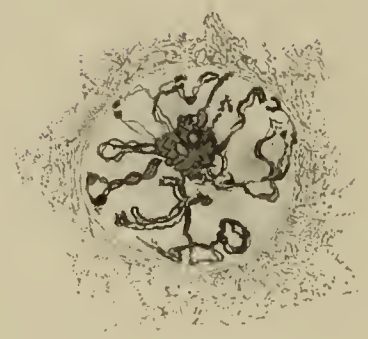

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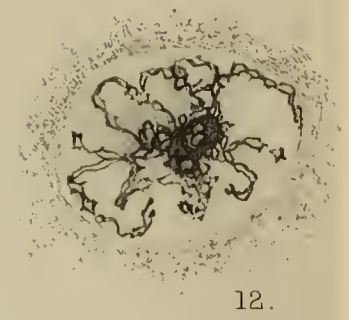

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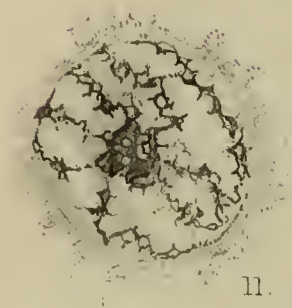

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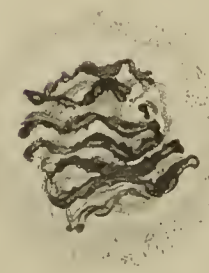

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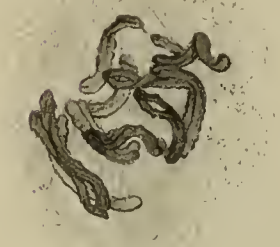

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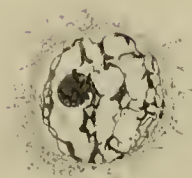

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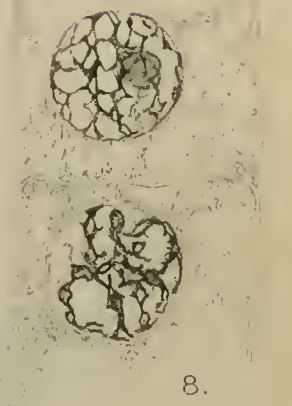

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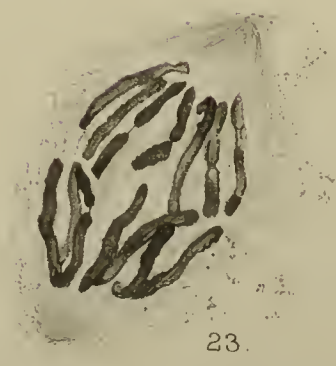

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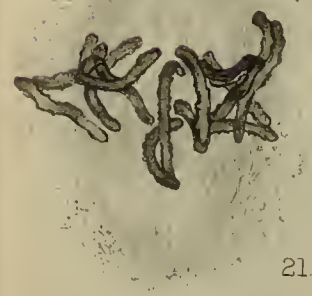

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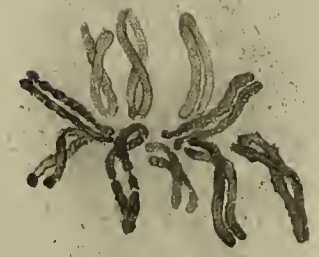

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FRASER AND SNELL —— VEGETATIVE DIVISIONS IN THE BEAN.

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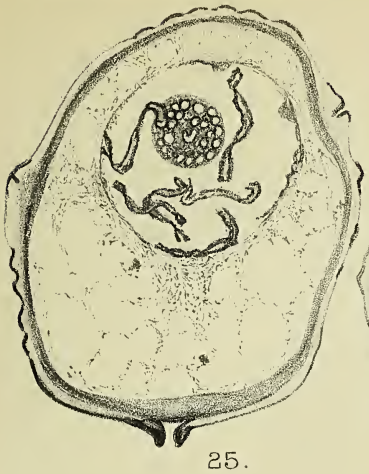

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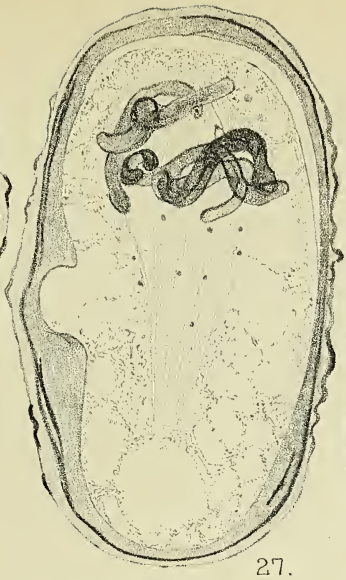

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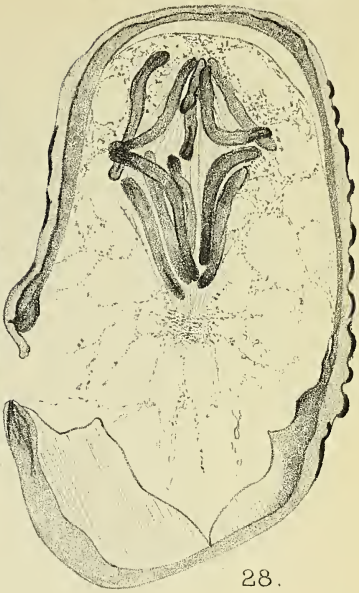

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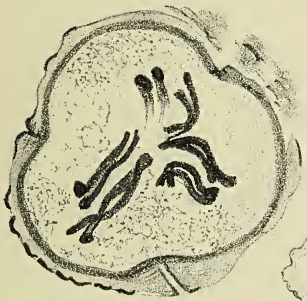

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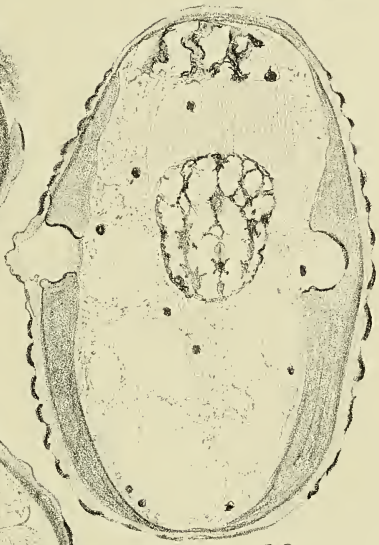

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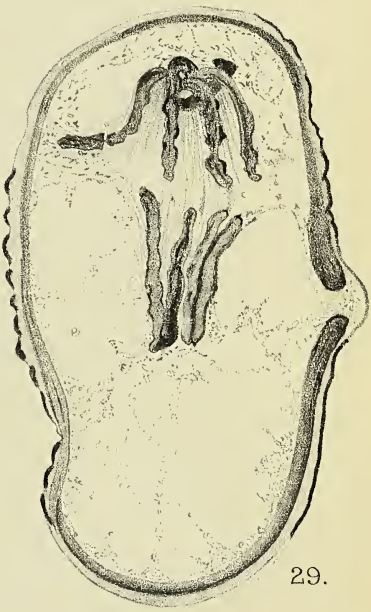

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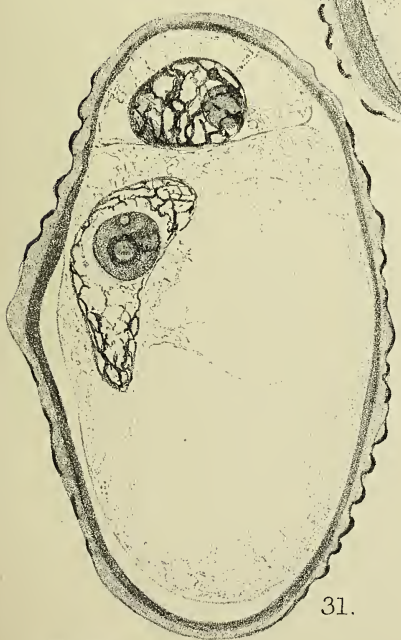

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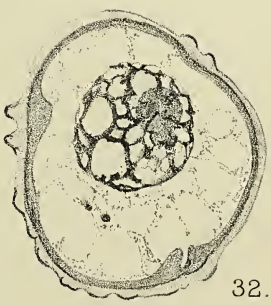

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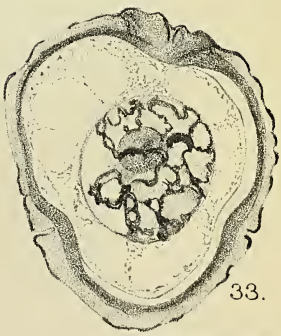

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FRASER AND SNELL.—— VEGETATIVE DIVISIONS IN THE BEAN.

Plant Distribution in the Woods of North-East Kent.

PART I.

BY

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With Plates LXIV–LXVI and four Figures in the Text.

ALTHOUGH the subject of Plant Geography has, in recent years, attracted considerable attention in this country, comparatively few detailed descriptions of plant distribution in small areas have appeared. While the vegetation of large districts in the north of England and Scotland has been described and mapped, little or nothing has been published on plant distribution in the southern counties. This is especially the case with regard to Kent. In a county of this kind where almost the whole area is under cultivation, the woodlands form perhaps the most suitable subject for investigation.

Moss, Rankin, and Tansley (13) have recently published a general account of the woodlands of England, and have drawn attention to the great prevalence of coppiced woods in this part of the country. Considerable areas in North-East Kent are covered by woods of this description. The periodic felling which takes place in these brings about great alterations in the physical conditions, and the following paper will deal largely with the resulting changes in the vegetation.

Woodhead (17) has made one of the most important contributions dealing with the distribution of woodland plants. In this paper a detailed description of the vegetation of several woods in the neighbourhood of Huddersfield is given, and the effects of differences in illumination and in the soil composition respectively are discussed.

Cieslar (3), in 1904, gave a full account of the part played by light during the growth of the forests near Vienna. He has shown the great effect of shade in influencing the number of herbaceous species in a given situation ; the origin of the ground flora is also discussed.

Fliche (6) has given an account of the reafforestation of areas in the Forest of Champfêtu, near Sens (France), and the consequent disappearance of certain species. This paper is of especial interest since it deals with

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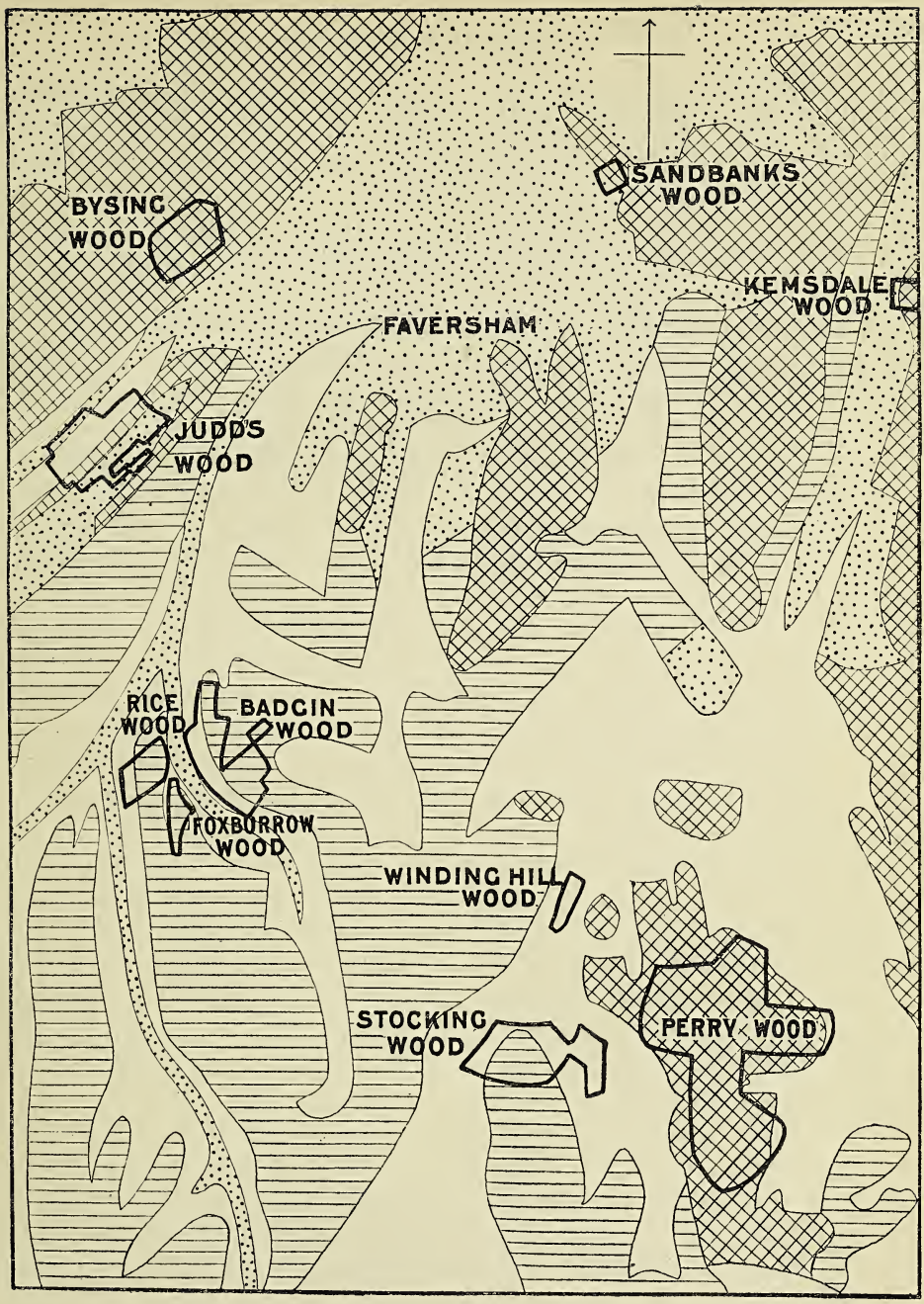
woods which, like those examined in the present investigation, occur on the Cretaceous and Eocene formations.

All the above communications deal with high forest, and, as far as can be ascertained, no papers have yet appeared describing the flora of coppiced areas and the changes consequent on the periodic felling which takes place in this type of woodland.

The woods on which the greater part of the investigations have been made occur in the neighbourhood of Faversham in North-East Kent. This district is especially suitable for an investigation of this kind on account of the great differences in composition of soils which are found in close proximity and in a comparatively small area. This part of Kent is included in maps Nos. 273 and 289 of the Ordnance Survey on the scale of one inch to one mile, and in Sheet 3 of the Geological Survey on the same scale. Additional observations have also been made on woods in the neighbourhood of Swanley in North-West Kent. This latter district is included in Map 271 of the Ordnance Survey, and Sheet 6 of the Geological Survey, both maps being on the scale of one inch to one mile.

The general underlying formation of the whole area under consideration is the Upper Chalk, but this is covered to a great extent by beds of clays and gravels, and by outliers of the Lower Eocene formations. In the Faversham district there is a gradual increase in altitude towards the south until the escarpment of the Chalk is reached about 10 miles distant; towards the north the area is limited by the Thames Estuary. The surface of the Chalk is deeply indented by numerous dry valleys which run down the dip slope towards the north. The bottoms of these valleys are generally occupied by river deposits, but on the sloping sides the Chalk is only covered by a thin layer of soil. One of these valleys is indicated on the lower left-hand side of the map shown in Text-fig. 1; its course can be traced by the deposit of alluvial material found along its bottom. On the ridges a deposit of stiff reddish clay is found, known as the 'Clay with Flints' on account of the numerous unworn flints occurring in it. Passing to the north towards the Thames Estuary the Clay with Flints gradually disappears, and is partially replaced by a deposit described as 'Loam on the Chalk' by the Geological Survey. The latter, in many places, is covered by deposits of brick-earth. Finally, in the close neighbourhood of the estuary, there is a comparatively deep layer of alluvium covering the earlier deposits (see Text-fig. 1).

In the immediate neighbourhood of Faversham the Tertiary (Lower Eocene) formations are only represented by small outliers, but farther to the east there is an Eocene deposit of considerable extent. The lowest of these beds, resting directly on the Chalk and known as the Thanet Sand, consists of a fine light-coloured sand typically without pebbles. The Woolwich and Reading series, which lie above and often pass almost imperceptibly into the



TEXT—FIG. 1.

Chalk.

Clay with Flints.

Alluvium.

Lower Eocene Formations.

Scale : 1 Inch=1 Mile.

The woods are shown in heavy outline.

former, are made up of alternating bands of coarse reddish clays and gravels with thin beds of clay; the uppermost layers are generally distinguished as the Oldhaven (or Blackheath) beds, and in the neighbourhood of Faversham consist largely of dark-coloured, well-rounded, flint pebbles. The map reproduced in Text-fig. 3 shows a Tertiary outlier made up of these three formations. The London Clay, the highest of the Lower Eocene formations, is usually not represented in the smaller outliers, but covers a large area to the east between Canterbury, Faversham, Whitstable, and Herne Bay. It consists of a stiff bluish clay which, on weathering, produces extremely heavy clay soils of a brown colour. The Swanley district only differs from the above in the relative areas of the various formations; here, while the Clay with Flints is almost absent, the Tertiary outliers are more numerous and larger in area.

Woods are of fairly general occurrence in both the above-mentioned districts, and their distribution offers some points of interest. As a general rule the Lower Eocene formations, with the exception of the Thanet Sand, do not produce soils especially suitable for agriculture, and, as a result, the larger woods are generally found occupying the Eocene outliers. This is particularly well shown in the Swanley district, where almost all the woods are confined to the Tertiary areas and cease abruptly at the boundary of the Chalk. In the Faversham district Perry Wood (Text-fig. 3) extends over the greater part of a Tertiary outlier, while the large London Clay area towards the east is almost entirely covered by the Forest of Blean. Woods are found both on the Chalk and the Clay with Flints, and although no general statement can be made for their distribution on these deposits, on the whole they are more abundant on the latter; wherever the soil on the Chalk is of sufficient depth cultivation has taken place. The river deposits and brick-earth beds are almost exclusively arable land, and are particularly suitable for the cultivation of Hops (Pl. LXIV; for explanation see description of plate). Woods are rarely found on the alluvial deposits in the neighbourhood of the Thames Estuary.

Since the general altitude of the district is low, rarely rising above 400 feet above sea-level, and the variations are comparatively slight, it seems probable that this factor has little or no influence upon the distribution of the types of woodland. With the exception of the shallow valleys previously mentioned, the surface of the Chalk is comparatively even and the hills are generally formed by the Lower Eocene outliers resting upon it. The Clay with Flints is a relatively shallow deposit, and on this account is only responsible for slight alterations in altitude.

The amount of rainfall is rather small; measurements taken at Selling, near Faversham, for the 13 years from 1897 to 1909 are given below:[1]

[1] These statistics were supplied by Sidney Neame, Esq., of Harefield, Selling, near Faversham, to whom I desire to express my thanks.

Year.	*In.*	*Year.*	*In.*	*Year.*	*In.*
1897	26·84	1902	24·4	1907	26·61
1898	23·68	1903	40·89	1908	28·35
1899	27·4	1904	28·09	1909	36·29
1900	30·61	1905	29·24		
1901	24·94	1906	29·22		

The mean annual rainfall calculated for the 42 years ending 1909 was 29·13 in.

Almost all of the woods under consideration are of the copse type, felling taking place usually at intervals of 14–15 years. The larger growths thus obtained are used extensively as Hop-poles, while the rest is made into faggots. In the cases where felling is complete and all the trees are cut off, the resulting woodland may be described as 'coppice', the term being used as suggested by Nisbet (12). In the majority of the woods standard trees occur amongst the underwood which are only felled at considerably longer intervals; a wood of the latter kind may be distinguished as 'copse'.

As a result of felling there is a great increase in the number of herbaceous plants. The factors bringing about this increase will be discussed later, but it is necessary to point out here that in many cases the species which appear are not typical woodland plants. Comparatively few species can exist under the deep shade cast during the later years of the coppice growth, and, as a result of felling, a stretch of almost unoccupied soil is produced, which is rapidly colonized by plants from various sources. In the following description of the types characteristic of various soils, all the plants occurring in the woods will be considered, whether they are true woodland species or not.

In this part of Kent, where cultivation is widespread and has gone on for a long period, little can be said as to the original condition of the woodland. Probably all the woods under consideration are of comparatively recent origin, and it is extremely doubtful whether any of the primitive forest remains. It appears that planting went on fairly extensively in these copses until quite recent times, but that this has been largely discontinued of late years on account of the great decrease in prices obtained for the underwood. At the present time it consists to a great extent of merely filling in the gaps left by the death of some of the 'stools'. In many cases, too, little consideration is paid to the suitability of the soil to the particular species planted. As a result of these facts, it is usually difficult to distinguish certain species as characteristic of particular soils when trees only are considered. The occurrence of herbaceous plants is a much better guide for generalizations of this kind.

In this district the distribution of the various types of woodland is intimately connected with the differences in the soil composition. In the ensuing description, therefore, the different geological formations are taken as the basis for classification.

In the present communication the relative prevalence of plants will be indicated by the use of certain terms. These will be employed in the following order:—

1. Very abundant.
2. Abundant.
3. Frequent.
4. Generally distributed.
5. Sparingly found.
6. Occasionally found.

No numerical value can be attached to these terms, but as far as possible the same term will always be used to express the same degree of prevalence. It will be noticed that the term 'dominant' has been omitted from the above list. The use of this term is considered unsatisfactory in this sense. In the following description the use of this expression will not necessarily indicate that the plant is very abundant, but that its presence has a great and well-marked effect on the immediately surrounding vegetation. Every plant obviously influences the plants in its vicinity to a certain extent, but the term 'dominant' is reserved for extreme cases in which the existence of the surrounding species is threatened or even rendered impossible.

In this paper the plant names adopted are those given in the 'Manual of British Botany', by C. C. Babington, ninth edition, edited by H. and J. Groves, 1904. The Mosses are named in accordance with the 'British Moss Flora', by R. Braithwaite, 1887. 'The Hepatics of the British Islands,' by H. W. Lett, 1902, has been used for naming the Liverworts.

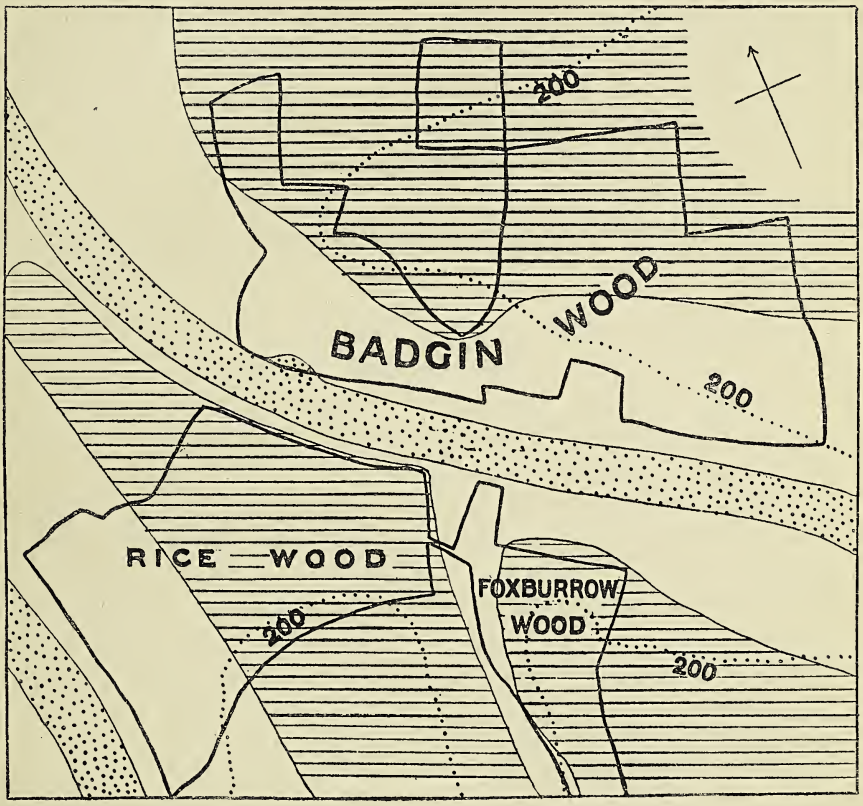
The Chalk.

The woods on the Chalk soils in both of the districts investigated are fairly numerous, but usually not of great area. In almost all cases they are found on the sides of the dry valleys already referred to, since it is only in these positions that the Chalk is free from the overlying deposits. The soils vary considerably in depth; while in some places the Chalk is barely covered, in others there is a considerable depth of soil upon it. Many of the woods examined extend over two or more different deposits, such, for example, as the Chalk and the Clay with Flints or the Chalk and the Thanet Sand, and the Chalk soils probably vary in composition with the proximity of these other deposits. Considerable differences in depth of soil may also be found in one small woodland area.

The composition of various Chalk soils is given by Hall and Russell in their 'Report on the Soils and Agriculture of Kent, Surrey, and Sussex' (7). The percentage of calcium carbonate, although variable, is usually high; as a result of this fact these soils, even in woodland areas, are rarely or never acid. Mechanical analysis shows that the amount of finely divided material (fine sand, silt, and true clay) is usually large.

The following woods on the Chalk have been investigated:—In the Faversham district: Winding Hill Wood, portions of Badgin, Rice, Foxburrow, Stocking, and Judd's Woods. In the Swanley district: portions of Rowhill and Joyden's Woods.

The woods on the Chalk consist typically of Beech interspersed with



TEXT—FIG. 2.

Chalk. Clay with Flints.

Alluvium. 200 Contours.

Scale. 6 Inches=1 Mile.

The woods are shown in heavy outline.

Yews. Moss, Rankin, and Tansley (13) have suggested that the original woodland of the Chalk was made up of Beech with an undergrowth of Yew, and the general occurrence of such woods in this neighbourhood bears out this conclusion. As already pointed out, the soil in these situations is often extremely shallow, and is therefore unsuitable for cultivation. On this

account it is possible that traces of the original woodland may remain in these spots, although its disappearance from the surrounding deposits is complete. The Yews give a characteristic appearance to the woods (Pl. LXIV ; for explanation see description of plate), being found in coppice as well as in high wood. In the former they frequently remain uncut during the felling, as Yew timber is at present almost useless.

The majority of the woods are regularly coppiced, but a few high woods are found. In these, although Beech is always abundant, a number of other species are found. Oak occurs where the soil is sufficiently deep, and in a few cases *Pinus sylvestris*, L., has been planted to a small extent. Small plantations of Larch are frequently found on the Chalk, these being, in most cases, of recent origin.

A detailed examination of Badgin Wood, near Faversham, has been made, and the southern part of this may be taken as an example of copse on the Chalk.

1. Badgin Wood, which lies about two miles to the south of Faversham (Text-fig. 1), is about half a mile in length, and varies from a quarter to a third of a mile in width. It may be noted that the boundaries of this wood shown in a map of the district published by Cowell (4) in 1839 in his 'Flora of Faversham' coincide exactly with those of the present day.

The wood is situated partly on the Chalk and partly on the Clay with Flints, while a very small area near the south-western corner lies on alluvial deposits (Text-fig. 2). The Clay with Flints forms a small plateau in the northern part of the wood, the whole of which lies at an altitude of rather more than 200 feet. The southern portion lies on a gentle slope, and here the Chalk is only covered with a thin layer of soil. The bottom of the dry valley, situated to the south of the wood, is occupied by alluvium (Pl. LXIV).

The whole of the wood consists of copse which is regularly felled at intervals of about fourteen years, and at the present time different parts of the wood show various ages of coppice growth. The depth of soil above the Chalk in the southern portion of the wood varies from six inches to two feet. Beech occurs most abundantly on the shallowest soil both as a standard and also as coppice growth, and at these spots the Yew is also found. These shallow soils are characterized by a considerable number of species among the underwood. The following occur frequently :—

Clematis Vitalba, L.
Acer campestre, L.
Euonymus europaeus, L.
Pyrus Aria, Ehrh.
Cornus sanguinea, L.
Viburnum Lantana, L.
Ligustrum vulgare, L.
Fraxinus excelsior, L.
Quercus pedunculata, Ehrh.
Betula verrucosa, Ehrh.
Corylus Avellana, L.

Towards the south-east corner the soil increases in depth to one foot to

eighteen inches, and here both Beech and Yew are absent, being replaced by Ash with occasional Hornbeam.

In addition to the above-mentioned species the following are generally distributed :—

Crataegus Oxyacantha, L.
Prunus spinosa, L.
Rosa canina, L.
Lonicera Periclymenum, L.
Ruscus aculeatus, L.

The following are occasionally found :—

Rhamnus catharticus, L.
Hedera Helix, L.
Viburnum Opulus, L.
Salix Caprea, L.

Betula alba, L., has been planted in one portion of the wood over a small area, and in another part *Acer Pseudo-platanus*, L., has been introduced ; the latter is regularly coppiced.

The distribution of herbaceous plants is greatly influenced by the age of the coppice, and in consequence different portions of the wood possess very different floras. In the tenth year after felling the coppice growth has attained a height of about twelve feet. A deep shade is produced, and the number of species which can exist under these conditions is comparatively small, the vegetation, as a whole, being poor and scanty. During the following four years before felling takes place again the conditions are fairly constant, the additional shade due to the increased height being almost inappreciable. The plants found during this period may be divided into two groups :—

(*a*) Plants little or not affected by the shade, in which flowering takes place early in the spring.

(*b*) Dwarf plants which persist in the vegetative state and rarely flower.

(*a*) The plants belonging to the first group are generally perennials possessing underground storage organs. Flowering takes place early in the spring before the leaves are produced by the trees, and during the later part of the season only the underground portions can be found. The following belong to this group :—

Primula acaulis, L.
Mercurialis perennis, L.
Endymion nutans, Dum.
Arum maculatum, L.

Mercurialis perennis is abundant, occurring in large patches and flowering and fruiting freely. This plant is the most conspicuous species of the shade flora as the stems and leaves persist until the late autumn. The remaining plants are found very sparingly where the soil is nine inches or less in depth, but occur more freely where the depth is greater; even on the deeper soils they are not plentiful. *Adoxa Moschatellina*, L., has been occasionally found on the outskirts of the wood, but its occurrence on the Chalk soils is too rare to justify its inclusion here. *Daphne*

Laureola, L., is often present, and on account of the similarity in its time of flowering may be placed in this group.

(*b*) The following are included in the dwarf shade flora:—

Anemone nemorosa, L.	*Fragaria vesca*, L.
Viola Riviniana, Reich.	*Potentilla Fragariastrum*, Ehrh.
Viola hirta, L.	*Veronica Chamaedrys*, L.
Geranium Robertianum, L.	*Lamium Galeobdolon*, Crantz.
Rubus fruticosus, L.	*Ajuga reptans*, L.
Geum urbanum, L.	

These plants persist under the shade conditions for a number of years, flowering rarely or not at all, and producing stunted leaves and stems. With the exception of *Geranium Robertianum* they are all normally perennial, and it is probable that the latter plant under these peculiar conditions persists for several years. The distribution of *Anemone nemorosa* varies considerably with the depth of soil and the density of the shade. It is completely absent on the shallowest soils under deep shade conditions, but appears on these soils in the vegetative form wherever the illumination is slightly greater. Its abundance increases on the deeper soils, and here, even in the deepest shade, it occurs frequently and flowers occasionally. Finally, on the deepest Chalk soils it is abundant and a large proportion of the plants produce flowers.

Of the remaining plants none are found abundantly, and in situations where the Anemone is absent areas of bare soil are often found. *Viola Riviniana* flowers more frequently than the other species, but even in this case the number of flowers produced is much less than in situations where the light intensity is greater; *Viola hirta* is of rare occurrence. The plants of *Rubus fruticosus* are small and few in number, and, as a general rule, flowers are not produced.

Dobner (5), who has mentioned the occurrence of a similar dwarf flora in the Hochwälder of Germany, refers to the persistence of *Rubus fruticosus* in the vegetative state under shade conditions (footnote, p. 46).

A great change takes place in the character and abundance of the vegetation after the cutting of the copse. Felling takes place usually during December and January, and, with the exception of the standards and Yews (when these are present), the whole of the underwood is removed. This results in a great increase of the light intensity, and the mean temperature of the surface soil is raised. As a consequence, increased evaporation goes on from the surface, and the upper layers of soil are much drier in the cleared areas than in those still covered by wood.

The plants which are found in the following spring and summer are

partly made up of those formerly present during the shade condition, but in addition to these many others appear.

The plants previously mentioned as flowering early in the spring are not greatly affected by the changed conditions. The short interval of increased illumination elapsing since felling has not been of sufficient duration to allow for the increase of those species, such as *Primula acaulis*, which benefit by the brighter light. The leaves and stems of *Endymion nutans* show a considerable decrease in size in consequence of the increased illumination; the greater dryness of the soil probably adversely affects this species.

On the other hand, the dwarf shade flora spreads rapidly, and at the same time the individual plants increase greatly in size. These plants form a considerable proportion of the vegetation found in the succeeding years, and are often of striking appearance. This is particularly the case with *Veronica Chamaedrys* and *Fragaria vesca*, both of which flower freely. *Rubus fruticosus* usually flowers sparingly in the first year after felling, but never becomes abundant on the shallow Chalk soils.

In addition to the above a large number of plants appear as seedlings, chiefly in the late summer. A considerable number of these are species commonly found in the open spaces of the woods, but many are not woodland plants in the ordinary sense, being normally found in fields, hedgerows, and on cultivated land. The great majority of these are biennials or perennials, which generally pass the first season in the vegetative condition and flower for the first time during the second summer.

The following annual plants are also found, and these flower in the first season:—

Arenaria trinervia, L.
Linum catharticum, L.
Alchemilla arvensis, L.
Torilis Anthriscus, Gaert.
Erythraea Centaurium, Pers.
Odontites rubra, Gilib.
Galeopsis Tetrahit, L.

Of the above *Linum catharticum* is the only species which occurs frequently. *Alchemilla arvensis* is generally distributed, and the remainder are occasionally found.

The annuals form only a small proportion of the vegetation, and at the same time the plants are mostly small. Consequently, the general effect is given by the biennials and perennials. During the first season, therefore, the flora is characterized by great vegetative development and a general absence of flowers.

During the second season the reverse is the case. In the early spring *Mercurialis perennis* is abundant, spreading in large clumps over the whole Chalk area and on the deeper soils forming a complementary association with *Endymion nutans*. The latter species is, however, not abundant; it is

probable that it is adversely affected by the competition of the light flora. *Primula acaulis* and *Anemone nemorosa* occur frequently and flower freely.

The dwarf shade flora develops luxuriantly in the second season and flowers more abundantly than during the first year. *Veronica Chamaedrys*, *Viola hirta*, and *Fragaria vesca* are abundant. *Viola Riviniana* is of much less frequent occurrence, although during the shade period the latter is the more plentiful of the two species.

The following perennials are very abundant :—

Helianthemum Chaemaecistus, Mill.
Hypericum hirsutum, L.
Myosotis sylvatica, Hoffm.
Teucrium Scorodonia, L.
Origanum vulgare, L.
Euphorbia amygdaloides, L.

The following are abundant :—

Hypericum perforatum, L.
Senecio erucifolius, L.
Echium vulgare, L.
Verbascum Thapsus, L.

The following are frequently found :—

Reseda lutea, L.
Polygala vulgaris, L.
Poterium Sanguisorba, L.
Dipsacus sylvestris, Huds.
Carduus palustris, L.
Inula Conyza, DC.

The following are generally distributed :—

Geranium columbinum, L.
Carduus nutans, L.
Arctium majus, Bernh.
Scrophularia nodosa, L.
Veronica officinalis, L.
Clinopodium Calamintha, O. Kuntze.
Prunella vulgaris, L.
Verbena officinalis, L.

The following are sparingly found :—

Aquilegia vulgaris, L.
Sagina procumbens, L.
Scabiosa columbaria, L.
Clinopodium vulgare, L.
Clinopodium Acinos, O. Kuntze.
Thymus Serpyllum, L.
Orchis pyramidalis, L.
Carex sylvatica, Huds.

The following are occasionally found :—

Daucus Carota, L.
Galium Mollugo, L.
Ophrys muscifera, Huds.
Habenaria bifolia, R. Br.

During the first year the shoots from the stools grow rapidly, and at the end of the season have attained a height of 3–4 feet. The growth during the second year is not so great, the average height at the end of the season being 5–6 feet. The luxuriance of the herbaceous vegetation during the third year is as great as it is during the second, and is similar to it in character. But during the fourth year the effect of the shade produced by the underwood, which now reaches 8 feet in height, is beginning to be felt, and the number of the herbaceous plants begins to decrease.

An attempt was made to determine the order of disappearance of the various species in consequence of the increasing shade, but this was found to be impossible. The depth of the shade varies greatly at different spots; where the stools are far apart or where one has died the light flora persists in almost unaltered composition for several years, while in situations of deeper shade the constituent species disappear practically simultaneously. *Hypericum perforatum* seems to persist longer than the majority of the species. By about the tenth year the whole of the light flora has disappeared, and from this time until felling takes place the vegetation is almost in a state of equilibrium.

The number of Mosses occurring is comparatively small, but a few are worthy of mention. In this neighbourhood *Anomodon viticulosus*, H.T., is confined to woods on the shallow Chalk soils; this species is not found in deeply shaded situations. *Neckera complanata*, Hueb., has an almost similar distribution, but can exist in deeper shade.

2. Winding Hill Wood, near Faversham, may be taken as an example of high wood on the Chalk. The majority of the trees are Beech; Oak is sparingly found. Sycamore, *Pinus sylvestris*, L., and *Picea excelsa* have been planted to a small extent, but the two latter species do not flourish on the Chalk soils. The shade cast by the trees is, on the whole, not so deep as that produced by close coppice. In consequence the ground flora is more abundant than that found in the copses during the shade period, although closely related to it in composition.

The Clay with Flints.

The distribution of the Clay with Flints has already been shortly described. In the Faversham district it covers large areas of the Chalk, the thickness of the deposit varying from a few inches up to 20 feet or more. It consists of a stiff reddish clay containing a very variable proportion of large unworn flints. In composition it differs from the soils of the Chalk formation which it overlies, particularly with regard to the proportion of lime. Hall and Russell (7), in analyses of the clay from two woodland areas in Kent, find the percentage of calcium carbonate to be 0·06 and 0·002 respectively. Mechanical analysis shows a large proportion of finely divided material, the average amount of fine sand being 27 %, the silt 27 %, and the clay 16 %. In spite of these great differences in composition, the vegetation of the Chalk and the Clay with Flints shows considerable agreement.

The whole of the woods on the Clay in this neighbourhood are regularly coppiced.

The following woods on the Clay with Flints have been investigated in the Faversham district:—

Portions of Badgin, Rice, Foxburrow, and Stocking Woods.

A detailed examination of the plant distribution in Badgin Wood has been made, and in the first place a description of this wood will be given.

(*a*) The northern portion of Badgin Wood is situated on the Clay with Flints, the deposit thinning out towards the south, while it is 15 feet or more in depth at the northern boundary (Text-fig. 2). The highest point in this area reaches an altitude of 215 feet, and the western portion falls slightly below the 200 foot contour. On the whole the surface of the deposit is approximately level.

Standard Oaks (*Quercus pedunculata*, Ehrh.) are found over the whole area, probably about sixty years of age and 30–40 feet in height; the lowest branches in these are from 12 to 15 feet from the ground, so that they clear the underwood when at its greatest height. The copse shows considerable differences in its component species, the variation being probably partly dependent on the depth of the soil. At the southern limit of the Clay, where the soil is only about 2 feet in depth, the underwood is almost similar to that of the deeper Chalk soil. Here the Hazel is the most abundant tree, but Ash and Hornbeam are found, as well as several other species. Passing towards the north there is an increase in the amount of Ash and Hornbeam, and, where the soil is approximately 3 feet in depth, these two species together equal the Hazel in abundance; at this point the Oak, Maple, and Birch show a corresponding decrease, while the Beech, *Pyrus Aria*, Ehrh., *Euonymus europaeus*, L., *Cornus sanguinea*, L., and *Ligustrum vulgare*, L., have entirely disappeared. On still deeper soil Ash and Hornbeam are the most abundant species, although the Hazel is still found. In some small areas Hornbeam only is found, while in others, although Ash is the most abundant species, it is never exclusively present.

The following occur sparingly:—

Crataegus oxyacantha, L.
Prunus spinosa, L.
Prunus avium, L.
Ilex aquifolium, L.
Populus nigra, L.
Populus tremula, L.
Castanea sativa, Mill.
Ruscus aculeatus, L.

In many parts of England Ash is characteristic of calcareous soils, well developed woods occurring on the Chalk of Hampshire, Dorset, and the Isle of Wight (Moss, Rankin, and Tansley (13), pages 137 and 140). On the other hand, in North-East Kent, Ash is only found sparingly on the Chalk, while it is much more abundant on the almost non-calcareous Clay with Flints. In this district, where the rainfall is considerably lower than in the more northern and western counties, it is possible that scarcity of water is the limiting factor on shallow rapidly draining soils such as the Chalk. The Ash is a species which makes large demands on the mineral salts of the soil for its full development. In a situation where the water supply is abundant the necessary food can be obtained even on a poor soil. The Chalk soils, how-

ever, contain a small proportion of potash and phosphates, and this circumstance, in conjunction with the deficient water supply, probably accounts for the scarcity of ash in these localities.[1]

It is probable that in this district the great majority of the woods on the Clay have been planted in comparatively recent times. In Badgin Wood the similarity in age of all the standard Oaks may be taken as evidence for this as well as the regularity in the distance between the stools. This fact, in conjunction with the considerable number of species now found occurring on this deposit, makes any statement as to the composition of the original woodland very difficult. The limited area studied in this investigation does not justify any conclusion.

The shade flora of the Clay resembles that found on the Chalk, but the species are found in considerably different proportions. The following plants are found flowering freely in the early spring :—

Anemone nemorosa, L.	*Arum maculatum*, L.
Adoxa Moschatellina, L.	*Endymion nutans*, Dum.
Primula acaulis, L.	*Orchis mascula*, L.
Mercurialis perennis, L.	*Orchis maculata*, L.

Endymion nutans and *Mercurialis perennis* occur abundantly, forming a complementary society, the rhizomes of the latter growing close to the surface some distance above the deeply buried bulbs of the Bluebell. *Arum maculatum* and *Primula acaulis* are present in considerably increased amount, while *Adoxa Moschatellina* is found frequently wherever the soil is more than 4 feet in depth. *Anemone nemorosa* is abundant and flowers freely ; it is interesting to note that under similar shade conditions on the shallow Chalk soils flowers are only sparingly produced by this plant.

Orchis mascula and *Orchis maculata* are occasionally found. *Daphne Laureola*, L., is absent, but sometimes *Monotropa Hypopitys*, L., a plant often abundant on the Chalk, is present.

All the plants of the dwarf flora given above (see page 866) are present on the Clay with the exception of *Viola hirta*, which is confined to the Chalk. Although *Lamium Galeobdolon* and *Ajuga reptans* are found in increased amount, the plants as a whole are not abundant.

The flora which appears after felling resembles, in general character, that already described on the Chalk. The majority of the plants which appear are biennial or perennial and flower for the first time in the second year. The number of annuals flowering during the first season is greater than that found on the Chalk.

[1] It is interesting to note in this connexion that Ash is found abundantly on calcareous alluvial soils which possess a good water supply. The copses occurring in the Valley of the Darenth in the neighbourhood of Farningham (North-West Kent) contain a large proportion of this species both as standard and as coppiced trees. The soil in this situation is largely derived from the washing of the surrounding chalk slopes.

The greater part of the vegetation found in the first summer is made up of seedlings of biennials and perennials, but amongst these several annuals may be found in flower. *Anagallis arvensis*, L., occurs frequently in some spots, and *Sonchus asper*, Hill, is spread sparingly over the whole area.

The following are occasionally found :—

Linum catharticum, L.
Alchemilla arvensis, Scop.
Gnaphalium uliginosum, L.
Senecio sylvaticus, L.
Lithospermum arvense, L.
Melampyrum pratense, L.
Galeopsis Tetrahit, L.
Polygonum Persicaria, L.
Polygonum aviculare, L.
Juncus bufonius, L.

Although the great majority of the biennials and perennials of the light flora only exist in the vegetative condition during the first season, a few may occasionally be found just coming into flower in the late summer of the first year. During the latter part of August, 1910, a few flowering plants of *Hypericum perforatum* and *Sanicula europaea* were found in an area felled in the previous January ; solitary plants of *Lychnis alba*, Mill., and *Hypochaeris radicata*, L., were also seen in a similar condition. *Hypericum humifusum*, L., is the only perennial plant discovered in which flowering regularly takes place during the season in which it first appears. This plant is not abundant on the Clay, but is easily noticeable on account of this peculiarity.

Although plant distribution on the Clay is largely correlated with the depth of the deposit, a number of plants are found occurring in equal abundance over the whole area. These are included in the following list :—

Anemone nemorosa, L.
Dipsacus sylvestris, Huds.
Senecio erucifolius, L.
Echium vulgare, L.
Myosotis sylvatica, Hoffm.
Euphorbia amygdaloides, L.

The following have a similar distribution and are frequently found :—

Polygala vulgaris, L.
Geranium columbinum, L.
Bunium flexuosum, Fr.
Arctium majus, Bernh.
Carduus nutans, L.
Scrophularia nodosa, L.
Veronica officinalis, L.

Others, while found over the whole area, increase in abundance with the increased depth of soil :—

Sanicula europaea, L.
Carduus palustris, L.
Nepeta Glechoma, Benth.

The following are only found on the shallow soil in the near vicinity of the Chalk :—

Linum catharticum, L.
Hypericum hirsutum, L.
Teucrium Scorodonia, L.
Habenaria bifolia, R. Br.

Similarly several plants occur only on the deepest soil near the northern boundary of the wood :—

Gnaphalium uliginosum, L.
Rumex obtusifolius, L.
Rumex sanguineus, L.
Polygonum aviculare, L.
Polygonum Persicaria, L.
Juncus bufonius, L.
Bromus erectus, Huds.
Bromus ramosus, Huds.
Poa nemoralis, L.

Viola Riviniana and *Viola Reichenbachiana* are both found, the former occurring in abundance on the greater part of the Clay, while the latter is almost confined to the deeper soils. Over a considerable part of the area the soil is approximately 4 feet in depth, and several plants are found here which are usually absent from the shallower and deeper soil :—

Hypericum perforatum, L.
Digitalis purpurea, L.
Anthoxanthum odoratum, L.
Holcus lanatus, L.
Holcus mollis, L.
Pteris aquilina, L.

Digitalis purpurea is scattered, while *Pteris aquilina* is found in a few small clumps. Several Grasses, on the other hand, are abundant, especially *Anthoxanthum odoratum* and *Holcus mollis*. This latter is a widely distributed woodland species and its occurrence here is not unexpected, but this is not the case with *Anthoxanthum*. These species each possess a shallow root system and would presumably come into direct competition with each other ; the time of flowering is, however, different, and the growth of *Anthoxanthum* is to a large extent completed before the flowering period of *Holcus mollis* commences. As a result of the Rothamstead manurial experiments, Lawes, Gilbert, and Masters (10) found that *Anthoxanthum odoratum* became prominent in unmanured plots where there was small growth of herbage and little activity of struggle. In experiments carried out at Chiswick, where *Anthoxanthum* was grown separately, the highest degree of vigour was obtained when both mineral and nitrogenous manures were supplied. The rapid decomposition of the humus accumulated during the shade period would probably produce somewhat similar conditions to those of the latter experiment, and at the same time the competition here would be considerably less than in a meadow. These considerations, together with the wide distribution of *Anthoxanthum*, perhaps partly explain its abundance in this situation. *Holcus lanatus*, another shallow-rooting species, also occurs in considerable abundance. This plant, in the Rothamstead grass plots, grows best under manurial conditions which do not tend to general luxuriance ; it attains its greatest percentage with liberal nitrogenous and mineral manure.

Hypericum perforatum occurs in considerable quantity over the whole Clay area, but is especially abundant on soil varying from 3 to 5 feet in depth. On the shallow soils it is partly replaced by *Hypericum hirsutum*, while on

the deeper ones the great abundance of *Carduus palustris* probably has a detrimental effect on its distribution..

In addition to the above-mentioned plants, the following biennials and perennials are occasionally found :—

Sagina procumbens, L.
Hypericum pulchrum, L.
Epilobium roseum, Schreb.
Daucus Carota, L.
Dipsacus pilosus, L.
Gnaphalium sylvaticum, L.
Solanum Dulcamara, L.
Veronica serpyllifolia, L.
Prunella vulgaris, L.
Orchis mascula, L.
Orchis maculata, L.
Gymnadonia conopsea, R. Br.
Listera ovata, R. Br.
Juncoides pilosum, O. Kuntze.
Brachypodium sylvaticum, Beauv.

A considerable number of weeds of cultivated ground, hedgerow plants, and plants of waste places (ruderal plants) are occasionally found on the Clay ; of these the following annuals flower in the first season :—

Alliaria officinalis, Andrz.
Stellaria media, Vill.
Arenaria serpyllifolia, L.
Torilis Anthriscus, Gaert.
Matricaria inodora, L.[1]
Senecio vulgaris, L.
Solanum nigrum, L.
Plantago lanceolata, L.

The following perennials flower for the first time in the second season and generally disappear in the later years :—

Lepidium heterophyllum, Benth.
Viola tricolor, L.
Lychnis alba, Mill.
Vicia sepium, L.
Chaerophyllum sylvestre, L.
Heracleum Sphondylium, L.
Helminthia echioides, Gaert.
Hypochaeris radicata, L.

Few Mosses are found in the shade produced by the older coppice growth, but after felling there is a considerable increase in number. Some of those which appear behave similarly to the biennial flowering plants with regard to fruiting. *Catharinea undulata* (L.), Web. Mohr, exists for the most part in the vegetative state only during the first season, but fruits abundantly during the second year.

Polytrichum juniperinum, Willd., *Polytrichum attenuatum*, Menz., and *Mnium hornum*, L., also show this, but in a less degree. The distribution of these species is associated with differences in the depth of soil. *Polytrichum juniperinum* forms a narrow band on the shallow soil in the neighbourhood of the Chalk, but is not found on the deeper soil. On slightly deeper soil it is replaced by a similar band of *Polytrichum attenuatum*. *Catharinea undulata* is abundant, but occurs only where the soil is more than about three feet in depth. *Mnium hornum* is usually confined to soil from three to four feet in depth, but is not found abundantly. Finally, *Mnium undula-*

[1] This plant is occasionally perennial.

tum, L., occurs sparingly only on the deepest parts of the Clay. In addition to the above-mentioned plants, a considerable number of other species occur; *Stereodon cupressiformis* (L.), Brid., *Hypnum rutabulum*, L., and *Thuidium tamariscifolium* (Neck.), Lindb., are frequently found.

In the eastern part of the wood the age of the coppice over the whole extent of the Clay nowhere exceeds three years, and here a definite zonation of the vegetation can be found in consequence of the above described distribution. The following zones can be distinguished :—

I. The soil is less than two feet in depth. The flora is in many respects similar to that of the Chalk, although several plants constantly found on the latter formation, such as *Reseda lutea*, *Origanum vulgare*, and *Helianthemum Chamaecistus*, do not appear. *Teucrium Scorodonia* is abundant, and *Hypericum perforatum* is frequent. Large patches of *Polytrichum juniperinum* are found, and *Cladonia pyxidata* occurs sparingly.

II. Depth of soil from 2 to 3 feet. *Teucrium Scorodonia* is less abundant, and there is a considerable increase in the amount of *Hypericum perforatum*. *Holcus mollis*, *Holcus lanatus*, and *Anthoxanthum odoratum* occur sparingly. *Polytrichum attenuatum* replaces *Polytrichum juniperinum* and *Mnium hornum* is frequently found.

III. Soil 3–4 feet in depth. The Grasses (*Holcus mollis*, *Holcus lanatus*, and *Anthoxanthum odoratum*) are abundant, giving a characteristic appearance to the flora. *Hypericum perforatum* is also abundant. *Carduus palustris* and *Sanicula europaea* are frequently found. Both species of *Polytrichum* are absent, and are replaced by *Catharinea undulata* and *Mnium hornum*.

IV. Soil 4–6 feet in depth. *Carduus palustris* is abundant, while there is a decrease of the Grasses and of *Hypericum perforatum*. *Adoxa Moschatellina*, absent from the shallower soils, is found, while *Sanicula europaea* shows a considerable increase. *Catharinea undulata* is abundant, while *Mnium hornum* has disappeared.

V. Soil over 6 feet in depth. *Carduus palustris* is very abundant, the plants being tall and luxuriant. *Sanicula europaea* and *Adoxa Moschatellina* are also abundant. *Rumex obtusifolius*, *Rumex sanguineus*, *Polygonum aviculare*, and *Polygonum Persicaria* are sparingly present. *Mnium undulatum* is found in addition to *Catharinea undulata*.

Analyses of soil from the different zones are not yet available, and until these are obtained no definite statements as to the cause of the above distribution can be made. Meanwhile it may be suggested that the zonation is largely influenced by the variations in the water content. In the shallow soils the drainage through the easily permeable chalk will be rapid, and the water content correspondingly small, but, with the increasing thickness of the clay, the drainage will be slower and the percentage of water retained by the soil will be greater. The lifting power also will be increased.

In support of this view the greater luxuriance of the vegetation on the deeper parts of the deposit may be cited; the similar increase in abundance of *Carduus palustris*, a plant commonly found in damp situations, may also be given.

The Moss distribution also leads to the same conclusion. *Polytrichum juniperinum*, found on the shallowest soils, is strongly xerophytic, possessing inrolled leaves provided with lamellae on the upper surface. In *Polytrichum attenuatum*, occurring on the slightly deeper deposit, the leaves are larger and flat but retain the lamellae. *Mnium hornum* and *Catharinea undulata* possess flat leaves of the usual structure and are typical mesophytic species. Finally, in *Mnium undulatum*, which is found on the deepest soil and is a commonly occurring species in damp woods, the leaves are large, flat, and very delicate in structure. These species form a series showing diminishing xerophytic characters with the increase in depth of the soil.

The Clay with Flints is known to vary considerably in composition and the percentage of lime may be another factor in determining the above zonation.

(*b*) Rice Wood lies a short distance to the south-west of Badgin Wood (Text-fig. 2). Here the Clay with Flints, instead of presenting a level surface, is found sloping to the north-east, and the flora occurring on it exhibits several differences from that found on the deposit in Badgin Wood.

Rice Wood consists of copse with standard Oaks, and while the species making up the underwood are generally similar to those occurring on the deeper Clay of Badgin Wood there is an increased amount of *Castanea sativa*, Mill. On the whole the wood is considerably damper, and although the soil is not more than eight feet in depth in any place the vegetation with some exceptions resembles that found on the deepest soil in Badgin Wood. *Anemone nemorosa*, L., occurs in great abundance and flowers freely under the shade condition. *Primula acaulis*, L., is found in only slightly smaller numbers. *Oxalis Acetosella*, L., a species not present in Badgin Wood, occurs sparingly.

A portion of the copse was felled during the winter of 1909–10, and this area is now at the commencement of the second year's growth. During the first season *Anagallis arvensis*, L., and *Gnaphalium uliginosum*, L., were abundant and flowered feely. *Hypericum perforatum*, L., *Carduus palustris*, L., and *Sanicula europaea*, L., occurred in abundance in the vegetative condition. Bryophytes are present in much greater numbers than in Badgin Wood. *Mnium undulatum*, L., is abundant, while *Plagiochila asplenioides*, L., and *Porotrichum alopecurum* (L.), Mitt., are frequently found.

Zonation of *Polytrichum juniperinum*, Willd., and *Polytrichum attenuatum*, Menz., similar to that already described in Badgin Wood occurs near the western limit where the deposit of Clay thins out in the vicinity of the Chalk (Text-fig. 2). The zone of *Polytrichum juniperinum* is found nearer to

the latter formation, while that of *Polytrichum attenuatum* occurs next to it on the deeper soil towards the north-east.

The differences in the flora of the two woods no doubt largely depend on the different aspects of the two areas. The north-eastern slope will receive less sunlight, and will in consequence be damper and colder than the approximately level area found in Badgin Wood. The increased humidity will account for the abundance of the bryophytic flora in this part of Rice Wood.

(*c*) A comparison has also been made with the vegetation of woods found on the Clay with Flints nearer to the Chalk escarpment and at a greater altitude. The most striking difference is due to the considerably greater amount of *Pteris aquilina* found in these woods; in some situations this species is the most abundant plant of the light flora. *Cardamine pratensis*, L., also occurs frequently in these woods and flowers freely during the second season after felling; this plant is not found in Badgin Wood.

The Loam on the Chalk.

The flora of the deposit described as Loam on the Chalk in the Geological Survey is usually indistinguishable from that found on the Clay with Flints. A portion of Stocking Wood near Faversham occurs on this deposit, and in this wood *Pteris aquilina* is generally distributed.

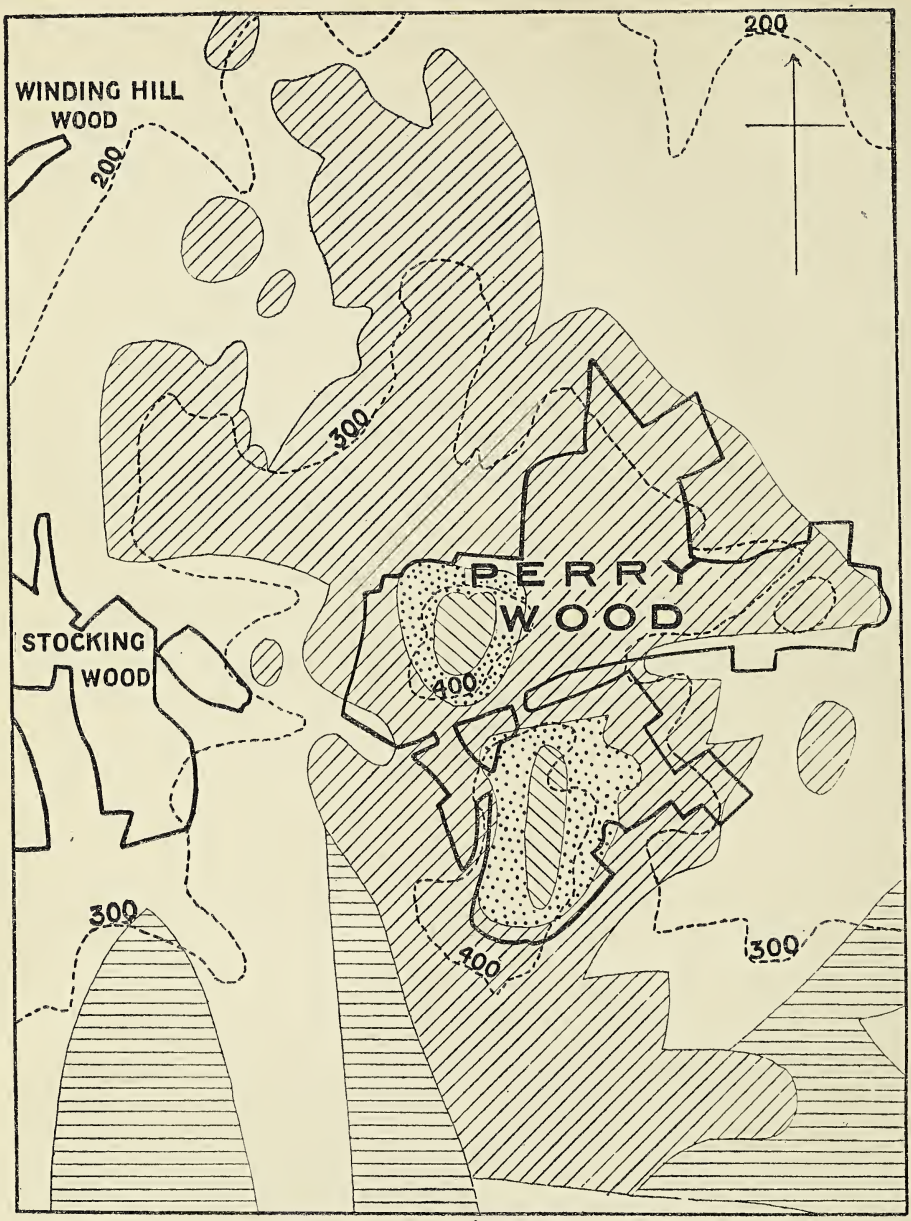
In Text-fig. 1 the Loam on the Chalk is included with the Clay with Flints.

The Lower Eocene Formations.

A considerable area of woodland is found on the Lower Eocene formations in both of the districts under consideration. In the neighbourhood of Swanley the greater part of the wood is confined to these deposits, little or none being found on the Chalk. The Eocene beds are generally found as outliers of variable extent which form small hills on the approximately even surface of the Chalk. The Thanet Sand, Woolwich and Reading series, and the Oldhaven pebble beds are frequently all represented in such an outlier, but often only the first or first and second of these formations are found. Occasionally there is also a capping of London Clay, but usually this deposit is found in the main mass of the Eocene formations.

The Thanet Sand.

The Thanet Sand is well represented in both districts, and gives rise to soil which on the whole is well suited for agriculture and is particularly favourable for the cultivation of fruit. In consequence the woods on this deposit are not usually extensive.



TEXT—FIG. 3.

Chalk.
Thanet Sand.
Oldhaven Beds.
Clay with Flints.
Woolwich & Reading Beds.
300 Contours.

Scale: 3 Inches=1 Mile.

The woods are shown in heavy outline.

The Thanet Sand consists of a fine light-coloured sand without pebbles, and gives rise to a light, frequently loamy soil. Chemical analyses show a small amount of calcium carbonate varying from 0·02 to 0·38 % (Hall and Russell (7)), and the soil in these woods generally gives an acid reaction with litmus paper.

The woods in almost all cases consist of coppice, generally without standards, frequently made up almost of Chestnut (*Castanea sativa*, Mill.). Hooker, in his 'Students' Flora of the British Islands' (8), places the Chestnut in the list of excluded species, and it is highly probable that the whole of these coppices have been planted. In this part of England mature specimens of the Chestnut only produce ripe seed in unusually long and warm summers, and in the case of the coppice, where felling takes place about every fourteen years, seed is only rarely ripened. No seedlings have ever been found. The regularity in the distance between the stools may also be given as evidence for the artificial origin of this type of woodland.

Although stones are not found in typical deposits of the Thanet Sand, in several cases soils described in the Geological Survey as belonging to this formation contain a considerable proportion of rounded pebbles. Soils of this kind are usually found in proximity to the Woolwich and Reading beds, and in these cases a certain amount of admixture has probably taken place between the two formations. The vegetation occurring on such soils differs in some respects from that found on the typical deposits. Certain differences are also found in the flora where the Thanet Sand thins out in the vicinity of the Chalk. These variations in the composition of the vegetation, although seen both in the light and in the shade flora, are more pronounced during the shade period. This circumstance is not unexpected when the conditions under which the light flora is produced are considered. During the shade period little or no change takes place either in the abundance of the ground flora or in its composition. The factors on which its existence depends are almost constant, and in consequence the vegetation is in a condition of equilibrium. As a result of felling, large areas of almost unoccupied soil are rendered available for plant growth, and these are rapidly seized upon by species which possess an efficient method of distribution.[1] Although many of these plants are doubtless well adapted to the soil composition a considerable number are probably enabled to develop, not because of the special suitability of the soil, but rather on account of the slight competition existing for a short period after felling has taken place. The occurrence of ruderal plants similar to those mentioned in the light flora of the Clay with Flints is probably rendered possible by the feeble competition during the first few years of the coppice growth.

It is improbable that the light flora ever attains a condition of equilibrium. The elimination of the more unsuitable species will proceed for

[1] The origin of the light flora will be discussed in a later communication.

some time, but before the process is complete the increasing shade cast by the coppice growth will begin to adversely affect the whole of the light flora. The latter will finally completely disappear, and the woodland will again present the original shade condition.

In the first place, therefore, the vegetation found on the various soils during the shade period will be separately described, and a general account of the light flora on the Thanet Sand will then be given.

I. *Flora found during the shade period.*

(*a*) Woods on the typical deposits of the Thanet Sand. The following woods of this type have been investigated in the Faversham district :—

Sandbanks Wood, portions of Kemsdale and Bysing Woods.

In these woods the soil is of considerable depth, and consists of a light sandy loam without pebbles. The coppice is made up of Chestnut with a small proportion of Elder (*Sambucus nigra*, L.), the latter forming an ill-defined lower stratum which, in the early spring, is very obvious, as the leaves of the Elder unfold some time before those of the Chestnut. The shade flora is well represented. *Endymion nutans*, Dum., is in all cases extremely abundant, while *Adoxa Moschatellina*, L., and *Ranunculus Ficaria*, L., are usually almost as plentiful. *Arum maculatum*, L., is also present, although not in such abundance. All these flower freely early in the season. It is interesting to note that all possess a more or less tuberous rootstock, and it seems that sandy soils of this nature are particularly suited to the tuberous habit. *Veronica hederaefolia*, L., and *Listera ovata*, R.Br., are found sparingly, and both of these plants bloom under the shade conditions, although in the case of the former the flowers are few in number.

The abundance of *Ranunculus Ficaria* in these coppices is striking, as this plant is rarely or never found in the woods on the Chalk, Clay with Flints, and remaining Eocene formations. The distribution of *Adoxa Moschatellina* is peculiar. Although occurring in great abundance in some of these coppices, in others, where as far as at present determined the conditions are identical, it is altogether absent. The occurrence of *Anemone nemorosa*, L., is somewhat similar, for this plant, although absent from some of these woods, occurs in considerable quantity in others. In the Chestnut coppices where it is found the Anemone flowers freely during the shade period. As already described, on the shallow soils of the Chalk this plant is not found during the later stages of the coppice growth, although it appears after felling has taken place. The factors determining the distribution of these plants are at present being investigated, and will be fully discussed in a subsequent communication.

In addition to the plants already mentioned, several are found which flower rarely or not at all, and are comparable to the dwarf shade flora of the Chalk and Clay with Flints. Of these, *Nepeta Glechoma*, Benth., is

abundant, while *Lychnis dioica*, L., *Viola Riviniana*, Reich., *Geum urbanum*, L., *Rubus fruticosus*, L., *Rumex sanguineus*, L., *Urtica dioica*, L., and *Holcus mollis*, L., are found in smaller numbers.

The Honeysuckle (*Lonicera Periclymenum*, L.) is a characteristic plant of the Thanet Sand, occurring in much greater abundance on this formation than on the Chalk and Clay with Flints.

It will be noticed that the majority of the plants already mentioned are species in which development is completed in the early summer. In consequence, in the late summer and autumn these Chestnut coppices possess practically no ground flora.

(*b*) Woods on the Thanet Sand in the neighbourhood of the Woolwich and Reading beds. The following woods of this type have been investigated :—

In the Faversham district : portions of Bysing, Judd's, and Perry Woods.

In the Swanley district : portions of Farningham, Jordan's, and Rowhill Woods. In all cases pebbles are present in the soil to a variable extent. In other respects the soil is similar to that already described on the typical Thanet Sand.

As before, Chestnut (*Castanea sativa*, Mill.) is the chief constituent of the coppices, and this species is sometimes present in the form of standards ; Oak (chiefly *Quercus sessiliflora*, Salisb., occasionally *Quercus pedunculata*, Ehrh.) is, however, the most frequently occurring standard tree. Elder (*Sambucus nigra*, L.) is not so abundant as on the typical soil. Beech, Hazel, Birch, and Holly are occasionally present. Both *Pinus sylvestris*, L., and *Pseudotsuga Douglasii*, Carr., are often planted in these copses, generally in small numbers, but sometimes forming clumps of considerable extent.

The ground flora is very scanty, and in some cases is made up entirely of Mosses. Of these, *Mnium hornum*, L., is the most abundant, and this, with smaller quantities of *Polytrichum attenuatum*, Menz., and *Leucobryum glaucum*, Schp., frequently covers more than half of the entire surface, giving a very characteristic appearance to these copses. In other cases, although the Mosses are still abundant, a few flowering plants are found. The following are generally distributed but never occur abundantly. These flower freely under the shade conditions :—

Anemone nemorosa, L.	*Primula acaulis*, L.
Oxalis Acetosella, L.	*Lamium Galeobdolon*, Grantz.
Asperula odorata, L.	*Endymion nutans*, Dum.

It is interesting to note that *Lamium Galeobdolon* flowers freely in the deep shade of the Chestnut copses on the Thanet Sand, although in the mixed copses of the Chalk and Clay with Flints it persists in the vegetative

condition only during the shade period. *Convallaria majalis*, L., and *Polygonatum multiflorum*, All., are found sparingly in the woods in the Swanley district; the latter blooms freely, but the former only occasionally produces flowers. *Neottia Nidus-avis*, Rich., is occasionally found.

The dwarf shade flora is only slightly represented; the following plants occur in small numbers:—

Rubus fruticosus, L.
Geum urbanum, L.
Solidago Virgaurea, L.
Lysimachia nemorum, L.
Nepeta Glechoma, Benth.
Ajuga reptans, L.
Rumex Acetosella, L.
Rumex sanguineus, L.
Holcus mollis, L.

These plants are present in a small stunted form and rarely flower. *Pteris aquilina* occurs on the more stony soils, producing stunted leaves destitute of sporangia.

(*c*) Woods on the Thanet Sand in the close vicinity of the Chalk. The following woods of this type have been investigated:—

In the Faversham district: portions of Bysing and Perry Woods.

In the Swanley district: portions of Farningham and Rowhill Woods.

Woods of this type generally occur on the outskirts of the Tertiary outliers and in situations where the deposit of the Thanet Sand is of slight depth. Analyses of these soils are not at present available, but it appears probable that the percentage of calcium carbonate is higher in these than in the typical soils of the Thanet Sand.

A considerable number of species make up the underwood of the copses. Chestnut (*Castanea sativa*, Mill.) is generally the most abundant, but Ash (*Fraxinus excelsior*, L.) and Hazel (*Corylus Avellana*, L.) are present in almost equal quantities, and in some cases form more than half of the underwood. Maple (*Acer campestre*, L.), Spindle (*Euonymus europaeus*, L.), Dogwood (*Cornus sanguinea*, L.), Oak (*Quercus pedunculata*, Ehrh., and *Quercus sessiliflora*, Salisb.), and Willow (*Salix Caprea*, L.) occur much less abundantly. *Viburnum Lantana*, L., *Ligustrum vulgare*, L., and *Clematis Vitalba*, L., are occasionally found. Oak standards (chiefly *Quercus sessiliflora*, Salisb.) are present in most of the copses.

The ground flora bears a close resemblance to that found on the deeper soils of the Chalk, but differs in the relative abundance of *Mercurialis perennis*, L. This plant, as already described, occurs in abundance on the Chalk soils, but on the Thanet Sand only forms isolated patches of small area. It is interesting to note that, apart from its occurrence on these soils of the Thanet Sand in the vicinity of the Chalk, *Mercurialis perennis* is altogether absent from the woods of the Lower Eocene formations.

II. *Flora produced during the light period.*

Felling takes place in the copses on the Thanet Sand at regular intervals of about fourteen years. The light flora produced agrees in general characters with that previously described in the copses of the Chalk and the Clay with Flints, but differs considerably in its specific composition. As before, it may be divided into—

(*a*) Plants of the shade flora persisting during the light period.

(*b*) Plants which can only exist during the light period.

(*a*) After felling has taken place the plants of the shade flora which flower early in the spring still persist, although in most cases in diminished numbers. The decreased abundance is particularly noticeable in the cases of *Adoxa Moschatellina*, L., *Endymion nutans*, Dum., and *Ranunculus Ficaria*, L. The coppices are generally cut in December or January, and in the spring of the following season little change can be observed in the composition of the flora. During the subsequent years, however, the species mentioned, which during the shade period were in almost undisputed possession of the soil, come into competition with the plants of the light flora. The decrease in number of these plants is due, not to the more brilliant illumination, but to the greatly increased competition. The Anemone, however, shows no decrease after felling has taken place, but increases both in the number of the individual plants and in the quantity of flowers produced.

The plants of the dwarf shade flora increase in abundance and flower freely during the first and subsequent years. *Lychnis dioica*, L., flowers during the first season, and in the next few years is one of the most striking species of the woods on the Thanet Sand; the plants are numerous and luxuriant, and flowers are produced in great abundance. On the more stony soils, *Solidago Virgaurea*, L., is widespread and flowers freely. *Nepeta Glechoma*, Benth., *Lysimachia nemorum*, L., *Rumex sanguineus*, L., and *Holcus mollis*, L., increase in amount and flower during the first summer. *Rubus fruticosus*, L., reaches its greatest development in the third and fourth year after felling. *Pteris aquilina*, L., develops rapidly with the increased illumination, and is present in abundance on the more stony soils.

(*b*) As already pointed out the flora which appears after felling agrees generally in its composition on the three varieties of soil already distinguished on the Thanet Sand. The plants making up this light flora are in many cases identical with those found during the light period in the copses on the Chalk and Clay with Flints. The resemblance between the vegetation of such dissimilar soils can no doubt be explained by the lack of competition during the years immediately following the removal of the underwood. As previously pointed out, under these conditions species with efficient seed distribution will seize upon the unoccupied areas and will

develop freely, although many of these plants under conditions of more severe competition would cease to exist, since they are not specially suited to the edaphic conditions.

As in the light floras already described, the majority of the species are biennial or perennial, and these flower for the first time in the second season after felling has taken place. A small number of annuals are found, and these flower during the first season. Of these the following are the most abundant :—

Gnaphalium uliginosum, L.
Anagallis arvensis, L.
Polygonum Persicaria, L.
Juncus bufonius, L.

The following are occasionally found :—

Cardamine hirsuta, L.
Galeopsis Tetrahit, L.
Chenopodium polyspermum, L.

Hypericum humifusum, L., occurs in considerable abundance, and, although perennial, flowers during the first season.

The following biennials and perennials are found abundantly :—

Hypericum perforatum, L.
Potentilla silvestris, Neck.
Circaea lutetiana, L.
Sanicula europaea, L.
Dipsacus sylvestris, Huds.
Senecio erucifolius, L.
Carduus palustris, L.
Echium vulgare, L.
Myosotis sylvatica, Hoffm.
Veronica officinalis, L.
Rumex obtusifolius, L.
Juncoides pilosum, O. Kuntze.
Holcus lanatus, L.
Holcus mollis, L.

The following are generally present, but do not occur in abundance :—

Hypericum pulchrum, L.
Fragaria vesca, L.
Arctium majus, Bernh.
Scrophularia nodosa, L.
Digitalis purpurea, L.
Stachys Betonica, Benth.
Anthoxanthum odoratum, L.
Agrostis vulgaris, With.
Aira caespitosa, L.
Aira praecox, L.
Melica nutans, L.

The following are occasionally found in small numbers :—

Cardamine pratensis, L.
Arenaria trinervia, L.
Lotus uliginosus, Schk.
Oenothera biennis, L.
Epilobium angustifolium, L.
Campanula Trachelium, L.
Hieracium umbellatum, var. *coronopifolium*, Bernh.
Veronica Chamaedrys, L.
Veronica serpyllifolia, L.
Juncus conglomeratus, L.
Carex sylvatica, Huds.
Arrhenatherum avenaceum, Beauv.

The following plants are generally found on the more stony soils:—

Senecio sylvaticus, L. *Aira flexuosa*, L.
Teucrium Scorodonia, L.

The following are found, generally in small quantities, on soils in the vicinity of the Chalk:—

Hypericum hirsutum, L. *Euphorbia amygdaloides*, L.
Verbascum Thapsus, L.

In addition to the above a small number of ruderal plants, weeds, &c., are found. These are present in greatest quantity during the first two years after felling and disappear later. Annual, biennial, and perennial plants are included in the list:—

Viola tricolor, L. *Solanum nigrum*, L.
Heracleum Sphondylium, L. *Polygonum aviculare*, L.
Senecio vulgaris, L. *Polygonum Convolvulus*, L.
Taraxacum officinale, Weber.

The great abundance of *Mnium hornum*, L., in some of the Chestnut coppices of the Thanet Sand has already been referred to. This is frequently associated with *Leucobryum glaucum*, Schp., *Polytrichum attenuatum*, Menz., and *Dicranum montanum*, Hed. On the drier, more stony soils, *Mnium hornum* is partially replaced by *Polytrichum juniperinum*, Willd., and *Dicranella heteromalla*, Schp. *Catharinea undulata* (L.), Web. Mohr., occurs abundantly on the sandy loam without pebbles, associated in the damper spots with *Pellia epiphylla*, L.; both these species are absent on the drier soils. *Georgia pellucida* (L.), Rabenh., and *Orthopyxis androgyna* (L.), Beauv., are generally distributed in small quantities; neither of these species has been discovered on the Chalk or Clay with Flints. *Isopterygium depressum* (Bruch), Mitt., is usually present. *Diplophyllum albicans*, L., occurs commonly on banks and cuttings associated with *Jungermannia ventricosa*, Dicks., and *Lepidozia reptans*, L.

The Woolwich and Reading Series.

The Woolwich and Reading formation is made up of beds of coarse sands and gravels alternating with occasional thin beds of clay. In consequence the character of the soil produced varies considerably in different situations, the gravels and sands giving rise to light sandy loam in which the drainage is rapid, while the bands of clay produce heavy, badly-drained soils. In all these soils the amount of calcium carbonate is very small. In an analysis published by Hall and Russell (7) the percentage of lime is given as 0·1, a proportion lower than that found in the majority of the soils

of the Thanet Sand. As would be expected, an acid reaction is constantly given by the soils in woodland areas. The proportion of mineral plant food also is generally lower than in soils of the Thanet Sand.

The areas of woodland occurring on the formation are not extensive in the districts under consideration. In the majority of cases the Woolwich and Reading beds form a relatively narrow zone in the Lower Eocene deposits bounded on one side by the Thanet Sand and on the other by the Oldhaven beds. Occasionally, however, in the smaller outliers in which the Oldhaven beds are not represented, the Woolwich and Reading areas are more extensive. It has already been pointed out that there is in many cases a gradual transition from the Thanet Sand to the Woolwich and Reading deposits.

(*a*) Woods on the sands and gravels of the Woolwich and Reading series. The following woods of this type have been investigated :—

In the Faversham district : portions of Bysing and Perry Woods.

In the Swanley district : portions of Jordan's, Farningham, and Rowhill Woods.

The woods in many respects are similar to those occurring on the Thanet Sand. In the coppices, Chestnut is frequently the most abundant species, but it is mixed with a considerable proportion of Oak and Beech. The occurrence of Beech on a soil containing so small a proportion of calcium carbonate is of interest. *Pyrus Aria*, Ehrh., is frequently found ; this tree, although widespread on the Chalk, is not confined to calcareous deposits, but is of general occurrence on light soils. In some of the woods *Pinus sylvestris*, L., has been extensively planted, and here the coppice is more open and felling does not take place at regular intervals. In consequence the sharp distinction between the light and shade vegetation already described in the case of coppice on the Chalk, Clay with Flints, and Thanet Sand cannot in these cases be maintained.

The flora of the Woolwich and Reading formation is distinguished by the abundance of *Pteris aquilina*, L. This plant, which occurs sparingly or not at all on the Thanet Sand, is dominant in all situations where the illumination is sufficient to allow for its development. *Adoxa Moschatellina*, L., and *Ranunculus Ficaria*, L., are absent, while *Primula acaulis*, L., *Anemone nemorosa*, L., and *Endymion nutans*, Dum., occur very sparingly.

The light flora generally resembles that already described on the more stony soils of the Thanet Sand, but is more xerophytic in character. *Carduus palustris*, L., is rarely or never found, while there is a larger proportion of *Teucrium Scorodonia*, L., and *Solidago Virgaurea*, L., *Calluna Erica*, DC., and *Aira flexuosa*, L., are usually present. *Polytrichum juniperinum*, Willd., occurs in addition to the Mosses already noted on the Thanet Sand.

(*b*) Woods on the Clay soils of the Woolwich and Reading series.

An area of this type has been investigated in Farningham Wood, near Swanley.

The coppice on the Clay differs from that on the sand and gravel in the larger number of woody species present. Chestnut, Hazel, and Ash are of frequent occurrence, while Oak, Birch, Beech, *Populus tremula*, L., and *Salix Caprea*, L., are found sparingly. Many of the herbaceous plants found on the sands and gravels also occur on the Clay, but the vegetation, as a whole, is of the mesophytic type. *Calluna Erica*, DC., and *Aira flexuosa*, L., are not found.

After felling, *Carduus palustris*, L., is produced in great abundance, and *Lysimachia nemorum*, L., *Scabiosa Succisa*, L., *Circaea lutetiana*, L., and *Juncoides sylvaticum*, O. Kuntze, are usually present in considerable quantity. Small ponds are of frequent occurrence, and in their vicinity *Lysimachia Nummularia*, L., *Polygonum Hydropiper*, L., and *Juncus conglomeratus*, L., are characteristic plants.

Clay soils of this type usually support an abundant bryophytic flora. Several of the more xerophytic species of the sands and gravels, such as *Polytrichum juniperinum*, Willd., and *Dicranella heteromalla*, Schp., are absent, and these are replaced by *Catharinea undulata* (L.), Web. Mohr., *Mnium hornum*, L., *Mnium undulatum*, L., and other mesophytic species. *Pellia epiphylla*, L., frequently covers large areas, and *Riccia glauca*, L., is often found.

The Oldhaven (Blackheath) Formation.

In the districts under consideration the Oldhaven deposits consist largely of rounded flint pebbles. The soils produced by these beds are poor and stony in character and in consequence are rarely cultivated. The proportion of calcium carbonate is lower than in the soils of the two Eocene formations already described, varying from 0·02% to 0·05% of the dry weight (Hall and Russell (7)). The acidity in woodland areas is very pronounced.

In these districts the Oldhaven beds are of small extent and are found capping some of the Tertiary outliers. Farningham Wood, near Swanley, covers almost the whole of an outlier of this kind. Towards the outside of this wood there is a continuous zone of Thanet Sand ; immediately within this is found a similar zone of Woolwich and Reading deposits, while the central and highest portion of the wood is situated on the Oldhaven beds. Perry Wood, near Faversham, has a similar conformation (Text-fig. 4).

The investigations made have been carried out in Farningham and Perry Woods. The woods occurring on the Oldhaven beds are of the Oak-Birch-Heather type referred to by Moss, Rankin, and Tansley (13). They consist for the most part of open coppice in which the trees are few in

number and are found at irregular intervals. In these coppices felling does not take place so regularly as in those on the formations already described.

The majority of the standards are *Quercus sessiliflora*, Salisb., although *Quercus pedunculata*, Ehrh., is occasionally found. *Pinus sylvestris*, L., has been planted extensively, and in some cases this gives rise to small areas of high forest, although it is generally intermixed with the deciduous species. In these situations *Pinus sylvestris* fruits freely and seedlings are of frequent occurrence. The Birch (*Betula verrucosa*, Ehrh.) is found in considerable quantity, although usually not so abundant as the Oak, while Beech, *Castanea sativa*, Mill., *Pyrus Aria*, Ehrh., and *Pyrus Aucuparia*, Ehrh., are usually present in small quantity.

Pteris aquilina, L., occurs in great abundance and is the dominant plant on these stony soils, except in deeply shaded situations. This, with *Calluna Erica*, DC., and *Teucrium Scorodonia*, L., makes up the greater part of the undergrowth. A society of this kind may be referred to as a *Xero-Pteridetum*, a term introduced by Woodhead (17) to describe the xerophytic type of vegetation, in which *Pteris aquilina* is dominant, found on the shallow sandy soils in the neighbourhood of Huddersfield. Although many of the species found on the Oldhaven beds differ from those described by Woodhead on the Millstone Grit, the general character of the vegetation is similar in the two areas.

The photograph shown in Plate LXVI gives a general view of the vegetation of the Oldhaven deposits. This photograph, taken in early spring, shows the dead stems and leaves of bracken in the foreground, the dark masses of *Calluna* in the middle distance, and Birch and *Pinus* in the background. On this soil *Endymion nutans*, Dum., *Anemone nemorosa*, L., and *Primula acaulis*, L., are almost absent. The abundance of *Rumex Acetosella*, L., demonstrates the acidity of the soil. The following plants are found in considerable quantity:—

Stellaria graminea, L.
Hypericum pulchrum, L.
Potentilla silvestris, Neck.
Solidago Virgaurea, L.
Juncoides pilosa, O. Kuntze.
Aira praecox, L.
Aira flexuosa, L.

Digitalis purpurea, L., and *Verbascum Thapsus*, L., occur sparingly. The Mosses found are of the xerophytic type; of these, *Polytrichum juniperinum*, Willd., and *Dicranum scoparium*, Hed., are the most widespread and cover large areas of the soil. *Cladonia pyxidata* (L.), E.Fr., is found in abundance amongst the Mosses, particularly in the vicinity of the groups of *Calluna*.

The London Clay.

In the neighbourhood of Faversham the London Clay extends over a large area between Canterbury, Whitstable, and Herne Bay, forming the main mass of the Eocene deposits. This area is almost entirely covered by woodland known as the Forest of Blean.

The soil is reddish brown in colour and contains a large amount of true clay, the proportion in some districts in East Kent rising to over 40 % (Hall and Russell (7)). It is, in consequence, of a particularly heavy nature and presents great difficulties in working for agricultural purposes. For these reasons it is probable that the area occupied by the Forest of Blean has never been brought under cultivation, but has always been covered with woodland. The proportion of lime in these soils is small; the analyses given by Hall and Russell (7) show 0·2–0·3 % of calcium carbonate, but these were obtained from arable soils which had probably been limed; the amount in soil covered by woodland is probably considerably less.

The observations made on the vegetation of the woods of the London Clay have been confined to portions of the Forest of Blean in the vicinity of Holly Hill, near Faversham. The altitude of these woods varies from 200 to 300 ft.; the slope of the ground is towards the north-west. The Oak (*Quercus sessiliflora*, Salisb.) is the most abundant tree found in these woods. The standards are well-grown trees and produce Oak timber of good quality. The Oak also forms the greater part of the coppice growth. Hazel, Chestnut (*Castanea sativa*, Mill.), Beech, and Birch (*Betula verrucosa*, Ehrh.) are usually present, but in considerably smaller numbers. Honeysuckle is frequently found in these copses. *Pinus sylvestris*, L., has been planted in some situations.

The ground flora in the shade of the old copse is very scanty. The following are found, but in all cases the plants are few in number. These flower in the early spring:—

Anemone nemorosa, L. *Primula acaulis*, L.
Endymion nutans, Dum.

In view of the abundance of the Bluebell on the Clay with Flints, its scarcity on the London Clay is striking. Although the two soils are very similar both in mechanical and chemical composition, the London Clay is much more compact and harder than the Clay with Flints; this is particularly the case with regard to the upper layers. The young bulbs of *Endymion nutans* would not meet with great resistance in their passage downwards, and this fact no doubt partially explains the scantiness of the species on this soil. *Mercurialis perennis* is absent from the London Clay in this district.

The dwarf shade flora is represented by the following plants:—

Viola Riviniana, Reich.
Rubus fruticosus, L.
Solidago Virgaurea, L.
Ajuga reptans, L.
Pteris aquilina, L.

All these species are found in small quantity. *Solidago Virgaurea* is the most abundant of the flowering plants, but this exists only in the vegetative condition. *Viola Riviniana* and *Ajuga reptans* occur sparingly and rarely flower. *Pteris aquilina* is found in considerable quantity, but only produces small stunted leaves without sporangia.

Felling takes place at intervals of about fourteen years, as in the copses on other soils in the neighbourhood. As before, the resulting flora can be divided into—

(*a*) The plants of the shade flora which persist under the light conditions.

(*b*) Species found only during the light period.

(*a*) After felling has taken place there is a great increase in amount of *Pteris aquilina*. This plant rapidly becomes dominant and in the late summer and autumn covers the whole of the soil. *Rubus fruticosus* also develops greatly, and flowers in the second and following years. *Solidago Virgaurea* becomes abundant, flowering to a small extent during the first season and freely in the later years. *Viola Riviniana* increases in amount, and flowers abundantly. *Anemone nemorosa*, *Endymion nutans*, and *Primula acaulis* show little or no alteration.

(*b*) The great majority of the plants which appear after felling are biennials or perennials, and these do not flower during the first season. Few annuals have been observed; of these, *Melampyrum pratense*, L., is generally distributed; *Myosotis collina*, Hoffm., is occasionally found. The following biennial and perennial plants occur in abundance:—

Hypericum pulchrum, L.
Calluna Erica, DC.
Teucrium Scorodonia, L.
Juncoides pilosum, O. Kuntze.
Juncoides sylvaticum, O. Kuntze.
Aira flexuosa, L.

The occurrence of *Calluna Erica* and *Aira flexuosa* on a heavy clay soil is unusual, and on this account a careful examination was made to determine whether the clay in this locality was overlaid by a sandy deposit. No such layer, however, was found. The upper layers of the clay become very hard and dry during the summer, and this fact perhaps accounts for the prevalence on the London Clay of species in which xerophytic characters are considerably developed. *Hypericum pulchrum* and *Aira flexuosa* flower at approximately the same date, and in the early summer form a conspicuous feature of the vegetation.

The following plants are of frequent occurrence :—

Sarothamnus vulgaris, Wimm.
Veronica officinalis, L.
Juncus conglomeratus, L.
Agrostis vulgaris, With.
Anthoxanthum odoratum, L.
Holcus mollis, L.
Festuca ovina, L.

The Broom is occasionally found in the lighter portions of the full-grown copse. After felling, plants frequently appear, but these do not flower until several years have elapsed.

Several species are generally distributed :—

Polygala vulgaris, L.
Senecio erucifolius, L.
Carduus palustris, L.
Erythraea Centaurium, Pers.
Stachys Betonica, Benth.
Prunella vulgaris, L.
Holcus lanatus, L.

The following species are scattered :—

Sagina procumbens, L.
Hypericum perforatum, L.
Euphorbia amygdaloides, L.
Lotus uliginosus, Schk.
Epilobium angustifolium, L.
Dactylis glomerata, L.

Hypericum Androsaemum, L., is occasionally found.

A good deal of the ground in the vicinity of the wood has obviously been cleared of the coppice growth within the last few years, and only the standard Oaks have been left. On this rough pasture *Aira caespitosa*, L., and *Pteris aquilina*, L., are of frequent occurrence.

The light flora of the London Clay is less abundant, both in quantity and in number of species, than those already described on the other soils of the neighbourhood. The most obvious feature of the flora is the great abundance of Bracken and Heather. These plants have a great and obvious effect on the surrounding vegetation, and the absence or rarity of many species commonly found on the Clay with Flints probably depends on their abundance.

The Moss flora of the London Clay is remarkable in the rarity or complete absence of several species which occur frequently on the neighbouring soils. *Mnium hornum*, L., and *Catharinea undulata* (L.), Web. Mohr., have not been found. On the other hand, *Polytrichum juniperinum*, Willd., *Polytrichum attenuatum*, Menz., and *Dicranium scoparium*, Hed., are abundant. *Thuidium tamariscifolium*, Lind., is generally distributed. *Cladonia pyxidata* (L.), E.Fr., and *Peltigera canina* (L.), Hoffm., occur frequently on this soil.

The Alluvial Deposits.

In the Faversham district several small areas of woodland occur on alluvial soils. In most cases these form portions of larger woods, of which the greater part is found on one or more of the soils already described.

Two types of vegetation can usually be distinguished in these woods :—

(*a*) The flora includes most of the species found in the woods on the Chalk and Clay with Flints and particularly resembles that found on the latter deposit.

(*b*) The flora includes most of the species found in the woods on the Tertiary deposits and closely resembles that found on the Thanet Sand.

It is highly probable that these differences in the vegetation depend on the origin of the alluvial deposit in question. A soil derived by the washing of Tertiary beds will in general agree in composition with the formation from which it originated. Similarly, an alluvial soil found at the bottom of a chalk slope will in all probability contain a large percentage of calcium carbonate. No analyses of these soils are at present available, but the matter will be further considered in a later communication.

(*a*) Woods on alluvial soils possessing a flora allied to that of the Chalk and Clay with Flints. The following woods of this type have been investigated in the Faversham district :—

Portions of Badgin, Judd's, and Bysing Woods.

In the first two woods each of the alluvial deposits in question is situated at the bottom of a chalk slope. In these areas only the shade flora has been investigated. In each of these cases the species making up the coppice growth are similar to those occurring on the Clay with Flints: Ash and Hazel predominate. The ground flora differs from that of the surrounding chalk soil in the increased amount of *Mercurialis perennis*, L., and *Endymion nutans*, Dum., and in the occurrence of *Adoxa Moschatellina*, L., a plant absent from the Chalk.

In Judd's Wood *Ranunculus Ficaria*, L., is abundant on the alluvium, and *Mnium undulatum*, L., is occasionally found. In Bysing Wood small areas of alluvial soil are found in a deposit of Thanet Sand which lies in the close vicinity of the Chalk. On these areas the underwood principally consists of Chestnut (*Castanea sativa*, Mill.) and Hazel. *Mercurialis perennis* occurs in isolated patches, and *Endymion nutans*, *Adoxa Moschatellina*, and *Anemone nemorosa* are generally distributed. The light flora is intermediate in character between that of the Clay with Flints and Thanet Sand. The following plants are particularly abundant :—

Lychnis dioica, L.
Gnaphalium uliginosum, L.
Dipsacus sylvestris, Huds.
Carduus palustris, L.
Echium vulgare, L.

(*b*) Woods on alluvial soils possessing a flora allied to that of the Tertiary deposits. The following woods of this type have been investigated in the Faversham district :—

Willow Wood, portions of Kemsdale and Sandbanks Woods.

(1) Sandbanks Wood. The alluvium occurs at the bottom of a steep slope of typical Thanet Sand (see p. 880). Only the shade flora has been investigated.

On passing to the alluvium from the Thanet Sand, Ash appears, and to a great extent replaces the Chestnut (*Castanea sativa*, Mill.) ; Hazel is occasionally present. *Ranunculus Ficaria*, L., is found in great abundance. *Adoxa Moschatellina*, L., and *Mercurialis perennis*, L., are absent from all parts of the wood.

(2) Willow and Kemsdale Woods. The soils of each of these woods abuts on an area of Thanet Sand. The woods consist of copse, the standard trees being Oak (*Quercus pedunculata*, Ehrh., and *Quercus sessiliflora*, Salisb.), while the underwood is made up chiefly of Chestnut (*Castanea sativa*, Mill.). Ash, Hazel, and Willow (*Salix Caprea*, L.) are also frequently found. The following plants are abundant in the shade flora :—

Anemone nemorosa, L.	*Primula acaulis*, L.
Adoxa Moschatellina, L.	*Endymion nutans*, Dum.

The light flora closely resembles that of the Thanet Sand, but includes the following species commonly found on the Chalk soils :—

Hypericum hirsutum, L.	*Inula Conyza*, DC.

Euphorbia amygdaloides, L., is often abundant.

Although two types of vegetation can usually be distinguished on the alluvial deposits, a consideration of the plants just mentioned will show that a certain amount of admixture of the species has taken place. This is not surprising as it is unlikely that any alluvial soil has been entirely derived from one deposit. It may be provisionally concluded that the composition of the flora varies with the composition of the soil.

General Observations and Summary.

Attention has already been drawn to the morphological peculiarities of the plants making up the greater part of the shade flora of the Chestnut copses on the Thanet Sand, and their general distribution in the neighbourhood can now be discussed. The plants in question—*Endymion nutans*, *Adoxa Moschatellina*, *Ranunculus Ficaria*, and *Arum maculatum*—all agree in the possession of a bulbous or tuberous rootstock. As pointed out by Woodhead (17 and 18), the mature bulbs of the Bluebell are found at a considerable depth in the soil; the rhizomes of *Arum maculatum* occupy an almost similar position. These two plants will, in consequence, come

into direct competition with each other. The rhizomes of *Adoxa Moschatellina*, on the other hand, are found at considerably less depth in the soil, while the tubers of *Ranunculus Ficaria* occur still closer to the surface. The society, as a whole, is complementary, the uppermost soil layers being occupied by *Ranunculus Ficaria*, the intermediate by *Adoxa Moschatellina*, and the lowest by *Endymion nutans* and *Arum maculatum*.

A full account of the life-history and morphology of *Endymion nutans* has been given by Woodhead (17 and 18), and this investigator has described the passage of the bulb downwards to the deeper layers of the soil during the development of the mature plant. A similar phenomenon has been mentioned by Oliver (14) in the case of *Adoxa Moschatellina*, and a similar process takes place in plants of *Arum maculatum* produced from seed (Scott and Sargant, 15). The soil found in copses on the Thanet Sand is particularly well adapted for the descent of the rootstock, since it consists typically of a loose sandy loam without stones of any kind. Little resistance is offered to the descent of the plant, and this circumstance probably partially explains the abundance of the species in question.

As already mentioned many of the areas described as Thanet Sand contain a considerable quantity of pebbles in the soil. The presence of pebbles evidently has an adverse effect on bulbous and tuberous species. In the case of *Endymion*, *Adoxa*, and *Arum* the sinking of the rootstock is obstructed both by the stones and the increased hardness of the soil. In consequence these species are absent or only occur in greatly decreased quantity wherever the soil contains a considerable proportion of pebbles. *Ranunculus Ficaria* also disappears from such soils, but in this case the limiting factor is, perhaps, the lower water supply. Of the four plants, the Bluebell can, apparently, best withstand the increase in quantity of the stones, for although absent on some soils of the Woolwich and Reading deposits, it is generally sparingly found both on these and on the Oldhaven beds.

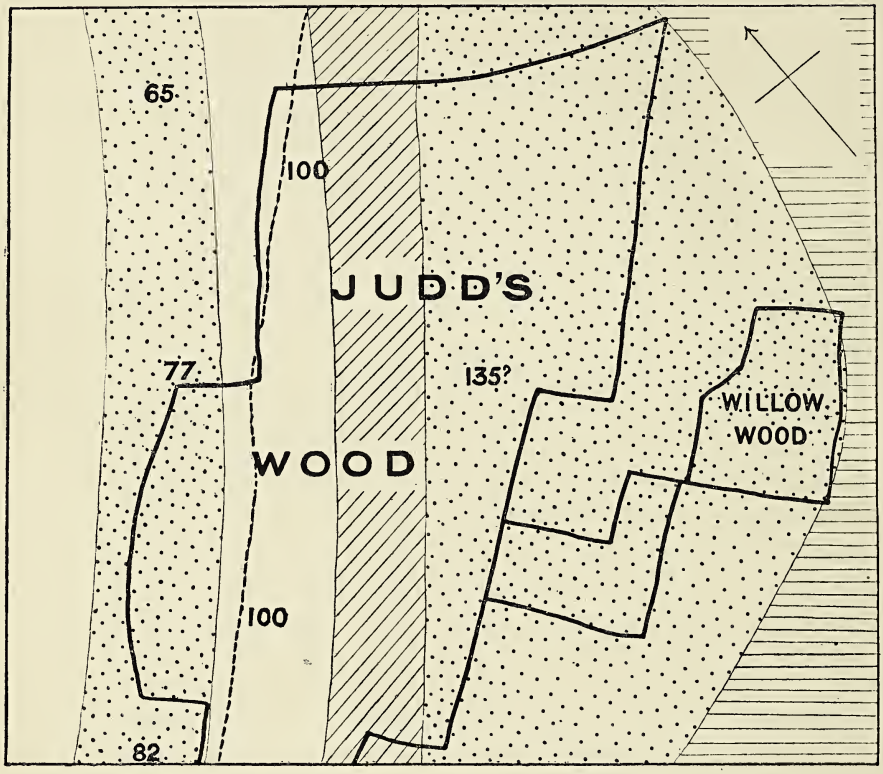
As would be expected, these plants are absent from or only sparingly found on the majority of the Chalk areas, since in these situations the soils are generally shallow and contain numerous fragments of chalk. *Endymion nutans*, *Adoxa Moschatellina*, and *Arum maculatum* do, however, occur frequently wherever the soil is of greater depth. These three species are also found on the deeper soils of the Clay with Flints, but are absent from or only sparingly found on the London Clay. This fact is somewhat surprising as these two soils are so nearly similar both in mechanical and chemical composition. The soil of the London Clay is, however, much stiffer and denser than that of the Clay with Flints and, in consequence, offers more resistance to penetration by the rootstock. This circumstance alone, however, seems inadequate to explain the abundance of these plants on one deposit and their scarcity or absence on the other.

It has already been indicated that, in the districts in which the investigation has been carried out, a number of soils of different structure and composition are found in close proximity. The areas covered by the various geological deposits are usually small and interpenetrate to a large extent, thus producing a system of great complexity.

Since this is the case it may be concluded that the absence of any species from a certain area is determined by conditions existing in that area and not by any defect in its method of distribution. An example will help to render this point clear. *Mercurialis perennis* is entirely absent from Perry Wood (see Text-figs. 1 and 3), but is found abundantly in Stocking Wood and in Winding Hill Wood, neither of which is more than a mile distant. This species produces abundant seed, and at the same time possesses an excellent method of vegetative propagation. Its absence from Perry Wood must therefore depend on some edaphic factor and not on its inability to reach this area.

In the majority of cases the limits of the various geological formations are well marked. With the few exceptions already noted this is also the case with the types of vegetation found on these deposits. In consequence, in a woodland area extending over several formations the changes in the composition of the flora met with in passing from one deposit to another are often very striking. The woodland area shown in Text-fig. 4 affords excellent examples of these sudden changes. The woods on the right-hand side of the figure (towards the south-east) are situated on alluvial deposits (brick-earth and gravels), and here the vegetation approaches the Tertiary type. The underwood is made up chiefly of Chestnut (*Castanea sativa*), Hazel, and Ash, while *Endymion nutans*, *Primula acaulis*, and *Adoxa Moschatellina* are abundant in the ground flora; *Mercurialis perennis* is absent. The light flora also closely resembles the Tertiary type. The central part of the wood is situated on the Thanet Sand, which here contains a considerable proportion of pebbles. The underwood consists of Chestnut, and the ground flora is made up almost entirely of Mosses; *Endymion nutans* and *Adoxa Moschatellina* are absent. This band of Thanet Sand occupies the crest of a ridge running in a north-easterly direction and extends for a short distance down the slope towards the north-west. Lower down on the slope the wood is crossed by a band of Chalk, and here the vegetation is typically of the Chalk type. The copse is made up of Hazel, Ash, *Euonymus europaeus*, *Cornus sanguinea*, and *Ligustrum vulgare*, with standard Oak and Beech; Yews are also present. In the ground flora *Mercurialis* is very abundant, *Endymion nutans* is generally distributed, and *Adoxa Moschatellina* is absent. The line of demarcation between the Thanet Sand and Chalk is very sharp and can be determined to within a foot by the distribution of *Mercurialis perennis*. The photograph in Pl. LXV shows the Thanet

Sand on the right covered with dead leaves of the Chestnut, and on the left the Chalk soil with abundant *Mercurialis perennis*. Finally, at the bottom of the slope, a deposit of alluvium (river gravel) is found. The



TEXT—FIG. 4.

Chalk. Clay with Flints.

Alluvium. Thanet Sand.

100 Contours. 65. Altitude in Feet.

135? Estimated altitude in Feet.

Scale: 6 Inches=1 Mile.

The woods are shown in heavy outline.

copse here consists principally of Ash and Hazel, and in the ground flora *Mercurialis perennis*, *Endymion nutans*, and *Ranunculus Ficaria* are abundant. It is interesting to note that the flora of the alluvium on the south-east of the wood is distinctly of the Thanet Sand type, while on the

north-west the alluvial deposit carries vegetation closely allied to that found on the Chalk.

The woodland area just described is of small extent, and the distance across the whole of the different deposits does not exceed half a mile. In a case of this kind it is obvious that the composition of the flora depends on edaphic conditions and is not affected by difficulties connected with the distribution of the species.

The sudden disappearance of *Mercurialis perennis* in passing from the Chalk to the Thanet Sand has also been observed in the eastern part of Joyden's Wood, near Swanley. In this case a similar change takes place in the flora to that just described, but here the line of demarcation is not quite so sharply defined.

Reference has already been made to the constant occurrence of Yew on the Chalk. Its restriction to this soil is well exemplified by a consideration of its distribution in the woods shown in Text-fig. 2. Each of these woods is situated partly on the Chalk and partly on the Clay with Flints. Yews are found in the southern part of Badgin Wood (Pl. LXIV), the south-west portion of Rice Wood, and in the north-west corner of Foxburrow Wood, but do not occur on any part of the Clay with Flints. Another point of interest in connexion with the distribution of species in Badgin Wood is the occurrence of *Adoxa Moschatellina* on the very small area of alluvium found in the south-west corner of the wood, this plant being absent from the surrounding Chalk soil.

The changes in vegetation met with in passing from one Tertiary formation to another, although not quite so striking as those just described, are still well marked. Perry Wood, near Faversham (Text-figs. 1 and 3), covers almost the whole of a Tertiary outlier which is made up of Thanet Sand, the Woolwich and Reading series, and the Oldhaven beds. The presence of this outlier on the Chalk gives rise to a hill reaching 500 feet in altitude. The outer and lower parts of the wood on the Thanet Sand consist chiefly of Chestnut coppice, and the flora is similar to that already described on the more stony soils of this deposit. On passing to the Woolwich and Reading beds the Chestnut is partly replaced by Oak; *Endymion nutans*, *Primula acaulis*, *Adoxa Moschatellina*, and other species abundant on the Thanet Sand decrease greatly in quantity or disappear. The central and highest parts of the wood exhibit the Oak-Birch-Heath type of vegetation, and here *Pinus sylvestris* has been extensively planted; *Pteris aquilina* is particularly abundant, although only found sparingly in the lower parts of the wood. Farningham Wood, near Swanley, is situated on a similar outlier, and here the distribution of the vegetation is almost identical.

In view of the great resemblance of the soils of the London Clay and Clay with Flints in both mechanical and chemical composition, a comparison

of the vegetation of the two soils is of interest. The following species are found on the London Clay, but are absent from the Clay with Flints :—

Potentilla silvestris, Neck.
Solidago Virgaurea, L.
Calluna Erica, DC.
Stachys Betonica, Benth.
Juncoides sylvaticum, O. Kuntze.
Aira flexuosa, L.
Aira caespitosa, L.
Festuca ovina, L.

The following are abundant on the London Clay, but are only occasionally found on the Clay with Flints :—

Hypericum pulchrum, L.
Teucrium Scorodonia, L.
Juncoides pilosum, O. Kuntze.
Pteris aquilina, L.

Mercurialis perennis, L., and *Adoxa Moschatellina*, L., while abundant on the Clay with Flints, are absent on the London Clay, and the following, while found abundantly on the former deposit, are only occasionally present on the latter :—

Anemone nemorosa, L.
Sanicula europaea, L.
Dipsacus sylvestris, Huds.
Myosotis sylvatica, Hoffm.
Echium vulgare, L.
Euphorbia amygdaloides, L.
Endymion nutans, Dum.

At present no definite reasons for the great difference in the vegetation of the two deposits can be given. It is, however, interesting to note that the flora of the London Clay is distinctly xerophytic in character, while that of the Clay with Flints shows few or no adaptations against drought. This matter will be further discussed in the second part of this paper.

The following species are, in this neighbourhood, found exclusively on the Chalk :—

Viola hirta, L.
Helianthemum Chamaecistus, Mill.
Reseda lutea, L.
Poterium Sanguisorba, L.
Origanum vulgare, L.
Clinopodium Calamintha, O. Kuntze.
Clinopodium Acinos, O. Kuntze.
Clinopodium vulgare, L.
Thymus Serpyllum, L.
Ophrys muscifera, Huds.
Taxus baccata, L.
Anomodon viticulosus, H.T.

The following are almost confined to the Chalk, but are occasionally found on other formations :—

Hypericum hirsutum, L.
Linum catharticum, L.
Euonymus europaeus, L.
Cornus sanguinea, L.
Viburnum Lantana, L.

In several cases plants found abundantly on the Chalk are replaced on the non-calcareous soils by closely-related species. Such parallel species have been described by Kerner (9) in the Alps. *Viola hirta* is abundant on

the Chalk, and is completely replaced on the Clay with Flints by *Viola Riviniana.* Similarly *Hypericum hirsutum* is replaced by *Hypericum perforatum* on the Clay. These plants exhibit the distinctions which Kerner has shown to exist between calcicolous and non-calcicolous species. Both *Viola hirta* and *Hypericum hirsutum* possess pubescent bluish-green leaves, whereas in *Viola Riviniana* and *Hypericum perforatum* the leaves approach grass-green and are glabrous; the flowers of the calcicolous species are somewhat lighter in hue than those found on the non-calcareous soils.

The two other species of *Hypericum* found commonly in this neighbourhood also show a decided preference for certain formations. *Hypericum humifusum*, although occurring occasionally on the Clay with Flints and Alluvium, is abundant on the Thanet Sand; *Hypericum pulchrum*, which is occasionally found on all the different soils, occurs in abundance on the London Clay.

A general consideration of the plant distribution in the districts investigated leads to the conclusion that two main types of vegetation can be distinguished:—

(*a*) That found on the Chalk and Clay with Flints.

(*b*) That found on the Tertiary formations.

The inclusion of the Chalk and Clay with Flints under one heading may at first appear remarkable, but a consideration of the vegetation already described on these deposits will justify this conclusion. Although the soils of the two formations differ greatly in chemical composition, many points of agreement are found in the respective floras.

It might be expected that the vegetation of the two clay soils found in the Faversham district would show considerable agreement. It has been proved, however, that this is not the case. In fact, the flora found on the Clay with Flints more nearly resembles that of the Chalk than that occurring on the London Clay.

Although the Tertiary soils range from light sands to heavy clays, a considerable number of species occur throughout the four formations. These are frequently absent from or only occasionally found on the Chalk and Clay with Flints. The following lists will emphasize the difference between the two types.

(*a*) Plants found either on the Chalk or Clay with Flints, and absent from or only occasionally found on the Tertiary deposits:—

Viola hirta, L.
Hypericum hirsutum, L.
Helianthemum Chamaecistus, Mill.
Reseda lutea, L.
Linum catharticum, L.
Euonymus europaeus, L.
Cornus sanguinea, L.
Viburnum Lantana, L.

Origanum vulgare, L.
Clinopodium Acinos, O. Kuntze.
Clinopodium vulgare, L.
Clinopodium Calamintha, O. Kuntze.
Thymus Serpyllum, L.
Mercurialis perennis, L.
Quercus pedunculata, Ehrh.
Ophrys muscifera, Huds.
Orchis pyramidalis, L.
Habenaria bifolia, R.Br.
Taxus baccata, L.

Anomodon viticulosus, H.T.
Neckera complanata, Hueb.

(*b*) Plants found on the Tertiary deposits but absent from or only occasionally on the Chalk and Clay with Flints :—

Hypericum pulchrum, L.
Oxalis Acetosella, L.
Potentilla silvestris, Neck.
Oenothera biennis, L.
Circaea lutetiana, L.
Asperula odorata, L.
Hieracium umbellatum, var. *coronopifolium*, Bernh.
Campanula Trachelium, L.
Calluna Erica, DC.
Stachys Betonica, Benth.
Lysimachia nemorum, L.
Lysimachia Nummularia, L.
Rumex Acetosella, L.
Quercus sessiliflora, Salisb.
Neottia Nidus-avis, Rich.
Juncoides pilosum, O. Kuntze.
Juncoides sylvaticum, O. Kuntze.
Aira praecox, L.
Aira flexuosa, L.
Aira caespitosa, L.
Festuca ovina, L.

Orthopyxis androgyna (L.), P. Beauv.
Leucobryum glaucum, Schp.
Georgia pellucida, Raben.
Diplophyllum albicans, L.

A general consideration leads to the conclusion that plant distribution in the districts investigated is greatly influenced by the composition of the soil. This, however, is not by any means the only factor involved. Whether a species shall or shall not exist in a certain situation depends on the whole of the conditions obtaining in that place. Some of these, such as soil composition, altitude, water supply, and light intensity, evidently have a preponderating influence, but, in addition, many others are no doubt present, of which the existence is unsuspected. The variation of any one condition may determine the presence or absence if all the other conditions remain constant.

Since this is the case it is necessary to emphasize the circumstance that the present investigation has been confined to a comparatively small district. It is not desired to make any general statements as to the distribution of species outside this area. It will be apparent to those familiar with other types of vegetation that many of the peculiarities of distribution here described do not hold good for other localities. The absence of a plant from a particular formation in the district investigated does not necessarily imply that the species is absent in other districts and under other conditions.

In the present contribution a general account has been given of the vegetation found in the woodland areas. In a second paper it is proposed to deal in detail with some of the factors influencing the distribution. In this latter communication the whole of the results obtained will be fully discussed.

In conclusion, I wish to express my thanks to Professor Farmer, F.R.S., for his help and advice throughout the progress of this investigation.

I also wish to acknowledge my indebtedness to the late John Rigden, Esq., for his kindness in permitting free access to the woods on his estate.

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DESCRIPTION OF PLATES LXIV–LXVI.

Illustrating Mr. Wilson's paper on Plant Distribution in the Woods of North-East Kent

PLATE LXIV.

Photograph of the south-west portion of Badgin Wood, near Faversham, taken from the middle of the north side of Rice Wood and looking north-east (cf. Text-fig. 2). The foreground shows a portion of Rice Wood on the Clay with Flints covered by copse which has recently been felled. The alluvium in the valley is under cultivation and carries a crop of Hops. The lower portion of Badgin Wood, on the Chalk, is covered by copse in which the Yews are well seen. The background shows coppice on the Clay with Flints with standard Oaks.

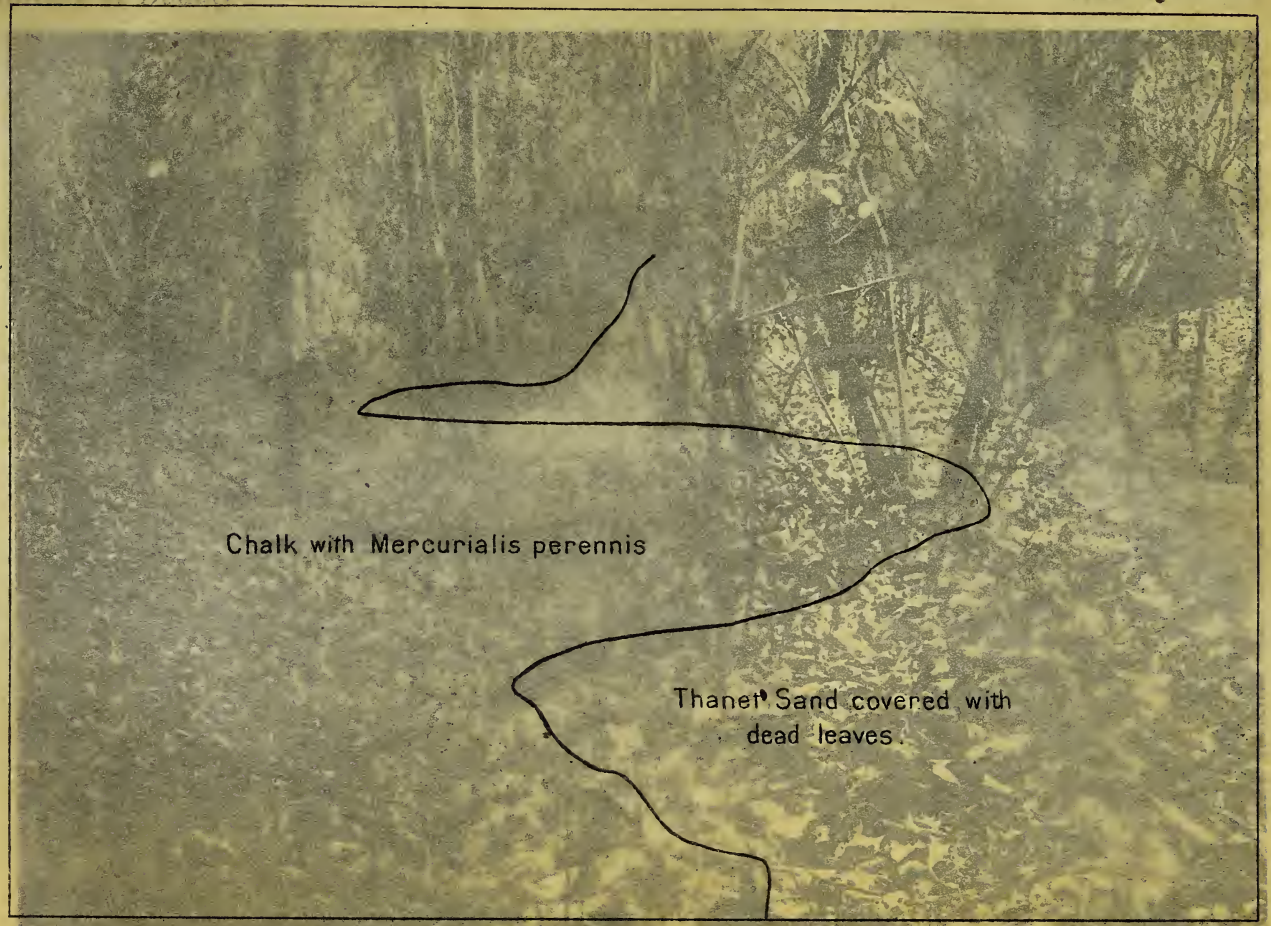
PLATE LXV.

Photograph taken in the western part of Judd's Wood, near Faversham, looking north-east and showing the junction of the Thanet Sand and Chalk (cf. Text-fig. 3). On the left the Chalk soil is seen covered with *Mercurialis perennis*. On the right the Thanet Sand is shown covered with the dead leaves of the Chestnut (*Castanea sativa*). The limit of the *Mercurialis* is sharply marked.

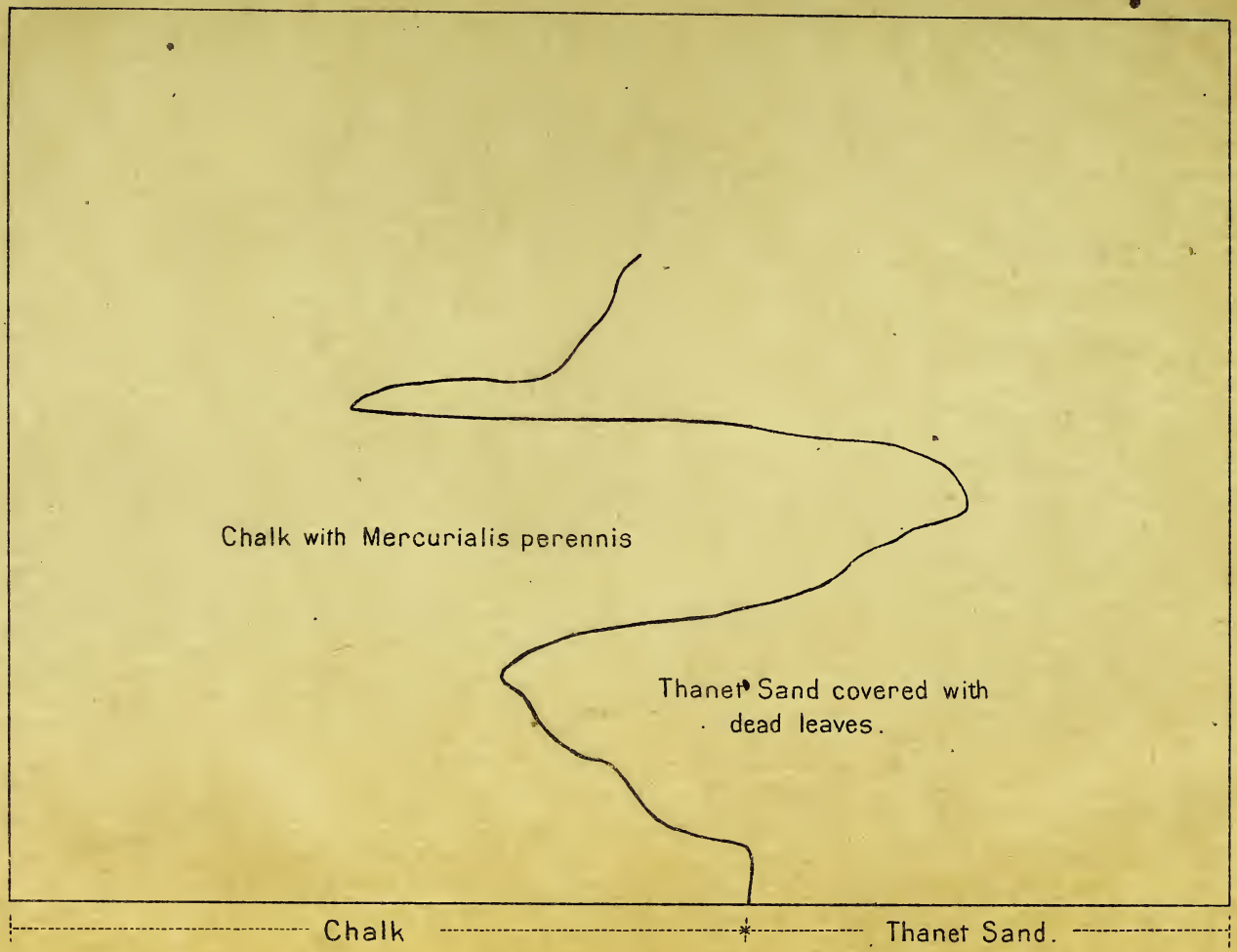
PLATE LXVI.

Photograph taken in the early spring in Perry Wood, near Faversham, showing the vegetation of the Oldhaven pebble beds. The dead leaves of Bracken are shown in the foreground. In the middle distance *Calluna Erica* and *Betula verrucosa* are seen. The background is occupied by *Pinus sylvestris*.

WILSON — PLANT DISTRIBUTION.



WILSON — PLANT DISTRIBUTION.


Chalk with Mercurialis perennis
Thanet Sand covered with dead leaves.
Chalk
Thanet Sand.

Chalk with Mercurialis perennis
Thanet Sand covered with
dead leaves
Chalk
Thanet Sand

M.W.phot.

WILSON — PLANT DISTRIBUTION.

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WILSON — PLANT. DISTRIBUTION.

On the True Nature of the Cretaceous Plant Ophioglossum granulatum, Heer.

BY

MARIE C. STOPES, D.Sc., Ph.D.

With two Figures in the Text.

OBJECT of the Paper:—To show that the Cretaceous impressions known as *Ophioglossum granulatum*, Heer, are wrongly attributed to this genus: that the American specimens are male cones of *Pinus*: that the specific identity of these with the Greenland specimens is uncertain: and, finally, to urge a simple method of distinguishing well-founded from problematical determinations, such as would be afforded by the use of Gothic type for the specific names of the latter.

The only *Ophioglossum* included in Knowlton's[1] useful list of the Cretaceous and Tertiary plants of North America, and indeed, so far as I am aware, the only recorded Cretaceous species of *Ophioglossum*, naturally attracted my attention when I was examining the Cretaceous plants for a larger work on which I am engaged. The Ophioglossums are of interest both from the exceptional nature of the living plants, and from the fact that we have almost no material from which to reconstruct their geological history.

In 1883 Heer[2] described the family Ophioglossaceae as being represented in the Patoot (Cretaceous) beds by a new species, *O. granulatum*. The materials on which this determination was based were one or two incomplete, slender aments, in which were oval, closely packed granules, which he held to be sporangia, and which he thought were much like those of *O. vulgatum* in size and shape. No leaves were found. The species was defined as 'O. spica fertili elongata, sporangiis distichis, ovalibus granulatis, $1\frac{1}{2}$ mm. longis'.

Reference to Heer's Fig. 8, Pl. LVII, will convince any one of the extremely problematic nature of the specimen, and of the danger that must lie in determining it from its external features. Notwithstanding this, how-

[1] Knowlton, U.S. Geol. Surv. Bull. No. 152, 1898.

[2] Heer, 'Flora foss. Arctica.' Die foss. Flora Grönlands, pt. ii, 1883. See p. 8 and Pl. LVII, Figs. 8, 9.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

ever, in 1895 Newberry[1] identified some specimens from the Amboy clays with Heer's species, saying, 'there can be no mistake about the identity of the plant,' though he adds that as to its true nature there can be great difference of opinion. In the first place, I think there is doubt as to the absolute identity of the plants from the two deposits, as comparison of Newberry's illustrations of his specimen (Pl. IX, Figs. 11, 12, 13) with Heer's figure, before cited, testifies. Heer's specimen is less than half the size of Newberry's, and the two do not agree in detail, beyond the fact that both are slender aments. To conclude, as Newberry does, that 'they are interesting as being the fruit of some of the plants which are common to the Amboy clays and the Cretaceous beds of Atane, Greenland', is an illustration of the extremely slender foundation on which palaeobotanical generalities have too often been based.

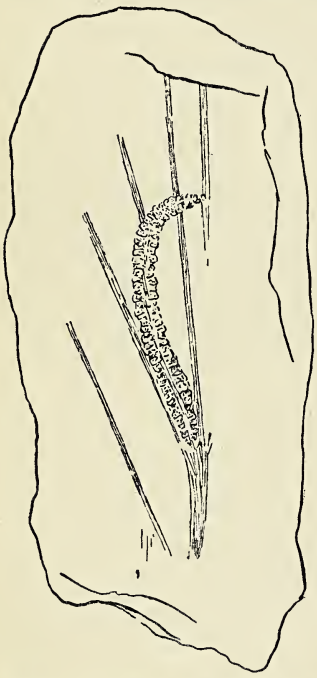

FIG. 1. Sketch drawn in New York from Newberry's original specimen for his Fig. 13. This shows the cone and axis with leaves attached, of what has hitherto been called *Ophioglossum*, and is really a *Pinus* ♂ cone.

Fig. 13 of Newberry illustrates his most perfect specimen, which is much more complete than those of Heer. It shows a curved spike on a stalk, with two pointed linear leaves or bracts springing from the same stem. It will be remembered that there were no leaves recorded from the Greenland specimens.

In New York, through the kind courtesy of Dr. Britton of Bronx Park, I had the opportunity to examine Newberry's own collection. The original of his Fig. 13 appeared to me to show rather more of the leaf-like structures than does his figure. Fig. 1 is a sketch I made from Newberry's original. It shows the two linear leaves attached, and evidence of a third, which looks as though it had belonged to the same fascicle, with others which were doubtless belonging to the same axis. The matrix is not very favourable, either for actual preservation or for photography, as it is a friable clay with no strong colour contrast between the specimen and the matrix; but what is given in my sketch is easily recognized with the naked eye.

The leaves attached to the axis are so similar to those of other specimens without cones which have been identified as three-leaved pines,

[1] 'Flora of the Amboy Clays,' posthumous, ed. by Hollick. U. S. Geol. Surv., Monog., vol. xxvi, 1895. See also p. 43, Pl. LX, Figs. 11–13.

that the external features alone of the present specimen are enough to suggest that it is simply a three-leaved (possibly four- or five-leaved) pine with its male cone. In the original specimen a number of the 'granules with which the axis of the fruit spike is invested' appeared to me to hold promise of showing some structure. One of these 'granules' Dr. Britton generously allowed me to remove with the point of a knife. The 'granules' are evidently the crushed sporophylls and sporangia, and after treatment and clearing the fragment liberated a number of spores which could be well studied under the microscope. Most of them, of course, were crushed or contracted, but a considerable proportion showed their structure remarkably well, and, as is shown in Fig. 2, bear such a close resemblance to the winged pollen spores of *Pinus* that there can be little doubt that this is their true nature. A duplicate specimen, which Dr. Britton kindly presented to me, also yielded similar spores.

Fig. 1 shows the actual size of the male cone, which is about 35 mm. long. It is thus larger than is usual among the species of *Pinus* common in this country; but several species of North American and Mexican pines have cones nearly as long, and *P. australis*, Michx., from Florida has ♂ cones very similar to the fossil except that they are more massive. There is thus no discrepancy as regards size of the cone between fossil and living members of the genus.

Fig. 2. Pollen grains from the 'granules' of the original specimen of Newberry's Fig. 13. These show the two wings clearly.

These facts should suffice to establish the true nature of the American 'Ophioglossum', and the generic name must be eliminated. The specific name should be retained for the plant unless the male cones are shown to belong to any previously established species of *Pinus*. This specific name might also with advantage be made to cover the three-leaved pine-leaf-fascicles from the same deposit described by Newberry as *Pinus sp.*?, for there is every chance that they belong to the same plant.

Pinus granulata, (Heer) nov. comb., may be defined as:—male cones of *Pinus* about 1·5–3·5 cm. long, slender, with or without attachment to leafy axis, which appears to be a three-leaved form. Pollen grains with two rounded 'wings'.

Cretaceous, Amboy Clays, U.S.A., and Patoot beds, Greenland.

Though it is my conviction that the cones of Heer belong to a different species of Pine, I do not propose now to add another name to the overburdened palaeobotanical literature, because it is perfectly possible that they do represent younger cones of the same thing, and in any case they are so imperfect that they are of little value.

One or two general considerations arise from this work. Moral reflections as to the value of most determinations of fossil plant-impressions are perhaps out of place, but it seems a good opportunity to urge that the lists published by palaeobotanists should be printed in two forms, and that the names of species of leaves, stems, &c., of which there is a reasonable security of determination, should be differentiated from those in which there is no guarantee at all that the actual nature of the plant has been discovered. Any tri-nominal system is cumbrous, but those who publish on fossil plants might print their names in type of two kinds, which would indicate which species are doubtful. I should like to suggest that, instead of using italics or ordinary capitals, as is usual in printing the names of species and genera, such doubtful plant-impressions should be printed in Gothic lettering. This would indicate that our knowledge about them is mediaeval, of the Dark Ages, and would further save the inconvenience of tri-nominals, while it would indicate immediately the difference between the established and the doubtful determinations. As information accrued about a specimen it could readily be transferred to the clear, Latin italics.

Thus the transference of **Ophioglossum granulatum,** Heer to *Pinus granulata* (Heer) would indicate that an exceedingly doubtful determination had been replaced by one with some scientific basis. Any worker in another branch of science, seeing **O. granulatum** in Gothic, would be warned at least to look into the grounds for the determination for himself before he—let us imagine—used the record for his stratigraphic work in correlating horizons, or in writing up the early history of the Ophioglossaceae, when he would otherwise assume that the living genus was represented in Cretaceous times in the Amboy clays of North America. This is merely an illustration of what is very widely spread in fossil botany, but it may serve to give point to the general proposition that the time has come when it would be of real service to the science to attempt a conscientious distinction between valuable and doubtful determinations, and that Gothic lettering might give us an easy indicator. The need for this is all the greater because the results of palaeobotany touch so many other fields of research in animal palaeontology, geology, and palaeogeography, as well as botany itself. Workers from these other fields are seldom able to estimate the evidence that they are taking to build into their own work, even had they the time to go into the details, and thus a single error gets widely disseminated. Often it is not entirely the fault of the one who originally described the fossil, for he may say in his text that the nature of the specimen is doubtful and that, in default of better evidence, he gives a certain name with hesitation. That name, however, once given, is quoted and put into lists without being in any way distinguished from the rest, and the results are detrimental to the advance of

true knowledge in every way. It is no solution to call every leaf, as some conscientious workers do, 'Phyllites': different things, however doubtful, must have distinct names, and the use of Gothic characters for the very doubtful ones would greatly tend to 'create confidence' in the science of palaeobotany.

The observations on the specimens in New York were made while travelling in America on a tour which was partly aided by a grant from the Royal Society, to whose help and encouragement I am much indebted.

Pollen Formation in Oenothera gigas.

BY

REGINALD RUGGLES GATES, M.A., PH.D.

With Plates LXVII–LXX.

INTRODUCTION.

IN a paper published two years ago ('09 *a*), the writer showed that the appearance of the tetraploid number of chromosomes in *Oenothera gigas* is accompanied by an increase in the size of the nuclei and cells of this mutant as compared with *O. Lamarckiana*, the parent form. This increase in size was found to vary for different tissues. The respective average volumes of the cells were approximately 2 : 1 in the case of the epidermal cells of the petals. This was in harmony with Boveri's law deduced from experimental cytological studies upon sea-urchin eggs, in which a doubling in the number of chromosomes was accompanied by a doubling in the volume of the cells, and in the *surface* (not the volume) of the nuclei. This numerical relationship was not found to hold, however, in *Oenothera gigas* in most of the tissues measured. Rather it was found that, in the pollen mother-cells, the *volume* of the nuclei was doubled, while the respective volumes of the mother-cells themselves were 1·5 : 1. In other tissues the ratios of the average cell volumes in *O. gigas* and *O. Lamarckiana* were in one case 3 : 1, in another tissue 3·67 : 1, and in still another 3·83 : 1. The volumes of the nuclei in the last cases have not yet been measured. Without outlining further the results of the paper mentioned, it may be stated that I concluded that the gigantic stature of *O. gigas* resulted from the larger size of its cells and nuclei, which in turn was in direct relation with the presence of the tetraploid number of chromosomes.

In complete harmony with the law for the nuclei in the pollen mother-cells of *O. gigas* and *O. Lamarckiana*, namely, that the doubling in the number of chromosomes is accompanied by a doubling in the volume of the nuclei, Tischler ('10) has recently found that in races of the Banana having respectively 8, 16, and 24 chromosomes, the volumes of the nuclei are as 1 : 2 : 3. The work of the Marchals ('09) has shown in the same manner in Mosses, that with the appearance of the tetraploid or octoploid

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number of chromosomes there is a corresponding increase in the nuclear volume. As is well known, Němec ('10) has obtained similar results in his experimental production of chromosome doubling in root-tips.

It does not necessarily follow, however, that a variety with larger cells will have the tetraploid number of chromosomes. This is shown by the case of a giant race of *Primula sinensis*, in which Gregory ('09) found that, though the cells were larger, the chromosome number was unchanged. There are therefore two types of giantism in plants, one in which the tetraploid chromosome number is accompanied by a doubling in the nuclear volume and an accompanying increase, in varying proportions, in the volume of the cells ; another, in which the amount of chromatin has not appreciably changed, while the cytoplasm has undergone a large increase. In the latter case there must be a marked change in the karyoplasmic relation, while in the former there is no change in the karyoplasmic ratio or, as my measurements show, a relatively small change.

A further consideration of the size relationships between *O. gigas* and *O. Lamarckiana* will not, however, be entered into in the present paper, the purpose of which is to give an account of the pollen formation in *O. gigas*. The process of chromosome reduction in *Oenothera* has already been described by me in some detail in several previous papers, including a study of *O. Lamarckiana* and several of its mutants, notably *O. rubrinervis* ('08) and *O. lata*, and the hybrid *O. lata* × *O. gigas* ('09 *b*) ; as well as a study of *O. biennis* and *O. laevifolia* ('09 *c*), in both of which the phenomena of reduction were found to be precisely the same as in *O. Lamarckiana* or *O. rubrinervis*.

Since diakinesis and certain other stages of the reduction process were studied with special care in a previous paper ('08), in which the method of chromosome pairing and the main events in the history of the chromosomes during meiosis were, I think, satisfactorily demonstrated, the present paper will not attempt to give another complete series showing all the events of meiosis, but attention will be concentrated upon certain stages which have been intensively studied. Of these, the series of stages or conditions which are usually grouped together under the term synapsis has received much attention, with the result that more changes in the nucleus are found to occur during this period than has previously been supposed. A type of pollen sterility which differs in certain respects from any previously known in plants will also be described, and certain variations in the phenomena of meiosis will be considered.

The material for these studies was all obtained from plants of *O. gigas* grown in connexion with my pedigree cultures, and which were derived originally through the kindness of Professor de Vries, from seeds descended through several generations from an original single mutant in his garden at Amsterdam.

The cytological methods employed need no description. For fixing solutions Bouin's fluid [1] was found satisfactory, in addition to various chrom-acetic solutions. Heidenhain's iron-alum haematoxylin was the stain most frequently employed, and a counter-stain with orange G was often useful in bringing out such structures as the cytoplasmic connexions between pollen mother-cells. The fact that the cells and nuclei are considerably larger in *O. gigas* than in the other forms has been a distinct advantage in the study of several of the stages of nuclear development. Several of these preparations were made in the laboratory of Professor Grégoire, to whom I am much indebted for laboratory facilities during my stay in Louvain.

Description.

Archesporium and Synapsis.

In a longitudinal section of a very young anther a single row of archesporial cells appears. The nuclear reticulum of these cells is at first extremely delicate, so much so that only traces of such a structure can be seen. It is always composed of finer threads than the cytoplasmic reticulum at this time. The tapetum is uninucleate and remains so until about the time of synapsis, when a simultaneous mitotic division occurs in all the tapetal nuclei. Frequently, though not always, the archesporial cells divide to form the cells which become the pollen mother-cells. In some cases, however, these cells, by subsequent growth and without division, form the pollen mother-cells directly. Simultaneous growth of the cells, nuclei, and nucleoli of the archesporial cells occurs, until they are enormously larger than their original size. Such a pollen mother-cell is shown in Pl. LXVII, Fig. 1. This cell is just ready for the beginning of the nuclear condition known as synapsis. Fig. 2 shows the beginning of this process, Fig. 3 a somewhat different condition occurring at this time, and Fig. 4 a slightly later stage as it sometimes occurs. As will be shown later, there is much variation in the relations between nucleus and cytoplasm at this period, but in all cases the result is a rather sudden increase in the size of the nucleus. Fig. 5 shows the typical synaptic condition after the nuclear expansion has been completed.

In another paper (Gates, '08) I have called attention to the fact that in *O. rubrinervis* there is a large amount of growth of the nucleus at the time of synapsis, without corresponding growth of the cytoplasm. With further measurements it has been shown in a recent paper (Gates, '11) that the appearance of contraction of the nuclear contents during the period of synapsis is largely, though not wholly, the result of this sudden nuclear expansion without a corresponding growth or change in position of the chromatin content of the nucleus.

[1] A note on my use of this fixing agent is to be found in Science, N. S., xxxi. 234, 1910.

A series of measurements of the pollen mother-cells and nuclei of *O. gigas* further confirms this view, and shows that while there is some growth of the pollen mother-cells during the period between the beginning and the completion of nuclear 'contraction', yet this is not in proportion to the very large increase in the size of the nucleus during this period. This result is in general agreement with Lawson's ('11) interpretation of synapsis from his study of *Smilacina*. The results of these measurements will not be presented in detail in the present connexion, but they show that, between the resting telophase of the last archesporial division and the synapsis (synizesis) condition, the pollen mother-cells and their nuclei undergo a simultaneous growth for a considerable period, during which a very large amount of increase in size takes place. In an earlier paper (Gates, '08) the same was shown to be true of the forms with fourteen chromosomes. This is followed by a rather sudden expansion of the nucleus at the beginning of 'synapsis'. The mother-cell having nearly completed its growth just before the beginning of the phenomenon of synapsis, its subsequent enlargement is relatively small, so that the ratio $\frac{\text{cell volume}}{\text{nuclear volume}}$ is conspicuously greater just before synapsis than in cells in the synaptic condition. This is clearly shown by a comparison of Figs. 1 and 5, Pl. LXVII. In Fig. 1, however, the nucleus is a trifle smaller than the average size at this time. Certain conclusions in connexion with this interpretation of synapsis have been discussed in another paper (Gates, '11) and need not be referred to here. A number of other features of exceptional interest have since developed in connexion with further study of synapsis, and these will be discussed in the present paper.

It is of course necessary to determine what pollen mother-cells are about to enter upon synapsis. One finds that in certain anthers of flowers at about this stage, some of the pollen mother-cells will be in the condition shown in Fig. 2, in which the synaptic process is just beginning. In such anthers other mother-cells do not show such 'clear spaces' adjoining their nuclear membrane, and such nuclei can safely be taken to be in the stage just preceding the beginning of synapsis.

There appears to be wide variation in the manner in which the nuclear expansion which produces the 'synaptic' condition comes about. In some cases (Fig. 2) its early stages are certainly very gradual, there being an expansion of the nuclear membrane which sets it free from the nuclear reticulum, leaving clear areas at one side of the nucleus, occupied only by karyolymph. Expansion of the nuclear membrane in this manner may continue until the nucleus has attained to nearly its definitive size. This may have been the case in the nucleus represented by Fig. 5. Sooner or later, however, in this growth period of the nucleus, the nuclear membrane is likely to become very delicate and indistinct, or it may break down

completely, leaving the nucleus in open communication with the cytoplasm. This is certainly true in fixed preparations, and is probably also true in the living cell. Fig. 3 illustrates a not uncommon condition at this time, in which there appears to have been a sudden forcible expansion or rupture of the nucleus on one side, following perhaps such a condition as shown in Fig. 2. This gives a clear area in the cell containing nothing but karyolymph or cell-sap. The main body of the nucleus is in such a case unaltered, and the amount of increase in the karyolymph can be estimated from the size of the pouch-like lateral enlargement of the nucleus. That this lateral expansion is relatively sudden is shown by the fact that the nuclear reticulum has not begun to undergo the changes which will transform it into the typical spireme condition of synapsis. Surrounding the clear area in such cases as Fig. 3, there may be a definite membrane, or it may be quite indistinct and indefinite.

Another condition very commonly found in mother-cells before the completion of synapsis is that shown by Fig. 4. Here the nuclear membrane evidently first began to enlarge, as in Fig. 2, on the left side of the nucleus. Then the membrane was ruptured, probably by the increasing pressure from within, which was causing its expansion. The result is an indefinite area on the right side of the original nucleus, which contains much karyolymph and little cytoplasm, but with no definite limiting membrane. The expansion of the nucleus in Fig. 5 probably took place in much the same way. There is thus a period during which, on one side or portion of the nucleus, the membrane partly or completely disappears. Later a definite membrane is again formed. It thus appears that the nuclear expansion, which is one of the main features of the period known as synapsis, may be gradual or relatively sudden, and that there is very commonly at this time a disappearance of the nuclear membrane on one side of the nucleus, leaving the latter and its contents in open connexion with the cytoplasm. This condition is brought about either by a rupture or by the solution of the nuclear membrane on one side of the nucleus, and there follows a period during which it is impossible to define accurately the limits of the nucleus. A portion of the membrane seems always, however, to remain intact. A little later the nuclear membrane is once more complete and perfect, the nucleus spherical, and we have the typical synapsis condition, as represented with a somewhat lower magnification in Figs. 6 and 7.

During the period when the limits of the nucleus are so poorly defined, the cytoplasm also shows a 'ragged' condition rather than a well-defined reticulum. This will be seen in comparing Figs. 3 and 5 with Figs. 1, 6, and 7. It might be concluded that these differences were due to differences in the treatment. To obviate the possibility of this conclusion, Figs. 1–7 were all drawn from the same preparation.

The two most characteristic features of this first period of synapsis

are therefore (1) the expansion of the nucleus, with frequently the partial disappearance of the nuclear membrane for a time, and (2) the rearrangement, without growth, of the structural content of the nucleus, from the reticulum to the spireme condition. This rearrangement has not begun in Pl. LXVII, Figs. 2–4, is partly completed in Fig. 5, and is quite completed in Figs. 6 and 7. It is not uncommon for threads to remain attached to the nuclear membrane, as in Figs. 4 and 5. Pl. I, Figs. 12 and 13 of a former paper (Gates, '08) show the same thing, and are also instructive (when compared with Pl. I, Figs. 6–11) in showing that expansion of the nucleus, rather than contraction of its contents, is taking place. In ordinary nuclear growth, such as occurs between successive mitoses, the reticulum also grows so that it continues to fill the nuclear cavity. But in the growth of the synaptic nucleus, the reticulum does not continue to grow, at least not to a corresponding extent, and probably not at all, and the result is that the reticulum soon occupies only a small part of the cavity of the synaptic nucleus.

Regarding the presynaptic nucleus, it will be seen from Figs. 1–4 that darker-staining portions of the threadwork frequently occur, especially near the periphery of the nucleus, but they show no features of regularity in size, shape, or number.

One feature which has been of much value in the study, particularly of the synaptic stages in *O. gigas*, is the occurrence of slightly different stages or conditions of synapsis in different anthers of the same flower, while all the mother-cells of each anther are in about the same condition. Thus in one flower the mother-cells of one anther were presynaptic, except a few which showed the beginning of the synaptic nuclear expansion. In another anther of this flower the mother-cells were all presynaptic and with numerous dark bodies lining the nuclear membrane as in Fig. 1, while other anthers showed the same condition except that the dark bodies were wholly absent. This probably indicated in this case a different physiological condition of the pollen mother-cells in these anthers rather than two successive stages during which these bodies made their appearance.

Figs. 6 and 7 show the typical synaptic condition, in which a rather dense aggregation of threads, usually with darker areas or bodies in its meshes, appears on one side of the nucleus. The threadwork appears to be more or less continuous and frequently shows a typical 'chromomeric' condition such as figured by Mottier for *Lilium* (Mottier, '09, Figs. 4 and 5). The threads are extremely delicate. This chromomeric condition of the threads will be referred to again later. The only difference between Figs. 6 and 7 is in the condition of the nucleolus. In the anther from which Fig. 6 was taken, the nucleolus was, in all the mother-cells, spherical and free in the nuclear cavity. Fig. 7 was drawn from a different flower, and in this anther the nucleoli were always flattened against the nuclear membrane as in the figure.

A study of many nuclei in the stage represented by Figs. 6 and 7 makes it evident that a certain amount of collapse together of the threadwork does take place. This is shown by the fact that the synaptic spireme not only occupies less space than the presynaptic reticulum, but has a greater density, showing that the material is more closely aggregated. That the spireme occupies less space may perhaps be partly a result of osmotic effects during the preparation of the slides. It is probable, however, that it is not to be accounted for as the result of contraction on the part of the threadwork. The threads of the presynaptic nuclear reticulum are anchored to the nuclear membrane, and this perhaps serves to keep the threadwork taut and occupying the whole of the nucleus. This is clearly shown by the fact that, when the nuclear expansion without chromatin growth begins, some of the threads frequently remain attached for some time to the membrane, and are pulled outwards with it as the pressure from within distends the membrane. Finally, when the nucleus has reached its definitive size, only a few such threads remain and the threadwork floats freely in the nuclear cavity. The expansion of the nucleus is completed before the rearrangement of its contents from the reticulum to the spireme condition takes place. It is probable that during this rearrangement a certain amount of collapse together of the threads, very few of which have any attachment to the nuclear membrane, takes place, so that the spireme, which is always placed unilaterally in the nucleus, occupies somewhat less space than did the reticulum. But while the spireme occupies usually somewhat less space than the original reticulum, yet this by no means accounts for all the empty space in the nucleus. This empty space is produced by a rather sudden expansion of the nucleus unaccompanied by any change in its reticulum. The transformation of the nuclear chromatin content from the reticulum to the spireme condition occurs apparently only after this nuclear growth is completed.

Nuclear Extrusions.

During the whole growth period of the pollen mother-cells, and the early synaptic stages (represented by Figs. 1–7), the nucleus occupies a central position in the pollen mother-cell. Then, while in the typical condition of synapsis or synizesis, the nucleus moves to one side of the cell until the nuclear membrane is in direct contact with the cell-wall. This condition has frequently been figured by previous investigators, e. g. in the papers of Overton ('09, Pl. III, Figs. 4–6) and Schaffner ('09, Pl. XII, Fig. 10), but no special significance has been attached to it. It is probable that this lateral movement of the nucleus always takes place, after its growth is completed and the transformation of its contents from a reticulum to the spireme of synizesis has occurred. This movement seems to be concerned

with a process which may prove to be of fundamental significance in connexion with the period of meiosis. This process will first be described.

After the synaptic nucleus of the pollen mother-cell has reached the side of the cell, an extrusion of material from the nucleus into the cytoplasm of the adjacent cell has been observed to take place. Pl. LXVII, Fig. 8 shows a row of pollen mother-cells in which this process is happening simultaneously in all the cells. In Figs. 9 and 10 the same process is shown under higher magnification. It will be seen that material is being extruded from the spireme of each nucleus, through an opening in the cell-wall into the cytoplasm of the adjacent pollen mother-cell. The nucleolus is always present at this time as a large spherical body, but in my observations I have never found it to take any part in this extrusion. The nucleus at this time is usually so placed that a considerable area of the nuclear membrane is in direct contact with the cell-wall, or perhaps the membrane may disappear over this area, leaving the nuclear contents in direct contact with the cell-wall and, through the openings in the wall, with the cytoplasm of the adjacent cell. The extrusion may take place through a single opening or (perhaps more frequently) through several.

In a previous paper (Gates, '08) I showed that rather large and conspicuous protoplasmic connexions occur between the pollen mother-cells in *Oenothera*. They doubtless occur in all the forms, as I have demonstrated them in several. Fig. 13 shows the connexions between two mother-cells of *O. gigas* in the condition of synizesis. It will be seen from this figure that they vary greatly in size. They are usually less numerous than in the figure, three or four being perhaps the most frequent number seen on one cell-wall. These connexions have never been observed on the walls separating the mother-cells from the tapetal cells, and apparently they only occur between mother-cells. Regarding the origin of these cytoplasmic connexions, they have not been traced to an earlier stage than the synaptic mother-cell because of the difficulty in demonstrating their presence. This requires a slight amount of plasmolysis, but not enough to rupture the connexions. When the mother-cells are very young, before the growth period begins, they are so small that no plasmolysis occurs, and the presence of connexions cannot therefore be observed in ordinarily fixed material at this stage of development. It seems most probable, however, that these connexions are formed by openings left in the cell-plate when it was originally laid down. These cytoplasmic connexions must form an important avenue of interchange between adjacent mother-cells. It is probable that during this process of extrusion the nucleus merely utilizes the connexions which happen to occupy the appropriate position.

If there are several of these openings near together, extrusion may take place through all of them. There may be a number of connexions on the

same wall, through which no extrusion takes place (Fig. 12). After extrusion has taken place, the nucleus moves back again towards the centre of the cell, and retains this position in the later stages of its development. In Fig. 12 the extrusion has evidently taken place, as shown by the dark-staining areas in the contiguous region of the adjacent cell, and the nuclei have begun to move back towards the centres of their cells. In this particular case it will be observed that two nuclei have extruded into the same mother-cell. The nuclear membrane is usually definite, sharp, and conspicuous at this time, but sometimes, as in one of the nuclei in Fig. 12, portions of it may be quite indefinite.

As already mentioned, in the cases observed (and the same process has been seen in certain anthers of several different flowers) the extrusion takes place simultaneously from all the mother-cells of a given anther, and so far as observed is always in the same direction in all the cells, indicating that the nuclear movement and extrusion are perhaps to bring about an equalization of pressure. It is very probable that the presence of these conspicuous openings or connexions between mother-cells allows of an equalization of pressure in the mother-cells from one end of an anther to the other. The movement of the nuclei and the nuclear extrusion may occur in connexion with this pressure-equalization, yet the latter seems scarcely adequate to account for it. Not infrequently the nucleus, instead of approaching the end wall of a mother-cell, moves to a side wall next the tapetum, which at this time is in direct contact with the row of mother-cells. In such cases no extrusion has ever been seen to take place, probably because there are no openings through which the extrusion could occur.

We may now trace the history of the extruded material. As shown in Figs. 8–10, extrusion of a portion of the chromatin material forming the spireme of synizesis takes place through the cytoplasmic connexions, just as a viscous fluid might, under pressure, flow through an opening or a tube, replacing the cytoplasm present. This material, which is forced through to the adjoining cell, first accumulates as a dense structureless mass or body. It is well known that, in the telophase of a nuclear division, when karyolymph begins to accumulate about the daughter group of chromosomes, a nuclear membrane is precipitated where this karyolymph comes in contact with the cytoplasm. In the same manner, an equally definite membrane is formed where the liquid accumulated about this extruded chromatin comes in contact with the cytoplasm. The result is a cavity containing chromatin and surrounded by a definite membrane. We may very well call this a pseudo-nucleus. The liquid contained in this cavity is probably the same as the karyolymph of the nucleus, and this liquid is either secreted by the extruded chromatin or at any rate produced in connexion with its activity. The chromatin in this pseudo-nucleus is at first in a compact mass, but this soon becomes looser in structure (Fig. 11, to the right), and finally (Fig. 11,

to the left) comes to appear strikingly like a threadwork or the 'spireme' of the nucleus from which it was extruded. The membrane surrounding this threadwork is at this time quite as definite as any nuclear membrane, so it is difficult to see how this differs in any particular, except its origin, from a real nucleus. It seems impossible to believe that any 'structure' persists through this series of transformations. This will be discussed later, for, without laying too much stress on this series of observations, it is obvious that they may have an important bearing on our present views of chromosome individuality and nuclear structure.

After the stage of the pseudo-nuclei shown in Pl. LXVII, Fig. 11, they become more indefinite, the membrane disappearing so that soon all that remains of the pseudo-nucleus is a densely-staining portion of the cytoplasm, as shown in Fig. 12. This becomes looser and more indefinite, taking many forms, and finally is probably entirely incorporated in the cytoplasm of the cell into which it was extruded.

This process of extrusion of chromatin from the nucleus of one pollen mother-cell into the cytoplasm of the adjacent mother-cell I call *cytomyxis*. Whether it always occurs at this stage of synapsis is not certain, but there are some observations which lead me to believe that it does not always occur. In some flowers one finds the nuclei of the mother-cells occupying positions almost or quite in contact with the cell-wall, but without any chromatin extrusion. As already stated, evidence that extrusion of chromatin has taken place remains for some time in the cytoplasm of the adjacent cell, so that in examining an anther it is easy to determine whether such extrusion has occurred. If the cytoplasm is entirely colourless (in iron-haematoxylin stain) it is evident that there has been no extrusion, no matter what the position of the nuclei. On the other hand, dark-staining cytoplasm does not always indicate that this process has taken place, for this is characteristic of degenerating cells. The position of these dark areas on the periphery and next to an adjacent mother-cell, together with their relation to the cytoplasmic connexions, serves to identify them.

It might be supposed that this process of chromatin extrusion was followed by degeneration of such mother-cells, but there are several reasons why this does not appear to be the case. In the first place, the nuclei appear perfectly normal after the extrusion has taken place, and later (Fig. 12) begin to move back towards the centre of the cell. The cytoplasm of such cells also appears wholly normal, and differs in no way from those in which no extrusion has occurred, except in having the dark areas on their periphery near certain of the cytoplasmic connexions.

At the time the movement of the nucleus to the cell-wall occurs, the spireme appears to be always on the side of the nucleus next the cell-wall, whether extrusion takes place or not. This is also true when the movement is to the wall in contact with the tapetal cells, with which there are no

observed cytoplasmic connexions. The nucleolus has never been observed to be in the 'sickle-stage' at this time, but is always spherical and floating freely in contact with the spireme. It takes no part in this extrusion.

It is probable that this lateral movement of the nucleus in the mother-cell always occurs, whether it be followed by an actual extrusion of chromatin or not. The conditions of pressure within the cells, and the presence of cytoplasmic connexions at the point of contact, probably determine whether such extrusion will occur.

It may be stated that the same process of chromatin extrusion occurs also in *O. biennis*, although Davis ('10), in his recent paper on this form, failed to observe it. In December, 1908, I showed (Gates, '09 *c*) that the phenomena of reduction are just the same in *O. biennis* and *O. laevifolia* as in *O. Lamarckiana* and its mutants. A recent re-examination of some of the preparations made at that time furnishes certain and conclusive evidence that the same process of chromatin extrusion occurs in *O. biennis*. Dark areas of cytoplasm had been noticed, but their significance was not understood, and it was supposed that they were connected with degeneration. They can now be clearly interpreted in the light of my observations on *O. gigas*, in which particularly favourable sections showing the extrusion were obtained. A few cases showing the actual extrusion taking place were also observed in *O. biennis*. This process, therefore, doubtless occurs in all the forms, and this is a further point showing the identity of the reduction processes in *O. biennis* and *O. Lamarckiana*.

Later Synaptic Stages.

After the nucleus moves back to the centre of the mother-cell, it retains this central position until the nuclear membrane disappears after diakinesis. The synizesis condition soon changes to one such as is to be seen in Fig. 14, in which the threadwork now occupies the whole of the nucleus once more, but the threads are beginning to show the progressive shortening and thickening which characterizes in part the succeeding stages of the nucleus. All the nuclei in one anther were in this particular condition, while other anthers of the same flower were in typical synizesis with the nucleus still central in position, and yet other anthers showed the beginning of the nuclear expansion which leads to the synizetic condition, as in Figs. 2–4. As shown by Fig. 14, the threadwork of the nucleus is fairly coarse, and the larger threads are characteristically straight and undeviating, quite unlike their usual meandering course. It is evident that a complete rearrangement of the contents of the nucleus must have taken place between this stage and the synapsis of Figs. 6 and 7. The interpretation of this stage will be referred to again later.

Pl. LXVII, Figs. 15–19 represent the same stage of the nucleus, in which a very characteristic structure is exhibited. This condition also follows soon after synizesis, and a complete rearrangement of the material from the synizetic condition has taken place. Figs. 15–17 are all sections of the nucleus at this time, representing its structure in different ways. The threads passing through the cavity of the nucleus are at this time comparatively few, as shown in Fig. 15, while the greater part of the chromatic material forms a rather delicate and very characteristic threadwork lining the nuclear membrane. Many of these threads appear in cross-section in Fig. 15. They adhere so closely to the nuclear membrane as to appear to be almost embedded in its substance. These threads are 'chromomeric' in structure throughout. They are nearly as delicate as in synizesis, and they are certainly single, not double, and contain *one* row of 'chromomeres'. This threadwork lining the nucleus frequently gives the appearance of a double nuclear membrane, i. e. one membrane within the other, but by careful focusing it can always be shown that the two apparent membranes in one focus merge into one. Figs. 18 and 19 are surface views of a portion of the nuclear membrane, to show the nature of this lining threadwork. Fig. 18 shows that free ends of the threads sometimes occur. This threadwork lines the whole of the nucleus in a remarkably uniform manner. The nuclear membrane itself is very definite at this time. As already stated, relatively few threads pass through the centre of the nucleus, and these are mostly different in structure from the threadwork lining the nuclear membrane. These central threads vary greatly in thickness, as shown by Figs. 15–17, some of them being coarse and heavy masses, while others are thin and delicate. In Fig. 17 a different representation of the chromomeric threads was attempted.

During this period the nucleolus varies in shape, frequently showing a certain amount of distortion, or being characteristically flattened as in Fig. 19.

Whether Figs. 15–19 represent a stage of development through which the nucleus always passes, or whether they represent rather a physiological condition of the nucleus which may or may not occur, is uncertain. In any case, it is a very characteristic condition, and exhibits a distribution of the chromatic material of the nucleus which is quite the reverse of that seen in synizesis, for instead of being rather closely aggregated in the synaptic ball it is in considerable part arranged as a threadwork lining the nuclear membrane. Davis ('09) has figured what is evidently the same stage in *O. grandiflora* (Pl. XLI, Fig. 13), but does not explain it.

The figures presented show how numerous are the stages or conditions of the nucleus during the period of synapsis. As I have shown elsewhere (Gates, '11), there is no reason to believe that synapsis accomplishes any special exchange of materials or influences between homologous chromo-

somes of the sporophyte. But it is well known that the synaptic period lasts for some time, and it is now evident that many changes in the condition of the nuclear contents may take place during this period.

The succeeding stages, up to the heterotypic mitosis, have not been studied in detail in *O. gigas*, and as they were investigated with special care in *O. rubrinervis* (Gates, '08), they will not be touched upon in the present paper.

Heterotypic Mitosis.

In *O. gigas*, as I have shown to be the case in the other *Oenothera* forms, the chromosomes are loosely scattered on the heterotypic spindle, so that it is frequently impossible to say which members constitute a pair. Pl. LXVIII, Figs. 20–22 represent pollen mother-cells in the metaphase of the heterotypic mitosis. In Fig. 22 the chromosomes show a rather irregular alinement in two series which have begun their journey to the poles. Usually the nucleolus disappears immediately upon the solution of the nuclear membrane after diakinesis, but occasionally it persists for a short time as a pale-staining body, as shown in Fig. 22. In connexion with a previous paper (Gates, '09 *a*) these stages were figured, but the plates were lost and I have since been unable to find again the particular cells from which they were drawn.

Fig. 23 represents an early anaphase in polar view, all the chromosomes but one being brought into view by focusing for a short distance in a vertical plane. A considerable amount of variation in the apparent size of the chromosomes is due to differences in the depth of stain. Figs. 24 and 25 are anaphases in somewhat oblique view. In both cases fourteen chromosomes can be counted in the group passing to the lower pole, while some of the chromosomes are missing from the upper pole. Fig. 26 is a later anaphase in side view.

As the chromosomes pass towards the poles of the heterotypic spindle there is much variation in their shape. Very often they appear square, oblong, triangular, or globular, with no indication of a bipartite structure. In other cases they may be somewhat elongated and with their arms bent in the form of a V. All these forms will be seen in Figs. 24 and 25. Many of the chromosomes in Fig. 26 and one of those in Fig. 24 show a sharp median constriction. In the chromosomes in Fig. 26 this appears as a definite lighter area through the median plane of the chromosome, and leads to the conclusion that this must be a transverse segmentation of the chromosome, if one can judge from their constant manner of orientation on the spindle. Other chromosomes in Fig. 26 show the 'tetrad' appearance so characteristic of the chromosomes in early interkinesis. This is due to a split (apparently longitudinal) of the more elongated chromosomes in the anaphase. There is thus wide variation in the time at which the split

which is characteristic of the heterotypic anaphase appears, and also in its direction, which apparently may be either longitudinal or transverse. The result is a very wide variation in the shape of the chromosomes as they pass to the poles of the heterotypic spindle. Davis states ('10, p. 644) that the chromosomes of the heterotypic mitosis 'in both *biennis* and *grandiflora* have the form of thickened V's and are not subglobular, as described and figured by Gates and Geerts'. With further observation he would have found that all the variations I have described occur in *O. biennis*, as well as in *O. gigas* and the other races of *Oenothera*. The split of the chromosomes for the homotypic mitosis may occur in the very early anaphase of the heterotypic, or it may be delayed so that the chromosomes retain a globular or subglobular shape with no evidence of fission even after the daughter nuclei of the heterotypic telophase have been formed. As will be seen from Pl. LXVIII, Figs. 24–26, the V-shape of the chromosomes in the heterotypic anaphase is frequently much less common than a more nearly globular shape.

When the chromosomes pass to the poles of the heterotypic spindle they at first form a very close group in contact with each other. The nuclear membrane very soon appears, as I have described elsewhere (Gates, '09 *b*, pp. 184–7), and as the karyolymph begins to increase the chromosomes continue to separate, and they remain hugging the nuclear membrane as the nucleus grows in size. They usually show clearly their bivalent character at this time, though the halves of these chromosomes are held tightly together and never appear in loose contact, yet the ends of the arms of the two halves are, usually at least, widely separated, as though they repelled each other like the strips of gold leaf in a Leyden jar. In my extensive observations of this interkinesis stage in a number of *Oenothera* forms, I have never found two chromosomes in contact at this time. They appear to be always equidistantly spaced just within the nuclear membrane, and it is probable that this manner of spacing is due to their mutual repulsion at this time.

During interkinesis there appears to be much variation in the extent to which the chromosome bivalents for the homotypic mitosis lose their compact condition and anastomose with each other. It is probable that in some cases the chromosome bivalents retain their compact condition with little change between the heterotypic telophase and the homotypic prophase. In other cases the chromosomes may become irregular in shape, with a considerable amount of anastomosis between chromosomes. Fig. 27 shows such a case in its earliest stages. In *O. gigas* this process frequently goes further, giving a series of coarse threads in which the boundaries of individual chromosomes can no longer be determined. In Fig. 27 the last of the heterotypic spindle is just disappearing. I have observed a number of times in *O. gigas* that an evanescent cell-plate may be formed

in the median plane of the heterotypic spindle. It seems to disappear with the spindle fibres, however.

Homotypic Mitosis.

Fig 28 shows the beginning of homotypic spindle formation, the first indication being the development of a weft of delicate wavy fibrils surrounding the nucleus. These become drawn out at certain points, as in Fig. 29, and then the nuclear membrane disappears. As will be seen from Fig. 28, the chromosomes in the homotypic prophase, before the disappearance of the nuclear membrane, show clearly their bivalent structure, which differs in no respect from that of the heterotypic telophase.

Just as in the anaphase and early telophase of the heterotypic mitosis the chromosomes not infrequently show no indication of a bivalent structure, so in the prophase of the homotypic mitosis this is sometimes the case. In Fig. 30 only one of the chromosomes is clearly bivalent, the rest showing little or no indication of a bivalent structure. Fig. 31 shows both the homotypic spindles in late prophase, just before the chromosomes are drawn into the equatorial plate. Nearly all of these chromosomes show their bivalent character. In this section all the chromosomes of the cell are present, there being sixteen on one spindle and twelve on the other. This means that on the heterotypic spindle in this mother-cell a 12–16 distribution of the chromosomes occurred, which is the widest departure from an equal distribution that I have found. Several cases of a 13–15 distribution in the reduction division were observed by counting the chromosomes in the early heterotypic telophase.

Fig. 32 is a side view of the homotypic metaphase, showing the chromosomes so closely massed that their boundaries are not clearly distinguishable. Fig. 33 shows all the chromosomes of a mother-cell in two rather scattered groups of fourteen each, probably in polar view, although the spindle fibres were not visible, and in this case there was no indication of the bivalent character of the chromosomes. Fig. 34 shows the homotypic spindles in side view, the chromosomes all showing their bivalent structure very clearly in this case. They are just being oriented in the equatorial plate, and several of them are still out of alinement. In Fig. 35 the chromosomes cannot all be separated from each other, and they are rather irregularly arranged on the spindles. In Fig. 36 only two of the chromosomes—one on each spindle—show their bivalent character. Their alinement is still somewhat irregular. Pl. LXIX, Fig. 37 is an early anaphase, in which the halves of the chromosomes have in most cases just separated. In this figure several of these anaphase chromosomes show a median constriction. I shall refer to this again later. In Fig. 38 the spindle to the left is in side view, showing several of the chromosomes whose halves have not yet separated, while the spindle to the right is seen in slightly oblique

polar view, and shows one of the early anaphase groups of fourteen chromosomes.

Pl. LXIX, Fig. 39 is a later anaphase of the homotypic spindle in oblique view, showing the group of fourteen chromosomes on their way to the lower pole. Only twelve of those approaching the other pole are to be seen, on account of the upper end of the spindle being obliquely cut. In that case it can be seen that the daughter chromosomes are partly looped or V-shaped, but some are long and rod-like, while others are short and rounded. There is thus also much variation in the shape of the chromosomes at this time, and this may apply to the chromosome group on a single spindle, as in Fig. 39. Pl. LXVIII, Fig. 40, shows a somewhat later homotypic anaphase in side view. The chromosomes here also show some variation in shape, but are mostly rather straight and somewhat elongated. Fig. 41 shows a homotypic spindle in anaphase, in which nearly all the daughter chromosomes are clearly constricted in the middle, and sometimes appear as though a definite transverse fission had taken place.

It has already been mentioned, in connexion with the synaptic stages, that different anthers of the same flower not infrequently show different conditions. A remarkable case of this kind has been observed in the pollen mother-cells during the homotypic mitosis. Pl. LXVIII, Figs. 32 and 35; Pl. LXIX, Fig. 37; Pl. LXVIII, Fig. 34; Pl. LXIX, Fig. 39; Pl. LXVIII, Figs. 40 and 41 are all taken from one flower. Of these, the first three are from one anther and the last four from another. A remarkable and constant difference was found between the pollen mother-cells of these two anthers. These differences (as can be observed from the figures) are (1) in the breadth of the spindles. In one anther (Pl. LXVIII, Figs. 32 and 35; Pl. LXIX, Fig. 37) these are very narrow, so much so that there is sometimes scarcely room for the chromosomes on the equatorial plate, and they project beyond the sides of the spindle, as in Pl. LXVIII, Fig. 32. These chromosomes also frequently fail to show their bivalent character at this time. In the other anther (Figs. 34, 40, and 41; Pl. LXIX, Fig. 39) the spindles are remarkably broad, and it would appear (although this is not certain) that the number of spindle fibres must be greater. The chromosomes usually appear as clear bivalents on the equatorial plate. This difference in the spindles was very striking and was found to be constant between different anthers. (2) While the walls of the mother-cells in anther no. 1 were smooth and even, as they ordinarily are, yet in anthers having the broad spindles already described the walls were always very irregular, showing extensive growths or proliferations subsequent to the separation of the mother-cells which takes place usually soon after synapsis. The character of these growths of the cell-wall can be judged from Pl. LXVIII, Figs. 28 and 34; Pl. LXIX, Figs. 38 and 39. This difference between pollen mother-cells is therefore not merely a matter of variation, but the broad homotypic spindles and

proliferated mother-cell walls were a constant feature of certain anthers in this flower, while certain other anthers showed with equal constancy very narrow spindles and smooth regular mother-cell walls. The cause or meaning of this difference is not evident. In other flowers the most usual width of the homotypic spindle is between these two extremes, as shown by Pl. LXVIII, Fig. 36, and Pl. LXIX, Fig. 38.

Pl. LXVIII, Fig. 42, shows an early telophase of the homotypic mitosis. Many of the chromosomes have the median constriction which appears to be characteristic of them at this time, not only in the homotypic telophase but in somatic telophases as well, as I have shown for *O. Lamarckiana* in a previous paper (Gates, '07, pp. 18–20). Figs. 43 and 44 are early homotypic telophases drawn with higher magnification, and nearly all the chromosomes show clearly a bivalent structure. Since the late anaphase stages of the heterotypic mitosis in *O. gigas* sometimes show a clear transverse constriction (Fig. 26), and since a similar constriction frequently appears in the early telophase of the homotypic mitosis, it appears probable that this may, in both cases, bear a relation to the succeeding karyokinetic division. It seems further probable that, in *O. gigas* at least, there is no significant difference between a longitudinal and a transverse fission of the chromosomes.

Fig. 45 is a slightly later stage in the homotypic telophase. The nucleus has grown and a nucleolus has appeared, while the chromosomes are beginning the process of 'stringing out' to form an anastomosis of coarse threads. In Pl. LXIX, Fig. 46, this process in the nuclei has gone somewhat further, the chromosomes having elongated considerably to form thick, somewhat ragged threads. This figure also shows the condition of the fibrillae in the cytoplasm just previous to the beginning of cell-plate formation. In comparing Pl. LXVIII, Figs. 42–45, and Pl. LXIX, Fig. 46, with those of the heterotypic telophase, it will be seen that while there has been no change in the size of the cell, the chromosomes, although the same in number, are only half the size of the bivalents in the heterotypic telophase, and the volume of the nuclei is correspondingly smaller. In this case the *amount* of chromatin evidently determines the size of the nucleus, for the number of bodies is the same in each case, and the size of the individual bodies is the differential factor.

The Pollen-tetrads and Pollen-grains.

Figs. 47 and 48 show the tetrad of young pollen-grains still within the mother-cell wall. After the tetrahedral walls come in, dividing the contents of the mother-cell into four pyramids with spherical base, whose apices meet at a central point, the walls begin to separate at the apex and the contents of each cell form a flattened disc, spherical in surface view, discoid in side view. This withdrawal of the cells leaves them arranged so

as to occupy nearly the whole periphery of the mother-cell, but with a large cavity in its centre. It would seem that this change in the shape of the young pollen-cells must be from forces inherent in the cells themselves, with which the mother-cell can have little concern. The discoid shape of the young pollen-grains, and afterwards of the older ones, is clearly self-determined.

Miss Lutz ('09) was the first to make the interesting observation that in *Oenothera gigas* the pollen-grains have characteristically four lobes or interstitial bodies, while all the other species of *Oenothera*, including *O. Lamarckiana* and its other mutants, are known to have usually three. These four interstitial bodies in the *O. gigas* pollen-grain are symmetrically placed at the corners of a square (Pl. LXIX, Fig. 49), showing that the four are as characteristic of *O. gigas* as three, placed at the corners of a triangle, are of *O. Lamarckiana* and its other mutants. Sometimes, however, one finds five interstitial bodies symmetrically placed, as in Fig. 50. At the age of the pollen-grain represented by Figs. 49 and 50, the cytoplasm still fills the whole cell, the nucleus is centrally placed, spherical, and with a definite membrane. Its reticulum is rather coarse, and two or three nucleoli are usually present.

Figs. 51 and 52 show a much later stage, in which the pollen-grain has undergone a great deal of growth, and the interstitial bodies give a more or less marked quadrangular appearance to the grain. The nucleus usually appears amoeboid in shape at this time, with very little chromatic content, and the cytoplasm is in coarse strands occupying only a portion of the cell. Beer ('06) has studied carefully various features in connexion with the growth and development of the triangular pollen-grains in *O. longiflora* and *O. biennis*.

In *O. gigas*, pollen-grains having more than four lobes are relatively common. Occasionally the lobes may then all be symmetrically placed, as in Fig. 50, but much more frequently the four original lobes are placed symmetrically, and the extra lobes are intercalated between these in various positions, frequently in a plane above or below the original ones. This is shown in Figs. 53 and 54, the first of which has four lobes rather regularly placed, and a smaller fifth lobe which is below the plane of the others. Fig. 54 has seven lobes, the three somewhat irregular and smaller additional lobes being all on the left side of the figure. It is therefore clear that in *O. gigas* the pollen-grain is constructed on a basis of four, just as in the other species it is on the basis of three, although grains with more than three lobes do occur. The reasons for this rather remarkable change are not clear, although one is tempted to speculate on the different size relationships of the cells and nuclei in the young pollen-grains, as a possible basis for this alteration. The doubling of the chromosome number, with the attendant doubling in the volume of the nuclei, but a different ratio of

increase in the cytoplasm, gives a new ratio for $\frac{\text{nuclear surface}}{\text{cell surface}}$, and this may conceivably have been the factor determining the appearance of a quartet instead of a triad of interstitial bodies.

This completes the account of the normal development. Many cytological features which were described in previous papers have not been repeated here, only the distinctly new features being emphasized.

Sterility in O. gigas.

I wish now to describe an interesting type of sterility in *O. gigas*, which differs in some respects from anything previously described in plants. Its peculiarity consists essentially in this: The cytological phenomena of reduction go forward in the normal manner, but the pollen mother-cells retain their archesporial appearance, their walls remaining in contact with each other and with the tapetum to form a compact tissue, so that they can never be set free or discharge pollen, even though the reduction processes are normally completed. This appears to be due primarily to a failure of the surrounding tissues of the anther to grow and form a cavity which allows sufficient space for the mother-cells to round off during synapsis and float freely in the cavity of the anther. Other features of this process will appear in connexion with the description. In all, four flowers, all from the same plant, were found to show this condition in various stages.

Fig. 55 is from a longitudinal section, showing some of the pollen mother-cells and their surrounding tapetal cells as they appear under a low power. Though the tapetal cells are binucleate and the mother-cells are in interkinesis, there is no tendency for the mother-cell walls to break apart or separate from the tapetum. The walls of the mother-cells frequently become much thickened and cutinized at this time. Fig. 56 is from another anther of the same flower, and shows a portion of a row of mother-cells. In the two long central cells of the row there has been an attempt at wall-formation after the heterotypic mitosis, which resulted only in an incomplete segmentation of the cytoplasm. The four cells so formed are each undergoing the second reduction division. There is frequently a more or less abortive attempt to form a wall after the heterotypic mitosis.

Figs. 57 and 58 are drawn with a little higher power. They each show two pollen mother-cells from a row, and all are in the heterotypic telophase. In all there has been a partially abortive attempt to form a wall separating the interkinetic nuclei, and in two cases this has resulted in a complete constriction of the cytoplasm. The lower mother-cell in Fig. 58, particularly, shows evidence of irregularity in the chromosome distribution during the heterotypic mitosis. The reduction processes are not entirely normal, and some of the evidences of irregularities will be seen in later figures. Pl. LXIX, Figs. 59–61, Pl. LXX, Fig. 62, represent stages of the homotypic prophase

under a high power. The chromosomes in these figures appear considerably larger than in the figures previously described. This is not due, however, to their containing more chromatin, but to the fact that many of the chromosomes are less dense and compact, and therefore stain less deeply. But although not alveolated, they occupy a larger space, and therefore of course, although they stain less deeply, appear to contain more chromatin. On account of this difference in compactness, the chromosomes differ greatly in apparent size, and in Pl. LXIX, Figs. 59–61, Pl. LXX, Fig. 62, while most of the chromosomes are in evident pairs, it is not possible in every case to say which are pairs and which single chromosomes.

Figs. 63 and 64 are anaphases of the heterotypic mitosis, showing the polyhedral character of the cells. Figs. 65–67 are later stages, showing the heterotypic telophase. All are from the same flower. In Figs. 65 and 66 the heterotypic spindle is in different stages of its disappearance, and a definite cell-plate is laid down. In Fig. 65 the interkinetic chromosomes are very compact and show no indication of bivalence, while in Fig. 66, which is a later telophase, the daughter nuclei have grown to larger size, the chromosomes are less compact, and most of them show clearly a bivalent structure.

In this telophase the chromosomes frequently undergo a sort of swelling or distension without vacuolation. They thus occupy a larger area, as already mentioned, but are less deep staining and less compact, though without any open spaces. Then stringing out at the corners occurs, and then the vacuolation process may begin.

Even the smaller nuclei in Fig. 66 afford evidence that their size depends upon the amount of chromatin they contain. In all the Figs. 65-67 there is evidence of irregularities in the chromosome distribution. It will be seen also that these mother-cells are polygonal, and still in contact with other cells on all sides.

One of the most striking features of these sterile anthers is the enormous variation in the size of the mother-cells. In normal development the pollen mother-cells, even when still in the archesporial condition, are approximately equal in size. In these sterile anthers, one mother-cell may have many times the volume of an adjacent mother-cell of the same anther.

Fig. 68 shows a mother-cell in the prophase of the homotypic mitosis. There was an incomplete attempt at segmentation (?) of the cytoplasm after the first division. These prophase chromosomes nearly all show their bivalent structure. Fig. 69 represents a homotypic telophase. The daughter nucleus in the higher focus on the left, being uncut, shows fourteen chromosomes. Here also there has been an incomplete attempt at a segmentation of the cytoplasm. Fig. 70 represents a condition sometimes occurring, in which a complete cell-wall is formed following the heterotypic mitosis. The two daughter cells are each in the telophase of another (the homotypic) mitosis,

and cell-plates are again being formed on these spindles. In Fig. 71 the same stage is to be seen, but no wall was formed after the first division. It is, of course, often difficult to determine just what stage is represented in a given mitosis, for the appearances are very different from those of ordinary pollen formation, and frequently can only be identified by the number and condition of the chromosomes.

Plate LXIX, Figs. 72–75 are homotypic telophase groups more highly magnified. Fig. 72 shows the chromosomes when they have just reached the pole of the homotypic spindle. Figs. 73–75 are slightly later, after the nuclear membrane has been formed. These nuclei are uncut and show all the chromosomes, fourteen in the first two cases and fifteen in the third. Thus one chromosome occasionally finds its way to the wrong pole, as I have shown in other forms of *Oenothera*, and such irregularities occur also in normal development in *O. gigas*, as pointed out previously in this paper.

In Plate LXX, Fig. 76, an evident cleavage of the cytoplasm of the mother-cell followed the heterotypic mitosis, and one of the daughter nuclei has divided again, this being followed by the beginning of a cleavage of the cytoplasm. Fig. 77 is a mother-cell in which there have been evident irregularities, and scattered spindle fibres appear in the cell. Fig. 78 represents two mother-cells in which there have been abortive attempts at cell-wall formation following division of the nucleus. In Fig. 79, in the upper mother-cell, no walls were formed and nuclei of varying size lie in the cytoplasm, while in the lower mother-cell degeneration of the nuclei is evidently taking place. The relationships of the cells, as well as other features, make it evident that these all represent mother-cells. The remarkable variation in the size of these mother-cells has already been mentioned, and is very well shown by comparing, e. g., Figs. 79 and 80, which are drawn with the same magnification. The latter tiny cell has undergone division, but several extra nuclei have been formed in the cytoplasm. In Fig. 81 a better attempt was made at completing the tetrad formation in the mother-cell, but three small nuclei were formed.

Figs. 82–84 show other more or less complete attempts to produce tetrads. In Fig. 84 apparently only one of the nuclei has undergone the homotypic division. Fig. 85 shows a characteristic condition which sometimes occurs after the formation of the tetrad nuclei. The mother-cell becomes scanty in cytoplasm, and the nuclei move together and form a group in the centre of the cell.

Of course, in all the mother-cells here described the tetrad of cells formed can never function as pollen-grains. All the mother-cells remain closely surrounded by the tapetal cells at their sides and by other mother-cells at their ends, and there is no tendency for them to become free and separate. As already stated, this appears to be due to the failure of the surrounding cells of the anther to continue their

normal growth. It is evident that this type of sterility is not due primarily to any failure of the cytological processes concerned with reduction, for these are followed through in many cases without irregularities. The tapetal cells, after the karyokinetic division of their nuclei which makes them all binucleate, do not undergo the further nuclear divisions and other changes which are characteristic of these cells in ordinary cases, but their later alterations seem to be suspended. Yet they seem well equipped for performing their glandular function, and the mother-cells are apparently not at all lacking in nourishment. This condition, in which the pollen mother-cells retain their archesporial condition and remain in contact although their nuclei undergo the normal reduction processes with few irregularities, has been found in four flowers of *O. gigas*, all taken from one plant. In one of these nearly all the anthers were in different stages of reduction, a condition already mentioned as being of much value in connexion with the interpretation of synapsis stages. In this flower, in anther no. 1 the pollen-tetrads had been more or less successfully formed, as in Pl. LXX, Fig. 83, but the walls of the mother-cells were very thick and cutinized. They still formed a compact tissue, and evidently could never round off and separate to form pollen-grains or free pollen-tetrads. The mother-cells and also the tapetal cells of this anther have very little contents and are evidently lacking in nourishment. In anther no. 2 of this flower the mother-cells are in interkinesis (Plate LXX, Fig. 65). In some cases a wall has been formed between the daughter nuclei, in others there is only a segmentation of the cytoplasm or an evanescent cell-plate. Anther no. 3 is in much the same stage as anther no. 2, some of the cells being in the homotypic prophase. In anther no. 4 the two reduction divisions had been completed, as in Plate LXX, Fig. 80, and small extra nuclei in the cytoplasm indicated frequent irregularities of the spindles. In anther no. 5 the mother-cells had undergone the reduction division, followed by segmentation of the cytoplasm, and the daughter nuclei had passed into the resting condition. In no. 6 the mother-cells were mostly in the early homotypic telophase, and a segmentation of the cytoplasm had followed the reduction division. In no. 7 the mother-cells were chiefly in interkinesis, showing the chromosome bivalents of this time. Anther no. 8 was in a stage just following synapsis, as illustrated by Plate LXVII, Figs. 15–19. In all the anthers except the first the tapetal nuclei are binucleate and well filled with cytoplasm, in the condition they normally exhibit about the time of synapsis. The other three flowers which showed this type of sterility were more uniform in the stage of development of the mother-cells throughout each flower.

Somatic Mitoses.

No figures of the somatic mitoses in *O. gigas* will be presented in the present paper, but the fact that they are much larger than in the forms with

fourteen chromosomes is a distinct advantage in the study of mitosis in nuclei, which are so small in comparison with other seed plants. Two points have been definitely determined from my study of these somatic divisions in *O. gigas.* (1) In the prophase the spireme segments into a single chain of twenty-eight chromosomes, all of which were arranged end to end in a single series. The spireme is usually wound regularly just within the periphery of the nucleus. (2) Soon after this segmentation, while still in the prophase condition, the chromosomes show, in many cases at least, a clear longitudinal split. Thus the split in the chromosomes occurs long before the equatorial plate stage.

Discussion.

Several special features in connexion with the data in this paper call for further discussion. Phenomena of chromatin extrusion, evidently very similar to those described here for *Oenothera*, have been described by Miss Digby ('09) in the pollen mother-cells of *Galtonia.* The chief difference exhibited by these processes in the two cases is that in *Galtonia* the nucleolus as well as the spireme extrudes bodies, while in *Oenothera* the nucleolus apparently takes no part in the process. In *Oenothera* the extrusion takes place, usually at least, through cytoplasmic connexions already present between mother-cells, the viscous chromatic material extruding through these pores and forming a solid mass in the other cell.

This may have an important bearing on our present theories of nuclear structure, for the exuded or extruded material quickly forms a pseudo-nucleus by the accumulation of karyolymph and the precipitation of a membrane where this comes in contact with the cytoplasm. Furthermore, the extruded chromatin, though it at first forms a body as solid as a nucleolus, soon spreads out and forms a structure closely resembling a reticulum or spireme. It seems probable from these observations that far too much objective morphological significance has been attached to various reticulum and spireme stages of the nucleus. It is evident that the greatest care must be exercised in interpreting spireme and reticulum stages as structures having a definite morphological meaning. Rather would it seem that these appearances depend upon the condition of physical aggregation of the chromatin material. For example, the spireme condition may be assumed as the result of the electrical charges held by the chromatin molecules. While cytological observation is of course very important as indicating different physiological conditions and stages of development in the nucleus, yet the chromosomes are not necessarily to be regarded as a congeries of definite bodies or particles which persist and multiply individually from stage to stage. This is an important modification of the ordinary method of cytological interpretation, for it assumes that we already see with our higher

powers the smallest nuclear elements which exist as distinct elements of organic structure. Many of the so-called structural features of the nucleus are such as might be assumed by any other colloidal material as well as chromatin, under similar physical conditions of aggregation.

Therefore it seems probable that our present microscope powers already disclose all of 'structure' that is to be found in the nucleus, and the search for structure within structure down to and beyond the limits of our present powers of magnification is probably a vain one. Chamberlain ('09) has found that in the enormous nuclei in the reproductive cells of Cycads, no more 'structure within structure' is to be seen than in the very much smaller nuclei of other plants. The appearance is rather that of a colloidal material loosely aggregated. It is not always sufficiently kept in mind that the 'structures' studied in the nucleus represent the coagulation and precipitation figures resulting from certain conditions of distribution of the viscous, colloidal chromatin materials in the living nucleus, although these different distributions are doubtless in themselves of the highest physiological significance.

This point of view does not imply that the chromosomes are chemically alike, and there is much evidence that they are not. Further, in the absence of definite observational evidence of self-propagating differential elements within the chromosome, it seems best at present to formulate hypotheses without making the assumption that such exist.

It is evident that this process of chromatin extrusion may also have an important bearing on our views of chromosome individuality, but it does not seem best to discuss this matter further until the significance of the process is better understood. Since this process, similar in the main essentials, has now been observed in two widely separated genera of Angiosperms, belonging respectively to Monocotyledons and Dicotyledons, it will probably be found to be of general occurrence. I have used the term cytomyxis to indicate that the process is probably one of equalization between different mother-cells. It is probable that actual extrusion of chromatin does not always occur, but that synapsis *may* be passed through without it, although the movement of the nucleus to the side of the cell probably always does take place. This process may have an important significance from the standpoint of heredity and the life cycle. A somewhat similar process has recently been described by Carruthers ('11) in the Ascomycete *Helvella*, although here the extrusion is from the nucleus into the cytoplasm of the same cell, and not into another cell.

The type of sterility here described is an interesting one. The plant which showed flowers in this condition also showed normal pollen development in other flowers, and its pollen was used successfully in making crosses. De Vries mentions (Mutationstheorie, ii, p. 59) that one plant of *O. gigas* in his cultures in 1899 was wholly sterile after repeated artificial

pollination. This was presumably due to a defect of the ovules, but it is not stated whether the pollen was good. In 1909 I observed one plant of *Oenothera* which was wholly sterile in its anthers, but produced plenty of large seed capsules, and therefore must have borne normal ovules and was presumably pollinated from adjacent plants. Regarding this plant, I need only say that it was one of a culture derived from near Liverpool, England, and resembling *O. grandiflora*, Ait., in many of its characters. Material was collected for a cytological study, but has not yet been examined, so it cannot be stated what was the nature of the sterility in this case. The plant differed in no other particular from other plants in the same culture and it bloomed abundantly, but the anthers in every flower examined at different times were dry and empty.

The observations on this type of sterility indicate the correctness of Tischler's ('08) conclusion, from his study of sterile hybrids, that the sterility does not depend upon any form of chromatin repulsion. It does not seem possible to define the general cause of sterility more definitely than 'lack of nutrition'.

In my previous paper on *Oenothera gigas* ('09 *a*), dealing with the size relationships of cells and nuclei, the point in the life cycle of *O. Lamarckiana* at which this mutant originated was discussed, and the evidence which is brought together in that paper pointed to the probability that the tetraploid chromosome number originated in the early divisions of the fertilized egg, through the failure of a nucleus to complete its division after the fission of the chromosomes had taken place. This gives two sets of fourteen chromosomes which are identical two by two. Strasburger ('10) agrees with this view as to the manner of origin of the *O. gigas* mutant, but Stomps ('10) thinks it more probable that the tetraploid number of chromosomes originated through the union of two germ cells in each of which reduction had failed to take place, which would be in accord with de Vries' theory regarding the origin of a mutation. As I have pointed out elsewhere (Gates, '09 *a*, p. 544), there are several reasons why this manner of origin is less probable than the one I have suggested. If Stomps' theory were correct, we should have a mutant occurring with twenty-one chromosomes, and it would be much more frequent in occurrence than in *O. gigas*. Such a mutant has never yet been found, and all the other mutants which are known have fourteen chromosomes, as in *O. Lamarckiana*. In the paper referred to (Gates, '09 *a*, p. 545) I made a list of cases of related species one of which has the diploid and the other the tetraploid number of chromosomes, the inference being that, as in the case of *O. gigas*, the tetraploid species had originated from a related diploid species. Although a number of such cases are now on record, there is not, so far as I am aware, a single case of a species whose sporophyte has the triploid number of chromosomes. But several such hybrids are known, notably

among plants *Drosera rotundifolia* × *D. longifolia* (thirty chromosomes), and *Oenothera lata* × *O. gigas* (twenty-one chromosomes). The former hybrid has, I believe, only been observed in the wild condition where both parent species occur in the same locality, so that it is probably a hybrid, although there is no experimental proof of its hybrid origin. Its characters agree with this supposition, and it has never been suggested that this form might be a mutant instead of a hybrid, nor would the suggestion have any probability. Stomps ('10, p. 59) cites an observation of Geerts ('09, p. 52), who found a single case of a megaspore mother-cell in *O. Lamarckiana* having twenty-eight instead of fourteen chromosomes. Stomps makes the assumption that such a mother-cell would undergo reduction, and that fertilization with a normal male cell would occur, giving a form having twenty-one chromosomes. It is probable that the tetraploid number of chromosomes originated in this cell through its failure to complete a mitosis. It does not follow that such a megaspore mother-cell would undergo reduction to fourteen chromosomes followed by embryo-sac formation and fertilization of the egg. From what we know of the close relation between the occurrence of the tetraploid condition and apogamy, it is at least equally probable that this tetraploid megaspore mother-cell would develop apogamously, without undergoing reduction or fertilization, and would therefore give rise to a form having twenty-eight chromosomes. Indeed, this may have been the manner of origin of *O. gigas*, instead of the failure to complete a mitosis in the fertilized egg as I previously suggested. A decision as to whether this suspended mitosis occurred in the megaspore mother-cell or in the fertilized egg or young embryo will only be possible with further observations, but either of these hypotheses seems at present more probable, and more in accord with related facts, than an origin from the union of two unreduced germ cells.

It further seems probable, though not certain, that whenever in the life cycle the duplication in the number of chromosomes occurred in *O. gigas*, other simultaneous changes took place in the germ-plasm. My previous paper ('09 *a*) on this subject was an attempt to explain as far as possible the peculiar characters of *O. gigas* on the basis of these changes in the dimensions of the cells. It is probable that such an explanation is inadequate to account for *all* the new characters of *O. gigas*, and that the appearance of the tetraploid number of chromosomes was accompanied by other changes. The further application of this method of analysis to *O. gigas* and other forms having a tetraploid number of chromosomes will aid in determining the nature and extent of any such change. It is to be hoped that an analysis of other cases from this point of view will be attempted.

Summary.

In this study of pollen development in *Oenothera gigas*, several special features of synapsis are emphasized. At the beginning of synapsis there is a rather sudden increase in the volume of the nucleus, there being in some cases a distension of the nuclear membrane and in other cases a rupture of the membrane and accumulation of karyolymph in the cytoplasm. The nuclear reticulum finally floats freely in the karyolymph, and then the rearrangement of the chromatin material from the reticulum to the spireme condition takes place.

Following this there is, at least in some flowers but probably not in all, an extrusion of chromatin from the nucleus of one mother-cell through cytoplasmic connexions, into the cytoplasm of an adjacent mother-cell. For this process I have suggested the name *cytomyxis*. Probably the nucleus always moves to one side of the cell at this time, but this may or may not be followed by extrusion of chromatin.

The extruded chromatin accumulates in a mass after passing through the cell wall. A clear liquid appears around these masses, and a membrane delimits the clear area from the cytoplasm, forming what I have called a pseudo-nucleus.

Later, these masses loosen up, and acquire an appearance very similar to a spireme. The membrane afterwards disappears and the extruded chromatin finally appears to be incorporated with the surrounding cytoplasm.

It is evident that this process may have an important bearing on current conceptions of the life cycle and heredity, and on the theory of chromosome individuality. It is not improbable that various features of the finer morphology of the nucleus depend upon the physical condition of aggregation of the nuclear contents, rather than upon autonomous self-propagating 'structures' as such.

After the chromatin extrusion has taken place, the nucleus moves back to the centre of the cell and undergoes various other transformations during the remainder of the period known as synapsis. The most conspicuous of these is one in which the larger part of the chromatin content of the nucleus is in the form of intertwining chromomeric threads which line the nuclear membrane.

The chromosomes are loosely arranged in the equatorial plate of the heterotypic spindle. As they pass to the poles they frequently undergo a split, which in some cases gives evidence of being transverse, in others longitudinal.

In one flower examined, two of the anthers exhibited constant differences in their mother-cells. In one anther all the mother-cells had smooth walls and the (homotypic) spindles were very narrow. In the other anther the

cell-walls all showed outgrowths or proliferations, and the spindles were constantly very much wider.

There is much variation in the shape of the daughter chromosomes of the heterotypic mitosis, and in the time when the fission of the chromosomes for the homotypic mitosis occurs. In the anaphase or telophase of the homotypic mitosis the chromosomes usually show a median constriction.

A type of pollen sterility was studied in certain flowers, in which the reduction processes are normal or nearly so but the surrounding tissues fail to grow, remaining in contact with the mother-cells, which therefore retain their polyhedral shape. In some cases the four nuclei resulting from reduction move together to form a group in the centre of the mother-cell. In other cases a wall may be formed after the heterotypic mitosis, or the cytoplasm of the mother-cell may undergo cleavage to form a tetrad. The usual irregularities observed in hybrids also sometimes occur.

It is considered most probable that the mutant *O. gigas*, with the tetraploid number of chromosomes, originated through a suspended mitosis, either in the fertilized egg or in the megaspore mother-cell.

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EXPLANATION OF PLATES LXVII–LXX.

Illustrating Dr. Gates's paper on Pollen formation in *Oenothera gigas*.

(The lenses used were a Leitz $\frac{1}{12}$ inch oil immersion, ap. 1·40 with compensating oculars, the figures being reduced one-fourth in reproduction. This gives magnifications which allow comparison of the size of the cells in *O. gigas* with those of other Oenotheras figured in my previous papers.)

PLATE LXVII.

Fig. 1. Archesporial pollen mother-cell just previous to the beginning of synapsis. Many dark bodies appear near the periphery of the nucleus. × 2,850.

Fig. 2. Nucleus showing the beginning of synapsis. The nuclear membrane is gradually expanding and being pushed away from the reticulum. × 2,850.

Fig. 3. Mother-cell in the beginning of synapsis. Apparently a rather explosive rupture has taken place on one side of the nucleus, producing a cavity in the cytoplasm filled only with karyolymph. The cytoplasmic reticulum presents a rather ragged appearance. No change has yet taken place in the nuclear reticulum. × 2,850.

Fig. 4. A slightly later condition, in which the distension of the nuclear membrane has been followed by its rupture at one place, producing an ill-defined area in the cytoplasm on the right side filled with cell-sap. On the left side a few nuclear threads remain attached to the membrane. × 2,850.

Fig. 5. Synapsis is nearly completed. A single nucleolus is enclosed in the meshes of the delicate threadwork, which is nearly transformed into a spireme. The nuclear membrane is very indistinct on one side and the cytoplasmic reticulum is ' ragged '. × 2,850.

Fig. 6. Pollen mother-cell in synapsis (synizesis). The nuclear threads are extremely attenuated. The nuclear membrane is intact and the cytoplasmic reticulum is not ragged. × 1,875.

Fig. 7. Same synaptic condition as Fig. 6. Darker areas appear on the nuclear threadwork, which is quite transformed into the spireme condition. The nucleolus is attached to the nuclear membrane. × 1,875.

Fig. 8. Row of pollen mother-cells, showing the extrusion of chromatin from the nucleus of one mother-cell into the cytoplasm of the next, to form pseudo-nuclei. × 600.

Fig. 9. Three pollen mother-cells, showing how the extrusion takes place. × 1,275.

Fig. 10. The same more highly magnified. × 1,875.

Fig. 11. Section of pollen mother-cell, showing the two pseudo-nuclei formed by extrusion from the adjacent cell. In the one on the right the chromatic material is just beginning to break up, and on the left this process has gone further, producing an appearance resembling a spireme. In the next section the nucleus of this cell and its cytoplasmic connexions are seen. × 1,875.

Fig. 12. Chromatin has been extruded from the nuclei to form dark masses in the cytoplasm of the adjoining cell. The nuclei are moving back towards the centre of the cell. × 1,875.

Fig. 13. Showing numerous cytoplasmic connexions between pollen mother-cells. × 1,875.

Fig. 14. A later stage in which the nucleus occupies a central position. The nuclear thread-work has been completely rearranged since synapsis, the threads now occupying the whole cavity, and being characteristically straight instead of meandering. × 2,850.

Fig. 15. A synaptic stage in which most of the threads line the nuclear membrane and are seen here in cross-section. A few threads, varying greatly in thickness, pass through the cavity of the nucleus. × 2,850.

Fig. 16. Same stage as Fig. 15. × 2,850.

Fig. 17. Same stage, differently represented. × 2,850.

Fig. 18. Section of nucleus at this time, showing only the 'chromomeric' threadwork lining the nuclear membrane. Some free ends of threads appear. × 2,850.

Fig. 19. Same as Fig. 18, showing also the nucleolus and several other bodies attached to the nuclear membrane. × 2,850.

PLATE LXVIII.

Fig. 20. Pollen mother-cell in heterotypic metaphase. Twenty-six chromosomes are visible, rather irregularly scattered on the spindle, several apparent pairs. × 2,100.

Fig. 21. Heterotypic spindle in metaphase, showing twenty chromosomes. × 2,100.

Fig. 22. Heterotypic spindle in early anaphase, showing nineteen chromosomes and a persistent nucleolus. × 2,100.

Fig. 23. Heterotypic spindle, early anaphase in polar view. Twenty-seven chromosomes visible. × 2,100.

Fig. 24. Heterotypic spindle, late anaphase, slightly oblique cut. All fourteen chromosomes present at lower pole, and twelve at upper pole of spindle. × 2,100.

Fig. 25. Oblique cut, later anaphase, 14 + 11 chromosomes visible. × 2,100.

In Figs. 22, 24, and 25 the anaphase chromosomes vary in shape, being mostly sub-globular, rod-shaped, or V-shaped, but very few show indications of their bivalent character.

Fig. 26. Late heterotypic anaphase. Six of the chromosomes show a clear median transverse constriction or segmentation, while several others have the characteristic appearance of 'tetrads'. It appears that both transverse and longitudinal divisions of the chromosomes may occur at this time. × 2,100.

Fig. 27. A late stage of interkinesis. The remnants of the heterotypic spindle still remain in the cytoplasm. The chromosomes do not always anastomose to this extent during interkinesis. × 2,100.

Fig. 28. Homotypic prophase, showing beginning of spindle formation and chromosome bivalents. The mother-cell wall has characteristic outgrowths. See text, p. 924. × 2,100.

Fig. 29. Slightly later stage than Fig. 28, showing fourteen bivalent chromosomes on the multipolar spindle. × 2,100.

Fig. 30. Slightly later stage of homotypic spindle. The spindle fibres are being rearranged and attached to the fourteen chromosomes, only one of which in this case shows its bivalent character. × 2,100.

Fig. 31. Homotypic prophase. The chromosomes are all present, sixteen on one spindle and twelve on the other. They vary much in size and shape, but most show their bivalent character. This unequal distribution must have occurred in the heterotypic mitosis. × 2,100.

Fig. 32. Homotypic metaphase. In all the mother-cells of this anther the spindles were very narrow, so that there was scarcely room for the chromosomes. × 2,100.

Fig. 33. Homotypic metaphase in polar view, showing the two scattered groups of fourteen chromosomes. × 2,100.

Fig. 34. Homotypic metaphase. In this anther all the mother-cells had proliferated cell-walls and very broad spindles. The chromosomes are all clearly bipartite. × 2,100.

Fig. 35. Another homotypic metaphase, showing narrow spindles and smooth mother-cell wall. × 2100.

Fig. 36. Homotypic metaphase, in which only two chromosomes, one on each spindle, show their bivalent character. Thirteen chromosomes visible in left-hand spindle, and a nucleolus persists in the cytoplasm. × 2,100.

Fig. 40. Homotypic spindle in late anaphase. Spindle cut, but 12 + 13 chromosomes present. × 2,100.

Fig. 41. Section of another homotypic spindle in anaphase, in which nearly all the chromosomes show a clear median constriction. There are also several rods and one V. × 2,100.

Fig. 42. Homotypic telophase, showing many of the daughter chromosomes transversely constricted. × 2,100.

Figs. 43 and 44. Somewhat later homotypic telophase nuclei, showing the bivalent appearance of many of the chromosomes. × 2,850.

Fig. 45. Later homotypic telophase, in which the nucleus has grown larger, a nucleolus has appeared, and the chromosomes have begun to lose their compact structure. × 2,850.

PLATE LXIX.

Fig. 37. Early homotypic anaphase. Spindles very narrow. Several of the daughter chromosomes show a median constriction. × 2,100.

Fig. 38. Homotypic mitosis. Spindles broad and cell-wall proliferated. The spindle on the left shows several of the chromosomes whose halves have not yet separated. The spindle on the right is an early anaphase, showing in oblique view one of the groups of fourteen daughter chromosomes. × 2,100.

Fig. 39. Later homotypic anaphase, slightly oblique cut, showing 14 + 12 chromosomes on the spindle. Chromosomes partly looped and partly rod-shaped or nearly globular. Cell-wall proliferated. × 2,100.

Fig. 46. Late homotypic telophase. The chromosomes are elongating and 'stringing out' to pass into the resting condition. Cell-plates not yet formed. Cell-wall proliferated. × 2,100.

Figs. 47 and 48. Tetrads of young pollen cells within the mother cell-wall. × 525.

Fig. 49. Young pollen grains of *O. gigas* with four interstitial bodies. × 1,275.

Fig. 50. Similar grain, with five interstitial bodies symmetrically placed. × 1,275.

Figs. 51 and 52. Typical four-lobed pollen grains of *O. gigas*. Nucleus amoeboid in appearance, cytoplasm in strands. × 1,275.

Figs. 53 and 54. Five-lobed and seven-lobed grains, with the extra lobes asymmetrically placed. × 1,275.

Fig. 55. Longitudinal section, showing a row of pollen mother-cells in interkinesis, and the surrounding binucleate tapetal cells. The mother-cell walls have remained in direct contact with the tapetal cells. × 600.

Fig. 56. A row of mother-cells still in contact with each other, and polygonal, although undergoing the reduction divisions. In the two middle mother-cells a cleavage of the cytoplasm follows the heterotypic mitosis. × 900.

Figs. 57 and 58. Each figure shows two mother-cells in interkinesis. In two of the cells a cleavage of the cytoplasm has appeared between the daughter nuclei, and in one a small extra nucleus appears in the cytoplasm. × 1,125.

Figs. 59–61. Homotypic prophase nuclei before the disappearance of the nuclear membrane. Most of the chromosomes are clearly in pairs, and they vary greatly in size owing to differences in their compactness. × 2,550.

Fig. 72. Early homotypic telophase group of chromosomes. × 2,850.

Fig. 73. A later stage of the homotypic telophase, after the formation of the nuclear membrane. Fourteen chromosomes. × 2,850.

Fig. 74. Slightly later stage, nucleus uncut, showing fourteen chromosomes and a nucleolus. × 2,850.

Fig. 75. Same as Fig. 73. Showing fifteen chromosomes and a nucleolus. In Figs. 72–75 the chromosomes show no sign of a median constriction as in normal development. × 2,850.

PLATE LXX.

The figures in this plate are all from flowers showing abnormal development, in that the pollen mother-cells retain their polyhedral shape.

Fig. 62. Homotypic prophase, showing spindle formation and the polygonal character of the mother-cell. × 2,550.

Figs. 63 and 64. Heterotypic anaphase, showing the polyhedral shape of the cells and the compact, univalent structure of the chromosomes. × 1,875.

Figs. 65 and 66. Heterotypic telophase, showing an attempt at cell-wall formation. In Fig. 66 small extra nuclei appear in the cytoplasm. × 1,875.

Fig. 67. Heterotypic telophase with extra nuclei in the cytoplasm. × 1,875.

Fig. 68. Homotypic prophase, showing bivalent chromosomes. The heterotypic mitosis in this mother-cell was followed by segmentation of the cytoplasm. × 1,875.

Fig. 69. Homotypic telophase. One nucleus uncut shows fourteen chromosomes. A cleavage of the cytoplasm appears in the line which was the equatorial plane of the heterotypic spindle. × 1,875.

Fig. 70. Homotypic telophase. A complete cell-wall has been formed, following the heterotypic division. × 1,125.

Fig. 71. Same stage as Fig. 70, but no wall formed after the heterotypic mitosis. × 1,125.

Fig. 76. Late homotypic telophase. Segmentation of the cytoplasm followed the heterotypic mitosis, and a cleavage of the cytoplasm is appearing between the two daughter nuclei on the left. × 1,125.

Fig. 77. This mother-cell shows evident irregularities, with extra nuclei, a cleavage of the cytoplasm, and scattered spindle fibres. × 1,125.

Fig. 78. Two mother-cells with abortive attempts at wall formation following division of the nucleus. × 1,125.

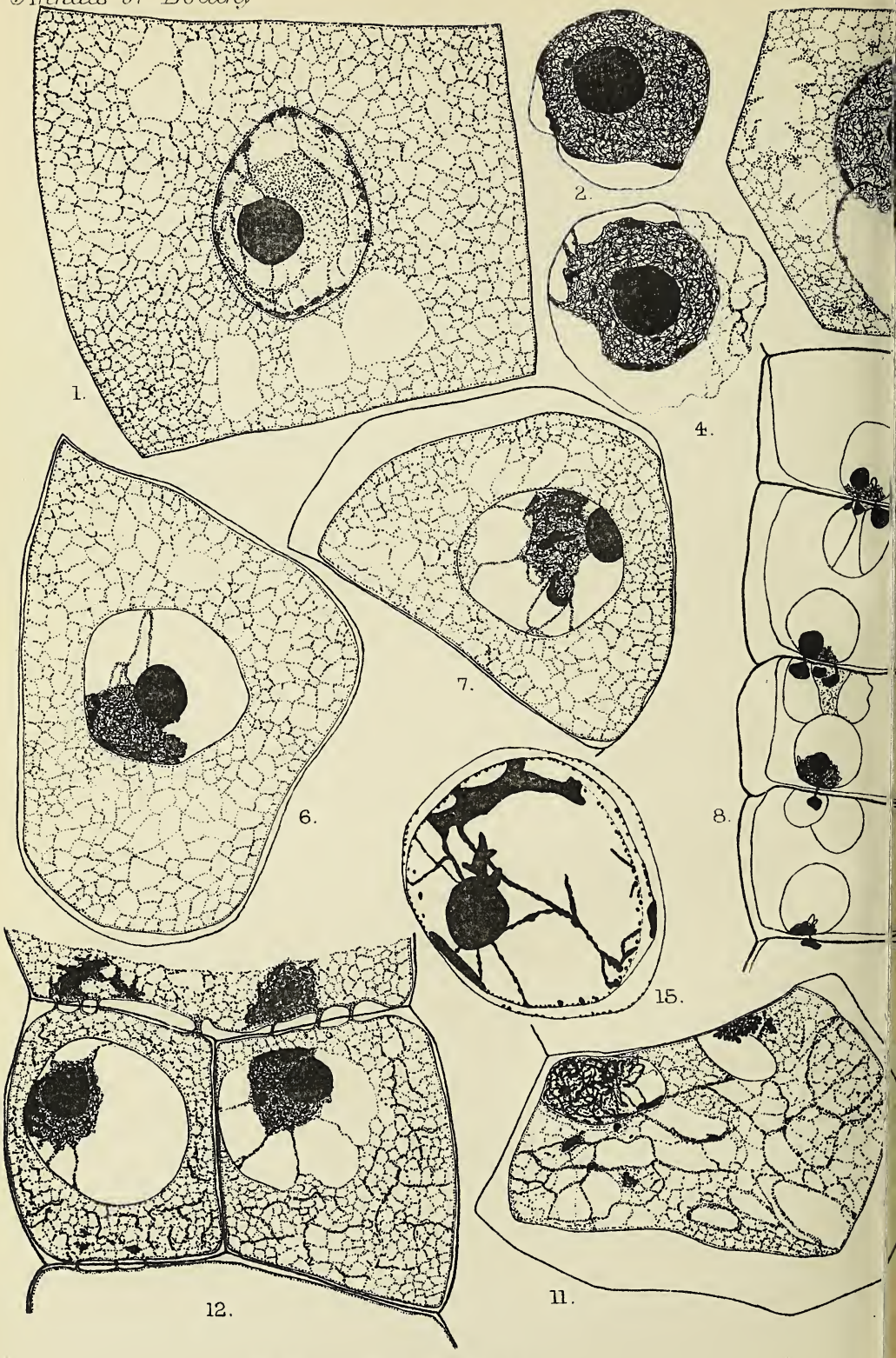
Fig. 79. Two mother-cells after irregular reduction divisions. In the upper cell small extra nuclei lie in the cytoplasm, while the nuclei of the lower cell are degenerating. × 1,125.

Fig. 80. A very small mother-cell (cf. Fig. 79), showing irregularities in the chromatin distribution. × 1,125.

Fig. 81. A completed tetrad, but three small nuclei formed in the cytoplasm. × 1,125.

Fig. 82–84. More or less complete attempts to form pollen tetrads. Walls are formed within the mother-cell, although the latter remains in contact with adjacent cells. In Fig. 84 apparently only one daughter nucleus has undergone the second mitosis. Mother-cell walls very thick. × 1,125.

Fig. 85. A condition sometimes found after the completion of tetrad formation. The four nuclei move together to the centre of the mother-cell, which contains little cytoplasm. In this case no walls were formed within the mother-cell. × 1,125.



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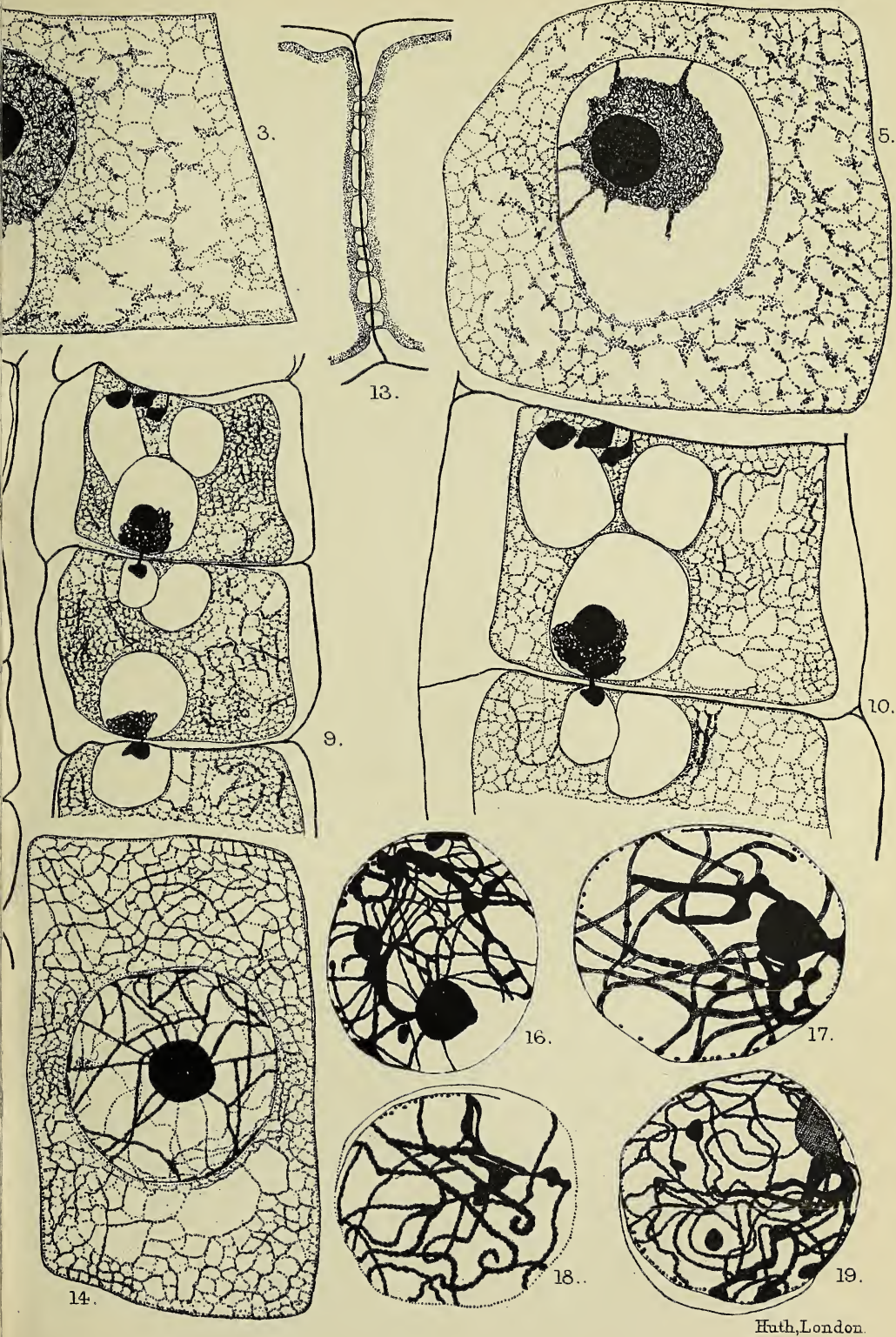

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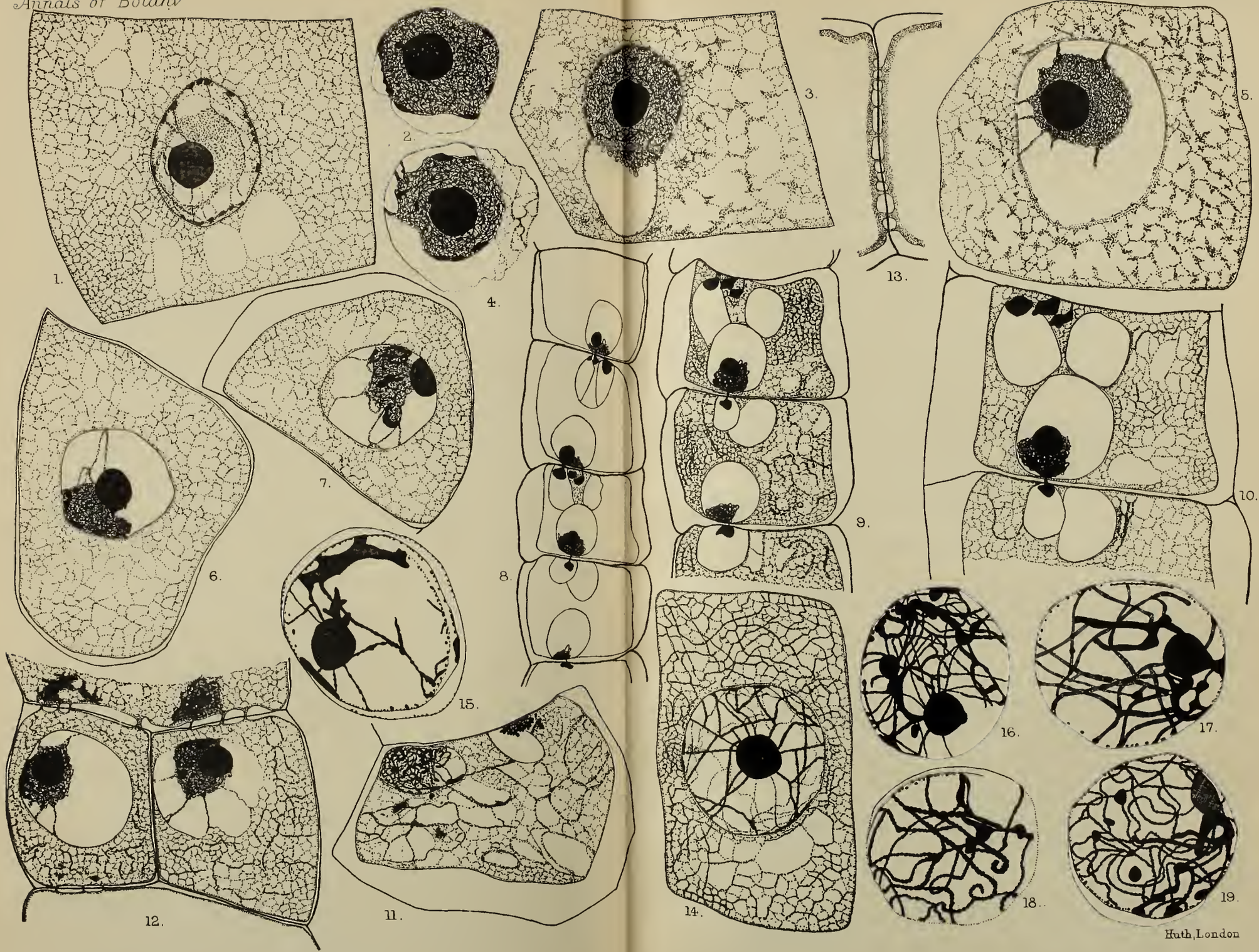

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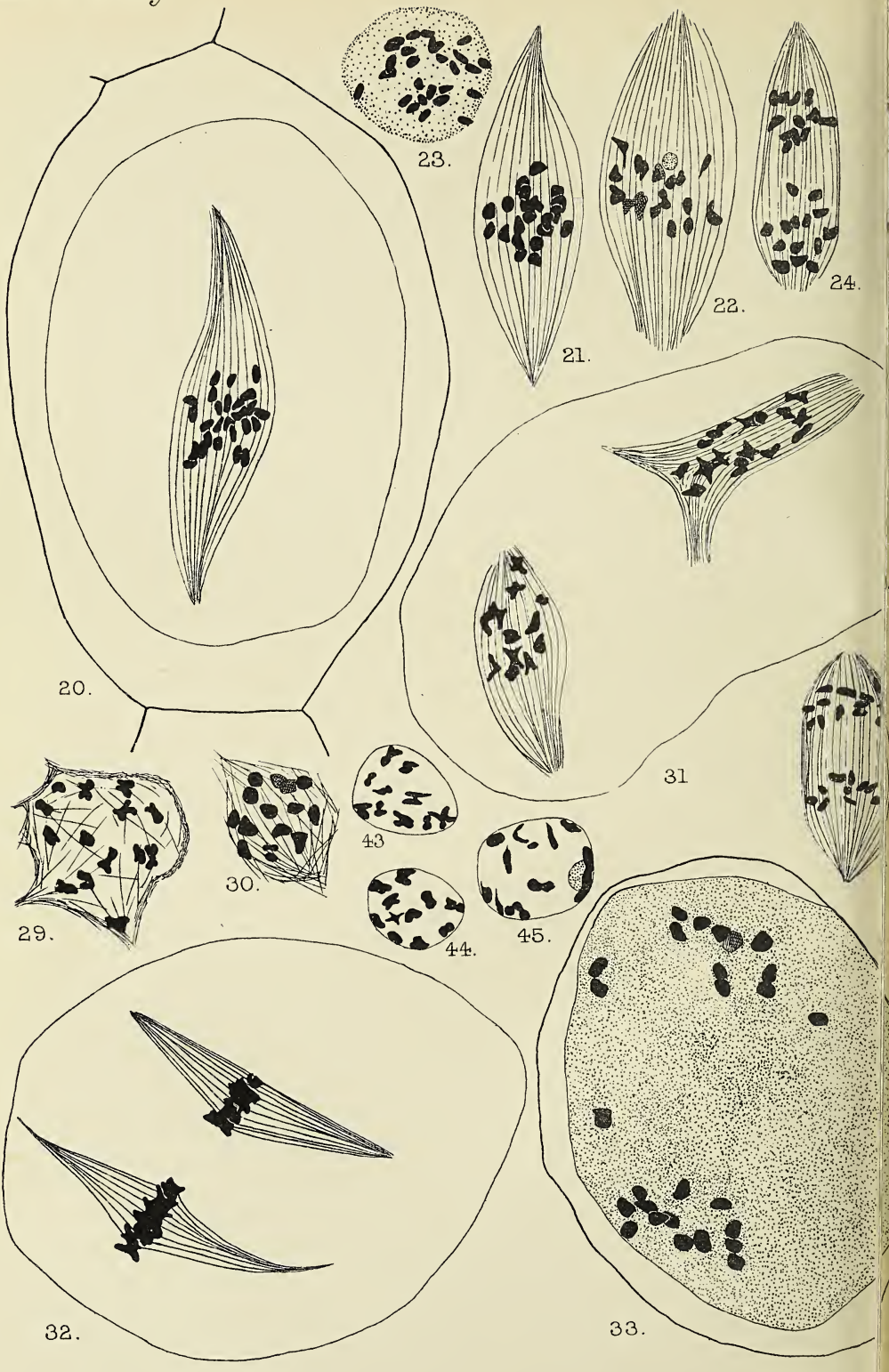
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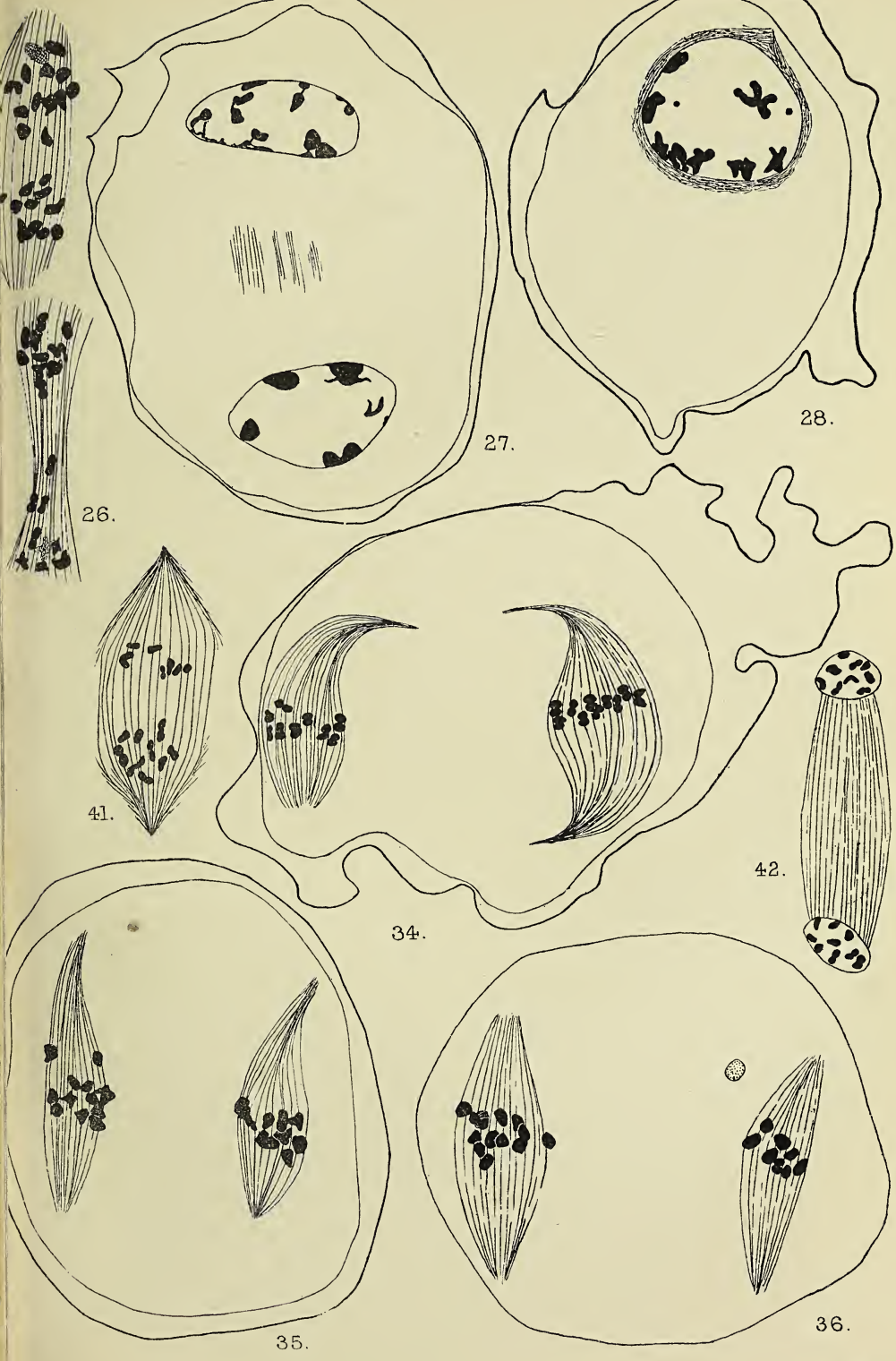

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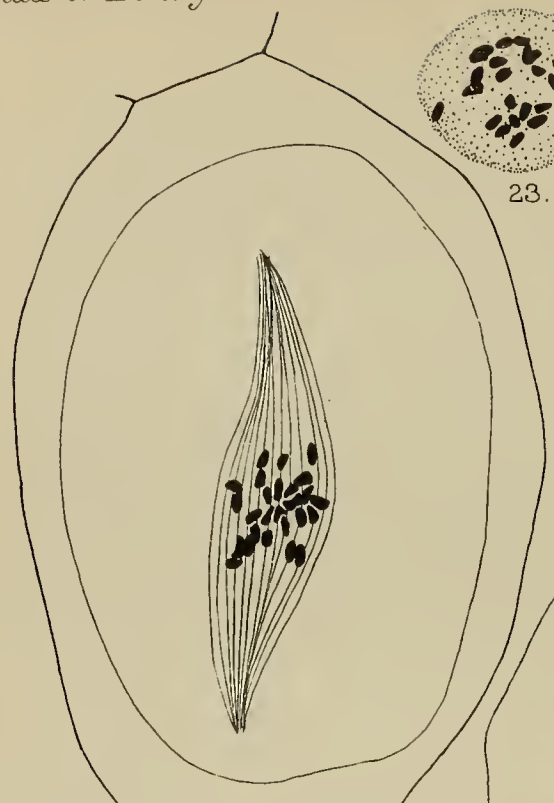

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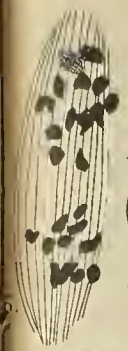

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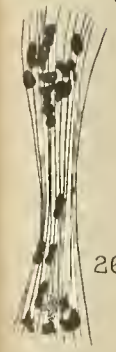

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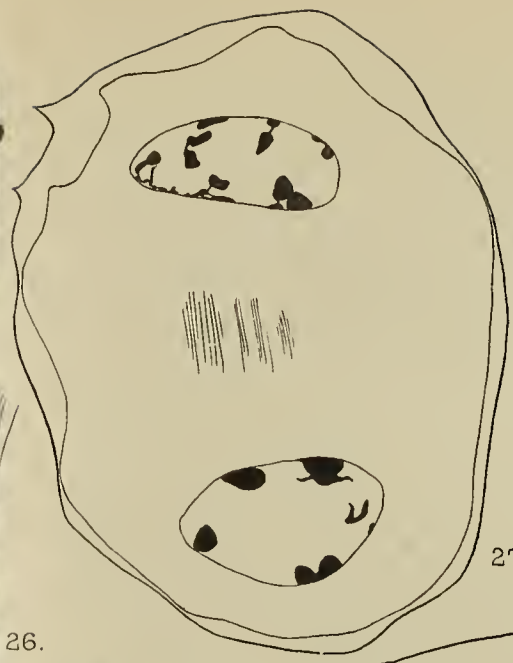

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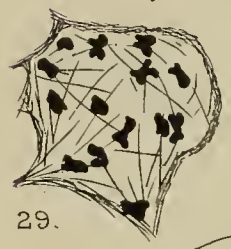

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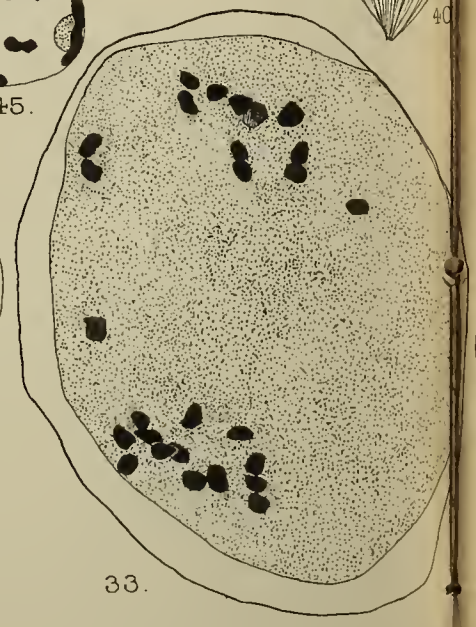

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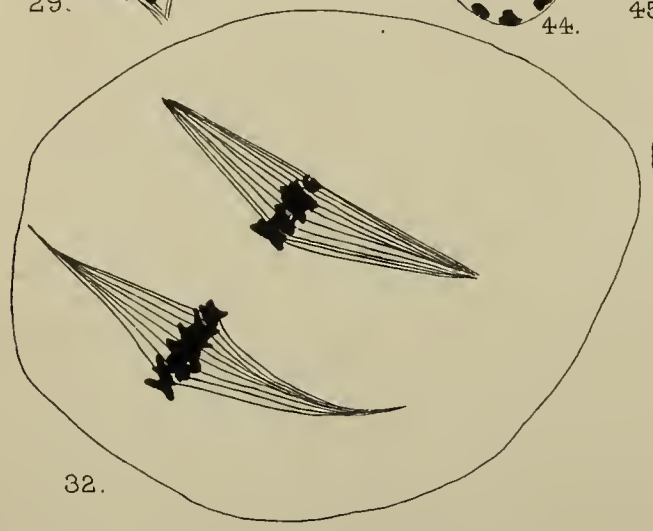

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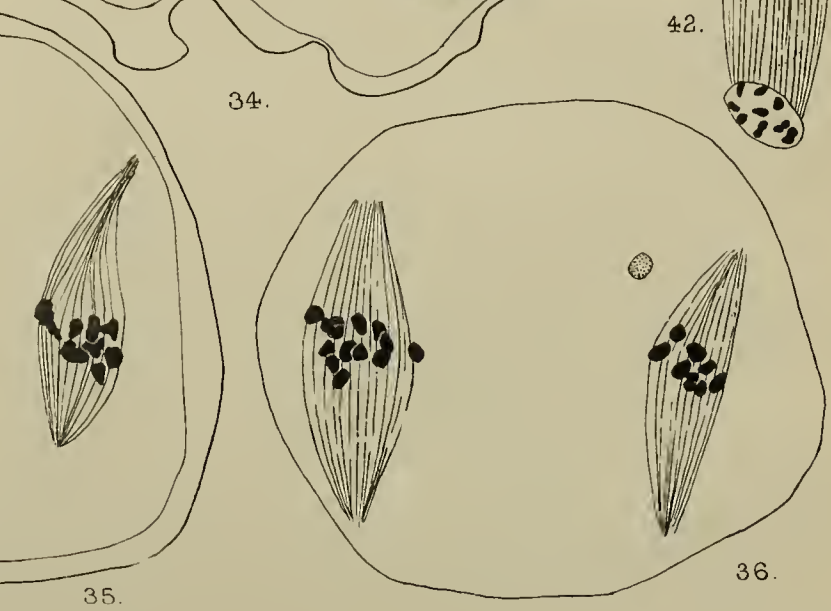

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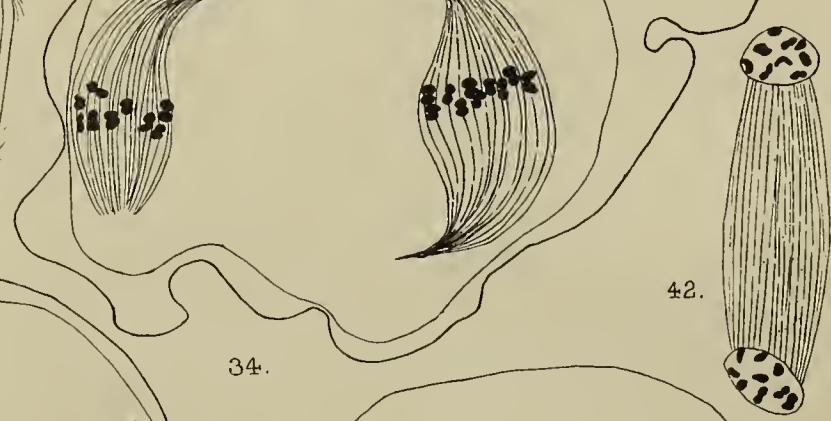

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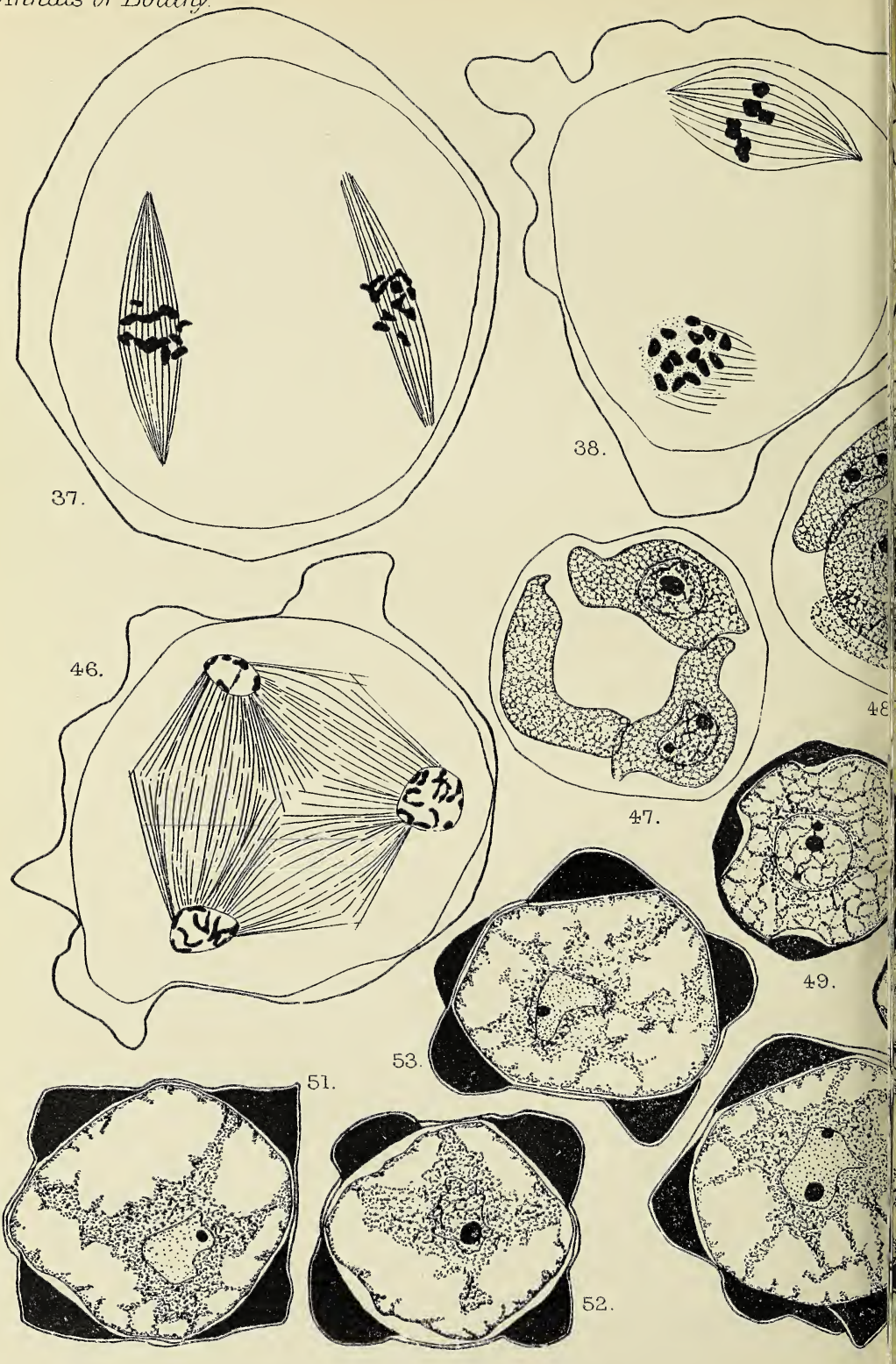

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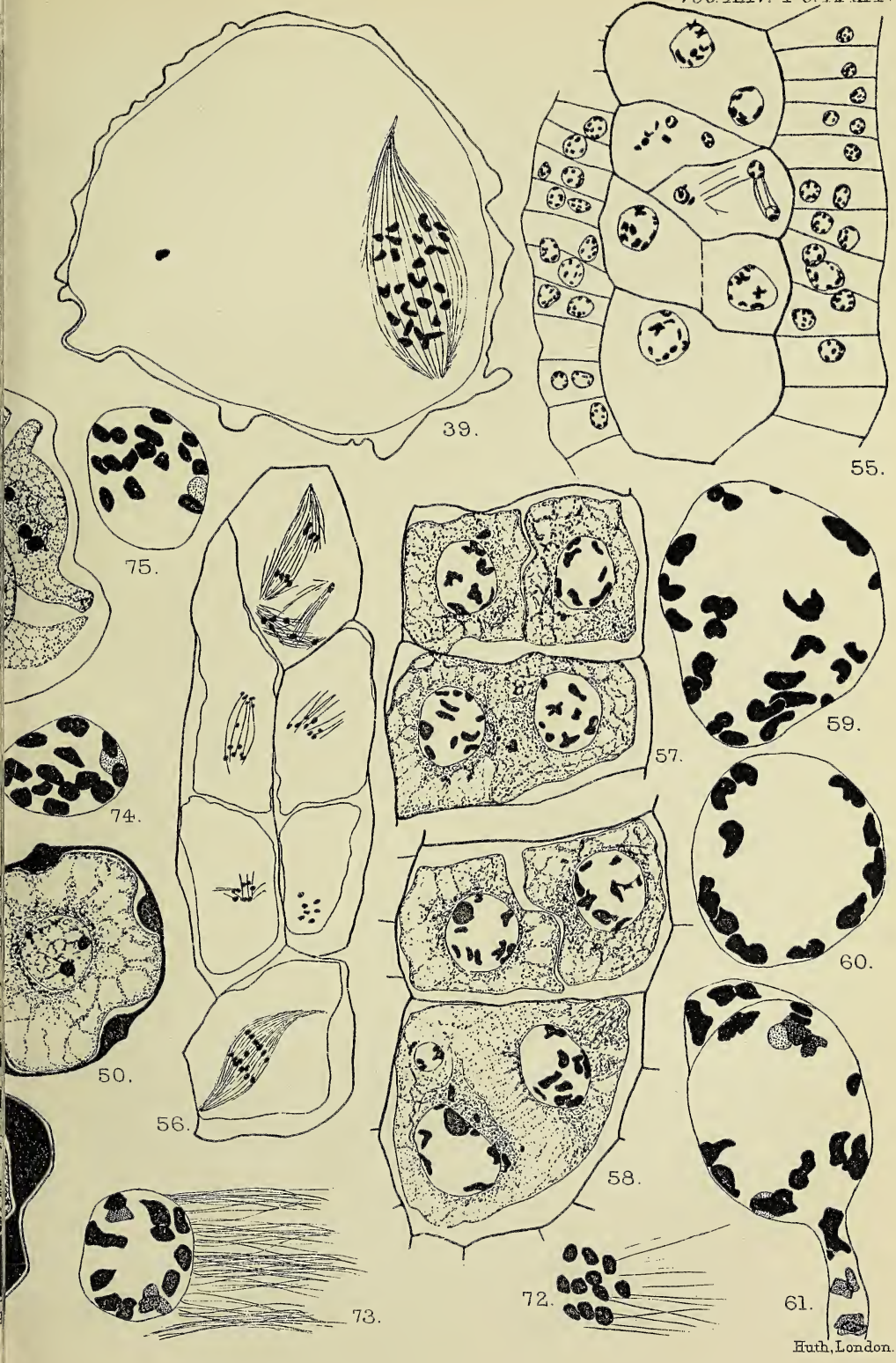

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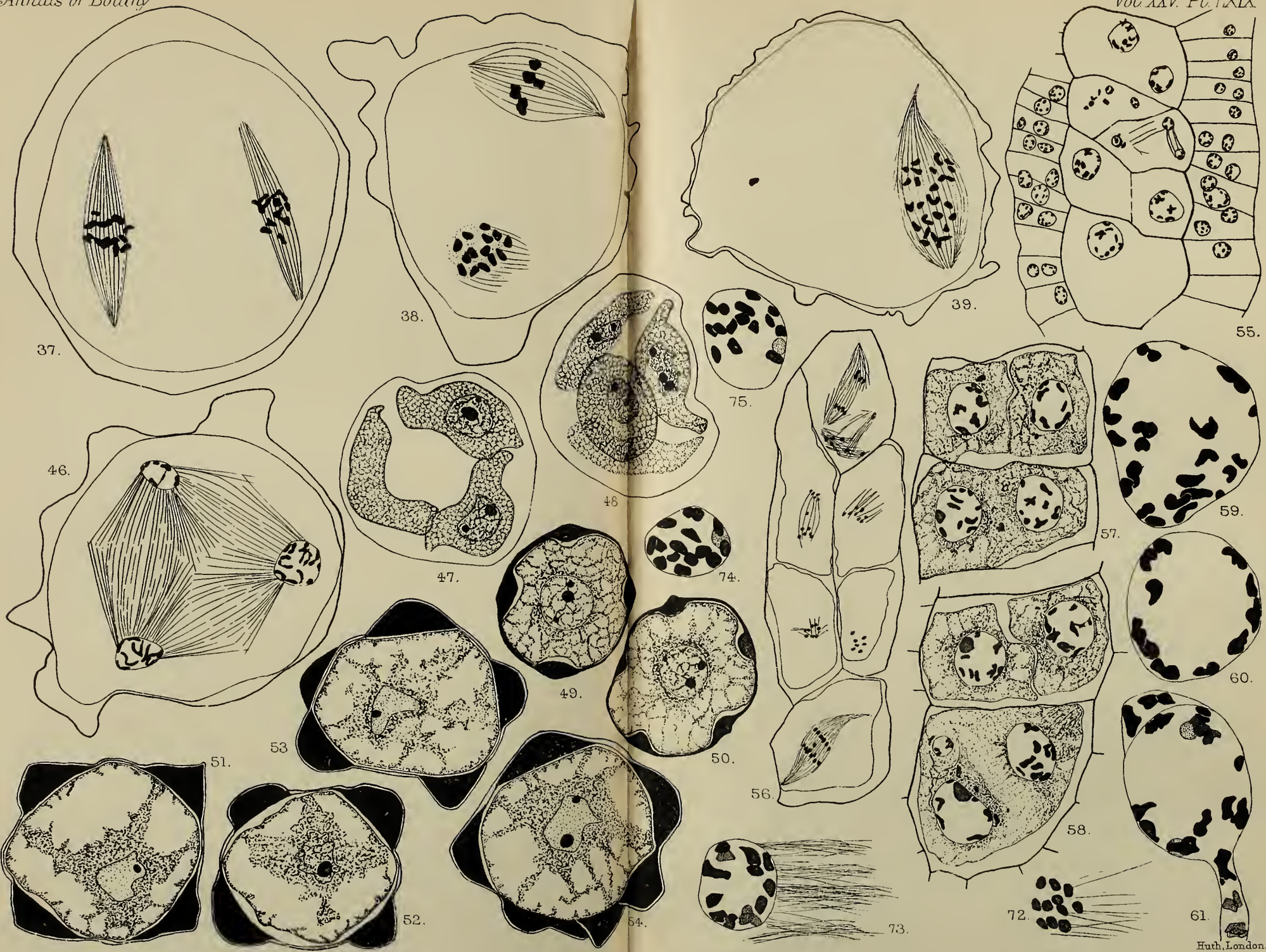

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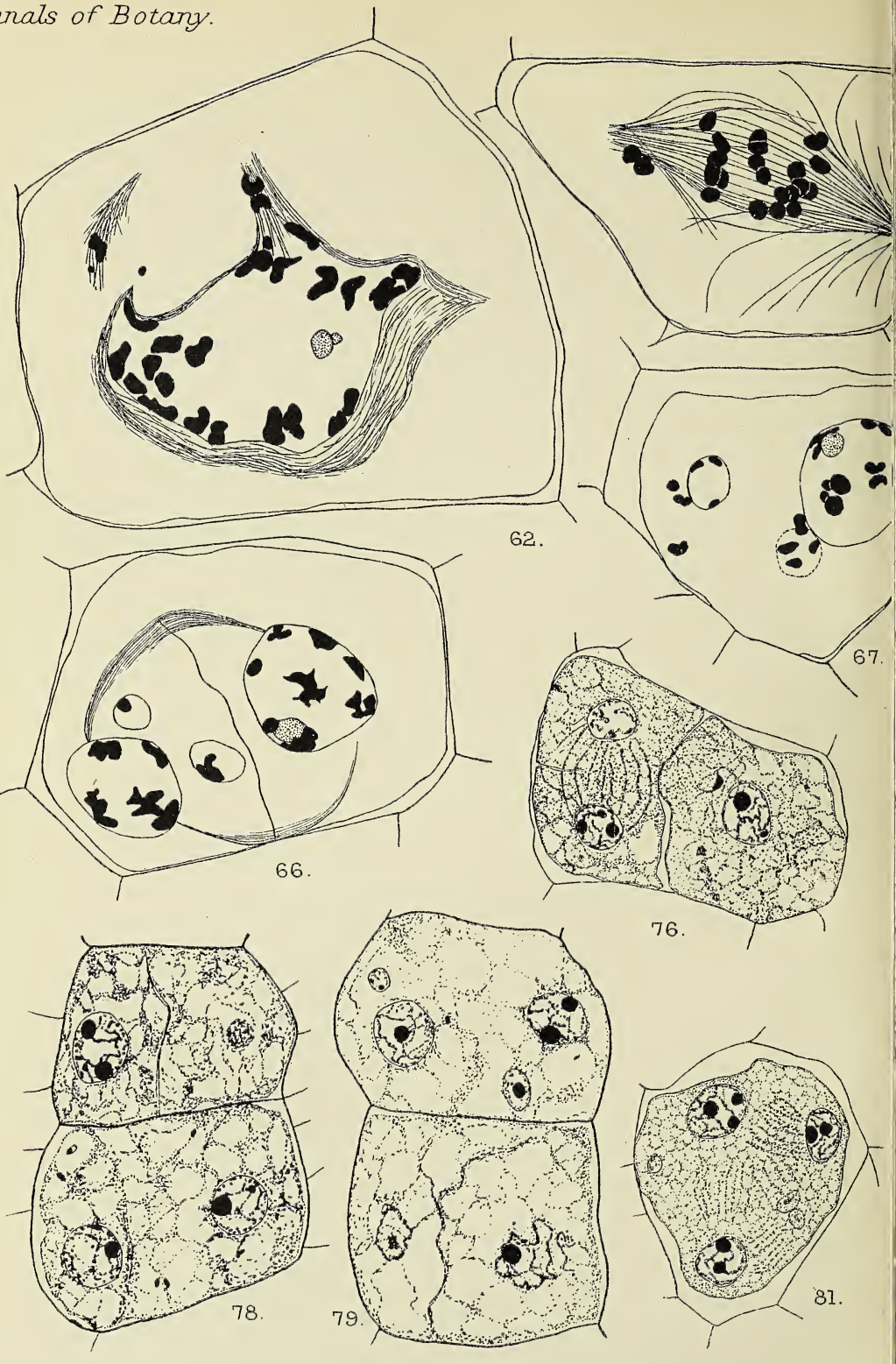
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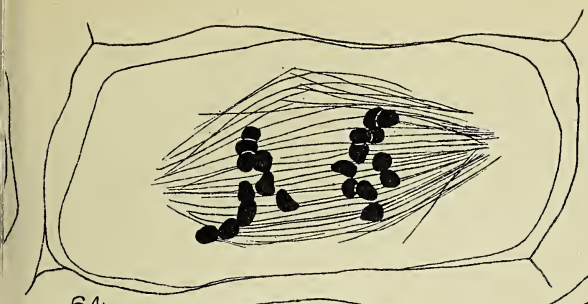

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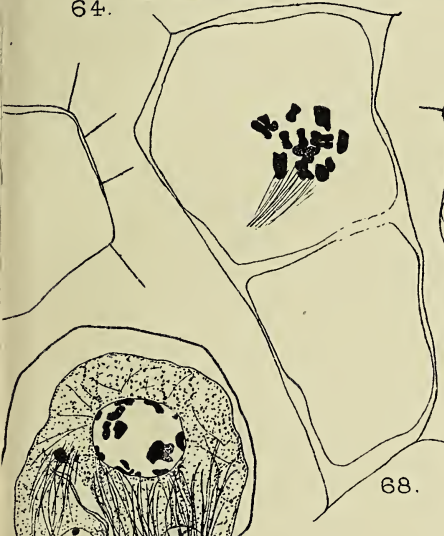

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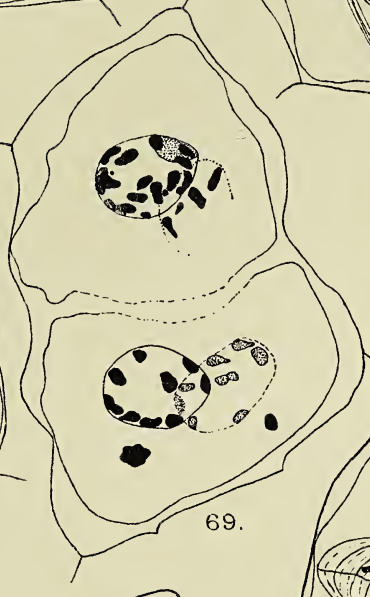

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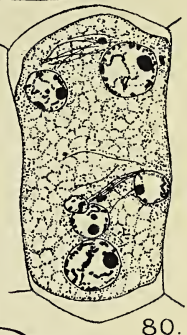

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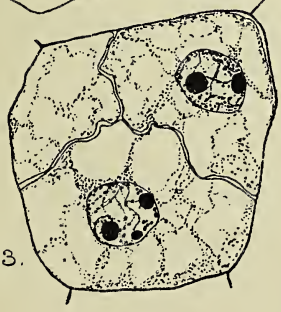

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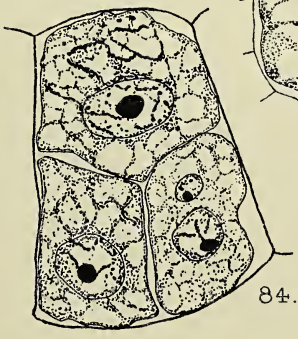

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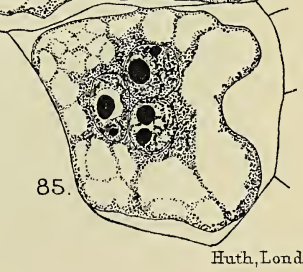

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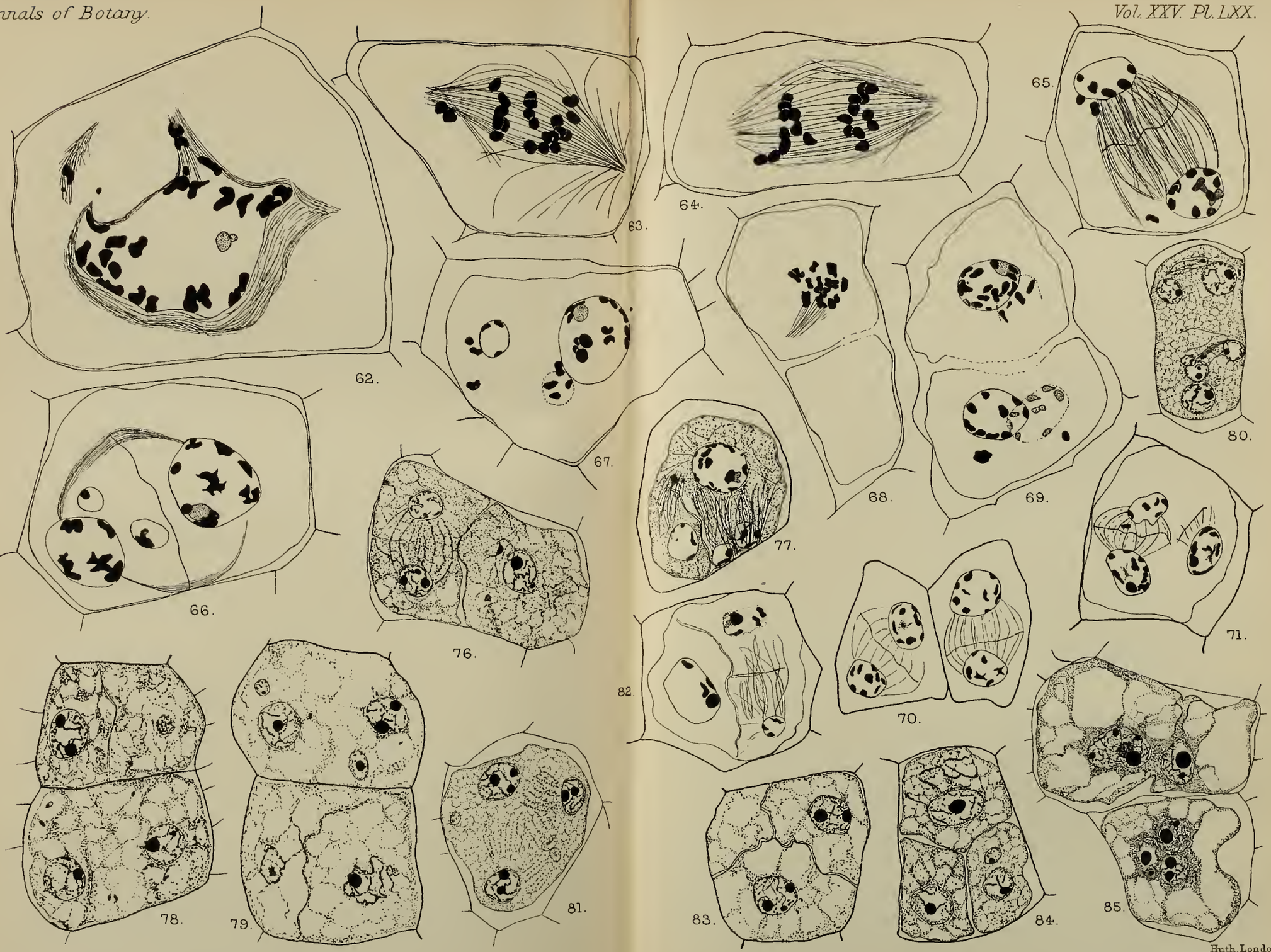

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Cytological Studies on Oenothera. III.

A Comparison of the Reduction Divisions of Oenothera Lamarckiana and O. gigas.[1]

BY

BRADLEY MOORE DAVIS.

With Plates LXXI–LXXIII.

OF all the forms derived from *Oenothera Lamarckiana* which have appeared in the cultures of de Vries and others, *O. gigas* is to the writer by far the most interesting for the reason that we have in *gigas* a type sharply distinguished from *Lamarckiana* in a large number of particulars and clearly different in its nuclear structure, since it has twice the number of chromosomes present in the parent form. Whether others of the so-called mutants of *Lamarckiana* will show characteristic and stable differences or peculiarities of the chromosomes or chromosome count becomes a matter of importance, and we are waiting with much interest for the results of investigations upon these forms.

The writer (Davis, '11) has recently published an account of certain hybrids between *Oenothera biennis* and *O. grandiflora* that resemble *Lamarckiana* to such a degree as to justify, in his opinion, the consideration of a working hypothesis that *Oenothera Lamarckiana* arose as a hybrid between some strains of these two characteristic wild American species. The reasons, both experimental and historical, for this hypothesis will not be repeated here, especially since my studies in these directions are to be continued with other strains of these species that seem likely to give hybrids that will approach more closely to the desired end—the synthesis of a hybrid so similar to *Lamarckiana* as to be practically indistinguishable from this form by the usual taxonomic tests.

Thus, although the bearing of the possible hybrid origin of *Lamarckiana* upon de Vries' interpretation of the behaviour of this remarkable plant will not be discussed at this time, it will be apparent to the reader that if the evidence should finally indicate *Lamarckiana* to be of hybrid origin, as

[1] An investigation conducted with aid from the Elizabeth Thompson Science Fund, for which the author desires to express his indebtedness.

a number of writers have already suggested, many of the so-called mutants are likely to be interpreted as segregates splitting off according to Mendelian expectations. Nevertheless, whatever shall be the final judgement concerning the significance of some or perhaps most of the forms derived from *Lamarckiana*, we have in *gigas* at least a type in which profound changes have taken place in the germinal constitution through the doubling of the chromosome number characteristic of the genus *Oenothera*.

Oenothera gigas appears to be a true mutant, or sport, since it is reasonable to believe that its strongly marked peculiarities are directly associated with that change in nuclear structure which increased the number of sporophytic (somatic) chromosomes from fourteen to twenty-eight. This peculiarity of nuclear structure involves a totally different form of origin from any that is likely to be established for the other derivatives from *Lamarckiana*, and *gigas* therefore among these derivatives probably stands in a class by itself, and this is also indicated by the extreme rarity of its appearance. Whereas many of the other of de Vries' 'mutants' have appeared in cultures in sufficient numbers and proportions as to suggest a Mendelian segregation of forms from a hybrid, *gigas* on the contrary has, as far as the writer is aware, been reported as arising only seven times among the thousands of cultures of *Lamarckiana* and its derivatives made by de Vries and the later investigators who have worked with these forms.

As described in 'Die Mutationstheorie', *gigas* first appeared in 1895 among a group of thirty-two rosettes of *Lamarckiana* selected by de Vries ('09, p. 227) from a very large culture of this type in the fourth generation. It is probable that all of the *gigas* material now in cultivation is descended from this plant. On two other occasions *gigas* is believed by de Vries ('09, p. 327) to have arisen in his cultures, once in 1898 from seed of *sublinearis* (a 'mutant' of *Lamarckiana*), and once in 1899 from a cross of *lata* × *hirtella*, but neither of these plants matured. MacDougal ('07, p. 3) reports the appearance of one plant of *gigas* in his cultures of *Lamarckiana*, from seed of de Vries, at the New York Botanical Garden. Lastly, Schouten ('08; Ref., Bot. Centbl., vol. cviii, 1908, p. 246), a student of de Vries, records three plants of *gigas* in cultures from commercial seed of *Lamarckiana*. Thus *gigas* at the most has been noted only seven times, and, since apparently the cytology of de Vries' strain alone has been studied, it is by no means certain that all of the forms reported later are the same as the first example from de Vries' cultures of 1895.

The extreme rarity of *gigas* as a derivative of *Lamarckiana* seems to the writer exactly what should be expected on the supposition that this type is a true sport, or mutant. The fact of its rarity, probably based on the marked peculiarities of its nuclear organization, indicates that *gigas* is an exception, and that other derivatives from *Lamarckiana* may finally be regarded as variants of quite a different sort from the 'mutants' of de Vries'

definition. For these reasons a detailed comparison between the reduction processes of *Lamarckiana* and *gigas* is a matter of importance in any attempt to establish the fundamental differences between these two forms, and the possible manner of origin of the latter type.

The writer is pleased at this opportunity to describe the conditions in *gigas*, since this plant has been employed as one of the parents in certain crosses with wild species of *Oenothera*, and the behaviour of the chromosomes in the hybrids of the first and second generation gives promise of some interesting results which, if worked out satisfactorily, will be presented in future papers.

Methods.

Experiments in methods of fixation have been continued since the recent study of *Oenothera biennis* (Davis, '10), where the effects of Carnoy's alcohol-acetic and chloroform-alcohol-acetic mixtures were contrasted with those of chrom-osmo-acetic acid, much to the advantage of the latter fluid. In the past summer of 1910 Bouin's fluid and Juel's fluid were given a similar trial. Anthers of *Lamarckiana*, after 4–5 hours in Bouin's fluid, were run quickly (1 hour) through grades of alcohol and washed in 80 per cent. alcohol until clear. This material exhibited a much greater degree of shrinkage than is present in anthers fixed in Flemming's fluids with the precautions outlined in my previous papers. The extent of the shrinkage, with no compensatory advantages of importance in the staining reactions, is in the writer's experience unfavourable to Bouin's fluid as a fixing agent for the anthers of *Oenothera*.

Juel's fluid (zinc chloride 2 gr., glacial acetic acid 2 c.c., 50 per cent. alcohol 100 c.c.) was tried upon the anthers of *gigas*, the material being left in the fluid 24 hours and then carried through grades of 50–85 per cent. alcohol, in which the material was thoroughly washed. The results were unsatisfactory. There was great shrinkage of the protoplasts and such changes in the structure of the cytoplasm that sharp differentiation of its structure was not possible with iron-alum haematoxylin. It was also difficult to stain the chromatin and nucleoli, which failed to exhibit the sharp outlines obtainable after fixation with Flemming's fluids.

Further experience with chrom-osmo-acetic acid has not modified the methods outlined in the previous papers (Davis, '09 and '10), but there seems to be little choice between the stronger and weaker formulae of Flemming. An essential for success with these fluids lies in such precautions as will facilitate the most rapid penetration possible, and to this end the writer knows of no better practice than that of brushing the anthers with water before immersion in the fixing fluid. The greater the attention paid to this somewhat laborious technique the less will be the amount of shrinkage

exhibited by the protoplasts, which in favourable material may be scarcely evident.

As in the previous studies, iron-alum haematoxylin was employed as the stain throughout the investigation.

Oenothera Lamarckiana.

Oenothera Lamarckiana has been made the subject of a lengthy study by Geerts ('09), which considers the development and fertilization of the embryo sac as well as the formation of the pollen. Gates's ('08) account of the reduction phenomena during pollen formation in *O. rubrinervis* considers a type so close to *Lamarckiana* as to make possible a close comparison with the latter form. There are important differences between the writer's conclusions and those of the investigators cited above in various details of the accounts of presynapsis, synapsis, and the form and behaviour of the chromosomes during the reduction mitoses. These differences become at once apparent in comparing our figures, and only certain of the most marked points of disagreement will be referred to in the text.

Presynapsis. The daughter nuclei following the last mitosis in the archesporium of the anther (Pl. LXXI, Fig. 1) exhibit clearly the fourteen sporophytic (somatic) chromosomes characteristic of the type. The chromosomes become smaller as the nucleus, increasing in size, passes into the resting condition, the development of the nuclear reticulum apparently drawing material from the chromosomes and so reducing their bulk that they can finally only be recognized in later stages as deeply staining bodies (Fig. 2). These are the chromatic bodies described by the author for *biennis* and *grandiflora*, and there can be no doubt that they are derived from the chromosomes of the preceding mitosis; they apparently correspond to the prochromosomes of Overton, Rosenberg, and others.

In the earlier presynaptic stages the chromatic bodies may frequently be counted as fourteen, but as the reticulum becomes more pronounced they cannot be distinguished from other structures, such as small nucleoli and granules which appear in the nucleus. The chromatic bodies of *Lamarckiana*, like those of *biennis* and *grandiflora*, are distributed irregularly throughout the nuclear cavity, and there is no evidence of groupings in pairs which might be interpreted as indicating the presence in the nucleus of two parallel spiremes such as have been described by Overton ('09), Lundegard ('09), and Rosenberg ('09 *a*). On the contrary, their position on the linin strands suggests rather an end-to-end relationship in keeping with the arrangement of the chromosomes in the spireme preceding the heterotypic mitosis.

The development of the nuclear reticulum makes it impossible to follow the chromatic bodies further, since their substance merges with the strands of the network and their form becomes lost. This is a gradual process

extending over a considerable part of the long period leading up to synapsis. It involves the gradual thickening of what are at first relatively few very delicate threads (Fig. 2) and the formation of new strands until the nucleus finally contains a close network (Figs. 3 and 4) in the meshes of which lie one or two large nucleoli, and frequently several smaller bodies of a similar nature. While on the evidence from their history it is reasonable to suppose that the chromatic bodies are chromosome centres or prochromosomes, and probably maintain their individuality in the nuclear reticulum, nevertheless such individuality is not clearly evident in the resting nucleus of *Oenothera*.

Synapsis. A contraction of the strands composing the nuclear reticulum away from the nuclear membrane (Figs. 5 and 6) indicates the approach of synapsis. This contraction, as in *biennis* and *grandiflora*, proceeds until almost all of the strands in the network are drawn together in a close mass generally at one side of the nucleus and near the nucleolus (Fig. 8), which is sometimes quite surrounded by the web of threads (Fig. 7). This contracted and confused mass of strands, the arrangement of which cannot be followed with precision, constitutes the synaptic knot, a sharply marked stage extending over a considerable period, probably of one or more days. Occasional free loops extend from the contracted mass into the nuclear cavity, and the study of these, together with such structure as is apparent in the less dense regions, makes it clear that the synaptic knot is a close association of looped threads frequently anastomosing so as to form what appears to be a tangled mass.

In such a complex association of strands it is not difficult to find threads that run closely parallel to one another for greater or less distances, or that are united at points. However, in a structure so involved and with elements so minute as are these threads, it is not safe to assume that these relations signify anything more than such a close association of strands as would naturally result from the contraction of the complexly looped and anastomosing thread or system of threads that constitute the nuclear reticulum. The evidence is entirely inadequate to support the interpretation that such parallel relations between the strands indicate the presence of two independent systems of threads (maternal and paternal spiremes), such as might be assumed to be associated with one another side by side. On the other hand it is impossible to say whether or not the coiled and twisted loops of the synaptic knot are parts of a single continuous thread or of a system of threads forming a reticulum; if the former, it must be a thread of great length.

As the process of synapsis proceeds it becomes evident that the threads thicken (compare Fig. 9 with Figs. 6 and 7), and from the later developments there is good reason for believing that this thickening is associated with a very material shortening of the thread system. The increased thickening of the strands, although clearly shown at the stages

of mid-synapsis (Fig. 9), becomes much more conspicuous in the post-synaptic stages leading to the development of the spireme. Thus with the loosening of the contracted coils of the synaptic knot there emerges a much more clearly defined system of loops than can be recognized in the complicated reticulum which entered the synaptic knot (compare Figs. 10 and 11 with Figs. 6 and 7). It is evident that the threads have become much thicker and that the thread system has grown much shorter, and the inference is natural that these developments are the result of the process of contraction so characteristic of synapsis. This history is the same as that for *biennis* and *grandiflora*.

Gates ('08) interprets the presence of parallel threads in post-synaptic stages of *rubrinervis* as indicating a splitting of the spireme, which is later closed by the union of the two threads. The writer, from his studies on *biennis* and *Lamarckiana*, cannot agree with this view, for although parallel threads may frequently be observed, there seems to him no evidence that this condition is other than such an arrangement as is to be expected when a complex system of threads has been gathered together by such a process as that of synaptic contraction. Since parallel threads may be observed in stages just previous to mid-synapsis it is but natural to expect that somewhat similar relations should be found in stages following the loosening of the synaptic knot. These threads in *Oenothera* are so delicate and their arrangement is so intricate that the establishment of such an interpretation as that of Gates is surrounded with great technical difficulties.

As in the study of *biennis*, the term 'synapsis' is reserved for this period of a first contraction of the synaptic material. A type of contraction that is frequently conspicuous at a much later period during the formation of the chromosomes (the 'second contraction') will be described in that connexion. The relation and significance of the two phenomena will be further considered in the 'Cytological Discussion'.

The Formation of the Chromosomes. Following the period of synapsis, which is of relatively long duration, there begins as described above a gradual loosening of the synaptic knot and a shortening and thickening of the thread system. Further developments now follow rapidly. The threads become more prominent (Fig. 12) and begin to take on the thickness and appearance of the spireme which is soon to be developed. During this process the complicated coiled arrangement, which because of the anastomoses exhibited the structure of a network, becomes more simple, and the threads may be followed for considerable distances in the looser convolutions. The threads remain, nevertheless, for a long time so complexly looped that their outline cannot be accurately traced throughout their entire length.

The process of shortening and thickening proceeds until an undoubted

spireme appears (Fig. 13), which is so short that the band may be followed distinctly in its convolutions throughout the nuclear cavity. The spireme is in some cases unquestionably a single thread, although the loops are frequently joined by delicate cross strands, and it is sometimes attenuated at points. In other cases it appears as though the spireme is not strictly continuous, but consists of two or more somewhat distinct portions.

Following the differentiation of the spireme (Figs. 12 and 13), a process of segmentation begins (Figs. 14 and 15) which transforms the spireme into a chain of fourteen chromosomes. This segmentation consists in a drawing apart of certain regions of the spireme as though by a process of constriction (Fig. 14), or perhaps more accurately by the condensation of the chromatin at fourteen chromosome centres on the spireme. This condensation results in a still further shortening of the spireme so that the complexities of its early looped arrangement largely disappear, and the chromosome segments may be followed for long distances as parts of a chain (Figs. 15, 16, and 17). The chain is sometimes broken as though a few segments had been displaced, which might readily result from such a looping of the spireme that parts would be thrown out of the general line of arrangement, or because portions of the original spireme were separated at points.

During the segmentation of the spireme and the condensation of its portions to form the fourteen sporophytic (somatic) chromosomes the segments are frequently found grouped at one side of the nucleus in a close mass (Figs. 18–20) that resembles superficially the chromatic contraction of synapsis. This condition is more conspicuous in *Lamarckiana* than in my material of *biennis*, and constitutes a very clearly marked stage in the reduction processes which will be described as the second contraction, since it appears to correspond closely in the time of its appearance with the 'second contraction' stage of a number of writers on different types. This stage in *Oenothera* takes place during the segmentation of the spireme or shortly after, so that the contracted material consists of chromosome segments either in process of formation (Figs. 18 and 19) or in a somewhat more advanced stage in their condensation into chromosomes (Figs. 20 and 21).

Gates ('08, pp. 11 and 12) places the stage of 'second contraction' in *rubrinervis* previous to the segmentation of the spireme, but his figures are not consistent with his account. Thus Fig. 20 exhibits a pair of chromosomes cut off from the remainder of the chromatin, which, in spite of the lack of detail shown, has the appearance of a contracted, looped, and segmented spireme, or perhaps even of a chain of chromosomes. Fig. 21, with an even greater lack of detail present in the drawing of the contracted chromatin, shows evidence of a segmenting spireme in the loop extending into the nuclear cavity. Fig. 18 may illustrate, as described, the shortened and thickened thread (spireme) entering upon the phase of second con-

traction, but there are here also indications that segmentation is about to take place.

The chromosome segments of the spireme when first formed are sometimes four to six times longer than they are broad and of slightly varying lengths even in the same nucleus, but as the process of chromatin condensation continues they become much shorter and gradually approach uniformity of size. In consequence of this process of segmentation the arrangement of the fourteen sporophytic chromosomes on the spireme is always end to end (Figs. 15–17), but as the spireme becomes older groups of chromosomes are frequently detached from the main chain. Pairs of chromosomes are thus occasionally separated which take the form of rings (Fig. 18), probably because they formed two sides of a loop in the spireme. The chromosomes of *Lamarckiana*, like those of *biennis*, are, then, not grouped regularly in pairs during the prophases of the heterotypic mitosis as was the case in material of *grandiflora* studied by the writer (Davis, '09). In that material a characteristic pairing of the chromosomes was found to result from the regular arrangement of the loops of the spireme by which adjacent chromosome segments in the chain were as a rule brought somewhat side by side, so that they remained united to form seven ring-shaped bivalent chromosomes. Such pairs or rings are not common in *Lamarckiana*, and as a rule the arrangement of the chromosomes is in the form of a single long chain or two or more shorter chains.

This method of the organization of the chromosomes in *Lamarckiana* as segments of a univalent spireme placed end to end is therefore the same as the history described by the writer for *biennis* and *grandiflora*, and in this conclusion the present paper is in full agreement with the account of Geerts for *Lamarckiana* and with that of Gates for *rubrinervis*.

The Heterotypic Mitosis. The arrangement of the chromosomes end to end in a single chain or in two or more shorter chains is frequently maintained through the prophases of the first, or heterotypic mitosis, although the chromosomes may be so closely grouped that it is difficult to follow them with precision. The cause of the intimate association of the chromosomes may readily be traced to the conditions of the second contraction from which the prophases develop, and where, as described above, the condensation of the chromosome segments appears to draw these elements together. This condensation continues throughout the prophases so that the chromosomes at the metaphase of this mitosis (Fig. 27) are very much smaller structures than the segments of the spireme from which they were derived (Figs. 15–21). As in *biennis* and *grandiflora*, the mature chromosomes are essentially similar to one another, and have usually the form of thickened V's due to the bending of the chromosome segments of the spireme; they are not rounded structures such as have been described and figured by Gates and Geerts.

Spindle formation begins, as in *biennis* and *grandiflora*, by the entrance of fibrillae into the nuclear cavity following the breaking down of the nuclear membrane. The fibrillae likewise push out into the surrounding cytoplasm and thus establish multipolar spindles (Figs. 23 and 24) similar to those described for a number of higher plants (e.g. *Equisetum, Larix, Lilium*, &c.); the large nucleolus disappears at the beginning of spindle formation. The chromosomes still joined end to end in one or more chains are brought by the development of the fibrillae to the centre of the spindle, where they generally lie in a rather dense group, although occasional chromosomes may be found somewhat separated from the main assemblage.

The bipolar spindle characteristic of the metaphase of the mitosis (Fig. 27) results from the rearrangement and gathering of the spindle fibres into two broad sheaves that end in granular areas which merge with the alveolar cytoplasm. The chromosomes may frequently be found still arranged in chains in the equatorial regions of the recently formed bipolar spindle (Figs. 25 and 26), but as the spindle grows older the chromosomes separate from one another, and by bending at the ends and thickening in the middle regions usually take on the form of thickened V's. There appears to be no system in the grouping of the chromosomes as they are brought to the equatorial plate just previous to the metaphase of the mitosis. Their arrangement is quite irregular, as shown in Figs. 25 and 26, and although occasional ring-shaped pairs of chromosomes may be noted which were undoubtedly derived from adjacent segments of the spireme, most of the chromosomes become so separated that it is impossible to determine with certainty what was their relation to one another on the spireme.

The V-shaped form of the chromosomes is especially evident as the two sets move away from one another towards the poles of the spindle (Fig. 28). However, during anaphase, as the chromosomes approach the poles (Fig. 29) their structure becomes complicated by a lengthwise fission of each chromosome (Fig. 30), which takes place in the plane of the page upon which the above letter (V) is printed. Thus the seven chromosomes that leave the equatorial plate of the heterotypic mitosis arrive at the poles and enter the daughter nuclei as seven split chromosomes (Pl. LXXI, Fig. 31, Pl. LXXII, Figs. 32, 33). This division is a premature fission of each chromosome in preparation for the second, or homotypic mitosis.

There was found, during the study of considerable material, only a single case that showed numerical irregularities in the distribution of the chromosomes by the heterotypic mitosis. In this example eight split chromosomes were present at one pole of a spindle (Pl. LXXI, Fig. 30), and six chromosomes were distributed between two sections of the other pole. Similar irregularities were reported by the writer (Davis, '10, p. 638) for

biennis. These cases in both *biennis* and *Lamarckiana* are of great interest, but it should be noted that they are rare, and it would be quite impossible to find sufficient material to determine whether or not fertile pollen-grains can be developed with nuclei bearing a greater or less number of chromosomes than the normal.

The group of seven split chromosomes is best observed in polar views of late anaphase, and at the time of the reconstruction of the daughter nuclei. Their history may be readily followed through the interkinesis between the heterotypic and homotypic mitoses. Following the organization of the daughter nuclei, the halves of each chromosome bend so that they separate at the ends, and thus come to have the form of two U's joined together in the middle region (Pl. LXXI, Fig. 31; Pl. LXXII, Figs. 32, 33). The seven split chromosomes become therefore seven evident pairs, the members of each pair remaining in close association until the metaphase of the homotypic mitosis. The chromosomes during the reconstruction of the daughter nuclei increase rapidly in size, and remain in this expanded state throughout the interkinesis. Nucleoli appear (Fig. 33) and sometimes the ends of the chromosomes proliferate and show a tendency to form an imperfect network. The period of interkinesis is of considerable length, with the individuality of the chromosomes generally maintained quite as clearly as is indicated in Figs. 31–33, and this history is the same in all essentials as that in *biennis* and *grandiflora* and agrees with the accounts of Gates and Geerts.

In summary of the events of the heterotypic mitosis in *Lamarckiana* it should be noted (1) that the 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 that 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 appearance of a web of fibrillae around the resting nucleus of the interkinesis together with the breaking down of its membrane indicates the approach of the homotypic mitosis. The fibrillae enter the nuclear cavity and establish a multipolar spindle (Pl. LXXII, Figs. 34 and 35), at the same time carrying the seven pairs of chromosomes towards the centre of the structure. A bipolar spindle is then developed by the rearrangement of the fibres, and the pairs of chromosomes become grouped upon an equatorial plate (Fig. 36) so that the members of the pairs will be separated and distributed by the mitosis with perfect regularity, seven chromosomes in each set. The two homotypic spindles in the

pollen mother-cell may lie side by side or at right angles to one another. The chromosomes, by condensation during the prophases, become very much smaller than in the expanded conditions of the interkinesis, and by the time that the homotypic spindle is fully developed they have returned to about the same size as when they entered the period of interkinesis. They have the form of short and slightly bent rods (Fig. 36).

During the anaphase of the homotypic mitosis (Fig. 37) and later when the chromosomes are gathered at the poles (Figs. 38–40) their form becomes more irregular, changing even more markedly after the organization of the daughter nuclei which are to enter the pollen-grains. These nuclei (Figs. 41 and 42) show at first clearly the seven chromosomes which enter into their composition, but the form of the chromosomes becomes attenuated as the nuclei enlarge. Presently a network becomes evident connecting the chromosomes, and the nuclei of the pollen-grains thus pass into typical resting conditions (Fig. 43) possessing one or more nucleoli and a delicate open reticulum in which lie deeply staining regions. These have the appearance of the chromatin bodies described for the nuclei preceding synapsis, but they are of course in the reduced number seven. The chromatic bodies become less and less evident as the nuclei increase in size, and by the time that the young pollen-grains are formed they can no longer be distinguished in the chromatic reticulum that has developed, unless some of the denser granules present may represent such chromatic centres or prochromosomes; such granules cannot, however, be counted with regularity.

This description of the homotypic mitosis in *Oenothera Lamarckiana* is in agreement in all essentials with the account of Geerts for the same form, with that of Gates for *rubrinervis*, and with the writer's accounts of *biennis* and *grandiflora*. The mitosis is clearly an equation division distributing the halves of chromosomes, the premature division of which takes place during the anaphase of the heterotypic mitosis, the halves remaining associated in seven pairs throughout the period of interkinesis.

The Partial Sterility of the Pollen in Oenothera Lamarckiana. De Vries noted the fact of partial sterility in *Lamarckiana* of both pollen-grains and ovules, and this subject is given considerable attention in the account of Geerts ('09, pp. 82–105), who reports that about 50 per cent. of these structures may be abortive. Geerts figures a number of pollen-tetrads and young embryo-sacs in which the nuclei give evidence of degeneration, but he does not clearly associate the phenomenon with irregularities in the distribution of the chromosomes.

Gates ('07) presents observations on the development of the pollen in the hybrid *lata* × *Lamarckiana*, showing clearly that the group of seven chromosomes which normally should enter each nucleus of the pollen-grain may be broken up with the result that extra nuclei are formed having

smaller numbers of chromosomes, and a similar case was also observed by the same author (Gates, '08) for *rubrinervis*. Both Geerts and Gates report evidence of nuclear degeneration in stages previous to the heterotypic mitosis, indicating that the causes of sterility are more deep-seated than irregularities of chromosome distribution, although the latter phenomenon is perhaps intimately associated as an effect of physiological disturbances previous to pollen formation.

The writer can only report for *Lamarckiana* the facts of certain irregularities in the distribution of the chromosomes. Thus, during the homotypic mitosis it is not uncommon to find that some of the chromosomes in a group of seven have failed to reach the poles of the spindle, and as a result form smaller supernumerary nuclei in the pollen mother-cell. Such a case is shown in Pl. LXXII, Fig. 44, where the chromosomes of three groups, a total of twenty-one, are distributed among five nuclei. Tetrads may even be formed in which large and small nuclei become associated in the same cell and pass into a resting condition, but it is not known whether such a cell can mature into a fertile pollen-grain. An irregularity in the heterotypic mitosis has also been described (Fig. 30) in which six or eight chromosomes respectively had been distributed to opposite poles, but here also we do not know whether or not fertile pollen-grains may be formed with chromosomes in a greater or less number than the normal.

An extensive field for cytological investigation is suggested by the recent paper of de Vries ('11) on double reciprocal crosses between *Oenothera biennis* and *O. muricata*. In explanation of his remarkable results de Vries postulates the development of two forms of germ cells, both male and female, carrying two different sets of hereditary tendencies. One of these two forms of gametes is eliminated from the male organ by the sterility of half of the pollen-grains, and the other form is eliminated from the female organ by the abortion of half of the ovules. As a result two groups of characters are strictly sex-limited in their inheritance, one being carried through the male and the other through the female gamete. It is too early to discuss this hypothesis from the cytological side, for the problems concerned will demand prolonged investigation. The writer's belief, based on preparations of pollen in which the entire contents of anthers were examined, is that the proportion of sterile pollen varies widely. Thus in certain races of *Oenothera biennis* from 20 to 30 per cent. of the pollen is sterile, while in other races the proportion of sterile pollen appeared to be fully 75 per cent.

It seems clear, however, that the total amount of sterility in the Oenotheras is much too great to be explained entirely by peculiarities of the chromosomes, or by such irregularities in their distribution as are known to occur during the reduction divisions. Thus, it is difficult to understand how the ovules of *Oenothera lata* can be fertile and the pollen

sterile when the chromosomes are of the same germ-plasm. There are other factors at work probably of an intricate physiological nature.

For these reasons the writer is not impressed with the importance of the criticism that the partial sterility of *Lamarckiana* indicates its hybrid origin. Partial sterility in *Oenothera* and related genera, as pointed out by Geerts, is a phenomenon so common that the significance of its presence or absence may readily be unduly emphasized. So far the hybrids of the crosses between *biennis* and *grandiflora* (Davis, '11) which approach closely to *Lamarckiana* have been fertile to a high degree in the F_1 generation and then have exhibited a great range in their fertility in the F_2, some of them being more than 50 per cent. sterile and others apparently perfectly or almost perfectly fertile. The writer will lay no emphasis on the partial sterility of *Lamarckiana* in the development of his views that *Lamarckiana* arose as a hybrid between strains of *biennis* and *grandiflora*. The behaviour of *Lamarckiana* in throwing off marked variants in such large numbers, together with the fact that *Lamarckiana* is unknown as a clear component of the American native flora, is the chief reason for his belief that this interesting plant is not, as assumed by de Vries, representative of a wild species.

Oenothera gigas.

The first announcement that the number of chromosomes in *Oenothera gigas* is double that of the parental form *Lamarckiana* appeared in a note of Miss Anne Lutz (Science, xxvi, 1907, p. 151), who reported the discovery of twenty-eight or twenty-nine chromosomes in mitotic figures of the root-tips. Gates (Science, xxvii, 1908, p. 193) shortly after confirmed this discovery in an examination of the reduction divisions in the pollen mother-cells which showed the sporophytic number of chromosomes to be twenty-eight and the reduced number to be fourteen. The present study is in agreement with the above conclusions. The material with which these two investigators have worked, and also that described in this paper, is all descended from the first plant of *gigas* which in 1895 appeared in the cultures of de Vries.

Gates ('09) has published a paper chiefly devoted to a comparison of the cell and nuclear measurements of *gigas* with those of *Lamarckiana*, to test Boveri's theory that in related forms the nuclear surface and cell volume in homologous tissues are proportionate to the number of chromosomes in the types compared. In this paper is given a short account of that period of the reduction processes from the metaphase of the heterotypic mitosis to the prophase of the homotypic. The unfortunate loss of the figures intended to illustrate this account renders impossible a close comparison between Gates's description and the conclusions of the writer,

but certain important differences in our conclusions, evident from the text, will be noted. The following account will be written with the view to comparison with the foregoing description of *Lamarckiana*.

Presynapsis. As in *Lamarckiana*, the daughter nuclei of *gigas* following the last mitosis in the archesporium (Pl. LXXII, Fig. 46) show clearly the sporophytic chromosomes, but the large number, twenty-eight, is naturally not easy to count with accuracy. As the nuclei increase in size a delicate reticulum is developed, the material of which is apparently drawn from the chromosomes since the latter decrease in size and finally can be recognized only as deeply staining granules (Figs. 47 and 48). These are the chromatic bodies and correspond to the similar structures in *Lamarckiana*.

The large number of the chromatic bodies, together with the difficulty of distinguishing them in these stages from other granules in the nucleus, makes accurate counts impossible, but there can be no doubt that the chromosomes are represented in the nuclei by deeply staining bodies upon the nuclear reticulum in such stages as are shown in Figs. 47 and 48. No evidence was discovered that the chromatic bodies are grouped in pairs and such relations to one another as appeared were rather those of an end-to-end arrangement on the strands of the network.

As the nuclear reticulum develops further (Figs. 49 and 50) the chromatic bodies become so lost in the thicker strands of the network that they can no longer be recognized. As in *Lamarckiana* the development of the reticulum is a gradual process involving the thickening of what were at first relatively few delicate threads (Figs. 47 and 48), and the development of many others until the nucleus finally becomes filled with a rather dense reticulum (Fig. 50) in which lie one or more nucleoli. In later stages and just previous to the beginning of the synaptic contraction, the network becomes more like a loosely tangled group of threads (Fig. 51), and this condition is generally more clearly shown in *gigas* than in *Lamarckiana*, *biennis*, or *grandiflora*.

Synapsis. The approach of synapsis is indicated here, as in other species of *Oenothera*, by a contraction of the nuclear reticulum away from the nuclear membrane (Fig. 52), and this contraction continues until almost all of the threads in the network are drawn together into a close mass, the synaptic knot (Figs. 53 and 54), generally at one side of the nucleus and near the nucleolus. The appearance of the synaptic knot is similar to that of *Lamarckiana* (compare Fig. 53 with Fig. 9); it consists of a contracted intricately coiled mass of threads whose arrangement cannot be followed, but from which occasional loops and strands extend into the nuclear cavity. The form of the knot may be almost spherical as in Fig. 53 or more irregular as in Fig. 54. As in *Lamarckiana*, *biennis*, and *grandiflora* the writer was unable to discover any relations between the threads other than

such a close association as would result from the contraction of a complexly looped thread or system of threads anastomosing to form a reticulum.

Following the period of mid-synapsis, when the contracted condition is most conspicuous, there begins a loosening of the synaptic knot, and it becomes at once evident that the threads have thickened and that the thread system has grown shorter (Fig. 55). Thus there emerges from synapsis a much more clearly defined system of loops than appeared in the involved tangle of threads that entered the synaptic knot (compare Fig. 55 with Fig. 53). The thickened thread system then begins to assume the appearance of the spireme, which shortly after may be clearly recognized (Fig. 56). The entire history of the formation of the spireme from the stage of the nuclear reticulum preceding synapsis seems to be a process of contraction and thickening of the threads in the reticulum, the phenomena of synapsis being an expression of this process.

The Formation of the Chromosomes. With the loosening of the synaptic knot following synapsis, as described above, it becomes at once apparent that the threads have grown markedly thicker and the thread system much shorter than at the beginning of the synaptic contraction (compare Fig. 55 with Figs. 51 and 52). Nevertheless, the thickened threads still present a very complicated coiled arrangement which, as in *Lamarckiana*, cannot be followed throughout the nucleus. The structure is, however, as far as could be determined, that of a single thread or a group of threads intricately looped and occasionally anastomosing. The spireme (Figs. 56 and 57) is developed by the continued shortening or condensation of the thickened thread system until the band may be distinctly traced throughout the greater number of its convolutions, although it is very difficult to determine whether the spireme is strictly continuous or composed of two or more portions.

There then begins the process of segmentation (Fig. 58) that transforms the spireme into a chain of twenty-eight chromosomes (Fig. 59), which because of their large number can be counted only in very favourable preparations. The process is the same as in other Oenotheras, and consists in the constriction of the spireme and condensation of the chromatic material at twenty-eight centres. The result of the condensation is a further shortening of the spireme, so that much of the complicated looped arrangement becomes simplified, and the segments may be followed for long distances (Fig. 59), arranged end to end like links in a chain. The chain of chromosomes is frequently broken, perhaps because the spireme is made up of somewhat distinct portions, or because certain segments become displaced or separated, as might readily take place at points in the loops.

The stage of second contraction is frequently very conspicuous in *gigas*, for the reason that so large a number of chromosomes is concerned with the phenomenon. It may appear as early as the segmentation of the spireme

(Pl. LXXII, Fig. 60), but it is most commonly noted after the process of condensation has brought the segments to a size closely approximating that of the chromosomes (Figs. 61–63, compare with Pl. LXXI, Figs. 19–21). The chromosomes are not with regularity brought together in pairs during the second contraction, although such relations are not uncommon in detached groups, and in this behaviour *gigas* agrees with *Lamarckiana* and *biennis*, in contrast to the usual formation of ring-shaped bivalent chromosomes in the writer's material of *grandiflora*.

The Heterotypic Mitosis. The prophases of the heterotypic mitosis in *gigas* give excellent stages in the development of the spindle, which is larger than that of *Lamarckiana* (compare Pl. LXXIII, Fig. 66, with Pl. LXXI, Fig. 27). The process of spindle formation begins, as in other Oenotheras, with the appearance of a web of fibrillae around the nucleus (Pl. LXXIII, Fig. 64), the fibrillae entering the nuclear cavity with the breaking down of the membrane. As the fibrillae also push out into the surrounding cytoplasm they establish a multipolar spindle (Fig. 65), which shortly becomes bipolar (Fig. 66) by the rearrangement of the spindle fibres.

The chromosomes may frequently be found end to end in chains during the prophases (Figs. 64 and 65), but the development of the spindle fibres finally brings the chromosomes to the centre of the spindle, where they generally lie in a crowded group until they form the equatorial plate of the heterotypic spindle. During the development of the spindle the chromosomes usually take on the form of thickened V's by bending at the ends and thickening in the middle regions, as in all other Oenotheras studied by the writer. They are similar in size to the chromosomes of *Lamarckiana*. There is no system apparent in the grouping of the chromosomes as they are brought to the equatorial plate. Although occasional pairs may be noted, the arrangement of the chromosomes is generally quite irregular until their alinement at the metaphase of the mitosis, when as a rule the two sets are clearly differentiated (Fig. 66).

The form of the chromosomes becomes complicated as they approach the poles of the spindle during anaphase by the lengthwise fission which is so characteristic of these forms, and which is illustrated in a number of cases shown in Fig. 67. Thus the fourteen chromosomes that pass to each pole of the heterotypic spindle generally arrive and enter the daughter nuclei as fourteen split chromosomes (Fig. 68), although the division is sometimes delayed. This division, which is a premature fission of each chromosome in preparation for the homotypic mitosis, was, however, always shown in the chromosomes of the nuclei during the interkinesis (Figs. 71 and 72). Gates ('09, p. 528) is uncertain whether the fission is always lengthwise or not sometimes transverse, but the writer has observed no evidence of departures from the manner of lengthwise division present in *Lamarckiana*, *biennis*, and *grandiflora*.

An interesting type of irregularity, present in the heterotypic mitosis, is shown in Fig. 69. In this case two pairs of chromosomes had lagged behind the main group in passing to the poles of the spindle, and were associated in a small secondary spindle within the main structure. That such stray chromosomes, as well as the main group with a chromosome content smaller than the normal, may form independent nuclei is illustrated in Fig. 70. It is, however, improbable that fertile pollen-grains can be developed under such abnormal conditions. The fact that the chromosomes in these nuclei have lost their usual form and by anastomosing appear to be developing a chromatic reticulum, indicates that the nuclei do not proceed further towards the homotypic mitosis, but probably pass into a resting condition.

The nuclei during the interkinesis between the heterotypic and homotypic mitoses show many interesting features in agreement with the conditions in *Lamarckiana* and other Oenotheras. This is the period when the count of the chromosomes can be most easily made, as illustrated in Figs. 71 and 72, which show fourteen pairs of chromosomes derived from the fourteen split chromosomes previously described. It is clear that the halves of the split chromosomes swing so that their ends separate and thus become a pair united only in the middle region. Although the chromosomes sometimes proliferate and show a tendency to form an imperfect network, the pairs generally remain clearly defined throughout the interkinesis. The writer has not observed cases in which the chromosome boundaries were no longer distinguishable, as reported by Gates ('09, p. 528) for certain 'semi-resting' conditions, and it may be questioned whether the latter are normal, i. e. whether such nuclei enter the homotypic mitosis and give rise to fertile pollen-grains.

The history of the heterotypic mitosis thus agrees in all essentials with that of *Lamarckiana*, some of the features being even more perfectly shown on account of the larger size of the spindles, the only complications resting with the double number of chromosomes, which are naturally more difficult to follow.

The Homotypic Mitosis. With the breaking-down of the nuclear membrane following the interkinesis (Fig. 73), the fourteen pairs of chromosomes, previously scattered through the nuclear cavity, are brought by the development of the fibrillae to the centre of the spindle, which is at first multipolar. At the equatorial plate of the final bipolar spindle (Fig. 74) these pairs of chromosomes become grouped very regularly, so that the members of the pairs are distributed in two sets of fourteen chromosomes each. The chromosomes at the metaphase of the homotypic mitosis have the form of short rods, having returned by the condensation of their material to about the same size as when they entered the period of interkinesis (compare Fig. 74 with Fig. 68).

The chromosomes become somewhat irregular in form in their passage to the poles of the spindle during anaphase (Pl. LXXIII, Fig. 75) and after the organization of the daughter nuclei that are to enter the pollen-grains (Figs. 76–78). The fourteen chromosomes may at first be easily counted in favourable preparations of the young pollen nuclei (Fig. 77), but as the nuclei enlarge the chromosomes become attenuated, and by anastomosis soon develop a chromatic network in which their outlines become lost. Deeply staining regions in the reticulum may at certain stages be recognized as chromatic bodies undoubtedly representing the chromosomes, but they cannot be positively identified in the nuclei of older pollen-grains (Fig. 79).

Thus it will be noted that the events of the homotypic mitosis in *gigas* are in agreement with those of *Lamarckiana* and the other forms of *Oenothera* that have been studied.

The size of the cells and nuclei of gigas as compared with that of Lamarckiana. It is important to compare the size of the cells and nuclei of *gigas* and *Lamarckiana* with reference to Boveri's well known conclusions from experimental studies on the eggs and larvae of sea-urchins. He was able to institute comparisons between larvae developed parthenogenetically (x chromosomes), normal larvae ($2x$ chromosomes), and giant larvae ($4x$ chromosomes) obtained by shaking eggs and thus arresting the process of mitosis after the division of the chromosomes, all of which become included in the same nucleus through the formation of a monaster. Among other conclusions, Boveri ('05) formulated a law that the size of the cells in these sea-urchin larvae is a function of the chromatin content, and that the cell volume is proportional to the number of the chromosomes.

Gates ('09) has brought together from a variety of tissues an interesting series of comparative measurements between the cells of *Lamarckiana* and *gigas*, which indicate that the cell volume throughout the *gigas* plant is uniformly greater than in *Lamarckiana*. However, the ratios between the cell volumes of the two plants in the homologous tissues studied by Gates vary greatly, ranging from a ratio of approximately 1 : 1·5 to a ratio of 1 : 3·8, instead of being in a uniform ratio of 1 : 2 as might be expected from Boveri's conclusions. The discrepancies between these results are perhaps difficult to understand, but it should be noted that the cells were measured from sections cut in paraffin (in which some distortion is inevitable), and also that no allowance was made for the size of the vacuoles, which in plant cells are large and variable, except as a rule in embryonic tissues. Thus it is extremely difficult to institute close comparisons between the size of the nuclei and plasma masses of most plant cells.

It is clear from Gates's results, nevertheless, that the cells of *gigas* are probably larger throughout the entire plant than those of *Lamarckiana*, and the greater size of many of the plant's organs (leaves, flower parts, pollen, &c.) is quite certainly correlated with this fact. An examination

of the figures accompanying this paper will illustrate the main principle. They were drawn for their cytological features alone, and without reference to comparative size, and many of the figures of nuclei do not give the outlines of the optical sections. Nevertheless, a comparison of the figures of *gigas* with corresponding figures of *Lamarckiana* will show almost uniformly the fact that the cells and nuclei in the pollen mother-cells of *gigas* are much larger than those of *Lamarckiana*. The point is brought out especially clearly in comparisons between Figs. 4 and 51, 7 and 53, 27 and 66, 32 and 66, 33 and 72, 40 and 78.

The origin and significance of the double chromosome number in gigas. As might be expected, we have no direct evidence of the manner in which *gigas* obtained its double quota of chromosomes at the time of its origin from *Lamarckiana*, and since the type arises at such exceedingly rare intervals, there is little hope of obtaining such evidence unless experimental means are devised for very greatly increasing its production. There is only one observation on the Oenotheras that can have any possible bearing on the problem, and this is the statement of Geerts ('09, p. 52) that he found an example of the heterotypic mitosis of *Lamarckiana* showing about twenty-eight chromosomes in place of the seven pairs (i.e. fourteen sporophytic chromosomes) normally present. This interesting preparation is not figured and there are no comments upon its possible bearing on the present problem.

If material of *Lamarckiana* should be found presenting a fair proportion of abnormalities such as that described above, it might be discovered that pollen-grains and embryo sacs are occasionally formed with nuclei containing fourteen chromosomes derived from twenty-eight chromosomes appearing during the heterotypic mitosis. Chance mating of germ-cells with this double number of chromosomes would give rise to individuals agreeing with *gigas* as to the chromosome count. The presence of twenty-eight chromosomes during a heterotypic mitosis of *Lamarckiana* might come about from a somewhat earlier appearance of that premature division of the chromosomes which normally takes place in *Lamarckiana* as early as the anaphase of this mitosis. Thus the pushing forward of this premature fission of the chromosomes from the anaphase to the metaphase of the heterotypic mitosis would result in the distribution of fourteen chromosomes to each pole of the spindle. Another fission introduced before the metaphase of the homotypic mitosis would make possible a group of four nuclei at the end of the reduction divisions, each with fourteen chromosomes. Such conceivable irregularities, however unlikely they may appear at first thought, should at least be borne in mind by those who study the Oenotheras.

Gates ('09, p. 544) has brought forward several suggestions as to the origin of the double number of chromosomes in *gigas*, not including the possibility given above. Of these, the one that seems most probable to

Gates and to the writer supposes a doubling of chromosome count in the fertilized egg or very young embryo by the division of the chromosomes preliminary to a mitosis which failed, however, to effect their distribution. It is known that such failures to effect the normal distribution of chromosomes may result from irregularities in spindle formation, as in a monaster, by which the full set of divided chromosomes is brought together in a single nucleus which contains of course the doubled number. There is much experimental evidence on the botanical side from the studies of Gerassimow, Němec, Miss Kemp, and others, and also on the zoological side, which shows that by various chemical and mechanical treatments mitoses may be interrupted at stages in their division, and two sets of divided chromosomes brought together again to form a single nucleus with twice the normal number of chromosomes.

It has long been known from the experimental studies of Gerassimow ('04), beginning as early as 1890, that the size of a cell bears a relation to the size of its nucleus. By subjecting the filaments of *Spirogyra* during cell division to low temperature, or treating them for a short time to the anaesthetic influence of ether, chloroform, or chloral hydrate, he showed that the portions of a partially divided nucleus may unite to form a single large nucleus, and that the cell increased in size. This line of investigation was later taken up by Němec in a series of studies on root-tips, the results being brought together in his recent work of 1910. When growing root-tips are immersed in solutions of certain drugs, chloral hydrate being a favourite reagent, partially divided nuclei may be made to fuse, with the result that tetraploid (syndyploid) nuclei are formed having double the normal number of sporophytic chromosomes. The effect of the reagent is to disorganize the achromatic portions of the mitotic figure, or otherwise disturb their functions, so that the movement of the divided chromosomes towards the poles is arrested, and they are gathered together to form a single nucleus.

Of especial interest is Němec's claim that the tetraploid number of chromosomes in some cases returns to the normal number by a heterotypic mitosis. This view is based chiefly on the peculiar appearances of the chromosomes, suggesting the diads and tetrads of the reduction division. Strasburger and others, after failing to confirm Němec's observations on the 'heterotypic mitosis', have criticized his conclusions on general grounds. Miss Kemp ('11), however, has found in chloralized root-tips of the pea, an especially favourable subject, figures very similar to those of the heterotypic mitosis, but her interpretations are not those of Němec. Although the chromosomes have the X, Y, and tetrad forms suggestive of the reduction division, Miss Kemp believes on good evidence that these peculiarities are due to the direct action of the drug.

The results and the interpretations involved in the investigations

mentioned above may have important bearings on the problem of the origin of such a type as *gigas*, for the origin of *gigas* was possibly concerned with the appearance of a tetraploid condition in the nuclei of *Lamarckiana*, and suggests an interesting line of experimental study upon this latter form.

It is of interest to compare *gigas* with *Lamarckiana* in view of the fact that the only cytological difference so far known concerns simply the presence in the former type of a double set of the same chromosomes which are present in the latter. *Oenothera gigas* is conspicuously larger and more robust in almost all of its parts than its parent *Lamarckiana*. The seeds and seedlings are larger; the rosettes are frequently much larger; the stems are thicker and stronger; the foliage leaves are broader; the buds are larger, as are all of the flower parts; the capsules are much thicker, although proportionately somewhat short. The height of *gigas* is, however, generally not so great as, or no greater than, that of *Lamarckiana*. The differences between the two forms thus concern the relative proportions of the organs. There are no obvious characters present in one plant that are not found in the other, and this would be expected since the inheritance is through chromosomes which are qualitatively similar.

Nevertheless, while the differences between *gigas* and *Lamarckiana* may depend chiefly on the greater vegetative vigour or luxuriance of the former type, it is clear that the expression varies in the different organs, since the results are not uniformly proportionate throughout the organization of *gigas*. That is to say, *gigas* is not a giant form of *Lamarckiana* proportionately larger in all of its parts, but it has distinct characters of its own due to different comparative relations in the form and measurements of its organs. *Gigas*, therefore, does not bear the same relation to *Lamarckiana* as the giant form of *Primula sinensis* seems to bear to the ordinary form, from which it appears to differ simply in the greater size or luxuriance of its parts. In this case as reported by Gregory ('09), the giant has the same number of chromosomes as the smaller parent form, but the cells and nuclei in the tissues studied were found to be slightly larger. These differences in size were relatively very small as compared with those between the nuclei and cells of *gigas* and *Lamarckiana*, and the two cases are evidently not of the same class. The large form of *Primula sinensis* is apparently a true giant variety of the parent type, while *gigas* is a progressive mutant, its peculiarities being clearly sufficient to separate it as a distinct species from *Lamarckiana*.

The conclusion seems unavoidable that *gigas* arose with its peculiarities fully developed as the result of profound changes in the germ-plasm of its parent *Lamarckiana*, and as far as cytological evidence goes, the doubling of the chromosome count was the most obvious cause of the changes. In this germinal modification lay the basis for the sudden appearance of a sharply marked mutant more distant from any possible parent wild species

(assuming *Lamarckiana* to be of hybrid origin) than from *Lamarckiana* itself. Furthermore, the trend of evolution in *gigas* is distinctly progressive as far as evidence is furnished by the greater size of its organs and vegetative vigour. There is apparently none of the retrogressive variation so conspicuously shown in almost all of de Vries' 'mutants' in which one or more characters of *Lamarckiana* have dropped out or are presented in reduced form in the 'mutants'. The remarkable rarity of *gigas* as a derivative from *Lamarckiana* should not, however, be forgotten in the acceptance of its being representative of a true mutant.

Cytological Discussion.

Including the results presented in this paper, the writer has published accounts of the reduction divisions in four species of *Oenothera*, viz.: *grandiflora* (Davis, '09), *biennis* (Davis, '10), *Lamarckiana*, and *gigas*. The material of the last three species was from strains that had been bred in 'pure lines' for two or more generations; the material of *grandiflora* was from plants grown from wild seeds. In addition to these species the history of the reduction divisions of *O. muricata* has also been traced, but this history agrees so closely with the account of *biennis* that a description would be scarcely more than repetition. From these studies we are perhaps in a position to take a general survey of the conditions presented by the Oenotheras in relation to certain problems of cytology. It is, however, probable that important evidence on some of the chief difficulties will come from the investigation of certain *Oenothera* hybrids which will be the next step in the writer's studies, and this brief review must be, therefore, somewhat tentative in character.

The attention which in recent years has been devoted to the study of the chromosome history during interkinesis is rapidly giving important results. The work of Overton ('05, '09), Rosenberg ('04, '05, '09), and Lundegard ('09, '10) upon a variety of plants has placed the prochromosome theory in a strong position. The last statement of this view by Overton (Science, vol. xxxiii, 1911, p. 193) reports that the chromosomes following a mitosis form by alveolization independent reticula in the resting nucleus which touch one another but do not anastomose, and which during the prophase of mitosis by condensation become again transformed into chromosomes. My studies on *Oenothera* seem to favour the above view, although I have as yet been unable to trace the outlines of the chromosome reticula in the mid-period of the resting nucleus. The chromosomes may readily be followed in the process of alveolization for a considerable time following the mitosis and chromatic bodies, or prochromosomes, may be recognized at a stage during the organization of the dense chromatic network which precedes the differentiation of the spireme, when their boundaries again become lost. There is, however, a mid-period

of the resting nucleus when the chromatic material is distributed over a network so delicate as so far to defy morphological analysis, but such negative evidence in view of the minuteness of the structures in *Oenothera* has, in the writer's opinion, little or no value.

The history of the chromosomes in *Oenothera* during the interkinesis between the heterotypic and homotypic mitoses presents very important evidence in favour of the theory of the individuality of the chromosomes of which the conception of chromosome centres or prochromosomes in the resting nucleus forms a part. The writer knows of no plant types in which the chromosomes may be more easily traced through this short resting period than in *Oenothera*. The paired chromosomes characteristic of this interkinesis may be followed with absolute precision, and this stage affords perhaps the most favourable opportunity in the entire life-history of making the chromosome count. The process of expansion following the anaphase of the heterotypic mitosis increases the size of the chromosomes, but alveolization does not set in to such a degree as to obscure their outlines, although there are sometimes slight tendencies to form reticula such as are conspicuously developed in many plants (e. g. *Lilium*) at the same stage. It is possible that the period of the interkinesis is too short to allow of the complete vacuolization of the chromosomes, or perhaps their size and form make their modification more difficult. As a result the conditions usual in the mid-period of a resting nucleus have apparently been dropped out of this interkinesis in *Oenothera*; the process of alveolization never proceeds far, the chief change of chromosome form being a slight enlargement followed shortly by the condensation in preparation for the homotypic mitosis.

The most difficult periods of the reduction processes as regards both observation and interpretation are those which lead to the differentiation of the chromosomes in preparation for the first, or heterotypic, mitosis. These periods include the synaptic and presynaptic stages leading to the condition called (when present) 'diakinesis', and the peculiar phenomenon of the 'second contraction'. It has become necessary to define one's usage of the term 'synapsis' since McClung ('05) has proposed the restriction of the term to the 'fusion of simple chromosomes into multiple ones usually of bivalent value'. Chromosomal associations are the exception rather than the rule in the Oenotheras, an important peculiarity which will be discussed presently. McClung has proposed the term 'synizesis' for 'the condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not'. Without questioning the value of McClung's discrimination of the stages in the Orthopteran material studied by him there does not seem to the writer sufficient reason for the limitation in plant cytology of the term 'synapsis' to chromosomal fusions, since such phenomena, even

when present, are not the conspicuous stages of chromatic contraction which are so well known to botanists from their apparently universal presence in higher plants. For these reasons the writer continues to use the term 'synapsis' for that characteristic and relatively long-enduring contraction of the chromatic reticula or thread systems that appears previous to the differentiation of the spireme of the heterotypic mitosis.

There is no question in the writer's mind but that the events of synapsis are the result of a true contraction, i. e. a shortening and thickening of the strands or threads of the chromatic reticulum. Lawson ('11) has recently expressed the view that in the phase of synapsis 'there is no contraction whatever of the chromatic substance', but that the appearance of a contraction is the result of an enlargement of the nuclear cavity due to a growth which carries the nuclear membrane away from the chromatic mass. This view can fortunately be readily tested as to the facts. It will be admitted that there is frequently marked nuclear growth throughout the period of synapsis which would undoubtedly emphasize a contraction of the chromatic material, but can such growth account for the differences, in some cases very great, between the size of the nuclear cavity and its contained synaptic knot? It is a simple matter to measure the size of the nucleus and of the chromatic mass in optical section through the various stages of synapsis. Lawson holds that the chromatic mass remains essentially unchanged in its volume and presents evidence for this view in a series of figures of *Smilacina*. These figures, however, are to the writer not convincing, inasmuch as the chromatic mass of advanced synapsis (Pl. LXXI, Figs. 10–17) may be included in a circle having the diameter of the presynaptic nucleus (Figs. 1 and 2) with considerable space left unfilled. An examination of the figures of any of the species of *Oenothera* studied by the writer will show the outlines of the chromatic mass very much smaller and consequently denser during the synaptic stages than in the full-sized presynaptic nuclei, and it is his opinion that Lawson's view will not be sustained by the numerous accounts of synapsis on various material.

The relations among the strands that compose the chromatic reticulum previous to and during the synaptic contraction have so far baffled my studies. There is apparently in their arrangement no system or order of especial significance. In such a complicated and dense mass of elements (see figures) it is not difficult to find threads that run closely parallel for greater or less distances, but the writer is not willing on the evidence at hand to attach significance to these relations. It has not been possible to recognize even the elements of a simple spireme and much less conditions that might be interpreted as indicating the presence of two parallel spiremes of paternal and maternal origin. Fortunately the later history of the reduction processes clearly shows that the entire group of sporophytic (somatic) chromosomes is formed end to end (telosynapsis) by the seg-

mentation of a univalent spireme, and there is no place evident in the reduction processes of *Oenothera* for the parallel conjugation of two spiremes (parasynapsis).

This can be said respecting the results of the synaptic contraction. From the dense reticulum of the presynaptic stages there is differentiated a thread system, and during the process the threads are materially thickened and the length of the system is apparently much shortened. This history may indicate nothing more than a process of condensation and contraction of the chromatic elements of a thread system such as is found during every mitosis among higher plants, in which case synapsis may have little significance except as associated with the peculiar conditions surrounding the growth and activities of spore mother-cells. On the other hand the actual results of the synaptic contraction would certainly bring prochromosomes or other chromatic elements into as intimate a relation or proximity, short of an actual homogenous mixing of substance, as could well be imagined by the most enthusiastic advocate for transfers or mutual interactions of idioplasms. The long period in which the nucleus lies in a state of synapsis also favours the importance of the process as one of a fundamental nature. In the history of reduction in the Oenotheras there is apparently no phase when the chromosomes or their representatives (chromatic centres or prochromosomes) are in such intimate association and for so long a time as during mid-synapsis.

We are now ready to pass to another phase of the history of reduction that also presents serious difficulties of observation and interpretation, and which must be considered with reference to later events when the chromosomes become differentiated. This puzzling phase comprises the history from the period of mid-synapsis, characterized by the fully contracted synaptic knot, to the formation of the mature spireme which segments into the full number of sporophytic (somatic) chromosomes. With the loosening of the synaptic knot there emerges a thread system the elements of which are markedly thicker than the chromatic strands of the early and mid-phases of synapsis. These conditions are clearly shown in Figs. 15 and 16 for *grandiflora* (Davis, '09), Figs. 9 and 10, 42 and 43 for *biennis* (Davis, '10), Figs. 10 and 11 for *Lamarckiana*, and Fig. 55 for *gigas*. With the thickening of the threads there also takes place a shortening of the thread system, and there can be little doubt that the two processes are the result of the general condensation and contraction of the chromatic material throughout the period of synapsis. The next developments which lead to the differentiation of the mature spireme are more difficult to follow, for the threads take on irregularities of form and thickness which might suggest various interpretations to investigators who have reported the stages of double threads (strepsinema) in certain material. The writer, however, for reasons stated below, believes these peculiarities

to be simply features of a continued chromatic condensation and contraction which transforms the system of thickened threads into the spireme.

Grégoire ('10, pp. 325 and 387) has expressed the opinion that the 'strepsinema' stage will be found in *Oenothera* exhibiting the characteristic longitudinal division or doubling of the spireme, which is so important a feature of his views on a side-by-side association of chromosomes (parasynapsis) leading up to diakinesis. He believes that the chromosomes will be found to arise side by side in pairs as a result of this longitudinal doubling of the spireme. Grégoire, then, suggests that the arrangement of the chromosomes in *Oenothera* in a chain is a later development due to the separation at one end of the chromosomes in each pair during diakinesis and the secondary union of the free ends of the pairs to form a chain not of $2x$ independent sporophytic chromosomes but of x pairs of chromosomes. This ingenious interpretation is intended to bring certain peculiarities of chromosome formation and arrangement in *Oenothera* into line with Grégoire's well-known theory, which he believes to be of general application throughout the animal and plant world.

The difficulties confronting Grégoire's interpretation described above seem to the writer insurmountable for the following reasons:—

First. With respect to the segmentation of the spireme, this is one of the most evident and direct processes in the entire history of reduction phenomena in *Oenothera*. It is illustrated in Pl. LXXI, Figs. 11–16 for *biennis* (Davis, '10), Figs. 13–17 for *Lamarckiana*, and Pl. LXXII, Figs. 57–59 for *gigas*. The spireme is not complexly coiled, the stages of segmentation are easily found, the form of the chromosomes is so simple that no especial difficulty is offered to the study of their arrangement, and the usual diploid (sporophytic) number, fourteen (twenty-eight in *gigas*), is so small that they may be counted with accuracy. It is perfectly clear that the spireme segments into the full set of $2x$ (diploid) chromosomes arranged end to end and not into x pairs of chromosomes arranged side by side. There is to the writer no doubt of the facts of the case or of the interpretation that these constitute the full set of sporophytic chromosomes formed end to end by the segmentation of a univalent and not a double spireme.

Second. There is no general pairing of the chromosomes in *biennis*, *Lamarckiana*, *rubrinervis*, or *gigas*. The occasional association in these species of chromosomes to form ring-shaped pairs, whether united at one or both ends, comes about through the approximation of chromosome segments forming the two sides of loops in the spireme. The exceptional conditions in certain material of *grandiflora* (Davis, '09, pp. 558 and 559), where seven ring-shaped pairs of chromosomes are formed, result from the presence of a spireme composed of complexly coiled loops which segment in such a manner that seven loops (later rings) are generally differentiated,

each composed of two chromosomes. It is to be expected that different material of *grandiflora* will show deviations from these conditions approaching more closely to those of the other species, since the differences depend on the degree of complication in the arrangement of the portions of the spireme.

It is best to approach the interpretation of the synaptic processes in *Oenothera* from the stage of the segmented spireme. We are, in the writer's opinion, on safe ground in our understanding of this condition as the segmentation of a univalent spireme into the full set of sporophytic chromosomes (diploid), and any explanation of the subtle events of synapsis must accord with this view. Should it be established that the spireme splits longitudinally before its segmentation, and that this fission is quickly closed, the most probable explanation of such a phenomenon would be the very early appearance of the division which is effected during the anaphase of the heterotypic division in preparation for the homotypic mitosis. But, as previously noted, the writer has found no evidence that such a division takes place or that there is a fusion of parallel spiremes. There is then left the interpretation of synapsis as a long-continued condensation of the chromatic elements from the stages of the presynaptic reticulum to the spireme which develops from the thickened threads that emerge from the synaptic knot, the stage of mid-synapsis (synaptic knot) being characterized by the very close association of the chromatic elements. It is possible that the above view may be modified by the study of the reduction phenomena in *Oenothera* hybrids, but the evidence so far seems in its favour.

With respect to the relations between the chromosomes in the spireme it is possible that they are alternately of paternal and maternal origin, or they may be arranged merely by the law of chance. On this point we shall probably have little or no evidence in *Oenothera* until suitable hybrids have been studied not only in the F_1 generation but also through selected forms of the F_2 and perhaps later generations. Whenever the chromosomes are associated in pairs during diakinesis it is evident that the members of a couple were adjacent chromosomes on the segmented spireme, but the phenomenon of pairing is relatively uncommon in the *Oenotheras*, and it is unsafe to lay emphasis on these conditions. Usually the chromosomes are distributed without apparent order during diakinesis, which in consequence is not so striking a stage in *Oenothera* as in those types where a regular pairing of the chromosomes obtains. The apparent lack of system in the arrangement of the chromosomes during diakinesis, however, may not be real since physiological conditions may determine a very precise distribution of the chromosomes by the heterotypic mitosis.

The phenomenon of the ' second contraction ' which takes place during the segmentation of the spireme and the condensation of its portions to form the sporophytic chromosomes is frequently a clearly defined phase of the

reduction processes in *Oenothera*. Some writers have interpreted this stage as one which brings the chromosomes into close association in pairs in preparation for their distribution by the heterotypic mitosis, or even results in their temporary fusion during the period of diakinesis. The second contraction in *Oenothera* gives no support to these views, since, as pointed out, there is no general pairing of the chromosomes (certain material of *grandiflora* excepted), and little or no opportunity for close associations. The period in *Oenothera* when the chromatic elements are in their most intimate relations to one another is that of mid-synapsis, when the thickened threads are so closely tangled and massed that contact would seem almost certain, although this need not of course involve the actual fusion of idioplasms. The second contraction in *Oenothera* may then have no further significance than the assembling of the segments (sporophytic chromosomes) of a spireme so complexly looped that its parts are necessarily drawn closely together by the continuation of the general process of condensation which operates throughout the entire phase of synapsis and ends only when the mature chromosomes are ready for distribution on the equatorial plate of the heterotypic spindle. This interpretation on the evidence at hand seems to the author the most reasonable for this stage in the Oenotheras.

Finally we must emphasize the conclusion, also stated in the author's previous papers, that the sporophytic (somatic) chromosomes of *Oenothera* are formed end to end by the segmentation of univalent spiremes which follow synapsis. In this conclusion Gates, Geerts, and the writer are in full accord. There seems to be no possibility of interpreting the spireme as bivalent, i. e. composed of two spiremes (paternal and maternal) united side by side. Even should it be established that the spireme in earlier stages of developments shows indications of a doubling or lengthwise fission (a strepsinema stage, of which the writer has seen no clear evidence), such a fission could have no relation to the hypothesis of the conjugation of parallel spiremes, since the chromosomes in the full diploid number are formed from as many segments of the spireme and are not formed in pairs from a haploid number of spireme segments. Such a doubling of the spireme (if present) might be related to the lengthwise fission of the chromosomes which appears during the anaphase of the heterotypic mitosis in preparation for the homotypic division, but it is difficult to see any other probable interpretation of importance. Thus it appears impossible to bring the facts into accord with the hypothesis of a side-by-side pairing of the chromosomes through the association of two parallel spiremes or two parallel sets of chromosomes as held by Grégoire, Allen, Rosenberg, Overton, Stomps ('10), and their followers. The results from the studies on *biennis*, *grandiflora*, *Lamarckiana*, *rubrinervis*, and *gigas* strongly support the view of Farmer and Moore, Mottier, Digby ('10), and others of an end-to-end

relation of the chromosomes when first formed by the segmentation of a univalent spireme.

Summary.

1. Chromatic bodies, probably representing chromosome centres or prochromosomes, may be recognized in the nuclei shortly after the last mitoses in the archesporium when the sporophytic chromosomes by alveolization have developed delicate nuclear reticula.

2. Shortly before synapsis the nuclear reticula become so dense in structure through the development and thickening of numerous strands that the chromatic bodies can no longer be distinguished, their substance apparently merging with that of the deeply staining networks.

3. The advent of synapsis is indicated by the slow contraction of the reticula which carries most of the strands towards the centre of the nuclei. During this process the threads become more evident in the reticula and come to lie in confused and complicated coils tightly gathered together to form the synaptic knot from which a few delicate strands and loops extend into the nuclear cavity. These threads appear at times to run closely parallel with one another, but the evidence does not justify an assumption that this relation indicates more than such a close association as would be brought about by the gathering together of a complexly looped thread or system of threads.

4. The synaptic knot, marking the period of mid-synapsis, generally lies close to the nucleolus, and is a clearly defined stage extending over a considerable period (perhaps one or more days). This is the time in the reduction processes of *Oenothera* when the chromatic elements (prochromosomes) are in their most intimate relation to one another. The results of the synaptic contraction are an evident thickening of the threads and shortening of the thread system.

5. Following the period of mid-synapsis, the chromatic material emerges from the synaptic knot by a loosening of the coils of the contracted thread system. It then becomes evident that the threads have continued to thicken and shorten although still complexly looped and coiled. From this condition is developed by further condensation the very much thicker thread that becomes the univalent spireme. Although irregularities in the form and thickness of this spireme may be observed during its development, no clear evidence has been noted that it ever becomes doubled by a lengthwise fission to give a 'strepsinema' stage.

6. A segmentation of the spireme gives rise to the full set of sporophytic (somatic) chromosomes arranged end to end. These numbers, fourteen in *Lamarckiana* and twenty-eight in *gigas*, are the diploid numbers for these species respectively. The spireme segments are always in the diploid number, which precludes the possibility of their being bivalent in

nature. The chromosomes take on their form and proportions by a shortening of the spireme segments marking the end of the long process of chromatic condensation which began in presynaptic stages.

7. The condensation of the spireme segments results in a further shortening of the spireme during which much of its complicated looped arrangement disappears. Associated with this condensation is a second contraction during which the segments of the spireme or chains of chromosomes are frequently drawn closely together in a cluster. It is doubtful whether this stage is of especial significance since the chromosomes are not regularly grouped in pairs, and it may be merely the result of the final condensation of the chromatin previous to the differentiation of the fully developed chromosomes.

8. The chromosomes separate during the prophases of the heterotypic mitosis, but, although adjacent chromosomes sometimes form rings, there is no general pairing of the chromosomes characteristic in some forms of the stage of diakinesis. The chromosomes by contracting and bending in the middle regions become V-shaped; they are all essentially similar to one another.

9. When grouped in pairs it is clear that such alternate (adjacent) chromosomes on the spireme are separated by the heterotypic mitosis, but the generally scattered arrangement makes it impossible to determine whether alternate chromosomes are always so distributed. The absence of a conspicuous stage of diakinesis with the chromosomes grouped in pairs makes the determination of this important point very difficult in *biennis*, *Lamarckiana*, and *gigas*.

10. The heterotypic mitosis is a reduction division distributing the fourteen chromosomes of *Lamarckiana* and the twenty-eight chromosomes of *gigas* in two sets of seven and fourteen chromosomes each for the respective species.

11. A lengthwise fission of each chromosome in preparation for the homotypic mitosis becomes apparent during the anaphase of the heterotypic division.

12. Thus seven split chromosomes in *Lamarckiana* and fourteen split chromosomes in *gigas* enter the nuclei of the interkinesis between the heterotypic and homotypic mitoses and may be followed as pairs of chromosomes throughout the period of the interkinesis.

13. The homotypic mitosis is an equation division distributing the members of the pairs of chromosomes present during the interkinesis.

14. Irregularities in the distribution of the chromosomes are not uncommon, and may account for a portion of the sterility of the pollen in these forms, but it seems probable that other factors of a physiological nature are operative.

15. The pollen mother-cells and their nuclei and also the pollen-

grains of *gigas* are much larger than those of *Lamarckiana*, thus supporting the view that in related types the size of the cells and nuclei are proportionate to the number of chromosomes.

16. *Oenothera gigas* is a progressive mutant, its peculiarities being clearly associated with the changes in its germ-plasm incident upon the doubling of its chromosome number.

CAMBRIDGE, MASSACHUSETTS,
March, 1911.

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EXPLANATION OF FIGURES IN PLATES LXXI–LXXIII.

Illustrating Professor Davis's paper on the Reduction Divisions of *Oenothera Lamarckiana* and *Oenothera gigas*.

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. Sections cut 6 μ thick, and stained with iron-alum haematoxylin.

PLATE LXXI.

Figs. 1–31, *Oenothera Lamarckiana.*

Fig. 1. Telophase following a mitosis in an archesporial cell, chromosomes still evident.

Fig. 2. Resting nucleus in a pollen mother-cell showing large nucleolus, chromatic bodies, and delicate reticulum.

Fig. 3. The chromatic bodies are no longer easily recognized in the denser reticulum.

Fig. 4. Nucleus filled with a dense reticulum shortly before the advent of synapsis; chromatic bodies no longer distinguishable.

Fig. 5. The beginning of the synaptic contraction, two nucleoli.

Figs. 6 and 7. The synaptic contraction well under way, the threads and meshes of the reticulum becoming drawn into a close mass.

Fig. 8. Two pollen mother-cells, their nuclei in mid-synapsis.

Fig. 9. A closely contracted synaptic knot.

Figs. 10 and 11. The thickened threads which emerge with the loosening of the synaptic knot.

Fig. 12. Further shortening and thickening of the threads to form the spireme.

Figs. 13 and 14. Spireme with constrictions which show that the process of segmentation has begun.

Figs. 15 and 16. Segmented spiremes, the chromosome segments for the most part clearly arranged end to end to form a chain.

Fig. 17. A segmented spireme in a somewhat complicated looped arrangement.

Fig. 18. Two chromosome segments in the form of a ring separated from the main group which is apparently in the stage of the 'second contraction'.

Figs. 19 and 20. These illustrate the stage of 'second contraction'. The segments of the spireme are gathered in a close mass that bears a superficial resemblance to the synaptic knot.

Fig. 21. Another form of the 'second contraction', the spireme segments more loosely associated.

Fig. 22. Early prophase of the heterotypic mitosis. The nuclear membrane has disappeared and the nucleolus is no longer present; fourteen chromosomes could be counted, most of them still clearly arranged end to end.

Figs. 23 and 24. Multipolar spindles; the chromosomes closely clustered as though still in the condition of the 'second contraction'.

Figs. 25 and 26. The spindles have become bipolar. Most of the chromosomes show the end-to-end arrangement; fourteen may be counted in Fig. 25.

Fig. 27. Metaphase of the heterotypic mitosis. The chromosomes, now bent in the form of thickened V's, show no close association in pairs.

Fig. 28. Anaphase of the heterotypic mitosis. Seven chromosomes are present in each of the two sets which pass to the poles of the spindle.

Fig. 29. A group of seven chromosomes, still undivided, at the pole of a heterotypic spindle.

Fig. 30. An exceptional case in which eight split chromosomes, instead of the normal number seven, are clearly present at the pole of a heterotypic spindle.

Fig. 31. Early telophase of the heterotypic mitosis showing the seven split chromosomes; nucleoli have not yet appeared.

PLATE LXXII.

Figs. 32–45, *Oenothera Lamarckiana.*

Fig. 32. Pollen mother-cell following the heterotypic mitosis.

Fig. 33. Resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. The seven pairs of chromosomes are shown, mostly in the form of U's joined in the bent middle region.

Figs. 34 and 35. Multipolar spindles in the prophases of the homotypic mitosis. The seven pairs of chromosomes now appear in a much more condensed form than during the interkinesis.

Fig. 36. Metaphase of the homotypic mitosis; seven pairs of chromosomes at the equatorial plate.

Fig. 37. Early anaphase of the homotypic mitosis; the chromosomes in two sets, seven in each group.

Figs. 38 and 39. Late anaphase and early telophase of the homotypic mitosis. The seven chromosomes at each pole exhibit irregularities of form.

Fig. 40. Pollen mother-cell in telophase of the homotypic mitosis; the seven chromosomes distinct in each nucleus.

Figs. 41 and 42. Telophases of the homotypic mitosis. The chromosomes are elongating and becoming irregular in form preparatory to the development of the chromatic network.

Fig. 43. Nucleus of a young pollen-grain, showing the open reticulum with certain deeply staining regions that probably represent chromosome centres or prochromosomes.

Fig. 44. Pollen mother-cell in which there was an irregular distribution of the chromosomes during the homotypic mitoses, resulting in the formation of two small extra nuclei each containing three chromosomes.

Fig. 45. Young pollen-grain with a small extra nucleus, the result of an irregularity in the homotypic mitosis similar to the sort illustrated in Fig. 44.

Figs. 46–63, *Oenothera gigas.*

Fig. 46. Telophase following a mitosis in an archesporial cell; chromosomes still evident. Compare with Fig. 1.

Figs. 47 and 48. Resting nuclei in pollen mother-cells, showing large nucleoli, chromatic bodies, and delicate reticula.

Figs. 49 and 50. Nuclei filled with dense reticula, chromatic bodies no longer distinguishable.

Fig. 51. Nucleus shortly before the advent of synapsis. Compare with Fig. 4.

Fig. 52. The synaptic contraction well under way, the threads and meshes of the reticulum becoming drawn into a close mass.

Figs. 53 and 54. Closely contracted synaptic knots. Compare with Figs. 8 and 9.

Fig. 55. The thickened threads which emerge with the loosening of the synaptic knot. Compare with Figs. 10 and 11.

Fig. 56. Further shortening and thickening of the threads to form the spireme. Compare with Fig. 12.

Fig. 57. A spireme just previous to the process of segmentation.

Fig. 58. Spireme with constrictions which show that the process of segmentation has begun. Compare with Fig. 14.

Fig. 59. A segmented spireme; the chromosome segments for the most part clearly arranged end to end in the form of a chain.

Figs. 60–62. Examples of the 'second contraction'. Segmentation has just begun in the spireme of Fig. 60. Compare with Figs. 18–20.

Fig. 63. Another form of the 'second contraction'; the spireme segments are more loosely associated. Compare with Fig. 21.

PLATE LXIII.

Figs. 64–79, *Oenothera gigas.*

Fig. 64. Early prophase of the heterotypic mitosis. Nineteen chromosomes are shown, some of them evidently paired; the remaining nine chromosomes were present in the adjacent section. The nuclear membrane has disappeared and fibrillae have entered the nuclear cavity.

Fig. 65. A multipolar spindle. Some of the chromosomes are still arranged end to end.

Fig. 66. Metaphase of the heterotypic mitosis. Fourteen chromosomes were present in each group at the equatorial plate. Compare with Fig. 27.

Fig. 67. Anaphase of the heterotypic mitosis. Fourteen chromosomes in each of the two sets which pass to the poles of the spindle, some of the chromosomes showing the fission in preparation for the homotypic mitosis. Compare with Fig. 28.

Fig. 68. Anaphase with split chromosomes at the poles of the spindle.

Fig. 69. An irregularity in the heterotypic mitosis. Two chromosomes have become separated from each of the main groups at the poles of the spindle.

Fig. 70. A condition following such an irregularity in the heterotypic mitosis as that shown in Fig. 69. The appearance of the chromatin indicates that such nuclei pass into a resting condition.

Figs. 71 and 72. Resting nuclei of the interkinesis between the heterotypic and homotypic mitoses. The fourteen pairs of chromosomes are shown, mostly in the form of U's joined in the bent middle region. Compare with Fig. 33.

Fig. 73. Early prophase of the homotypic mitosis; fourteen pairs of chromosomes. The nuclear membrane has disappeared.

Fig. 74. Metaphase of the homotypic mitosis. The chromosomes are now in a much more condensed form than during the interkinesis.

Fig. 75. Early anaphase of the homotypic mitosis. Compare with Fig. 37.

Fig. 76. Late anaphase of the homotypic mitosis.

Fig. 77. Telophase of the homotypic mitosis, the fourteen chromosomes beginning to show irregularities of form.

Fig. 78. Pollen mother-cell in telophase of the homotypic mitosis. Compare with Fig. 40.

Fig. 79. Nucleus of a young pollen-grain, showing the open reticulum.

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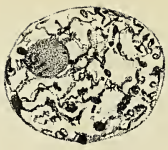

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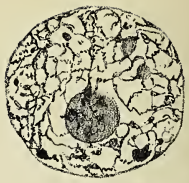

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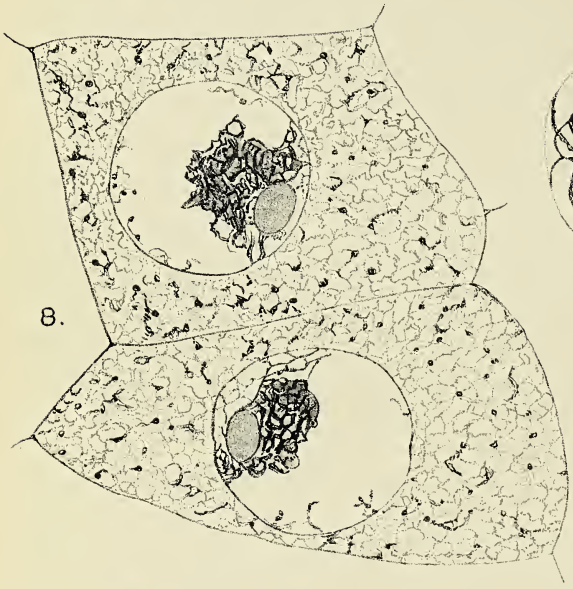

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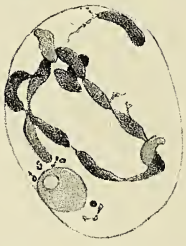

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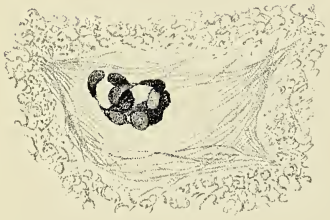

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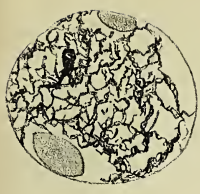

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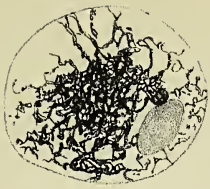

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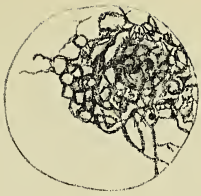

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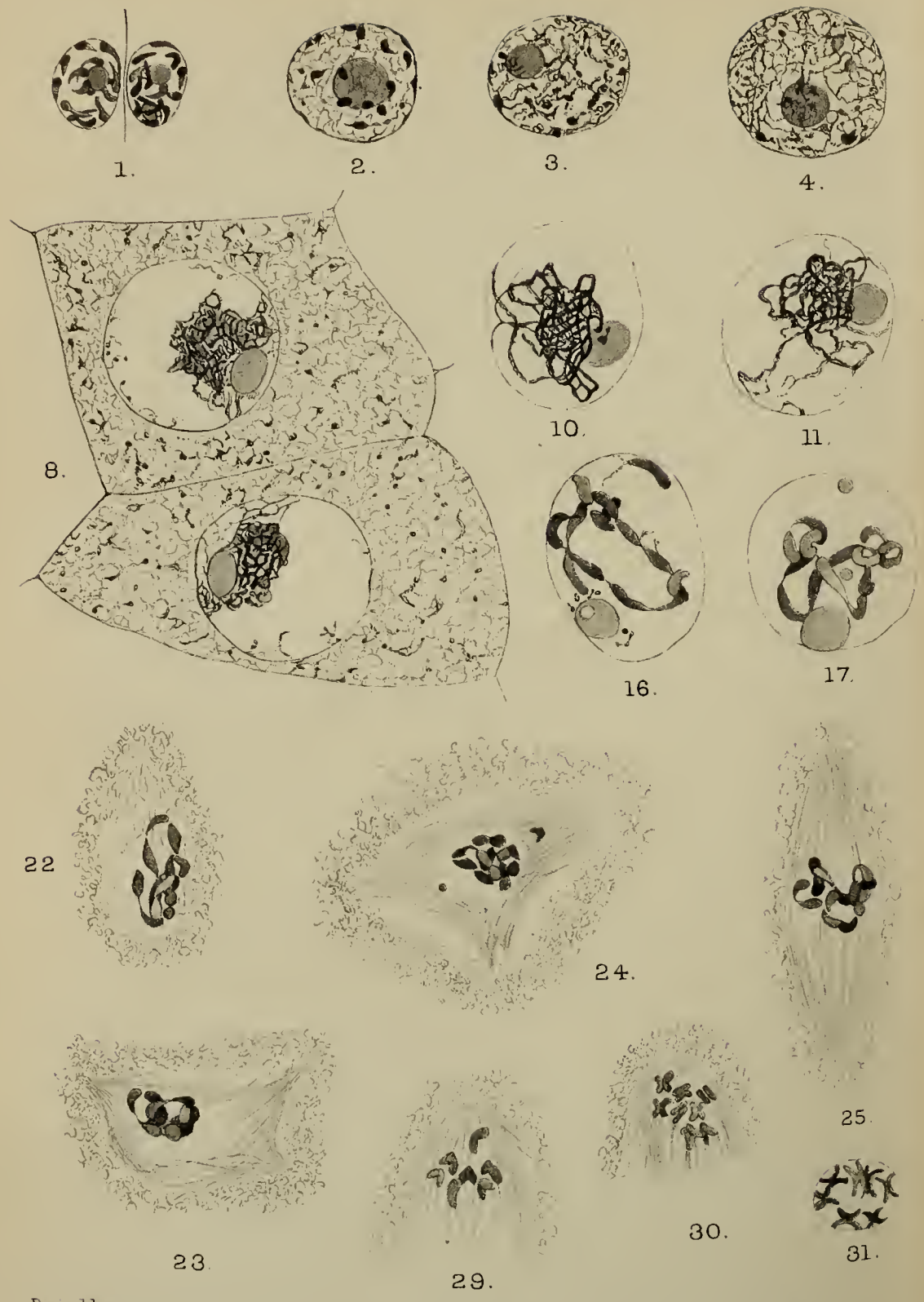

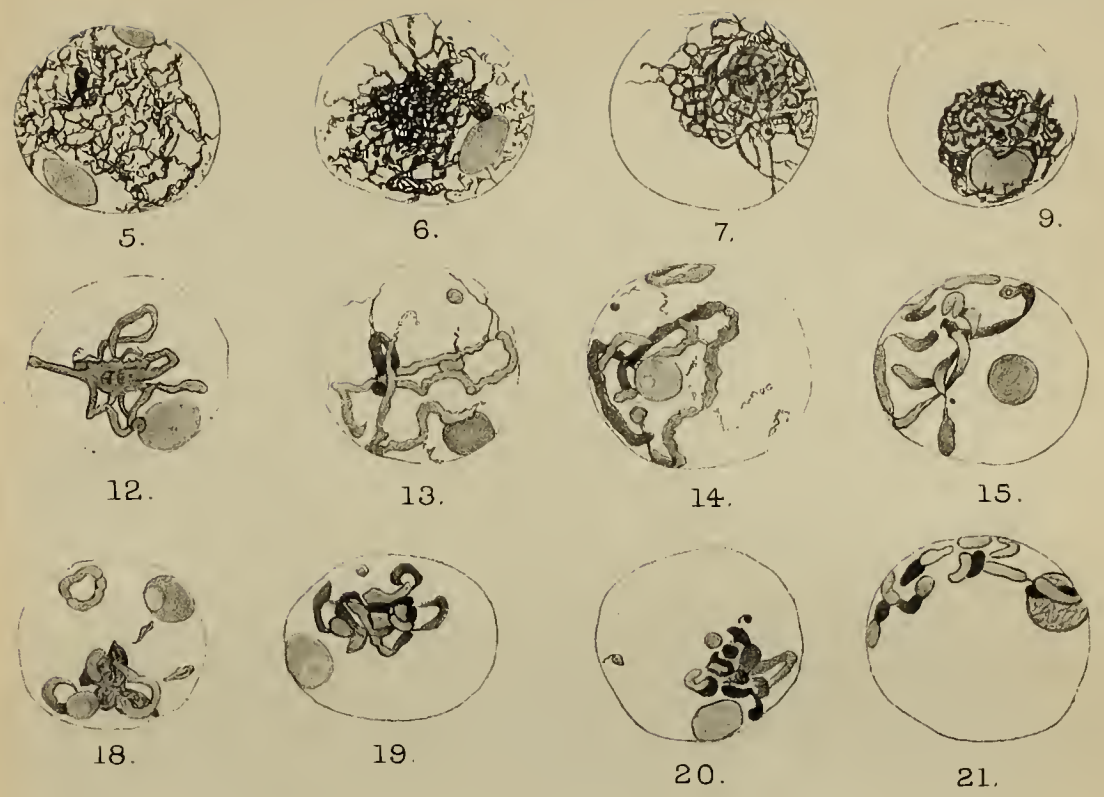

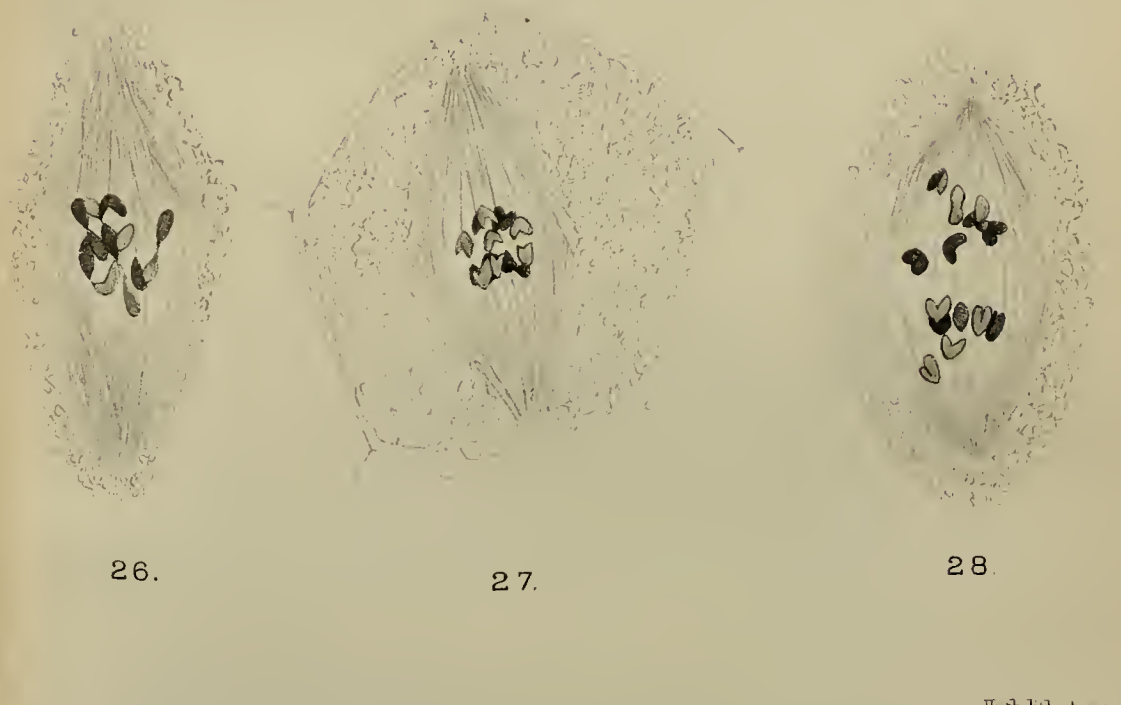

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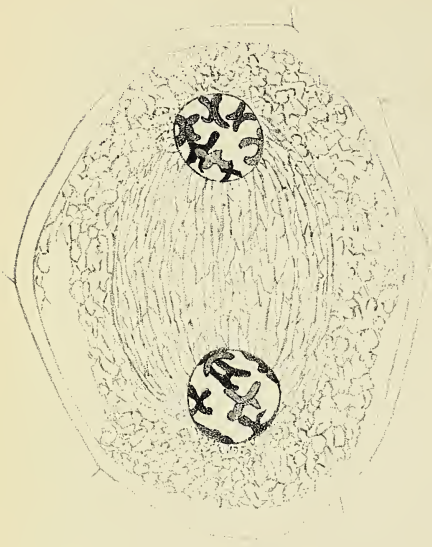

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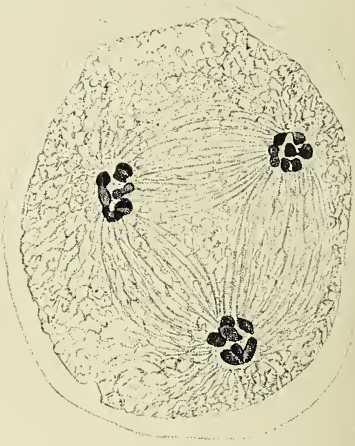

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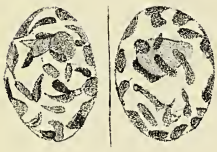

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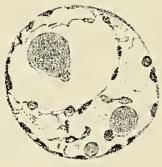

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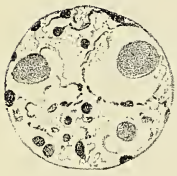

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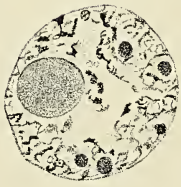

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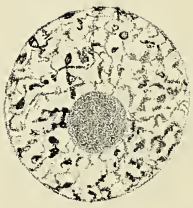

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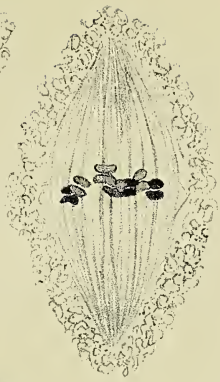

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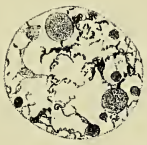

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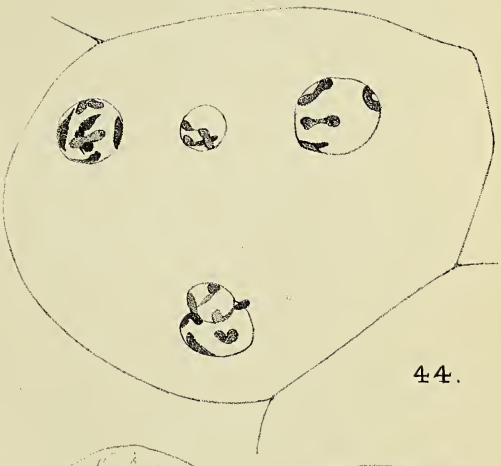

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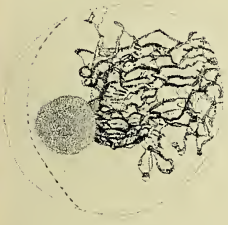

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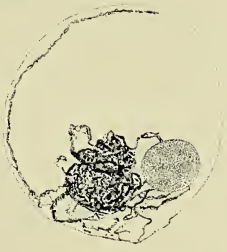

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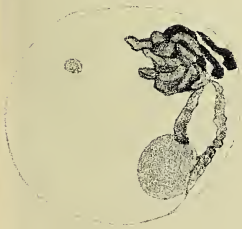

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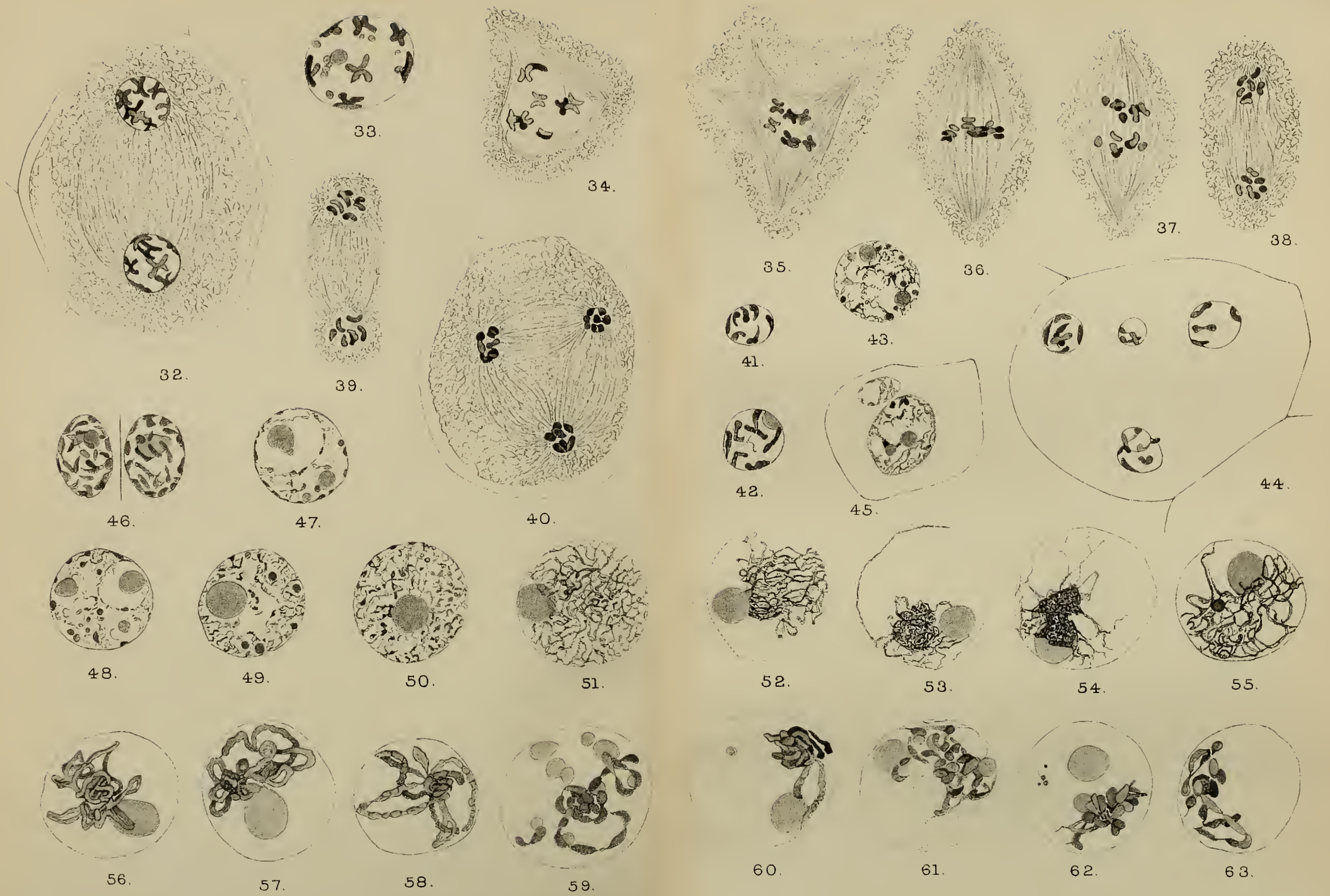

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DAVIS—— REDUCTION DIVISIONS OF OENOTHERA LAMARCKIANA AND O. GIGAS.

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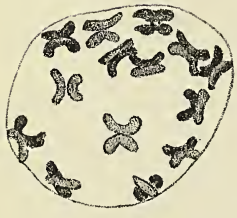

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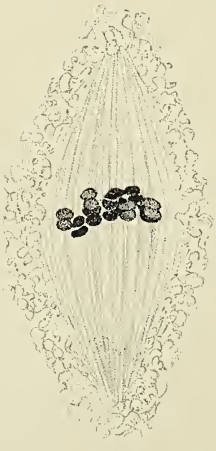

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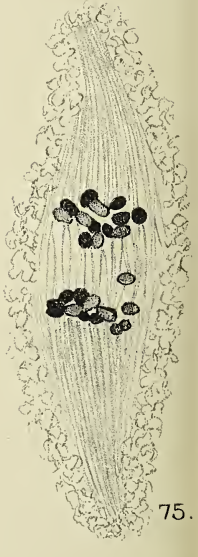

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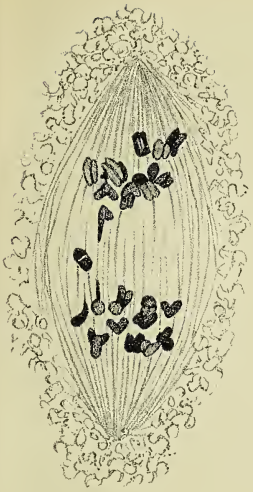
DAVIS —— REDUCTION DIVISIONS OF OENOTHERA LAMARCKIANA



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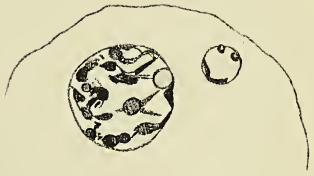

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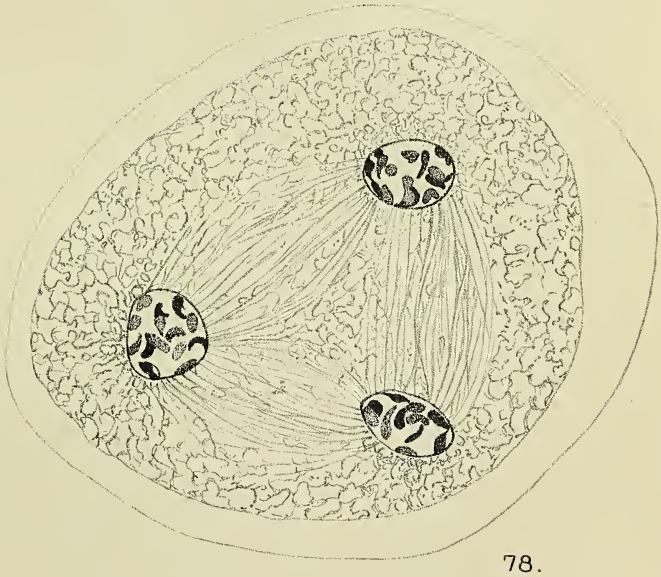

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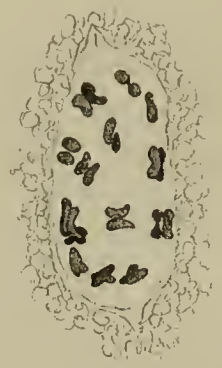

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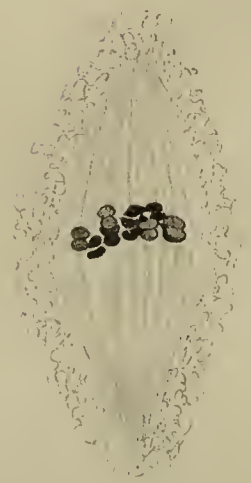

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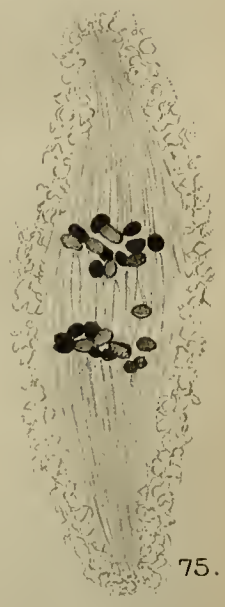

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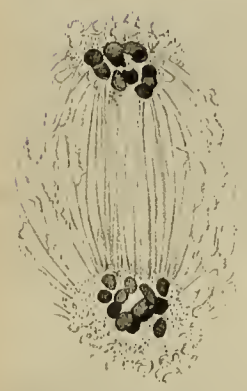

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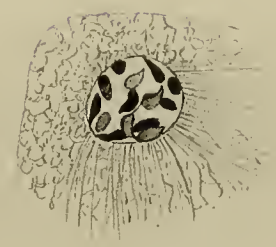

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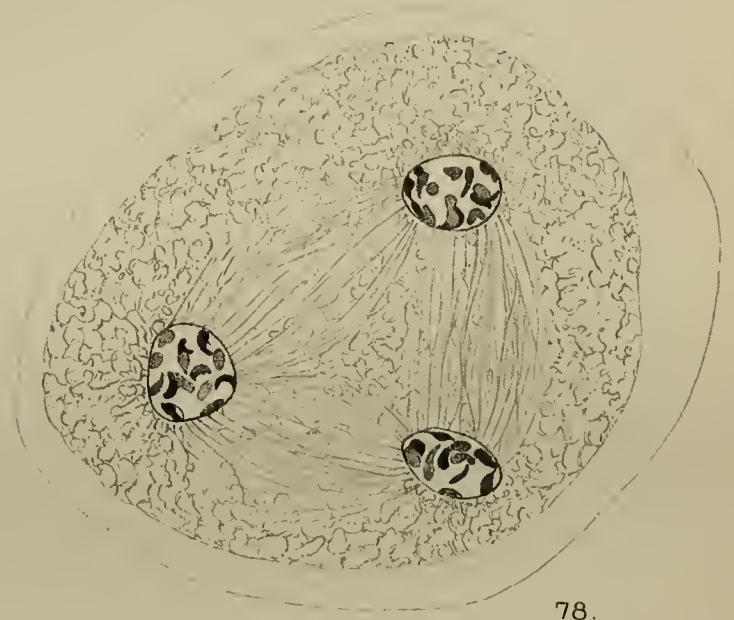

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DAVIS —— REDUCTION DIVISIONS OF OENOTHERA LAMARCKIANA AND O. GIGAS.

A Reconsideration of the Origin of 'Transfusion Tissue'.

BY

M. GERALDINE CARTER, B.Sc.

Goldsmiths' College, University of London.

With four Figures in the Text.

TRANSFUSION tissue in gymnospermous leaves has been described by a large number of botanists during the last sixty to seventy years. Zimmermann, in a paper on this subject published in 1880,[1] gave the names of Mohl, Karsten, Thomas, Hartig, Frank, Sachs, Bertrand, and de Bary, as those who had written about it before that date, and he himself described the position and nature of the thickening of the transfusion tissue in twenty-one species of Coniferae. Towards the end of the paper he stated that he found its varied orientation and great increase towards the tip of the leaf difficulties in the way of accepting Mohl's assumption that it served for the conduction of sap.

Transfusion tissue of the ordinary kind, as distinguished from the accessory transfusion tissue of *Cycas* to which this paper does not further refer, has been found by Stopes [2] in *Cordaites* also, so that it is known in fossil Gymnosperms as well as living ones.

But it is not only in Gymnosperms that transfusion tissue occurs; Scott [3] describes it in the leaves of Lycopods and thinks that even gymnospermous transfusion tissue may be comparable with what is found there,[4] and Boodle and Worsdell in a paper on *Casuarina* [5] pointed out that 'transfusion elements occur in the ridges of the young stem and of the leaves'. They considered, however, that these had a different origin and nature from the transfusion tissue so well known in the leaves of Gymnosperms. The pits of this transfusion tissue are, moreover, simple, while in gymnospermous transfusion tissue bordered pits are found.

Other writers since Zimmermann have shown interest in the last-named kind of tracheidal elements. Strasburger devotes some pages to it in the

[1] Ueber das Transfusionsgewebe. Flora, 1880.
[2] New Phytologist, vol. ii, 1903, p. 91.
[3] Studies in Fossil Botany, pt. i, pp. 160, 224.
[4] Studies, pt. ii, p. 655.
[5] Annals of Botany, vol. vii.

'Leitungsbahnen'.[1] Haberlandt[2] describes it, while Worsdell[3] and Miss Chick[4] are now to be referred to.

A paper entitled 'On the Origin of "Transfusion Tissue" in the Leaves of Gymnospermous Plants' was read before the Linnean Society in March, 1897. In it the author, Worsdell, suggested that transfusion tissue took its origin in 'the successive unlimited centripetal development of the tracheides' of the centripetal xylem found in the cotyledonary bundles of *Cycas* and *Ginkgo*. This view was mainly founded on the facts—

1. That in the cotyledons of *Ginkgo biloba* and of *Cycas revoluta* a good deal of centripetal wood was found, and that this gradually shaded off into typical transfusion tissue.

2. That centripetal xylem as well as transfusion tissue is present in the leaves of certain Conifers, i. e. *Cephalotaxus* and *Taxus* as well as in *Dammara*, *Araucaria*, *Widdringtonia*, and *Pinus*.

Centripetal wood is no longer regarded as a rare phenomenon in the leaves and cotyledons of Gymnosperms.

A paper of much interest in this connexion is that of Miss Chick,[5] who found in *Torreya* that at the base of the cotyledon the protoxylem was central; the more internal elements were crushed and could be traced down to the metaxylem, while other elements which were not crushed were in contact with the primary and secondary xylem of the bundle. At this level centripetal xylem elements were found only very occasionally, and there were very rare transfusion elements on the flanks of the bundle. Both increased upwards, but the centripetal xylem was greatest in the middle and the transfusion tissue at the tip. The writer went on to say that it appeared as though the transfusion tissue were being formed from the parenchyma outside the bundle, but emphasized the transition in size and other characters between it and the xylem. Bernard,[6] though he disagrees with Worsdell with regard to the petiole of *Ginkgo*, agrees with him in the general view that transfusion tissue represents centripetal wood.

So far as is known to the writer, no one but Worsdell has investigated the cotyledons of Gymnosperms exclusively with regard to the origin of transfusion tissue; and it was suggested by Mr. T. G. Hill that such an investigation would be profitable; in addition to this suggestion Mr. Hill supplied the material and has been most generous with his help throughout. The seedlings used were mostly grown at the Chelsea Physic Garden through the kindness of Mr. Hales. Hand sections (transverse and longitudinal) of the cotyledons were made and stained with gentian violet and vesuvian brown; others were treated with phloroglucin and others

[1] P. 100 et seq. [2] Physiologische Anatomie, p. 332.
[3] See reference in text.
[4] The Seedling of *Torreya Myristica*. New Phytologist, vol. ii, 1903.
[5] Loc. cit. [6] Le bois centripète dans les feuilles des Conifères.

with aniline sulphate. Microtomed sections lent by Mr. Hill were also looked through for comparison.

Preparations were made of each of the following species: *Taxus baccata, Cephalotaxus pedunculata, C. Fortunei, Araucaria brasiliensis, Pinus sylvestris, Sequoia sempervirens, Sciadopitys verticillata, Cupressus Lawsoniana, C. tortulosa, Thuja orientalis, T. spheroidea,* and *Juniperus communis.*

Microtomed sections of these and of *Podocarpus chinensis* were also examined.

The work was undertaken without immediate reference to the literature, and the following questions were kept in view throughout:—

1. When very little transfusion tissue is present, e. g. at the tip of the cotyledon, is it on the adaxial side of the bundle or does it form the 'wings' referred to by Haberlandt?

2. Is it ever more abundant on the adaxial side than towards the flanks of the bundle?

3. Is there any proportion to be observed between the amount of centripetal xylem and of transfusion tissue in a cotyledon?

The following observations were made:—

1. *Taxus baccata,* Linn. In some cotyledons no transfusion tissue was found. The earliest beginning of it which was noted was a group of two (soon increasing to three) elements prolonging the incipiently crescent-shaped mass of xylem at one of the horns. At that level no centripetal xylem was present, though a single element in an almost median position occurred lower down.

2. *Cephalotaxus pedunculata,* Sieb. and Zucc. Transfusion tissue begins in the adaxial position. In one case a transfusion tracheide was present before centripetal xylem was formed. It is not contiguous with the protoxylem, and is larger than any of the xylem elements, though there is not the great disproportion in size that is seen in some other genera.

3. *C. Fortunei* differs from the above in having much less transfusion tissue. The beginnings were not observed.

4. *Podocarpus chinensis.* A single microtomed series was available. Transfusion tissue began with a single element of large size similar in position to that of *Taxus,* in both bundles of one cotyledon, and in the other it was also first formed at the side; though the shape of the xylem here was less regular.

5. *Araucaria brasiliensis* proved to be one of the most difficult species to investigate. The transfusion tracheides were not very numerous, were more difficult to distinguish from the xylem than in some other forms, and showed no constancy of position with regard to it. Sometimes two or three arose at the side of the bundle; once at least the first one came on the ventral side, which is unique amongst all the cotyledons examined.

6. *Pinus sylvestris*, L. Not much transfusion tissue was observed. In one case the first transfusion tracheide to appear was quite at the side and level with the metaxylem. In no case was any found distinctly on the ventral side of the strand.

7. *Sequoia sempervirens*, Endl. No adaxial transfusion tracheides were found. About the middle of the cotyledon these elements form a chain leading out from one side of the bundle.

8. *Sciadopitys verticillata*, Sieb. and Zucc. As noted by Hill and de Fraine in their paper 'On the Seedling Structure of Gymnosperms, I',[1] transfusion tissue is here exceptionally abundant. It is also very remarkable for its size and arrangement; it forms two chains leading from the

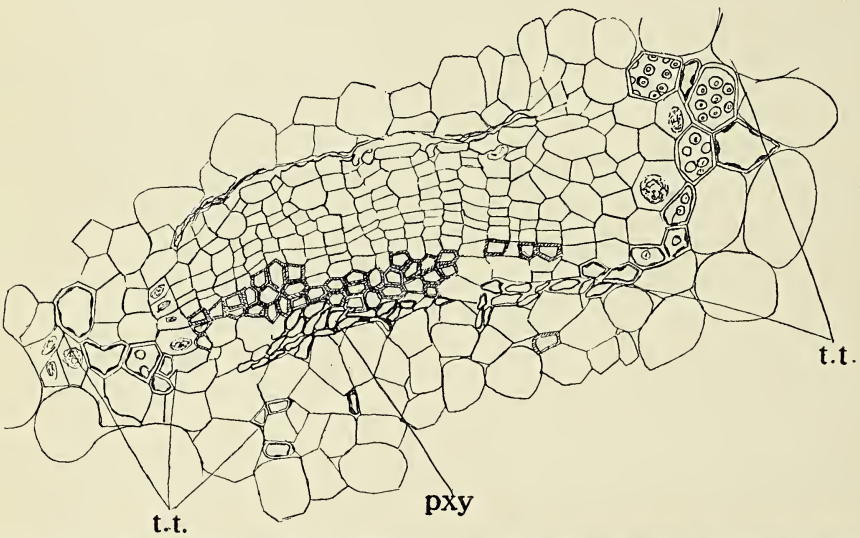

FIG. 1. *Sciadopitys verticillata.* Transverse section of cotyledon near the middle, showing abundant transfusion tissue. *pxy*, protoxylem; *t.t.*, transfusion tissue. In this and succeeding figures, the adaxial side of the leaf is towards the bottom of the page.

flanks of the xylem in the vascular strand and bending round towards the phloem (see Fig. 1). Its arrangement here is very like that seen in the leaves of Conifers, and from its appearance there would be little difficulty in regarding it as modified centripetal xylem. At the tip of the cotyledon, however, a very different state of affairs is found. Here, as was the case with *Torreya*,[2] at the extreme tip, no other sort of vascular tissue is present. A little lower down, where protoxylem is distinguishable, the transfusion tracheides are abundant before any metaxylem appears.

9. *Cupressus Lawsoniana*, Murray. Here transfusion tissue is found very near the tip of the cotyledon. It begins in a lateral position, and is quite typical of many such beginnings (Fig. 2).

[1] Annals of Botany, vol. xxii, p. 708.

[2] Chick, loc. cit.

10. *C. torulosa*, D. Don. These sections showed no transfusion elements, or only one near the side of the bundle.

11. *Thuja orientalis.* This is an interesting subject, as in large cotyledons a good deal of transfusion tissue is present, and it extends

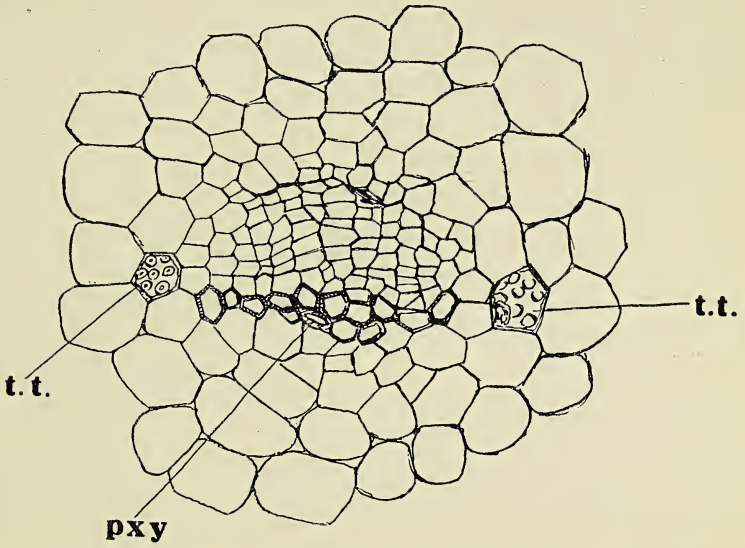

FIG. 2. *Cupressus Lawsoniana.* Transverse section of middle of cotyledon, showing transfusion tissue beginning at the sides of the bundle. *t.t.*, transfusion tissue; *pxy*, protoxylem.

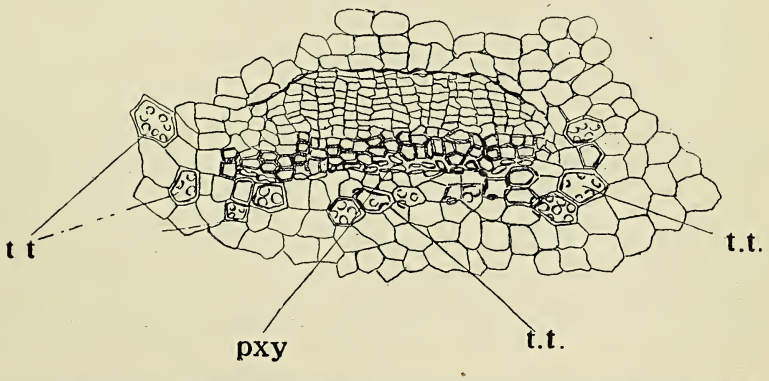

FIG. 3. *Thuja orientalis.* Transverse section of cotyledon from plant six months old, showing much transfusion tissue. *t.t.*, transfusion tissue; *pxy*, protoxylem.

in a more or less complete chain across the adaxial side of the xylem, as well as appearing at the sides (Fig. 3). If this sort of condition only were seen, it would appear very reasonable to suppose that centripetal wood (which was, however, not observed in this genus) had given rise by modification to these tracheides. The beginnings, therefore, were all the more carefully

sought for, but as the individuals examined were about six months old and had their cotyledons well developed, these beginnings were not found. *Thuja orientalis*, var. *aurea*, however, of which younger specimens were available, showed them well, and they were invariably found to be lateral (Fig. 4).

12. *T. spheroidea*, Spreng. The transfusion tissue begins with a single large typical element at the side of the bundle. Such an element is very much bigger than those forming the xylem, and quite unmistakable.

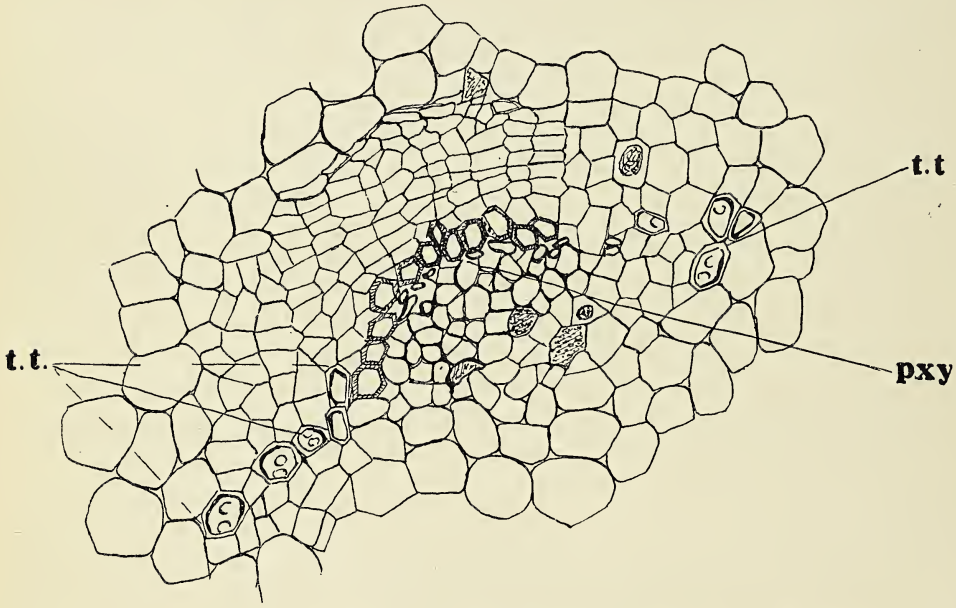

FIG. 4. *Thuja orientalis*, var. *aurea*. Transverse section of cotyledon, showing transfusion tissue arising first at the side of the bundle. *t.t.*, transfusion tissue; *pxy*, protoxylem.

13. *Juniperus communis*, L., had a few transfusion tracheides, though none were seen in the adaxial position.

It is therefore seen that the above questions may be answered as follows:—

1. In no case are the earliest formed tracheides in such a position that they may be regarded as surviving links between a vanished mass of orthodox centripetal xylem and a modification of this tissue which has gradually become more abundant at the sides of the bundle.

2. The transfusion tissue is never more abundant in the adaxial position in the cases examined. Indeed, it is only in *Cephalotaxus* and in *Thuja* that more than one element is found on the ventral side, and in the latter, at any rate, it arises there later than in the lateral position.

3. The relative amounts of centripetal xylem and of transfusion tissue may be summarized thus:—

Genus.	*Amount of centripetal xylem.*	*Amount of transfusion tissue.*
Taxus	Fair amount	Fair amount
Cephalotaxus	More than in other genera	About as in *Taxus*
Podocarpus	None	Fair amount
Araucaria	None	Not always easy to distinguish. It was found by Hill and de Fraine (loc. cit., II)[1]
Pinus	None	Little
Sequoia	None	Fair amount
Sciadopitys	Little	More than in other genera except *Thuja*
Cupressus	Little	Little
Thuja	None	Much
Juniperus	Fair amount	Little

It thus appears that there is no correlation between the amounts of the two tissues. One does not wax as the other wanes. On the other hand the amount of transfusion tissue is greatest in those cotyledons which are largest and which perform assimilatory functions for the longest time unaided by other leaves.

In contradistinction to what has been noted in Chick's paper on *Torreya*,[2] the transfusion tissue of these cotyledons was not more abundant at the tip, except as noted under *Sciadopitys verticillata*. It was at first thought that ease in distinguishing it would be gained by treating the sections with phloroglucin, but the only result that this led to was the conclusion that the transfusion tissue must lignify late, since even when it was distinguishable in a section it did not always take on the red colour. Treatment with aniline sulphate confirmed this opinion. The middle of the cotyledon almost invariably showed most of the tissue.

In addition to the above facts two others emerged with some clearness during the investigation.

1. That the size of the transfusion elements is enormously disproportionate to that of the centripetal wood when present, and also to that of the centrifugal wood. At the same time it is necessary to observe that those nearest to the xylem of the bundle are as a rule smaller than those further out. The transfusion tracheides are largest of all in *Sciadopitys verticillata* (Fig. 1).

2. The transfusion tissue when it appears in the adaxial position never abuts directly on the xylem of the bundle; it is always separated by at least one layer of parenchyma. This seems a point of some importance in view of the transition emphasized by Worsdell; it agrees with the remark of Miss Chick that the transfusion tissue appeared to be developing from the parenchyma.

Although not directly concerned with the question of the origin of

[1] Annals of Botany, vol. xxiii, p. 213. [2] Loc. cit.

transfusion tissue, that of its function is one as to which curiosity would naturally be felt during the course of an examination of it. An attempt to throw some light on this was made by placing living seedlings of various species with their roots in a watery solution of eosin. After standing there one to three days sections of the cotyledons were cut and placed in a mixture of acetic alcohol and glycerine on a slide, and examined at once. The seedlings usually withered badly under this treatment, and when the eosin was replaced by aniline blue the internal tissues were not coloured at all. With those in eosin it was sometimes possible to see, in spite of the rapidity with which they were decolorized, that the transfusion tracheides contained the eosin solution, but this unfortunately was indecisive with regard to the question whether their function is that of conduction or storage, so that the point remained open. The mesophyll was also coloured by the eosin in most cases. Scheit[1] has pointed out that transfusion tissue is much less abundant in the leaves of those species which grow in dry places than of those which have a damper habitat. The short and stumpy form of the tracheides which has been pointed out by all who have described them in leaves, and which is equally noticeable in the cotyledons, seems to suggest storage tissue rather than conducting elements. It is also interesting to note that when transfusion tissue is found in Gymnosperms or elsewhere, it is always in structures exposed to xerophytic conditions.

Summary and Conclusions.

1. Since the distribution and nature of transfusion tissue in the leaves of Conifers make the hypothesis that it owes its origin to a modification of centripetal xylem a tenable one, investigation was made to see whether its point of origin in the cotyledons could throw any light on the matter.

2. It was found that the first-formed transfusion tracheides in the cotyledons examined appeared in such positions and were of such a size as to make it appear improbable that they arose, in these organs at any rate, as an extension of the development of the centripetal wood. A comparison of these tracheides with the other tissues of the vascular strand suggests rather that they develop from the parenchyma.

3. No direct light is thrown by the examination upon the question whether the transfusion tissue serves for conduction or storage, but there is a slight bias in favour of the latter view.

[1] Die Tracheidensäume der Blattbündel der Coniferen. Jenaer Zeitschrift für Naturwissenschaft, 1883.

The Evolution of the Annual Ring and Medullary Rays of Quercus.

BY

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With Plates LXXIV–LXXVI.

THE typical structure of an annual ring of a deciduous oak, as represented, for instance, by *Quercus sessiliflora*, is as follows:—

The inner part of the ring shows a so-called 'pore-zone', formed by numerous large vessels, which lie in a tissue mainly constituted of tracheides, with parenchyma and a few 'fibres' interspersed among these, and numerous medullary rays.[1]

Outside this zone the vessels decrease in calibre, at first suddenly and then gradually, attaining a minimum in the outer zone of the summer-wood ('autumn-wood'). These smaller vessels are ranged in radial series, and are surrounded by rather short, copiously pitted tracheides together with some parenchyma.

At an increasing distance from these vessels the short pitted tracheides ('parenchyma-tracheides') give way, first, to typical elongated tracheides, and secondly to elongated fibro-tracheides, whose walls are very thick and have scanty little pits. (There may be fibres with very few simple pits or devoid of all pits; but I am not convinced of the correctness of statements to that effect.) Traversing the intervasal masses of tracheides and fibro-tracheides are rays, and tangential lines of wood-parenchyma which link the circumvasal parenchyma with the ray-parenchyma. The tracheides and fibro-tracheides are polygonal or rounded in transverse section, and thus may be referred to as 'rounded'. At the outer boundary of the annual ring they are replaced by radially shortened fibro-tracheides whose thick tangential walls have numerous bordered pits, so that the outermost part of the ring is formed of several concentric series of flattened fibro-tracheides. Thus there are four distinct kinds of tracheides, which are connected by intermediate forms: (1) parenchyma-tracheides; (2) ordinary tracheides;

[1] For the sake of brevity hereafter the 'annual ring' and 'medullary ray' will often be referred to under the respective names of 'ring' and 'ray'.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

(3) rounded fibro-tracheides ('fibres') with few and small pits; (4) flattened fibro-tracheides.

The *medullary rays* exhibit themselves in two extreme forms: (1) The *multiseriate* rays are thick, high, gradually tapering wedges, which are several cells in thickness and many cells in height. (2) The *uniseriate* rays are thin, shallow ribbons, one cell in thickness, but several cells in height. Between these extreme forms transitional stages occur in certain parts of the wood.

The wood of other, and especially evergreen, species of *Quercus* may show a widely different type of architecture, so that in some cases the annual ring is unrecognizable by the naked eye and all the rays are thin. The detailed histology also varies in different species, but this matter will not be discussed in the present paper.

Here I propose to consider only the evolution of the annual ring, especially in regard to the vessels and the medullary rays.

Evolution of the Annual Ring.

In some evergreen species of *Quercus* the vessels in the spring-zone of the ring show little or no preponderance in size over those elsewhere, and are separated by wider tangential intervals: moreover, the band of flattened tracheides forming the outer boundary of the ring may become obscure or partially obliterated. Thus, in some evergreen species the ring is widely different from the typical one of deciduous species; and in all evergreen species the vessels are smaller and the pore-zone less marked than those of at least allied deciduous species.

To ascertain in which direction the evolution of the annual ring has moved, it is obviously necessary to know whether the ancestral type of *Quercus* was evergreen or deciduous. Thus the course of evolution may have been :—

1. Regressive from a typical ring with a marked pore-zone.
2. Divergent from a ring with a feebly marked pore-zone.
3. Progressive from a ring with no indication of a pore-zone.

Without subscribing to the opinion that the first has been the direction of evolution of the obscure type of ring, I will describe the series of that assumption; this course will have the advantage that the first stage will thus be the most familiar.

The annual ring in *Quercus* may be identified by the presence of one or more of the following structural features :—

1. A marked 'pore-zone' is seen in the inner zone ('spring-wood') of the ring, because here the vessels are widest.

2. The vessels near the outer boundary of the ring are of the minimum diameter.

3. The fibro-tracheides forming the outer boundary of the ring are flattened and copiously pitted on their tangential walls.

4. The outline of the ring is continued across the broad medullary rays, and usually dips inward in the ray.

5. The cells of the uniseriate rays at the boundary of the ring are shortened, have their terminal (tangential) walls specially thickened, and contain more abundant coloured contents. Less clearly the same is often the case in the multiseriate rays.

6. The parenchyma near the outer part of the ring contains more abundant coloured contents.

7. When the tangential bands of parenchyma are multiseriate in the middle parts of the ring, they may be thinner and even uniseriate in the outer part.

All or any of these distinctive features may be wholly or locally wanting. Yet by applying these tests I have found no species of *Quercus* devoid of annual rings, or at least of growth-rings which may or may not represent annual increments of growth. Abromeit ('84) fully appreciated the first three methods here enumerated of identifying rings, and by their means showed that in most evergreen types annual rings obscure to the naked eye are recognizable with the aid of the microscope. He stated that rings often cannot be detected in the cases of *Q. dilatata*, *Q. lamellosa*, and *Q. chrysolepis*. I find annual rings can be traced in the last two though the outline may be obscure locally; *Q. dilatata* I have not examined.

A. Deciduous Types. Class I.

Typical deciduous species differ in the exact structure of their pore-zone of wide vessels, as regards the tangential distance apart of the vessels and the number of concentric series of wide vessels present; but the latter feature varies with the environment in one and the same species. The manner in which the vessels outside this zone decrease in size also varies in different species, the transition being more or less gradual or sudden.

The following Table briefly indicates the nature of the pore-zone. The terms 'ordinary', 'moderate', 'loose', 'very loose' refer to the tangential distance apart of the vessels; their tangential distance apart is measured (merely by inspection) in diameters of the vessels. The suffix 'seriate' with the prefixed figures denotes the number of concentric layers of wide spring vessels, while the terms 'sudden' or 'gradual' describe the mode of transition from these wide vessels to those outside them by decrease in size.

These statistics show that even in deciduous species the pore-zone may be loose by reason of the tangential separation of the vessels. It may be added that in deciduous species, *Q. rubra* for instance, the first-formed vessel in the spring-zone may be smaller than some vessels immediately succeeding

on or near the same radius. Both these features foreshadow more marked ones in evergreen types.

PORE-ZONE.

Species.	*Density.*	*Distance apart of vessels.*	*Number of concentric series.*	*Transition from wide to narrow vessels is:—*
Q. lobata, Née	moderate	0–1 diam.	4-seriate	gradual
Q. rubra, Linn.	ordinary	½–1 „	4–5-seriate	moderate to sudden
Q. obtusiloba, Michx.	moderate	½–1 „	2(–4-) seriate	sudden
Q. coccinea, Wangenh.	moderate	0–1 „	(1-)2(–3-) seriate	sudden
Q. garryana, Dougl.	moderate	½–1½ „	1–4-seriate	gradual
Q. Prinus, Linn.	moderate	0–2 „	1–2-seriate	sudden
Q. macrocarpa, Michx.	loose	0–2 „	(1–)2(–3-) seriate	rather sudden
Q. californica (*Q. Kelloggii*, Newb. ?)	loose	1–2 „	1–3-seriate	gradual
Q. Macdonaldi, Greene	very loose	1–4 „	multiseriate	very gradual

B. Evergreen Types.

The annual rings of evergreen species show all transitions from the preceding (deciduous) type, to that in which the vessels near the inner and outer boundaries of the ring are smaller than those nearer the middle of the ring. Hereafter, I will refer to the annual ring as being composed of an 'inner', a 'middle', and an 'outer' zone, though the three zones are not sharply delimited.

Class II. Sub-evergreen or Sub-deciduous.

Some evergreen species of *Quercus* possess annual rings marked by a pore-zone and by a decrease in the calibre of the vessels in an outward direction until a minimum is attained in the outer zone of the ring. The main difference between such types of rings and those of typical deciduous species lies in the absolutely smaller calibre of the widest vessels. In this type of annual ring the limiting zone of flattened tracheides is well marked. The evergreen species of *Quercus* showing this structure of the annual ring approaching closely that of deciduous species also approach the latter in duration of their foliage, and hence may be termed sub-evergreen or sub-deciduous, for they shed all their foliage gradually during winter (*Q. dumosa*, Nutt), or throw off all their older leaves in spring either immediately before (*Q. arizonica*, Sarg.), together with (*Q. Emoryi*, Torrey), or immediately after (*Q. hypoleuca*, Engelm), the unfolding of the new leaves.[1]

In two of these species interesting deviations in the annual rings present themselves. *Q. hypoleuca* (Pl. LXXIV, Fig. 1) in addition to its ordinary rings has others that are much narrower, are devoid of wide vessels, and show a maximum calibre of vessel in the middle zone. In *Q. Emoryi* (Fig. 2), a still narrower ring rather feebly delimited from its immediate

[1] For particulars concerning the defoliation I rely upon Sargent's 'Manual of the Trees of North America'.

and normal predecessor may occur, and at first sight presents the appearance of being merely an extension of the outer zone of this, as all its vessels are small. The question arises in these and other cases as to whether these narrow structures are true annual rings formed during an unfavourable season, or merely growth-rings representing a 'doubling' of the annual ring. It would be interesting if they were autumn- or winter-rings in these two species, which grow on mountains in Arizona and adjoining regions.

Class III. **Transitional Sub-evergreen and Evergreen.**

A number of sub-evergreen and evergreen species of *Quercus* possess wood whose rings represent transitions from the condition of distinctness to that of absence of the pore-zone in the spring-wood.

Q. chrysolepis, Liebm. (Fig. 3), is an evergreen species, the leaves of which live for three or four years. The ring often shows a general gradual decrease in the calibre of the vessels from the inner to the outer boundary, and in such cases the pore-zone is indicated by wide vessels, which are often of the maximum diameter and contrast with the outermost of the immediately preceding ring. Nevertheless, in the inmost zone of the ring narrow vessels also occur, while in the middle zone there are vessels of various sizes, and the contrast between the inner and the outer halves of the ring is not so great as in Class II. The ring is delimited by a layer of flattened tracheides.

In *Q.* (*Pasania*) *densiflora*, Hook. et Arn. (Fig. 4), an evergreen species whose leaves live for three or four years, and *Q. Engelmanni*, Greene, whose old leaves are all shed as the new ones appear in the spring, the ring is at about the same stage as in *Q. chrysolepis*, though the layer of flattened fibro-tracheides is more distinctly marked. But in *Q. densiflora* the somewhat feebly indicated pore-zone is much looser.

The sub-evergreen *Q. virginiana*, Mill., has rings marked by a pore-zone which is represented by widely separated vessels of maximum size contrasting with those of the outer zone of the ring immediately within them (Abromeit ('84) noted the occasional presence of widest vessels in the spring-zone). But frequently here and there in the annual ring a vessel in the inmost zone is smaller than those farther out in the same radial series, so that there is already a tendency for the maximum calibre of vessel to be transferred to the middle zone. The layer of flattened tracheides is less marked than in *Q. chrysolepis*. *Q. virginiana* also possesses narrow rings whose vessels are all small; their nature is as unknown as in the similar cases of *Q. hypoleuca* and *Q. Emoryi*.

In *Q. agrifolia*, Née (Fig. 6), whose old leaves all fall during winter and early spring, and *Q. tomentella*, Engelm (Fig. 7), whose leaves live for two or three years, the pore-zone is denoted by a very loose single concentric series of isolated wide vessels, which are often of the maximum calibre

and contrast with the outermost ones of the immediately preceding ring. The remaining part of the ring is rather uniform as regards the size of its vessels, for larger and smaller ones are distributed apparently indifferently. In both species a layer of flattened fibro-tracheides marks the outer boundary of the ring.

In all the species so far described in the three classes the limiting layer of flattened tracheides is visible and continuous. But in the next two types this layer becomes here and there obscure, because the flattening of the fibro-tracheides ceases over certain tangential distances.

In contrast with all the species so far dealt with—which are American—*Q. semecarpifolia*, Sm. (Pl. LXXIV, Fig. 8), is Indian. It is apparently sub-evergreen, as Gamble describes it as 'evergreen', while Brandis states that it is 'sometimes leafless for a few weeks in spring'. The pore-zone in the spring wood is indicated by widely separated vessels that are often the largest in the ring and nearly always larger than the outermost ones of the immediately preceding ring. Yet in some of the radial series of a ring the widest vessels lie in the middle zone. The limiting band of flattened tracheides is interrupted only for short distances, and even at these places the boundary of the ring is recognizable by reason of the shorter length of the ray-cells, their thicker terminal walls, and their richer contents.

Class IV. **Evergreen.**

In Class III there does not appear to be any parallelism between the longevity of the leaves and the distinctness of the pore-zone, for the transitional types of wood belong to evergreen and sub-evergreen types. Yet the Indian species, which is sub-evergreen, shows more definite tendencies towards the production of a pore-zone than do the four truly evergreen Indian species about to be described, for these reveal, coupled with the absence of a pore-zone, a frequent transference of the maximum diameter of vessel to the middle zone of the ring, or show no recognizable relation between diameter and position of the vessels in the ring.

Q. glauca, Thunb. (Pl. LXXV, Fig. 9), and *Q. lamellosa*, Sm. (Fig. 10), have rings that are recognizable by reason of the bounding line of flattened tracheides. Frequently the vessels in the outermost part of the ring are distinctly smaller than their predecessors, but apart from this there appears to be no rhythmic succession in the ring as regards calibre of vessel. In both species the layer of flattened fibro-tracheides is never very distinct; and in places the fibro-tracheides forming the boundary of the ring have not the flattened form (as was noted by Abromeit in *Q. lamellosa*); yet, especially in *Q. glauca*, these bounding fibro-tracheides show a greater number of bordered pits than do the fibro-tracheides immediately preceding them.

The remarks made in reference to these two species also apply to the rings of *Q. incana*, Roxb. (Fig. 11), in which, however, the partial

eclipse of the bounding line of tracheides is more complete. Thus *Q. incana* is the species with the most indistinct rings.

But the most remarkable ring is that of *Q.* (*Pasania*) *fenestrata*, Roxb. (Fig. 12), in which the vessels show no detectable distinctions in size that are associated with their position in the ring. The outer boundary of the ring in most places is clearly defined by a line of flattened fibro-tracheides, though the line becomes obscure here and there. This ring, however, presents the appearance of being broken up into separate segments; for here and there are to be seen pairs of larger medullary rays which are separated by a mass of tissue that is devoid of vessels, and in this separating tissue the tangential line of flattened tracheides marking the boundary of the ring is so much nearer the centre of the stem that it presents the appearance of being continuous with the middle zone of the ring on the other sides of the rays. This peculiar apparent disruption of the ring will be explained later in the present paper.

Comparison of the different types.

The annual rings of typical deciduous species are marked by three features:—

1. Presence of large vessels contrasting greatly with the smallest vessels.
2. The concentration of the largest vessels in the spring zone.
3. The approximation of the vessels in the spring-zone to form concentric series.

In evergreen species there is a tendency for decreased size of the vessels, decreased difference in the sizes of the vessels in the ring, centrifugal transference of the maximum size of vessel to the middle of the ring or irregular distribution of the wider vessels in the ring, and finally increased tangential separation of the vessels in the spring-zone.

In order to give a statistical basis to my observations I made a number of measurements of the diameters of the vessels and of the tangential distances apart of the vessels in the inner (spring) zone. Having already arranged the species in order according to distinctness of the pore-zone as judged by inspection, I tested this order by means of the measurements made, and was able to form some idea as to the extent to which the various changes in the arrangements, sizes, and number of vessels move along parallel lines in evolution.

The measurements are given in the succeeding table, all being given in millimetres representing magnifications of twenty-five diameters. The diameters of the vessels were obtained by taking the mean of two diameters at right angles.

Commencing at the left-hand side, the first column gives the name of the species and its habit. Evergreen (E.), sub-evergreen (SE.); the letters

				Vessels in spring (inner) zone.					*Vessels in outer zone.*			*Max. Diam. of vessel in midd. zone. M*	$\frac{Y}{M}$	$\frac{V}{v}$	*Order of succession according to—*					
				Mean Diam. V	*Max. Diam. Y*	*Max. Dist. apart tang. D*	$\frac{D}{V}$	*Min. Diam.*	*Mean Diam. v*	*Min. Diam.*	*Max. Diam.*				$\frac{V}{v}$ *sm.*	$\frac{V}{v}$ *lar.*	$\frac{D}{V}$ *sm.*	$\frac{D}{V}$ *lar.*	$\frac{Y}{M}$ *sm.*	$\frac{Y}{M}$ *lar.*
(1)	SE.	*Q. arizonica*		5·5	8·25	15	2·7	1·5	2						2·75	3	5	5–6	2	1–4	1–4
(2)	SE.	*Q. hypoleuca*	A	1·9	2·5	16·5	8·7	1·5	1·4	1	2				(1·36)						
		,,	B	6	8·25	12·5*	2·1	3	1·6	1	2				3·75	7	4	2	9–10	1–4	1–4
(3)	SE.	*Q. dumosa*	A	3·1	4·5	26·5	8·5	1·75	1·1	1	1·5	3	1·5		(2·8)	2	3	7	8	1–4	1–4
		,,	B	4·3	5·75	14	3·3	1·75	1·1	1	1·5				4						
(4)	SE.	*Q. Emoryi*	A	6·6	7	7·5	1·1	6·25	2	2	2				(3·3)	1	2	1	1	1–4	1–4
		,,	B	7·15	7·75	9	1·4	5	1·75	1·5	2				4·1						
(5)	E.	*Q. chrysolepis*	A	5	6·25	14·5*	2·9	2·25	2·66	1	3·5	6·75	·93		1·9	5	8	3–4	3	7	5
		,,	B	3·8	5·5	9·5	2·5	1	2·25	1·75	3	4·5	1·2		1·7						
(6)	E.	*Q. densiflora*	A	4·8	6	22·5	4·7	3·75	3·1	1·75	4	5·5	1·09		(1·5)						
		,,	B	6·4	7·75	17·5	2·7	4·75	2·4	1	3·75				2·6	6	6	5–6	4	5	7
		,,	C	6·4	8·25	17·5	3	3·5	2·5	1·25	3·5				2·6						
(7)	SE.	*Q. Engelmanni*	A	1·6	2	12	7·5	1·5	1·9	1·5		6·25	·32		(·8)	12–13	1	3–4	7	11	6
		,,	B	6	7	15	2·5	4·75	1·3	1		6	1·17		4·6						
(8)	SE.	*Q. virginiana*	A	4·75	6·25	31*	6·5	3	4	3·75	4·25	7	·89		1·19						
		,,	B	3		36·5*	12·2		3·75						·8	12–13	12	11	12	8	11
		,,	C	7·2	8·5	39*	5·4	6·75													
(9)	SE.	*Q. agrifolia*	A						1·75	1·5											
		,,	B	5·4	5·5	30	5·5	5·25	4	3·75		5·25	1·05		1·35	8–9	10	9	6	9	9–10
		,,	C	4·8	5·25	22·5	4·7	4·25				7	·75								
(10)	E.	*Q. tomentella*		4·9	5·5	43	8·8	4·5	2·63	1·25	3·5					4	9	13	11		
(11)		*Q. glauca*		6·3	8	30	4·8		4·75	3		7·5	1·06		1·3	10	11	10	5	6	8
(12)		*Q. lamellosa*	A	2·25	2·75	14	6·2	1·75	2·75	2·5		5·5	·5		·8	12–13	13	12	13	12	12
		,,	B	1	1	24	24	1	absent	absent		5·5	·17								
(13)		*Q. incana*	A	4·5	5·5	47·5	8·7		2·3	1·5		7·5	·73		2	11	7	8	9–10	10	9–10
		,,	B	5	5·75	21	3·7		4·6	3·5		5·5	1·05		(1·05)						
		1		2	3	4	5	6	7	8	9	10	11		12	13	14	15	16	17	18

A, B, C, refer to different annual rings of one specimen; the second to the sixth columns refer to the spring-zone and record respectively the mean diameter (*V*) of the innermost vessels, their maximum diameter (*V*), their maximum tangential distance (*D*) apart (or those marked with an asterisk the maximum tangential distance from a large medullary ray), the maximum distance apart (*D*) divided by the mean diameter (*V*) of the vessels, and the minimum diameter of the vessels; the seventh to the ninth columns refer to the outer zone of the annual ring and record respectively the mean (*v*), minimum, and maximum diameters of the vessels; the tenth column gives the maximum diameter (*M*) of vessels in the middle zone; the eleventh column gives the maximum diameter (*V*) of vessel in the spring-zone divided by the maximum diameter (*M*) of vessel in the middle zone; the twelfth column gives the mean diameter (*V*) of the vessels in the spring-zone divided by that (*v*) of the vessels in the outer zone.

The thirteenth to eighteenth columns give the resultant order as calculated respectively by the columns 12 (small measurements), 12 (large measurements), 5 (small measurements), 5 (large measurements). Measurements in reference to *Q. semecarpifolia*, *Q. fenestrata* are not given, and are incomplete in the case of *Q. tomentella*.

An examination of columns 1 and 13 to 18 show that the order of succession of the species when judged by the different criteria does not exactly agree. This is no doubt partly due to the limited number of observations, but it is probably also an indication that the various kinds of changes have not travelled along parallel lines in evolution. Yet when the last six columns are averaged the order attained agrees largely with that assigned by inspection (the greatest exception being *Q. virginiana*).

Name of species and habit.	*Order when judged:—* *By inspection.*	*By columns 13–16.*	*By columns 13–18.*	*By columns 16–18.*
SE. *Q. Emoryi*	1–4	1	1 (1–6)	1 (1–5)
SE. *Q. arizonica*	1–4	2	2 (1–6)	2 (1–5)
SE. *Q. dumosa*	1–4	4	3 (1–6)	3 (1–5)
SE. *Q. hypoleuca*	1–4	6	4 (1–6)	4 (1–5)
E. *Q. chrysolepis* [1]	5	3	5 (1–6)	5 (1–5)
E. *Q. densiflora*	6	5	6 (1–6)	6
SE. *Q. Engelmanni*	7	7	7	8
SE. *Q. virginiana* [1]	8	12 (13)	11 (12)	11 (12)
SE. *Q. agrifolia*	9	8	9	9
E. *Q. tomentella*	(11)	(11)	—	—
SE. *Q. semecarpifolia*	(10)	—	—	—
E. *Q. glauca*	11	10	8	7
E. *Q. lamellosa*	12	13 (14)	12 (13)	12 (13)
E. *Q. incana*	13	9	10 (11)	10 (11)

[1] The anomalous position of the evergreen *Q. chrysolepis* and the sub-evergreen *Q. virginiana* would vanish if, as I cannot help suggesting, the wood specimens of the two had been inadvertently interchanged in Professor Sargent's collection or before reaching me.

The indistinctness of the annual ring in certain evergreen oaks, so far as it is due to lack of pore-zone, has long been known and is specifically mentioned by Sanio ('63) in his description of the wood of *Q. Ilex*. Abromeit (loc. cit.) did not, however, fully realize the close connexion between leafing habit and existence of a pore-zone, because he was misled as to the habit of certain species. For he states that of the species examined by him only in the following evergreen species is the spring-wood marked by wider vessels: *Q. Wislizeni*, *Q. castaneaefolia*, *Q. glandulifera*, *Q. serrata*, and to some extent *Q. cuspidata* and *Q. agrifolia*. This list requires correction. On the one hand *Q. serrata* is not evergreen, and on the other hand *Q. hypoleuca* is evergreen yet possesses a distinct pore-zone; and curiously Abromeit in the special part of his paper mentions both these facts correctly.

Evolution of the Medullary Rays.

The wood of all species of *Quercus* has uniseriate, thin, shallow rays, but that of only some species possesses in addition multiseriate, thick, high rays. It is possible that the ancestral types of *Quercus* belonged to the thick-rayed or to the thin-rayed type. I propose to trace the series of types on the momentary assumption that the line of evolution has been from the type with broad high rays to that with exclusively narrow uniseriate ones, not because I adopt this view, but because the broad-rayed type of *Quercus* is the familiar one, and because in the solitary type that appears to point very strongly in one direction, that direction is from the broad-rayed to the narrow-rayed type.

In some types, *Q. Macdonaldi* (Pl. LXXV, Fig. 13) for instance, the broad rays are high multiseriate plates of parenchyma, showing, in the tangential section, deep vertical expanses of ray-parenchyma devoid of any fibres.

In other types fibres run in the broad ray in various directions. These subdivided rays may be ranged into two classes, according to the main direction of the fibres:—

(*a*) *Fibres transverse or transversely oblique*, forming in tangential section lines or bands that are uniseriate, biseriate, triseriate, up to multiseriate. With increased thickness of the bands of dividing fibres a climax is reached when in tangential section there is a series of obliquely or vertically superposed rays more or less fusiform, so that it becomes difficult to decide whether to describe the series as a single divided ray or as a number of superposed rays.

(*b*) *Fibres vertical or vertically oblique*, leading finally to the division of the broad ray into a series of small rays arranged side by side.

The broad rays often show transverse, vertical, and intermediate dividing fibres, but usually one type of division is more prominent.

I will first trace out a series in which the *transverse* division by fibres is increasingly marked. The following descriptions refer to the tangential view of the rays, and describe only certain of the several types of rays occurring in each species.

Series I (Deciduous with high rays).

Q. Macdonaldi (Fig. 13): ray very high and broad.

Q. Durandii, Buckl.: ray very high, crossed at long intervals by thin interrupted transverse lines of fibres.

Q. aquatica, Walt.: ray very high, thinner than those of *Q. Durandii*, divided by transversely oblique bands of fibres. The bands are separated by very long or by shorter intervals; they are thin, often biseriate, though sometimes they consist of more than two ranks of fibres and are then 'frayed', that is to say, they include gaps which are occupied by ray-parenchyma.

Q. acuminata, Sarg.[1] (Fig. 14): rays at about the same stage as in *Q. aquatica*, but vertically superposed rays occur here and there.

Q. bicolor, Willd. (Fig. 15), and *Q. garryana*, Dougl., have high vertical rays, as well as vertical series of rather broad rays, which are separated from one another by thinner or thicker bands of transversely oblique fibres, so that in the latter case it is difficult to decide whether the ray should be described as divided or the rays as superposed.

Series II (Deciduous with slender fusiform rays).

The high rather slender fusiform rays seen in places in *Q. bicolor* and *Q. garryana* lead to the high somewhat plumper ones of *Q. sessiliflora*, Salisb. (Fig. 15), some of which are feebly divided by a few fibres. At about the same stage are the rays of *Q. heterophylla*, Michx., and some in *Q. undulata*, Torr., but the latter also shows rays divided at intervals by thinner or thicker bands of transversely oblique fibres. *Q. georgiana*, M. A. Curt. (Fig. 17), has true undivided narrow fusiform rays which are often ranged in vertical series, the successive rays being separated by solid or frayed bands of fibres.

Series III (Deciduous with broader fusiform rays).

Series II with rather slender fusiform rays is paralleled by another series with broader fusiform rays. These are undivided in *Q. Kelloggii* (cp. Pl. LXXVI, Fig. 19), but are feebly divided by occasional transversely oblique thin bands of fibres in *Q. texana*, Buckl., and *Q. Douglasii*, Hook. et Arn. (Pl. LXXV, Fig. 18), both of which also show vertical or slightly obliquely vertical series of close-set superposed rays. The difficulty of distinguishing descriptively between a divided ray and a series of superposed

[1] I do not know what the correct name of this American oak should be, as the true *Q. acuminata* is an Indian species described by Roxburgh.

rays reaches a climax in *Q. serrata*, Thunb., which has high columns of ray tissue divided at moderate intervals by fibres ranged into bands which vary from thin biseriate lines to thick multiseriate frayed or solid masses.

So far all the species whose rays have been described are deciduous. They all possess well-developed multiseriate rays, which, however, differ considerably in height and in degree of division (or aggregation).

Series IV (Evergreen).

In tracing out the series in evergreen species it is of interest to consider the extent to which distinctness of pore-zone and subdivision of the broad ray go side by side. The result of the inquiry is to demonstrate that in the first ten species as arranged in order in the second Table there is an unmistakable correspondence between the structure of annual ring and medullary ray, and that only two suggestive exceptions occur. To render this clear, in the succeeding paragraphs the figures denoting the order of succession in the Table are given in brackets after the name of each species.

As regards broad rays, the simplest occur in three species that are also in the first four in the Table, namely, *Q. Emoryi*, Torr. (1–4), *Q. arizonica*, Sarg. (1–4), and *Q. hypoleuca*, Engelm (1–4). The first of these (Pl. LXXVI, Fig. 19) has high true undivided rays, also high fusiform rays divided here and there solely at the ends by obliquely transverse fibres. *Q. arizonica* (Fig. 20) possesses plump true fusiform rays, as has *Q. hypoleuca* (Fig. 21), some of whose rays are traversed by infrequent transverse uniseriate lines of fibres. In *Q. hypoleuca* fusiform rays are superposed to form vertical series, the individual rays being separated by thick strands of fibres, also occasionally by a vessel. This species may show a few vertical fibres projecting into the ray from the margin.

Q. dumosa (1–4), which is among the first four species according to structure of ring, is fourth as regards ray. The rays are smaller than in the three other species, fusiform, undivided or divided near the ends by oblique uniseriate lines of fibres. In addition, there are still smaller superposed fusiform rays that show more extensive subdivision.

Q. Engelmanni, Greene (7) (Fig. 22), possesses rays that are divided by transverse, oblique, and curling fibrous partitions.

Q. chrysolepis, Liebm. (5) (Fig. 23), has rays about as thick as those of *Q. Engelmanni*, divided by obliquely transverse biseriate bands of fibres and penetrated by a few vertical marginal fibres.

In *Q. agrifolia* (9) some of the rays (Fig. 24 *a*) are broader than those of the two species just described; these are divided by nearly transverse thick bands of fibres into shallow superposed segments (or rays). In addition there occur narrower rays (Fig. 24 *b*) that are divided by transverse or oblique thinner strands of fibres, which become so

numerous near the ends of the ray as to dissect it into numerous small fusiform rays: in addition, vertical marginal fibres extend into the ray.

From this point the next two types seem to diverge along different lines.

Q. virginiana (8) (Fig. 25) has broad rays more divided than in any of the species so far described; they are transversely crossed by thick frayed bands of fibres, which differentiate the ray into superposed segments. The segments in turn are thoroughly permeated by a complex system of thin oblique or vertical lines of fibres.

In *Q. tomentella* (11) (Fig. 26) the rays are very much narrower than any of those so far described, and are repeatedly divided by uniseriate to multiseriate bands of fibres into shallow, slender, oblique fusiform segments (or rays).

In the series of rays so far given *Q. virginiana* stands out as the most dissected type as regards broad rays, and this position in the Series IV agrees with that as indicated by the statistics provided in the second Table, though not so closely with the position as judged by mere inspection of the distinctness of the pore-zone.

But when *Q. densiflora* (6) (Fig. 29) is considered, the correspondence between indistinctness of pore-zone and subdivision of the ray vanishes, for the broad ray in this species is repeatedly divided by numerous *vertical* fibres and shows an excellent series of stages of vertical subdivision of the ray. The meaning of this exception becomes clear when it is noted that the species belongs to an entirely different section (*eu-Pasania*) or genus (*Pasania*), and the anomaly vanishes when it is compared with the other species belonging to the same section or genus, namely, *Q. fenestrata*, which will be discussed later in this paper. Thus genetic affinity as well as habit influence the structure of the broad rays.

Yet when the remaining species are considered, namely, *Q. semecarpifolia*, *Q. glauca* and *Q. lamellosa*, and *Q. incana*, the correspondence between division of the ray and indistinctness of annual ring ceases.

From these, *Q. lamellosa* (12) and *Q. glauca* (11) might be excluded, because they do not, like the first series of forms, belong to the section *Lepidobalanus* or *Erythrobalanus* but to *Cyclobalanus*. The rays of *Q. lamellosa* (Fig. 29) are broad, show transverse frayed partitions formed by several series of fibres, and are penetrated at their margins by occasional thin vertical strands of fibres: they are thus at about the same stage of subdivision as those of *Q. hypoleuca* or the less divided ones of *Q. agrifolia*. *Q. glauca* (Fig. 29) possesses rays shallower than those of *Q. lamellosa*, and these are divided by thicker closed bands of fibres into segments that are here and there crossed by isolated strands of fibres, so that the rays are comparable with the more divided kinds of *Q. agrifolia*.

But it is *Q. semecarpifolia* (Fig. 30) that destroys parallel sequence of

ray and annual ring, although it belongs to the section *Lepidobalanus* ; for its broad rays are moderately high, yet slender fusiform structures, which are divided near the ends by obliquely vertical lines of fibres into more or less separate little rays, and at a greater distance from the ends are undivided or deeply penetrated by obliquely vertical thin lines of fibres. Moreover, several complex rays of this kind may be almost vertically superposed, being separated from one another by only a narrow strand of fibres. Thus in this species the rays are more divided than are those of *Q. lamellosa.*

In *Q. incana* (Pl. LXXVI, Fig. 31 *a*) some of the rays are broad and high, and repeatedly divided by many thin vertical, uniseriate or biseriate strands of fibres, so that they recall the rays of *Q.* (*Pasania*) *densiflora.* Other broad rays of this species (Fig. 31 *b*) are slightly thinner and, while traversed by less numerous vertical fibres, have oblique, frayed or closed bands and uniseriate lines of fibres dividing the ray more or less into superposed segments.

Though there is a general correspondence between the condition of the rays and of the annual ring in the different species of *Quercus*, and especially the American species, there are some deviations from this tendency. These deviations may be due to :—

1. The fact that the broad rays in one and the same species differ in the same annual ring, and in the different annual rings. A single broad ray in a ring may be represented farther inwards by a complex of separate rays, as will be explained later ; and possibly in some cases the reverse may be true. Thus, in comparing different species of *Quercus* by this method, equal-aged annual rings should be selected for observation.

2. Difference of climate may in part be indirectly or directly responsible for the differences between the rays of the American and Indian species of *Quercus* that are at the same stage as regards duration of leaves or structure of annual ring.

3. The sections *Lepidobalanus* and so forth may represent not natural affinities but collections of relatively non-allied types converging as regards structure of fruit. This suggestion, however, is not supported by such anatomical evidence as we possess regarding the structure of wood, for Abromeit (loc. cit.) showed that in the 'White Oaks' (*Leucobalanus*, a division of *Lepidobalanus*) the walls of the vessels are thin, while in the 'Black Oaks' (*Melanobalanus*, or a division of *Erythrobalanus*) the walls of the vessels are thick.

The question now arises as to the direction in which the evolution of the rays has taken place in *Quercus* and in the Fagales, that is, whether the archetype possessed broad rays or only narrow ones. As it is possible that evolution can take place in both directions, the answer may be different in the case of the genus and the cohort.

There are three possibilities, namely, that in addition to the narrow uniseriate rays the archetype had :—

1. Rays broad and high from their commencement (including broad primary rays).

2. Exclusively narrow rays in the inner annual rings, and broad rays farther from the centre,

3. No multiseriate rays.

In either of the first two cases broad rays have given way to narrow ones in some types of *Quercus*.

The condition of the rays in the two families of the Fagales gives no reliable clue to the primitive state, for in each family there are broad-rayed (*Quercus*, *Fagus*, *Alnus*) as well as narrow-rayed (*Castanea*, *Castanopsis*, *Alnus*) types. It is thus evident that whichever direction evolution has followed, the change has taken place independently in both families, and in both families transitional rays occur ; for instance, in *Quercus*, *Alnus*, *Carpinus*, and *Corylus*. In *Castanea vesca* the rays are shallow and uniseriate. In *Fagus sylvatica*, in addition to uniseriate rays, there are more or less undivided or divided multiseriate, as well as grouped or isolated small rays varying from uniseriate linear to pluriseriate fusiform (2–15 cells thick). In *Alnus glutinosa*, besides the numerous uniseriate rays, there are ' false rays ' composed of densely aggregated largely uniseriate, but also biseriate rays. In *Carpinus Betulus* the individual rays are thicker than in *Alnus glutinosa*, and vary from uniseriate to thin fusiform rays, which often have uniseriate ends and a median fusiform portion 2–3 cells thick : there are also rays that are 2–3 cells thick near their ends, but uniseriate in the middle, and thus represent two superposed fused rays (or one that has not yet divided) : the ' false rays ' are aggregates of such rays. In *Corylus Avellana* both the individual and ' false rays ' are thicker in turn than in *Carpinus Betulus*, the biseriate and triseriate being more numerous relatively and being supplemented by quinqueseriate rays. In *Betula alba* biseriate and triseriate fusiform rays are abundant.

If we assume, as seems most probable, that the Fagales represents a reduction-series as regards floral characters, and that *Castanea* is in this respect the most primitive of the cohort, it would seem not unlikely that the wood of *Castanea* should also represent the primitive condition in the cohort. Now, *Castanea* has narrow rays : in *Castanopsis indica* the abundant uniseriate rays are supplemented by occasional groups of small fusiform (up to 5-seriate) rays, which are ranged in tall vertical bands that agree structurally with the ' vertically divided broad rays ' of (*Pasania* and) *Quercus*, and the wood of the latter shows many stages that may be interpreted as denoting a compounding of fine rays to form broad ones. (*En passant*, it may be remarked that if in the ancestral *Quercus* the rays were all narrow, then if we are to judge by modern types this archetype

was evergreen. But the existence of deciduous species of *Castanea* with solely narrow rays prevents us from fully relying on such a judgement. The change from or to an evergreen condition appears easy in the family, since it has occurred independently in three genera—*Castanea*, *Nothofagus*, and *Quercus*.)

The view that the primitive condition in *Quercus* (and *Alnus*) was that in which only narrow rays existed has been strongly supported by Bailey ('10 (1), '10 (2)) and Eames ('10) in a number of papers. Their view is that the broad high rays have become established by a compounding process. The evidence upon which they rely is geological, ontogenetic, and pathological. The geological evidence based upon the structure of the wood of a few isolated specimens showing 'false rays' (i. e. many fine rays closely aggregated), though suggestive, is not convincing, as the species examined were too few. Very interesting on the other hand is Eames's discovery that in seedling oaks all the rays are narrow, and that later in life, in the later-formed rings, there is a more or less gradual 'compounding, that is, grouping and fusion' of the narrow rays, until eventually the group of rays is represented by a single broad high parenchymatous ray, more or less free from interrupting fibres. Eames regards this evidence as important from the standpoint that 'the seedling is known to be a seat of ancestral characters'. And Bailey has found the same kind of phenomenon in certain species of *Alnus*, in which the inner annual rings of the seedling have merely evenly distributed uniseriate rays, while annual rings farther out possess aggregated rays. From the point of view of the assumption that the seedling is a seat of phylogenetically early structure features, these facts lose their significance if it be shown that a similar linking up of small rays to form large ones takes place in older parts. I find that such a linking up does take place, at least in connexion with some of the rays of the inner rings of the twigs of *Quercus Robur*. Again, in *Quercus Robur*, apart from the broad rays whose initiation commences at the centre of the stem, there are secondary [1] broad rays that take their origin in annual rings distant from the centre. How do these actually arise? I have made no serial sections of such broad secondary rays, but the fact that in tangential sections of various oak woods there are to be seen in the same annual ring, side

[1] Both Bailey and Eames seem to employ the terms 'primary' and 'secondary' as synonyms of 'broad' and 'narrow' in reference to the rays. A 'primary ray' is in reality one that establishes radial parenchymatous continuity between pith and the wood farther out, since it is initiated in connexion with the original 'primary rays' separating the vascular bundles. A 'secondary' ray is formed later and is not in parenchymatous continuity with the pith, but it may be as thick as a primary ray. The term 'compound' as applied to the broad rays by the same authors seems unfortunate, first because the term has already been employed in two different senses, both of which, however, imply that the ray is actually composite in structure; to use the same term for an undivided ray of *Quercus* is not only to assume that it represents a fusion of separate rays, but also to render the term 'compound' as a descriptive term useless in case of other rays whose real structure is divided or aggregated.

by side with broad, high, more or less undivided rays, others that are much divided (or smaller aggregated ones), arouses the suspicion that these secondary broad rays, however far from the centre they arise, are continuous at their inner extremities as a group of shallow thin rays. There are other possibilities, including the one that the divided ray may represent one undergoing division in an outward direction ; and such division does take place in *Fagus*. In regard to the matter, Eames ('10) states that in such later wood the origin of the broad ray is nearly always abrupt, and that 'the transition from the lignified elements to [ray] parenchyma occurs' generally within one to three annual rings ; but from this description it is not possible to form a mental picture of the exact mode of origin of such a ray. Again, in another genus, namely *Alnus*, branches show features similar to those in the seedling : in *Alnus glutinosa* it is well known that 'false rays', which are so regular a feature in the main stem, are absent or rare in the branches. These facts suggest that the mode of origin of broad rays in *Quercus* and *Alnus* by a process of linking up of small rays may be merely of physiological significance.

Yet putting aside the hypothesis that the seedling is the seat of ancestral characters, the ontogeny of the broad ray, as demonstrated by Eames, and its inception as a pencil of fine rays, does seem to suggest that it is phylogenetically compounded of these. But the same linking process would take place in entirely different circumstances. Supposing that the oak stem had always been characterized by the possession of broad high rays in at least rings at some distance from the centre, if we now consider the tangential (or more truly cylindrical) projection of the tangential view of a high broad ray in an outer annual ring upon the inmost annual ring, then not only all the small rays visible on that elongated (inmost) projection, but also all the rays arising outside the inmost ring and lying within the truncated wedge formed by drawing lines parallel to the rays from the tangential outer outline of the thick ray to its outline in the inmost ring, would necessarily link themselves to the large ray or must end blindly outwards before reaching this. Again, if in the ancestral *Quercus* the primary rays were originally from their very commencement high and thick, but in the descendants had been split up only in the inner rings into a number of fine rays by reason of the cambium producing normal longitudinal constituents of the wood, then the same linking up of fine rays into the broad rays would be seen.

This possibility of the existence of broad primary rays in *Quercus* and their gradual excavation to form a number of fine rays leads to the consideration of two fagaceous species—*Fagus sylvatica* and *Quercus* (*Pasania*) *fenestrata*.

L. Jost ('01) states that in *Fagus sylvatica* the primary rays in their inmost region (near the pith) are high and undivided, but traced outwards

each ray becomes divided up by fibres into superposed 'partial' rays, and this division becomes more extensive outwards because the partitions formed by the fibres become more numerous and thicker. Thus, according to L. Jost, the condition as regards division of the rays is exactly the reverse of that described later by Eames and Bailey in connexion with the seedlings of *Quercus* and *Alnus*. It seemed possible that in seedlings of *Fagus* the relation of the rays might be as in *Quercus* according to Eames. Accordingly, R. Tabor [1] investigated the matter and found that even in the seedling stem of *Fagus sylvatica*, the large primary rays, when traced outwards, undergo division in the manner described by Jost. Thus, on the hypothesis that the seedling is the seat of primitive characters, *Fagus* and *Quercus* point in opposite directions. But Tabor's preliminary observations strongly suggest that in the seedling of *Fagus*, secondary rays do become gradually linked up and give way farther outwards to broad rays. Such a fact, if established, that side by side in the same annual rings disintegration and integration are taking place in the rays, would point to the processes as being determined by physiological needs, not by phylogenetic characters.

The Indian oak, *Q.* (*Pasania*) *fenestrata*,[2] is of interest in relation to the problem under discussion, and appears to suggest strongly that, at least in some cases, the bundles of narrow rays have been produced phylogenetically by disintegration of broad rays. The material I examined was a trunk whose diameter was twenty-eight centimetres. It possesses thin rays several cells in thickness, which are characterized by their disproportionate height (Pl. LXXVI, Fig. 32); or in other words the height corresponds to rays very much broader than these. Now in many (most?) species of *Quercus* the boundary of the annual ring dips in where it crosses a broad ray, and the extent of the inward bend is at least often proportional to the width of the ray. In *Q. fenestrata* these characteristic high rays are often approximated in pairs, and between the two rays forming a pair, the boundary of the annual ring is much nearer the centre of the stem than it is elsewhere (Pl. LXXV, Fig. 12). Moreover, the space between the two rays forming a pair is devoid of vessels. These three sets of facts denote that in some way the pair of high rays, together with the tissue between them, is equivalent to a single broad ray; and the very considerable inward dip of the annual ring between the two rays finds simplest explanation in the assumption that in the ancestor this complex was represented solely by ray tissue. This case of *Q. fenestrata*, while clearly suggesting that disintegration of the broad rays into narrow rays can take place, is not

[1] Mr. Tabor's work is as yet incomplete and unpublished.

[2] The specimen from India arrived labelled *Q. spicata*, but the Herbarium specimen presumably obtained from the same individual tree was determined at Kew to be *Q. fenestrata*. Yet the structure of the wood does not agree with Gamble's description of that of *Q. fenestrata*, and Mr. Gamble suggests that my specimen may be the wood of *Q.* (*Pasania*) *lanceaefolia*.

necessarily in favour of the view that in *Quercus* the direction of evolution has been from the broad to the thin rays by disintegration: for it is possible to interpret this case in accord with the theory supported by Bailey and Eames, by assuming that in *Q. fenestrata* the thin rays have ceased to broaden and heighten at their original rate, so that they do not link up as they did in the ancestor, and thus represent a case of arrested development.

So far, then, there is no crucial evidence deciding whether the primitive condition in *Quercus* was broad-rayed or narrow-rayed.

Nor can it be said that Bailey's interesting observations ('10 (2)) on traumatic oak woods supply decisive evidence. Even if we admit that traumatic structure may sometimes be reversionary in essence, Mr. Bailey himself points out that severe injury causes solely uniseriate rays to be produced at first near the wound, but that slight injuries may cause the reverse, namely, a precocious production of broad rays.

The disintegration of broad rays into narrow ones phylogenetically or ontogenetically is conceivably possible by two means. Either the cambium, in place of producing ray-tissue, gives rise in certain spots to fibres and wood-parenchyma, or fibres and lines of wood-parenchyma invade the rays by sliding growth (and this ontogenetically may eventually give way to the first method). Either of these hypotheses would equally well explain the facts that in the broad rays there occur cells transitional between fibres and wood-parenchyma or ray-parenchyma, and that fibres are to be seen projecting from the margins into the broad rays. In favour of the invasion view may be mentioned the fact that longitudinal wood fibres (belonging to the body of the wood) are often bent sharply at right angles to their course where they come into contact with a ray, so that each assumes an L shape.

Summary.

The annual rings so distinct in deciduous species of *Quercus* become less marked in evergreen species. They are to be recognized by one or more of the following structural features:—

(i) Maximum calibre of the vessels in the inner zone of the annual ring;

(ii) Minimum calibre of the vessels in the outer zone of the ring;

(iii) Shorter length, nature of the terminal wall, and richer coloured contents of the cells of the medullary rays at the boundary of the annual ring;

(iv) Changed direction (usually inward dip) of the boundary of annual ring where it crosses a thick medullary ray;

(v) Difference in the number of parenchyma cells forming tangential series in the middle and outer zones of the annual ring, and richer coloured contents in the outermost zone;

(vi) One or more layers of flattened fibro-tracheides with abundant tangential bordered pits forming the outer boundary of the annual ring.

Any one or a number of these features may be wholly or totally lacking.

There is a certain degree of correspondence between the habit and the arrangement of the large vessels in the annual ring of *Quercus*. Species showing the most striking pore-zone are deciduous; those showing it regularly and distinctly but not having so marked a disproportion in size between the innermost and outermost vessels are sub-evergreen; whilst those species with no trace of a pore-zone are truly evergreen; finally, the transitional forms, including both evergreen and sub-evergreen species, have feebler or more irregular indications of the porous spring-zone, which in its simplest condition assumes the form of a simple concentric series of widely separated vessels. In a previous paper ('10) I have shown that in American species the maximum calibre of vessel varies with the habit, being greatest in deciduous, least in truly evergreen, and intermediate in sub-evergreen species.

In none of the species examined by me are all traces of annual (or growth) rings lacking, though in some evergreen species the boundaries of these are in places difficult to follow, and in *Q. fenestrata* the annual ring presents the appearance of being broken up into distinct arcs differing in the length of their radii.

All species of *Quercus* possess uniseriate shallow medullary rays, while some species also have broad high multiseriate rays.

Between multiseriate and uniseriate rays there exist in various species numerous transitional stages representing either disintegration of the broad ray into a number of narrow ones or integration of numbers of narrow rays to build up the broad ray.

It is impossible at present to decide whether in *Quercus* the broad-rayed or the narrow-rayed type was primitive. Pointing in favour of the latter view are the observations of Bailey and Eames that in seedlings of *Quercus* and *Alnus* the narrow rays gradually link up to form broad ones. But pointing in the opposite direction are Jost's statements that in the inner rings of *Fagus* the broad high rays when traced outwards undergo division into smaller ones, while Tabor's observations on the seedling of *Fagus* show that both kinds of change are going on simultaneously in the rays of the same annual ring. In *Quercus fenestrata* the structure and paired arrangement of certain high rays strongly suggest that disintegration of the broad rays in a phylogenetic sense has taken place.

In American evergreen species of *Quercus*, excluding *Pasania*, there is a striking parallelism between, on the one hand, the distinctness of the annual ring as judged by the size of the vessels in the spring-zone, and, on the other, the thickness and degree of subdivision of the broad rays (or the thickness and degree of aggregation of the narrow rays). The parallelism is disturbed in American species by differences due to genetically distant

relationship, so that *Q.* (*Pasania*) *densiflora* falls out of line with the other species: the parallelism does not hold good of Indian species, possibly because of climatic differences.

It is a pleasure to thank Professor Jeffrey of Harvard University, and Mr. R. S. Pearson, Imperial Forest Economist in India, for their kindness in supplying the material used in this investigation from America and India respectively.

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EXPLANATION OF FIGURES IN PLATES LXXIV–LXXVI.

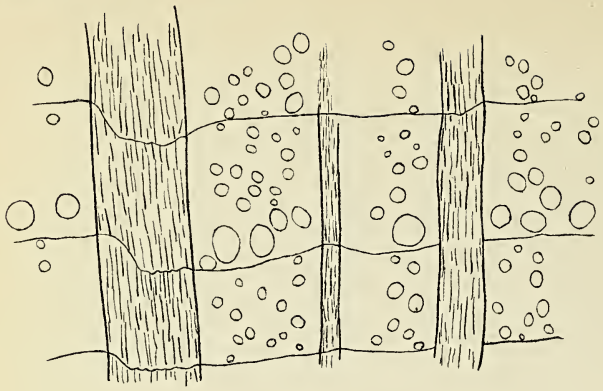
Illustrating Prof. Groom's paper on the Evolution of the Annual Ring and Medullary Rays in *Quercus*.

Figs. 1–12 are of transverse sections with a magnification of 12·5. Figs. 13–32 are of tangential sections of the broad medullary rays, with a magnification of 12, excepting where special magnifications of 25 are recorded.

Figs.
1. *Q. hypoleuca.*
2. *Q. Emoryi.*
3. *Q. chrysolepis.*
4. *Q. densiflora.*
5. *Q. virginiana.*
6. *Q. agrifolia.*
7. *Q. tomentella.*
8. *Q. semecarpifolia.*
9. *Q. glauca.*
10. *Q. lamellosa.*
11. *Q. incana.*
12. *Q. fenestrata.*
13. *Q. Macdonaldi.*

Figs.
14. *Q. acuminata.*
15. *Q. bicolor.*
16. *Q. sessiliflora.*
17. *Q. georgiana.*
18. *Q. Douglasii.*
19. *Q. Emoryi.*
20. *Q. arizonica.*
21. *Q. hypoleuca.*
22. *Q. Engelmanni.*
(*a*) × 12.
(*b*) × 25.
23. *Q. chrysolepis.*

Figs.
24. *Q. agrifolia.*
(*a*) × 12.
(*b*) × 25.
25. *Q. virginiana.*
26. *Q. tomentella.* × 25.
27. *Q. densiflora.*
28. *Q. lamellosa.*
29. *Q. glauca.*
30. *Q. semecarpifolia.*
31. *Q. incana.*
(*a*) vertically divided ray.
(*b*) obliquely divided ray.
32. *Q. fenestrata.*



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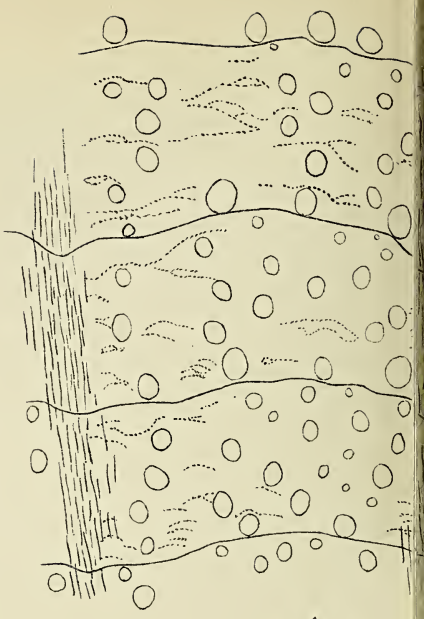

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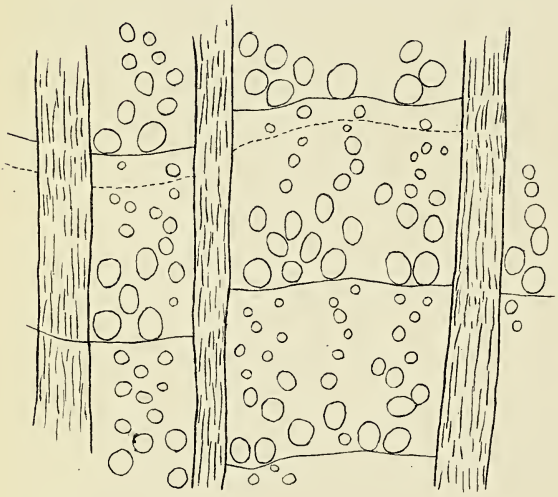

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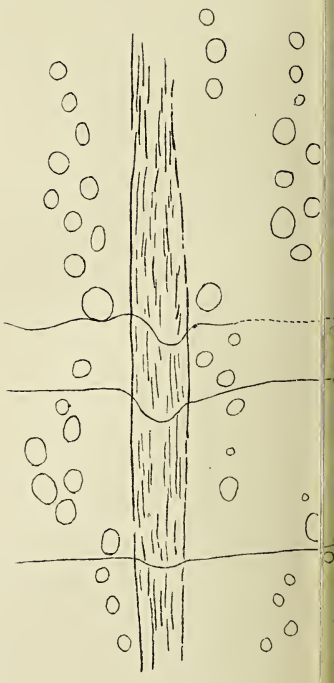

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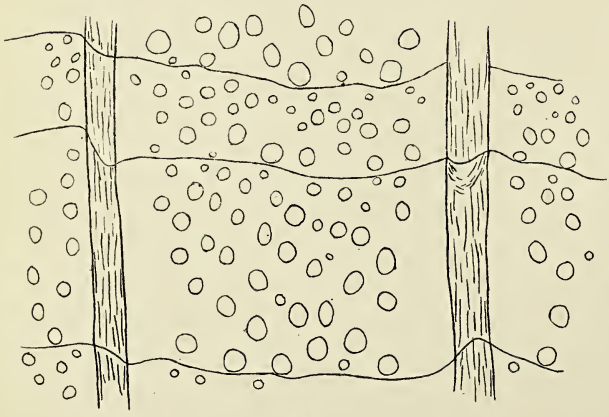

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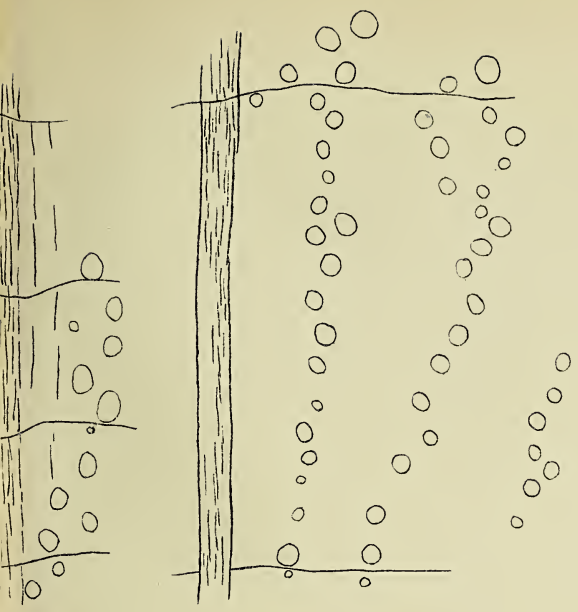

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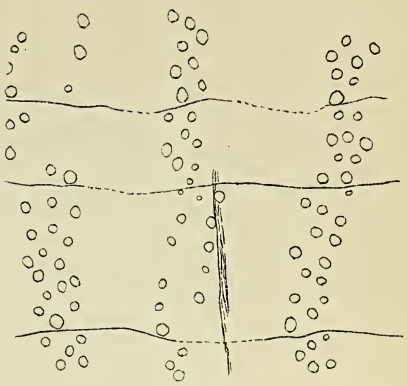

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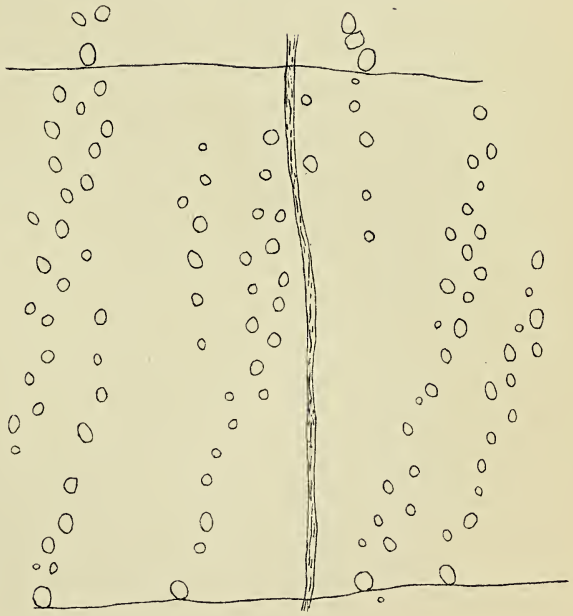

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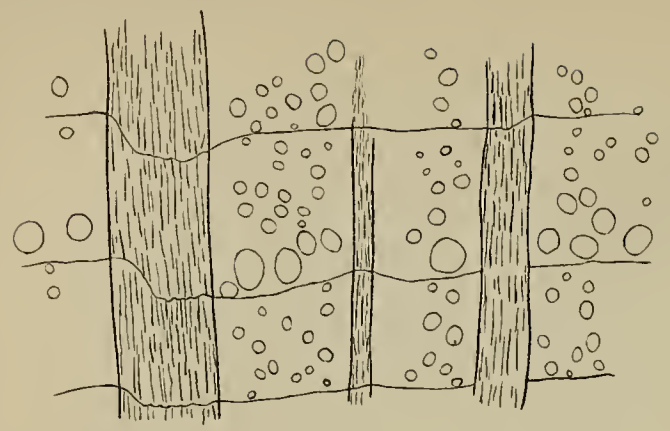

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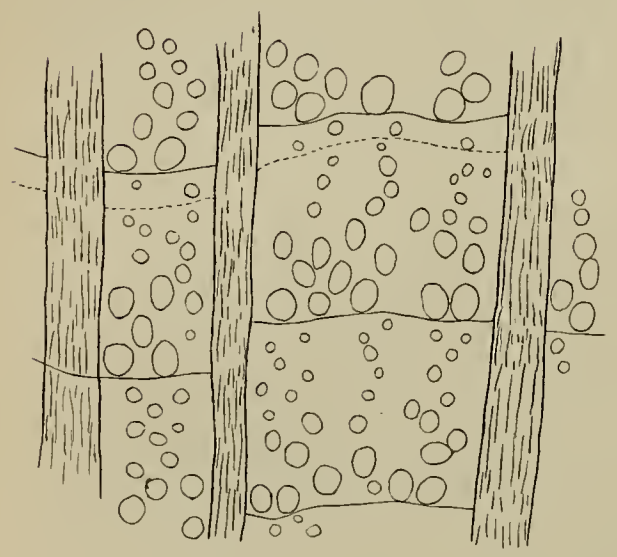

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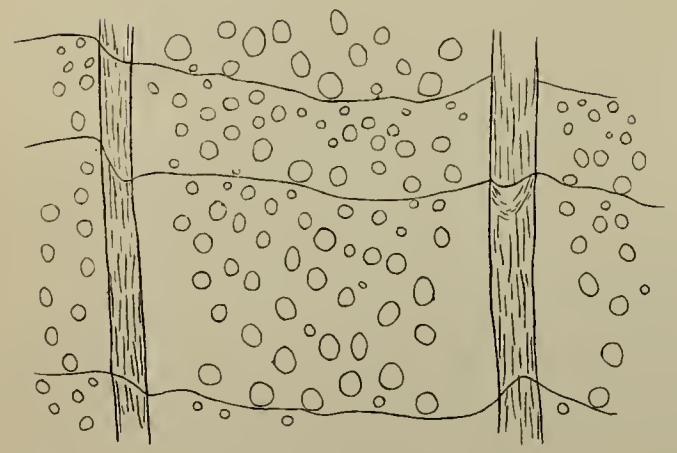

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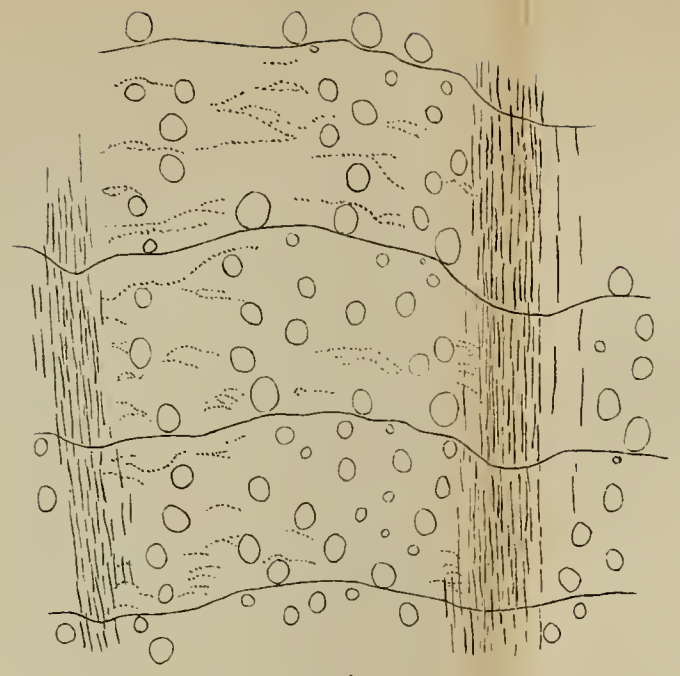

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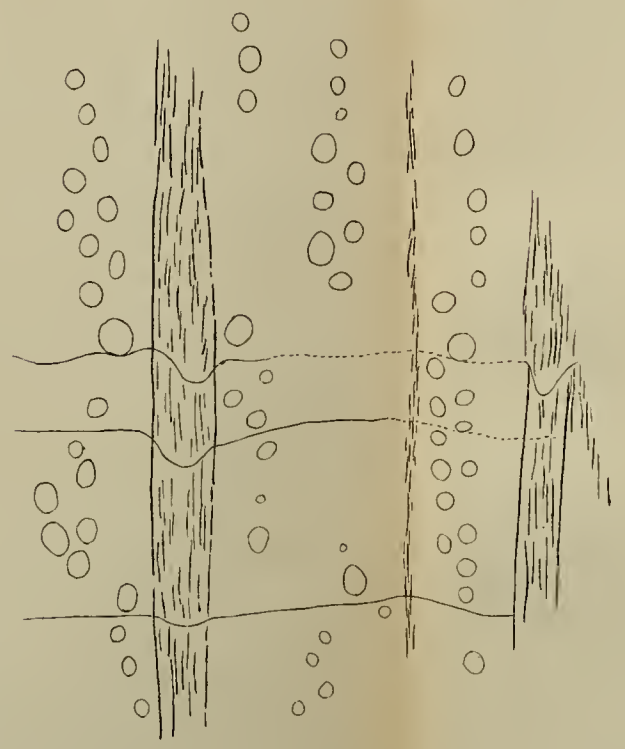

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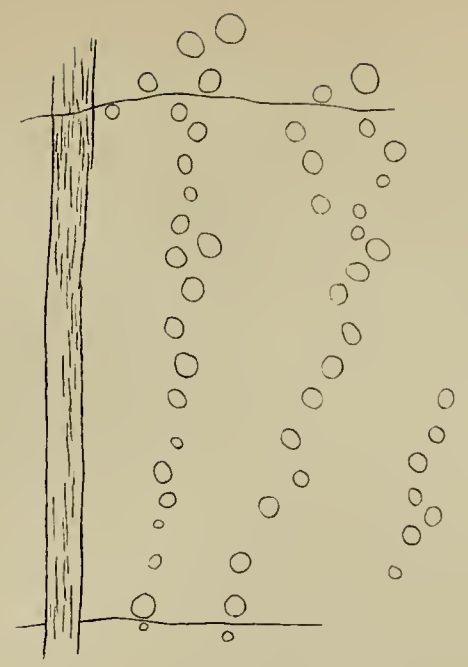

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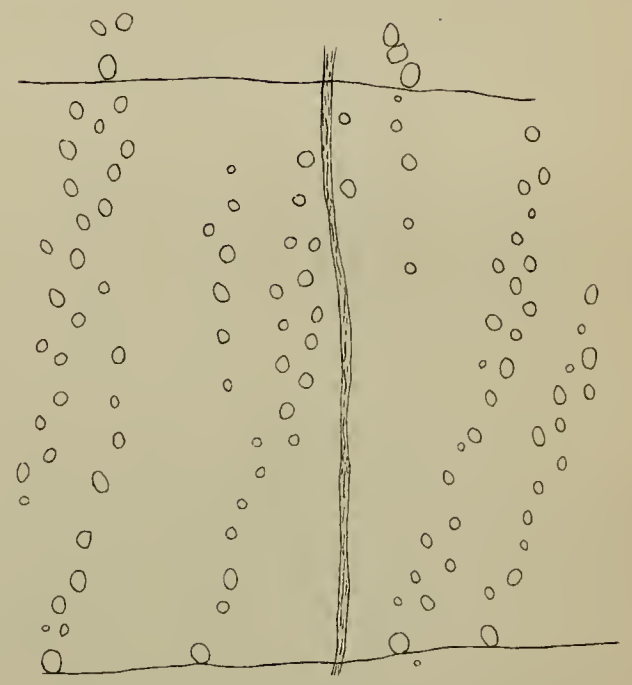

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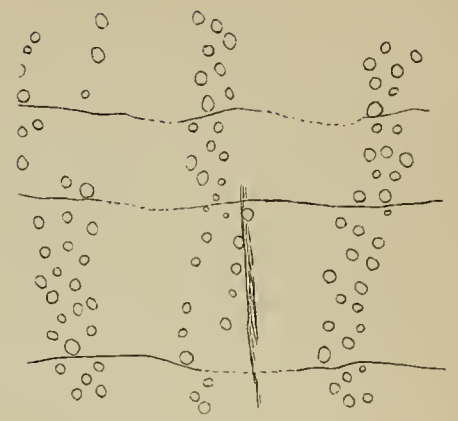

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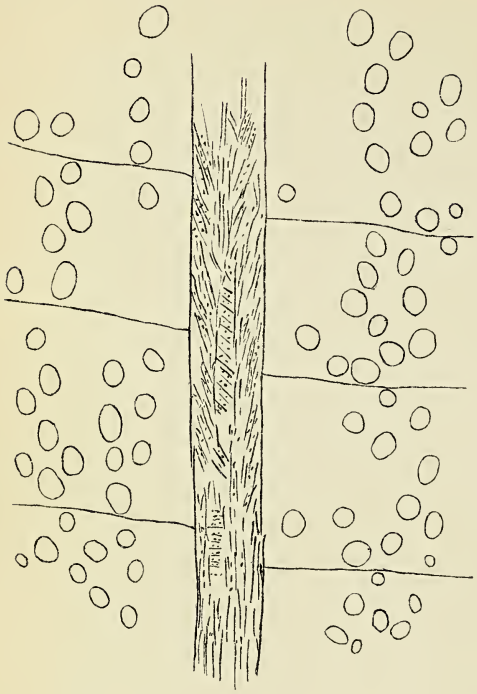

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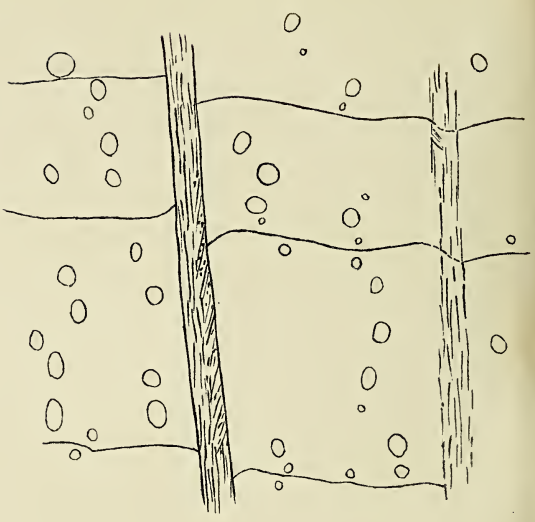

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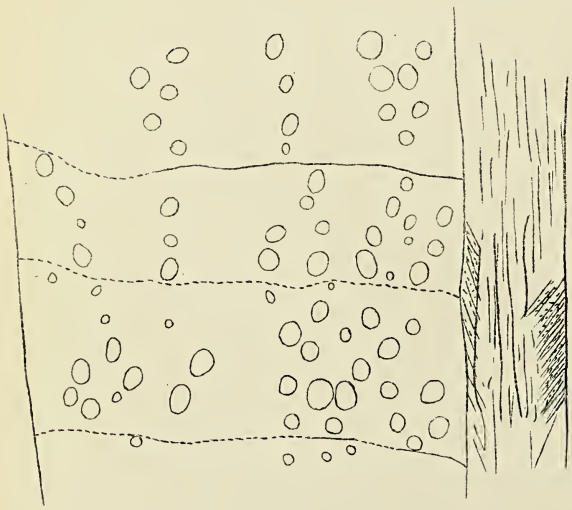

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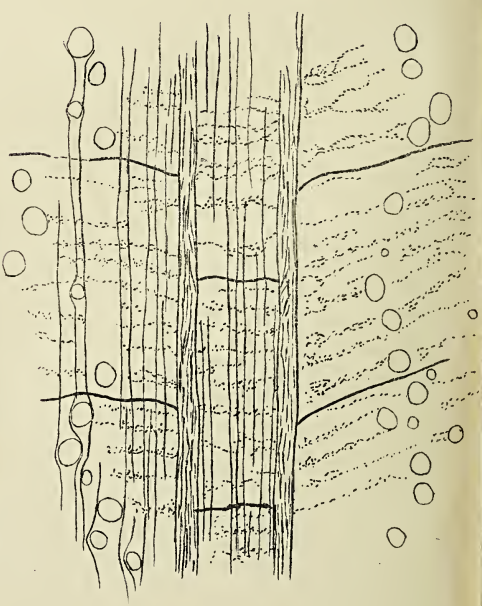

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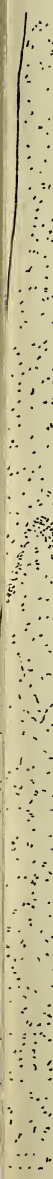

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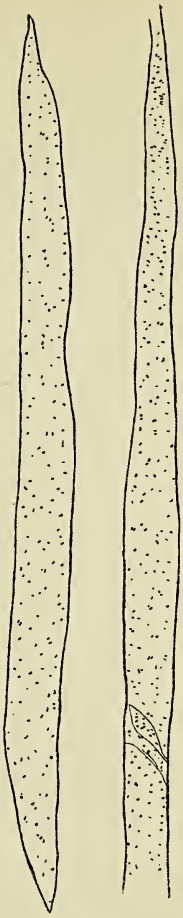

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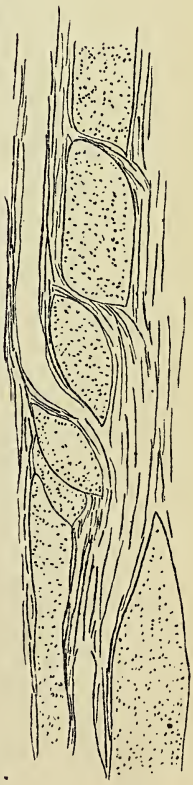

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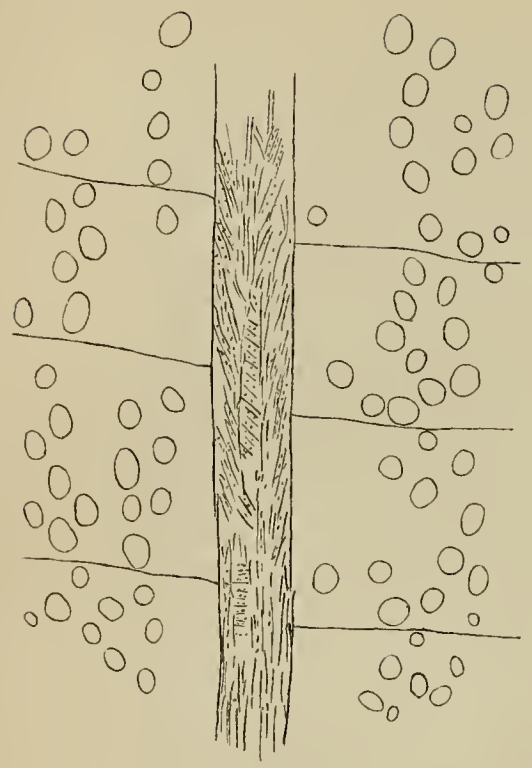

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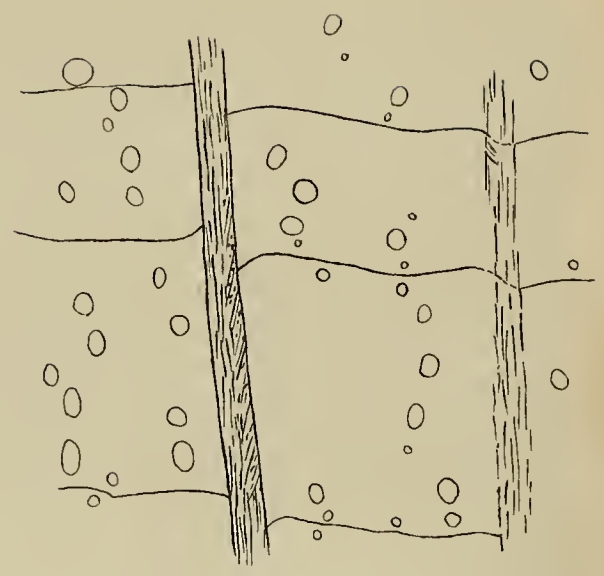

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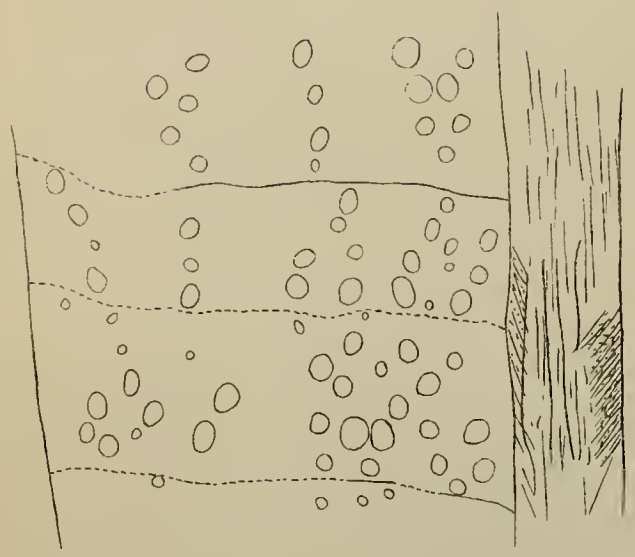

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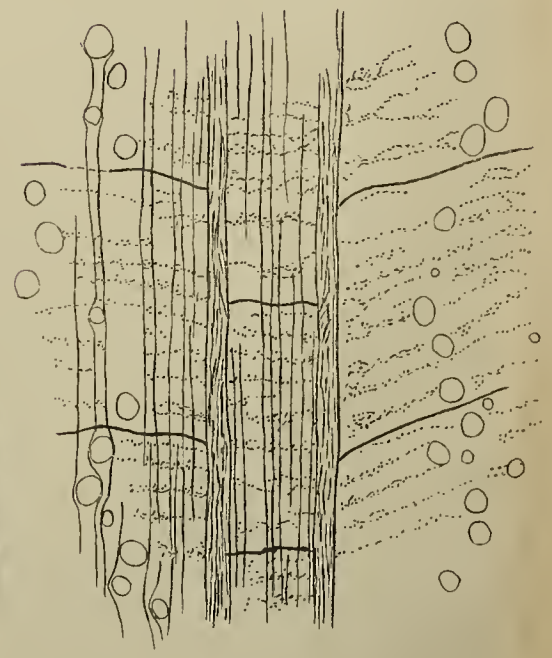

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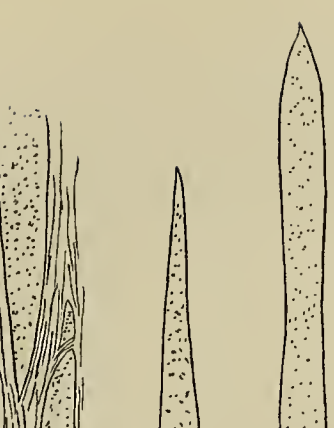

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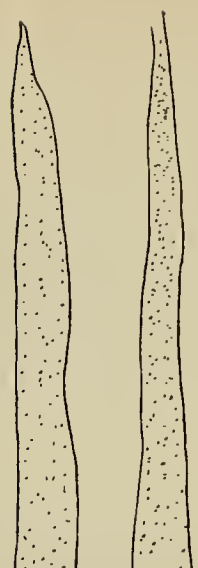

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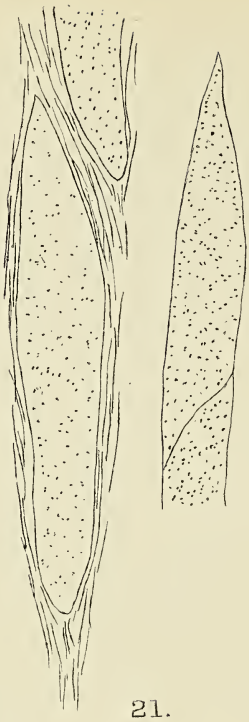

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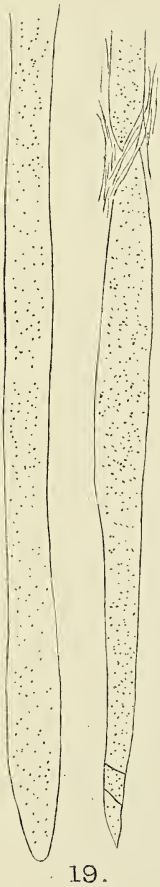

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GROOM — ANNUAL RINGS.

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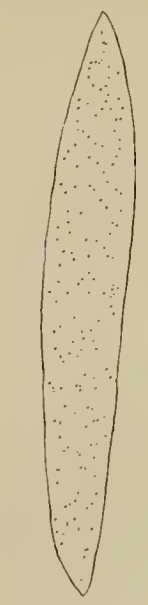

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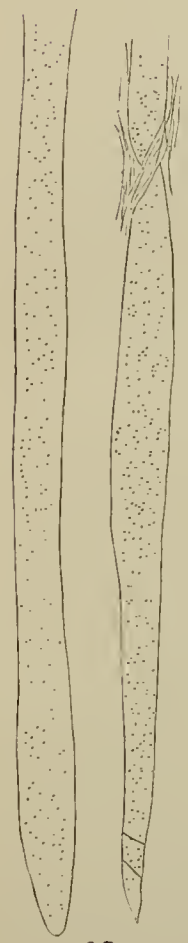

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On the Origin of the Multiseriate Ray of the Dicotyledons.[1]

BY

W. P. THOMPSON,

1851 *London Exhibition Science Research Scholar, University of Toronto, at Harvard University.*

With Plates LXXVII and LXXVIII.

THE medullary rays of dicotyledonous plants present several distinct types. The simplest of these is but a single cell wide, and for this reason is called a *uniseriate* ray. It is similar to, and, in part at least, homologous with, the usual type of ray found in gymnospermous plants. A second type is two or more cells wide, and accordingly is known as *multiseriate*. Such a ray is of widespread occurrence in the Dicotyledons. Still another type, recently designated the *compound* ray, is much broader than either of the foregoing, and consists of an extensive homogeneous mass of parenchyma. This is the type which gives to the oak wood, for example, its characteristic grain. Finally, closely associated with the compound ray, one finds in certain woods an aggregation of uni- and multiseriate rays, to which the term '*false*' ray has been applied.

The inter-relationships and evolution of these different types of rays have recently formed the subject of a series of investigations in this laboratory. Eames[2] has demonstrated from a study of the structure of fossil and seedling oaks, as well as from comparative evidence, that the broad type of ray has arisen by a progressive fusion of uniseriate rays and an accompanying transformation of the included fibres and wood parenchyma into ray parenchyma. That is to say, in the ancestral oaks, and in primitive regions of modern oaks, only uniseriate rays may be found, and these by a process of fusion have given rise to the modern broad oak ray. Bailey[3] has emphasized the perfect series of transitions in this process furnished by the false rays of the Cupuliferae, and has shown that the factor at work in the development of the compound ray has been the influence of the leaf-trace. The most natural and convenient place for

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 43.

[2] Eames, A. J.: On the Origin of the Broad Ray in *Quercus*. Bot. Gaz., xlix, March, 1910, No. 3, pp. 161–7.

[3] Bailey, I. W.: The Relation of the Leaf-trace to the Formation of Compound Rays in the Lower Dicotyledons. Ann. Bot., Jan. 1911, pp. 225–241.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

the storage of the food manufactured in the leaf is, of course, in the stem adjacent to the leaf-trace. Accordingly the storage organs, i. e., the rays, at this point in the stem have been enormously developed, especially in view of the probable persistent habit of the leaves of Angiosperms during the warmer Mesozoic times. Then, following its development at the leaf-trace, the broad ray has spread throughout the plant. Bailey has also suggested that the further development of compound rays at the expense of the woody cylinder has given rise to the herbaceous habit among Angiosperms. The same conclusion was reached by Eames[1] after an investigation of herbaceous and semi-herbaceous forms. This aspect of the work has resulted in a conclusion exactly the reverse of the commonly taught Sachsian hypothesis of the development of the woody cylinder from the herbaceous type by the filling in of the interfascicular regions through the activity of the so-called interfascicular cambium.

It will be observed that the only kind of ray not included in these investigations is the multiseriate type, that intermediate in size between the uniseriate and compound types. It is the object of this paper to determine the origin of this ray and to establish its position in the general scheme of ray development in the Dicotyledons. In carrying on the investigation it was found that the Ericaceae presented very favourable conditions, and this family was studied in detail. Confirmatory evidence was found in many other groups.

The origin of the multiseriate ray is epitomized in Pl. LXXVII, Fig. 1. This is a photograph of a transverse section of *Rhododendron punctatum* (Andr.) just above the level of exit of the leaf-trace. All the rays in the section are seen to be uniseriate except the conspicuous group at the top, which is directly above the leaf-trace. At this point we see next to the pith a broad compound ray which during its course through the wood breaks up progressively into several smaller multiseriate rays. Otherwise stated, the group of multiseriate rays has originated by the breaking up of a compound ray.

A tangential view of this group of rays is presented in Fig. 2. At the bottom of the field is the leaf-trace, and extending upward from it completely through the field and for a long distance beyond is the aggregation of multiseriate rays. A small group also extends for a very short distance below the trace. Complete series of such tangential sections through the node from the bark to the pith show that all the multiseriate rays in the branch are associated in this manner with the traces. Complete series of transverse sections through the node show that the solid type of broad ray is not present at the pith through the whole vertical extent of the group, but at the upper extremity multiseriate rays arise directly from the pith.

[1] Eames, A. J.: The Origin of the Herbaceous Type in Angiosperms. Ann. Bot., Jan. 1911, pp. 215–224.

In this species, then, almost the whole of the woody cylinder possesses only uniseriate rays ; the multiseriate ones occur only in association with the leaf-trace and arise by the dissection of compound rays.

A similar condition is found in *Ledum groenlandicum* (Oeder) and is represented in Fig. 3. Only one side of the woody cylinder is shown, with the edge of the pith at the extreme bottom of the figure. The horizontal spreading of the multiseriate rays with the increase in diameter of the stem is almost diagrammatic. Following the breaking up, the resulting rays may widen by their own growth and split again. The tangential view is given in Fig. 4. This section was taken further from the pith than Fig. 2, and consequently more horizontal spreading of the rays has taken place. As in *Rhododendron punctatum*, all the multiseriate rays in the stem arise in association with the leaf-traces.

An important additional step is seen in Fig. 5, which is a photograph of a section of *Kalmia angustifolia* (L.), at the nodal region. In this species three traces arise at approximately the same level. One of their corresponding large rays is seen unbroken at the lower left of the figure. Above and at the lower right the breaking-up process has taken place. The result is that considerably more multiseriate rays occur in the adult wood than in the aforementioned species. Furthermore, at the right of the figure are to be seen two multiseriate rays which are not associated with the others. A series of sections would show, however, that in reality these are associated with a leaf-trace of a lower node. As may be seen in the tangential section (Fig. 6), the group of rays extends for a long distance above the trace, in fact up to and beyond the next trace. This overlapping of the groups of rays gives the appearance in question and assists in distributing the multiseriate rays throughout the wood. Another important fact in this connexion is that the groups of rays in their course outward through the wood extend continually higher vertically, so that in a tangential section as far out as the bark they are much higher than in one at the pith.

Still more conspicuous overlapping and vertical extension of the rays may be observed in *Chaemadaphne calyculata* (Moench). In this species and in *Kalmia angustifolia* it is often difficult to observe the solid oak type of ray even at the pith. This stage is apparently omitted, the multiseriate rays often arising in a group from the pith above the leaf-trace.

In other species of the Ericaceae, for example in *Vaccinium corymbosum* (L.), multiseriate rays may be found which are not in association with the leaf-trace but which arise quite independently at the pith or in the wood. The natural inference is that the habit of forming multiseriate rays, arising as indicated, has become firmly fixed upon the cambium, which may then form this type quite apart from the influence of the leaf-trace. The uniform distribution of the multiseriate type throughout the wood will then

be a matter of course. Nevertheless, one still finds the breaking-up process taking place at the leaf-trace. Pl. LXXVII, Fig. 7, which is a photograph of a section above the node in *Vaccinium corymbosum*, shows a broad ray at the bottom being dissected into the numerous multiseriate ones seen at the top. In addition to these, other multiseriate rays are visible at the left and right which are quite independent of the broad ray. In *Vaccinium corymbosum*, therefore, the breaking up of the broad ray at the leaf-trace takes place as previously described, and in addition new rays arise quite independently of the leaf-trace. The adult wood will therefore be characterized by a rather uniform distribution of multiseriate rays, although here and there one may observe a slight grouping, the result of the breaking up of a compound ray.

A tangential view of the condition existing in *Vaccinium corymbosum* is given in Fig. 8. The leaf-trace is visible at the extreme lower edge of the figure, and the ray in the process of dissection above it. Independently arising multiseriate rays are to be seen at the left and right.

A similar condition is present in *Gaylussacia resinosa*, except that the solid type of ray is more evanescent.

It is obvious that the net result of the process of dissection of compound rays and the fixation on the cambium of the habit of forming the dissected type will be the uniform distribution of the latter type throughout the wood. Such a condition one finds to exist in many species of the Ericaceae; for example, *Clethra alnifolia* (L.) (Fig. 9). At the bottom is the pith, and throughout the wood are multiseriate rays intermixed with uniseriate ones. Fig. 10 shows the condition in tangential section, the multiseriate rays being strikingly uniform in distribution. Furthermore it is important to notice that the leaf-trace at the bottom of the figure is no longer accompanied by a compound ray or by an aggregation of multiseriate rays. This plant presents, therefore, the completely developed condition of the multiseriate ray of the Dicotyledons.

Among other Ericaceous plants presenting equally well developed conditions may be mentioned *Andromeda floribunda* (Pursh), *Lyonia ligustrina* L. (DC.), *Leucothoe racemosa* L. (Gray).

To summarize the conditions found in the Ericaceae: The breaking up of compound rays into smaller multiseriate ones is of common occurrence. In some species only one such group may be seen in a given section. Then by the occurrence of several traces at the same level, by the overlapping of rays from traces at different levels, by the vertical extension of groups of rays with the increase in size of the stem, and above all by the acquired habit of forming multiseriate rays independently of the leaf-trace, the multiseriate rays become uniformly distributed throughout the stem, and the dissection process is lost.

Turning now to other groups of Dicotyledons in which the compound

ray is well developed, one finds the evidence equally conclusive. Among the Proteaceae, *Grevillea* and *Hakea* have been examined. The wood of *Grevillea robusta* for some distance from the pith possesses typical large rays of the oak type, which extend vertically for long distances. They are not confined to the vicinity of the leaf-traces as in the Ericaceae, but are uniformly distributed throughout the wood. A short distance from the pith they become divided into two or three smaller rays by the intrusion of fibres. Fig. 11 shows a broad ray at the bottom being split in this manner into two smaller rays. In any given section two or three such rays can usually be seen undergoing division.

In *Hakea* sp. the conditions are almost identical with those described for *Grevillea robusta*. Fig. 12 shows the division taking place in this species, three smaller rays being formed at the top.

These two examples are sufficient to show that the dissection process occurs in the Proteaceae and that it is probably of widespread occurrence in the family. Importance is added to this by the fact that the broad rays are much better developed here than in the Ericaceae.

In regard to the Casuarinaceae the story is much the same. The early formed wood of *Casuarina glauca* possesses very large solid compound rays, as may be seen in Pl. LXXVIII, Fig. 13, which is a photograph of a tangential section of this species taken very close to the pith. The homogeneous rays immediately become traversed by strands of fibres in the form of a network (Fig. 14). The strands increase in size and number, with the result that the remnants of the original broad ray gradually become smaller and scattered uniformly through the wood. The ultimate condition is shown in Fig. 15, in which there is no indication of a broad ray, but in its place a typical multiseriate-rayed wood. This condition is reached at a distance of two or three centimetres from the pith.

In *Casuarina equisetifolia* the broad rays are never so well developed as in *C. glauca*, and become broken up much sooner. Fig. 17 shows the typical broad ray for this species, and Fig. 18 the aggregation of smaller multiseriate rays resulting from its dissection. A section still further from the pith would show a uniform distribution of small multiseriate rays.

An undetermined species of *Casuarina* presented the same phenomenon. It appears, therefore, that the conditions described are normal for the family. The obvious conclusion is that the Casuarinaceae formerly possessed broad rays and are replacing them by smaller multiseriate ones.

In the broad-rayed genera of the Fagaceae the breaking-up process is sometimes apparent and resembles slightly that found in *Casuarina*. At the pith of *Fagus ferruginea* broad rays extend for extremely long distances vertically, as may be seen in Fig. 19. The two rays visible here extend far beyond the limits of the figure. A short distance from the pith strands of fibres begin to cross the rays obliquely and divide each into

a vertical series of lower rays. The beginning of this process is shown in Pl. LXXVIII, Fig. 20, which is a photograph at the same magnification of a section taken further from the pith of the same branch. Still further out the separating strands increase in size, with the result that the low rays or segments of an original high ray are gradually pushed apart. This continues until the condition found in the adult (Fig. 21) is attained. Here the rays are scattered through the wood and give little suggestion that they have originated from the oblique dissection of vertically elongated compound rays. Owing to the obliquity of the dissecting fibres the process cannot usually be well observed in transverse sections. Occasionally, however, the fibres are sufficiently vertical to make the process visible in favourable sections. This is especially true in the European Beech, *Fagus sylvatica*. In Fig. 22, taken from a section of the latter species, the broad ray in the centre of the field is undergoing the oblique division.

Even in certain species of the oak itself, in which the broad rays reach their maximum development, the beginning of their dissection may be seen. In *Quercus bicolor*, for example, in the later formed wood of very old trees the rays begin to undergo oblique division.

In *Platanus occidentalis* the conditions are almost identical with those described for *Fagus ferruginea*.

If the smaller multiseriate rays have originated as suggested, it is to be expected that typical multiseriate-rayed forms would exhibit some evidence of their derivation from a broad-rayed ancestry. Such evidence has been found in many forms. As a first example we may take the case of *Carpinus japonica*. This species is characterized by the possession of multiseriate rays. Yet occasionally one may find in the seedling the broad oak type of ray. Fig. 23 shows such a ray at the bottom of the field dividing to form the two smaller rays at the top. Obviously we have in this primitive region a recapitulation of the process by which multiseriate rays have originated.

Ostrya virginiana, the hornbeam, which is likewise characterized by the possession of multiseriate rays, furnishes a second example. Fig. 24, from the root of a seedling, shows the retention in this species also of the broad type and its division into the smaller type. Moreover, wounded seedlings of *Ostrya* occasionally exhibit broad rays in the wood formed immediately after injury. This phenomenon is to be regarded as a traumatic reversion and, together with the observation just recorded, is interpreted to mean that the multiseriate-rayed *Ostrya* is descended from forms which possessed broad rays.

The conditions in the genus *Betula*, which has been investigated by Professor Jeffrey, lead to exactly the same conclusion. Species such as *B. populifolia* (Marsh) possess compound rays of the so-called false type throughout the ordinary wood. Others, such as *B. alba* (L.), possess them

only as a passing phase in the seedling, and have thereafter only smaller multiseriate rays. Others again, such as *B. papyrifera* (Spach), do not normally possess compound rays even in the seedling, but a wound in the latter region will recall them. Finally, *B. lutea* (Michx.) and *B. lenta* (L.) never have them even in severely wounded seedlings. The various species of *Betula* thus exhibit in an admirable manner the loss of compound rays and their replacement by those of the multiseriate type. The actual breaking up cannot be observed so well as in the forms previously described, for the compound rays usually die out rather abruptly.

Mr. I. W. Bailey, who is now investigating the loss of compound rays, finds among other things that the chestnut (*Castanea*), though possessing in the adult wood only uniseriate rays, shows undoubted evidence of having once possessed compound rays. He also finds that in *Castanopsis* the evidence is even more striking.

All these examples from the Cupuliferae show that, upon investigation in the proper regions, relics in one form or another of a broad-rayed ancestry may be found. Nor is the evidence lacking in other groups. In the Juglandaceae broad rays have been observed in seedlings of *Carya*. A tangential view of such a ray undergoing division is presented in Fig. 16. It is probable that investigation in the proper regions will also reveal their presence in many other families of the Dicotyledons.

In this connexion the work of Miss Holden[1] on the uniseriate-rayed Sapindales is noteworthy. She finds that *Aesculus*, which is normally characterized by the possession of uniseriate rays, may possess rays of much larger size in the petiole of the leaf, in the root, and in the reproductive axis. From these observations she concludes that this simple-rayed form is simple not primitively but by degeneration from a broader-rayed type.

Conclusions.

We have seen that the members of the Ericaceae exhibit in an extremely diagrammatic manner the breaking up of compound rays into smaller multiseriate ones, and the stages by which typical multiseriate-rayed plants completely lacking compound rays in the normal adult wood have been derived. Among forms with broad rays the dissection process is apparently widespread, being figured in the Casuarinaceae, Proteaceae, and Fagaceae, as well as in the Ericaceae. Among plants possessing normally only the smaller multiseriate rays the actual process may be observed in the regions of retention of ancestral characters, and where the actual process is lost, relics are still retained of the broad-rayed ancestry. From all sides, then, the conclusion seems inevitable that the multiseriate

[1] Ruth Holden: Some Features in the Anatomy of the Sapindales. Botanical Gazette, ined.

rays of the Dicotyledons have originated by the breaking up of the compound type.

That the elaborate system of compound rays should have been modified seems but reasonable. The influence at work in the development of the compound, aggregate, or foliar ray was originally the demand for the storage of assimilates descending from the large leaves of the earlier Angiosperms. It was natural that in the warmer climate of Mesozoic time, when the leaves probably persisted for a number of seasons, the storage devices of the wood should be mainly centred about the leaf-traces. In later epochs, however, with the advent of a severer winter season and the consequent acquirement of the deciduous habit in connexion with the leaves, the organization of the storage system about the leaf-traces as permanent centres would no longer be advantageous or desirable. Besides being less unwieldy, the system of smaller rays affords equally large capacity for storage and a more convenient general relation between conducting, supporting, and storage tissues.

It should also be emphasized that there has been abundant opportunity in point of time for the complete evolution of the system of multiseriate rays, not only because the compound type is characteristic of the most primitive Dicotyledons, but also because multiseriate rays were already in existence in the Cretaceous. Stopes and Fujii[1] have described many woods of this type from the Cretaceous of Japan.

The general course of ray development would then be as follows: Starting with a primitive uniseriate-rayed condition, the broad rays have been built up by a process of compounding primarily about the leaf-trace and extending from here throughout the wood. From this condition two courses take their origin. On the one hand the continued development of the compound ray at the expense of the woody cylinder has resulted in the ultimate production of the herbaceous condition. On the other hand the forms which remained arborescent replaced the compound rays by a system of smaller multiseriate rays with its more uniform distribution of ray parenchyma. The typical ray structure of the more recent arborescent Dicotyledons accordingly appears to be the multiseriate condition. It is obvious that ray structure will supply a valuable criterion in considerations of phylogeny and classification.

Summary.

1. The production of multiseriate rays by the breaking up of the ancestral broad compound type may be observed in many families of the Dicotyledons (Ericaceae, Casuarinaceae, Fagaceae, and Betulaceae).

[1] Stopes, M. C., and Fujii, K.: Studies on the Structure and Affinities of Cretaceous Plants. Phil. Trans., Series B, vol. cci. pp. 1–90.

2. Originating from the breaking up of compound rays, the multiseriate ray has spread throughout the wood in the higher dicotyledonous Angiosperms.

3. Reversion to the ancestral compound type of ray may be observed in the seedling, root, &c., of dicotyledonous plants which are characterized by the multiseriate type.

4. The multiseriate ray represents the most recent development in ray structure among the Dicotyledons.

The writer has carried on this investigation in the Phanerogamic Laboratories of Harvard University as an 1851 Exhibition Science Research Scholar from the University of Toronto. He is greatly indebted to Professor E. C. Jeffrey for direction and material, and to Mr. I. W. Bailey for innumerable kindnesses.

EXPLANATION OF PLATES LXXVII AND LXXVIII.

Illustrating Mr. Thompson's paper on the Origin of the Multiseriate Ray.

PLATE LXXVII.

Fig. 1. *Rhododendron punctatum.* Transverse section above the node, showing broad ray breaking up into several multiseriate rays. × 12.

Fig. 2. The same. Tangential section, showing leaf-trace and group of rays above it. × 25.

Fig. 3. *Ledum greenlandicum.* Transverse section above node, showing the splitting up of broad ray and horizontal spreading of the multiseriate rays. × 12.

Fig. 4. The same. Tangential section, showing the multiseriate rays above the leaf-trace. × 25.

Fig. 5. *Kalmia angustifolia.* Transverse section through the nodal region, showing unbroken broad ray, two groups of multiseriate rays resulting from the breaking up of broad rays, and two multiseriate rays extending up from a lower node. × 12.

Fig. 6. The same. Tangential section, showing the group of multiseriate rays above the leaf-trace. × 25.

Fig. 7. *Vaccinium corymbosum.* Transverse section, showing breaking up of a compound ray and independently arising multiseriate ones. × 45.

Fig. 8. The same. Tangential section. × 45.

Fig. 9. *Clethra alnifolia.* Transverse section, showing uniform distribution of multiseriate rays. × 50.

Fig. 10. The same. Tangential section, showing the same uniform distribution, and also absence of aggregation above the leaf-trace. × 50.

Fig. 11. *Grevillea robusta.* Transverse section, showing splitting of broad ray. × 60.

Fig. 12. *Hakea* sp. Transverse section, showing splitting of broad ray as in *Grevillea robusta* (Fig. 11). × 60.

PLATE LXXVIII.

Fig. 13. *Casuarina glauca.* Tangential section close to the pith, to show the large compound ray. × 45.

Fig. 14. The same. Tangential section some distance from the pith, to show the network of fibres which breaks up the compound ray. × 45.

Fig. 15. The same. Tangential section of the adult wood, to show the absence of broad rays and presence of multiseriate type. × 45.

Fig. 16. *Carya* sp. Tangential section of seedling, showing presence of broad rays. × 45.

Fig. 17. *Casuarina equisetifolia.* Tangential section close to the pith, showing broad ray. × 45.

Fig. 18. The same. Tangential section further from the pith than Fig. 17, to show the group of multiseriate rays resulting from the breaking up of a broad ray. × 45.

Fig. 19. *Fagus ferruginea.* Tangential section near the pith, to show the broad unbroken rays. × 45.

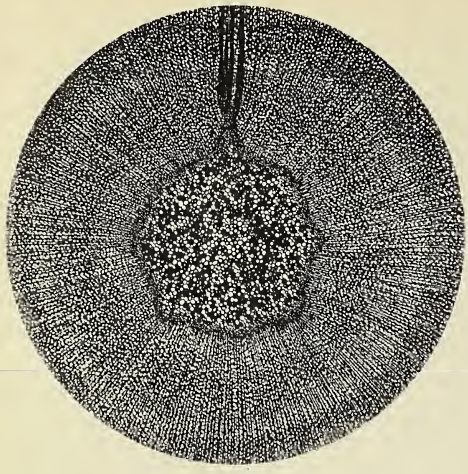
Fig. 20. The same. Tangential section, showing the vertical dissection of a broad ray by oblique fibres. × 45.

Fig. 21. The same. Tangential section of adult wood. × 45.

Fig. 22. *Fagus sylvatica.* Transverse section, showing oblique dissection of a broad ray. × 60.

Fig. 23. *Carpinus japonica.* Transverse section of a seedling, showing a broad ray splitting into two of the multiseriate type. × 60.

Fig. 24. *Ostrya virginiana.* Transverse section of the root of a seedling, showing a broad ray splitting into three of the multiseriate type. × 60.



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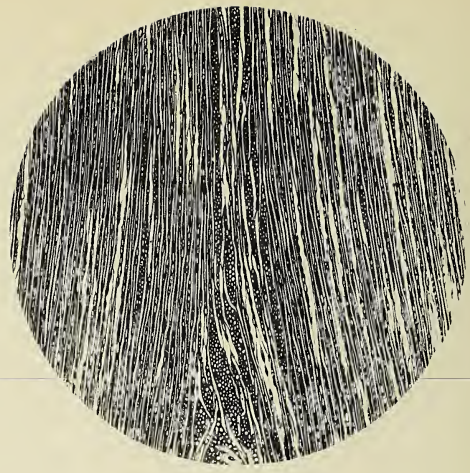

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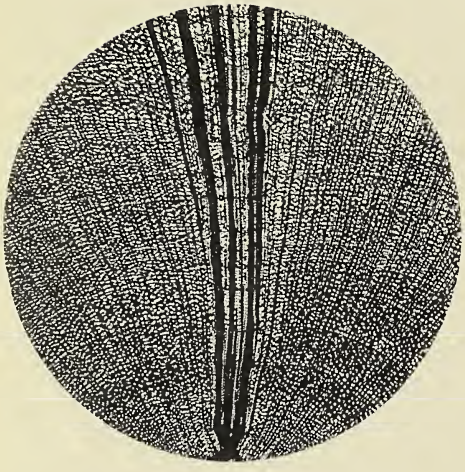

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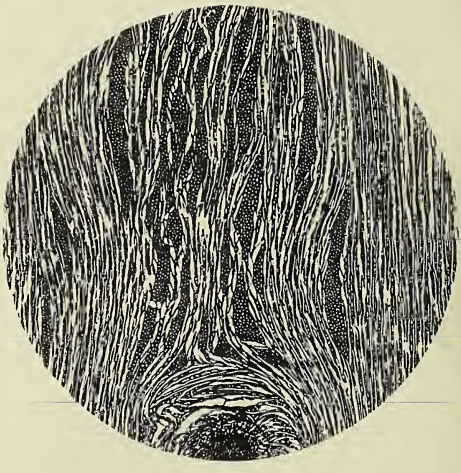

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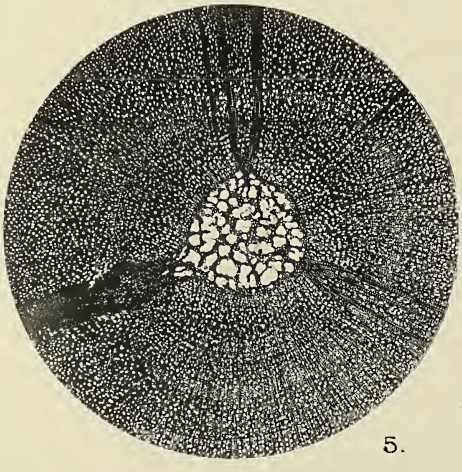

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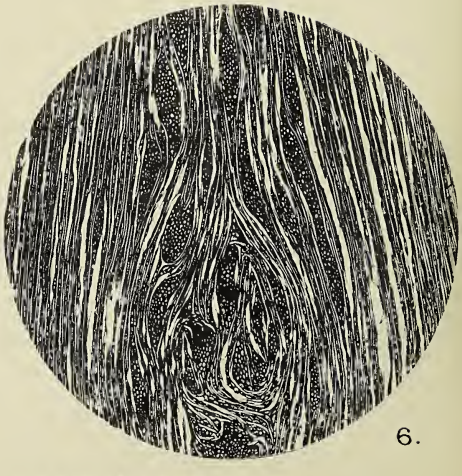

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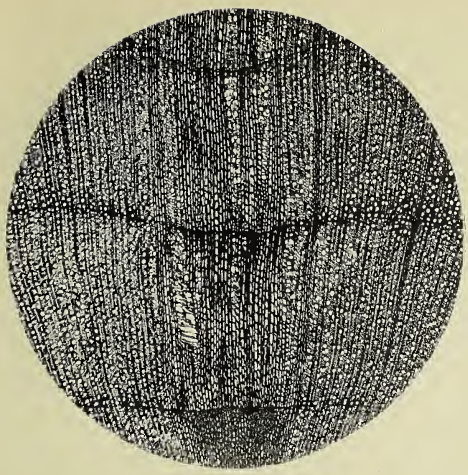
THOMPSON —— MULTISERIATE RAYS



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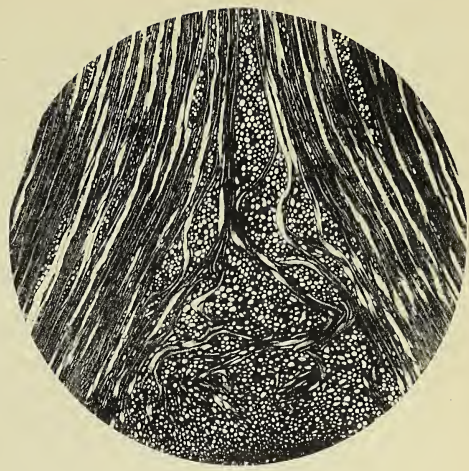

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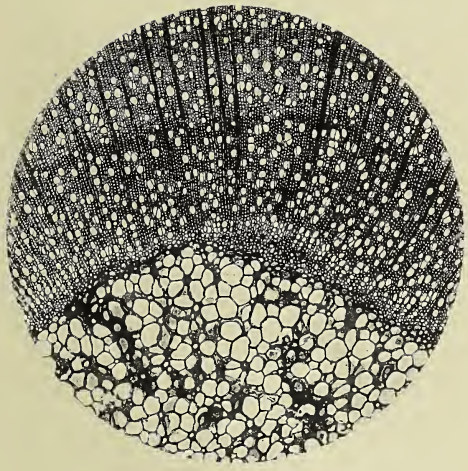

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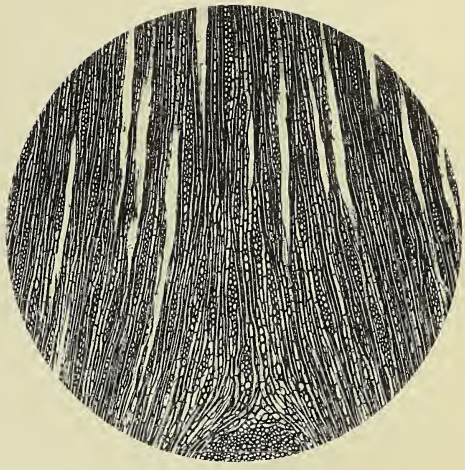

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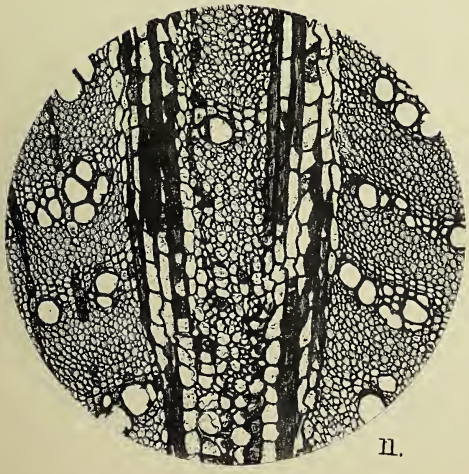

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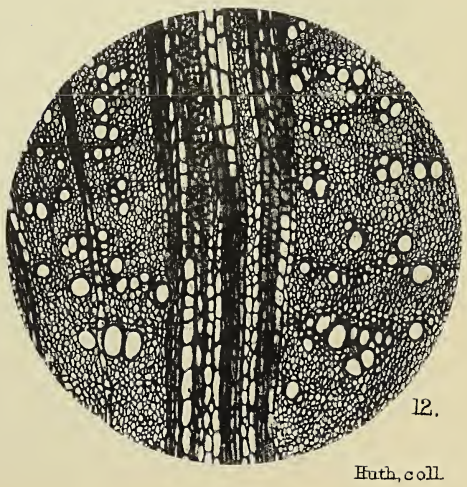

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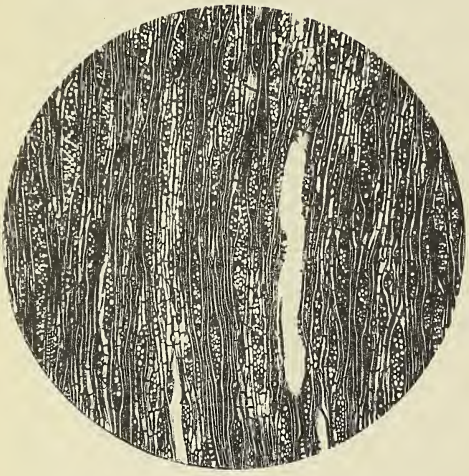

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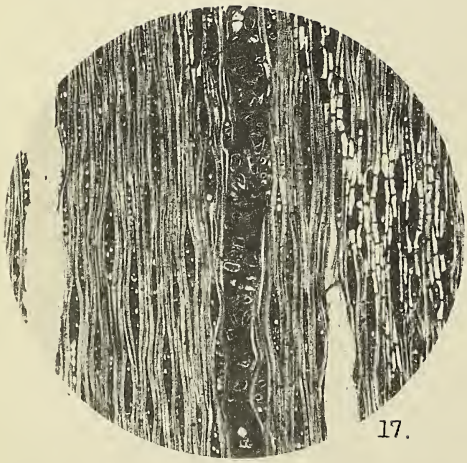

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THOMPSON—— MULTISERIATE RAYS.

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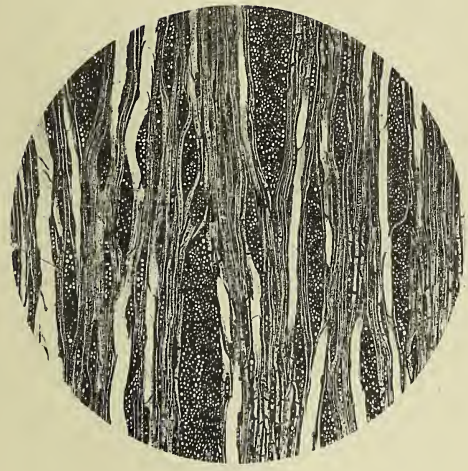

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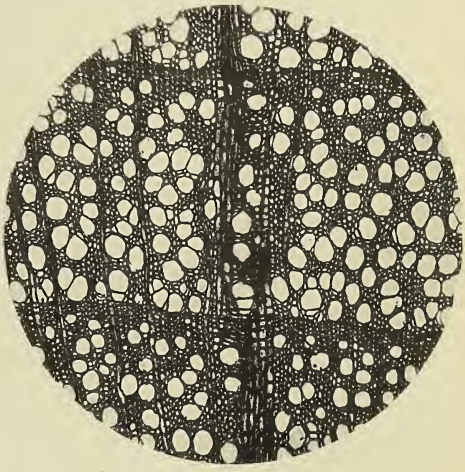

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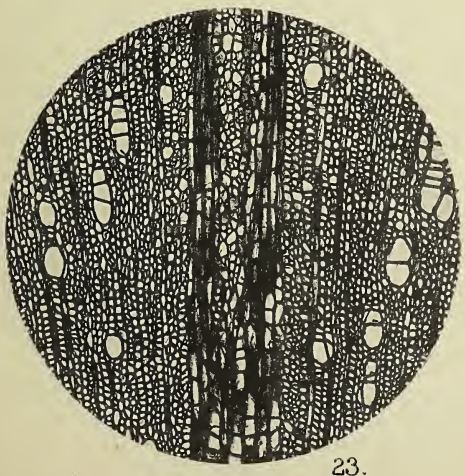

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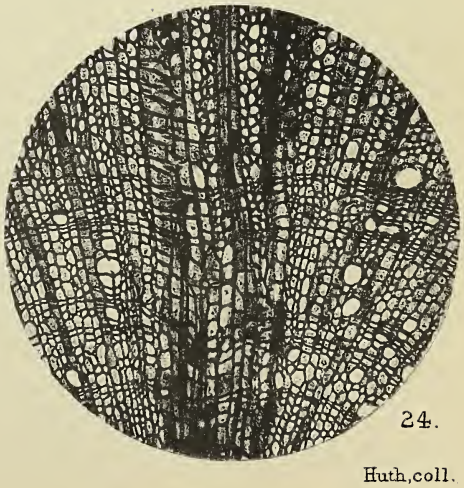

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The Leaf Buds of Archytaea alternifolia.

BY

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Demonstrator in Botany, Royal College of Science, London.

With Plate LXXIX.

ARCHYTAEA is a small genus of the Ternstroemiaceae comprising only four species, of which two are American and two Malayan. The material under investigation belongs to one of the Malayan species, and was collected in the neighbourhood of Singapore.

A. alternifolia, Szyszyl., is a glabrous evergreen shrub with alternate, oblanceolate, leathery, sessile leaves. The buds are not invested by scale leaves, stipules, or other protective structures. The young leaves are convoluted in the bud, the outer ones unrolling gradually from above downwards; the base of each leaf closely invests the stem for some little distance above its node.

The bud has in consequence the form of a trumpet or a funnel (Pl. LXXIX, Fig. 1), the upper portions of the younger leaves within being freely exposed. All danger of desiccation is, however, apparently obviated by the fact that the buds are borne in an upright position and are filled with a watery fluid.[1]

As a consequence the development of the young leaves takes place under water, which must either be collected from the atmosphere in the form of rain or dew, or must be secreted within the bud.

In the well-known case of *Spathodea*, described by Treub (10), the water which fills the unopened calyx of the flower bud is secreted by glands borne on the inner surface of the sepals. Kraus (4) has described flower buds filled with water in *Parmentiera*, and Raciborski (6) has come to the conclusion that very probably a number of genera belonging to the Bignoniaceae and Melastomaceae have a similar protective device. So far as I am aware no leaf buds have been previously described in which the young leaves are protected by immersion in water.

The leaf. The mature leaf is oblanceolate in form, the largest leaf in the material measuring 13 cm. in length and $2\frac{1}{2}$ cm. at its maximum breadth. The surface is glabrous and the margins slightly revolute. A main vein

[1] For this information I am indebted to Prof. Groom, who collected the material.

runs from the base to the apex of the leaf. The secondary veins at some distance from the margin are united by a system of bundles, running parallel to the edge of the leaf. From these bundles short branches run directly towards the margin, to become joined up in their turn by another system of connecting bundles. Finally, short branches from the outer marginal series pass out towards the edge of the leaf and end blindly in the mesophyll. In the lower part of the leaf the margin is quite entire, but in the upper quarter—from the broadest part to the apex—it exhibits a number (40–44) of shallow notches. Towards each notch one or more ultimate branches of the marginal bundles are directed. No water stomata were found in or near the notches. On the other hand, the cells of the mesophyll, among which the vascular bundle ends, are closely compacted and filled with darkly stained contents. The opacity of the cells makes it practically impossible to ascertain the nature of their contents. The presence of tannin in other parts of the leaf favours the suggestion that the contents of these cells included tannin, which has interacted with traces of iron salts, derived from the cans in which the material was preserved. The significance of these notches in the mature leaf will be apparent when the structure of the young leaves is described.

The upper epidermis is devoid of stomata, but these are numerous on the lower side of the leaf. They are of the Rubiaceous type—that is, they show from two to five subsidiary cells arranged parallel to the guard cells (Pl. LXXIX, Fig. 2).

The mesophyll is bifacial, with one, sometimes two, layers of palisade tissue of ordinary type. No sclerotic cells, such as occur in *Thea*, *Camellia*, and other members of the order, are found in the mesophyll of *Archytaea*. The upper epidermis in the young leaf is one-layered, but early divides into two layers, the lower and larger cells forming a conspicuous hypoderm in the mature leaf. The inner walls of the hypoderm cells are strongly gelatinized, the swollen stratified wall encroaching on and occupying the greater part of the lumen of the cell (Pl. LXIX, Fig. 3). Rarely these hypoderm cells undergo a second division. The epidermal cells, on the other hand, invariably become further divided by vertical walls into a group of four to eight cells. The cells of the lower epidermis are not divided, but, with the exception of those connected with the stomata—the guard cells and subsidiary cells—they undergo gelatinization of the inner walls.

Cells with mucilaginous walls also occur scattered throughout the lower part of the mesophyll, and are abundant in the outer cortex of the stem.

The young leaves. The buds examined were composed of four or five leaves. The outermost leaf, which is generally three-quarters grown, has in all essentials the structure of the mature leaf; its basal part is tightly rolled around the inner leaves of the bud. The next younger leaf is clearly visible (Pl. LXXIX, Fig. 1), its apex often extending well above the tubular

base of the outer leaf, and showing very distinctly the teeth with which the margin is beset.

These teeth occupy a position corresponding to the notches on the mature leaf. They are hyaline, conical in form, slope sharply forwards, and have a slight inclination towards the upper surface of the leaf (Pl. LXXIX, Fig. 4). On the next younger leaf—and frequently also on the one following—the teeth are as well developed as on the one described, though, owing to the small size of the leaf, they are more closely set. On the youngest leaf the teeth, though conspicuous, are obviously immature.

One fact is evident: the teeth are ephemeral structures which come rapidly to maturity, attain their full development on the second and third leaves, and shrivel and disappear, as soon as the leaf which bears them becomes, in its turn, the outermost envelope of the bud. Their function, whatever it may be, must be performed whilst the leaves are still immersed in their bath of watery fluid. Portions of the leaf—cleared with eau de Javelle—show clearly that each tooth is served by one or two, sometimes three, of the smaller veins (Pl. LXXIX, Fig. 4); these run to within a short distance of the base of the tooth, and there fray out into a number of tracheides. These are, however, continuous with a strand of epithemoid tissue which runs up into the core of the tooth. No water-pores are borne on the teeth, nor are any stomata found in their immediate neighbourhood. If the teeth are concerned with the excretion of water, the latter must find some other exit than a stomatal pore.

The epidermis of the young leaf has a moderately thick outer wall, though this is feebly—if at all—cuticularized. In the second leaf of the bud it has already divided to form the hypoderm, though the upper cells have not undergone the further division by vertical walls. The epidermis which covers the teeth, however, consists of thin-walled cells with rounded contours, not covered by an incipient cuticle, and with numerous definite *intercellular spaces* between them, communicating with the interior of the tooth (Pl. LXXIX, Figs. 5 and 8). The cells forming the core of the tooth are thin walled; those towards the outside are isodiametric, those in the middle are elongated in the direction of the length of the tooth (Pl. LXXIX Fig. 6). The tissue throughout is fairly closely compacted, but narrow intercellular spaces occur at the points where three or more cells meet (Pl. LXXIX, Fig. 7). The central elongated cells of the tooth are continuous with a strand of similar cells, which communicate directly with the terminal tracheides of the vascular bundles. This strand of epithem is not marked off from the mesophyll by a definite sheath of cells.

The leaf teeth of *Archytaea* are in fact hydathodes, though of a somewhat unusual type. The epithem tissue which forms the core, or even the whole tissue of the tooth, has the same relation to the vascular bundle as has been shown to exist in *Tropaeolum*, *Primula*, *Geranium*, and

many Rosaceae, Saxifragaceae, &c., by Haberlandt (2), Reinke (7), von Minden (5), and others.

There is, however, one important point of distinction, and that is that, instead of the water escaping by a well-defined pore between two epidermal cells of special construction, or by a group of such pores, it finds its exit by numerous small intercellular spaces. These are found between all the epidermal cells of the upper two-thirds of the tooth (Pl. LXXIX, Figs. 5 and 8).

At the base of the tooth no intercellular spaces are found between the epidermal cells or the two or three subjacent layers. These cells, moreover, have thicker cell-walls (Pl. LXXIX, Fig. 5), and sometimes show indication of divisions (Pl. LXXIX, Fig. 7).

It is these cells, forming a shallow plinth for the tooth, which later acquire the dark-coloured contents already referred to. They are able to resist the fate which overtakes the thin-walled cells of the tooth, when freely exposed to desiccation by the later growth of the leaf.

Solereder (8, page 133) describes *Camellia* as having leaf teeth which 'figure as secretory organs . . . they contain the termination of a vascular bundle in connexion with an epithemoid tissue, and above the latter a secretory epidermis of palisade-like radially elongated cells'.

This probably refers to an observation of Reinke's (7), who described the structure of the leaf teeth in *Camellia japonica*, and considered the epidermis of the tooth as a glandular tissue, which excreted a mucilaginous secretion through the cuticle.

It was unfortunately impossible to confirm this observation; the only material of *Camellia* available was in a condition too advanced to show functional leaf teeth. In some shoots of *Thea viridis*, kindly supplied by Messrs. Veitch, the young leaves showed prominent teeth with a clear hyaline, viscid apical portion. This terminal portion of the tooth consisted of a central core of cells, covered externally by an epidermis of palisade-like cells; the whole structure strongly resembling the glandular 'villi' figured by Hanstein (3), Groom (1), and others, and obviously corresponding to the teeth described by Reinke (7) in *Camellia*. No excretion of water was observed when a shoot was placed under a pressure of 60 cm. of mercury for four hours, but no definite conclusion can be drawn from this isolated observation.

Similar teeth have been described and figured by Virchow (12) and Tschirch (11) for *Thea sinensis*. In *Thea* and *Camellia* the teeth—even if they should function as hydathodes, which does not seem probable—cannot subserve the same functions as those of *Archytaea*. The buds are small, and the shoot elongates rapidly, so that the young leaves are early separated, and the teeth are apparently functional on leaves which no longer invest the bud.

The liquid which fills the space within the bud of *Archytaea* has been

referred to as water. Unfortunately no information is available as to the nature and reactions of the fluid. In the material, which was preserved in spirit, the space between the young leaves was partly occupied by a soft greyish substance, quite structureless, insoluble in water, alcohol, and ether, staining fairly readily with aniline dyes. There is every probability that this substance is coagulated mucilage, and that the fluid carried by the bud was a weak mucilaginous solution.

At any rate ample means exist for the secretion of the mucilage, in the shape of numerous colleters, which are found in the axils of the leaves, borne on the leaf bases (Pl. LXXIX, Figs. 9 and 10). These glands are club-shaped multicellular bodies, borne on narrow stalks, and consisting for the most part of small isodiametric cells with thick walls and abundant contents. There is no indication of columnar epidermal cells such as were described by Hanstein (3) in *Coffea* and *Viola*, and by Groom (1) in *Coprosma*, *Hoffmannia*, and *Gardenia*.

In the club-shaped part of the glands large irregular cavities occur close to the surface, which are occupied by a deeply staining contracted mass—possibly mucilage (Pl. LXXIX, Fig. 11). In some instances these cavities may be individual cells of large size, but in most cases they seem to have arisen by the fusion of a number of cells, possibly by the mucilaginous degeneration of their walls. The glands are always closely surrounded by the greyish coagulated substance above described, and it seems highly probable that they are largely concerned with its production.

The facts described above all point to the conclusion that the various structures are admirably adapted to serve for the protection of the young leaves from drought and excessive insolation. The structure of the teeth and their relation to the vascular system indicate very clearly their probable function as hydathodes, and water is certainly present in the living buds. The general construction of the bud and its upright position enable the water to be readily retained. The narrow spaces between the leaves and the mucilage secreted by the colleters obviate the danger of desiccation to which the young leaves and axillary buds would be exposed, if the bud became emptied of water, or for any reason the hydathodes failed to maintain a supply. The thick cuticle and numerous mucilaginous cells protect the leaves from excessive loss of water, after their emergence from their watery bath.

It is obvious that these conclusions can only be fully sustained by actual demonstration with living material, which is unfortunately not available to me.

Summary.

I. The leaf buds of *Archytaea alternifolia*, Szyszyl., have a trumpet-shaped structure, are upright and filled with water.

II. The young leaves possess a number of marginal teeth which are probably hydathodes.

III. The hydathodes are connected with vascular bundles by epithemoid tissue, and provision is made for the escape of water by intercellular spaces between the epidermal cells of the teeth.

IV. Colleters are borne in the axils of the leaves on the leaf bases. They are probably concerned in the secretion of mucilage.

V. A mucilaginous hypoderm is present towards the upper side of the leaf, and mucilaginous cells in the mesophyll and in the epidermis of the lower side.

In conclusion I desire to record my appreciation of Prof. Groom's kindness in handing me the material for investigation, and to express to him, and to Prof. Farmer, my thanks for much helpful advice and criticism.

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EXPLANATION OF PLATE LXXIX.

Illustrating Mr. Tabor's paper on *Archytaea alternifolia.*

Fig. 1. Entire leaf bud; leaf teeth are seen on the second leaf at *t*. Nat. size.

Fig. 2. Lower epidermis in surface view. Stomata with 3–5 subsidiary cells. *g* = guard cells, = subsidiary cells. × 200.

Fig. 3. Transverse section of mature leaf. *m.h.* = mucilaginous hypoderm cells with swollen stratified walls, *m.e.* = mucilaginous epidermal cells, *m.m.* = mucilage cell, *g* = guard cell, *s* = subsidiary cell. × 175.

Fig. 4. Portion of young leaf showing three teeth and related vascular strands. Cleared with eau de Javelle. × 28.

Fig. 5. One tooth from the same preparation as that from which the preceding figure was drawn. *i.* = intercellular space, *p* = thick-walled cells forming a plinth for the tooth. × 240.

Fig. 6. Horizontal section of margin of leaf, with tooth in nearly median section. The terminal tracheides of two vascular bundles are seen associated with narrow elongated cells of epithem tissue. *t* = tracheide, *ep.* = epithem, *i* = intercellular space. × 265.

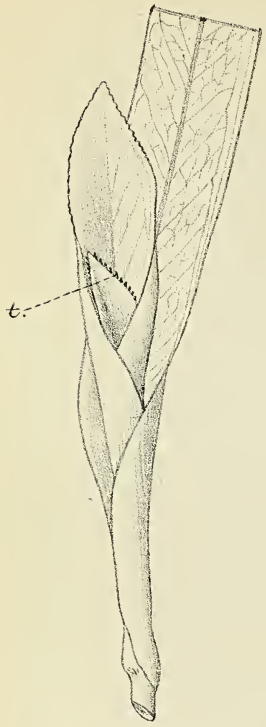
Fig. 7. Similar section in which the tooth was cut almost transversely near its base. In this and in the preceding section the tracheides and surrounding cells were torn by the razor. *p* = thick-walled cells at base of tooth, other lettering as in Fig. 6. × 300.

Fig. 8. Tangential section of the epidermis of a tooth with intercellular spaces. *e* = epidermal cell, *i* = intercellular space. × 600.

Fig. 9. Transverse section of bud immediately above the insertion of the outermost leaf. *st* = stem, *l.t.* = leaf traces, *l.b.* = leaf base, *b* = axillary bud, *c* = colleters. × 10.

Fig. 10. Longitudinal section of base of bud showing portions of five leaves. *t* = row of immature teeth on fourth leaf, other lettering as in Fig. 9. × 10.

Fig. 11. Transverse section of colleter. (Cell contents omitted in most cells.) *m.c.* = mucilage cavity. × 265.



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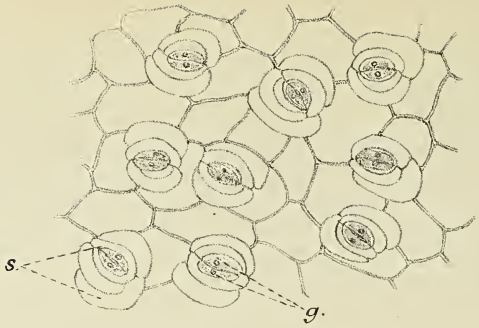

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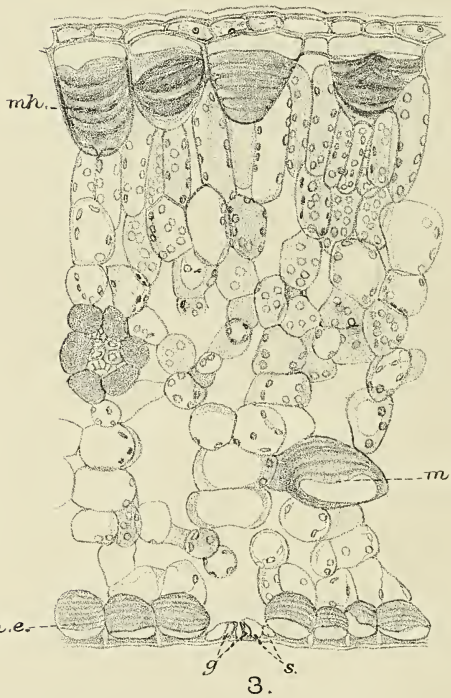

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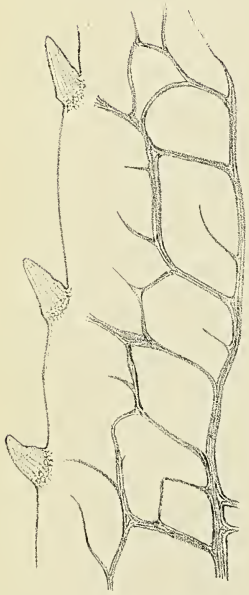

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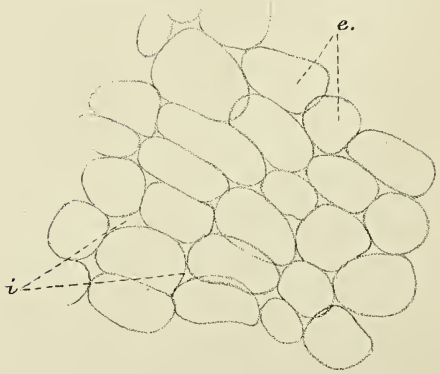

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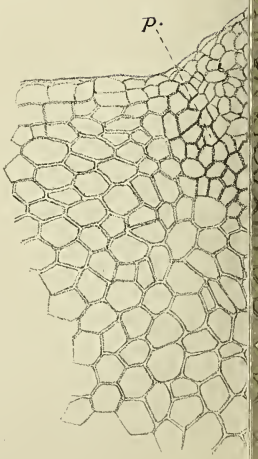

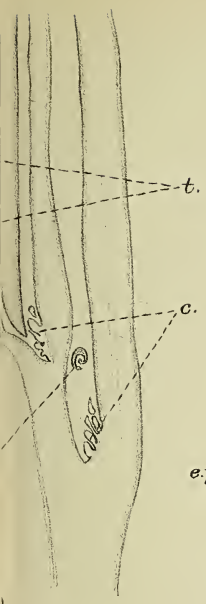

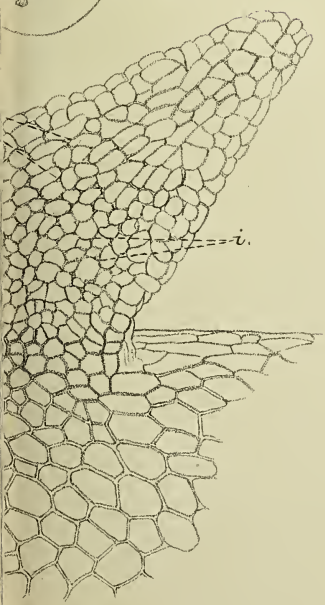

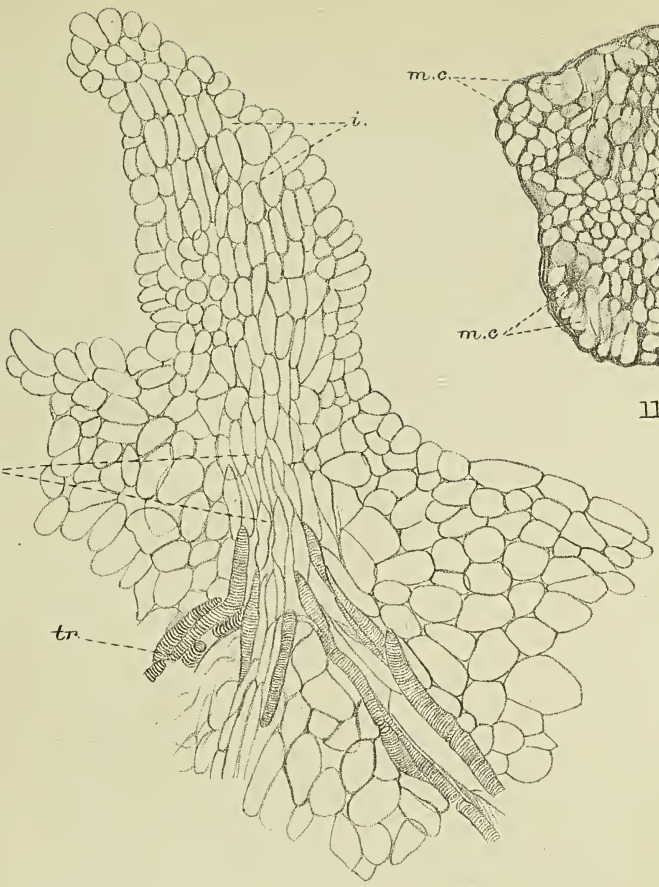

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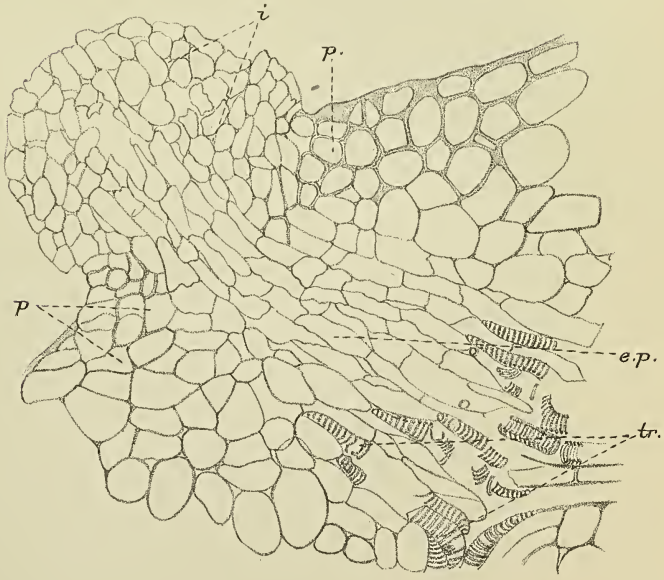

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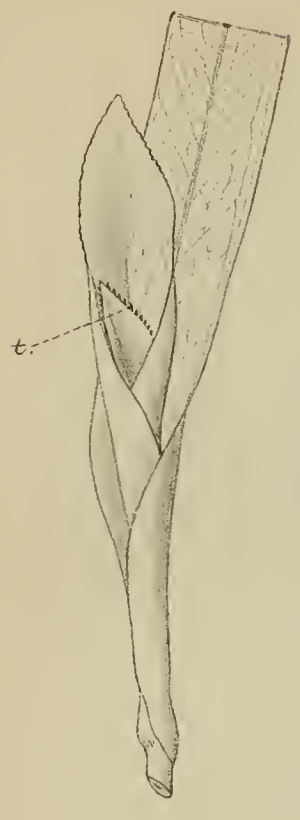
Huth, lith. et imp.



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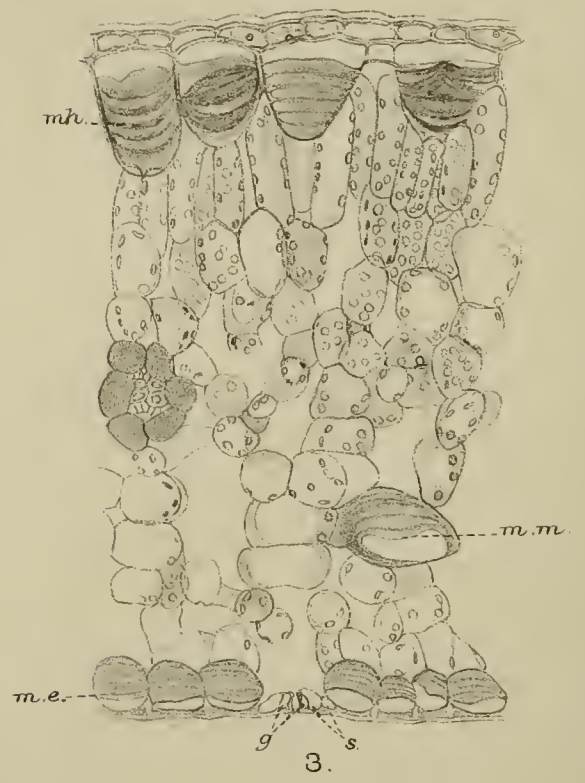

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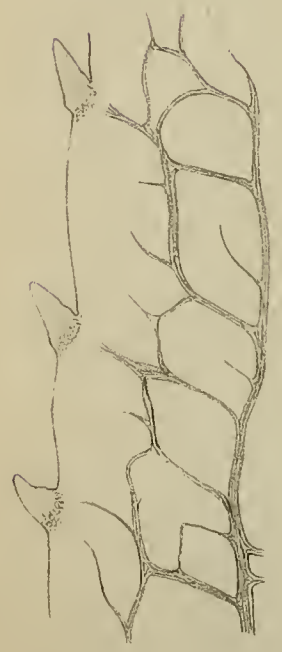

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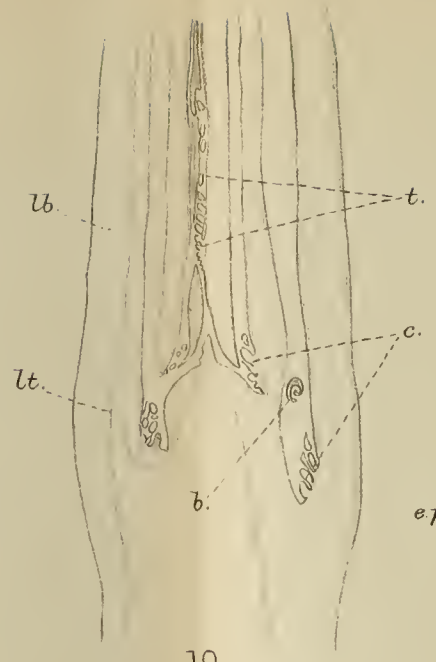

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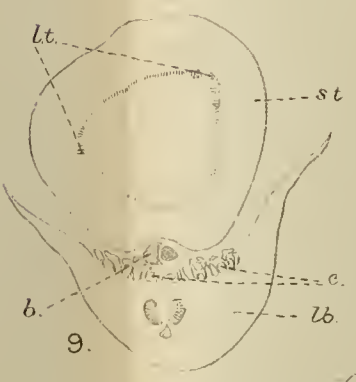

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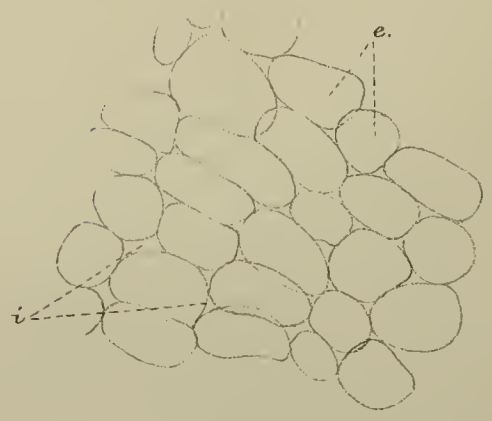

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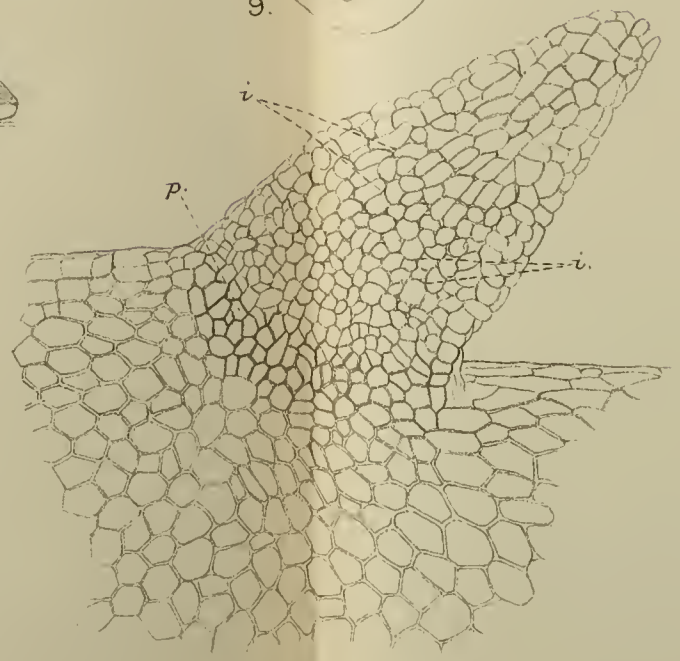

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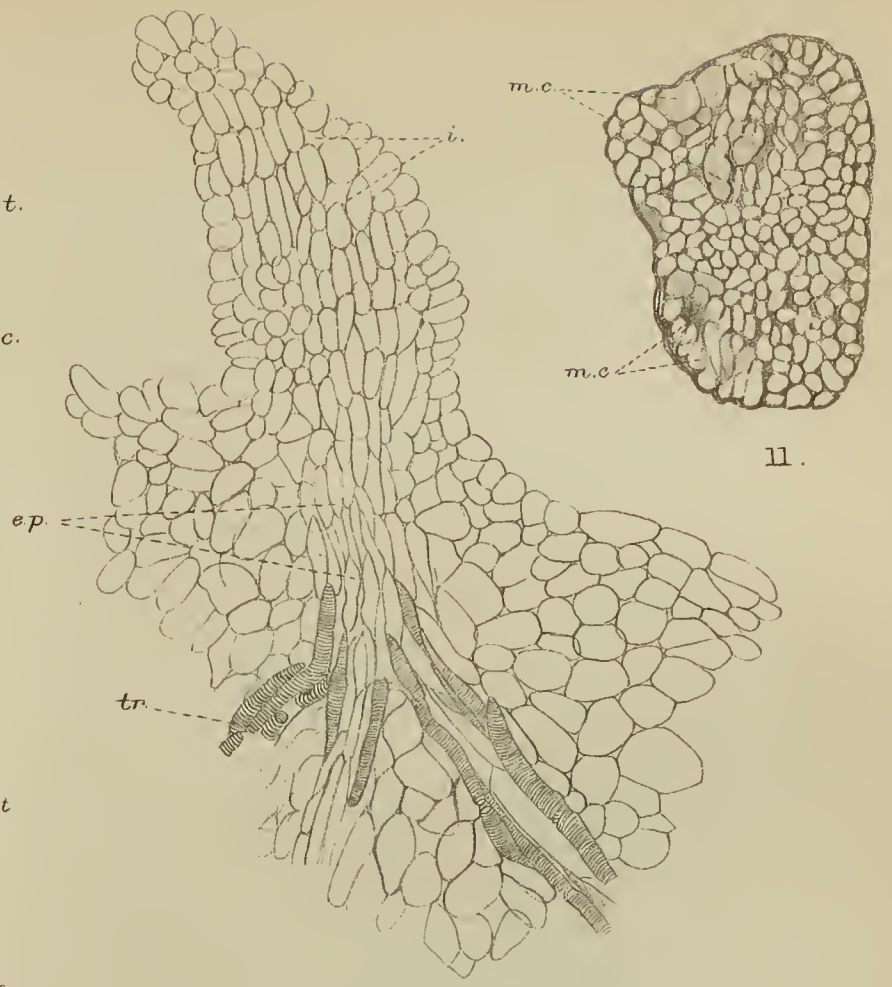

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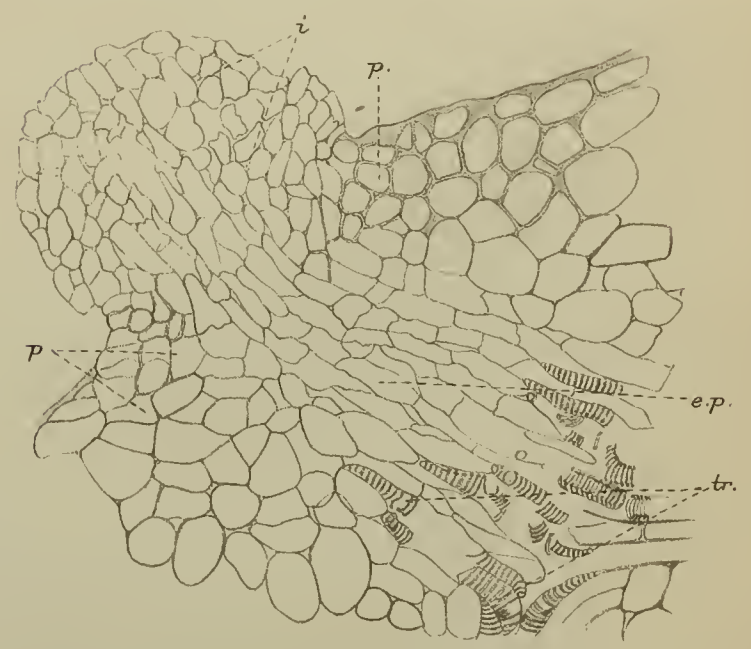

7.

TABOR — ARCHYTAEA.

Huth, lith et imp.

On Allomyces, a new Aquatic Fungus.

BY

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With Figures 1–18.

AMONG the aquatic Phycomycetes few are more interesting than the members of the family Leptomitaceae of Schroeter, filamentous Oomycetes characterized by the filaments being segmented through the presence of successive constrictions, with oogonia, when present, containing a single oosphere surrounded by periplasm. The family was included by Schroeter with the Saprolegniaceae and Pythiaceae in the order Saprolegniineae, but Thaxter ('06, p. 324) subsequently pointed out that its affinities with the Pythiaceae must cause it to be transferred to the Peronosporaceae if *Pythium* be united to the latter family.

It is probable, however, that the Leptomitaceae are more primitive forms than either the Saprolegniaceae or *Pythium*, and I have suggested ('07, p. 58) that they may show indications of affinity with the curious genus *Monoblepharis* and through it with the green Algae (Siphoneae or Oedogoniaceae). The number of forms known to belong to the family is small, and it is therefore of decided interest to encounter a new one with distinctive characters.

The Fungus occurs in Pusa in still (but not stagnant) water, and in Poona in river water, in both cases growing on dead flies, &c., which have fallen in. The plants may be single or two or more may occur together, the growth presenting to the naked eye the familiar appearance of a *Saprolegnia* colony. It can be readily isolated from accompanying Phycomycetes by suspending dead sterile flies or ants near the surface of a dish full of water in which a few sporangia ready to discharge are placed. It grows freely in such cultures, and is not much hindered by the development of Bacteria as in some of the allied forms.

The plant consists of a distinct basal part formed of several very large cells, sometimes arranged in a single column, sometimes in a dichotomously branched system. From the distal end of this, whorls of more slender

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fertile branches arise, which bear the reproductive organs, sporangia and resting spores. From the basal cell numerous fine branched rhizoids penetrate the substratum. The most striking character of the plant, in view of its being an undoubted Phycomycete, is that it is distinctly multicellular, all parts, except the rhizoids, being regularly septate.

The various parts may now be described in detail.

The basal portion is anchored to the substratum by a branching system of rhizoids arising from the lowermost cell. The lowest cell itself is variable in shape, sometimes almost square, sometimes oblong or clavate (Figs. 1–3). It measures from 100 to 200 μ in length by 60–100 μ in breadth, and is thick-walled, but not rigid. Above, it is either continued into a short column of three or four similar cells (Fig. 3), or branches dichotomously to form a fan-shaped body of large cells (Fig. 2). These again branch upwards, the succeeding branches being more slender and the tendency to dichotomy being usually lost. Frequently, whorls of three branches arise from the uppermost of the basal cells, but there is no regularity about this; dichotomy may be preserved for some time or the branches may arise at different levels from near the end of the basal cells. Each branch is cut off by a septum at the point of origin, and after the mass of basal cells is left, any branch may terminate in a sporangium, growth being usually renewed just below the sporangium, and the latter being then pushed a little to one side.

The fertile branches radiate from the basal cells and are composed of septate hyphae, the cells reaching an extreme length of 250 μ, with a breadth of 15–25 μ. Usually a terminal sporangium is formed before growth has proceeded very far, and the axis is continued by a branch arising just below the sporangium, somewhat as in *Phytophthora vexans*. This may be repeated many times, each fertile axis thus forming a sympodium, as in Fig. 5; or branching of a more or less dichotomous type may occur, as in Fig. 4. In this case, as probably in all other cases of the sort amongst the Fungi, one of the branches arises a little behind the apex of the filament, apparent equality of origin being only subsequently reached. Ultimately the apex of every branch ends in a sporangium or chain of sporangia or a resting spore. The walls of the fertile branches are thin. The septa are formed regularly behind the growing apex, but secondary septation may occur at a later stage. Usually one develops at or near the origin of each branch. Sometimes they occur at more or less regular intervals throughout the axis, sometimes several are formed close together below a sporangium (Fig. 1). They appear to be laid down in interrupted plates from the periphery of the tube (Fig. 17, *c*). Usually they are complete, but rarely a small pore appears to be left in the centre, as in some, at least, of the higher Fungi. In no case observed was the septum reduced to a mere annular thickening, such as occurs in *Gonapodya*. The segments

are usually slightly constricted at the septum, except at the origin of a branch.

The sporangia are formed in great abundance, either singly or in basipetal chains, at the ends of the hyphae. They are ovoid or barrel-shaped cells measuring 40–70 by 30–40 μ, with thin walls and granular hyaline contents. They are always inserted by a broad base on the ends of the fertile hyphae, from which they are cut off by a well-developed septum. Rarely, after a sporangium has formed at the end of a hypha and the axis has continued to grow from below it, a segment immediately beneath the origin of the branch becomes transformed into a sporangium. Fig. 1, *a*, shows such a case, the lowest sporangium having been formed after the upper was cut off and a new branch had arisen below it. Similarly, two sporangia may appear to arise side by side from the lowest of a chain so as to result in the production of a branched chain. In this case also the lowest is the last formed. By a repetition of subsporangial branching with early formation of new sporangia, rather complicated clusters of these organs may be formed, Fig. 6 showing one of the types met with. The contents of the mature sporangium are hyaline and granular. The papillae of discharge appear quite soon and are usually several in each sporangium. Fig. 7 shows a chain of ripe sporangia with the papillae formed and ready to burst. Usually there are two to four, less often only a single terminal one. They are large, very brightly refractive, and evidently formed of a protusion of the softened sporangial wall. They generally rupture simultaneously, or almost so, to emit the zoospores.

The zoospores are formed before the rupture of the papillae, being visible as rounded masses tightly filling the sporangium. The papillae then dissolve without any trace of the formation of a vesicle such as occurs in other Leptomitaceae (*Rhipidium*, *Araiospora*, *Sapromyces*). At first the zoospores pass out rapidly, though singly, separating immediately on exit. Later, as the crowd within becomes less dense, they emerge slowly, often with a more or less creeping movement. The last few left within the sporangium wander slowly, and with frequent amoeboid movements, around the walls and cavity, ultimately becoming engaged in one of the openings and creeping out. After exit, the zoospore lies near the sporangium for some time, up to five or ten minutes, and goes through a series of remarkable amoeboid changes. As it emerges it has usually the shape shown in the upper part of Fig. 10, a hyaline cap occupying the anterior end of the elongated body, and a single long and moderately stout cilium the posterior. The subsequent changes in shape are shown on the left-hand side in Fig. 10, drawn from a single spore. During these changes it is sometimes difficult to see the cilium, but it appears to be persistent. At the end of the amoeboid period the zoospore takes on the shape shown at the bottom of Fig. 10, and swims rapidly away. The swarming period

may last up to about half an hour, when the spores come to rest, still waving slowly their cilium, round off, the cilium disappears, and the spore surrounds itself with a wall. Germination is visible often within an hour of discharge, the germ-tube being single, slender, and branched. The zoospores are monoplanetic. I have made a most careful search for biciliate zoospores, the cilia being well fixed, and stained with iodine, and easy to see, and there is not the slightest doubt that in my cultures the spores were uniciliate. The point is necessary of emphasis on account of the importance sometimes attributed to this character, and also because of the observations made on *Blastocladia*, which appears to be a nearly allied genus. The latter has usually biciliate zoospores, but in some cases Thaxter, who has most closely studied it, failed to make out more than one cilium. Reinsch, who first observed it, did not describe the characters of the zoospores. The only other observer who appears to have encountered it is Petersen, who states that the zoospores are amoeboid and uniciliate. The remarkable triangular nucleus described by Thaxter in the zoospores of *Blastocladia* was not observed in the Indian Fungus.

Besides sporangia a second type of reproductive body occurs which is of much interest. This is a sort of resting spore of distinctive shape and structure. It is formed invariably at the end of peripheral branches of the fertile hyphae, and appears later than the sporangia, though mature cultures bear both organs. The early stages of its formation are just as in the sporangium, the end of the hypha swelling up into a club-shaped body which is cut off by a septum. Within this the resting spore develops as a thick-walled, brown cell, truncate pear-shaped and with a sculptured wall. Frequently this cell fills the swollen end of the hypha so completely that the investing wall is merged in the exospore of the resting spore. In other cases a space is visible between them which is generally most distinct on the side of the septum, but is also often seen right round the spore. The resting spore is thus formed free within the terminal cell of the hypha, a character which removes it far from the category of conidia such as those of the Peronosporaceae. It is set free by the rupture of the enclosing membrane at a late stage in the life of the plant (Fig. 15) or, in those cases in which the membrane is closely applied to the exospore of the resting spore, by the abjunction of the whole upper segment of the hypha. The resting spore is yellowish brown when young, becoming a rich deep brown when mature. Its wall is composed of two layers, an outer, thick, brown and with distinct markings and an inner, thin, structureless and probably hyaline, though the latter character would only be determinable in an actual section which was not obtained. The structure of the exospore is exactly similar to that described by Thaxter for the resting spore of *Blastocladia*, and, as his description is full, need not be further referred to. In those cases where the wall of the containing cell of the spore is closely

applied to the exospore and thrown off with the spore, it is visible on careful focusing as a thin hyaline third wall external to the others (Fig 13).

The diagnosis of this Fungus, for which I propose the name *Allomyces arbuscula*, n.g., n.sp., is as follows:—

Allomyces, n.g. Plant consisting of a large-celled basal body or foot, branched above, from which arise the slender fertile filaments; filaments septate, branched sympodially or dichotomously, branches terminating in sporangia or resting spores; sporangia single or catenulate, ovoid, with several papillae of discharge; zoospores emerging singly, without formation of a vesicle, large, oblong or elliptical, 1-ciliate, amoeboid at first, monoplanetic; resting spores terminal, ovoid, thick-walled, formed within the terminal cell of the filament and set free by its rupture, or sometimes completely filling it and then thrown off with the terminal cell, germination not seen; membranes of all parts without cellulose.

Allomyces arbuscula, Butl. Basal body of large cells 100–200 by 60–100 μ, attached below by rhizoids to the substratum, and one or more times dichotomously branched above; fertile filaments arising in groups of two or more from the end segments of the basal body, septate, cells up to 250 by 15–25 μ, sympodially or less often dichotomously branched, each branch terminated by a sporangium or chain of sporangia or a resting spore; sporangia broad elliptical, or ovoid, 40–70 by 30–40 μ, ends bluntly rounded or truncate, with one to four papillae of discharge; resting spores single, terminal, truncate below, rounded above, with thick brown pitted exospore, 40–60 by 30–45 μ.

The affinities of this Fungus must now be considered. The differentiation of the thallus into a dilated basal part and more slender fertile branches is a feature common to several genera of the Leptomitaceae (*Rhipidium*, *Araiospora*, *Sapromyces*) and also *Blastocladia*, the position of which is doubtful. This basal cell of certain Leptomitaceae is a structure which has appealed to all who have studied the group as of great taxonomic importance. Nothing like it is known in the other Phycomycetes. Usually it is a single cell which may be variously branched or lobed. In *Rhipidium americanum* the branching is more or less dichotomous, and if septation were to occur the result would resemble in all essential respects the basal body of the new Fungus. The same holds good for *Blastocladia* to an even greater degree. Hence, in spite of the multicellular structure of the basal body of *Allomyces*, it must be held to represent the basal cell of these genera.

The segmentation of the mycelium is a distinguishing feature of the Leptomitaceae, including *Gonapodya*, but not *Blastocladia*. It is unknown in other Phycomycetes except the Ancylistaceae, where every segment

becomes a reproductive organ. The nearest approach to it is found in the Saprolegniaceae, in several of which the old mycelium may segment to form 'gemmae' and in one case, *Saprolegnia torulosa*, de Bary, segmentation is normal. This segmentation is, however, of quite another order to that of the Leptomitaceae (cf. Maurizio, '96). In the latter group the segmentation is not a phenomenon of budding and only rarely is the interruption between successive filaments complete; usually a central passage, through which the protoplasm of adjacent segments is in direct communication, is left. In *Rhipidium continuum* and some plants of *Gonapodya polymorpha* the segmentation is obscure or lost. In *Apodya lactea*, however, the passage may be entirely closed by a deposit of cellulin; the process has been fully described by Pringsheim ('85). In *Gonapodya* something similar occurs, according to Thaxter, while Petersen's figures ('10, pp. 533–4) show complete interruption. In the cases examined by Pringsheim the passage is blocked by one or more cellulin corpuscles which move into it from the adjacent segments and become fused with the walls. In the new Fungus the process is different, a distinct membrane being thrown across the cell by an ingrowth or deposit which begins at the periphery and gradually extends to the centre (Fig. 17, *c*). Nevertheless, in its formation it is probable that the cellulin granules take part. These bodies are numerous and distinct and become massed at the point where the septum is developing (Fig. 17, *a*, *b*). They are ultimately divided into an upper and a lower group by the formation of the septum. The resemblance to what occurs in the differentiation of the sporangium in *Saprolegnia*, as described by Rothert ('90), is considerable, though in the latter case the whole septum appears simultaneously and not by an ingrowth from the periphery. It is clear that the case represents a more advanced stage in the segmentation of the Leptomitaceae and is not comparable to the true septation of the higher Fungi. The resulting septum is usually thin and similar to the cell-walls, but it is sometimes thick and irregular, as in *Apodya* (Fig. 17, *e*).

The sympodial axes of the fertile hyphae, the basipetal development of the sporangia, and the position of the reproductive organs are minor, but interesting points of affinity between the Fungus above described and the Leptomitaceae. *Rhipidium* and *Apodachlya* should be compared on these points.

The peculiar resting spore is more important and its homology with that of *Blastocladia* evident. *Apodachlya*, an undoubted member of the Leptomitaceae, is the only other Oomycete with thick-walled resting spores which can at all be compared with these. The conidia of *Pythium* and the higher Phycomycetes are merely modified sporangia, and hence belong to another spore category.

In view of the above characters, the inclusion of the new Fungus amongst

the Leptomitaceae is inevitable if we are to unite it to any of the recognized families of the Phycomycetes.

There are, however, two characters of considerable weight which the new Fungus possesses in common with *Gonapodya* and less definitely with *Blastocladia*, which separate these three forms widely from the other Leptomitaceae. These are the composition of the cell membrane and the character of the zoospores.

Of the authors who observed the two latter genera, Reinsch and Thaxter do not mention the composition of the membrane. Cornu ('72, p. 15) stated that *Monoblepharis* (in which he included Reinsch's *Saprolegnia siliquaeformis* = *Gonapodya siliquaeformis*, Fischer) is devoid of cellulose. Recently Petersen states that this is the case with both *Blastocladia* and *Gonapodya*. The new Fungus gives a reddish yellow colour with chloriodide of zinc, the wall of the resting spores being particularly deeply stained, and can be sharply distinguished by this means from Saprolegniaceae (*Saprolegnia* and *Achlya*), with which it is growing, the latter taking a clear blue colour. It is therefore in this respect more closely allied to *Blastocladia* and *Gonapodya* than to the other Leptomitaceae. *Monoblepharis* is the only other Oomycete giving a similar reaction.

As regards the zoospores, the form observed in Pusa was constantly 1-ciliate. *Gonapodya* also has 1-ciliate zoospores, according to Cornu and Thaxter, though the latter states that they have sometimes seemed to be 2-ciliate. Reinsch and Petersen do not specifically remark on this point. In *Blastocladia* there appears to be some doubt. Thaxter describes the zoospores as normally 2-ciliate, but sometimes only one cilium can be found; Petersen says that they are 1-ciliate. In shape and behaviour all three are allied, those of *Gonapodya* in particular having, like *Allomyces*, a short period of amoeboid movement after emerging from the sporangium. In the other Leptomitaceae the normal 2-ciliate zoospore of the Oomycetes is found.

These three forms are therefore similar in that they possess certain important characters differentiating them from the other members of Schroeter's family Leptomitaceae. Whether *Gonapodya* and *Blastocladia* were rightly included by Schroeter in this family is uncertain. Thaxter ('96, p. 325) doubtfully includes *Gonapodya*, but excludes *Blastocladia*. Petersen makes a separate family for each, Gonapodyaceae and Blastocladiaceae. *Allomyces* is clearly allied to the latter and serves to unite it more closely with the true Leptomitaceae. On the whole, though the character of the membrane is such as to prevent dogmatism, the new form tends to strengthen Schroeter's view and to indicate real affinity within the limits of the family as defined by him.

In 1907 I suggested that the peculiar Phycomycete *Monoblepharis*, the only known Fungus with motile sperms, was related more to the Leptomi-

taceae than to any other family. Is there any hint in the Fungus above described of affinity with this isolated genus? I think so. The resting spore, formed free within a cell of the mycelium, is something new amongst the Oomycetes, so much so that the possibility of there being a fertilization by antherozoids, as in *Monoblepharis*, requires to be carefully considered. I do not think that anything of the sort can occur. These bodies were formed in great abundance in the cultures, so that fertilization even by small motile sperms was unlikely to have been overlooked. Besides, all the sporangia give rise to zoospores of uniform type, and nothing was seen suggesting antheridia and sperms. In *Blastocladia* the same idea was present to Thaxter, but he failed to find anything in favour of it. Yet the resting spore of *Blastocladia* is certainly of the same type as that above described. What then are these bodies, so unlike any asexual spore known in the Oomycetes? I think they are parthenogenetic oospores, derived from a form resembling *Monoblepharis*.

In *Monoblepharis*, oospores sometimes develop without fecundation. Lagerheim figures such cases ('00, Figs. 55, 56), the spore remaining within the oogonium, though the fertilized oospores of the same species (*M. brachyandra*) emerge after copulation. In other species (e. g. *M. insignis*, Thaxter) the fertilized oospore remains within the oogonium. A consideration of the published figures of these species will I think show that there is nothing which militates against the homology here suggested. Certainly it is difficult to think of any other spore form in the Phycomycetes with which to compare these bodies. In other respects, *Monoblepharis* has strong points of resemblance to the three aberrant genera of the Leptomitaceae whose affinities have been referred to above. Like them its zoospore is, at least sometimes, 1-ciliate; its very variability in this respect recalls Thaxter's difficulty with the cilia of *Blastocladia* and *Gonapodya*; all other Oomycetes have 2-ciliate zoospores. Like them its membrane is devoid of cellulose; all other Oomycetes have cellulose walls. Like *Allomyces* its sporangia are basipetally formed, and its axis is often markedly sympodial (cf. Lagerheim, '00, Taf. ii, Fig. 16). The peculiar alveolate protoplasm of the vegetative hyphae or *Monoblepharis* is not marked in *Gonapodya* or *Blastocladia*, but is sometimes visible in the new form (Fig. 18). As further strengthening the relationship we have the imperfectly known Fungus, called by Lagerheim *Monoblepharis regignens*. This has the alveolate protoplasm of the latter genus, but the proliferous siliqua-shaped sporangia of *Gonapodya*. It has 1-ciliate zoospores, other reproductive organs being unknown. Whether this is a true *Monoblepharis* or not, it is a further connecting link between that genus and *Gonapodya*. There seems to be no longer any reason for assigning to *Monoblepharis* an isolated position amongst the Phycomycetes. It is connected, not very distantly, with a group of genera with non-cellulose walls, and these again are con-

nected by many essential characters, through *Allomyces*, with the typical Leptomitaceae.

Of this series *Monoblepharis* must be taken, I think, to preserve the most primitive characters in its peculiar sexual processes. Of recent years algologists have been more ready to admit its affinities with the green Algae than mycologists. Fischer ('92), Schroeter ('99), Dangeard ('06), and Atkinson ('09) in particular derive the whole of the Phycomycetes from the Monadineae, Flagellatae, or Protococcoideae. The most recent and perhaps the most thorough exposition of this view is Atkinson's. He vigorously combats the older idea that the Ancylistaceae and Chytridiaceae have been derived from saprophytic Phycomycetes through the debasing influences of parasitism. This is clearly not capable of direct proof, and there are numerous instances to be drawn from other groups in support of either argument. He attaches great importance to the phenomenon of diplanetism of the zoospores and traces its development from a simplified process in true Chytridiaceae, such as *Rhizidiomyces*, to its full perfection in the Saprolegniaceae. But a similar origin may be found for it amongst Algae, such as *Chlorosphaera*, *Chlorochytrium*, *Ulothrix*, &c., and it is in any case doubtful if the simplified process referred to has anything to do with true diplanetism. He does not attach much importance to the number of cilia on the zoospores as a basis of classification, nor does he consider that variability in this character need militate against the monophyletic view. A consideration of the conditions found in the Leptomitaceae in this respect certainly supports him here. *Monoblepharis* is not in his opinion a primitive Fungus, but may have developed its peculiar sexual processes independently, through change in the function of the zoospores. In the other Phycomycetes the sexual processes of *Pythium* and the Ancylistaceae are obviously allied to one another, but it is difficult to follow him when he connects these with the Chytridiaceae through *Polyphagus* and *Zygorhizidium*. He has possibly overlooked Dangeard's ('00) account of the cytology of *Polyphagus*, which is monoenergid in the vegetative and gametic stages. *Zygorhizidium* again appears to be monoenergid, while the sexuality of other Chytridiaceae is doubtful. Finally, after a review of the developmental characters of the two groups, Atkinson concludes that there are more points of similarity between the higher Phycomycetes and the Chytridiaceae than between the former and any of the Algae which have been suggested as their probable ancestors. This is undoubtedly true so long as *Monoblepharis* remains in the isolated position usually assigned to it by mycologists.

Petersen ('10) also considers that it is possible to unite the Chytridiaceae and Oomycetes in direct relationship, but he inverts the developmental line above suggested, and believes that the Chytridiaceae are degenerate members derived from the level of the Saprolegniineae (including the

Leptomitaceae) or *Monoblepharis*. This suggestion was first made by de Bary and has since been adopted by many botanists. Petersen admits that possibly the 1-ciliate series is derived from certain Algae, without any intermediate stage of myceliated Phycomycetes. If the latter, then the algal ancestors of the 2-ciliate series must have been closely related to those of the 1-ciliate forms. It would take too long to follow him here, but one point deserves special attention. Like Atkinson he does not consider that the 1-ciliate and 2-ciliate species need necessarily indicate two distinct groups, but there are nevertheless differences between them of great importance. He makes the general statement that the membrane of all the species with 2-ciliate zoospores contains cellulose, while most of the 1-ciliate forms are without this substance, and only a few (endophytic) forms have traces of it. If this is true (and we have seen above that it holds good for the Leptomitaceae) it is probably a generalization of great significance.

Vuillemin ('07) and Lotsy ('07) have been still more impressed by the differences between the 1-ciliate and 2-ciliate groups. They refer the latter to the series of the Isocontae of the green Algae. But Vuillemin adopts the Monadine theory of the origin of the 1-ciliate series, and Lotsy derives them from many lower organisms, more simple than the Isocontae. To both, *Monoblepharis* is a stumbling-block. Vuillemin ('07, p. 106) says of this genus: 'C'est un genre exceptionnel par ses organes reproducteurs comme par ses organes sexuels. Il a des allures de genre primitif avec ses spermatozoïdes, uniques parmi les champignons filamenteux. Il n'a pas de parenté plus plausible avec les autres champignons qu'avec les Algues vertes, telles que les *Œdogonium* auxquels Lagerheim le compare, ou avec les *Vaucheria* qui en seraient les ancêtres d'après Thaxter. Il est donc à souhaiter que ses affinités soient précisées par la découverte de formes nouvelles.'

I think that a consideration of the characters of the Leptomitaceae brought into evidence by the new Fungus above described may help to diminish these difficulties. On the one hand, *Monoblepharis* is brought into probable relationship with a group of aquatic Phycomycetes in which the zoospore may be 1- or 2-ciliate, the thallus segmented or not, and the membrane with or without cellulose. On the other, the members of this group show marked signs of affinity with the green Algae. The form described by Ernst ('02) as *Dichotomosiphon*, an ally of *Vaucheria*, is extraordinarily like *Allomyces* in several respects. From a common stalk fertile branches arise, chiefly dichotomously. These branches are constricted at their origin and segmented at intervals by a ring which does not entirely occlude the passage. The cell-wall gives the ordinary reactions of cellulose, except that it is coloured yellow by chloriodide of zinc. Though this Alga forms starch, its nearest ally *Vaucheria* does not. The position of

its sexual organs resembles that found in several Leptomitaceae, but fertilization is effected by 2-ciliate spermatozoids, and so far as is known resembles the process in *Vaucheria*. The asexual reproduction is so far only known through the formation of 'gemmae', which are held to be sporangia reduced to conidia as occurs in the higher Phycomycetes.

While, therefore, it is not safe to be dogmatic in regard to the origin of the Oomycetes, the discovery of new forms (*Dichotomosiphon*, *Allomyces*) strengthens the old view of their derivation from the Siphoneae. Only by further discoveries can this view be proved or disproved. Perhaps also the equally obscure question of the origin of the Chytridiaceae can only be solved in the same manner.

Summary.

In the above paper a description is given of an Indian aquatic Fungus, *Allomyces arbuscula*, n.g., n.sp., belonging to the family Leptomitaceae of Schroeter. It is an aberrant member of the family, inasmuch as it has a completely segmented thallus. Its nearest ally is probably *Blastocladia*, and with this genus and *Gonapodya* it constitutes a group of forms with non-cellulose walls and predominantly 1-ciliate zoospores. This group is believed to show decided indications of affinity to *Monoblepharis*, the peculiar resting spores of *Allomyces* and *Blastocladia* in particular being held to be parthenogenetically developed oospores derived from the *Monoblepharis* type. The Leptomitaceae are probably a more primitive family than either the Saprolegniaceae or *Pythium*, and have perhaps been derived through forms resembling *Monoblepharis* from the Siphoneae amongst the green Algae.

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EXPLANATION OF FIGURES 1–18.

Illustrating Dr. Butler's paper on *Allomyces*.

Fig. 1. Single plant showing basal body, rhizoids, and fertile branches which bear sporangia and a few resting spores. At *a* the lowest sporangium was formed after the upper was cut off and the axis had continued to grow from below it.

Fig. 2. Much branched basal body; branching dichotomous.

Fig. 3. Unbranched columnar basal body.

Fig. 4. Sub-dichotomously branched fertile hypha.

Fig. 5. Sympodial fertile hypha.

Fig. 6. Cluster of sporangia at the end of a fertile hypha.

Fig. 7. A chain of ripe sporangia with papillae formed, just before discharge.

Fig. 8. A chain of irregularly shaped sporangia.

Fig. 9. Sporangium discharging zoospores through three openings.

Fig. 10. Zoospores. At the top five zoospores just after emergence, showing the elongated shape, hyaline cap, and single posterior cilium; on the left amoeboid changes in shape of a single zoospore shortly after emergence, cilium not shown; at the bottom four zoospores in the final shape taken during the period of active swarming.

Fig. 11. Zoospore germinating with a branched germ tube.

Fig. 12. Resting spore in surface view.

Fig. 13. Details of the wall of a resting spore which completely filled the terminal cell of the hypha. The outer thin wall is that of the hypha; next is the thick exospore with pits (?) seen in optical section as dark bands; inside the structureless endospore.

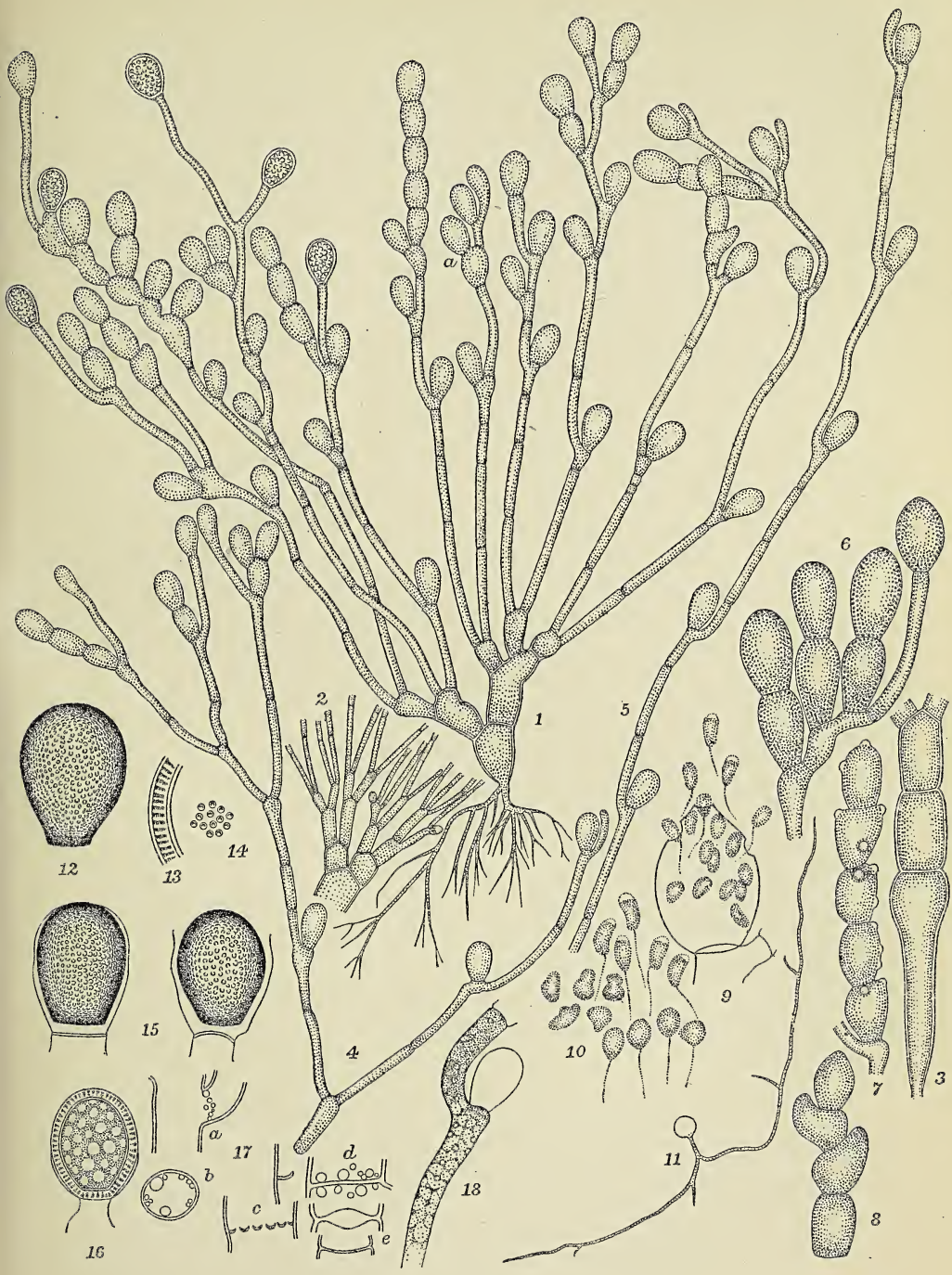
Fig. 14. Surface view of the wall of the exospore showing the pits (?).

Fig. 15. Two resting spores formed free within the terminal cell of the hypha; that on the right is being set free by the rupture of the hyphal wall.

Fig. 16. Resting spore completely filling terminal cell, the wall of which cannot be made out except at the lower part of the spore. Optical section through middle of spore.

Fig. 17. Details of septum formation. (*a*) Cellulin bodies collected at the origin of a lateral branch. (*b*) Same in surface view. (*c*) Peripheral interrupted plate in surface and profile view. (*d*) A septum of the basal body completed, with cellulin bodies grouped above and below. (*e*) Mature septa, upper unusually thickened, lower of the type ordinarily found in the fertile hyphae.

Fig. 18. Structure of protoplasm of a fertile hypha; lower portion reticulate, upper finely granular.



ALLOMYCES ARBUSCULA

The Vestigial Axillary Strands of Trichomanes javanicum, Bl.

BY

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With Plate LXXX and five Figures in the Text.

Introduction.

In some specimens of *Trichomanes javanicum* collected by Dr. M. Benson in Fiji, there were observed numerous examples of the curious vestigial axillary strand recorded in an allied species by Boodle.[1] Boodle mentions the axillary strand, together with the fact that it fuses with the leaf-trace during its passage through the cortex; but he does not give any details of its structure nor of its ultimate fate beyond the following general statement: 'Axillary branches occur very generally in the Hymenophyllaceae; at many nodes, however, the rudiment of the axillary branch may remain undeveloped.'

The axillary shoot first discovered by Stenzel, and later recorded in other species of *Zygopteris*, is discussed by Scott in his Studies, where he mentions (p. 312) that 'the axillary strand passed out into a cylindrical appendage placed exactly in the axil between stem and leaf'. The presence of a comparable structure has been suggested by Gwynne-Vaughan[2] in *Helminthostachys zeylanica*, and by Bruchmann[3] in *Botrychium lunaria*.

As the phenomenon seemed worthy of further investigation, especially with reference to the origin and ultimate fate of the strand, I determined to make a detailed examination of the new material. For this purpose microtome sections have been made through the apex of one plant, and through the bases of petioles. From some of the series longitudinal diagrams have been constructed.

Habit of Trichomanes javanicum, Bl.

Trichomanes javanicum possesses an upright stem, about 2·6 mm. in diameter, which bears strong bipinnate fronds and many interlacing roots.

[1] Boodle: Anatomy of the Hymenophyllaceae. Annals of Botany, vol. xiv, 1900. Whether this strand develops or not is left an open question by Boodle, loc. cit., p. 470.

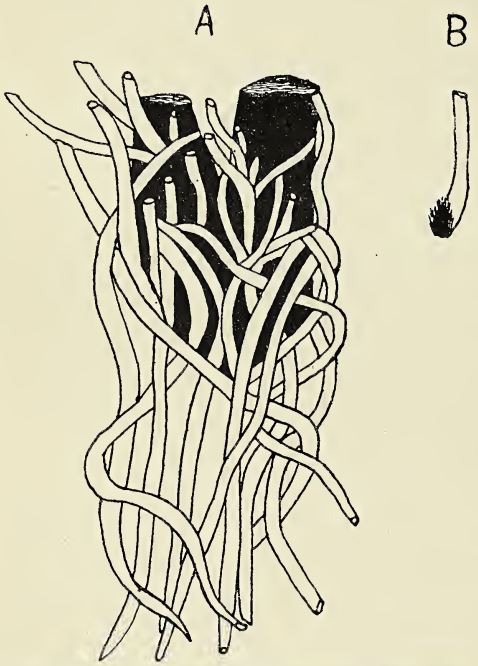
[2] Gwynne-Vaughan: Annals of Botany, vol. xvi, p. 170.

[3] Bruchmann: Flora, 1906, p. 226.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

No cases of normal branching have been observed in *T. javanicum*. Text-fig. 1, A, shows a curious case in which two stems, bearing about a dozen petioles, appear to dichotomize owing to the fact that they have grown in close proximity, and have become connected by the interweaving of their roots.

This erect unbranched habit seemed at first to give the clue to the meaning of the abortive axillary branch. The presence of axillary strands is described by Boodle [1] in *T. Prieurii*, which has an erect stem. But Gwynne-Vaughan [2] has described the occurrence, in a dorsiventral rhizome of *Helminthostachys zeylanica*, of canals leading from the axil of each leaf to the stele of the rhizome. Here each is met by a conical projection of parenchyma surrounded by endodermis. These, he suggests, represent vestigial axillary buds. Bruchmann [3] found similar bodies on young plants of *Botrychium lunaria*, which develop into lateral branches. He thus confirmed Gwynne-Vaughan's suggestion. The phenomenon is not, then, confined to forms with erect habit. Bower [4] suggests that there are two ways in which underground-growing organisms can secure perennation; the first is by repeated branching and the appearance of some of the branches above ground for a time; the second method is by suppression of branching in the stock and enlargement of the few leaves. The suppression of branching in the forms under consideration is probably connected with growth underground, and is not correlated specially with either erect or dorsiventral habit.



TEXT-FIG. 1. A. Habit of *Trichomanes javanicum*. B. Petiole, showing tongue of tissue at the base.

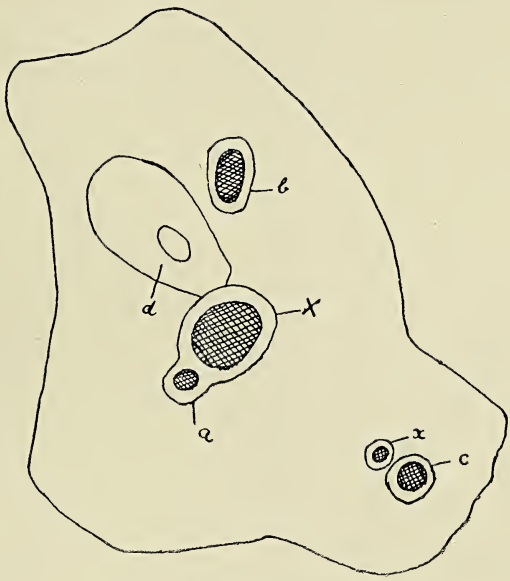
STELAR ANATOMY OF T. JAVANICUM.

The stem possesses a central stele composed of a mass of xylem interspersed with parenchyma. The tracheides are nearly uniform in size, so that it is impossible to distinguish the protoxylem. The xylem is surrounded by a continuous ring of phloem, outside which is the pericycle and

[1] loc. cit., p. 470. [2] loc. cit. [3] loc. cit. [4] Origin of Land Flora, p. 431.

endodermis. This species then resembles the *T. spicatum* type described by Boodle.[1] Text-fig. 2 is a diagram of a transverse section of the stem. The central stele, X, is giving off a meristele, *a*, which will later divide to form a branch and a leaf-trace; *b* is such a meristele which has travelled some distance in the cortex. A leaf-trace, *c*, has come off from the axillary branch, *x*, and lies on the same radius with it. The root *d* is just going off from X, the stem stele.

In structure the meristele closely resembles that of the stem as it is given off. It consists of a solid mass of xylem and parenchyma surrounded



TEXT-FIG. 2.

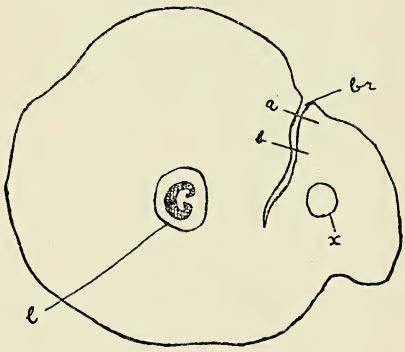
by phloem, pericycle, and endodermis. It differs from the stem in having central protoxylem elements (Pl. LXXX, Fig. 1). This character is kept by the meristele as it passes through the cortex until it divides into branch and leaf-trace. This division takes place in some cases just before the meristele curves into the petiole, and in other cases the division is delayed until it has reached the petiole base or travelled a short distance up the petiole. The resulting strands lie on the same radius; the inner smaller strand is that of the axillary branch and the outer strand is the leaf-trace. In *T. radicans*, where the axillary branch develops normally, the inner strand is larger than the leaf-trace. Its diminution in size in *T. javanicum* is due to the fact that it eventually perishes.

[1] loc. cit., p. 477.

Morphology of the Axillary Branch.

It was found to be difficult to follow the axillary strand into the petiole in sections of the whole stem, so petiole bases were separated from the stem and examined. Examination with a lens showed, at the base of the petiole on the adaxial surface, a small tapering mass of tissue covered with brown hairs (Text-fig. 1, B). This tissue came off as a whole in hand sections, so the petiole bases were embedded and cut with the microtome.

In several cases it was found that the division into axillary branch and leaf-trace had already taken place, in the cortex of the stem probably, so that the two were some distance apart. Other cases showed the meristele still undivided, and exhibiting the same characters as when it first came off from the stem. The meristele becomes somewhat oval in shape and soon shows a band of parenchyma separating off the xylem of one rounded end



Text-fig. 3 A. Diagram showing the break (*br.*) in the cortex separating off the axillary strand (*x*). *l*, leaf-trace.

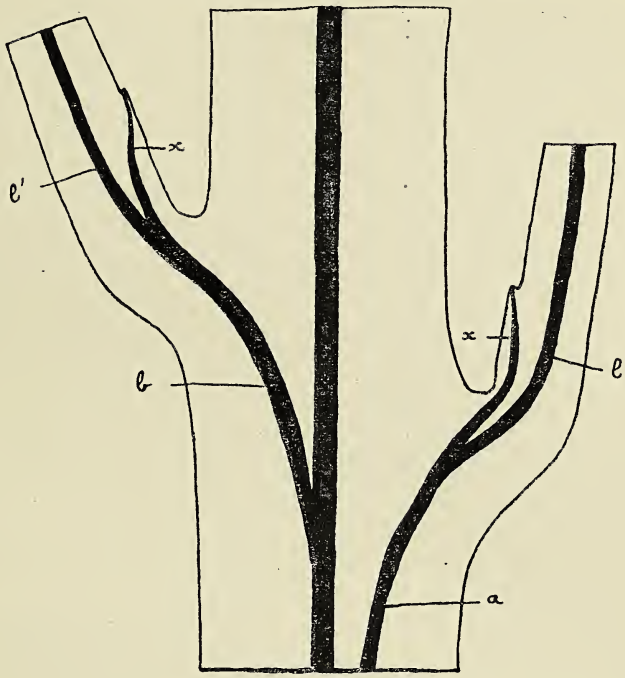
Text-fig. 3 B. Enlargement of *a–b* from 3 A.

to form the axillary strand (Pl. LXXX, Fig. 2). This separation goes on gradually until the axillary strand consists of a mass of xylem with parenchyma surrounded by phloem, pericycle, and endodermis. In every respect this strand resembles in structure the stem stele.

The leaf-trace has its xylem arranged round two groups of parenchyma with the thinner portion of the xylem on the flattened adaxial surface. Two groups of protoxylem can be distinguished in the parenchyma (Pl. LXXX, Fig. 3). Gradually the parenchyma aggregates into one group, and the thin band of xylem is interrupted by parenchyma cells, so that the metaxylem takes the arched form characteristic of the leaf-trace in *Trichomanes*. The protoxylem is in two groups, one at each incurved arm of the metaxylem.

The axillary branch strand passes very gradually through the cortex and diminishes in size. Pl. LXXX, Fig. 4, gives its structure when it is 0·075 mm. away from the leaf-trace. As it moves further away the amount

of xylem diminishes; for instance, at a distance of 0·16 mm. from the leaf-trace the branch stele possesses six lignified elements (Pl. LXXX, Fig. 5). This number is further reduced to two, and then to one lignified element at a distance of 0·23 mm. from the leaf-trace (Pl. LXXX, Fig. 6). The phloem also loses its differentiation until the strand is composed of a small mass of deeply-staining parenchyma (Pl. LXXX, Fig. 7). At this stage there is a distinct bulge in the cortex in which the strand is situated, and in some cases a break begins to appear between this portion and the rest of the cortex. Text-fig. 3 A gives a diagram showing the axillary branch, *x*, situated in a portion of the cortex which is separating from the rest by a break, *br*,



TEXT-FIG. 4.

in the cortex cells. This break is apparently brought about by the dissolution of the middle lamella of these cells as shown in Text-fig. 3 B. Thus this tissue is gradually separated from the cortex of the petiole, and it disorganizes along with the remains of the branch stele. In other cases the break is not so distinct; the portion of the cortex containing the axillary strand projects beyond the rest. It gradually diminishes in size, and the tissue becomes disorganized. There is no doubt that this is the tapering mass of tissue visible on the adaxial surface of the petiole base.

From the serial sections of the stem and petiole bases it has been possible to reconstruct a longitudinal diagram of the course taken by the

meristele. Text-fig. 4 shows that the meristele, *a*, passes slowly through the cortex until, just before it curves into the petiole, it divides to form the axillary branch, *x*, and the leaf-trace, *l*, which passes out into the petiole with *x* towards the adaxial and *l* towards the abaxial surface. The other meristele, *b*, figured in the diagram, reaches the petiole and there divides into branch strand, *x'*, and leaf-trace, *l'*. Each axillary branch strand gradually curves away from the leaf-trace and ends in a small protuberance of tissue on the adaxial surface.

Conclusion

An examination of fossil and recent forms has led Scott [1] to mention a suggestion that dichotomy may have been the primitive type of branching, and that axillary branching may have arisen at first as a reduced dichotomy, the subtending leaf being really borne on one arm of the dichotomy. Tansley[2] applies the dichotomy theory also to axis and leaf. He brings forward in support of this theory the fact that there is a certain similarity in structure between the stele of the axis and the leaf-trace in the Botryopterideae and the Hymenophyllaceae. In *T. javanicum* the meristele is radial in structure, but differs from the axis in having central protoxylem. The meristele divides into two unequal parts to form branch and leaf-trace, which are not alike in structure, since the branch strand exactly resembles the axis and the leaf-trace shows signs of dorsiventrality and has two distinct groups of protoxylem. This does not really militate against Tansley's theory, since *Trichomanes* is a recent form, and has undergone a certain amount of specialization. In the ancient forms included in the Botryopterideae it is often very difficult to distinguish between axis and leaf-trace. Dr. M. Benson has pointed out this difficulty and the great similarity between axis and petiole in her paper on *Botryopteris antiqua*, Kid.

The conical mass of parenchyma in which the axillary strand of *T. javanicum* ends recalls the parenchymatous mass, described by Gwynne-Vaughan [3] in *Helminthostachys zeylanica* as projecting from the stele of the axis to meet the base of each axillary canal. This tends to confirm his suggestion that these bodies 'represent the last indications of vestigial axillary buds'.[4] Bower [5] has recently pointed out in his paper on *Ophioglossum palmatum* that the trend of modern work is to relate the Ophioglossales to the Botryopterideae on account of the similarity in sporangial dehiscence, stipular structure, and heterophylly. To this list may now be added the

[1] Studies in Fossil Botany, p. 318.

[2] The Evolution of the Filicinean Vascular System. New Phyt. Reprint, 1908, Lecture 1.

[3] loc. cit.

[4] Renault, in his publications in 1878, regarded the Botryopterideae as related directly to the Hymenophyllaceae and Ophioglossaceae. See Studies in Fossil Botany, p. 640.

[5] Annals of Botany, April, 1911.

presence in both groups of vestigial axillary branches. The Botryopterideae and Hymenophyllaceae show great similarity in anatomy, and also possess vestigial axillary branches found in an increasing number of forms. It is obvious then that the Ophioglossales, Botryopterideae, and the Hymenophyllaceae are in one circle of affinity.

I wish to thank Dr. M. Benson for the material she has placed at my disposal, and for her helpful criticisms during the investigation recorded in this paper.

DESCRIPTION OF FIGURES IN PLATE LXXX.

Illustrating Miss H. S. Chambers's paper on the Vestigial Axillary Strands of *Trichomanes javanicum*.

Fig. 1. Transverse section of a meristele given off from the rhizome. The xylem shows central protoxylem (*px*) and is surrounded by phloem (*ph*), pericycle (*pr*), and endodermis. × 180.

Fig. 2. Transverse section of the meristele, showing the xylem of the axillary branch (*a*) just separated from the leaf-trace (*l*) by a layer of parenchyma (*p*). × 125.

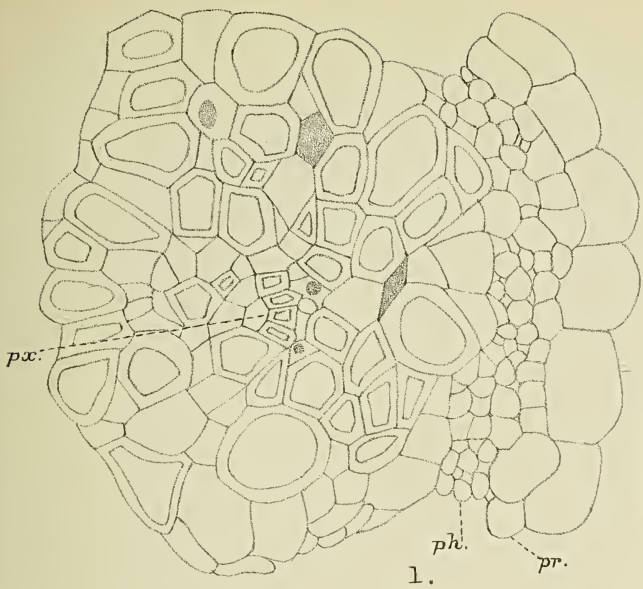
Fig. 3. Transverse section of the leaf-trace after the axillary strand has separated, showing the first signs of the change from radial to dorsiventral structure. The two groups of protoxylem (*px*) are near the flattened side of the leaf-trace. × 125.

Fig. 4. Section of the axillary strand separated from the leaf-trace by a distance of 0·075 mm. × 180.

Fig. 5. The same at a distance of 0·16 mm. from the leaf-trace. × 180.

Fig. 6. The same at a distance of 0·23 mm. from the leaf-trace. × 180.

Fig. 7. Section of the mass of parenchyma representing the last stage in the axillary strand when it is situated in the tongue of tissue on the adaxial surface of the petiole. × 180.



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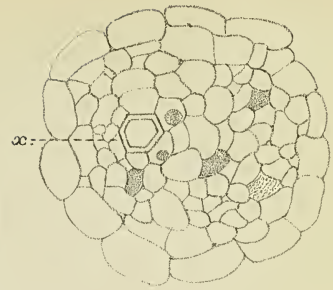

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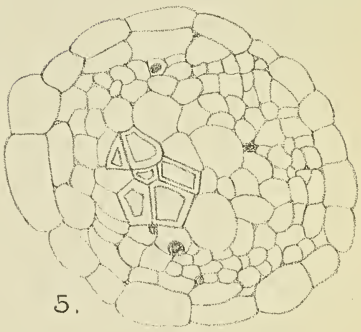

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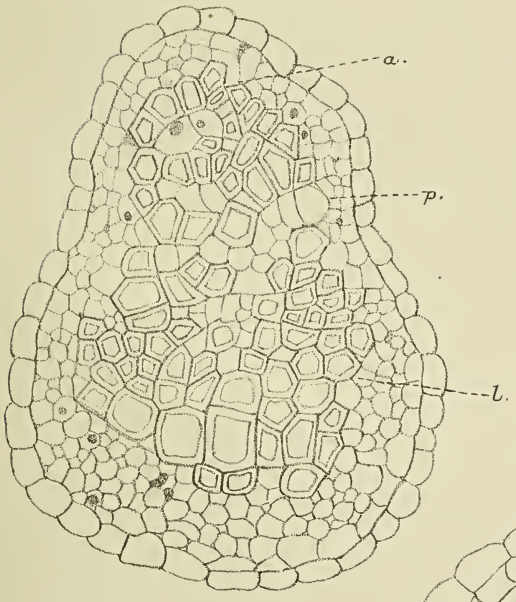

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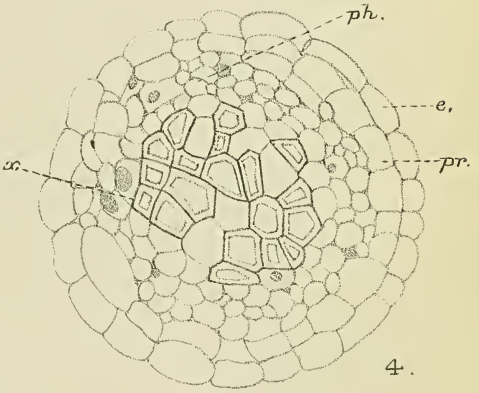

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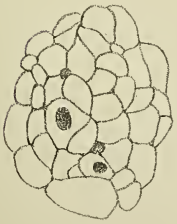


7.

H. S. Chambers, del. Hanh, lith et imp.

CHAMBERS — TRICHOMANES.

New Observations on Botryopteris antiqua, Kidston.

BY

MARGARET BENSON, D.Sc., F.L.S.

Royal Holloway College, University of London.

With Plates LXXXI–LXXXIII and three Figures in the Text.

CONTENTS.

I. INTRODUCTION.

IN 1908 Dr. Kidston published a short account[1] of a new species of *Botryopteris* from Pettycur, Fife. He gave it the name of *Botryopteris antiqua*. Unfortunately, he had inadequate material for a full description, and especially states that 'none of the stems yet met with show the departure of the leaf-trace from the stele'.[2]

A more abundant supply which was recently collected at Pettycur, and has been cut in the Botanical Laboratory at the Royal Holloway College, makes it possible to note the range of structure of the various organs, and affords, therefore, a better ground for comparison of this early species with those of later date.

Since the publication of Dr. Kidston's diagnosis, Professor Bertrand

[1] Kidston: On a New Species of *Dineuron* and *Botryopteris* from Pettycur, Fife. Trans. Roy. Soc., Edinburgh. vol. xlvi, Part II, p. 362.

[2] Kidston: loc. cit., p. 363.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

and M. Cornaille have published a note[1] on the *Botryopteris* type of leaf-trace in which they refer to a form which they call *Botryopteris antiqua*, 'd'Esnost'. Professor Bertrand kindly sent me a photograph of the transverse section of the petiole of this form, and states that Dr. Kidston identifies it with his species. In this photograph one of the two protoxylem groups is excellently preserved, and agrees in structure with the more detailed account I have given of the new specimens, but the other is not clearly shown. Professor Bertrand tells me that this form from Esnost has been known to him for many years and was known to Renault, but there were difficulties in diagnosing it as a species distinct from those of the Upper Carboniferous Rocks. The reasons for this difficulty I will refer to later (*vide* p. 1048).

The early date and wide distribution of the species, its variable but simple anatomy, make it an interesting subject of investigation, and I have therefore thought the following observations worthy of record.

II. General Morphology and Range of Variation.

The plant consisted of a rhizome which in one region gave rise to numerous adventitious roots and was covered with uniseriate hairs. After a short interval the rhizome bore petiolar structures whose traces came off at a two-fifth phyllotaxy. Often after only two nodes the rhizome gave off more adventitious roots, which sometimes arose very close to a node. Hairs are not generally found except on the root-bearing part of the stem, but this may be an accident of preservation. The petioles varied not only in size but in character. The larger ones contained a diarch trace, the smaller only a monarch trace. The latter forms generally left the stem at the same level with a curious uninerved sheathing body, probably to be homologized with the so-called aphlebiae of the Zygopterideae. It will be treated of in a later section (*vide* § VI).

The stem stele varies very much in size and constitution in different parts of the same plant. In the purely leaf-bearing zone the protoxylems are concentrated into one or two centres, but in the region of root formation there are many scattered and peripheral protoxylem elements, and the transverse section of the stem is increased in size.

As in the later species of *Botryopteris* the large metaxylem elements of the petiole trace are porose, while the smaller elements and protoxylem show all intermediate phases from porose to reticulate. As a result, where no leaf-traces are being differentiated in the stem, it presents quite a different appearance from that at a node. (Cp. Pl. LXXXI, Fig. 1, and Pl. LXXXII, Fig. 13 *a*.)

Lastly, the Botryopteridean sporangia which are associated with these

[1] C. E. Bertrand et F. Cornaille: Les caractéristiques de la trace foliaire botryoptéridienne, t. cl, note, p. 1019. Comptes rendus des Séances de l'Académie des Sciences, 1910.

stems vary very considerably in size. Thus the sporange photographed in Fig. 18 is nearly double the size of any member of the group of four in Fig. 17. The latter show the same dimensions (275 μ) and arrangement as those described recently by Dr. Scott.[1]

The specimens considered occur in three different blocks of calcite, but in spite of all the range of variation they exhibit, it has not been found possible to demonstrate the presence of more than one species.

III. Node and Internode.

In that part of the stem which is not complicated by the emission of roots, the structure of the stele throughout the node and internode is characterized by a very simple series of changes. As the leaf-trace separates from the stem xylem, the latter consists of a mass of primary tracheides with but one protoxylem group placed laterally very near the surface of separation of the trace (Pl. LXXXI, Fig. 1). In cases where the leaf-trace is equal in size to the stem stele there is little to distinguish the trace from the stele (cp. Figs. 7 and 8 *a*). The trace separates off along one margin first, and gradually increases its angular divergence until it lies at 90° to its plane of attachment (Pl. LXXXII, Figs. 11 and 12).

The protoxylem group left in the stem bifurcates, and after a very short interval (within three millimetres sometimes) two protoxylem groups are to be seen, now surrounded by metaxylem. Photographs of stem sections in this plane are shown in Figs. 2, 8 *a*, and 9 *a*. These are each from a different specimen and agree in demonstrating the formation of peripheral metaxylem elements which restores the mesarch position of the two protoxylem groups. One of these groups is the new leaf-trace group, which is thus clearly shown to be mesarch in origin.

IV. The Leaf-trace and Petiolar Bundle.

One of the two above-mentioned protoxylem groups, lying at two-fifth angular divergence from the point of departure of the last leaf-trace, may now be looked upon as destined for the next leaf. As already stated, it may pass out undivided, in which case we have a monarch trace and monarch petiolar bundle (Pl. LXXXI, Figs. 4 *a* and 9; Pl. LXXXII, Fig. 14; Pl. LXXXIII, Figs. 19 and 21).

Very frequently in the specimens under consideration the protoxylem of the leaf-trace is duplicated (Fig. 11) while still in contact with the stem xylem. An early stage in this duplication is indicated in Fig. 6 *a*, where separation is just being initiated. While the leaf-trace is passing through the cortex of the stem, all formation of strictly centrifugal metaxylem ceases, but the protoxylems line the bottom of adaxial grooves owing to the differentiation of elements of metaxylem laterally. These

[1] Scott: Sporangia attributed to *B. antiqua*, Kidston. Annals of Botany, 1910, Note, p. 820.

grooves, which appear like shallow pits bordered by a pair of minute cusps in transverse sections of the petiole, persist after the branching of the main rachis (Pl. LXXXIII, Fig. 24). Each groove with its projecting margins probably represents a so-called tooth of the later species, *B. hirsuta* and *B. ramosa*, although in these species the adaxial protoxylems are clearly endarch.

In this view I differ from MM. Bertrand and Cornaille, who refer to the *Botryopteris antiqua* type of petiole as tridentate.[1] This interpretation may possibly have prevented the earlier diagnosis of *B. antiqua*, 'd'Esnost'.

As is shown in the succeeding paragraph, it is only during incipient phases of branching that the Pettycur species could be described as tridentate, and it was due to the prevalence of petioles in this condition in my earlier material that I was unable to clearly distinguish the earlier from the later species until comparatively recently.

V. The Branching of the Diarch Petiolar Bundle.

When a diarch petiolar bundle is preparing to branch, a third pair of cusps appear, exactly like the original two. This, which probably owes its origin to the bifurcation of one of the two existing protoxylem groups, travels for a considerable distance in the petiole before the separation of the two metaxylem masses takes place. Hence, as already stated, in a long series of sections (Slides 390. 11–21, *vide* Fig. 24) of a branching petiole, we may have a fair semblance of the triarch type of petiole characteristic of later species. Finally, the branch, which is generally smaller than the parent rachis, passes off laterally. No equal dichotomy of a diarch petiole has been met with—the only record of a case being apparently due to an oversight.[2]

VI. The Monarch Petiolar Bundle and Aphlebiae.

I have no instance of two successive nodes giving off diarch traces—monarch traces apparently precede and follow the diarch trace. The monarch traces, however, apparently never come off, pass through the cortex and enter the petiole, without being accompanied by aphlebiae.

In four of the specimens figured (Figs. 8–9, 10, 19–20, and 21), monarch petiolar traces are obviously associated with minute monarch traces which come off from the stele almost simultaneously with their respective petiole traces.[3] In three of these cases (Figs. 8–9, 19–20, 21), the monarch petiolar trace with its aphlebia trace follows a diarch trace, whereas in Fig. 10 there is evidence that a large trace succeeded such a pair. This figure also indicates that the aphlebia trace diverged from the petiole trace

[1] Bertrand et Cornaille: loc. cit., p. 1022. 'La trace réalise alors le facies tridenté des *B. hirsuta*, *B. antiqua*.'

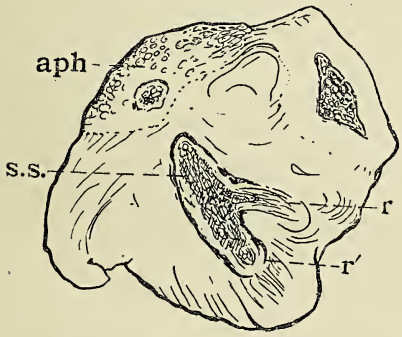
[2] Kidston: loc. cit., Figs. 11 and 12, which appear to represent a node of a stem.

[3] Cp. Kidston: loc. cit., Fig. 4, where '*pet*'' appears to represent the aphlebia corresponding to the petiole trace *pet*''.

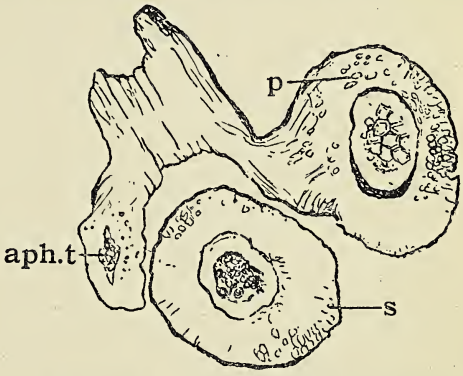
when they both became free from the cortex. This is also shown in other preparations.

That the angle between the two bundles was filled by a lamella is shown in Text-figs. 1 *a* and 1 *b*, which are camera drawings from sections showing the sheathing character well. In Fig. 14 the aphlebia trace is still unseparated from the stem stele (*vide* Fig. 14, *px*). We have in this series of sections an example of the aphlebia trace coming off (Slide 393. 25, Text-fig. 1 *a*) at a considerable interval after the corresponding petiole trace (Slide 393. 28, Fig. 15).

Another curious case is afforded by the series partly reproduced by Figs. 1–5. A badly developed diarch trace comes off just before the level of Fig. 1 (Slide 407. 14). A monarch trace comes off at the next node



TEXT-FIG. 1 *a*. A camera sketch from a stem node of which the previous section is shown in Fig. 14, Pl. LXXXII. The Text-figure shows the aphlebia (*aph.*) with its sheathing base, which is attached to the monarch petiole, *p*. The stem stele (*s.s.*) is giving off various roots (*r.*, *r'*). Slide 393. 25. × 19.



TEXT-FIG. 1 *b*. A camera sketch from a comparable node at a slightly higher level. *p* = petiole, *aph.t.* = aphlebia trace, *s* = stem. From a slide kindly lent for the purpose of this figure by Dr. Gordon. C. N., 1301. × 19.

(*vide* Fig. 4 *a*, which is a photograph from Slide 407. 9). No evidence of an aphlebia appears with the latter until Slide 407. 7, where the petiole, now free from the cortex, shows a small branch coming off from its vascular bundle (*vide* Fig. 23). If one follows up this small branch through a series of sections of the petiole (Slides 407. 7–2), we find it flattens out and disappears, never entering an appendage of the petiole. We must, I think, regard this as a belated and abortive aphlebia trace. The phenomenon is very like the belated formation and abortion of the axillary strand in the leaf of *Trichomanes javanicum* described by Miss Chambers.[1] In this species it was shown that the axillary strand, described by Boodle[2] as occurring in many of the Hymenophyllaceae, may defer its separation from the leaf-trace until the latter has entered a petiole.

[1] Chambers: in the present number of the Annals of Botany.

[2] Boodle: Anatomy of the Hymenophyllaceae. Annals of Botany, xiv, 1900, p. 455.

There is no evidence that the vascular bundle supplying an aphlebia ever branched. In no single case has an aphlebia been seen in connexion with a diarch trace (cp. Pl. LXXXII, Figs. 11–13). Thus we may regard it as fairly established that diarch petioles without any aphlebia occur, succeeded by petioles with a monarch vascular bundle and a monarch aphlebia trace. This contrast between the petioles of *B. antiqua* is interesting, and probably will prove to indicate some distinction in function to which we have as yet no clue.

The aphlebia is simpler in structure than the bodies described under this name in the Zygopterideae, for the latter receive a bundle which 'divides into two, three, or four strands',[1] whereas in *Botryopteris antiqua* I have met with no example of the branching of an aphlebia bundle.

In one respect the diarch petiole resembles the monarch petiole with its aphlebia, e.g. they each receive a vascular supply, including two protoxylem groups. Perhaps on this ground we may regard the diarch petiole as equivalent to the monarch with its appendage.

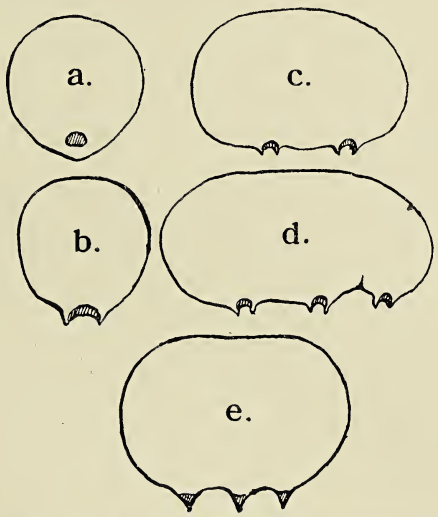
As is well known, Dr. Paul Bertrand regards *Botryopteris* as having been derived from Anachoropterid ancestors by the shifting of the two fundamental poles of the leaf-trace to an adaxial position. Such a theory involves the assumption that the leaf-trace in *Botryopteris* was primarily diarch. The new observations appear to introduce some difficulty in the acceptance of this view because, if the diarch type were the older, we should be under the necessity of regarding it as already undergoing disintegration or reduction in *B. antiqua*—the oldest known species of the genus.

The argument in Bertrand and Cornaille's recent account of the characteristics of the Botryopteridean trace is based primarily on the '*forensis*' type. They suggest the '*ramosa*' type to be a reduction form, since they compare it [2] with the reduction form met with in the upper part of the frond of the '*forensis*' type. They also refer to the *B. antiqua* trace as having 'le facies tridenté'. If *B. antiqua*, 'd'Esnost,' has a tridentate leaf-trace it cannot be identified with *B. antiqua* of Pettycur. Except in branching diarch petioles, I have never seen a tridentate trace from Pettycur. If the authors of the above note homologize each of the minute cusps limiting a group of protoxylem with the tooth of *B. ramosa*, then the *B. antiqua* trace is respectively either quadri- or bidentate. The identification of a single protoxylem group in *B. antiqua* with a correspondingly single protoxylem group in the latter type of trace seems absolutely necessary, even though in the former it may be slightly mesarch or sunk between two cusps, and in the latter it is endarch and projects as a single point. It will be simpler, therefore, to refer solely to the number of pro-

[1] Scott: Studies in Fossil Botany, p. 314.
[2] Bertrand et Cornaille: loc. cit., p. 1022.

toxylem groups, in which case *B. antiqua* may be said to have either a monarch or diarch, while *B. ramosa* and *B. hirsuta* have a diarch or triarch trace.

Since this is the case the trend of evolution in the genus *Botryopteris* would seem to be towards a multiplication of the protoxylems rather than towards a reduction. If we accept the monarch type of leaf-trace as the older, and regard the diarch type as derived from it, we may explain the diarch type as due to arrest of branching at a primary phase. Such a view could be extended to the derivation of the later triarch forms, which may well bear the same relation to the diarch forms as the latter to the monarch. The series would then be continuous, and is represented diagrammatically in Text-fig. 2. In this figure the gradual protrusion of the protoxylem is simultaneously indicated.



TEXT-FIG. 2, *a*, *b*, *c*, *d*, *e*. A series of diagrams of transverse sections of petiole bundles or leaf-traces of *Botryopteris* (*vide* text). *a* = the mesarch form of the monarch trace which is found while the trace is still only partially separated from the stem stele (cf. Fig. 2) in *Botryopteris antiqua*. *b* = the very common monarch petiole bundle of *B. antiqua*. *c* = the diarch trace of *B. antiqua*. *d* = the branching diarch petiole bundle of *B. antiqua* (cf. Fig. 24). *e* = the unbranched petiole bundle of *B. ramosa*. The position of the protoxylem is indicated by hatching, and is shown to be mesarch at one end of the series and endarch at the other.

In support of this view we may add the facts (*a*) that diarch petioles are known fairly commonly among the later triarch forms, and (*b*) that with the exception of the slightly mesarch position of the protoxylems (Fig. 24), it would not be easy to distinguish, as already stated, a branching diarch petiole of *B. antiqua* from a non-branching triarch petiole of *B. ramosa*.

VII. The Root-bearing Zones of the Stem Stele.

Sections of stems bearing numerous roots occur both in transverse and longitudinal planes. This part of the stem is covered with delicate

uniseriate hairs with a slightly enlarged base. The roots pass out of the stem rather abruptly, and resemble those of later species of *Botryopteris*. When the xylem of a stem stele is about to give rise to roots, the diagrammatic arrangement of protoxylems and metaxylem described above for the leaf-bearing stem (Pl. LXXXI, Figs. 1–3) is interfered with. The protoxylem becomes at first more scattered (Figs. 12 *a* and 13 *a*), and small peripheral groups are formed at irregular intervals. Meanwhile the xylem increases considerably in bulk, and consists mainly of elements of a smaller size and less definitely porose marking than those supplying the leaf-trace (*vide* Fig. 22). Different examples of such stems are shown in Figs. 4, 6 *a*, and 9 *a*. It is in this region that sections of the stem, e.g. Fig. 13 *a*, show considerable resemblance to those of the root-bearing regions in *Botryopteris ramosa*, and possibly admit of some comparison with those of *Diplolabis* and *Metaclepsydropsis* recently described by Dr. Gordon.[1]

VIII. Pseudo-secondary Thickening of the Stem Xylem.

As already observed, the xylem of the stem increases its dimensions very rapidly after giving off a leaf-trace, especially if it is about to give off roots (cp. Figs. 11 and 13).

In Fig. 13 two roots are being given off, and we may safely conclude that in Fig. 14 (cp. Figs. 15 and 14) and in Fig. 9 (cp. Figs. 7 and 9) roots would have been demonstrated if the series could have been prolonged.

This increase in size is brought about partly by the differentiation of parenchymatous elements which divide irregularly, as commonly occurs in primary xylem. Partly, however, the serial mode of division of a common mother-cell obtains, examples of which are seen in Figs. 4, 12 *a*, and 15. This mode of developing new xylem appears to be intermediate between the irregular division of normal primary mother-cells and the regular formation of secondary wood from a definite meristem. The resemblance is more striking when several contiguous cells lay down tangential walls in the same plane, as may be seen in Fig. 12 *a*. There is no reason to think this is a vestigial character, as no older nor contemporary form of the Botryopterideae has been described with normal secondary thickening.[2] If primitive it is very suggestive of the way in which secondary meristems may have arisen, and thus seems worthy of record. For such cases of cellular increase as these the term 'pseudo-secondary' is suggested. They are of course exceedingly common, occurring in the stem of *Metaclepsydropsis duplex* and *Diplolabis Römeri*,[2] among contemporary Ferns and in many recent plants.

[1] Gordon: *Diplolabis Römeri*, 1910; *Metaclepsydropsis duplex*, 1911. Trans. Roy. Soc., Edinburgh.

[2] Gordon: loc. cit., 1911, Fig. 8. Since writing this Dr. Gordon tells me he has found a striking case in a new stem from Pettycur.

In some cases it may coexist in the same bundle with the more advanced type, i. e. that brought about by the divisions of a definite meristem. Thus pseudo-secondary wood is found at the nodes of *Protocalamites*, where an abrupt increase in the centripetal primary wood takes place, but the centrifugal primary wood of the same bundle is added to by the agency of a normal cambium (Fig. 25).

IX. Bearing of the New Observations on the Affinity of the Genus.

In Dr. Paul Bertrand's admirable monograph[1] on the frond of the Zygopterideae, he not only accepts the view that *Botryopteris* is a reduced type, but suggests the mode of its origin. Thus on page 278 he says: 'The Anachoropterideae seem to be derived from the ancient Zygopterideae by the loss of the accessory plane of symmetry. The Botryopterideae form a series parallel to the Anachoropterideae, from which they are derived by the approximation of the two fundamental poles to the anterior face of the foliar trace.'

If this view be accepted we must interpret the simplicity of structure met with in *Botryopteris antiqua* as due to reduction, and not to its relatively primitive character. As already pointed out, this view involves the assumption that the diarch type of petiole is older than the monarch, and the species is in process of simplification. This result is not easy to harmonize with the fact that later forms of *Botryopteris* petiole are triarch.

If we hold Bertrand's view we must grant that not only do the two Anachoropteridean 'fundamental poles approximate to the anterior face of a foliar trace', but that very frequently they are represented by one pole only throughout the main rachis of the petiole in the oldest known species of the genus (Fig. 14).

Our new knowledge of the stem structure and nodes of the older Zygopteridean Ferns, *Metaclepsydropsis duplex* and *Diplolabis Römeri*, which we owe to Dr. Gordon, leads us to regard these forms as possessing a less specialized type of stem structure than later Zygopteridean species, e. g. *Ankyropteris corrugata*. The nodal form of the petiolar bundle, i. e. the leaf-trace, is also comparatively simple, exhibiting in transverse section a tangentially extended band of primary xylem with a mesarch protoxylem group at either end. This structure appears to offer the best basis of comparison between the Zygopterid and Botryopterid types.

The section of the stem of *B. antiqua* shown in Fig. 13 *a* is not unlike the type found in *Metaclepsydropsis*, except that the peripheral larger centrifugal elements are continuous all round the periphery in the latter.

[1] P. Bertrand: Études sur la fronde des Zygoptéridées, 1909.

There is no vestige of a stellate character in the transverse section of the stem stele of either genus. The smaller internal elements do not contribute to the trace in either. In *Botryopteris* there are far fewer mesarch groups of protoxylem; hence the giving off of a leaf-trace approximates very closely to a simple dichotomy, and resembles what one would expect to find in a primitive Pteridophyte.

We cannot but regard *Botryopteris* as having a close affinity with the Zygopterideae, in spite of the outstanding contrast in the mode of branching of their petioles. The presence of aphlebiae, now for the first time recorded in a species of *Botryopteris*, is a new feature of resemblance. If the *Botryopteris* type does not owe its simplicity to reduction, then the Zygopterid type must owe its complexity to elaboration.

The fundamental difference may possibly be traced back to the origin of the Zygopterid leaf-trace, from parts which in their ancestors gave rise to more than one trace, but we must wait for more facts before the problem of the common origin of the mono- and bisymmetric type of petiole can be solved. That the more complex type is the derived type is suggested by the ontogeny; the gradual series of changes which occur in a single petiole of *Metaclepsydropsis* or *Diplolabis* (so beautifully represented in Dr. Gordon's figures[1]) as it passes from the nodal form to the upper part of the main rachis may possibly be regarded as representing in some degree the phases in its evolutionary sequence.

X. Summary.

A considerable amount of new material of the Pettycur species of *Botryopteris* having become available, it has been possible to give details of its structure, especially with regard to its stelar anatomy and the presence of aphlebiae at the base of its smaller leaves.

Botryopteris antiqua, Kidston, is a form which, though variable, is often as large and well differentiated as *Botryopteris ramosa*. The protoxylem elements of the leaf-trace before it leaves the axis are mesarch, and later are aggregated into either one or two adaxial grooves, and are not evenly distributed. Heterophylly obtained, for those petioles which are supplied by a monarch trace are found to possess a uninerved sheathing organ which is regarded as of the nature of an aphlebia. The diarch petioles show no trace of this organ.

The axis was of the nature of a rhizome which at intervals gave off numerous adventitious roots.

The xylem of the stem stele is differentiated differently in the leaf-bearing part and the root-bearing part. The distribution of the protoxylem has been clearly shown in both types of structure and the departure of

[1] Gordon: loc. cit.

leaf-traces, both monarch and diarch, has been traced in detail. The preservation of the phloem elements is unsatisfactory, but indications of the characteristic large sieve tubes seen in later species can be made out surrounding the xylem of the petiolar bundle.

Reasons are adduced against the theory that the *Botryopteris* type of petiole is derived from the *Anachoropteris* type.

XI. Conclusion and Acknowledgements.

I have had the privilege of discussing the problem of the aphlebiae with Dr. D. H. Scott.

All the slides were cut and, with two exceptions, all the micrographs taken by C. H. Wells, my laboratory attendant.

Help towards the expenses of the work was received from the Royal Society Government Grant.

The boulder from which the sections shown in Figs. 16, 22, 24, and 25 were cut was kindly presented to me by Professor I. B. Balfour.

EXPLANATION OF PLATES LXXXI–LXXXIII.

Illustrating Dr. Margaret Benson's paper on *Botryopteris antiqua*.

Abbreviations used: *aph.* = aphlebia, *c.* = cusp limiting the protoxylem group of a leaf-trace, *m l.t.* = monarch leaf-trace, *d.l.t.* = diarch leaf-trace, *p.s.* = pseudo-secondary thickening, *px.* = protoxylem, *r.* = root, *s.s.* = stem stele.

All the figures are photomicrographs from slides in the Royal Holloway College Collection.

PLATE LXXXI.

(Figs. 1–9.)

Figs. 1–5 represent successive transverse sections through a stem stele. Slides 407. 12–8.

Fig. 1 shows the condition of the stele in a leaf-bearing zone immediately after the liberation of a diarch trace. The single protoxylem (*px.*) is about to bifurcate. Slide 407. 12. × 95.

Fig. 1 *a* is a low-power micrograph of the whole section from which Fig. 1 was taken. × 20.

Fig. 2 shows the completed duplication of the protoxylem (*px.*, *px.'*) shown in Fig. 1. Slide 407. 11. × 95.

Fig. 3. The next leaf-trace (*m.l.t.*) is preparing to leave the stem stele. Slide 407. 10. × 95.

Fig. 4. The leaf-trace shown in the stem stele in Figs. 2 and 3 has now just become free (*m.l.t.*). The stem stele is rapidly increasing in size by pseudo-secondary thickening (*p.s.*). Slide 407. 9. × 95.

Fig. 4 *a* is a low-power micrograph of the whole section from which Fig. 4 was taken. It shows both the leaf-traces still in the cortex. × 20.

Fig. 5 is a low-power micrograph of the next section of the same series, showing the two petioles supplied by the above traces just leaving the stem. Slide 407. 8. × 20.

Fig. 6. A micrograph of a stem giving off a root and a leaf-trace. The stem stele which shows an early stage in the liberation of another trace is shown enlarged in Fig. IV *a*. Slide 405. 16. × 20.

Fig. 6 *a*. An enlarged view of the stem stele shown in Fig. 6. The protoxylem of the leaf-trace appears to be dividing (cp. a corresponding stage in the protoxylem of the stem in Fig. 1).

The contrast in character between the metaxylem of the leaf-trace and that of the stem is well shown. The stem is partially in a root-bearing phase. × 95.

Fig. 7. A transverse section of a diarch petiolar bundle. This petiole should be compared with the stem in Fig. 8 *a*. The cusps are not as well preserved as in the petiole shown in Fig. 16. Slide 407. 8. × 95.

Figs. 8 and 9 are low-power micrographs from two successive sections transverse to a stem which first gives off a large diarch trace and later an aphlebia trace (Fig. 9), to be followed by a monarch petiolar trace. This succession is shown also in Figs. 19, 20, and 21. Slides 407. 24–25. × 20.

Fig. 8 *a* is a high-power micrograph of the stem stele from Fig. 8 and is comparable with that shown in Fig. 2. × 95.

Fig. 9 *a* is a high-power micrograph of the stem stele from Fig. 9, and shows the separation of an aphlebia trace (*aph.*) and origin of a monarch petiolar trace, while the rest of the stem stele is preparing for the emission of roots. This series should be compared with that shown in Figs. 19 and 20, and with Fig. 21. × 95.

PLATE LXXXII.

(Figs. 10–18.)

Fig. 10. An obliquely longitudinal section through a stem bearing a small petiole with its aphlebia (*aph.*). In the upper part of the section can be seen a large leaf-trace in its attached part. Slide 416. 4. × 20.

Figs. 11–13 represent three sections from a series through a stem bearing at first a diarch trace and later roots. Slides 417. 2–5. × 30.

Figs. 11 *a*, 12 *a*, and 13 *a* are high-power micrographs of the respective stem steles. × 95.

The series shows diagrammatically the change in character of the stem as it passes from a purely leaf-bearing zone into a root-bearing zone, and should be compared with Figs. 1–4.

Fig. 11 *a* shows the duplicated protoxylem of the stem which is characteristic of internodal structure, and corresponds with that shown in Figs. 2 and 8 *a*.

Fig. 12 *a* shows the rapid increase in diameter of the stele in preparation for root formation and pseudo-secondary (*p.s.*) development of new metaxylem.

Fig. 13 *a* shows a stem section in marked contrast to such a one as is shown in Figs. 1, 2, 8 *a*, and 11 *a*. It may be regarded as bearing some resemblance to the stem of *Diplolabis Römeri* and *Metaclepsydropsis duplex*. The bulk of the tracheides have a small diameter.

Figs. 14 and 15 represent two sections from the same series. Slides 393. 26 and 28.

Fig. 14 shows a monarch trace entering the base of the petiole and the stem in a root-bearing phase. At *px.* can be seen the protoxylem of the aphlebia trace. Cf. Text-fig. 1. (Roots were numerous in the next section, 393. 25.) Slide 393. 26. × 20.

Fig. 14 is a high-power micrograph of the node from which the above monarch trace took its origin. It shows the single protoxylem group characteristic of a purely leaf-bearing node (*vide* Fig. 1) and the addition of new primary xylem by pseudo-secondary formation. Slide 393. 28. × 95.

Fig. 16 is a high-power micrograph of the adaxial part of a diarch petiolar bundle. The minute cusps with intervening protoxylem elements can be clearly seen. The phloem elements are as usual badly preserved. This figure should be compared with the branching diarch petiole seen in Fig. 24. Slide 320. 11. × 95.

Fig. 17 is a high-power micrograph of part of a group of four 'Botryopteridean' sporangia measuring on an average 275 μ across. Slide 326. 2. × 95.

Fig. 18. A single larger 'Botryopteridean' sporange represented at the same magnification as those of Fig. 17. Such sporangia occur in all the three blocks from which the specimens figured have been obtained. Slide 400. x. × 95.

PLATE LXXXIII.

(Figs. 19–24.)

Figs. 19 and 20 are micrographs of two successive sections of a stem at a node. The leaf-trace which is just becoming free in Fig. 19 is shown as the petiole bundle in Fig. 20, and the small aphlebia trace (*aph.t.*) not yet detached from the stem stele in Fig. 19 is now (in Fig. 20) found passing into the base of an aphlebia.

Fig. 19 shows in the upper part of the photograph evidence of the stem having recently borne a large trace which has perished. Slides 402. 35 and 34. × 20.

Fig. 19 *a* is a high-power micrograph of the stem stele from Fig. 19. It shows the details of the aphlebia trace which is destined to go off almost simultaneously with the monarch petiolar trace just given off. Slide 402. 35. × 95.

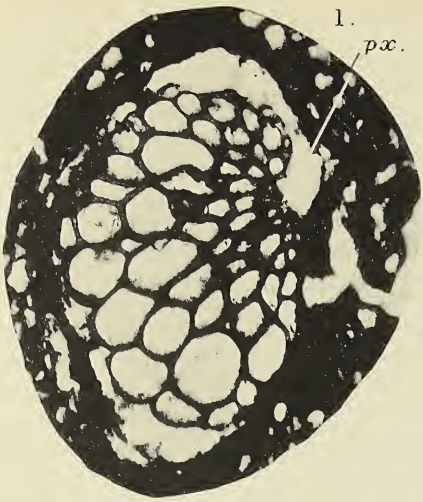
Fig. 21. A low-power micrograph of a stem stele (*s.s.*) showing simultaneously a diarch leaf-trace (*d.l.t.*), a monarch leaf-trace (*m.l.t.*), and an aphlebia trace (*aph.*). Slide 417. 15. × 11.

Fig. 22. A longitudinal section of the tracheides of a well-developed stem stele in a root-bearing region. They are reticulate and show various intermediate conditions between the porose marking met with in the metaxylem of the leaf-trace and the spiro-scalariform marking of the protoxylem elements. Slide 302. 19. × 95.

Fig. 23. A transverse section of the petiole which may be seen leaving the stem on the left of Figs. 4 *a* and 5. The vascular bundle gives off a small group of tracheides which later become flattened out and do not leave the rachis (*vide* text, where it is suggested they represent an abortive aphlebia trace). Slide 407. 7. × 20.

Fig. 24. A transverse section of a diarch petiole showing an early phase of branching. The pinna-trace separates off in the next section and eventually supplies a branch. Slide 390. 12. × 20.

Fig. 25. A transverse section of one bundle or a node of *Protocalamites* showing in the upper centripetal part pseudo-secondary thickening. Slide 306. 7. × 95.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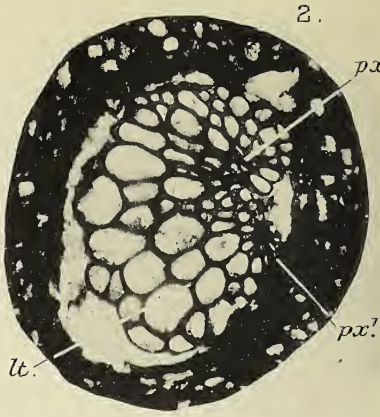
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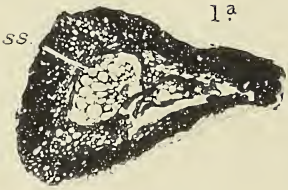
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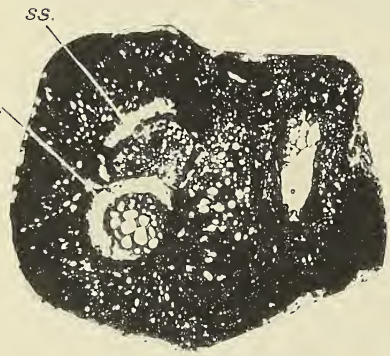
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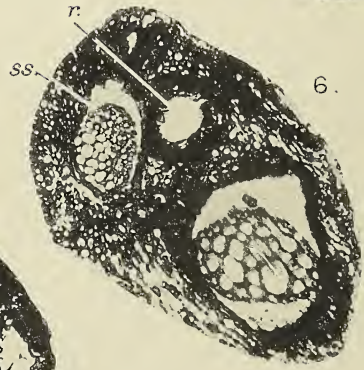
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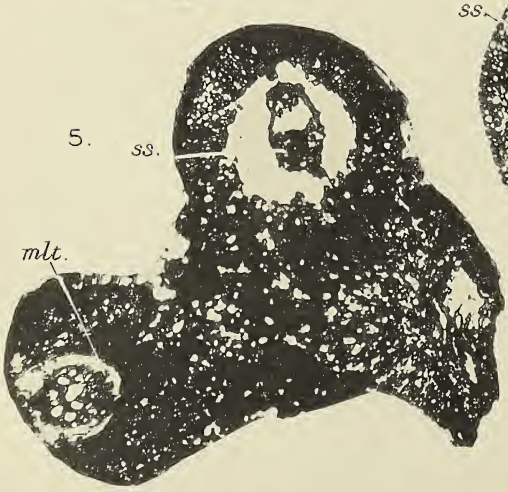
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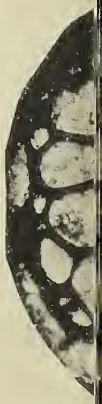

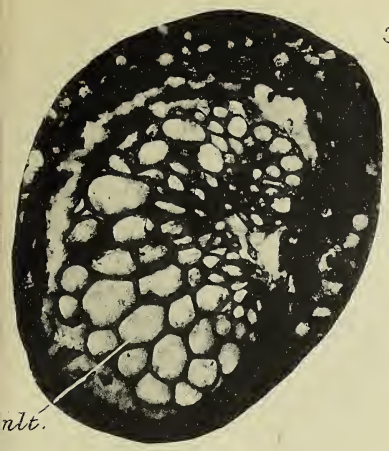
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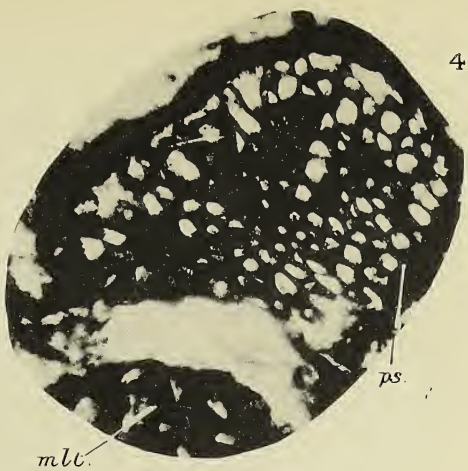
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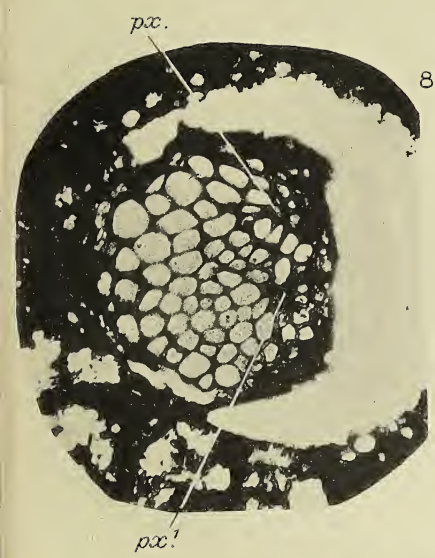
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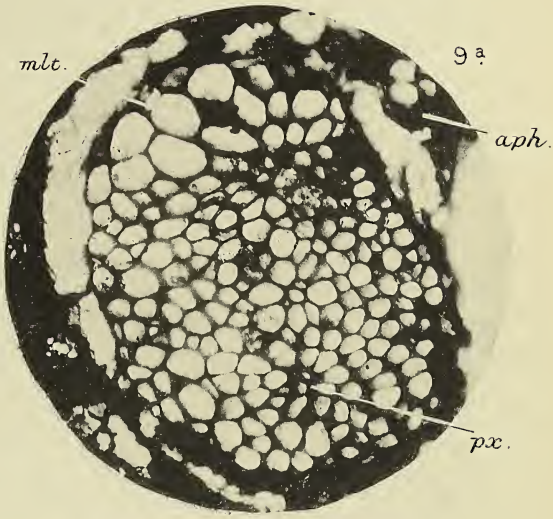
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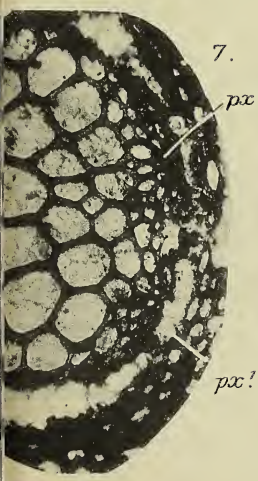
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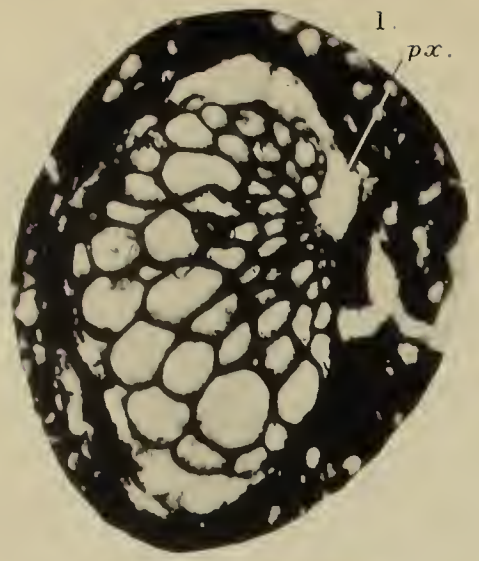

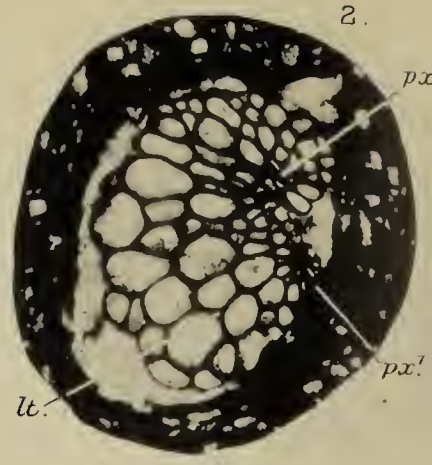

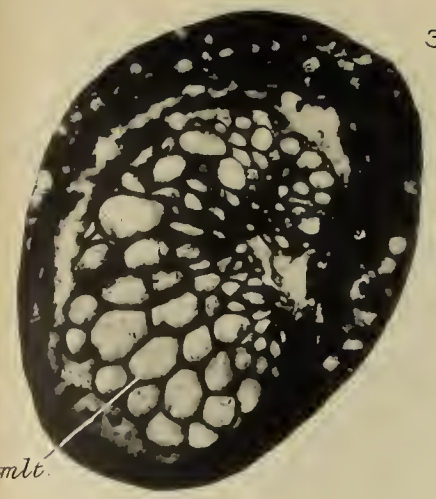

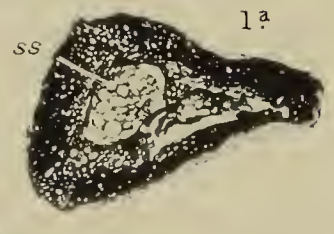

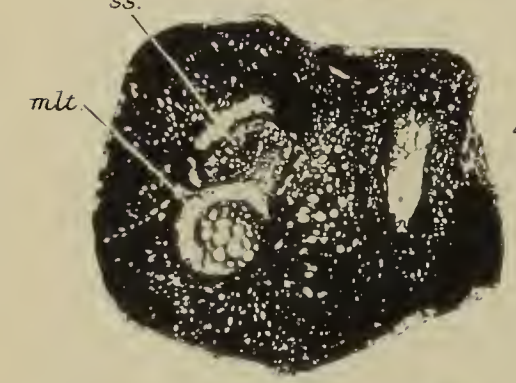

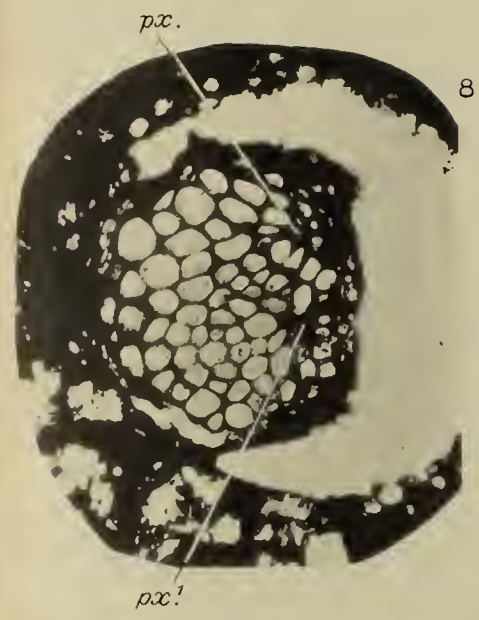

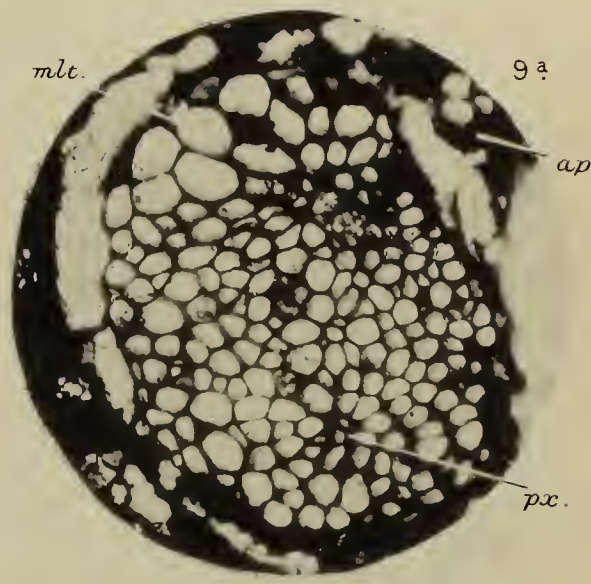

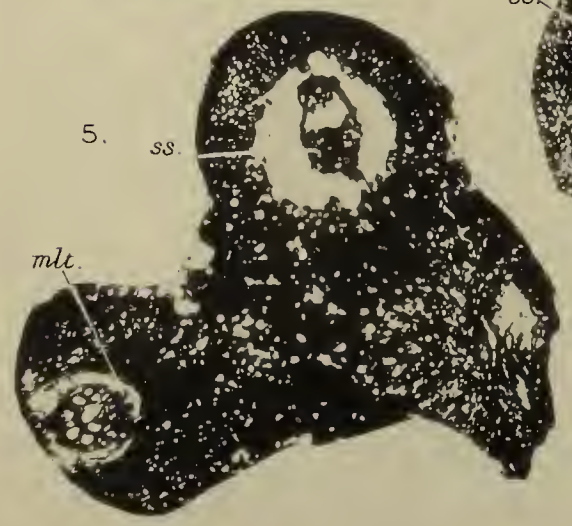

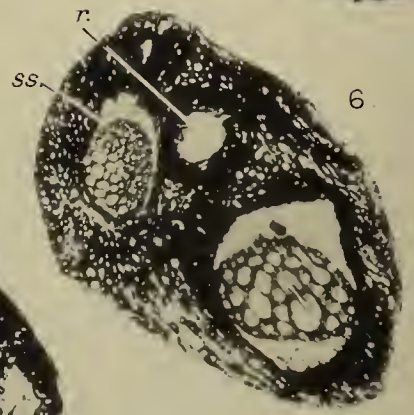

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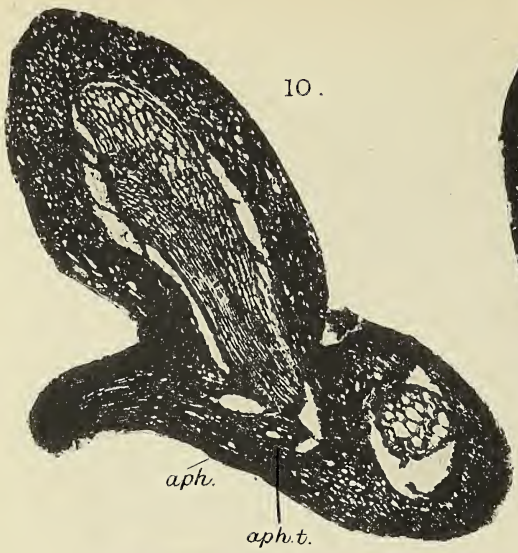
BENSON — BOTRYOPTERIS.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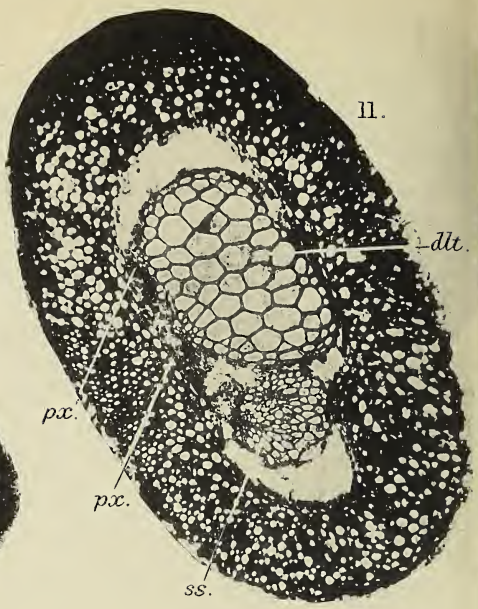
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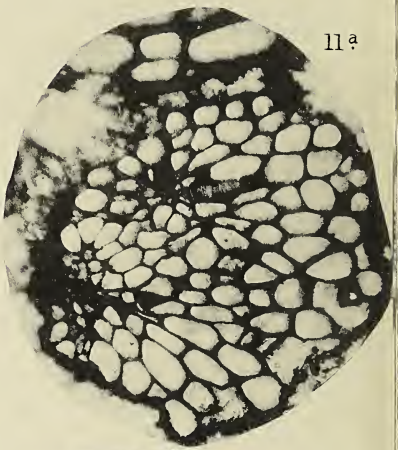
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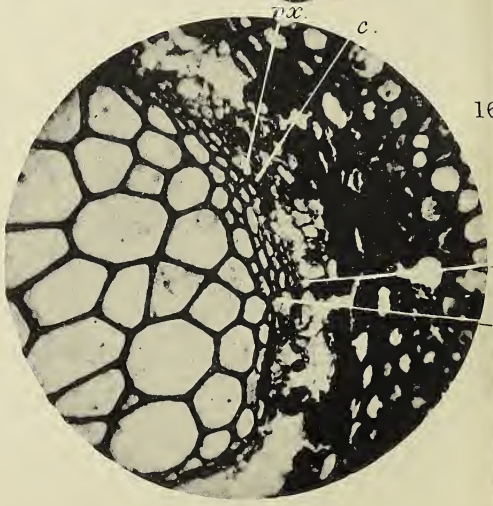
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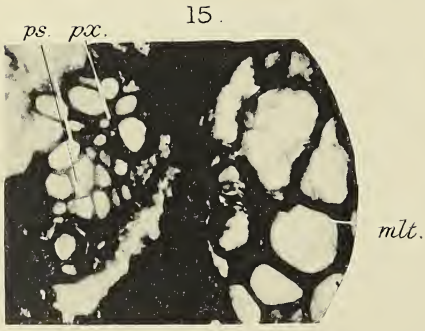
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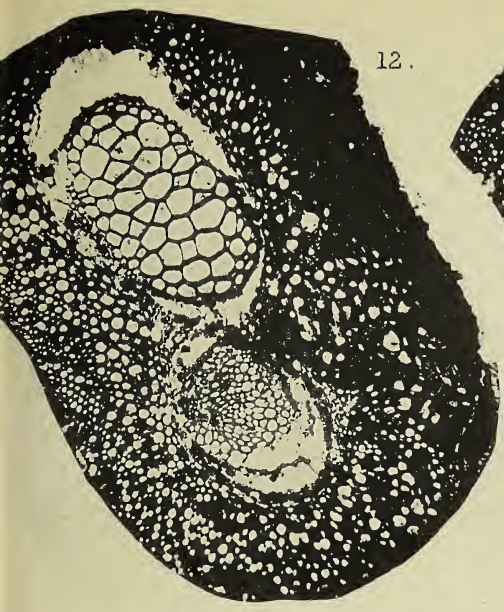

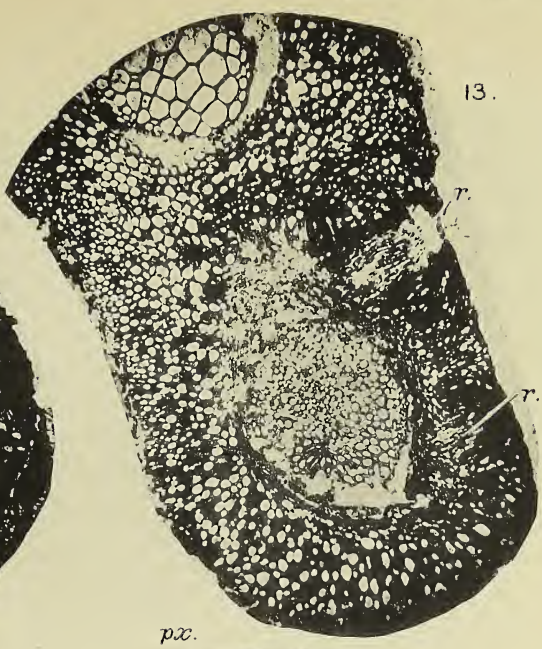

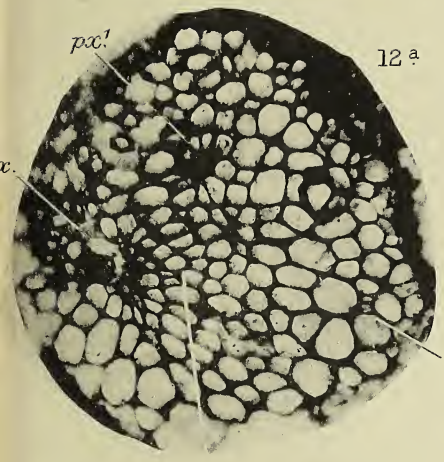

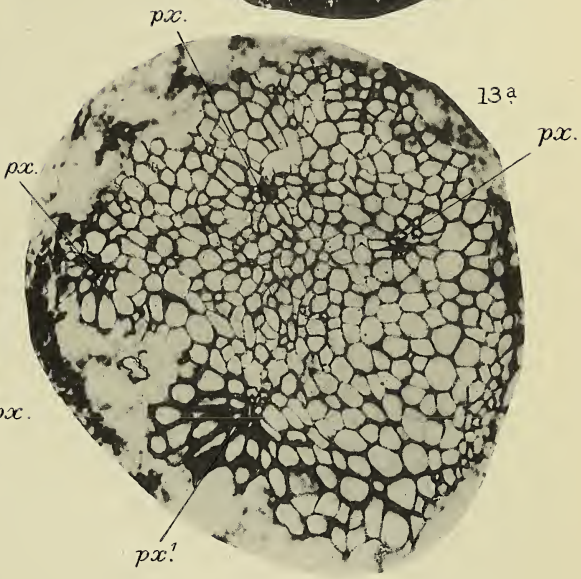

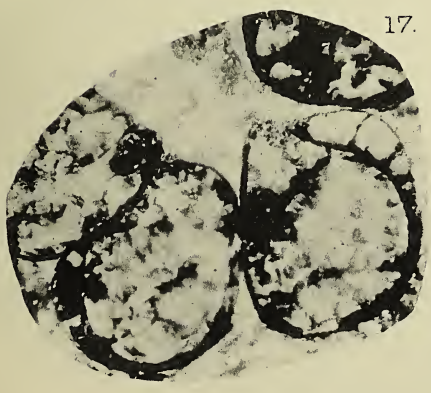

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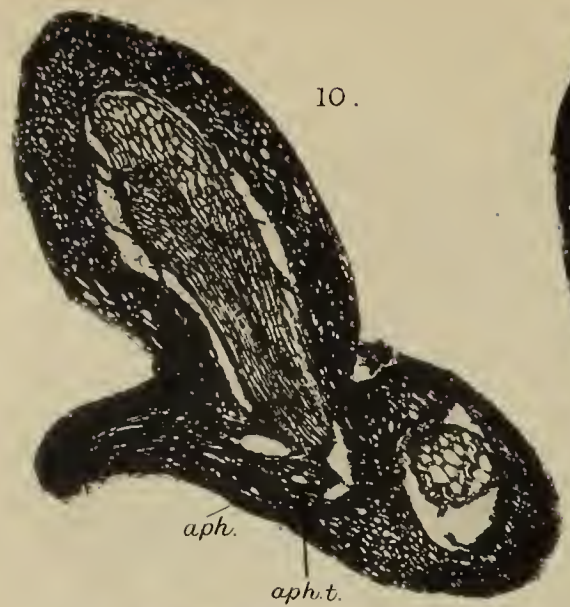
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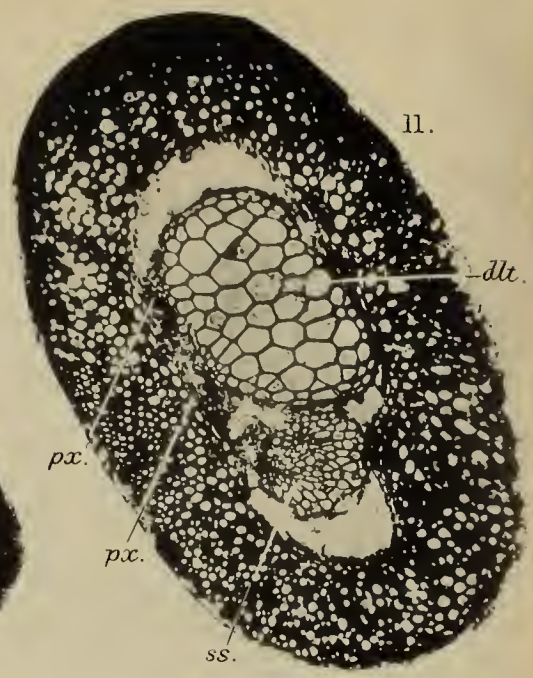
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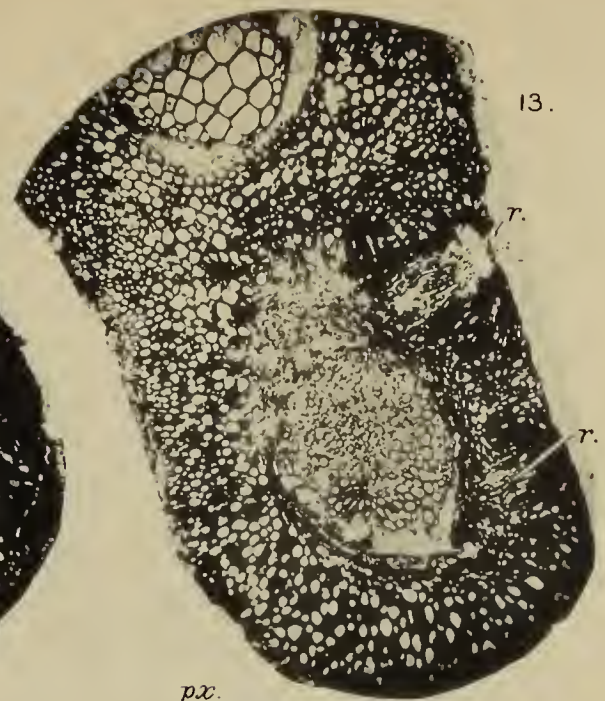
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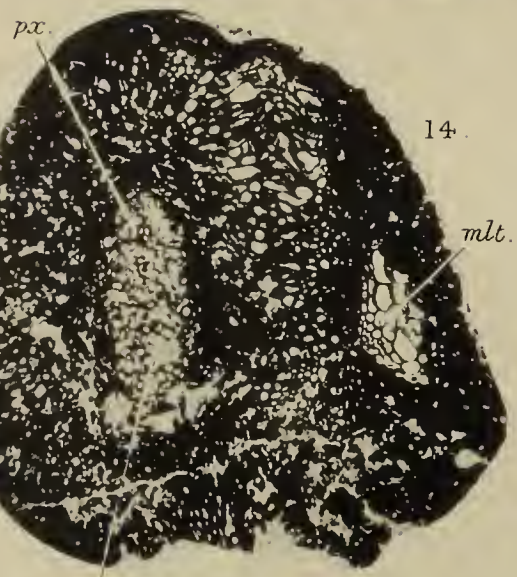
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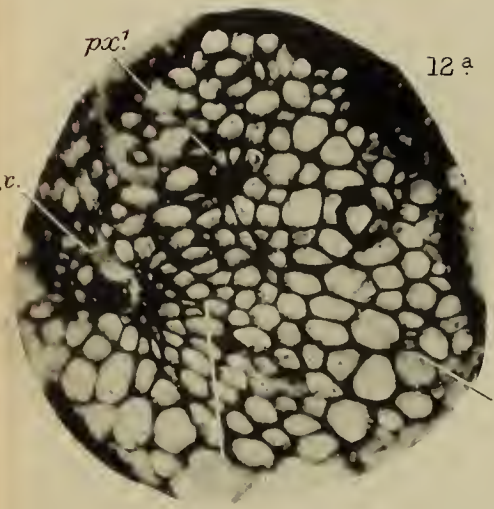
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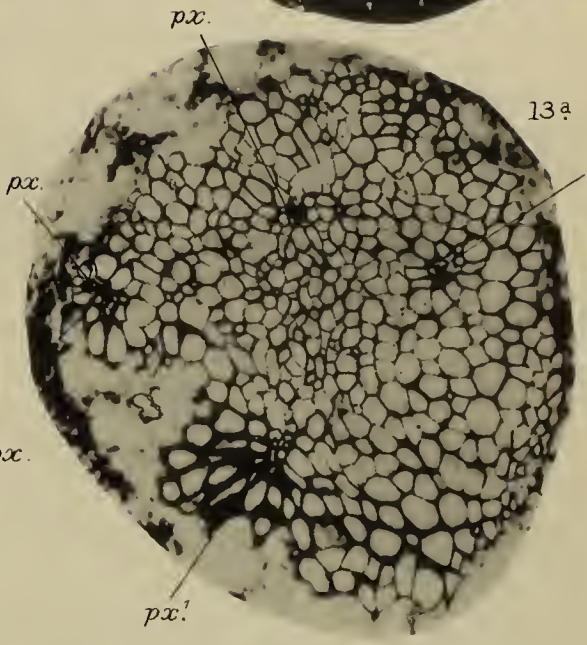
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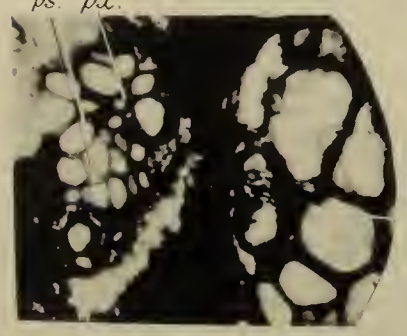
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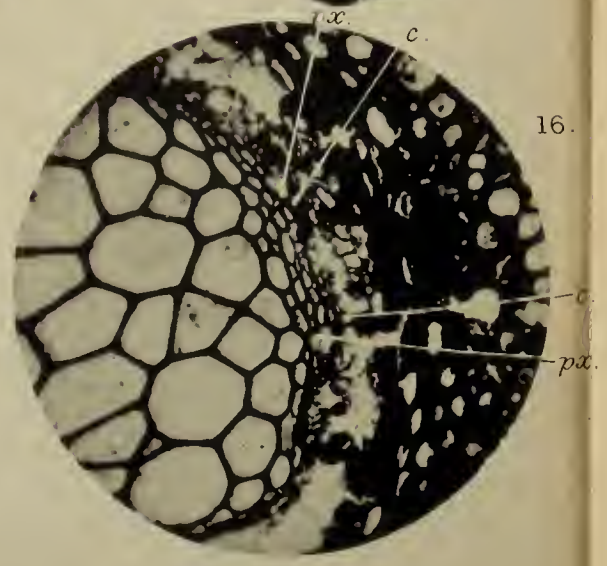
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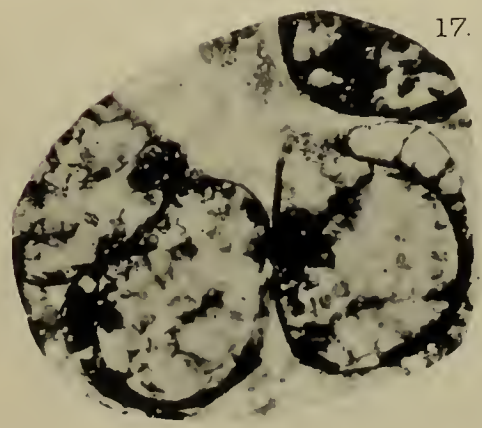
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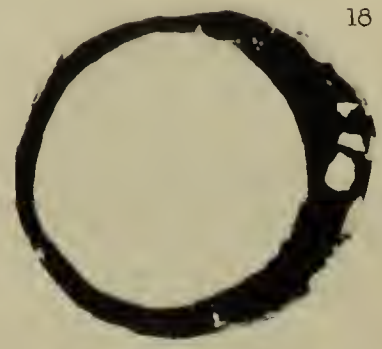
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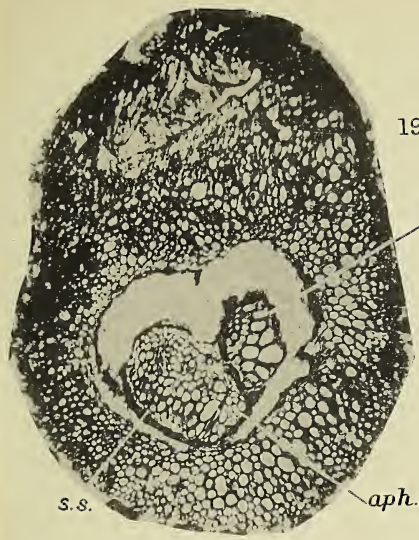
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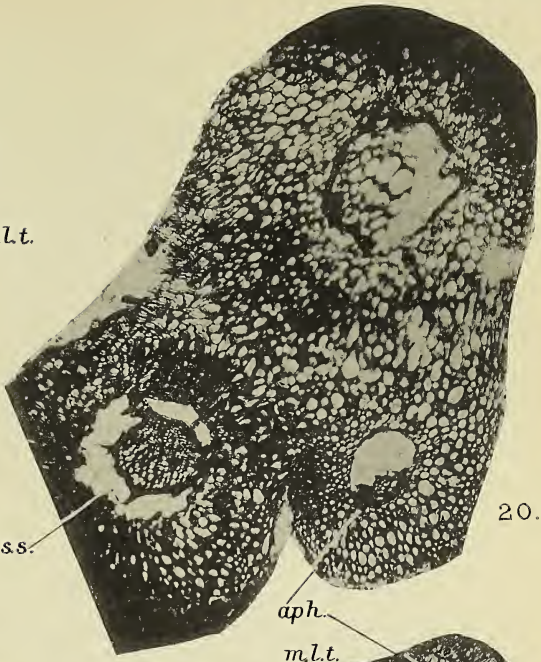
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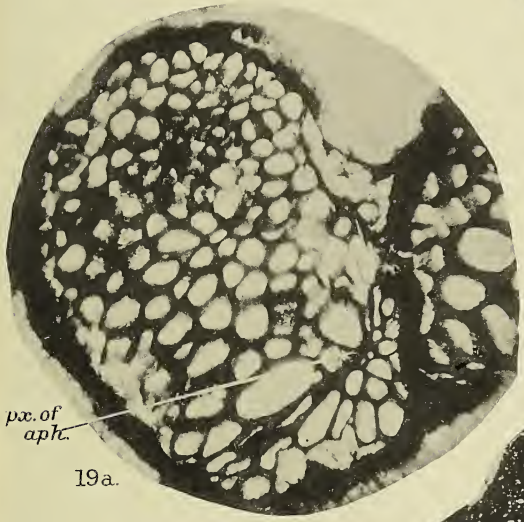
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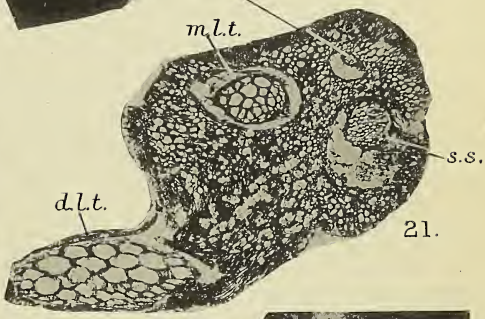
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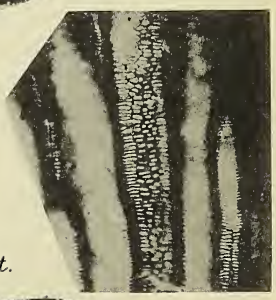
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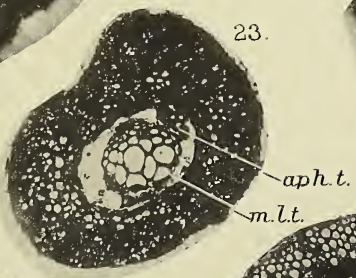
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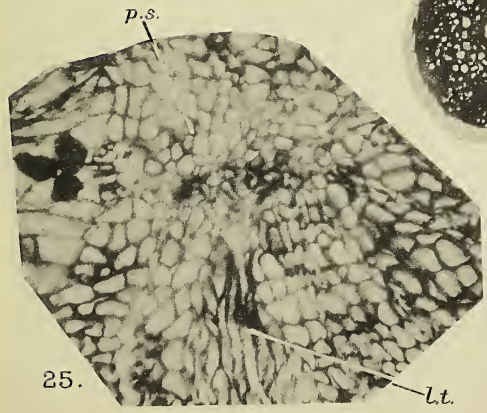
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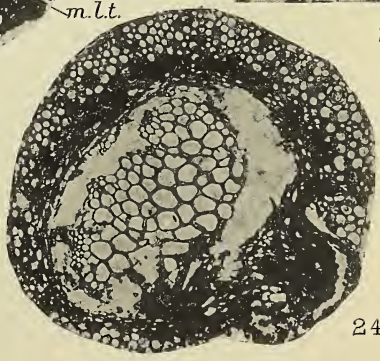
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On the Development of the Sporangia and Spores of Aneimia phyllitidis.

BY

WILLIAM CHASE STEVENS,

Professor of Botany in the University of Kansas, U.S.A.

With Plates LXXXIV and LXXXV.

IN a contribution to the Botanical Gazette, Binford ('07) has advanced our knowledge of the Schizaeaceae by a careful study of the sporangia of *Lygodium*. He confirms the work of Prantl ('81) in all essential respects, and carries his research on into the development of the tapetum and sporogenous tissue.

Material which I put through Flemming's fixative some years ago in Strasburger's laboratory affords me opportunity to give an account of *Aneimia phyllitidis* also in more detail than Prantl has related it. Prantl begins with the earliest stage of development of the sporangium, and shows that it arises from a single protodermal cell not far from the growing apex of the sorophore. A series of sporangia is thus formed in acropetalar order along the margins of the sorophore ; and while this is going on the sorophore grows faster on its upper than on its lower surface, and so shifts the sporangia from their marginal to a dorsal position. This unequal growth at the two surfaces is shown in Pl. LXXXIV, Fig. 6. As the sorophore elongates, new sporangia continue to be started ; but not all of these come to maturity, evidently because, in part at least, those first formed divert nutriment to themselves to such an extent that those coming later are starved.

Prantl reports that the protodermal cell that is to produce a sporangium grows out beyond the general level before dividing. My sections do not show this. Rather I would conclude from them that a sporangium arises from several protodermal cells ; but I do not have all stages complete enough for a critical discussion of this point. At the early stages shown in Fig. 1, *a* and *c*, we find a few initials of the sporangium wall, and a central cell or initial of the archesporium and tapetum. Anticlinal divisions in the wall initials multiply them many times (Figs. 2, 4, &c.). At the same time the central cell by periclinal divisions cuts off an outer layer

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of cells or initial layer of the tapetum, and these surround a single cell, which is proved to be the archesporium.

The tapetal initials now multiply by anticlinal divisions, and finally each undergoes a periclinal division to form a two-layered tapetum (Pl. LXXXIV, Fig. 1, *d*, and Figs. 2, 4, and Pl. LXXXV, Fig. 8). This agrees essentially with the formation of the wall, tapetum, and archesporium of *Lygodium* as described by Binford, excepting that he finds a three-layered tapetum in places. Turning to the Ophioglossaceae for comparison, Bower ('96) has found that in *Helminthostachys* the tapetum is formed by division of the wall-cells alone, and Campbell ('05) reports that in *Botrychium* the tapetum comes partly from the wall-cells and partly from the sporogenous cells.

While the tapetum is thus increasing, the archesporium is undergoing divisions that result in a sporogenous tissue, or group of grandmother-cells of the spores (compare Pl. LXXXIV, Fig. 4, and Pl. LXXXV, Fig. 8). The sporangium is growing rapidly during these stages, the number of tapetal cells increasing step by step with the growth of the wall.

About the time the number of grandmother-cells of the spores, or gonotokonts, to use the term proposed by Lotsy ('04), is complete (approximately 65) the outer tapetal cell layer begins to break down, and this is soon followed by a similar behaviour of the inner layer, leaving the solid mass of gonotokonts suspended in a tapetal cytoplasm (Pl. LXXXIV, Figs. 4, 5, and 6). Prantl found a different behaviour in the sporangia of *Aneimia phyllitidis* studied by him, in that the inner layer of the tapetum formed a plasmodium while the outer layer remained intact (see his Fig. 120); and Binford finds the outer layer persisting in *Lygodium* (his Fig. 30). In some instances I find the outer tapetal layer breaking down even before the archesporial divisions are far advanced (Fig. 3).

There seems to be a consensus of opinion that in all such cases the tapetal cells serve as nurse cells to the gonotokonts through their meiotic divisions, and to the spores through their developmental processes; and there are some considerations which suggest that this function can be carried on better when the entire tapetum is in the condition of a plasmodium, since the nuclei are then free to distribute themselves amongst the gonotokonts after these have separated into relatively small groups or into single free individuals, and also since the tapetal cytoplasm in the form of a plasmodium is free to circulate, and so facilitate the distribution of nutritive materials that in part are held in solution in the cell sap, and in part are suspended in the cytoplasm, or even enter into its composition. The conclusion of de Vries ('85) that, since diffusion has been found inadequately slow, the transportation of materials must be assisted by the rotation and circulation of the protoplasm, is given recent support in the researches of W. Bierberg ('08), who found cytoplasm rotation to more than treble the rate of transportation above that achieved by diffusion alone.

Pfeffer ('97), however, contrary to de Vries, sets forth that within the cells a sufficiently rapid distribution of materials could take place with the assistance of mechanical bendings, and variation in cell-turgor, tissue tensions and temperature. While all these things must be operative, in the light of Bierberg's results there seems great probability that the plasmodium habit of the tapetal cells may be for the distribution of food by cytoplasmic movements, as well as for setting free the nuclei that they may assemble immediately about the gonotokonts and their descendants (Figs. 5, 6, and 7).

Fig. 7 shows that such movement has actually occurred, for here the cytoplasm has to a large extent forsaken the periphery of the sporangium and accumulated about the gonotokonts. On comparing Pl. LXXXIV, Figs. 6 and 7, and Pl. LXXXV, Figs. 11 and 13, we have evidence that the cytoplasm migrations begin when the tapetum first breaks down and continue through the subsequent stages of sporangial and spore development up to the maturity of the spores. It seems, therefore, highly probable that in this particular instance a distribution of nutrient materials within the sporangium may be aided by a more or less constant plasmodial circulation.

It will be noted, on comparing Pl. LXXXIV, Fig. 2, and Pl. LXXXV, Figs. 8 and 9, that the sporangia and sporogenous cells increase in size up to the time of synapsis in the nuclei of the gonotokonts; then growth is lessened while the meiotic divisions are taking place. After this it appears from the increased size of the vacuoles in which the young spores lie (Fig. 11) that the sporangia resume their growth for a time, while the young spores remain in size about as they were when first formed. In this stage no vestige appears of a wall of the gonotokonts, and the young spores lie widely separated in the vacuole that formerly held their respective gonotokonts. After a time, however, the plasmodium creeps in between the spores and holds each one separately embedded (Figs. 11 and 13). Miss Twiss ('10) finds a similar early disappearance of the gonotokont wall in *Lygodium*, but in *Aneimia hirsuta* she still finds traces of it after the walls of the spores appear in the tetrad.

The separation of the gonotokonts from each other is initiated by their assuming a spherical form (compare Pl. LXXXIV, Figs. 4 and 7). Thereupon it appears that the tapetal plasmodium creeps in between them and shoves them more or less widely apart, while at the same time the nuclei of the plasmodium become dispersed among and around them, as shown by Pl. LXXXIV, Fig. 7, and Pl. LXXXV, Figs. 9 and 10. That the gonotokonts are in a condition of growth at this time is seen on comparing Pl. LXXXIV, Fig. 4, and Pl. LXXXV, Fig. 9, and that it is also a critical nutritive period is attested by the fact that one finds not infrequently whole sporangia now beginning to disintegrate.

When the last division is complete, as shown in Pl. LXXXV, Fig. 10, the plasma membrane of a gonotokont persists as the common outer membrane

of the spores as they lie still connected in the tetrad. It appears that the plasma membranes bounding the inner surfaces of the spores now become dissolved out in a median plane, so that the spores are free to separate. That such disjunction actually soon takes place is shown in Fig. 11.

The processes concerned in the separation of the spores are not entirely evident; some observable facts, however, give basis for a conclusion as to the main events. By comparison of Figs. 10 and 11 it is seen that after the last meiotic division the vacuole in which a gonotokont was lying increases considerably in size, while the young spores just issuing from the tetrad decrease in volume. Evidently it is this shrinkage which initiates the separation of the spores. In some rare instances thin films of the tapetal cytoplasm flow in between the spores before their contraction is far advanced (Fig. 12). Prantl, l. c., considers the clear space around the spores (seen in my Figs. 11 and 12 and his Fig. 77) to be a greatly swollen cell-wall; and the delicate strands extending between the spores, as shown in my Fig. 12 and his Fig. 122, he concludes are planes of differentiation in the swollen wall. Evidently the nature of his preparations led him to the wrong conception.

After the spores have separated the plasmodial cytoplasm creeps in and completely embeds each one, as shown in Fig. 13; and then the spores enter upon the final period of growth in size and formation of cell-wall. Figs. 11, 18, 19, 20, and 21 are drawn to the same scale, and show the progression of these events as the spores advance to maturity. Figs. 18 and 19 were taken from the sporangial stage shown in Fig. 13. Here the wall of the spore has begun to thicken; and, whereas with the triple stain, safranin, gentian-violet, and orange G, it was when first formed stained purple, it now is stained red. At this stage the sculpturings on the surface of the wall characteristic of this species (shown in Fig. 21) have not yet appeared. Fig. 19 is a surface view giving the three flattened surfaces where the other three spores of the tetrad joined it. There is a line of cleavage down each of the three angles, one of which is seen in cross-section in Fig. 18. Even in these immature spores one often finds in his preparations the three flaps parted and revealing the nucleus within.

The wall now thickens, heaping up ridges at its outer surface and adding an inner layer which comes out almost colourless from the triple stain (Fig. 20). The inner coat (endospore, or intine) is not continuous at the three angles, as shown in cross-section through one of the angles in Fig. 20, neither does it follow the protruding lips of the outer coat (exospore, or extine) at this place, as Beer ('06) finds to be the case in *Helminthostachys*.

When the spore is of the age shown in Fig. 18 a thin film of cytoplasm can be followed out from the mass which holds the nucleus embedded, around the inside of the wall; but when the spores have arrived at the stage shown in Fig. 20 the entire protoplast seems to have receded to one corner

of the spore, either from plasmolysis caused by the reagents or from natural causes. The balled-up condition of the nucleus in Fig. 20 indicates that the former alternative is the more probable.

We find in the literature speculations as to how the growth in thickness of spore coats comes about. In *Helminthostachys*, Beer, l. c., found that the spore protoplast, at the time when the exospore first appeared, was so poor in substance as to make it unlikely that this could be employed in the growth of the wall. The protoplastic substance of *Aneimia* spores also is very little, even relatively less than in the spores of *Helminthostachys* (compare my Figs. 18 and 20 with Beer's Fig. 11), and entirely inadequate, it seems to me, to supply materials for the increase in wall thickness that takes place between the stages represented in Figs. 18 and 20. As Beer suggests, it appears that the substance for this increase must come from, or through the agency of, the tapetal cytoplasm. Fitting ('00) comes to a similar conclusion for the spores of *Isoëtes* and *Selaginella*. Beer holds it impossible to decide whether this material is applied to the wall by the plasmodium or by the spore protoplast. He suggests that in the latter event the nutrient substance would have to enter the spore protoplast in liquid form before it could be applied to wall construction. As was stated above, in *Aneimia* the exospore takes the safranin stain strongly while it is yet quite thin. This reaction, according to Thomson ('05), indicates that the exospore has undergone the cutin or suberin modification; and this would make difficult the passage of materials to and from the spore protoplast. This fact adds plausibility to the theory that the plasmodium, working from without, supplies the materials, and possibly the stimuli also, for the characteristic growth of the wall.

In his studies of various species of *Isoëtes* and *Selaginella*, Fitting, l. c., finds the exospore widely separated from the mesospore and the spore protoplast withdrawn from contact with the latter, excepting at the spore apex, before these coats have attained their characteristic thickness, and before the endospore has been laid down. Although he finds no indication of protoplasm inhabiting the spore coats, he nevertheless comes to the conclusion that these have the power of constructing their own substance from the materials that come to them from the tapetum. He says: 'Ich glaube also, dass gewichtige Gründe zu der Annahme vorliegen, dass die neuen Hauttheilchen erst innerhalb der wachsenden Membranen entstehen oder, mit anderen Worten, dass diese Membranen die Fähigkeit besitzen, ihre constituirenden Verbindungen selbst aus einer Nährlösung aufzubauen.' And again: 'Ich halte es für denkbar, ja für wahrscheinlich, dass die Sporenhäute von Isoetes und Selaginella nicht von dem Sporenplasma aus, sondern direct von den Tapetenzellen ernährt werden.'

A more recent study of *Selaginella* by Florence Lyon ('05) suggests, however, that where gaps occurred between the membranes and spore

protoplast in the sections studied by Fitting, Campbell ('05), and herself they were due to shrinkage during the preparation of the sections. In sections which showed no signs of shrinkage anywhere, Miss Lyon found the region of the gaps filled with a substance that seemed to be a part of the spore membrane.

In *Aneimia* the spore protoplast is found in contact with the wall all around after the thickening and cutinization of the wall are well advanced (Fig. 18). In older spores the recedence of the protoplast away from most of the wall (Fig. 20) may be due to plasmolysis during the processes of fixing and embedding ; or it may be that the thin film of cytoplasm shown in Fig. 18 has been used up in nourishing the spore coats, as Strasburger ('82, '89) has suggested for similar appearances in many pollen-grains.

Miss Twiss, l. c., finds for *Lygodium circinatum* and *Aneimia hirsuta* that soon after the spore wall has been laid down, and before its thickening has set in, it begins to stain red with the safranin-gentian-violet combination; then as the wall thickens its outer portion stains yellow, indicating pectin, while the inner part reacts to safranin as before. While this differentiation of the exospore is going on, or before, an endospore layer of cellulose is laid down which remains thenceforth chemically unaltered. This differentiation of the exospore into a pectinized and a cutinized or suberized layer does not appear in my preparations.

When the spore walls of *Aneimia phyllitidis* are first formed, we find the cytoplasm and nuclei of the tapetal plasmodium in a well-nourished condition, while at a later stage when the spores are more fully matured, and with the growth of the walls essentially completed, the tapetal cytoplasm is very noticeably impoverished and its nuclei are found in various stages of disintegration.

As might be expected, many of the phenomena of sporangial development have to do especially with the nutrition of the spores. Mention has already been made of the advantages due to the surrounding of the gonotokonts and spores by the tapetal plasmodium, and attention should now be called to the provision for the storing of food and water. I find that at the stage shown in Pl. LXXXIV, Fig. 7, the wall-cells of the sporangium are usually loaded with starch. The cells of the sorophore also, adjacent to the sporangium, contain starch, although in less amount. The very large cells on the marginal upper surface of the sorophore doubtless serve for the storage of water as well as food. Their reaction to the stains shows them to be filled with a very finely granular substance that presumably is some form of food. Within the tapetal and sporogenous cells, however, stored food is not evident. Beer, l. c., finds the wall-cells and tapetal plasma of *Helminthostachys* to contain reserve starch. Burlingame ('09) finds an abundance of starch in the plasmodium, and 'food granules' in the sporogenous cells of *Ophioglossum reticulatum* ; and Binford, l. c., finds the

wall-cells filled with reserve food in those sporangia of *Lygodium* whose sporogenous cells have failed to produce spores. This is true of *Aneimia* also, but not of their sterile sporangia alone. I find, rather, that there is on the whole less starch in the sterile than in the fertile sporangia.

The fact that there is such an abundance of stored food in the sporangia in all stages of their development, and that sterile sporangia contain reserve starch, suggests that sterilization may not be due to insufficient nutriment alone.

Summary.

1. Starting from one, or possibly more than one protoderm cell, a few initials of the sporangium wall are soon formed surrounding a central cell, which proves to be the initial of the archesporium and tapetum.

2. This initial by periclinal walls cuts off a layer of tapetal cells, each of which in its turn undergoes one periclinal division, and forms a two-layered tapetum. The single cell surrounded by these is the archesporium.

3. The archesporium gives rise to approximately sixty-five gonotokonts or grandmother-cells of the spores.

4. Before the archesporial divisions are complete the outer tapetal layer begins to break down, and this is soon followed by the similar behaviour of the inner layer, and the mass of gonotokonts thus becomes suspended in the common tapetal cytoplasm now forming a plasmodium.

5. There is evidence that circulation of the tapetal cytoplasm takes place, which probably assists in the distribution of nutrient materials.

6. The sporangium increases in size up to the time of synapsis in the gonotokonts; then apparently growth is retarded during the progress of the meiotic divisions.

7. The mature gonotokonts separate from each other, and the tapetal plasmodium flows in and entirely surrounds each one.

8. From the time of synapsis to the completion of the meiotic divisions seems to be a critical nutritive period, for then it not infrequently happens that sporangia cease their growth and begin to disintegrate.

9. No wall is seen around a gonotokont as it lies separately embedded in the plasmodium, and when the last meiotic division is finished the plasma membrane of the gonotokont persists as the external membrane of the tetrad.

10. The separation of the young spores of a tetrad from each other is initiated by their expelling water and shrinking apart, and thereupon the tapetal plasmodium flows in between them.

11. At a very early stage in its thickening the spore coat reacts as cutin does to the triple stain. Down the three angles of a spore this coat shows lines of fission. Very often in the preparations the exospore breaks apart down these lines into three distinct flaps.

12. The early cutinization of the exospore, which would make difficult the passage of materials through it, is in favour of the theory that the increase in thickness of this coat is the work of the tapetal plasmodium.

13. While the exospore is thickening an endospore is laid down in contact with it, which gives the cellulose reaction with the triple stain. This coat is continuous excepting in front of the lines of cleavage in the exospore, so that the endospore would offer no resistance to the breaking apart of the spore-wall into the three flaps above spoken of.

14. At first the spore protoplast lines the wall throughout; but after the endospore has been laid down, either its substance has been used up over the larger area of the wall, or it shrinks away from the wall and is found balled up at one end of the spore; or the alternative remains that this condition is due to plasmolysis during the preparation of the materials.

15. As the growth of the spores progresses the gradual depletion of the tapetal cytoplasm and nuclei is evidence that the substance of these has been diverted to the nutrition of the spores.

16. Storage of food, largely in the form of starch, takes place in the sporangium wall and in the cells of the sorophore at the base of the sporangium.

17. The tapetal plasmodium and the gonotokonts do not appear to contain stored food.

18. Even sterile sporangia contain an abundance of stored food in their walls, and this suggests that their sterilization has been due to other causes than insufficient nutritive materials alone.

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EXPLANATION OF PLATES LXXXIV, and LXXXV.

Illustrating Professor Stevens's paper on *Aneimia*.

PLATE LXXXIV.

Fig. 1. Longitudinal section through the apex of a young sorophore, showing sporangia in successive stages of development. × 393.

Fig. 2. Median longitudinal section through young sporangium, showing wall-cells, initial cells of tapetum, and archesporium. × 860.

Fig. 3. Median longitudinal section through young sporangium, slightly older than in Fig. 2. The initial cells of the tapetum have divided, producing a two-layered tapetum, and the archesporium has undergone division. Even at this early stage the walls of the tapetum have begun to be absorbed. × 308.

Fig. 4. Still older stage than in Fig. 3, showing young gonotokonts or grandmother-cells of the spores surrounded by the two-layered tapetum. × 860.

Fig. 5. Section remote from the centre through a sporangium, showing tapetal cells completely broken down and tapetal nuclei migrated from the periphery to cluster about the gonotokonts, two of which are here shown. × 308.

Fig. 6. Longitudinal section through young sporangium and sorophore, showing the epidermal cells of the upper surface greatly enlarged, resulting in the turning of the sporangia, which are at first marginal, to the under side of the sorophore. × 393.

Fig. 7. Section through sporangium, showing mature gonotokonts previous to the synaptic stage. At this stage the wall-cells are filled with starch grains. × 540.

PLATE LXXXV.

Fig. 8. Section through young sporangium. All of the mother-cells of the tapetum have divided but one, and this is in the late prophase of division. Even at this early stage the meshes of the cytoplasm of the wall-cells are being filled with reserve starch. × 860.

Fig. 9. Portion of tapetal plasmodium and embedded gonotokonts in the synaptic stage. One of the gonotokonts disintegrating. × 860.

Fig. 10. After second meiotic division, showing young spores still in contact. × 860.

Fig. 11. A later stage. Young spores separated and dispersed within the cavities previously occupied by their respective grandmother-cells. × 860.

Fig. 12. The same as Fig. 11, but showing thin strands of cytoplasm that evidently had flowed in between the spores while they were yet in close proximity. × 860.

Fig. 13. Longitudinal section through a sporangium, showing young spores after the tapetal plasmodium has surrounded each one separately. The wall-cells, excepting the cap-cells, are loaded with reserve starch. × 308.

Fig. 14. One of the wall-cells of Fig. 13, showing reserve starch assembled about the nucleus. × 860.

Fig. 15. Polar view of late prophase of the first meiotic division. × 2,000.

Fig. 16. Early anaphase of the first meiotic division. × 2,000.

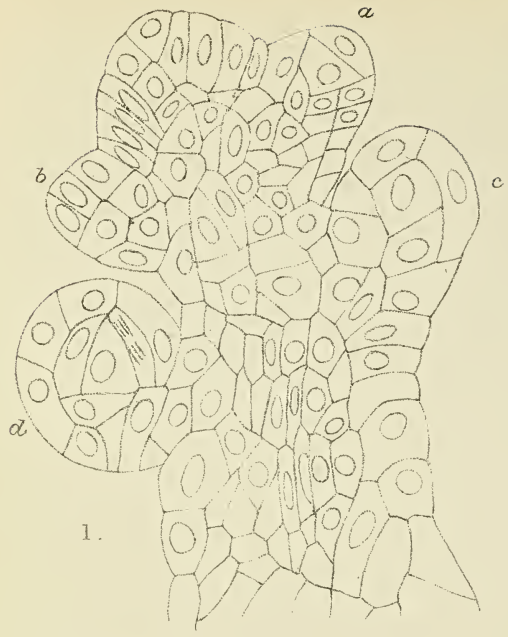
Fig. 17. Telophase of second meiotic division. Nuclear membrane not yet formed. × 2,000.

Fig. 18. Section of immature spore through its apex, showing the spore coat thinner there than elsewhere. Endospore not yet laid down. × 860.

Fig. 19. Surface view of immature spore of the age shown in Fig. 18. × 860.

Fig. 20. Section through mature spore, showing exospore and endospore, the former with a line of cleavage at the angle and the latter not lining the exospore at this place. The protoplast has receded entirely to one end of the spore. × 860.

Fig. 21. Surface view of mature spore, showing wall sculpturings and lines of fission through the spore coats down the three angles. × 860.



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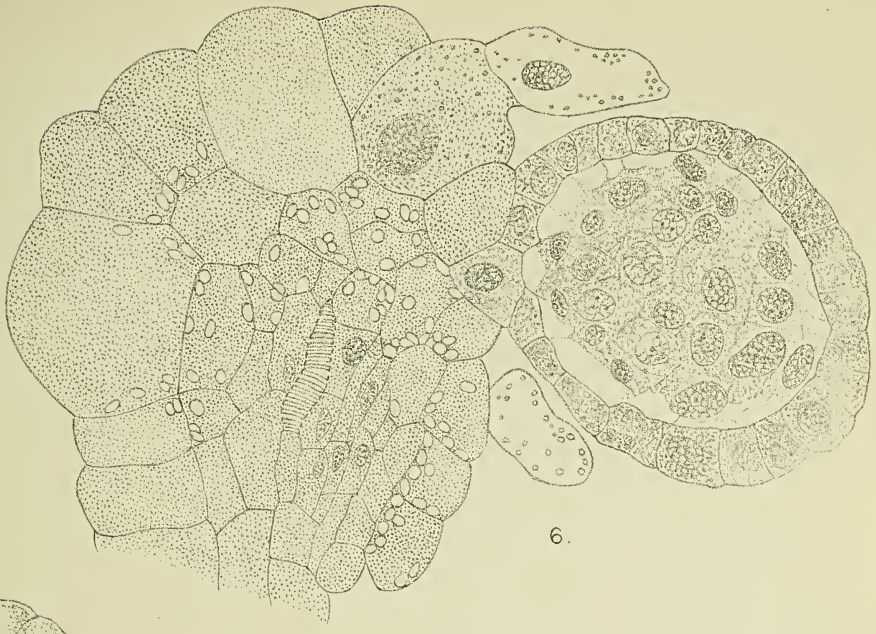
STEVENS — ANEIMIA.



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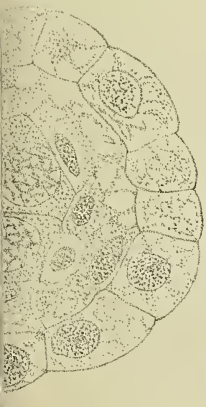

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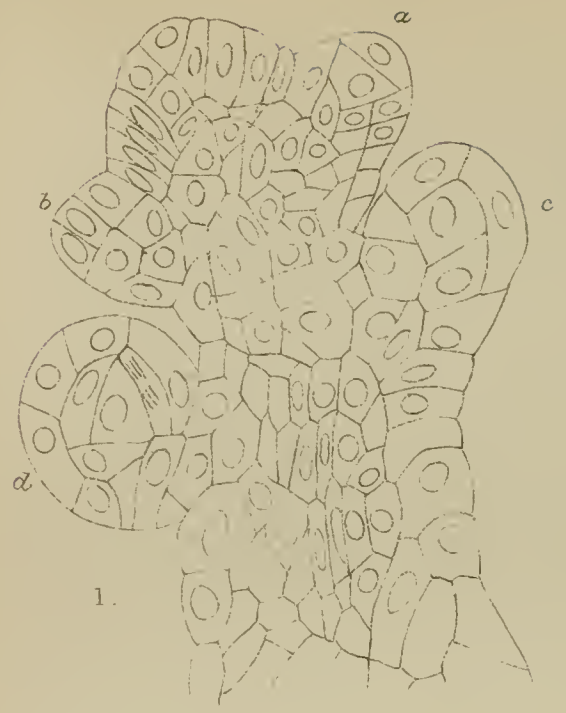

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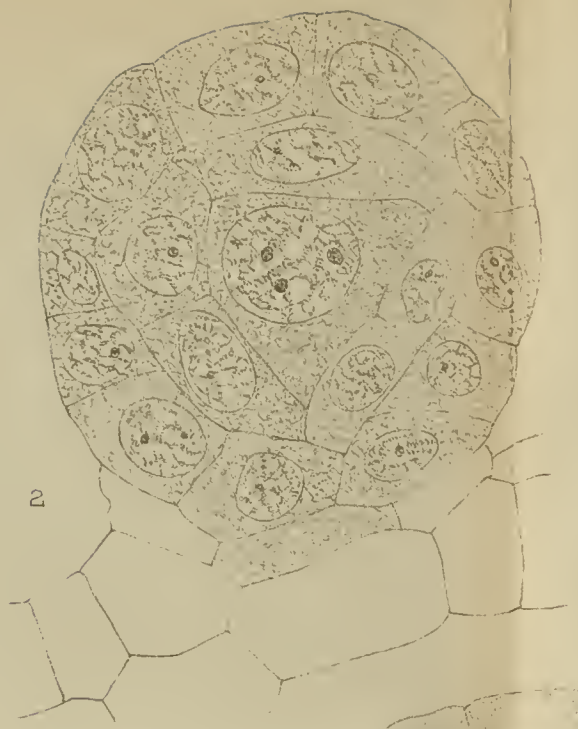

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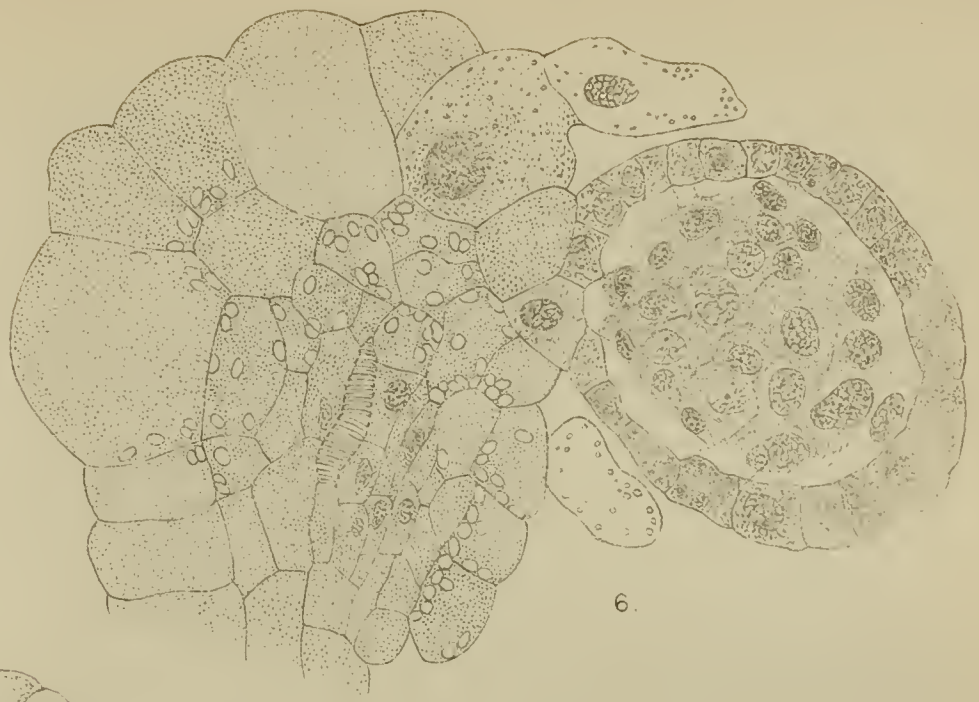

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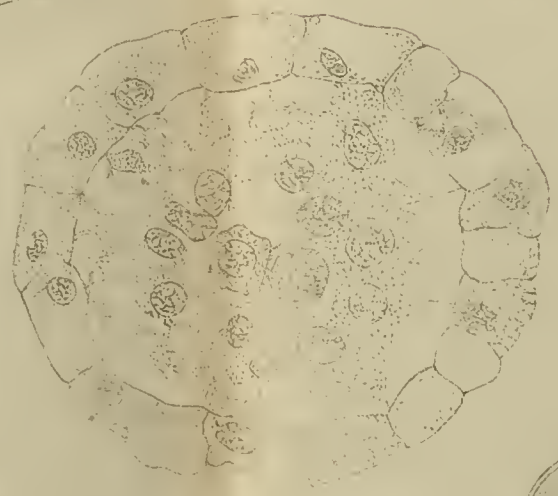

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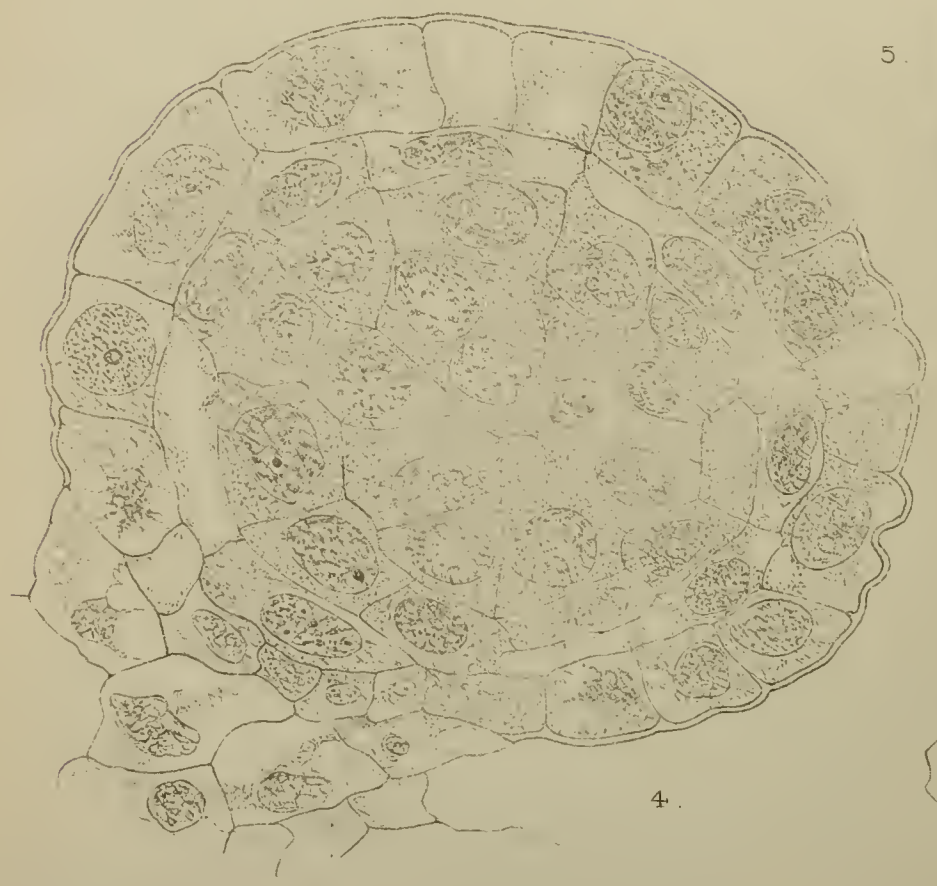

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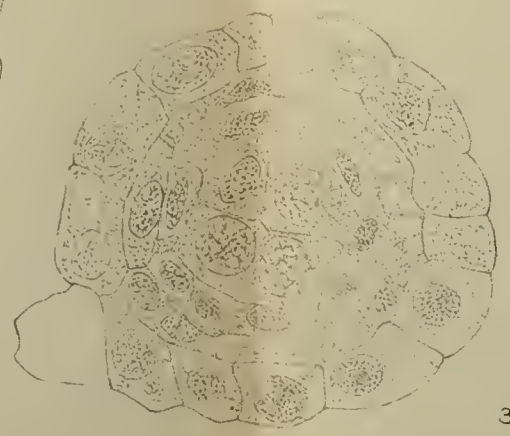

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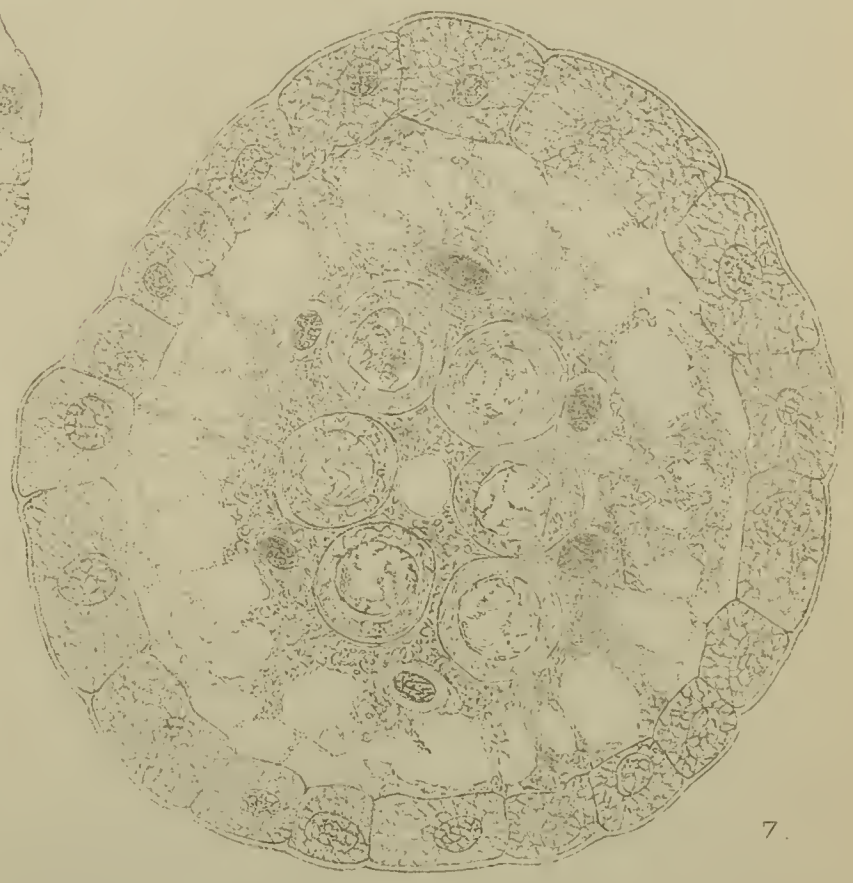

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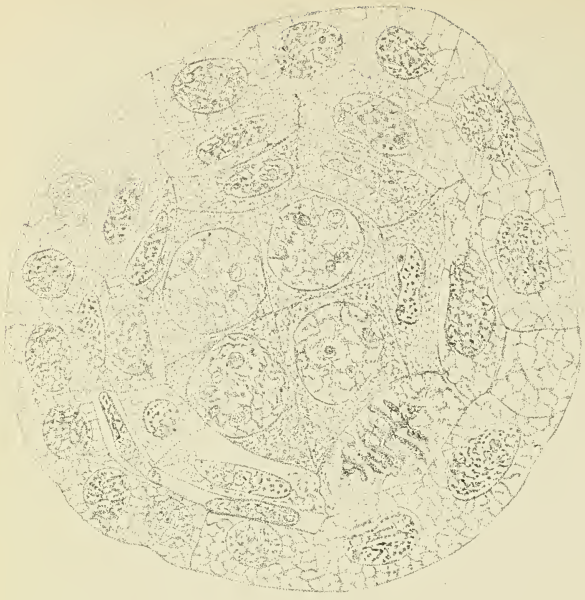
STEVENS — ANEIMIA.



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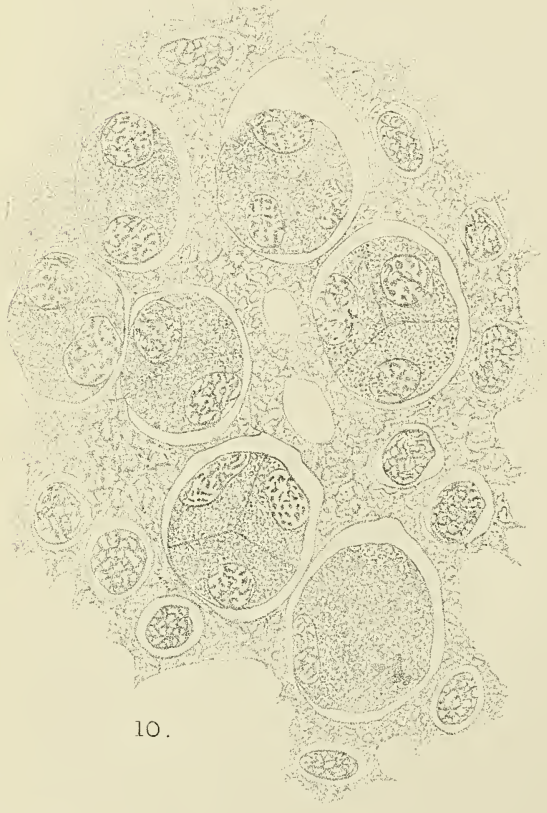

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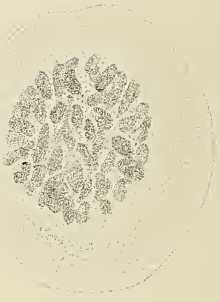

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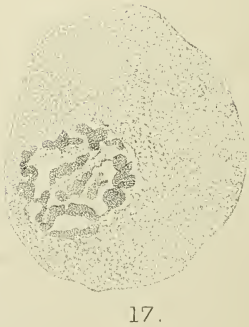

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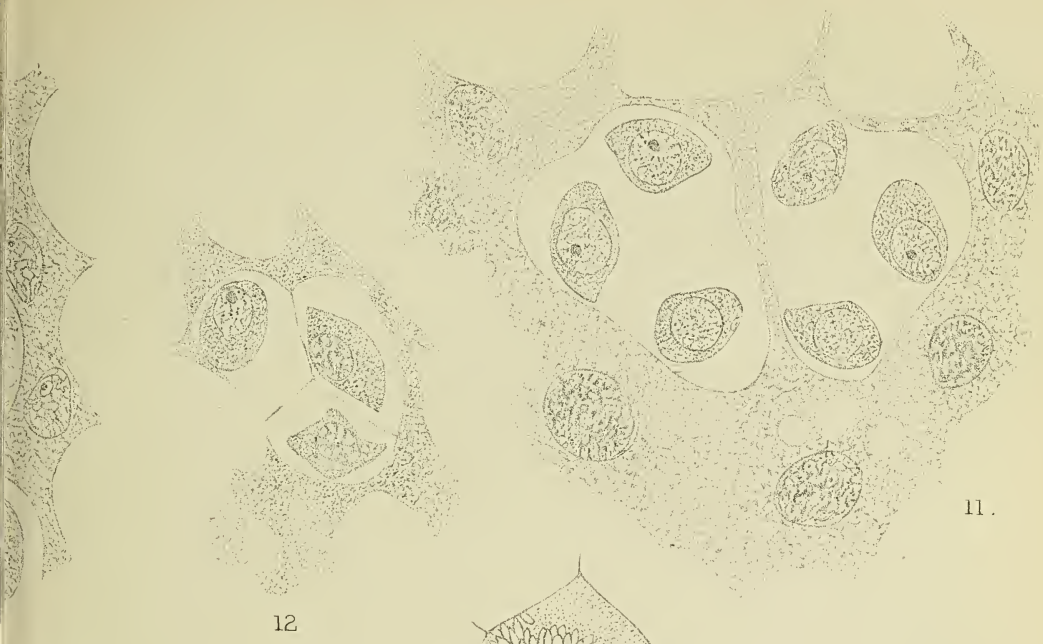

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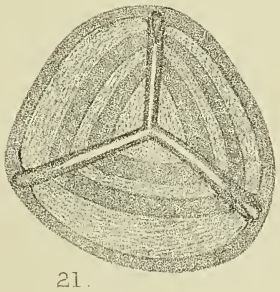

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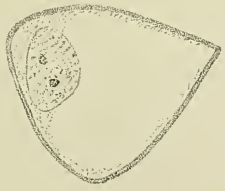

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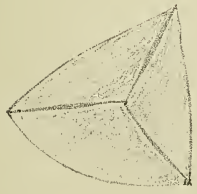

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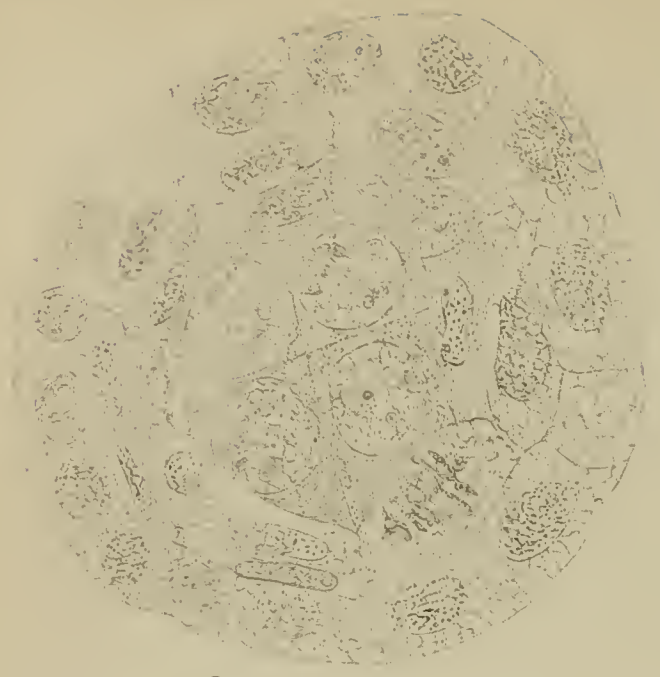

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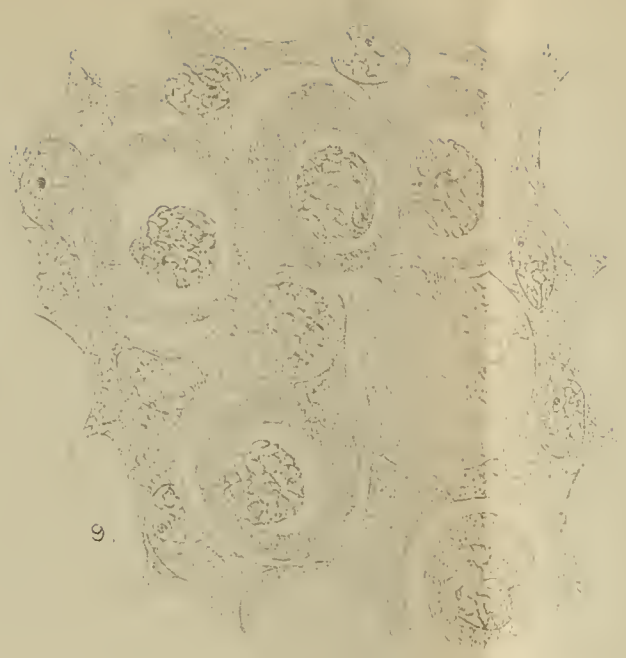

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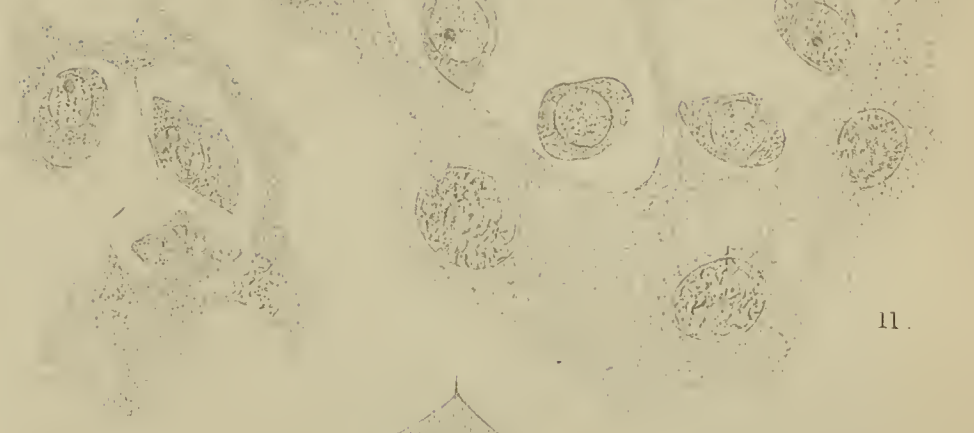

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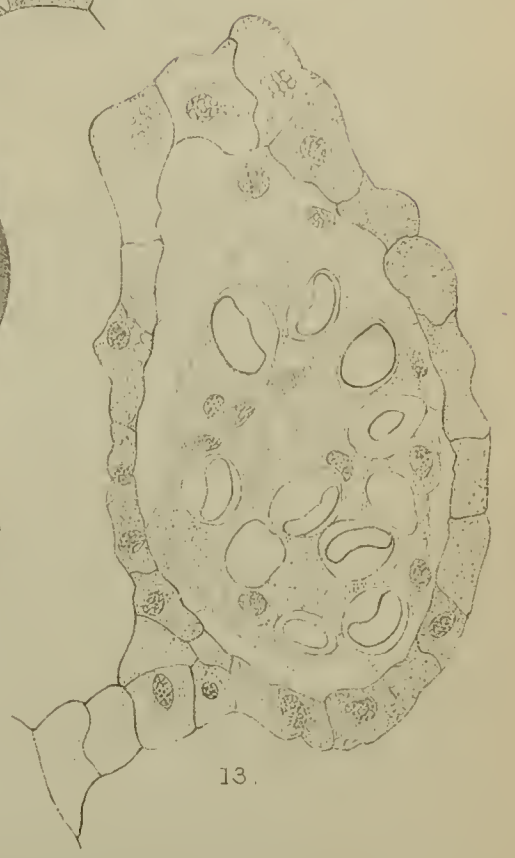

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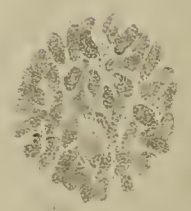

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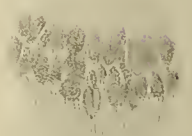

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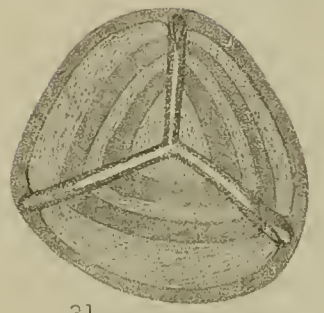

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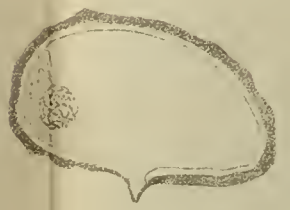

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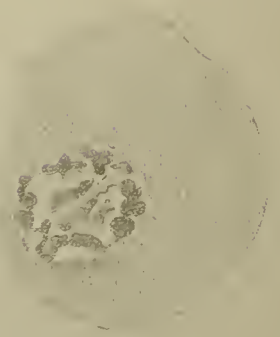

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Stevens, del.

Huth, lith.

STEVENS — ANEIMIA.

Note on the Action of Strychnine upon some Somatic Cells.

BY

HELEN P. KEMP,

Royal College of Science, London.

THE question of the nature of the alkaloids and of their action upon the living cell is one bearing closely upon that of absorption in general. Recognition of the fundamental character of the latter process, as a biological phenomenon of the first importance, has led to a considerable activity in this particular field of investigation; marked by the appearance, during the last three decades, of various accounts of the action of certain of the alkaloids upon different types of cell. There has been, however, so little co-ordination of work that not only is the advance made along this line relatively small, but the results also in some cases are ambiguous. The ambiguity appears to be largely due to the fact that similar methods have been used and similar phenomena studied with different ends in view, and cross-references made without a sufficient indication of the dissimilarity of the latter.

In 1887 Oscar and Richard Hertwig (3) published the results of a series of experiments on the action upon Echinoderm eggs of certain alkaloidal poisons, among others quinine, chloral, nicotine, and strychnine. They immersed the eggs in solutions of varied strength (0·005 %–0·1 %) and for varied lengths of time (10 mins.–3 hrs.) and then placed them in sea-water containing spermatozoa. As an indicator of the action of the various alkaloids, and as a standard of comparison between them, they chose the condition of polyspermy in the egg, this being dependent upon a modification of the latter under the influence of the poisons, by which it is rendered permeable to more than one spermatozoon. They used eggs at three distinct physiological stages of development, (1) non-maturated, (2) maturated but unfertilized, (3) fertilized; and obtained differences of result according to the stage chosen. Thus class (1) was found to be extremely sensitive to the modification indicated by polyspermy, such eggs after treatment with the poison being filled with spermatozoa, class (2) to be less sensitive, and class (3) almost impervious, the eggs developing, as far as could be seen, normally up to the pluteus stage, subsequent to their

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

immersion in strychnine. These differences are significant in a comparison of the results obtained with eggs, with those obtained with somatic cells.

The authors classed the alkaloids roughly according to the nature of their action, and stated that whereas quinine and chloral seemed to diminish the contractility of the protoplasm, destroyed the achromatic fibres, and seriously inhibited karyokinesis and the subsequent division of the cell, strychnine (and nicotine) on the contrary greatly increased the contractility of the protoplasm, and exercised no injurious effect upon either the achromatic structures or the processes of karyokinesis and cell-division. They further showed that with a stronger solution of strychnine(0·01%–0·25%) and prolonged immersion (2–3 hrs.) further development of the egg was arrested, and regressive metamorphosis ensued, terminated by fragmentation. They concluded that strychnine (as also nicotine and morphine) did not affect karyokinesis in any specific way, but only, if used in a sufficiently strong solution, by inducing a general inhibition of the metabolic processes, resulting, if severe, in complete degeneration of the egg.

The occurrence of tripolar and quadripolar division figures after treatment with chloral or quinine, i. e. the class of alkaloids opposed to that containing strychnine, is noted by the authors as being perhaps due to the condition of polyspermy, rather than directly to the action of the drugs themselves. This introduction of a confusing factor is certainly a serious disadvantage attaching to the use of polyspermy as an indicator of the action of the poisons. But it appears probable that the figures in question arise as a direct result of the drugs, rather than of the condition of polyspermy, since in 1893 Galeotti (2) described similar irregular mitoses, occurring in regenerating epithelial cells of the salamander on treatment with quinine and certain other alkaloids; and mitoses of the same character also occur in the somatic tissue of plants which have been subjected to the action of chloral (Němec (8), Kemp (5)).

In 1896 R. Hertwig (4) published the results of further experiments with strychnine upon Echinoderm eggs. He showed that by treating them with solutions of different strengths, the unfertilized eggs could be induced to form spindles of different degrees of completion; also that on pushing the process further and using one of the stronger percentages (over 0·1 %) for a longer time (3–5 hrs.), inhibition of the artificially induced activity took place, followed by degeneration of the egg. He noted that the connexion between the mitosis of the nucleus and division of the cell was uncertain, and that the former was frequently followed by a series of abnormal imperfect divisions, giving rise to various multinucleate or anucleate conditions. He also noted that fragmentation of the cell might take place apart from any true mitosis of the nucleus, and that such fragmentation was preceded by dissolution of the nuclear membrane and scattering of the chromosomes throughout the cytoplasm to form swollen vesicular bodies.

He concluded that the effect of the strychnine was confined to causing the onset of an activity of division latent in the unfertilized egg-cell, and that the above abnormal figures were of too obscure an origin to throw light upon the process of normal division and were consequently not worth following out, particularly as they generally heralded degeneration of the cell.

In 1900 Morgan (7) published the results of experiments with strychnine on the eggs of *Arbacia*. The emphasis of the paper, however, is chiefly upon the occurrence of cytoplasmic striation under the influence of the poison, and although the author quotes Hertwig at length, he contributes no fresh data concerning the abnormal nuclear figures described by the latter.

In 1902 Wasilieff (10) brought out a paper on the action of strychnine and certain other alkaloids upon the eggs of *Strongylocentrotus lividus*, but here again the writer is chiefly concerned with another point than that of the occurrence of abnormal divisions, namely, with the origin of the centrosome. He states, however, that spindles of peculiar shape arise after treatment with strychnine, and notes Hertwig's observation of subsequent irregular divisions.

Hertwig's paper of 1896 was quoted again in 1903 by Delage (1) in an account of the behaviour of the eggs of *Asterias* on exposure to carbon dioxide. Delage stated that the gas was capable of exciting to division approximately 100 % of eggs, provided the latter were in a state of incipient activity, i. e. about to form polar bodies, or having just done so. He noted that full development was never reached, but did not describe any abnormal divisions occurring during the retrogressive changes in the eggs.

From the year 1899 until the present time, investigations have been carried by Loeb (6) far along the line indicated by Hertwig's discovery of artificial parthenogenesis. Loeb treated the problem less from the point of view of abnormalities arising under particular conditions, than from the side of its wider interest, that depending on the nature of absorption in general; though he can perhaps hardly be said to have studied absorption *per se* so effectively as the results of absorption. He performed upon various kinds of egg extensive and detailed experiments with the salts of magnesium, potassium, sodium, and calcium, and also with some members of the fatty-acid series, and was successful in inducing development to an advanced stage. In 1906 he suggested that the direct effect of the sperm in normal development, and of the methods of artificial parthenogenesis, was the starting of a definite chemical process; and pointed out in support of this view that the process of segmentation is as entirely regular in parthenogenetic development as in that of fertilized eggs.

The present note gives briefly the data obtained from some experiments with strychnine, undertaken with a view to examining the mitoses described by Hertwig in 1896. It has been necessary to leave the work unfinished,

and the results are consequently incomplete, but their publication in that state seems justified by the fact that, as far as they go, they are of a nature sufficiently definite to afford a basis for possible further advance. Roots of Pea and Bean seedlings were chosen for experiment as affording a readily procurable supply of young tissue in a state of activity. Incidentally the use of somatic instead of reproductive tissue has this advantage—that it precludes any possible confusion between the spindle formation of the two rapidly successive polar-body divisions of the unmaturated egg-cell, and the spindles of irregular shape said to arise under the influence of the poisons. The seedlings were grown and manipulated during experiment in the following way. They were started in sawdust, and on their roots attaining a length of some inches were placed with the tips of the tap-roots in the poison; on removal from the latter they were replanted in sawdust, after washing. Several series of experiments were worked out. In the first two or three strychnine hydrochloride, but subsequently also the sulphate, was used, in various percentages and for various lengths of time (0·0001 %, 0·001 %, 0·01 %, 0·1 %, 0·25 %, and 0·5 % for 10, 15, 20 mins., half an hour, and 1 hour). Fixation of the roots, either directly after treatment with strychnine or at different points of further growth, failed to reveal any of the abnormal mitoses in question. The only visible effect of the strychnine was a shrinkage in the cytoplasm of the outer cell-rows, varying roughly in amount with the strength of the solution used, and an apparent slight increase of karyokinetic activity in the more central layers of the tissue, the latter point, however, being difficult of exact proof. The mitoses were normal in appearance, with strongly marked achromatic fibres and clearly divided groups of chromosomes.

It seemed possible from these results that the poison either had not entered the roots at all, or had not penetrated beyond the outer layers of cells, and on the indication of Loeb's (6) work with alkalies upon eggs, and of Overton's (9) observations on narcosis in tadpoles, a modification was made in the experiments, and the solutions of strychnine rendered slightly alkaline with sodium carbonate or hydrate. In an alkaline solution, however, some of the strychnine base was thrown down, and it was doubtful what percentage of it remained in solution. Microscopical examination showed also no features essentially different from those seen in the earlier experiments, but a more severe shrinkage of the cytoplasm, a condition seen also in the control experiments with tap water containing the same proportions of the alkali as were used in the strychnine solutions, i. e. $\frac{N}{10}$, $\frac{N}{20}$, and $\frac{N}{40}$. A wide range of percentages and of periods of immersion was used (0·001, 0·01, 0·1, 0·25, and 0·5 % of strychnine, in solutions of sodium carbonate or hydrate from $\frac{N}{10}$, $\frac{N}{20}$ to $\frac{N}{40}$, for 10, 15, 20 mins., half an hour, 1 hour), but no result was obtained beyond distortion of the nuclei and shrinkage of the

cytoplasm after treatment with the stronger solutions. The experiment was then tried of first placing the roots in the above percentages of strychnine for 15 mins. or half an hour, and then transferring them to the alkaline strychnine solution. This was done on the supposition that it might be essential for the poison to secure a hold upon the tissue previous to the dissociating action of the alkali. Again, however, the results were negative, and no physiological reaction of the tissue was observable other than the apparent increase of karyokinetic activity noted above. This method was therefore abandoned and attention concentrated upon ascertaining whether the poison had penetrated the roots.

It was considered that whereas the experiments described above dealt essentially with static conditions, the introduction into the latter of a dynamic factor, such as transpiration, might yield more decisive results, the poison being sucked up forcibly through the roots. With this end in view water-cultures of the Pea were made, and on sufficient growth of stem and leaves to ensure the occurrence of transpiration, the intact plants were placed with their roots in solutions of strychnine ranging in percentage from 0·5 to 0·05 and 0·005 %. On removal of the plants from the poison after various periods of immersion (18, 20, 48 hrs.), the root-tips were fixed, either at once or after further cultivation in a salt solution, for microscopical examination; the stems and leaves were extracted for strychnine. In fixing the plants in the poison care was taken to immerse the roots only to a point which left several inches below the hypocotyl exposed above the surface of the solution. The hypocotyl, cotyledons, and a couple of inches of root and stem below and above the latter, were coated with vaseline. The hypocotyl was then wrapped in soft asbestos and the plant inserted, by removal of a wedge, into the cork of the culture bottle as during cultivation. When the experiments were performed in an open glass, the mouth of the latter was covered with strong paper or thin macintosh. By this method the possibility of capillary movement of the solution up the outside of the roots and over the stem was obviated, and any strychnine found in the stems and leaves must therefore have reached the latter through the vascular tissue of the roots, after penetrating the outer cells.

The extractions were made as follows: The plant was cut off above the vaselined area of the stem, chopped up, and extracted with alcohol at about 30° C. for several hours. The extract was then filtered off, and evaporated to dryness; the residue picked up with water, neutralized, and shaken with ether after again filtering. The ether was evaporated and the small residue which remained tested for the presence of strychnine. The chemical tests used for detection of the latter were :—

1. Mandeline's reagent (a solution of vanadium chloride in strong sulphuric acid), giving a violet colour in the presence of strychnine, turning to cherry pink.

2. Sulphuric acid and potassium bichromate, giving a deep violet in the presence of strychnine.

The residue was also examined for crystals and for the intensely bitter taste characteristic of strychnine.

The result of the tests was as follows. The presence of strychnine was demonstrated in the stems and leaves of the plants examined, in amounts varying very roughly with the strength of the original solution. Thus, while only to be detected by bitterness to the taste and by faint, though distinct colour reactions, in the plants treated with a solution containing 0·00 5% of strychnine, in those which had been subjected to the action of a 0·5 % solution the base was obtained in a crystalline form from the ether residue and the colour reactions were vivid.

The absorption of strychnine through the roots of the above plants being proved by the appearance of the poison in their stems, a microscopical examination was made of the root-tips fixed in parallel to the stem extractions. The periods of immersion in these transpiration experiments having been somewhat prolonged, in order to allow ample time for the rise of the poison in the stem, the effect observed microscopically in the roots was naturally severe, but the general results were similar to those of the earlier experiments with strychnine. In those which had been treated with the stronger percentages the nuclei were shrivelled, diffusely stained, and apparently dead; and also in the roots fixed immediately after removal from the poison, whether of weaker or stronger percentage, they were seen to be in a completely inactive and apparently pathological condition. In the roots which had been subjected to the 0·5 % solution no recovery was observed, but where weaker solutions had been used (0·05 and 0·005 %) there was a gradual return of activity, after the plant had been replaced in its culture solution for a few days, the nuclei staining more clearly and here and there undergoing mitosis. Such mitoses were to all appearance normal and none of the irregular figures described by Hertwig were found.

It seems possible that the difference between the results obtained in these experiments with somatic cells, and those obtained by Hertwig with eggs, may be due to an essential difference in the physiological character of the two kinds of tissue. It was noted above that O. and R. Hertwig, in working with Echinoderm eggs at three distinct stages of development, obtained a characteristic type of response at each stage. Again, it has been found that between two kinds of egg—such as those of *Asterias* and *Strongylocentrotus*—there is a considerable difference of sensitivity to external stimuli; those of the former being more easily excited to parthenogenetic division than are the latter. Further, it has been shown that in many hybrids, whereas the nuclei of the sexual cells show remarkable irregularities in their division figures, those of the somatic tissue are perfectly

normal. It is conceivable that between the animal egg and the plant-cell a similar physiological difference may be the cause of the difficulty in inducing, in the plant, types of mitosis which occur readily in the egg-cell of the animal.

Summary and Conclusion.

Somatic tissues of the Pea (*Pisum Sativum*) and Bean (*Vicia Faba*) were treated with the sulphate and hydrochloride of strychnine with a view to inducing the occurrence of the abnormal divisions described by Hertwig in 1896. No such figures were found.

In order to promote dissociation of the salts, and thereby possibly their absorption by the roots, the strychnine solutions were made slightly alkaline with sodium carbonate or hydrate. These alkaline solutions had no greater effect than those of natural reaction. The penetration of the poison into the tissue therefore appeared doubtful.

A series of experiments with water-culture Peas was carried out with two objects: (1) in order to utilize the factor of transpiration, and so promote absorption of the strychnine; (2) in order to make extractions of the stems and leaves for strychnine, and so obtain a positive datum as to the entry of the poison into the root-cells.

The presence of strychnine in the stems was demonstrated by this method, and correlatively its absorption by the roots. Microscopical examination of the latter, however, yielded results similar to those obtained in the earlier experiments, and no abnormal divisions were found.

These results indicate that strychnine exercises no specific effect upon the tissues used in the above experiments, exciting in them no definite physiological response, but only, if used in sufficient strength, producing a general disturbance of metabolism, from which recovery is doubtful and which generally precedes degeneration.

In conclusion, I take the opportunity of thanking Professor J. Bretland Farmer, F.R.S., for his advice and direction in this investigation; and also Dr. M. A. Whiteley for most kindly giving me detailed help in making the necessary chemical examinations.

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The Effect of Chloroform upon Respiration and Assimilation.[1]

BY

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With Twenty-four Figures in the Text.

NUMEROUS scattered observations have been made on this subject, but there has been no systematic study of the effect of different doses of an anaesthetic upon respiration and assimilation. The object of this research has been to obtain satisfactory data on this subject. In work of this kind a *single* estimation of the respiration after a dose of anaesthetic is of very little value; therefore in all cases the effects of the dose have been followed for many consecutive hours. These long sequences of observations were rendered possible by using the apparatus designed by Dr. Blackman, and referred to in my previous paper, No. VII of this Series.[1]

Throughout the work isolated leaves only have been employed, chiefly those of young Barley shoots and of Cherry Laurel. These were freshly cut for each experiment, enclosed in glass chambers through which a continuous current of air was drawn, and experimented upon at a temperature of 25° C.

The data obtained for the effect of different doses of chloroform will be presented in three separate sections. The two sections constituting Part I deal with (i) the effect of single doses of chloroform on respiration, and (ii) the effect of continuous treatment with chloroform. Part II deals with the effect of chloroform upon assimilation.

As the series of respiration estimations in each experiment lasts some time and the leaves are in most cases in the dark, starvation effects cannot be neglected. Numerous control experiments without anaesthetics have made clear the normal course of the respiration of the leaf starved in the dark. Under such conditions there are always considerable fluctuations in the successive respiration-readings, but in the large number of controls

[1] This paper constitutes Part X of Experimental Researches on Vegetable Assimilation and Respiration. The recent papers of this Series, carried out at Cambridge under the general direction of Dr. F. F. Blackman, are: VII, A. A. Irving: The Beginning of Photosynthesis and the Development of Chlorophyll, Ann. of Bot., vol. xxiv, Oct., 1910; VIII, Blackman and Smith: A New Method for estimating the Gaseous Exchanges of Submerged Plants, Proc. Roy. Soc., B, vol. lxxxiii, 1911; and IX, Blackman and Smith: On Assimilation in Submerged Water-Plants, and its Relation to the Concentration of Carbon Dioxide and other Factors, Proc. Roy. Soc., B, vol. lxxxiii, 1911.

carried out, it is always possible to see that the respiration remains approximately uniform for a few hours, and then begins to fall off in a curve, at first quickly and afterwards slower, towards a small constant value. This holds for all the leaves employed; therefore unless there is any actual need for mentioning the controls, which were often made, they will be omitted from the figures.

It has been thought unnecessary to print full tables of all the actual estimations of CO_2, and so throughout the paper each experiment is represented only by a graphic figure.

In these figures the ordinates are grammes of CO_2 actually given off per two hours, this being the usual duration of each single reading. These amounts are not reduced everywhere to a uniform weight of leaf, but they refer to a weight of fifteen grammes in the case of all Barley experiments, and to the variable weight of a single leaf in the case of Cherry Laurel.

The abscissae are hours from the beginning of the experiment, and the hatching at the base of the figures indicates the time and duration of application of the chloroform vapour. In Part II, where the leaves are sometimes in the light sometimes in the dark, this is indicated by the white or black band along the top of the figure.

PART I. SECTION I.

The Effect of Single Doses of Chloroform on Respiration.

For all the experiments in this section young shoots of Barley were employed.

The Barley was grown in pots under similar conditions and cut off just above the surface of the ground when the shoots were six inches tall. Enough shoots to weigh just 15 grms. were collected for each experiment, so that the CO_2-numbers are directly comparable in this section.

The cut shoots were weighed and at once placed in the leaf-chamber, which, after being sealed, was in its turn placed in the water-bath already at the temperature of 25° C. The leaf-chamber itself was a tall glass jar of about 970 c.c. in volume, and square in section, with a glass lid which was waxed on at the beginning of each experiment; through this lid passed the air-inlet tube, which almost reached the bottom of the chamber, and the short exit tube. The Barley leaves were arranged to stand round the sides of the chamber, in water about $\frac{1}{4}$ inch deep. At a certain time after the beginning of the experiment the dose of chloroform was run in from a fine pipette through the inlet tube into a small open dish standing in the middle of the bottom of the chamber, from which it readily vaporized.

At the beginning of every experiment the air-current was allowed to run for a preliminary 2–3 hours before any actual estimations of CO_2

were taken. Unless otherwise stated, each CO_2-estimation represents a reading lasting two hours.

Very small doses of chloroform may have no visible effect upon Barley leaves, but moderate and strong doses bring on two obvious changes: one, a change in the leaf-colour so that the bright green becomes a dull grey green, and the other an exudation of drops of water through the stomata on to the surfaces of the leaf. As a result of this loss of water from the mesophyll cells the leaf-blade becomes more or less flaccid.

Experiment I. Chloroform dose, 0·1 *c.c.* Two separate lots of 15 grms. of Barley-shoots were observed simultaneously, one of them, the lower curve in Fig. 1, serving as the control. As regards the other one, the upper curve, after the preliminary, a single reading was taken to get the normal respiration value before chloroform was administered; this was found to be

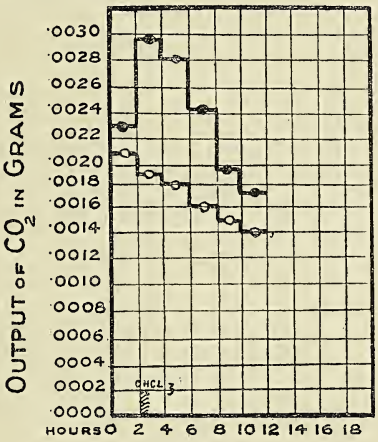

FIG. 1.

0·0023 grm. CO_2 per hour. The dose of chloroform was run in at the beginning of the second reading; such a small quantity would be vaporized at once at 25° C. The second reading gave 0·0029 grm. CO_2, showing a clear rise in the respiration, and this was followed by a gradual fall to 0·0017 grm. This small dose of chloroform does not cause a visible change in the bright green colour of the leaves, but it brings about exudation of water so that the surface is covered with minute drops and the leaf becomes flaccid. Upon comparison with the normal curve of the control leaves it is seen that the respiration of the chloroformed leaves never falls below the normal output throughout the experiment. The effect of this small dose of chloroform was therefore to increase the CO_2-production by a temporary augmentation, after which the production declined to about normal. On p. 1083, where the effects of different doses are compared, this type of effect is represented in the *schema* as B (see Fig. 7).

Experiment II. Chloroform dose, 0·2 *c.c.* The procedure in this experiment was similar to that in Exp. I in all respects, save that double the amount of chloroform was added. The results are given in Fig. 2. Upon

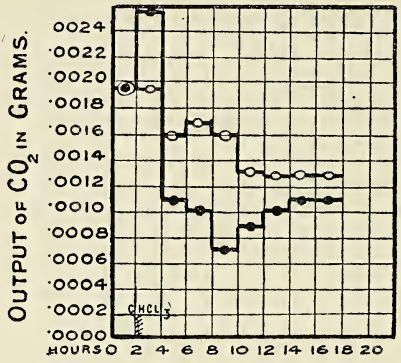

FIG. 2.

comparison with the control (open circles for each reading) it is seen that the effect of this dose of chloroform is to produce a temporary outburst of CO_2, quickly followed by a depression below the normal, from which recovery takes place; after fourteen hours the CO_2-output is about normal. This type of curve will be called C (see Fig. 7).

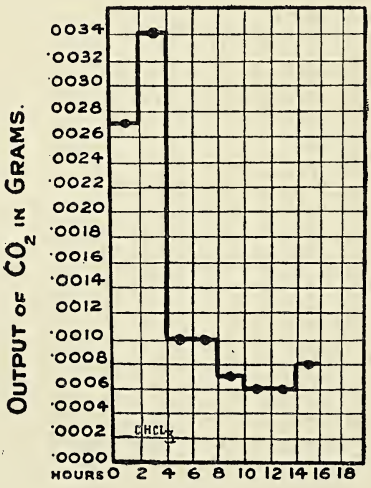

FIG. 3.

Experiment III. Chloroform dose, 0·5 *c.c.* The procedure was the same, but an increased dose given at the second hour of the experiment. Fig. 3 shows the effect on the output of CO_2. After the outburst of CO_2

following the addition of the chloroform the production dropped quickly to less than half its normal value and did not rise again appreciably, though possibly the last reading may be looked upon as a very slight recovery from the chloroform effect and the curve scheduled as intermediate between C and D.

Experiment IV. Chloroform dose, 1·0 *c.c.* Procedure similar: 1·00 c.c. of chloroform caused a similar outburst of CO_2, which is shown in Fig. 4. The normal output of CO_2 was 0·0036 grm.; upon the addition of 1·00 c.c.

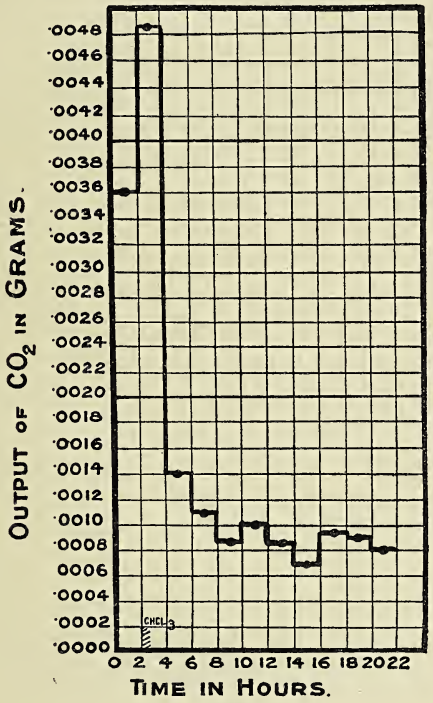

FIG. 4.

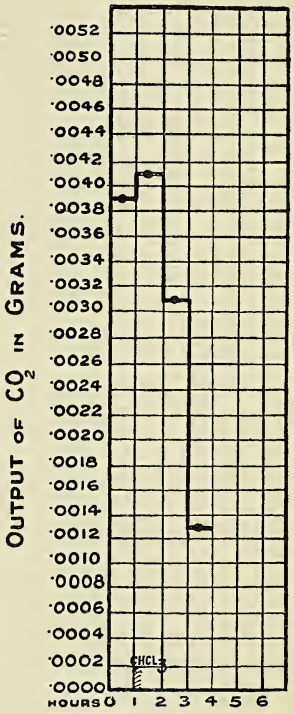

FIG. 5.

of chloroform this rose to 0·0048 grm., then fell in the next reading to one-third of its original value. After this the fall of CO_2-output continued at a slower rate, tending to reach a small, level or slightly falling value without any sign of recovery. This is called type D.

Experiment V. Chloroform dose, 2·0 *c.c.* Fig. 5 shows the effect of a further increase in the dose of chloroform; the outburst of CO_2 is now very brief, but is made clear when, instead of two-hour, one-hour readings are taken as they are in this experiment. The CO_2-production falls off very rapidly after the introduction of such a dose of chloroform. This curve may be regarded as intermediate between D and the next type, E.

It will be noticed that the slope of this curve cannot be directly compared with those of the other experiments, because the abscissae are twice as extended in this figure.

Experiment VI. Chloroform dose, 10 *c.c.* This dose of chloroform is very much greater than that used in the preceding experiments. The result is a new type of curve, without any detectable initial outburst of CO_2 occurring. There may no doubt be a very brief outburst of CO_2,

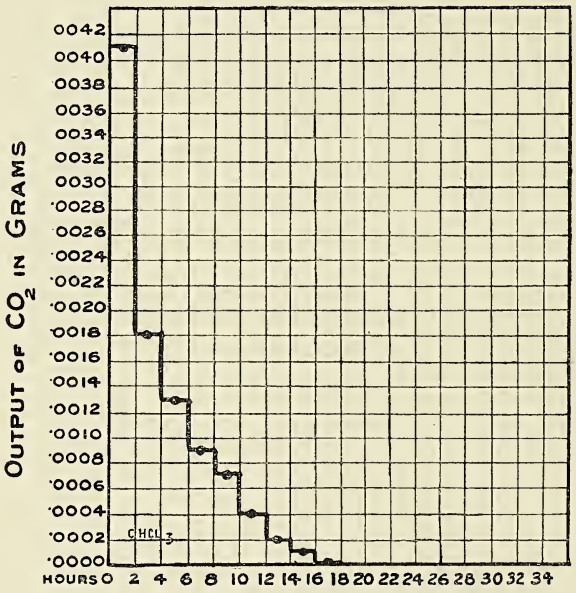

FIG. 6.

lasting such a short time that it is masked by the fall during the rest of the two-hour reading. A series of one-hour estimations did not, however, give any evidence of an outburst of CO_2 having occurred.

The CO_2-production continued to fall off rapidly, and fourteen hours after chloroform had become nil. This is the extreme type of curve, E, characteristic of the largest doses of chloroform.

We may now pass to a general comparison of these different types of chloroform-effect provided by Exps. I–VI.

The first point for comment is the great difference in the values given for the normal respiration of 15 grms. of Barley leaves at 25° C., even though the shoots were of the same height and age in each experiment. After these differences were observed the matter was investigated, and was found to be due to the different depths of planting the seeds on different occasions, causing greater or less amounts of stem to be above the ground

and included in the cut-off shoots. Those shoots which were cut off immediately above their seed gave a much higher value for their respiration than those cut off half an inch above the seed. Presumably the actively growing stem respires more energetically than the nearly mature leaf-blades.

For the above reason the curves cannot be compared directly by superposing them all with the same base line, but in Fig. 7 they are superposed all starting from the same point—that of the normal respiration just when the dose of chloroform is given.

It is at once seen that the effects of the different doses fall into a progressive series from B to E, which we can easily compare by means of this *schema*.

The falling interrupted line in the *schema* represents the curve of CO_2-production in a normal leaf cut off and kept in the dark. This serves as a standard of the respiration uninfluenced by chloroform.

The smallest dose given in this section, 0·1 c.c., causes the CO_2-production to rise at once with an outburst which gradually dies away, but yet leaves the CO_2 as great as in the normal leaf, even after twelve hours, type B.

Type C is given by a dose of 0·2 c.c.; here there is a similar initial outburst, but this falls off more quickly, so that it gets below the normal leaf after six hours; then recovery sets in and the value of the normal leaf is attained.

One c.c. of chloroform produces type D, in which the falling off from the initial outburst is still earlier and steeper, and is followed by no sign of recovery; instead, the CO_2-production slowly diminishes hour after hour. With a much stronger dose, 10 c.c., we get type E, in which there is no detectable initial outburst, but the CO_2 falls off rapidly from the beginning and the curve sweeps quickly down to zero. No doubt there is a brief outburst of CO_2 here also, but this augmentation is more than masked by the great diminution of the CO_2 during the remainder of the one- or two-hour reading taken immediately after chloroforming.

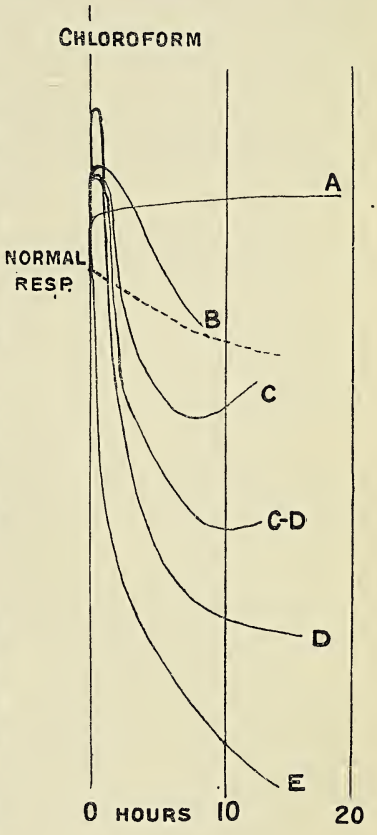

Fig. 7.

Of these doses of chloroform all except the weakest caused the leaf

to turn colour and set up irreversible changes. With 0·1 c.c. of chloroform alone—type B—could there be perfect recovery from the dose.

In this section of experiments no smaller dose was tried, but in the next section we shall find that there is yet another type of reaction, type A, characteristic of still smaller doses. In this case the initial augmentation of the respiration does not fade away, but is maintained. This result is however not really comparable with the others, as here the dosing with chloroform is continuous.

PART I. SECTION II.

The Effect of Continuous Treatment with Chloroform upon Respiration.

As the exact concentration of chloroform in the chamber is not known at any time with the procedure of Section I, this work was followed up with experiments in an air-current continuously charged with a constant amount of chloroform.

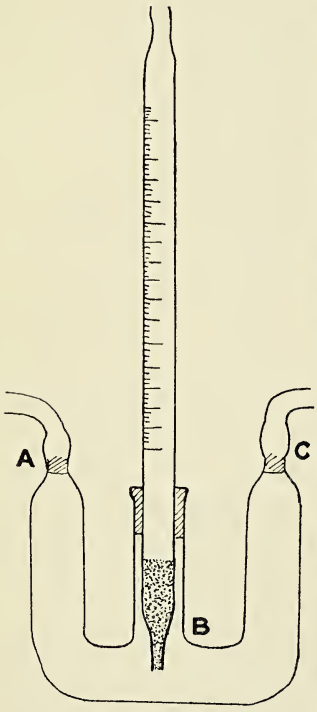

Fig. 8.

To control the supply of chloroform a piece of apparatus was constructed by which different concentrations could be maintained. It took the form of a fine graduated pipette very tightly packed at its lower end with powdered chalk, through which the chloroform in the pipette percolated. This pipette was inserted into the middle arm of an anchor-shaped tube. See Fig. 8.

The stream of CO_2-free air entered this tube at *A* and passed along by *B*, taking up as vapour the chloroform that percolated through the chalk. It then passed out at *C* laden with the vapour. By taking readings of the fall of the upper surface of chloroform across the graduations of the pipette, the quantity of chloroform passing through the chalk plug into the air-current could be found.[1] The rate at which the chloroform passed through the chalk core depended upon its closeness and depth. A set of pipettes was prepared which gave a fairly wide range of filtration-rates, and to obtain intermediate rates, air-pressure

[1] A control pipette of chloroform, with its lower end sealed, was placed in a similar position, and readings taken to find the correction for evaporation at the top. This was found to be so small that for most experiments it could be neglected.

was employed to hasten the filtration. For this purpose the top of the pipette was connected by stout tubing to a bulb of air, the pressure on which was adjusted by a head of mercury.

The air-current through the apparatus travelled at the rate of a litre per hour, and the amount of liquid chloroform percolating per hour is therefore a measure of the concentration per litre of air in contact with the leaf.

This apparatus was not satisfactory for giving very strong concentrations, because when more than 1 c.c. of chloroform filtered through in an hour, it did not all vaporize at the ordinary temperatures, but collected in the anchor tube in the liquid form. To give stronger concentrations the 'anchor tube' was abandoned and the air-current led through a bulb half full of chloroform and sunk in the water-bath so as to be at a temperature of 25° C. The rate of vaporization in this case was determined by control experiments.

The experiments in this section are carried out partly with Barley shoots, in fifteen-gramme lots, as before; and partly on Cherry Laurel. In this latter case a single leaf was used for each experiment, and as the weights of these vary, the CO_2-numbers are not directly comparable either with other Cherry Laurels or with the Barley shoots. The single leaves were not enclosed in a glass jar, but were set up in the oval leaf-chamber figured in 'Assimilation and Respiration, VII' (op. cit.).

The series of CO_2-estimations fluctuates much more with single leaves (Cherry Laurel) than with groups of leaves (Barley) in which the individual fluctuations partly equalize one another.

Experiment VII. Concentration of Chloroform = 0·046 *c.c. per litre of air-current.* A single leaf of Cherry Laurel weighing 1·55 grms. was

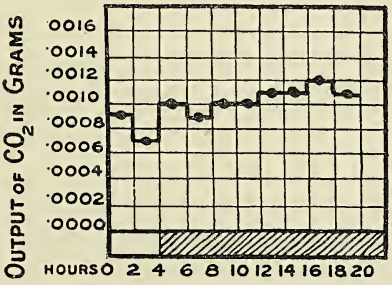

FIG. 9.

set up in the flat leaf-chamber and two normal respiration readings taken before chloroform was given. These gave values of 0·0009 and 0·0007 grm. respectively (Fig. 9). The chloroform was then administered, and in

the first two hours the output of CO_2 rose to 0·0010 grm. and during eighteen hours did not fall below the initial. The output of a normal leaf during that time would have fallen considerably, as the curves in Fig. 7 show. Type A in that *schema* is the mean between the results of Exp. VII and Exp. VIII, and illustrates the continued augmentation of respiration that results from a small continuous dosing with chloroform.

The leaf was slightly brown, in parts, at the end of the experiment, but mostly green.

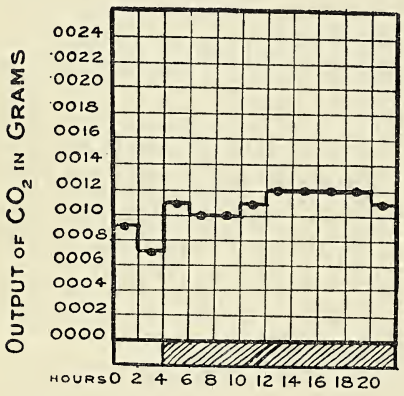

FIG. 10.

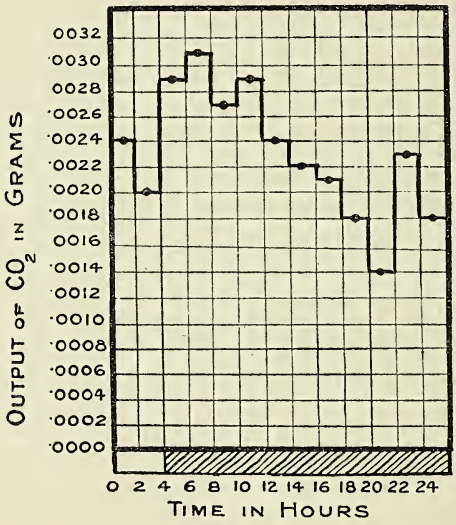

FIG. 11.

Experiment VIII. Concentration of Chloroform = 0·05 *c.c. per litre of air-current.* This is another experiment with a single Cherry Laurel leaf quite similar to Exp. VII.

Fig. 10 shows that this leaf, which weighed 1·8 grms., responds in the same way to continuous treatment with this concentration of chloroform, giving a curve of type A.

Experiment IX. Concentration of Chloroform = 0·08 *c.c. per litre of air-current.* This experiment is carried out with Barley shoots, fifteen grammes in the glass jar used in Exps. I–VI, and after two normal respiration readings the air-current was charged continuously with 0·08 c.c. chloroform.

The effect of this was to raise the output of CO_2 in the characteristic outburst, after which the CO_2 declined in a way very much like type B. The CO_2 rose somewhat in the last two readings, so that we may style it intermediate between B and C.

Experiment X. Concentration of Chloroform = 0·22 c.c. per litre of air-current. Barley leaves were again used, the chloroform being started immediately after the first normal respiration reading. The output of CO_2 then rose from 0·0024 to 0·0049 grm. in the second reading. This outburst

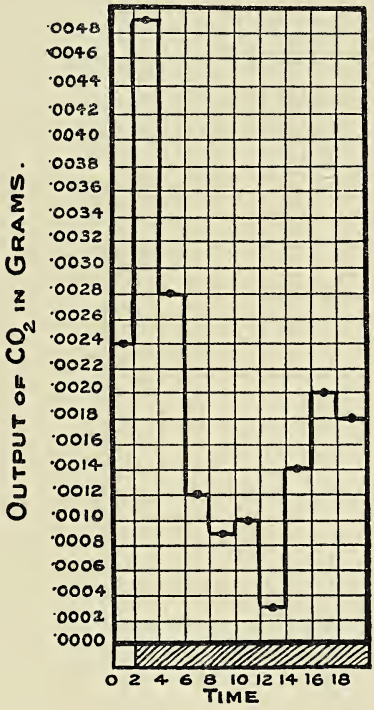

FIG. 12.

was followed during the next few hours by a rapid fall, so that it only lasted a brief time (Fig. 12). This in itself is an approach towards the later types. The rapid fall is followed by a well-marked recovery, so that in its later stage it recalls C and in its earlier D; we may therefore regard it as C–D.

Experiment XI. Concentration of Chloroform = 1·5 c.c. per litre of air-current. Barley shoots were used for this experiment, and as it was found impossible to charge the air-current with so much chloroform by means of the 'anchor tube', the bulb apparatus (p. 1085) was employed under such conditions that about 1·5 c.c. chloroform was vaporized per hour. One reading of the normal respiration was taken and then the bulb-tube of chloroform was put in the circuit.

This concentration of chloroform caused an outburst of CO_2 in the second reading, the output rising from 0·0019 to 0·0029 grm. After this

it fell steadily, but gradually, giving a curve of a type D–E. This is seen in Fig. 13. The hatching at the bottom of the figure shows that the 12 c.c. of chloroform put in the bulb-tube was all vaporized in eight hours.

Experiment XII. Concentration of Chloroform = 4 c.c. per hour per litre of air-current. As 1·5 c.c. did not give the extreme form of curve, type E, still stronger concentration was obtained. For this the bulb-tube was inadequate and the procedure of Section I was employed, 4 c.c. of chloroform being introduced *every hour* into the dish at the bottom of the glass jar. This amount was repeated for four consecutive hours,

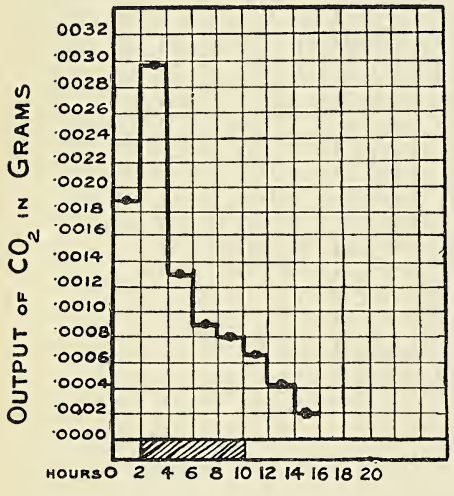

Fig. 13.

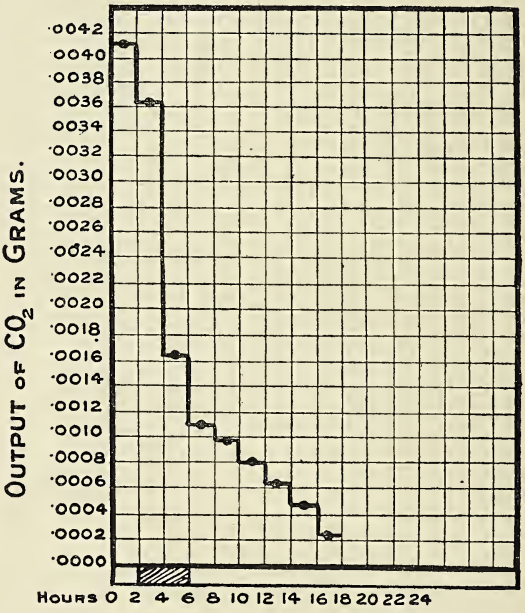

Fig. 14.

i.e. four lots of 4 c.c. of chloroform were run into the leaf-chamber: subsequent to that no more chloroform was given.

With this strong dose, the Barley showed no sign of an initial outburst of CO_2, but fell off steadily towards zero, reaching it about sixteen hours after the beginning of chloroforming.

The type of curve is the same as that found for Exp. V and called type E. The results of this experiment are plotted in Fig. 14. This experiment completes our series and we have passed from A to E by different concentrations of chloroform.

We may now refer to two experiments, XIII and XIV, performed to bring out certain special points.

When the outburst of CO_2 caused by chloroform is very transitory and followed by a rapid fall, then its occurrence will not be easy to

demonstrate, as the CO_2-number for the whole two-hour reading may be quite low. Even when the two-hour reading, immediately after chloroform, shows a fair augmentation over the normal respiration, yet if the outburst lasted only one hour then the curve of two-hour readings would not show so sharp a peak as a curve of one-hour readings. Still shorter readings might show an even more acute and high peak. Exp. XIII was intended to bring out the intensity and brevity of the outburst of CO_2 following a suitable dose of chloroform. A series of one-hour readings with a group of six Cherry Laurel leaves, weighing 13·82 grms., was started, and after five readings of normal respiration, 0·63 c.c. of chloroform per litre of air-current was given. Fig. 15 shows the result. The CO_2-output for one hour rises to 0·0063, just three times the normal respiration in the previous hour, and then falls off rapidly. Had two-hour readings been taken, the peak after chloroform would have only attained 0·0052, not more than double the normal, as in Exp. X.

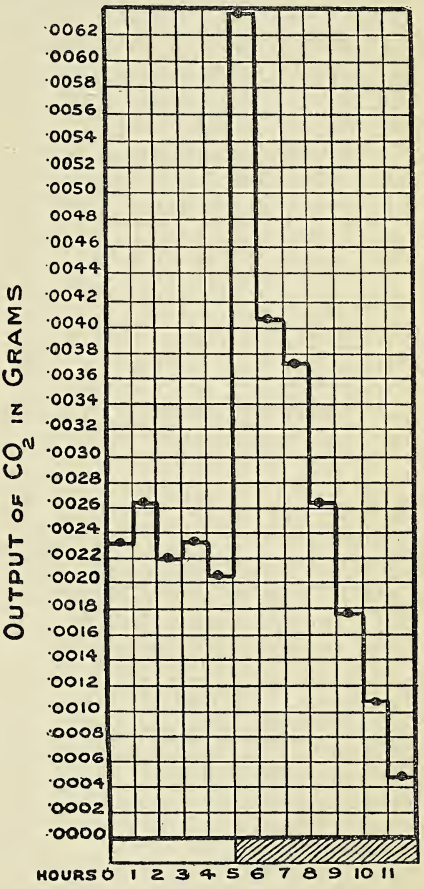

FIG. 15.

Fig. 15 is not comparable with the other figures as regards the slope of the curve, as the abscissae are here extended to double.

Exp. XIV was intended to throw light on another particular point.

It was just possible that the fall in the output of CO_2, after chloroform had been given to the leaves, might be due to the closure of the stomata during the experiment, owing to the action of this agent. Two leaves of Cherry Laurel were therefore taken, and through the lamina of one numerous cuts were made pinnately with a sharp scalpel. Respiration readings were then taken for both leaves separately, chloroform being subsequently given to each at a concentration of 0·1 c.c. per litre of air-current. If the stomata closed owing to the action of the chloroform, it is to be expected that there would be less CO_2 given out from the whole leaf than from the cut leaf. This was not found to be the case. The slight extra rise seen in the broken curve for the cut leaf, Fig. 16, was no doubt due to a slight increase

in respiration, possibly from the wounding of the leaf. The rest of the two curves are almost identical.

Each leaf weighed 2·3 grms. and both were brown at the conclusion of this experiment.

It will now be interesting to compare the types of curve obtained in this section with those recorded in Section I. We find again the series from B to E following one another in the same order with increasing concentrations of the chloroform. We see, however, that a continuous exposure to the vapour is somewhat more effective than an initial dose of the same amount. Thus a curve which may be located as B–C would be given by an initial dose of 0·15 c.c. and by a continuous dose of 0·08 c.c. per litre; a curve of type C–D by initial dose of 0·5 c.c. and continuous concentration of 0·22 c.c. per litre. Roughly speaking, an initial dose of between $2n$ and $3n$ has the same effect as a continuous dose of n per hour when the experiment takes place in an air-current of a litre per hour.

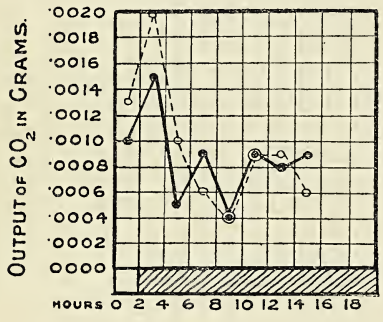

Fig. 16.

It is clear that it is the initial stages of action of the vapour which are most significant, and that, when these changes are once set going, the final stages run their course much the same whether the vapour is still being applied or has been stopped.

Some other effects of chloroform upon leaves.

Moderate doses of chloroform bring about a destruction of chlorophyll and an exudation of sap in Barley leaves (cf. p. 1079). In Cherry Laurel there are two further symptoms easily observed, liberation of hydrogen cyanide from the cyanogenetic glucoside and the browning of the leaf.

The browning would mask the chlorophyll even were it unchanged in the metabolic breakdown, but extraction tests with alcohol show that destruction of the chlorophyll goes on at the same time as browning, and no trace of green pigment can be extracted from a fully brown leaf.

A few observations on the effect of temperature and of water-content on the rate of browning and of the breakdown of the cyanogenetic glucoside were made by using Guignard's test-paper,[1] which turns from yellow to red in the presence of HCN.

Leaves of Cherry Laurel treated with equal amounts of chloroform, in bottles, were kept at different temperatures, and the time taken for the

[1] Filter paper soaked in 1 % picric acid, dried, and then soaked in 10 % sodium carbonate.

appearance of the red colour noted. It was found that at temperatures between 35° and 55° C. the presence of HCN became evident in 50–60 seconds, while at 30° C. two minutes elapsed, and at 11° C. quite twenty minutes.

Some observations on the effect of partial or complete removal of the water from leaves were also made. A fresh Cherry Laurel leaf in chloroform vapour will turn brown and evolve HCN in about half an hour, but the more the water is removed by allowing the leaf to dry up in the air the longer the time required for these signs, which seem to go closely together. The following times were found :—

Leaf dried to	90 %	of fresh	weight—brown	in	1	hour
„	„ 80 %	„	„	„	6	hours
„	„ 75 %	„	„	„	26	„
„	„ 72 %	„	„	„	30	„
„	„ 50 %	„	„	„	36	„

A leaf completely air-dry retained its green colour indefinitely in chloroform vapour and also evolved no trace of HCN.

PART II.

The Effect of Chloroform upon Assimilation in Prunus Laurocerasus.

Several observers, Claude Bernard,[1] Bonnier,[2] Ewart,[3] have brought forward evidence that the process of photosynthesis in plants is arrested by chloroform, but there has been no systematic study of the action of different doses, except that of Kegel.[4] Kegel, who worked with water plants, concluded that chloroform under certain conditions increases the evolution of oxygen, while mostly it depresses it. His evidence for the acceleration of assimilation is not very convincing, and the present work was undertaken to get satisfactory data for the action of the chloroform in different concentrations.

It was found that the smallest dose of chloroform tried so depressed the assimilatory power, that the leaf was not able to assimilate even the whole of its own CO_2 of respiration. It was therefore unnecessary to supply air laden with CO_2 to the illuminated leaf, and the experimental procedure was thereby much simplified. It sufficed merely to measure the

[1] Leçons sur les phénomènes de la vie. Paris, 1878.

[2] Recherches sur l'action chlorophyllienne séparée de la respiration. Ann. Sci. Nat., sér. vii, tome iii, 1886.

[3] Assimilatory Inhibition in Plants. Journ. Linn. Soc., 1895.

[4] W. Kegel : Ueber den Einfluss von Chlorofo m und Aether auf die Assimilation von *Elodea canadensis*. Inaug. Diss. Göttingen, 1905.

CO_2-output from the illuminated leaf; this of course was nil with the normal leaf, but became considerable when chloroform was given; if on darkening the CO_2-output rose still further, it was evidence that the power of assimilation had not been completely inhibited, but if it remained stationary then the assimilative power had been abolished.

Single leaves of Cherry Laurel were alone used in this part of the work, and they were set up in the oval leaf-chamber previously mentioned. The experiments were all carried out in the water-bath at 25° C., and the leaf in its chamber was lighted through the glass window of the bath by the Keith high-pressure incandescent light. When a leaf in the dark was compared with a lighted leaf, its leaf-chamber was wrapped in black cloth and placed behind a light-screen in the same bath.

The experimental procedure was precisely the same as in Part I, and the chloroform was supplied by the special tube described on p. 1084. This was placed in the circuit just before the CO_2-free air entered the leaf-chamber, and when two leaves, one in light and one in dark, were used simultaneously, it was inserted just before the stream of air was bisected. Then, as the two aspirators were arranged to drop at equal rates, each leaf received exactly half the total chloroform vapour.

As assimilation is much more sensitive to the presence of chloroform than respiration, the doses used in this section are smaller than those in Part I. It will perhaps be best to take the experiments in order of increasing chloroform concentration.

Experiment XV. Chloroform concentration = 0·0014 *c.c. per litre of air-current.*[1] The Cherry Laurel leaf, weighing 2·3 grms., was placed with its stalk in water in the oval leaf-chamber which was sealed and put in the bath, already at the required temperature, 25° C. The illuminating burner was then lighted, and after a preliminary of three hours, a series of two-hour estimations of the CO_2 given off by the leaf was started. The first reading showed that the leaf was assimilating all its respiratory CO_2 (Fig. 17) and that none was escaping from the leaf. The chloroform apparatus was then placed in the circuit, left in for four hours, and then removed. The two CO_2-estimations taken during this time showed the immediate effect of this very minute dose of chloroform; CO_2 escaped from the leaf to the amounts of 0·0002 and 0·0006 grm. respectively, showing that assimilation of its respiratory CO_2 by the leaf was partly inhibited. This effect continued after the chloroform had been removed, and the figure shows the subsequent output of CO_2; it will be noted that although this fell to 0·0002 grm. it did not again reach zero by assimilation, in eight hours.

[1] The chloroform sank in the graduated tube at the rate of 0·0045 c.c. per hour, but a control with a tube closed at the lower end showed that 0·0031 of this was due to evaporation from the upper surface. Therefore, only 0.0014 c.c. chloroform filtered through each hour to the air-current.

To see how much assimilation was now actually taking place, the leaf was darkened, when the output rose at once to 0·0013 grm., showing that the leaf had been assimilating some two-thirds of its respiratory CO_2. This trace of chloroform therefore had at once very much reduced the function of assimilation, after which the leaf partially recovered. The amount of chloroform was not enough to completely abolish this function, as the CO_2-output in the light never rose to be as great as the respiratory output in the dark. It is very striking that so small a dose, which would presumably have no effect upon respiration, is sufficient to reduce assimilation nearly to nothing, and that too when the dosing only lasts four hours. The leaf remained bright green throughout the experiment.

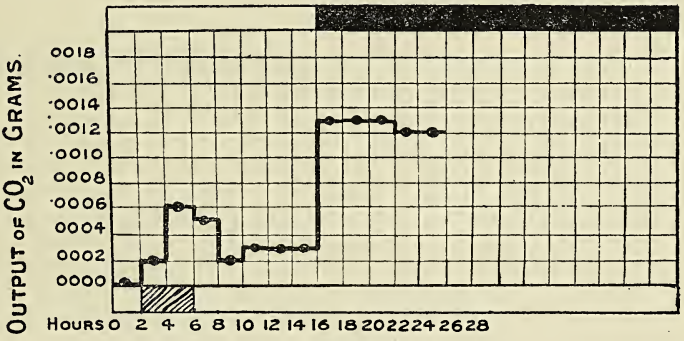

FIG. 17.

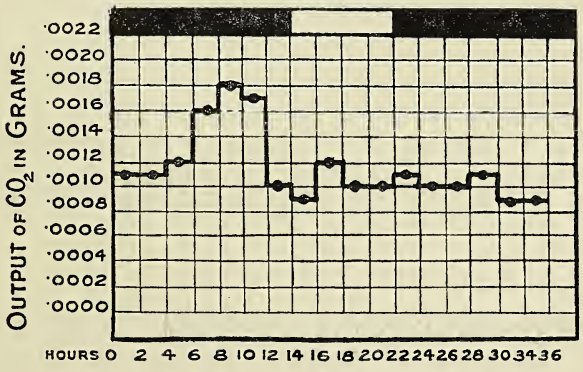

FIG. 18. The hatching which indicates chloroform has been accidentally omitted at the bottom of this figure: it should extend from the fourth hour to the end of the experiment.

Experiment XVI. Chloroform concentration = 0·02 *c.c. per litre of air-current.* In this experiment it was decided to chloroform the leaf in the dark first, and then to illuminate it and see if there would be a fall in the CO_2-output as evidence of some residual power of assimilation.

The leaf weighed 2·1 grms., and was set up in the chamber in the usual way.

Two normal respiration readings were taken in the dark and then chloroform was given; after which five more readings were taken, still in the dark. Fig. 18 shows that during this time the reaction started to be of the A–B type. At the end of the seventh reading the leaf was illuminated. No further fall in the output of CO_2 took place during the readings in the light. When the leaf was darkened once more, the output of CO_2 was still found to be keeping fairly uniform.

From this we see that 0·02 c.c. of chloroform per litre of air-current, given continuously, abolished completely the power of assimilation.

The green colour of the leaf here also remained unchanged throughout the experiment.

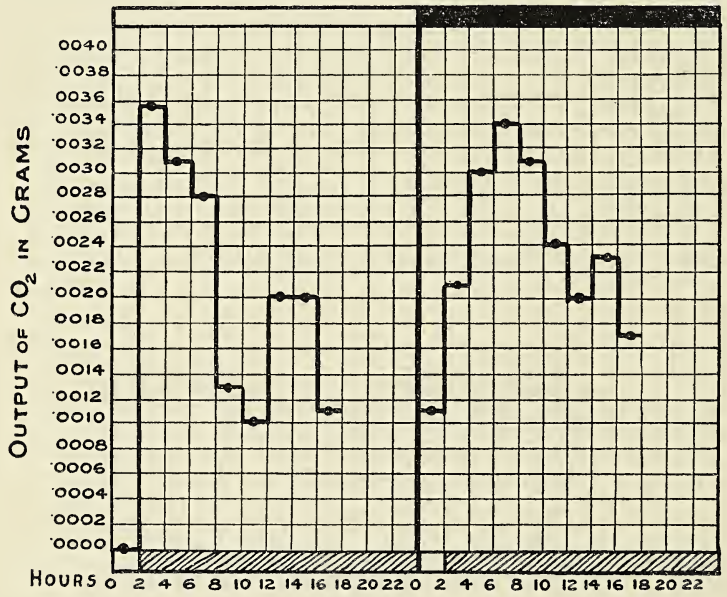

FIG. 19.

Experiment XVII. Concentration of chloroform = 0·091 *c.c. per litre of air-current.* In this experiment simultaneous readings were taken of two separate leaves, one in the light (weight, 3·30 grms.), the other in the dark (weight, 3·31 grms.). Both were given preliminaries of 3 hours. After normal respiration and assimilation readings, respectively, had been taken, chloroform was given to both in equal concentrations. The results are seen in Fig. 19.

It will be noted that in the lighted leaf not only was all assimilation of CO_2 arrested by the chloroform, but an outburst of CO_2, comparable

with that which took place in the dark, occurred. The highest readings from both leaves were approximately equal.

The delay in the full development of the outburst of CO_2 which took place in the darkened leaf here and in a few other cases seems most probably due to some diminution of rate of penetration of the chloroform.

The leaves were still green at the end of the experiment.

Experiment XVIII. Concentration of chloroform = 0·12 *c.c. per litre of air-current.* The procedure was identical with Exp. XVII, but the concentration of the chloroform was increased. The leaf in the light weighed 3·55 grms., that in the dark 3·50 grms. The results are embodied in Fig. 20

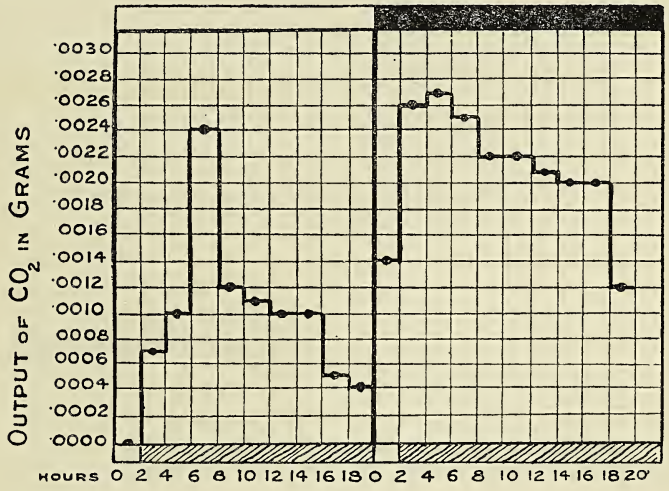

FIG. 20.

and need no further comment, save that in this case both the leaves were brown at the end of the experiment and upon opening the leaf-chambers there was a distinct smell of hydrogen cyanide.

Experiment XIX. Concentration of chloroform = 0·137 *c.c. per litre of air-current.* This experiment followed a somewhat different course; a single leaf (weight 3·00 grms.) was illuminated for forty-four hours continuously, being chloroformed after ten hours of illumination (Fig. 21).

The readings taken before the chloroform showed that the leaf constantly assimilated all its respiratory CO_2 (only the last appears in the figure). When the chloroform was introduced 0·0010 grm. CO_2 was given out during the next reading. Later, there was a very considerable but abnormally retarded outburst of CO_2, showing that the function of assimilation was completely arrested.

Subsequently the output of CO_2 fell fairly rapidly towards zero; when

the leaf was removed from the chamber it was of a dark chocolate colour with drops of exuded moisture on its lower surface, and smelt strongly of HCN.

The curve of this experiment is rather irregular and slow in its response, but is of the same type as that of the previous one.

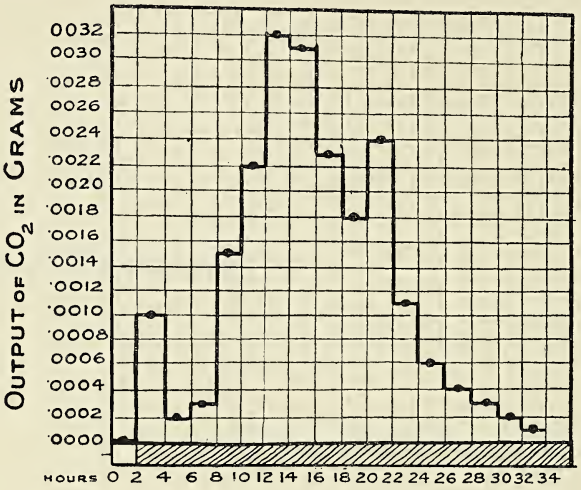

FIG. 21.

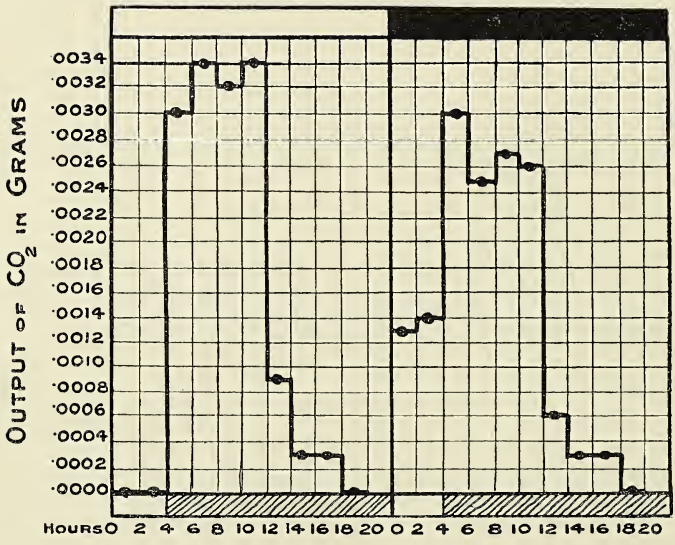

FIG. 22.

Experiment XX. Concentration of chloroform = 0·2 *c.c. per litre of air-current.* A further increased concentration of chloroform vapour in the entering stream of air produced still more accentuated outbursts of CO_2 both in light and in dark; and these fell off rapidly to zero. Fig. 22 shows

that after a sufficient moderate dose of chloroform, the curves of output of CO_2 became practically identical, whether the leaf was illuminated in a light strong enough for assimilation in a normal leaf, or was completely darkened. The leaf in the light weighed 2·95 grms., that in the dark 2·80 grms.

Both leaves turned brown and had drops of moisture on their under-surface at the conclusion of the experiment.

Experiment XXI. Chloroform concentration, about 0·25 *c.c. per litre of air-current for one hour.* To continue the series by giving still stronger doses, 0·5 c.c. of chloroform was introduced straight into the anchor tube of the chloroform apparatus, the middle arm being then corked. This amount was completely vaporized in less than one hour.

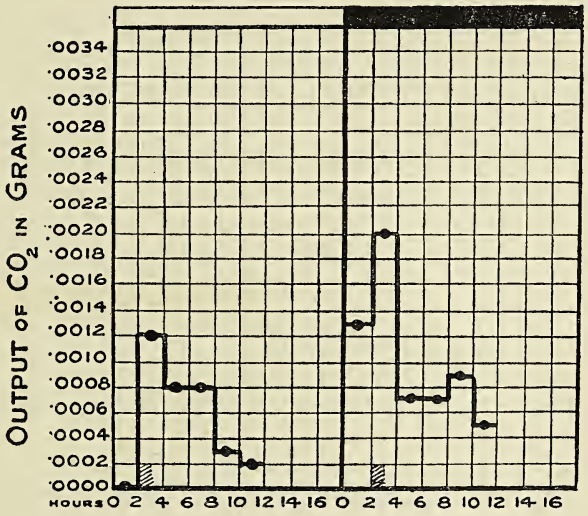

FIG. 23.

As this experiment was a double one (Fig. 23), with one leaf in the light (weight 2·1 grms.) and the other in the dark (weight 2·15 grms.), each leaf was subjected, for about an hour, to a concentration of chloroform of about 0·25 c.c. per litre of air. The usual outburst of CO_2 was produced in both leaves. Although the chloroform supply then ceased, the output of CO_2 fell rapidly to zero, as it would have done had the chloroform been kept on continuously. The leaves turned a chocolate colour, and exuded a quantity of water, so that they were very flaccid at the end of the experiment.

Experiment XXII. Chloroform concentration, about 0·75 *c.c. per litre of air-current for one hour.* The procedure for this double experiment was the same as in Exp. XXI, but 1·5 c.c. of chloroform was introduced into the anchor tube and vaporized in an hour. The leaf in the light weighed 2·5 grms., that in the dark 2·31 grms.

The effect upon the CO_2-production and upon the condition of the leaves was practically the same as in the previous experiment (Fig. 24).

Stronger doses of chloroform would no doubt have reduced the CO_2-output to zero still more quickly, but sufficient has been shown in this direction in the experiments in Part I.

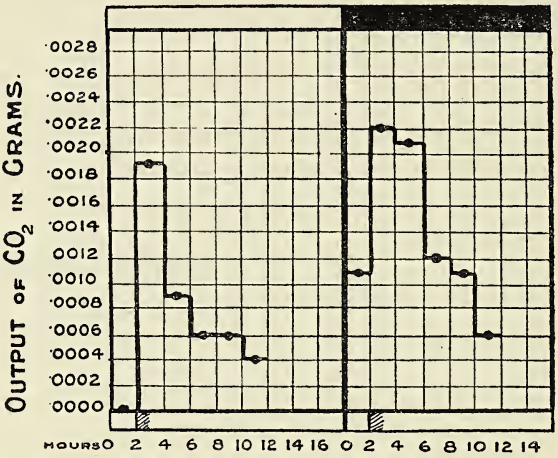

FIG. 24.

CONCLUSIONS.

Effect of chloroform upon respiration of leaves.

1. The effect depends upon the dose of chloroform, and some five types of curve may be distinguished, forming a regular progression between the augmentative effect of minute doses and the inhibitory effect of large doses. These types of curve are grouped together in a schema (Fig. 7, p. 1083).

2. Very small doses of chloroform increase the respiration; this effect may be maintained if the chloroform is given continuously, but the respiration reverts to normal when the chloroform is withdrawn.

3. Medium doses of chloroform cause an initial outburst of CO_2, which is followed by a decline in the CO_2-production to much below normal; the rate and extent of this decline increase with the dose.

4. Strong doses of chloroform abolish the initial outburst and the CO_2-production at once rapidly falls to zero.

5. The early period of the application of chloroform is the effective time; and the same subsequent curve of CO_2-production may result whether the chloroform is then withdrawn or kept on continuously.

On comparing the single doses of chloroform given in Section I with

the continuous chloroforming of Section II, it will be found that a continuous concentration of n c.c. of chloroform per litre of air-current produces the same type of effect as an initial dose of $2n$ or $3n$ c.c.

6. The other effects of chloroform upon Barley leaves are (1) destruction of chlorophyll and (2) exudation of water through the stomata. In Cherry Laurel leaves we find in addition (3) browning of the leaf and (4) decomposition of the cyanogenetic glucoside.

Effect of chloroform upon assimilation in leaves.

7. Very minute doses of chloroform, which have no detectable effect in the dark, arrest assimilation in a lighted leaf and cause CO_2 to be given out in the light.

8. If the chloroform is given for a short period and at a very low concentration, then upon its removal the leaf may partially recover its assimilative power.

9. Quite moderate doses of chloroform completely abolish the assimilative power.

10. Larger doses of chloroform abolish all traces of assimilation so quickly that a leaf in the light reacts exactly the same as a leaf in the dark and shows the characteristic initial outburst of CO_2 and the other stages described under the heading of respiration.

In conclusion, I should like to express my thanks to Dr. F. F. Blackman for the use of his special apparatus and for his help in correlating these experimental results.

The Female Inflorescence and Ovules of Gnetum africanum,[1] with Notes on Gnetum scandens.

BY

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(*Report No. 5 of the Percy Sladen Memorial Expedition in South-West Africa*, 1908–9.)

With Plates LXXXVI and LXXXVII and sixteen Figures in the Text.

FOR the material on which this investigation is based I am indebted to Professor H. H. Pearson, of the South African College, Cape Town, who collected and preserved it during the recent Percy Sladen Memorial Expedition in South-West Africa.

The material of *G. africanum* includes many stages in the development of the ovules (most of the younger ovules are, however, unfortunately aborted); also large fertilized ovules containing well-developed endosperm, but no embryos. As other authors have given comparatively few details of their investigations of the ovules of various species of *Gnetum*, it is not possible to say how far this species, here for the first time described, differs from those already known. In the morphology of the inflorescences, and in the general structure of the ovule and its integuments, it agrees with the species described by Strasburger[2] and Lotsy.[3] Strasburger's material appears to have included only young stages of the ovule, and he was not able to give an account of the mature seed.

Some material of *G. scandens* (comprising small portions of the female inflorescences with young, unfertilized ovules all nearly at the same stage, one mature fertilized seed on its stalk and male inflorescences) has been examined for comparison, and Miss Berridge's recent description of the ovule of *G. Gnemon* appears opportunely. It is hoped that the ovules and inflorescences of other species may be investigated later. From Karsten's

[1] A short preliminary account of the ovules of this species was given at the Sheffield Meeting of the British Association, 1910. The Morphology of the Ovule of *Gnetum africanum*. Report, p. 783.

[2] Strasburger, 1872, 1879.

[3] Lotsy, 1899.

description of the macroscopic features of the ovules, it appears likely that the detailed structure of the integuments in the various species [1] would differ widely, and such differences may well prove of value in comparison with other seeds both fossil and recent.

The present paper includes an account of the vascular system in the female inflorescences of *G. africanum* and *G. scandens*, and of the male inflorescence of *G. scandens*. Several interesting points serve to recall the structure in the allied genus *Welwitschia* and in the Cycads.

The structure of the ovules in *G. africanum* has many points of interest. A short description of these was given at the Sheffield meeting of the British Association, where attention was also drawn to the remarkable detailed resemblance between the ovules of this species and those of *Bennettites*.

I. Recapitulation of the General Morphology of Inflorescence and Ovule in Gnetum.

The inflorescence in most species [2] is a spike, bearing at its base two bracts, in the axils of which other inflorescences, buds or flowers may occur. At each node of the inflorescence axis is a cupule derived from two fused bracts,[3] in the axil of which the flowers are borne on a ring-shaped swelling of the inflorescence axis. The swelling or cushion arises from a ring of meristematic tissue in the axil of the young cupule, and the flowers are developed by further localized growth of portions of this tissue.[4] Between and below the flowers the cushion is covered with numerous multicellular hairs (Fig. 3 A, Pl. LXXXVI).

In the female inflorescence, the presence of five to eight flowers in a ring is general throughout the genus. Five to seven are common numbers in *G. africanum* and six appears to be usual in *G. scandens*.

In the early stages the cupules with their axillary swellings are crowded together, but later they are usually more or less separated from one another by the growth of the internodes. In *G. scandens*, the nodes become very widely separated (Fig. 1, Pl. LXXXVI); in *G. africanum*, on the other hand (Fig. 2, Pl. LXXXVI), the internode is very short and the nodal swelling extends over a relatively very large area.

The bases of the young flowers are sunk deeply into the swollen axis, so that the portions of the cushion intervening between them appear in transverse section as peltate projections (Pl. LXXXVI, Fig. 3 B, *G. africanum*).

[1] Karsten, 1893 (1) and (2). One difference remarked is that seeds may be angular and pointed at the apex, or smooth and rounded at the apex. The species described here both belong to the latter class.

[2] See Strasburger, 1872, 1879; Karsten, 1893; Lotsy, 1899, &c. In some species, such as *G. scandens*, *G. Ula*, and *G. Rumphianum*, the stalks of the mature seeds are so much elongated that the inflorescence becomes at length a raceme.

[3] Karsten: Cohn's Beiträge, 1893, p. 340, &c.

[4] Lotsy, 1897, p. 84.

In *Gnetum scandens* the fertilized ovule is later carried out on a stout stalk (Fig. 1 A, Pl. LXXXVI). Each female flower consists of an ovule enclosed in three coverings, the outer of which is, according to the species, green, orange or red,[1] and more or less succulent, resembling in texture the bracts and cupule, while the two inner are more specialized and are referred to in this paper as the outer and inner integument.

The female inflorescence is said[2] commonly to terminate in a flower; this has been once observed to be apparently the case in *G. africanum*.

In the male inflorescence the cushion bears a ring of stamens, and above these a number of abortive ovules each consisting of a nucellus with two coverings.

II. Anatomy of Inflorescence Axis and Cupules in G. africanum and G. scandens.

(*a*) The *general structure* in the node and in the internode of the inflorescence axis is strikingly different.[3] In the internode of a young inflorescence the structure resembles in all particulars, except a greatly increased diameter, that of the naked peduncle on which the cone is borne.

The peduncle and the internodes of the young inflorescence axis are made up of thin-walled parenchymatous tissue, in which a few branched fibres with lignified walls are scattered at intervals,[4] and are traversed by a ring of simple collateral vascular bundles, each of which grows in thickness by means of a cambium. In the older inflorescences there are a few concentric groups of bundles outside the main ring; these may be continuous throughout the internode.[5]

The simple main ring of bundles characteristic of the internode runs up into the node; the number of bundles in the ring increases to some extent with the age of the inflorescence, but there is no regular interfascicular cambium. In *G. africanum* there are generally 8–12 bundles in the ring, and there does not appear to be any further increase in number in the oldest inflorescences in my material; in *G. scandens* as many as 16–20 may occur in quite young inflorescences, and new bundles are still being inserted between the old ones, according as the appendicular organs require a larger vascular supply.

[1] Lotsy, 1899, p. 53; Griffith, 1859, p. 303, &c.; Karsten, 1893 (1).

[2] Strasburger, 1879, p. 118.

[3] This account applies mainly to the female inflorescence axis of *G. africanum*; the material of *G. scandens* showed much the same features, but, owing to the inflorescence being younger, they were less marked.

[4] These fibres resemble the 'spicular cells' in *Welwitschia*, but have no crystals embedded in their walls; Sykes, 1910 (1), p. 181.

[5] Cf. Worsdell, 1901, p. 772. In *Welwitschia* such concentric strands are numerous; see ibid., and also Sykes, 1910 (1), p. 194, &c., and (2) p. 341.

Each bundle is made up chiefly of secondary elements; the crushed primary phloem and the torn spiral protoxylem are, however, clearly recognizable.[1] The secondary xylem throughout the inflorescences is mainly composed of narrow reticulately thickened elements.

The structure at the node is much more complicated. Besides the main ring of bundles there are two outer rings concentric with it, the middle one being orientated inversely to the other two. The two outer rings are concerned with the supply of cupules and flowers; they are connected with the main series, and in the older inflorescences are also continuous with the small additional concentric bundles found in the internodes. Below the bracts at the base of the spike and below each bract cupule, the pith tends to become lignified, the lignification spreading centripetally from the xylem of the main bundles.[2] In the older inflorescences of *G. africanum*[3] this tendency to lignification of the pith extends throughout the internode; while at the nodes, probably owing to the tissue tensions brought about by the rapid growth of the cushion, the pith is torn and hollow (Text-figs. 2 and 5). The tension under which the node has increased in size has also resulted in the development of large spaces in the pericyclic region of each bundle; these spaces are crossed at intervals by trabeculae (Fig. 4, Pl. LXXXVI). Throughout the node the thick-walled fibres increase greatly in number, both in the pith and more especially in the outer layers of the cortex, where they form a more or less continuous strengthening band.

Text-fig. 3 is a longitudinal section through a node, and shows the hollow pith with its numerous fibres, and the ovular bases sunk in the swollen cushion in the axil of the bract cupule. Fig. 3 A, Pl. LXXXVI, is taken from a section through the base of the node and shows the cushion, covered with hairs, surrounded by the free apex of the cupule. Fig. 3 B, Pl. LXXXVI, through the middle of the node, shows the sunken ovule and the interovular portions of the cushion which are peltate in transverse sections.

Bracts and Cupule. Both the basal pair of bracts and the cupule are specialized for protective functions. The epidermis is thick-walled, and corky excrescences appear in places. Stomata are very rare, occurring mainly on the decurrent bases of the cupules and bracts, but being entirely absent from the free tip. Numerous branched fibres with lignified walls occur throughout the tissues. The free portion of the cupule has a mucilaginous subepidermal layer on its inner surface (M, Fig. 5, Pl. LXXXVI).

The vascular bundles supplying the cupules pass out horizontally from the main bundles and then run downwards, afterwards turning sharply upwards into the free tip. They are thus often cut twice in a transverse

[1] Contrast the absence of protoxylem in the cone axis of *Welwitschia*; Sykes, 1910 (2), p. 339.

[2] See Worsdell, 1901, p. 772.

[3] The material of *G. scandens* was too young for comparison with this stage.

section. They branch freely, they are small and numerous, and associated with them are many transfusion tracheides which occur chiefly laterally on the phloem side of the bundle (*Tr.*, Fig. 5, Pl. LXXXVI).[1]

(*b*) *The Course of the Bundles.* The bundles run straight up the peduncle and internodes of the inflorescence without anastomosing. As they reach the base of the inflorescence they branch, and from two opposite points in the stele two single, usually concentric bundles arise. Each of these first bundles forms the median bundle of a bract supply and is speedily supplemented by numerous other bundles, arising on each side of it from others of the bundles of the main axis. Many of the bundles, including the single median one, have a double origin from two of the bundles of the main axis, as shown in Text-fig. 1.[2]

TEXT-FIG. 1, A, B, C, shows the origin of one of the concentric bract bundles from two bundles of the main axis.

I had only two whole spikes among my material and in both cases there were no buds in the axils of the two basal bracts, so the vascular supply of axillary organs other than the flowers could not be investigated.

The bundles supplying the bract cupules arise in a manner very similar to those supplying the bracts; the median bundle is, however, not often clearly distinguishable, the bundles are more numerous and the general arrangement more irregular. The chief interest in the vascular system of the inflorescence of *Gnetum* lies in the origin of the two outer nodal rings of bundles and the method of formation of the bundle supply of the flowers. Worsdell[3] compares 'these transitory extra-fascicular rings of bundles with the more fixed extra-fascicular rings of bundles in the vegetative axes of the Cycads'; the former are almost all used up at each node by the bract and flower supplies, the latter are concerned with the leaf supplies and are therefore not persistent above the youngest leaf-trace.

The course of the bundles through the node of the older inflorescences of *Gnetum africanum* is exceedingly complex, so the younger inflorescences of *G. scandens* will first be described. In *G. scandens* the simple ring of fibrovascular bundles has a long undisturbed course through the elongated internode, and the small size of the cushion at the node brings the insertion of the ovules closer in the axil of the cupule (see Fig. 1, Pl. LXXXVI).

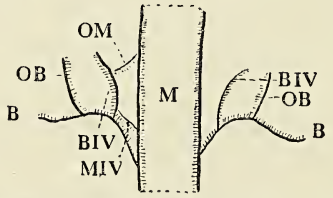
[1] As in *Welwitschia*; Sykes, 1910 (1), p. 186. Cf. Worsdell, 1901, p. 767, on *Ephedra distachya*, where he found a few tracheides of centripetal xylem in the bract of the female inflorescence.

[2] A similar double origin of bundles is common throughout the inflorescence. Cf. *Welwitschia*, Sykes, 1910 (1), pp. 193, 204, &c.

[3] Worsdell, 1901, pp. 767–8.

In the simplest case[1] examined (Text-fig. 2, right-hand side), a ring of bract bundles (B) arose from the main axial bundles (M) and passed out into the cupule, first running upwards and then turning sharply downwards. During their outward course two series of bundles were given off, the outer of which (OB) was orientated in the same way as the main bundles and supplied the lower half of the ovular bases, and the inner of which was inversely orientated (BIV), running up higher into the cortex of the main axis and then turning outwards to supply the upper half of the ovular base. The inverse series arises from the bract bundles at some distance from the main bundles and is never closely attached to the latter as it is in *G. africanum* (Text-fig. 4).

In a rather older node of *G. scandens*, the inverse series (Text-fig. 2, BIV, left-hand side) receives further contributions (OM and MIV) from the bundles of the main axis, some of which become more or less inversely concentric.[2]



TEXT-FIG. 2. Course of bundles in female inflorescence of *G. scandens*. The right-hand side represents a somewhat younger ovule than the left. M = bundles of main axis; B = supply of bract cupule; OB = outer series of bundles supplying ovule and arising from B; BIV = inner series of inversely orientated bundles arising from B and supplying ovule; MIV and OM = bundles arising from main bundles and augmenting the inverse series supplying ovule. (Black lines = phloem; stroked lines = xylem.)

It is probable that in older inflorescences than are found among my material a more complicated structure approaching that seen in *G. africanum* would be developed.

In the male inflorescence of *G. scandens* all the cases examined had a simple arrangement similar in essentials to that shown on the right-hand side of Text-fig. 1. Numerous bundles supply the bract cupules as in the female inflorescence, and from them arise an outer series of bundles, mostly concentric, which give off branches to the stamens and then supply some of the abortive ovules;[3] and also, nearer the main bundles, an inner inversely orientated series which also supplies both stamens and ovules. As the bundles divide to form staminal and ovular traces, an anastomosing ring is formed such as is shown in the photograph in Fig. 17, Pl. LXXXVII. The stamens each receive a simple concentric bundle,[4] the abortive ovules a little ring of bundles.

[1] Strasburger, 1879, pp. 116–18, Figs. 15–23, Taf. XXI.

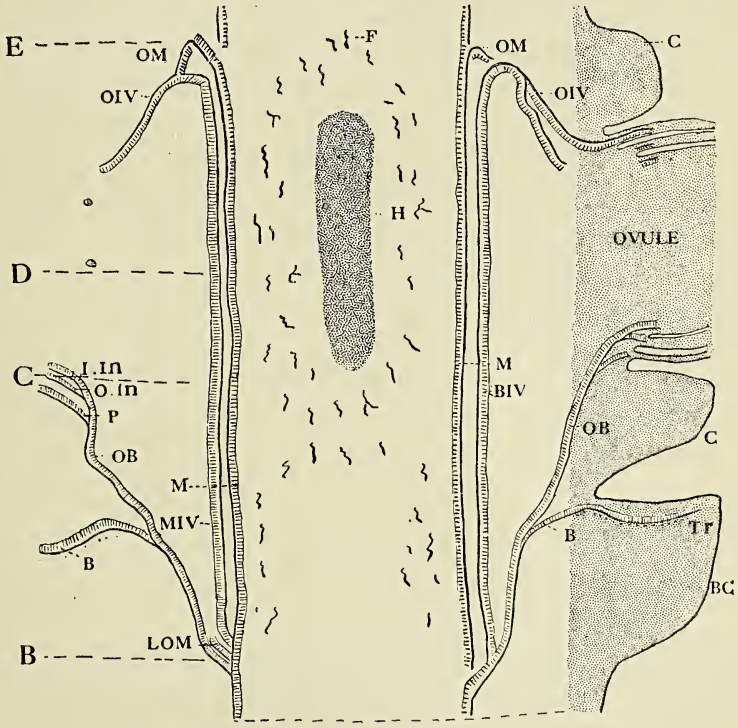
[2] See Sykes, on *Welwitschia*, 1910 (1), p. 192.

[3] As described by Strasburger, 1879, p. 106, and Taf. XXV, Figs. 65 and 67.

[4] Cf. *Welwitschia* stamens and *Bennettites* ovules, Worsdell, 1901, p. 763, and Sykes, 1901 (1), p. 187.

In the younger examples of female inflorescences of *G. africanum*, the state of affairs is similar to that in *G. scandens*. The great extent of the node in this species (Fig. 2, Pl. LXXXVI) is, however correlated with a disturbance of the arrangement of the vascular system over a greater distance, and indeed the course of the undisturbed ring characteristic of the internode is in this species relatively very short.

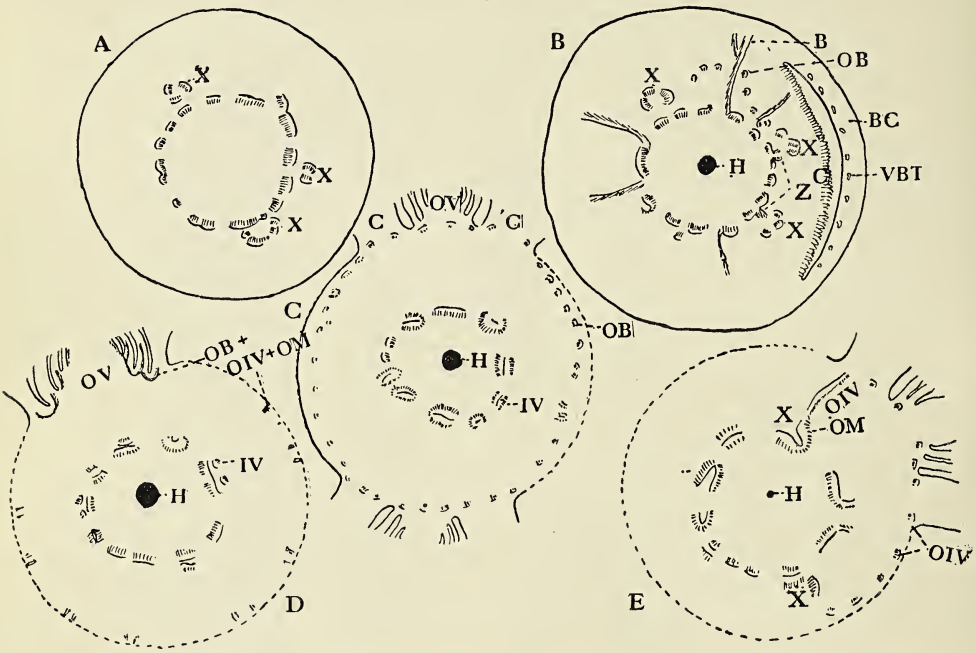
In Text-fig. 3 is seen a diagram of the course of the bundles



TEXT-FIG. 3. Letters as before; also F = branched fibres; H = hollow stem opposite ovule; BC = bract cupule; C = nodal cushion; TR = transfusion tissue; LOM = small bundles arising from main series and augmenting series OB; OM = bundle arising from main series and augmenting series OIV; OIV = inverse series turning down to supply ovule; P, O.IN, I.IN = bundles supplying perianth, inner integument and outer integument respectively.

through the node of an old female inflorescence of *G. africanum*. As before, the bract bundles (B) are seen to originate from the main bundles, and as they pass out they give rise first to a series of inversely orientated bundles (BIV) and then to some normally orientated ones (OB), both of which supply the ovule. Both series are augmented from the main series. In the oldest inflorescence I examined, only a few small bundles (LOM) were added to the outer series, which still is chiefly composed of bundles derived from the bract bundles. But a large number of the inversely orientated bundles forming the inner series come directly (MIV) from the main bundles

at intervals throughout their long course up the node and remain closely associated with them till at the top of the node they pass out to the ovules. There they are joined by a number of additional bundles from the main axis (OM), at about the level at which they turn downwards (OIV), making a sharp angle with their former course, and run into the upper edge of the ovule. In *G. scandens* the inverse series does not make this sharp bend, but



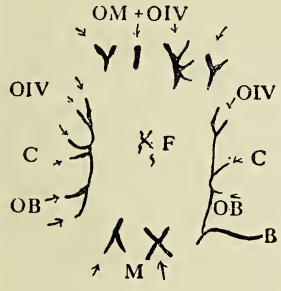
TEXT-FIG. 4. A–E. Series of transverse sections through a node and internode of an inflorescence of *G. africanum*. (Letters as before; also X = concentric bundles in internode; Z = bundles with double origin[1]; VBT = bract bundles with transfusion tissue; OV = ovule.) A is from the base of an internode; some of the bundles are already dividing in preparation for the next node. B = base of bract cupule, cut obliquely, with axillary cushion covered with hairs. C = lower part of cushion, three series of bundles, some of the outer series are concentric. D is through the median plane of the ovules, showing some of the ovular supply cut in longitudinal section, but only two series in main axis. E = near top of ovule, showing some of the bundles supplying the upper part of ovule, cut both in their downward course near the edge of the section, and in their upward course attached to the main bundle as part of the inverse series. A concentric bundle is being re-formed in the cortex at the level of fusion between an ovular bundle and a main bundle.

its whole course is in a straight line; sloping gently outwards from the point of origin from the bract bundles (Text-fig. 3).

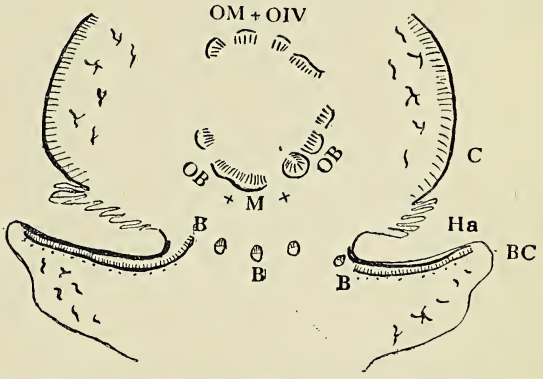
A series of transverse sections through the node shows, first, above the level of the origin of the bract bundles, three concentric series of bundles; then the median sections through the ovules show only the two series, the outer being in the act of passing out into the ovules; finally, above this level three series again appear (the new outer ring being formed by the

[1] See p. 1105.

bundles which enter from the upper part of the ovule and, after passing upwards, turn down again to join the inverse series, the bundles of which are thus cut twice in these upper sections). Text-fig. 4 is a series of transverse sections illustrating this; the sections are obtained from an older part of the inflorescence than that shown in Text-fig. 3, and the pith has become hollowed out to a greater extent, only a very small part of the internode remaining solid, but the levels of the sections roughly correspond to B, C, D, E respectively in Text-fig. 3. The most interesting feature in this series is the concentric groups of bundles (XX), outside the main ring, which are persistent throughout the internode. At the base of the node these concentric groups become involved with the bract bundles and the lower part of the



TEXT-FIG. 5 is from a tangential section of the female inflorescence axis, showing the ring of bundles destined for a single ovule in process of formation. Letters as before; C = bundles entering from intermediate portion of cupule.



TEXT-FIG. 6 is a still more tangential section, showing the now fully formed ring nearly at the base of the ovular stalk. Ha = hairs on cushion; OM and OIV may be taken to represent bundles entering from above the ovule, OB and M those entering from below the ovule.

ovular supply, and are not continued as such into the node. They are formed again at the top of the node from the inverse series and main bundles at the level at which the former turns downwards towards the ovule (Text-fig. 4 E, X). Text-figs. 4 C and D show the close connexion formed in many cases between the inverse series and the main bundles, many of them being fused together so as to constitute a large inversely concentric bundle.

In tangential sections through the inflorescence axis it is seen that the bundles destined for each ovule are collected into a little ring (see Text-fig. 5). The upper and lower bundles, those derived respectively from the inverse series (OM and OIV) and the outer series (OB), form the upper and lower groups of the ring. At the sides of the ring are smaller bundles (C), which, derived at first from the upper and lower groups, have moved outwards into the intermediate portions of the cushion and then inwards again into the ovular base. The entry of such a bundle into the ovule is seen in Text-fig. 13 C, p. 1121.

The completely formed ring of bundles is seen in the still more tan-

gential section shown in Text-fig. 7, and the further course of the ring of 8–10 bundles thus entering the ovular axis may be followed in the series of sections through the ovular stalk shown in Text-fig. 7.

III. Anatomy of Stalk of Ovule.

The young ovule is almost sessile in the axil of the cupule, and its base is deeply embedded in the tissues of the cushion. In even the oldest ovules among my material of *G. africanum* the outermost covering originated very near the base of the short stalk, as in *G. Gnemon*. I do not know whether in *G. africanum* the stalk ever grows any longer; Professor Pearson tells me he has seen no signs of any further growth. The fully mature ovule of *G. scandens* had a stalk equal in length to about a third of the total length of the ovule.[1]

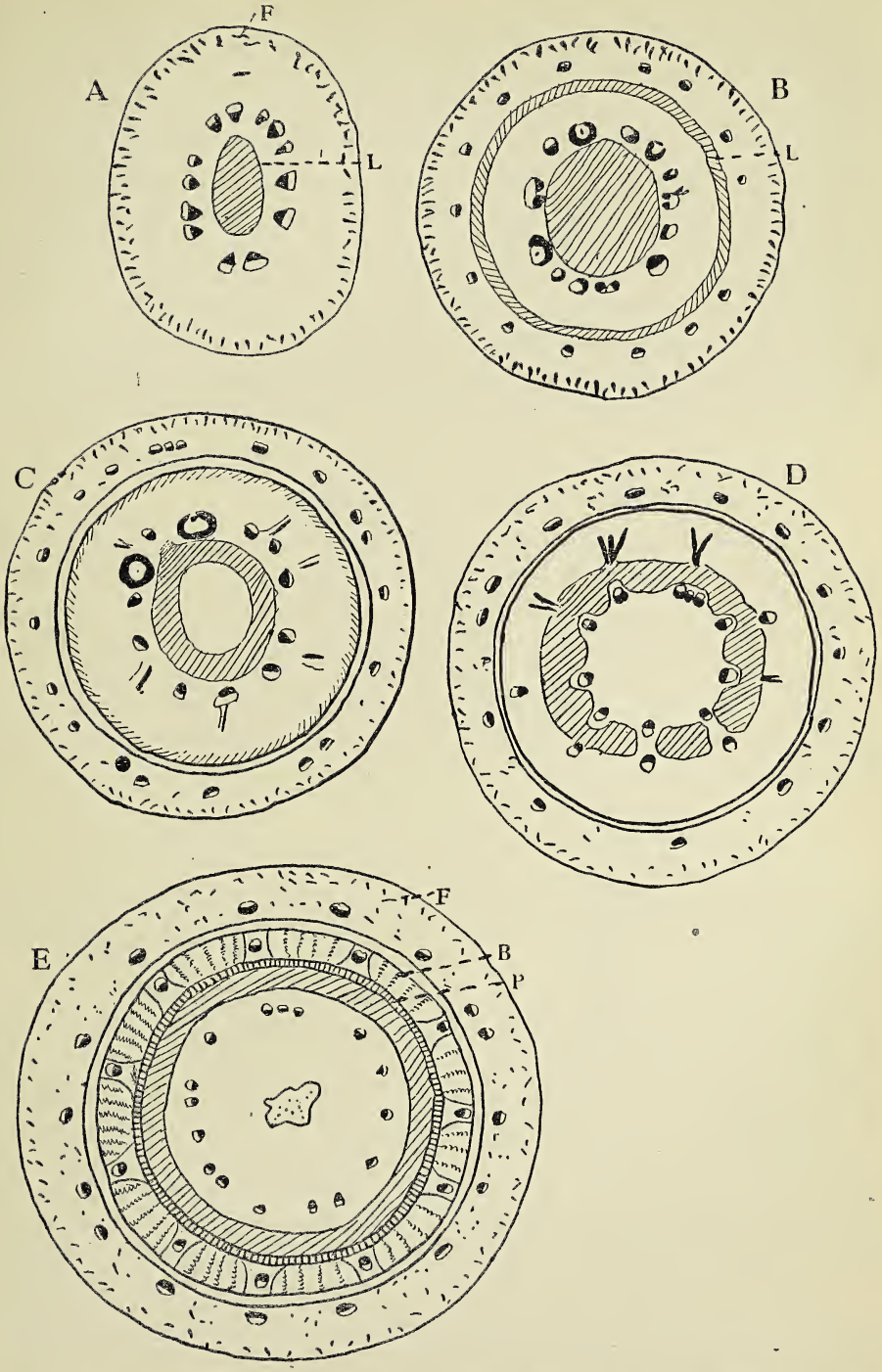
The flower-stalk receives from the main axis a simple ring of bundles, varying in number in very young flowers from about ten to thirteen (Text-fig. 7 A). Numerous sclerenchymatous fibres with lignified walls are gradually developed in the peripheral layers of the cortex, and lignification appears in the pith at a very early stage, becoming more and more strongly pronounced, till in the short stalk of the old ovule of *G. africanum* the pith is entirely composed of sclerified elements. In the long seed-stalk of *G. scandens* this lignification is found only in the upper portion of the stalk.

Below the base of the outer flower-covering the vascular bundles branch, and a number of small bundles pass out to supply the outer covering. Many of the bundles left behind in the axis become inversely concentric and irregularly orientated (Text-fig. 7 B). A ring of lignified cells appears in the cortex between the two rings of bundles, and the outermost covering is separated off along the outer edge of this ring.

Above the origin of the outer covering parenchymatous cells appear in the centre of the lignified pith. Text-fig. 7 C shows the parenchymatous pith surrounded by a ring of lignified tissue. The vascular bundles are now divided up rather irregularly into numerous smaller bundles, which become separated into an outer and an inner ring. During this process many more of the bundles become concentric or more commonly inversely concentric, or a few collateral bundles may be arranged to form a concentric group.

The ring of lignified tissue surrounding the pith next moves outwards and separates the two rings of bundles (Text-fig. 7 D, and Fig. 6, Pl. LXXXVI, *lf*); it finally becomes the inner layer of the outer integument. Text-fig. 7 E represents the base of the ovule just below the level of freedom of

[1] It would appear that *G. Ula*, figured by Karsten, Cohn's Beitrage, 1893, resembles *G. scandens* in having a long stalk; in that species the abortive ovules fall off, and the old inflorescence presents the appearance of a raceme.



TEXT-FIG. 7, A–E. Series of transverse sections through the stalk of an ovule of *G. africanum*. F = fibres, L = lignified tissue, B = tissue with darkly coloured walls, P = palisade layer.

the outer integument; the figure passes through the base of the embryo-sac, and the inner ring of bundles is seen running in the tissues of the fused nucellus and inner integument. The complex outer integument is already differentiated into three zones: (1) the innermost composed of fibrous cells, which are unlignified in the very young ovule, but become partly lignified at an early stage; (2) a palisade layer; and (3) the outer layer, containing strands of parenchymatous tissue each traversed by a bundle, alternating with larger strands of cells with brownish indurated walls. The structure of the outer integument shown in this diagram is continued upwards for some distance above its level of freedom, and the chief difference as far as the level of origin of the inner integument is that in the upper half of the ovule the fibrous zone, and consequently the palisade layer, assumes a rayed outline, projecting outwards opposite each vascular bundle (Text-fig. 10, p. 1114).[1]

IV. The Seed.

The seed in both species of *Gnetum* is straight and cylindrical, circular in transverse section, and tapering slightly towards the apex where a conical projection marks the position of the micropyle. The oldest seed of *G. africanum* among my material was about 1·2 cm. long (Fig. 2, Pl. LXXXVI), the single mature seed of *G. scandens* was about 2·7 cm. in length (Fig. 1 A, Pl. LXXXVI). It will be best to describe these seeds separately, though their development is similar and the older stages only differ in minor details.

G. africanum.

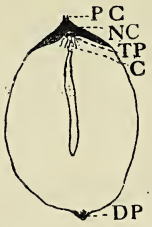
Text-fig. 9 is a diagrammatic representation of one of the oldest seeds examined; it is of course possible that further development may take place later.

The *nucellus*, which is fused for about two-thirds of its length with the inner integument, is composed at this stage of a few layers of thin-walled parenchymatous tissue. The inner series of vascular bundles traverses the fused region of nucellus and inner integument, clearly in the region of the integument, and there is no vascular supply to any part of the nucellus itself. The latter is almost filled by the embryo-sac, which is full of endosperm. The endosperm has a slightly corrugated outline, and at the apex of the embryo-sac it projects upwards and forms a sort of small 'tent-pole'[2] supporting the tip of the nucellus

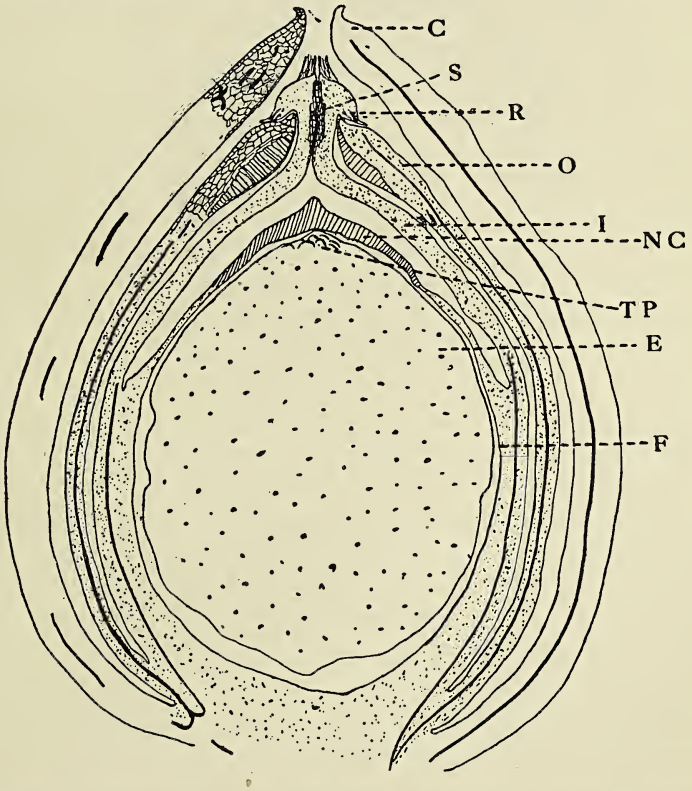
[1] Exactly the same stages occur in *G. scandens*; but as the lignification of the pith is only developed in the upper part of the long stalk of that species it will be realized that the lignified tissue which forms the sort of cup at the base of the ovule is there exactly comparable to the cup of fibrous tissue at the base of the long-stalked ovule of *Bennettites*; see pp. 1125, 1126.

[2] Cf. *Ginkgo*, Seward and Gowan, 1900.

(TP, Text-fig. 9); the cell-walls of the 'tent-pole' are often thickened and lignified, to fit them for their supporting function (Fig. 8, Pl. LXXXVI). The



TEXT-FIG. 8. Oldest ovule of *G. africanum*. Endosperm and adhering nucellar cap from which integuments have been removed by dissection. about twice natural size. NC = nucellar cap; PC = remains of pollen chamber; TP = tent-pole; DP = lignified cells in basal projection of endosperm; C = paths made by pollen tubes.



TEXT-FIG. 9. Diagram of median longitudinal section through seed of *G. africanum*. C = outermost covering; O = outer integument; I = inner integument; F = fused nucellus and inner integument; E = corrugated endosperm; TP = tent-pole; NC = nucellar cap; S = closed micropyle; R = down-growing flange of micropylar tube.

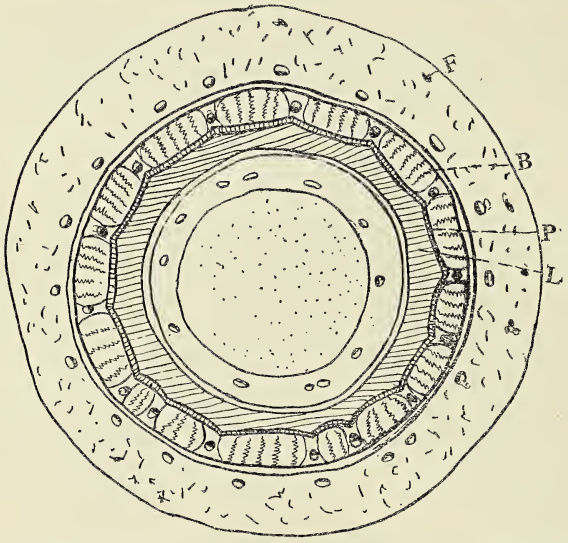
cells of the nucellus in this region have thick lignified walls and form a sort of nucellar cap [1] (NC, Text-figs. 8 and 9) which, being attached at its base to

[1] Griffith, 1859, p. 303, Taf. LV, Figs. 25 and 26; Coulter and Chamberlain, p. 383, Fig. 430.

the inner integument only by two or three layers of thin-walled cells, is very easily lifted off, when the seed is dissected, like a tent from its supporting pole (see Text-fig. 8). At the apex of the nucellar cap some signs of disintegration are generally visible, and represent the remains of the pollen chamber; in the tissues both of the cap itself and in the apical region of the endosperm it is generally possible still to distinguish traces of the paths made by the pollen tubes.

The seed has three coverings.

The *outermost covering* arises from the base of the seed and is free to



TEXT-FIG. 10. Transverse section through upper half of ovule. Part of same series as Text-fig. 7 A–E, and Text-fig. 11. For lettering see Text-fig. 7.

its apex; it is green, fleshy, and succulent, and is chiefly composed of homogeneous parenchymatous tissue among which are scattered numerous branched fibres with lignified walls (Fig. 11, Pl. LXXXVI). Numerous repeatedly branched vascular strands run up to its tip, and stomata are occasionally found in its outer epidermis; altogether it resembles the bracts forming the cupular ring in the axil of which the ovule is borne.

The middle covering or *outer integument* is complex in structure, and contains the hard protective layer of the seed. It arises just above the outermost covering and continues upwards, uniform in thickness till it expands at the apex and surrounds the micropylar tube. It is traversed throughout its length by numerous vascular bundles which branch at intervals and run almost up into the tip. Text-fig. 10 represents a transverse section through the upper half of the ovule. The structure of the outer integument at this level is very similar to that already described, at the base of the ovule, before it was free from the nucellus (p. 1112, Text-fig. 7 E);

Fig. 11, Pl. LXXXVI (01) shows the structure of the free outer integument between these two levels.

The outer zone of the outer integument is traversed by the vascular bundles, each of which is accompanied by a strand of parenchyma and alternates with strands of tissue composed of isodiametric cells with brown indurated walls (Text-fig. 10, B, Text-fig. 11, and Fig. 11, Pl. LXXXVI, D). Next comes a continuous palisade layer which at the base of the ovule is smooth, but in the upper part of the ovule projects outwards and makes an angle opposite each bundle. Inside this is the inner fibrous zone, the outline of which corresponds with that of the palisade layer. In my oldest ovules of *G. africanum* the palisade and fibrous layers were unlignified from the base of the ovule to near the apex, or only very slightly lignified in places, but in the mature ovule of *G. scandens* both layers were lignified throughout, and it is probable that there would be more lignification in *G. africanum* when mature.

As the upper part of the seed is approached the hypodermal strands of indurated tissue diminish in size and prominence, while the fibrous layer increases. The outward projections of the fibrous and palisade layers opposite each vascular bundle become individually more and more strongly pronounced, but as the vascular bundles die out the projections are also gradually reduced in number, there being always a projection opposite each remaining vascular bundle (Text-fig. 11).[1]

When the integument thickens at the apex and clasps the micropylar tube the hypodermal strands are lost sight of, and the palisade layer entirely disappears. By this time the fibrous layer occupies the greater portion of the thickness of the integument and its rays are reduced to four or five, opposite some of which the last remaining tracheides of the vascular bundles may still be detected. A characteristic star-like structure (Fig. 16, Pl. LXXXVII, and Text-fig. 11, p. 1116) is thus obtained, which reminds one of Lignier's figures of *Bennettites* and also of *Trigonocarpus*. At this level the radial section of the ovule (Text-fig. 9, p. 1113, Fig. 7, Pl. LXXXVI, and Fig. 15, Pl. LXXXVII) shows that the ends of the cells constituting the fibrous zone have arranged themselves to form a close series of cells elongated at right angles to the surface of the integument, and having their walls strongly lignified and pitted; the walls of the inner epidermal cells are similarly lignified and pitted (Fig. 7, Pl. LXXXVI).

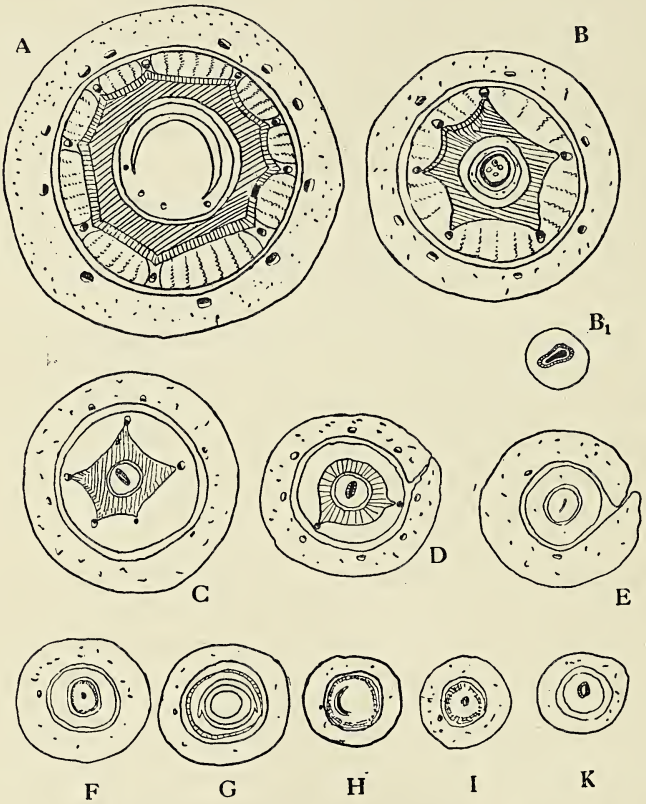
Outside the fibrous zone the expanded portion of the integument is composed of parenchyma only, the cells of which show some tendency towards arrangement in rows at right angles to the epidermis, though not so clearly as in *G. Gnemon*.[2] The general configuration of this layer

[1] For similar ribbed fibrous layers in integument, see *Conostoma* (Oliver, 1911), *Trigonocarpus* (Scott, 1907, &c.), and other ancient seeds. A similar tendency to the reduction in number of the angles towards the seed apex is seen in *Conostoma anglogermanicum*, Oliver and Salisbury, 1911, p. 26.

[2] Berridge, 1911, Fig. 1.

reminds one of the 'assise rayonnante' in *Bennettites Morierei* as described by Lignier.[1]

At the extreme tip of the integument the fibrous layer dies out, and only a thin band of parenchymatous tissue remains, tightly clasped round the micropylar tube.



TEXT-FIG. 11, A–K. Transverse sections through apex of ovule. A (slightly oblique) level of freedom of inner integument and termination of its vascular bundles: outer integument already shows some reduction in the number of its bundles. B through nucellar cap; B_1 is through extreme tip of nucellar cap and base of micropylar tube. C shows star-like arrangement of fibrous layer in outer integument; palisade layer and hypodermal strands have died out. D = termination of fibrous layer. E = parenchymatous tip of outer integument; micropylar opening reduced to a mere slit. F = micropylar tube almost solid. G = solid micropylar tube, enclosed by tip of outer integument, surrounded by base of down-growing flange. H = solid micropylar tube with wide flange, separate on one side, showing tip of outer integument projecting upwards. I = cavity beginning to reappear in micropylar tube; radial lines indicate divisions to form flange. K = just below withered tip of micropylar tube.

The *innermost covering or inner integument* is coalescent with the nucellus for about two-thirds of the length of the latter; its free portion encircles the nucellar cap and projects for some distance beyond the nucellus as the micropylar tube. At this stage the tip of the tube

[1] Thoday: Brit. Ass., Sheffield, 1910; also Berridge, 1911.

is withered; it only projects a little distance beyond the outer integument and does not overtop the outermost covering as it does in the younger stages.[1]

In my oldest ovule of *G. africanum* the innermost integument is composed almost entirely of parenchymatous cells, though in the micropylar tube (Fig. 11, Pl. LXXXVI) a few short cells with lignified walls are scattered; and it is probable that these would increase in number later, as they do in *G. scandens*.

A ring of numerous well-developed and branching vascular bundles traverse the common base of the integument and nucellus, running, however, distinctly in the region of the integument; as a rule the principal part of the bundles terminates just at the level of freedom of the integument, but one or more spirally thickened tracheides have been in several cases traced quite into the base of the free portion of the integument (Fig. 10, Pl. LXXXVI), and in most cases a few elongated cells with dense contents, such as accompany the xylem elements throughout the course of the bundles, continue past their termination for some little distance into the integument.[2] The integumentary nature of the vascular strands [3] is still more clearly seen in the younger ovules (see photograph shown in Fig. 13, Pl. LXXXVII), where the procambial strands extend right into the base of the integument.

The micropylar tube is closed at this stage [4] for a short distance opposite the tip of the outer integument. Above this level the cavity can still be distinguished in the withered tip; the cells lining the cavity are cuticularized. Below this level the cavity again appears and is at first slit-like, thus imparting to the seed a small amount of bilateral symmetry. A projecting flange [5] extends downwards from the closed part of the micropylar tube and is wrapped over the tip of the outer integument, locking the two firmly together.

This method of closing the micropyle, of which details will be given later, resembles that recently described by Miss Berridge in *G. Gnemon*. The tip of the inner integument appears to be withered lower down in my species, and all the structures at the apex are less conical and pointed than in *G. Gnemon* (cf. Text-fig. 9, p. 1113, with Berridge, Fig. 1, p. 140). In *G. africanum* the withering of the micropylar tube seems very rapid; in most of my smallest ovules the micropylar tube had already withered below the level of the outermost covering (Fig. 14, Pl. LXXXVII).

[1] Griffith, p. 302, Taf. LV, Fig. 18, shows the long tube of the inner integument before it withers; also Lotsy, 1889, Fig. 18, Pl. III.

[2] See also Lignier, 1911, p. 3.

[3] Miss Berridge tells me that isolated tracheides occur in the inner integument of *Ephedra*.

[4] Griffith, p. 302; Karsten, p. 200; Berridge, 1911.

[5] Described by Karsten in *G. Rumphianum* and *G. ovalifolium*. Cohn's Beiträge, 1893, p. 377; Thoday, 1910; Berridge, 1911.

Gnetum scandens.

The resemblance between the oldest seeds of *G. africanum* and the mature seed of *G. scandens* is very close in most respects. The chief difference is the fusion of the two outer coverings of the seed to form one, the single covering thus formed having a double vascular supply. Karsten [1] noted the variation in number of the integuments in various species of *Gnetum*, and enumerates three species with their two outer coverings fused (*G. verrucosum*, *G. Rumphianum*, *G. ovalifolium*); the other species he examined had an independent middle covering. It is probable that these three species, like *G. scandens*, would have three separate coverings when young.

The middle covering (outer integument) is entirely composed of lignified cells, and forms a hard, stony layer; the different kinds of tissues, palisade layer, hypodermal strands, fibres, &c., being now only recognizable by the shapes of the cells composing them.

The level of freedom of the inner integument is nearer the apex of the ovule than in the oldest seed of *G. africanum*, and the cavity between the micropylar tube and the nucellus is thus proportionately smaller.

The micropyle is closed, as in *G. africanum*; the solid mass of cells in the closed region is entirely lignified, as is also the greater part of the micropylar tube. There is altogether a great deal more lignification in this species than in *G. africanum*. It is not possible to say whether lignification of the integumentary tissues would ever develop to such an extent in mature ovules of the latter species, but it seems probable that it would not, since the young ovules of *G. scandens* have already more thickened tissues than older ovules of *G. africanum*.

V. Development of the Ovule.

A. The youngest ovules among my material were those of *G. scandens* shown in Fig. 1, Pl. LXXXVI. In these (Fig. 13, Pl. LXXXVII) the micropyle is widely opened and fertilization has not yet taken place. The embryo-sac contains a few nuclei in a peripheral position, and disorganization at the apex to form a pollen chamber has hardly begun. The three coverings arise close together from the base of the seed. The outer covering is already well developed, and is traversed by a network of vascular bundles. The middle and inner coverings are still undifferentiated; more or less definite strands of meristem traverse the middle covering and run into the base of the inner.

[1] Karston: Cohn's Beiträge, 1893, p. 377.

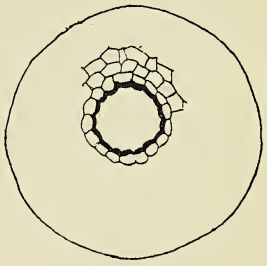
The nucellus is here composed of a very small basal region, from which arise the three coverings and a comparatively very large free apical portion.

The inner series of bundles is hardly yet developed. It is interesting to find that, at this very young stage, the open micropylar tube is circular in transverse section and is lined by cells whose outer walls are already thickly cuticularized [1] (Text-fig. 12).

B. The next stage was seen in the youngest ovules among my material of *G. africanum*. Their micropyles are already either closed or nearly so. The three integuments still arise near together at the base of the ovule. The vascular system, except in the outermost covering, still consists only of strands of cambial tissue, which extend to the tip of the outer integument, and run for some distance into the inner integument. The integumentary nature of the inner series of vascular bundles is clearly seen in the photograph shown in Fig. 14, Pl. LXXXVII.

The tip of the micropylar tube is already withered, and so firmly interlocked by means of the flange with the outer integument, that it is often difficult to make sure that there is no actual continuity of tissue between them.

The lining of the micropylar tube has probably never been so thickly cuticularized as it was in the young ovule of *G. scandens*. In the closed region there is often little or no sign of cuticularization to be seen at this stage, but in the withered tip, and often for a short distance below the closed region, the epidermis has a thinly cuticularized wall.



TEXT-FIG. 12. Transverse section of micropylar tube of young unfertilized ovule of *G. scandens*, showing thick cuticularized lining.

A well-developed pollen chamber is present at the apex of the nucellus [2] (Fig. 14, Pl. LXXXVII). At this stage the embryo-sac is considerably enlarged, and extends from the level of origin of the middle covering to some distance above the origin of the inner integument.

C. The *later stages of development* were mostly seen in abortive ovules (see Text-figs. 13 A, B, C). The greatest change which takes place consists in the gradual stretching and growth of the region between the inner and middle coverings. The basal region develops hardly at all in length, and the apical or free region of the nucellus also develops comparatively very little, but becomes gradually encroached upon by the growing endosperm: thus nearly all the growth in length of the seed

[1] The thickness of the cuticle is here more comparable with that in *Welwitschia* than in other species of *Gnetum*; Sykes, 1910 (2), p. 196, Fig. 10, Pl. XVII.

[2] Lotsy, 1899, p. 94, Fig. 35, Pl. V.

between stage B and the mature ovule takes place in the middle region between the two integuments.[1]

The other changes in the development of the ovule consist in the differentiation of the elements of the vascular-bundle system and of the tissues of the outer integument, the complete closure of the micropylar tube and further withering of the tip, the disintegration of the pollen chamber and hardening of the apex of the nucellus.[2] A diagram of the mature ovule is given on p. 1113.

Text-fig. 14 is drawn from part of a section through an abortive ovule in which these changes were almost complete. The ovule was about 1·5 mm. long, and a good deal of stretching of the region between the two integuments had already taken place. It is possible to distinguish the remains of the pollen chamber.

D. *Details of method of closing micropyle.* The method of closing the micropyle in *G. africanum* is in some respects different from that described recently in *G. Gnemon* by Miss Berridge;[3] in *G. scandens* I only saw very young stages and the mature stage with fully closed micropyle, but it would appear as if in that species the resemblance is closer. The closed micropylar tube of the mature ovule, of which the tissues are strongly lignified, is shown in Fig. 12, Pl. LXXXVI. The epidermal cells lining the canal appear to have elongated and interlocked with those opposite them, and thus to have obliterated the cavity.

In *G. africanum* the actual ingrowth of separate epidermal cells as hairs or papillae plays only a small part in the closure of the micropyle. This is due mainly to the rapid division of the epidermis to form radial rows of cells, the pressure of which rapidly closes the micropyle. The innermost elements of the radial rows occasionally project inwards, and sometimes appear to have therefore got crushed together. As a rule, however, this only happens above the region of the flange—that is to say, where the micropylar tube is clasped only by the outermost covering, and is not so tightly compressed as lower down.

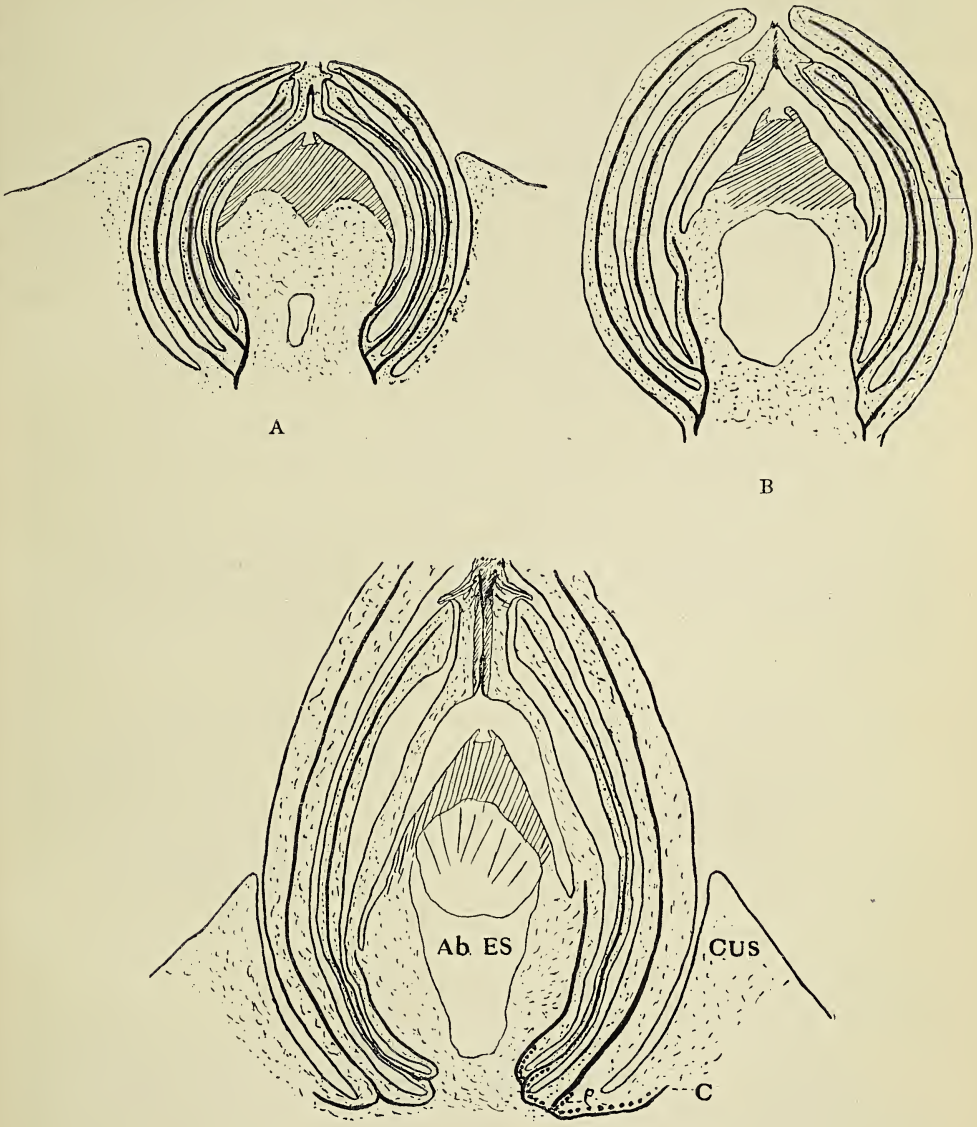
The down-growing flange is the result of rapid cell-division in the outer layer of the micropylar tube; it is fringed with short hairs. It adheres so closely to the outer edge of the outer integument that without close examination the tissues appear to be continuous.

[1] Earlier authors have noticed that the depth of attachment of the inner integument varies in *Gnetum*, but they appear to have regarded the difference as specific and not to have realized that they were dealing with different developmental stages. E. g. Karsten: Cohn's Beiträge, 1893, p. 397. The stretching of the ovule and consequent change in position of the level at which the inner integument becomes free is indicated in Coulter's diagrams (Figs. 2 A, 3 A, 6 A), but not referred to in his text; Coulter, 1908; see also Strasburger, 1879, p. 101.

[2] No sign of the development of a pedicel between the bases of the two outer coverings which is described by Coulter (1908, p. 381, Fig. 6 A) in *G. Gnemon* was seen by Miss Berridge or by me.

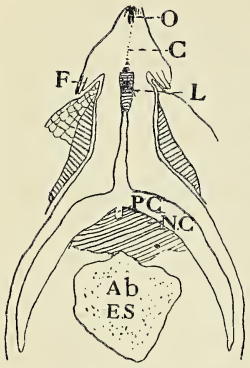
[3] Berridge, 1911. This method of closing the micropyle is analogous to that described in some Conifers, e. g. *Widdringtonia*, figured by Saxton, 1910, Fig. 2, Pl. I.

Text-fig. 15 represents a series of sections through the micropylar tube of an ovule of *G. africanum*, probably preserved soon after fertilization.

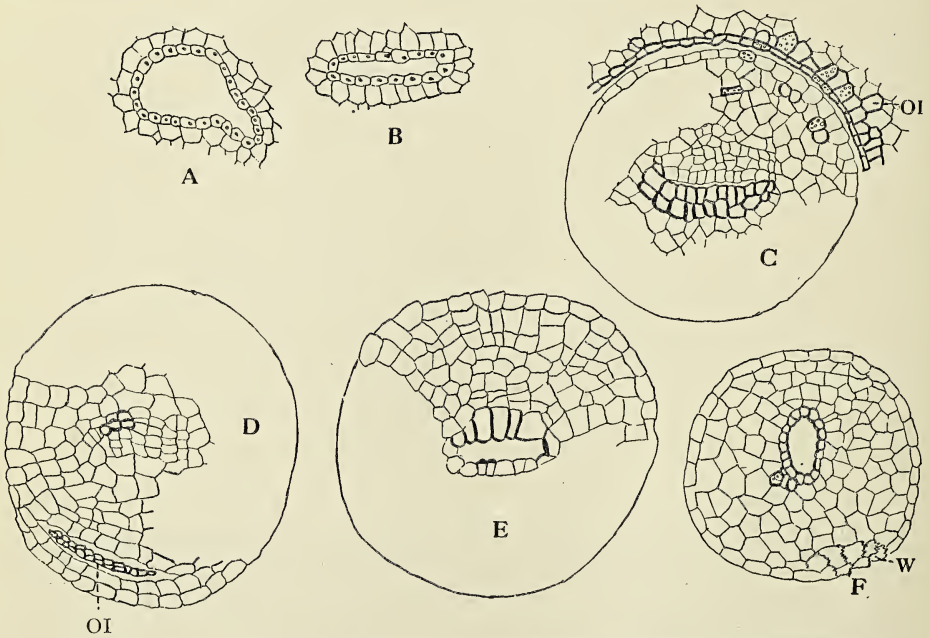


TEXT-FIG. 13. A. Ovule in which the micropylar tube is closed, and the nucellar cap is hardening, but stretching has hardly yet begun. B. The region between the two integuments has undergone considerable extension. C (shown *in situ*) is about the same stage as B, but the free nucellar apex is unusually long and narrow. The closing tissue of the micropyle forms a conspicuous lignified rod. *Ab.ES.* = abortive embryo-sac; CUS = interovular portion of cushion; C = bundle which has bent outwards into the interovular portion of cushion, and is now bending inwards again to enter ovule (see p. 1109); large black dots (*p*) = phloem of bundles.

Above the closed region of the tube there are already signs of withering (W, Text-fig. 15 F); the canal is open and lined by a thin cuticle. Text-fig. 15 E shows the beginning of radial divisions in the outer epidermis to form the flange, and the ingrowth of some of the cells of the inner epidermis. Text-fig. 15 D passes through the closed region and shows the radial rows of cells which have been derived from the inner epidermis. At the lower edge of the figure is seen the free part of the flange and the tip of the outer integument (OI) projecting upwards between it and the central portion of the micropylar tube. Below the flange (Fig. 15 C) the tube is nearly closed, but there is a narrow crevice between the epidermis of the two sides. The micropylar canal lower down is always slit-like (Fig. 16, Pl. LXXXVII). It gradually loses its lining of columnar cuticularized cells and its epidermis becomes thin-walled, made up of cells which are elongated in the tangential direction (Text-fig. 15 B). Just above the nucellar tip it becomes irregular in outline (Text-fig. 15 A). Nowhere is the epidermis torn or decayed, so that there is nothing com-



TEXT-FIG. 14. Longitudinal section through apex of abortive ovule of *G. africanum*, 1·5 mm. long. *Ab.ES.* = part of abortive embryo-sac; NC = nucellar cap; PC = remains of pollen chamber; F = flange. The micropylar canal is open at the withered tip O, closed at C; it is almost closed and lined with a cuticularized epidermis at L, and is open below.



TEXT-FIG. 15, A–F. Series of transverse sections through micropylar tube of fertilized ovule of *G. africanum*, described in text. OI = outer integument.

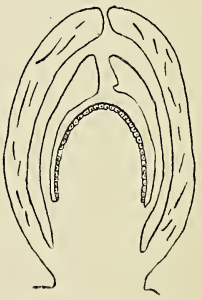
parable with the 'lysigenous' region described in *G. Gnemon* by Miss Berridge.

In the above series of sections it will be seen that some of the cells composing the micropylar tube are already lignified, especially the radial rows of cells derived from the inner epidermis. Lignification of these often begins on one side only, as in Text-fig. 15 C. Fig. 9, Pl. LXXXVI, shows a transverse section through the micropylar tube of an older ovule, in which there is a solid central core of lignified cells surrounded by a zone of regular thin-walled cells. Presumably this solid core was derived from the radial rows of cells closing the micropyle and formed by the division of the epidermis, while the regular zone of cells outside (S.C.) is the sub-epidermal layer.

In none of the ovules of *G. africanum* examined was the whole tube lignified as in the mature ovule of *G. scandens* (Fig. 12, Pl. LXXXVI).

VI. The Abortive Ovules of the Male Inflorescence of G. scandens.

The abortive ovules of the male inflorescence resemble those of other species of *Gnetum*. The nucellus is undifferentiated and composed of thin-walled cells with large nuclei. The outer covering contains many fibres and is obviously homologous with the outer covering in the fertile female flower. The inner covering appears to be generally homologized with the inner integument, the outer integument being absent. I have no evidence one way or the other, except that the general form of the inner covering in the abortive ovule of the male inflorescence is more comparable with the outer than the inner integument of the fertile ovule,[1] as will be seen from the figure.



Text-fig. 16. Abortive ovule from male inflorescence of *G. scandens*.

On the other hand, Karsten[2] states that the middle envelope always appears in a rudimentary condition during the development of the abortive ovules, and Lotsy[3] also makes a similar statement, though in the figure which he gives of the developing ovule the rudiment of the middle envelope is almost negligible.

[1] Cf. *Welwitschia*, Sykes, 1910 (1), pp. 207–8.

[2] Karsten, 1893, (1) and (2).

[3] Lotsy, 1899, Fig. 14, Pl. II.

VII. Comparison of the Female 'Flower' of Gnetum with that of Welwitschia. Primitive Features.

The ovule of *Gnetum*, while in some respects, at any rate, not so primitive as that of *Ephedra*, is certainly more primitive than that of *Welwitschia*. It is not intended now to enter into a comparison with *Ephedra*, as some work on that species is in progress.

The differences between the general form of the ovule in *Gnetum* and *Welwitschia* are probably dependent largely on the different positions of the two. The ovule of *Welwitschia* is closely appressed in the axil of a bract, and it is therefore from the beginning bilaterally compressed. Hence the bilateral symmetry of the outer covering, both external and internal, with its two wings, each traversed by one large group of fibrous cells and one vascular bundle. The inner integument and nucellus still retain the probably more primitive radial symmetry. The ovule of *Gnetum*, which is not laterally compressed, but protected all round by the nodal cushion and its outgrowths, is radially symmetrical, and the fibrous cells in the outer integument form an uninterrupted layer which has an outward projection corresponding to each vascular bundle.

Both *Welwitschia* and *Gnetum* have two integuments; the outer is free, the inner is in the mature ovule fused for about two-thirds of the length of the ovule, while its free apical portion forms the elongated micropylar tube with its characteristically cuticularized lining. There was no material of *Welwitschia* young enough to show whether in the earlier stages, here as in *Gnetum*, the inner integument arises near the outer from the base of the seed. The outer integument in both seeds has a bundle system throughout its length, but the small bundles supplying the inner integument terminate at the base of the free portion in *Welwitschia*, while in *Gnetum* (*africanum*) they are well developed and run for a short distance into the base of the free portion. This greater development of the inner system of *Gnetum*, also the more complex outer integument with its stony layer and the development of a pollen chamber, are all probably primitive features which mark the wide gap between the two genera.

The short free apical portion of the nucellus in *Gnetum* is also in great contrast with the elongated apical region of the nucellus in *Welwitschia*. This latter is probably a specialized development to form a spacious brood chamber for the embryo, and may be regarded as a step towards the Angiosperms.

An attempt has recently[1] been made to show that the *Welwitschia* ovule is built up on the same primitive ground plan as such a seed as *Lagenostoma*. It will be seen that all that has been said applies with still greater force to *Gnetum* the prolongation of the inner system of strands into

[1] Sykes, 1910 (1).

the inner integument proves them to be without doubt integumentary. Further, the complex nature of the outer integument in *Gnetum* makes the comparison there made between the seeds of the Gnetales, the Cycads, and some of the older types more striking,[1] and supports the attempt to refer them all to the same ground plan. The pollen chamber in *Gnetum* is clearly comparable with that described in the Cycads.[2] Other Cycadean features in the *Gnetum* inflorescence are the presence of the three concentric rings of bundles at the nodes, the middle one being inversely orientated to the others, and the occurrence of occasional concentric single bundles.[3] Similar features have been described in the 'peduncles' of *Welwitschia*. The seed characters, however, of the recent Cycads suggest that any connexion between the two families is remote, probably only through common Pteridospermic ancestors.

The seed characters in *Gnetum* also give most striking support to the other comparison drawn, in a former paper, between *Welwitschia* and the Bennettitales.

VIII. Comparison between the Seeds of Gnetum and those of the Bennettitales.

Conflicting and confusing as the accounts of the seeds of the Bennettitales at present necessarily are, the points of resemblance between them and *Gnetum* are even so most remarkable.

(i) It will be well first to recall the *detailed structure of the integument* in *Bennettites Morierei*. There is an outer layer which is expanded at the apex of the seed to form a palisaded tissue, which is considerably thickened ('assise rayonnante').[4] The inner layer of the integument is complex; next to the outer layer is a palisaded layer with thin walls (Lignier's 'assise plissée'), and internal to it are thin-walled cells which are brownish in colour ('tissu charnu') and a distinct fibrous layer. The fibrous layer, composed of elongated cells, extends downwards into the peduncle, and passes inside the vascular bundles, forming the pith of the upper portion of the peduncle; in this region it is composed of much shorter cells with very thick walls. *Bennettites Gibsonianus* would appear to agree with *B. Morierei*, except that the expanded portion of the outer layer is there composed of oblong cells which are not so distinctly palisaded.

Cycadeoidea Wielandii has a much thinner outer integument, also com-

[1] Coulter, 1908, p. 47, compares the seeds of *Gnetum* with those of *Cycas* and *Ginkgo*, since they all have a two-layered integument, the inner layer of which is stony, and both layers have a vascular supply. He regards the differentiation of the inner layer as a separate integument in *Gnetum* as a special development. I should regard it as a primitive feature, reminiscent of the *Lagenostoma* ground plan from which I believe all these seeds to have been derived.

[2] Lang, 1900. [3] *Ante*, pp. 1103, 1119; cf. concentric rings in the stems of Cycads.

[4] According to Lignier this outer layer is composed of 'tubular' cells, but Wieland refers the 'assise tubuleuse' to the interseminal scales, and gives no special description of the outer layer in *Bennettites* except at the apex of the seed.

posed of two layers: the outer, thick-walled and more or less clearly palisaded throughout, shows some sign of expansion at the tip of the ovule (Fig. 4, Pl. XXVIII, Wieland); the inner layer, which is composed of 'string elements', probably corresponds to the fibrous layer in *Bennettites*, and is similarly prolonged downwards into the peduncle.

In *Gnetum* the integument is also made up of two layers. The outer layer contains alternating vertical strands of parenchyma and dark coloured tissue, and is expanded at the apex of the integument, but is not thickened. In *G. africanum* and *G. scandens* there is only an indication of a radial arrangement of the cells at the apex, similar to that occurring in *Bennettites*, but in *G. Gnemon*[1] this is much more marked. The inner layer in *G. africanum* and *G. scandens* consists of a palisaded thin-walled layer, like that recorded in *Bennettites*, and a fibrous or stony layer, also as in *Bennettites* and *Cycadeoidea*: the arrangement of the latter layer appears to be exactly similar to that described in the fossil seeds; it extends downwards into the stalk of the ovule, where it passes inside the vascular bundles and forms the pith of the upper portion of the peduncle, in the integument its cells are elongated and run irregularly in the horizontal direction, while in the peduncle they become short and very thick-walled. The fibrous layer is lignified in the peduncle of the quite young ovule, and in *G. africanum* at the apex of the seed also, where it forms a palisade layer. In the old ovule of *G. africanum* this layer shows signs of lignification throughout: in the mature ovule of *G. scandens* the whole inner integument is lignified.

The distribution of the fibrous layer at the apex of the seeds in *Bennettites Morierei* is another point of resemblance to *G. africanum*.[2] It will be remembered that the former seed has four or five wings, while the latter has not. In both, however, the fibrous layer develops four or five wings which project outwards, and in *B. Morierei* extend into the outer wings. A comparison between Fig. 16, Pl. LXXXVII, of this paper and Lignier's Fig. 6, p. 41, &c., speaks for itself.[3] The wings of fibres in both seeds decrease in prominence lower down, forming small projecting angles which gradually die out, till the layer of fibres forms a smooth zone; cf. Text-figs. 7 C, and 10 of this paper with Lignier's Fig. 7, p. 42, Fig. 45, Pl. IV, &c.

Opposite each of the wings of fibres in *G. africanum* is a vascular bundle; Lignier could not certainly distinguish any vascular bundles in the upper part of the seed of *B. Morierei*, but states that there were suggestions of their presence opposite the outer point of each wing of fibres.

To sum up, the integuments of these three seeds agree in the possession

[1] Berridge, 1911.

[2] In *G. scandens* the star-like distribution of the fibres at the apex of the outer integument was not so striking as in *G. africanum*. In *G. Gnemon*, Miss Berridge tells me that she was also able to make out the star-like distribution, but no lignification had occurred.

[3] In *G. africanum* it is only in the extreme apex that the number of angles is reduced to five; lower down the seed these increase in number.

of an inner fibrous layer which is similarly distributed in the peduncle, main body, and apex of all three seeds, except that in *Gnetum* it forms at the tip of the seed a more definite palisade, which in *G. africanum* is lignified very early. *B. Morierei* and *Gnetum* have a middle palisade layer. In the seeds of the Bennettitales the stony layer tends to be formed from the outer layer of the integument; in *Gnetum* the inner layer shows the earliest signs of lignification.

(ii) The comparison of the *nucellus and inner integument* of the seed of *Gnetum* with the main body of the seed in *Bennettites* involves more difficult questions. In both seeds there is a projecting *micropylar tube*, which in *Gnetum* is derived from the inner integument and projects beyond the outer, but is fastened firmly on to it by means of the downward projecting flange. Lignier's figures of *Bennettites* are suggestive of the same arrangement in that seed, the relation [1] of the micropylar tube to the expanded portion of the integument being identical with that found in *Gnetum*. Lignier attempts to trace continuity between 'the integument' (outer integument?) and micropylar tube; he states, however, that the micropylar tube is homogeneous in structure, sclerified in the fertile seeds, not sclerified in the aborted, and shows no resemblance to the complex structure of the rest of the integument; continuity with the rest of the integument could not be demonstrated. This description would equally apply to *Gnetum*. The difficulty of investigating the apex of the seed may possibly be due to a similar withering of the apex after fertilization, and if it be true that the micropylar tube in *Bennettites* is also fastened by a flange to the top of the outer integument, as in *Gnetum*, it is easy to understand the confusion as to the boundary between the two. The description of the micropylar tube agrees with that of *Gnetum*, the canal being cylindrical below, with an epidermis composed of small thin-walled cells, irregularly slit-like above, with an epidermis which consists of narrow radially elongated cells, and is cuticularized (cf. Figs. 31, 32, Pl. III, Lignier, with Fig. 16, Pl. LXXXVII, of this paper). Opposite the expanded portion of the outer integument the canal is closed as in *Gnetum*.

The micropylar tube of *Cycadeoidea Wielandii* is not clearly comparable with either *Bennettites* or the mature seed of *Gnetum*. It is open, and it may be, as Miss Berridge has suggested, that the seeds figured are at an earlier stage and should be compared with unfertilized ovules of *Gnetum*. A greater difficulty is that the outer thickened layer of the (outer?) integument extends upwards and clothes the micropylar tube. It is, of course, possible that the seed is so young that the inner integument does not yet protrude beyond the outer, but this does not seem likely in view of the differentiation which has already taken place in the integument. It is more

[1] This comparison was made in detail by the author at the Sheffield meeting of the British Association, 1910; and a similar one has also been drawn by Miss Berridge, 1911.

probable that there is in this seed a different relation between the integument and tube.

That the micropylar tube in *Cycadeoidea* also, however, corresponds with the inner integument in *Gnetum*, there seems to me little doubt. Wieland says that 'the interior layer of the micropylar tube is regarded as a continuation of the wall of the nucellus'; that is to say, that it becomes free from the nucellus at a given level, but is fused with it below. It may be that in *Cycadeoidea* the external layers of the micropylar tube were fused with the palisaded outer integument, as in the mature ovules of such species as *G. scandens*. The outlines sketched in Wieland's Fig. 63 (2), p. 122, suggest this. It appears that the palisaded stony layer must have been prolonged upwards in this form, clothing the tube.

Owing to the difficulties in interpreting the material, the account of the *relations of the nucellus with the micropylar tube* and integument in *Bennettites* is very confusing.[1] Lignier describes a small cavity, 'corpuscular mass,' in the apex of the main body of the nucellus and also, above the main body, a large 'lysigenous' pollen chamber, beyond which the nucellus is prolonged upwards as a beak.[1] This beak projects into the micropylar tube. Miss Berridge has suggested that Lignier's 'corpuscular mass' is the true pollen chamber, agreeing in position with the pollen chamber of *Gnetum*. On the other hand, she regards the 'nucellar beak' as probably homologous with the secondary closing tissue which fills up the micropylar tube in *Gnetum*, and which has here become separated from the tube by an accident of preservation; and she regards the space between the beak and main body of the nucellus, not as a pollen chamber, but as comparable with the large space between the micropylar tube (or inner integument) and nucellus in *Gnetum*, which occurs also in Wieland's *Cycadeoidea* where no nucellar beak is figured.[2] In *G. Gnemon* the lower part of the micropylar tube below the closed portion is lined by a ragged torn layer of cells, and is very suggestive of Lignier's 'pollen chamber'; in *G. africanum* the thin-walled epidermis is still intact, but it is easy to see how, if not well preserved, it would become torn and ragged.

[1] Wieland does not describe any such complex structure in *Cycadeoidea*.

[2] Here the micropylar tube is open and the ovule is probably young, so that if the nucellar beak corresponds with closing tissue its presence would not be expected. Another point of difference between Wieland and Lignier's account supports Miss Berridge's theory. Lignier's Fig. 35, Pl. II, passes through the base of the so-called 'mucron nucellaire' and the 'pollen chamber'. It is clearly surrounded only by the (outer) integument, and there is nothing at this level representing the micropylar tube, which, according to him, has fused on to the (outer) integument. But Wieland describes the continuity of the inner layers of the micropylar tube and the nucellus. If Wieland's account is true this hollow 'beak', which is fused below with the nucellus, must represent the base of the micropylar tube below the closed portion. It corresponds exactly with Miss Berridge's figure of *G. Gnemon* at this level, and even its small transverse section, so striking in contrast with Figs. 32 and 31 of the upper portion of the micropylar tube, is another suggestive point, since the latter sections would pass through the flange and the former would be taken from the lower, narrower portion of the tube.

The closed upper portion of the micropylar tube in *Gnetum* is similar to the part of the tube which Lignier describes as closed in *Bennettites*, but it is not at first sight easy to see how below this the nucellar beak, which, it is suggested, represents the closing tissue, could have become so sharply separated off as an individual organ, as it is in Lignier's figures, apparently surrounded by a distinct epidermis of its own. Miss Berridge drew my attention to a point in some of my series of slides of *G. africanum* which affords a possible explanation of this difficulty. In Fig. 9, Pl. LXXXVI, a transverse section through the lower part of the closed region of the tube, the closing tissue derived from the original epidermis of the tube forms a solid cylinder of lignified cells which is separated off from the rest of the tube by the still intact and regular subepidermal layer. It would only be necessary for this delicate layer to be split off from the central rod in order to get the effect shown in Lignier's Fig. 32, Pl. III, a solid rod enclosed by the hollow micropylar tube.

In the absence of more perfectly preserved material of *B. Morierei*, we cannot regard this theory of the nucellar beak as proved, but in view on the one hand of the other resemblances between the seed and that of *Gnetum*, and on the other of Wieland's account of the young *Cycadeoidea* without a nucellar beak, it must be regarded, at any rate, as a brilliant hypothesis which has every appearance of probability.

(iii) The *structures surrounding and protecting the ovule* in *Gnetum* and *Bennettites* may be compared. It will be seen that while the ovule in the latter is enclosed at the base by the 'coque' or basal husk, this extends upwards only for a short distance, and it is the interseminal scales, with which the whole is surrounded, that give the ovule efficient protection. On the other hand, the interovular portions of the nodal cushion in *Gnetum*, which completely protect the young ovule, and in transverse sections of the axis remind one forcibly of the interseminal scales in *Bennettites*, do not continue to enlarge with its growth; and it is the extra outer covering, comparable in position with the basal husk in *Bennettites*, which is the efficient protective organ of the mature ovule. It is not necessarily intended here to homologize the basal husk with the extra outer covering or the interseminal scales[1] with the projecting portions of the cushion, but physiologically the comparison between them has some force.

The long stalk of *G. scandens* is suggestive of the long pedicel of the *Bennettites* ovule. Coulter[2] states that there is a short pedicel between the 'perianth' and outer integument in *G. Gnemon*, but neither Miss Berridge nor I have seen any trace of it.

[1] The interseminal scales have already been compared morphologically with the bracts in *Welwitschia*; Sykes, 1910 (1).

[2] Coulter, 1908, p. 381.

IX. Other Resemblances between Gnetum and the Bennettitales.

The method of vascular supply of the peduncle of the whole cone of *Cycadeoidea Wielandii*, and of the single *Gnetum* flower is very similar, as may be seen when Text-fig. 3, p. 1107, is compared with Wieland's Fig. 59, p. 113. The two figures are both drawn from radial sections, and two sets of bundles are seen running out into the axillary stalk. Wieland says that the lower bundles, which are seen from transverse sections to be orientated similarly to the main bundles, are 'either directly connected with, or envelop the supply of the leaf-base';[1] he calls them 'the axillary portion of the leaf bundles'. The upper series is orientated inversely to the other two and is chiefly derived from the main bundles. This description is strikingly like that of *Gnetum* given on pp. 1106 ff. of this paper. Transverse sections show that the bundle supply of each peduncle[2] is arranged in a ring, the outer members of the ring orientated like the main bundles, the inner inversely orientated. In *Gnetum* the outer series passes out more rapidly, and the whole of the ring supplying any given ovule cannot be cut in transverse sections of the stem, but only in tangential.

X. Summary.

1. The vascular system of the female inflorescences and flowers of *G. africanum* and *G. scandens*, and of the male inflorescence of *G. scandens* is described. The most interesting points are the presence of *three concentric rings* of bundles in the nodes, the middle one of which is *orientated inversely* to the other two. The method of vascular supply to the single female flower in *G. africanum* bears a remarkably close resemblance to the method of supply to the axillary inflorescence in *Bennettites*.

2. Concentric bundles occur fairly frequently in the two outer rings of bundles and are sometimes continuous throughout the internode.

3. Each ovule receives a ring of bundles; these traverse the stalk and branch at the base of the ovule to form three series; the two outer series traverse the outer and middle coverings to their tips, the inner series run up to the level of freedom of the inner covering and sometimes for a little distance into its base. In the young ovule *strands of meristem* run a considerable way up *into the free portion of the inner integument*.

4. Throughout the short ovular stalk of *G. africanum* and in the upper part of the long stalk of *G. scandens*, the centre of the pith is lignified. At the base of the ovule the lignification spreads outwards, while parenchyma appears in the centre of the pith. The layer of lignified tissue then passes outwards between the bundles into the region separating the bases of the two integuments, and is higher up continuous

[1] Wieland, p. 113.

[2] Ibid., pp. 69–71.

with the fibrous layer of the outer integument (middle covering). Thus, at the base of the ovule the fibrous layer forms a sort of cup just as it does in *Bennettites*.

5. The outer integument (middle covering) is complex in structure: in *G. africanum* it remains independent, but in *G. scandens* it becomes fused with the outermost covering. In *G. africanum* it is composed of an outer zone which is made up of alternating strands of parenchyma, each containing a vascular bundle, and indurated tissue; and an inner zone which becomes the stony layer of the seed. The inner zone contains a palisade layer and a fibrous layer. The fibrous layer is angled in the upper part of the seed, there being an angle opposite each vascular bundle. These angles, along with the number of vascular bundles, are gradually reduced in number, till at the tip of the integument there are only four or five. Here the integument clasps the micropylar tube, and the five-angled fibrous zone forms a sort of star, of which all the cells are lignified at a very early stage. A similar arrangement of the fibrous layer and a similar palisade layer is found in *Bennettites Morierei*.

6. A well-developed pollen chamber is present in the young ovule; as it decays the apex of the nucellus hardens and forms a pointed cap of lignified tissue.

7. In the young ovule the three coverings arise close together at the base of the nucellus, and the free apical portion of the nucellus is by far the largest part of the ovule. As the seed grows the region between the two integuments becomes much stretched and forms the greater portion of the mature ovule, so that the innermost covering becomes free about two-thirds of the way up the nucellus. The free portion of the nucellus in the mature seed is very small in proportion to the part developed by intercalary growth. The embryo-sac develops between the levels of freedom of the two inner coverings; it is as it expands that the intervening region stretches. In the mature seed it projects into the free portion of the nucellus.

8. The open micropylar tube of the youngest ovules (*G. scandens*) is circular in section, and its lining is strongly cuticularized. After fertilization the tube is closed for some distance by means of a tissue formed by division of the epidermal cells, and a 'flange' is also produced which fits tightly over the outer integument. In the older ovule and seeds the micropylar canal, where it is still open, is slit-like. The tissues of the tube become gradually lignified; the closing tissue becomes lignified first and forms a solid thick-walled central rod. The micropylar tube is remarkably similar to that of *Bennettites Morierei*, and this central rod is compared with the 'nucellar beak' in that seed.

9. The radial structure of the seed, the short free apical portion of the nucellus, the presence of a pollen chamber, the extension of the bundle system into the free portion of the inner integument, the complex structure

of the outer integument are all points of contrast with *Welwitschia* and probably indicate the more primitive nature of the *Gnetum* ovule.

10. The attempt to refer the seed of *Welwitschia* to the same ground plan as that of such Pteridosperms as *Lagenostoma* and such recent seeds as the Cycads is thus supported by the study of *Gnetum*. Between *Gnetum* and *Bennettites* there are still more obvious resemblances. None of these forms are of course regarded as directly derived from one another, but the seed characters render it probable that they derive their origin from common ancestors.

My thanks are due to Mr. Mangham for the five photographs forming Pl. LXXXVII illustrating this paper.

Botany School, Cambridge,
July 28, 1911.

ADDITIONAL NOTE.

Since the above was written two publications have appeared which bear on the subjects discussed, and which require some notice here.

I. Wieland's most recent paper on the American Fossil Cycads, 'Further Notes on Seed Structures,'[1] is chiefly concerned with the minute structure of their integuments. His account of *Bennettites Morierei*, especially his description of the base of the seed, is full of resemblances to *G. africanum*. But he seems quite convinced that the middle or stony layer of the seed, at any rate in *Cycadeoidea*, is continuous with the micropylar tube (see especially Fig. 5, p. 144), which is therefore not the prolongation of the inner flesh only, as it should be were it to be closely homologized with the inner integument in *Gnetum*. On the other hand, he gives a diagram (Fig. 3 C, p. 141) showing the micropylar tube clothed with the stony palisaded layer, but made up mainly of thin-walled tissue. The latter may be chiefly closing tissue, but must consist partly of prolonged inner flesh; he does not discuss the question fully. There appears to me nothing incredible in the suggestion (p. 1128 of the present paper) that the micropylar tube, so delicate in most species, should in *Cycadeoidea* have become protected by a firm hard layer derived from the outgrowth of some of the tissues of the outer integument.

Whether or no the attempts at homologies between Gnetalean and Bennettitalean seeds made in the present paper prove of any ultimate value, the remarkable resemblances in the detailed structure of the integuments and micropylar tubes are too great to be lost sight of.

II. Schuster's paper[2] on 'Weltrichia und die Bennettitales' contains

[1] The American Journal of Science, xxxii, 1911.

[2] Kungl. Svenska Vetenskapsakademiens Handlingar, Band 46, No. 11. Stockholm and Upsala, 1911.

a hypothesis of the origin of the Bennettitalean 'flower' which is given in so graphic a manner and is so fully in accordance with the forthcoming evidence that it carries conviction with it. That the interseminal scale finds its progenitor in the fern sporophyll is altogether probable, and is of course a belief which is commonly held, but it is harder to follow the writer in his derivation of the angiospermous carpel from the actual interseminal scale now found in the Bennettitales. The carpel of such Angiosperms as the Ranalean series seems more naturally related to the sporophyll of Schuster's 'Urtypus' of *Bennettites*, which is almost a Pteridophyte, than to the specialized scale of the known types. In view of the growing belief in the polyphyletic origin of the Angiosperms it may well be that in other forms without marginal ovules the carpel found its origin in a more specialized organ; possibly resembling more nearly the 'bract' in *Welwitschia*, as has been already suggested in a previous paper.

Oct. 10, 1911.

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DESCRIPTION OF PLATES LXXXVI AND LXXXVII.

Illustrating Mrs. Thoday's paper on the Female Inflorescence and Ovules of *Gnetum*.

PLATE LXXXVI.

Fig. 1. Single node of inflorescence of *Gnetum scandens*, showing young ovules with projecting micropyles borne on a cushion (*Cus.*) in the axil of the cupule of bracts (*Cup.*). *In.* = internode. In the right-hand figure are shown the gaps in the cushion from which two of the sunken ovules have been removed. (Rather larger than natural size.)

Fig. 1 A. Single mature ovule of *G. scandens* on stalk. (Natural size.)

Fig. 2. Lower portion of inflorescence of *G. africanum*, borne on naked peduncle. At the base of the inflorescence are two bracts in the axil of which was a cushion, but no buds or ovules. *In.* = the very short internode; B.C. = the cupule of bracts; C = large swollen axillary nodal cushion; *A.Ov.* = abortive ovules; *F.Ov.* = fertile ovules. (Natural size.)

Fig. 3 A. Diagram of transverse section through lowest part of node, showing free edge of cupule, and base of axillary cushion covered with hairs. Shaded patches on cupule (*Cup.*) represent corky tissue; C = hairs; shaded lines on inner edge of cupule = mucilaginous layer of cells. × 6.

Fig. 3 B. Diagram of transverse section through middle of node. The two inner series of bundles are seen in pith of main stem; the outer series has just passed out into the ovules. A.O. = abortive ovule with free integuments arising from base; C = peltate projecting portion of cushion; F.O. = fertile ovule; P = outer covering; O.I. = outer integument; C.M. = closed micropylar tube; N.C. = nucellar cap; E = embryo-sac. × 6.

Fig. 4. Single bundle from peduncle, showing space in pericycle (*Pcy.Sp.*) crossed by trabeculae; small space on inner edge of bundle (*Sp.*). V.B. = vascular bundle; F = fibre. × 400.

Fig. 5. Section through portion of bract cupule. M = mucilaginous layer under upper epidermis; *Tr.* = transfusion tissue at sides of bundles; F = fibres. × 78.

Fig. 6. Part of transverse section through base of ovule, just below level of freedom of outer integument (O.I.). N + *I.i.* = nucellus and inner integument; *l.f.* = lignified fibres, here marking the line of separation of outer integument and becoming later the inner zone of the outer integument; *p* = palisade layer; *v.b.* = vascular bundle; to the left of the bundle is a hypodermal strand of tissue with dark-coloured cell-walls. × 78.

Fig. 7. Longitudinal section through apex of expanded portion of outer integument. F = parenchymatous tip; L = lignified series of cells at the termination of fibrous layer; *a.r.* = outer

parenchymatous zone showing arrangement into radial rows (cf. Lignier's 'assise rayonnante'). × 116.

Fig. 8. Longitudinal section through apex of ovule, showing nucellar cap and 'tent-pole' of endosperm. T.P. = tent-pole; N = remains of nucellar tissue; T = tapetum; *I.i.* = inner integument. × 78.

Fig. 9. Transverse section through micropylar tube at the base of the closed region; a minute slit is just reappearing. The ovule is rather older than the one figured in the text (Text-fig. 9, p. 1113); the almost solid central core of cuticularized cells is probably due to the division and subsequent cuticularization of the epidermal cells originally forming the lining of the tube; the beginning of the cuticularization is seen in Fig. 15, Pl. LXXXVII. The more or less definite layer surrounding the solid core represents what was originally the subepidermal layer (*s.e.*). × 336.

Fig. 10. Longitudinal section passing through base of inner and outer integuments at level of origin from nucellus. I = inner, M = outer integument; *Tr.* = tracheides at termination of a bundle running into the base of the inner integument. × 116.

Fig. 11. Part of transverse section through mature ovule near its base. E = endosperm; N = remains of nucellus still fused with the inner integument; O.I. = outer integument; P = outermost covering; *St.* = branched fibres; V.B. = vascular bundle; D = hypodermal strand of brownish tissue; P = palisade layer; F = fibrous zone. × 116.

G. scandens.

Fig. 12. Transverse section through closed micropylar tube of mature seed just below flange (Fig. 1 A). All the cell-walls are lignified. The epidermal cells are elongated and now fit tightly together. × 400.

PLATE LXXXVII.

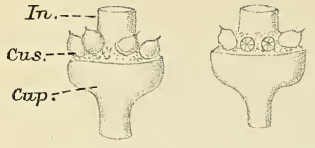
Fig. 13. Longitudinal section through young ovule of *G. scandens*. Shows open micropylar tube with cuticularized lining.

Fig. 14. Longitudinal section through young fertilized ovule of *G. africanum* in situ. Shows pollen chamber, and prolongation of strands of meristem into inner integument.

Fig. 15. Longitudinal section through apex of older ovule (abortive). Shows closed micropylar tube, flange, beginning of sclerified nucellar cap, lignification of inner fibrous palisade in outer integument.

Fig. 16. Transverse section through apex of outer integument of old fertilized ovule. Shows star of lignified tissue in outer integument, and slit-like micropyle.

Fig. 17. Transverse section through node of axis of male inflorescence of *G. scandens*, showing anastomosis of bundles.



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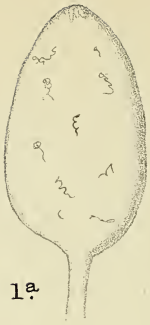

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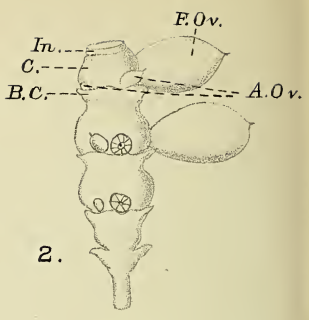

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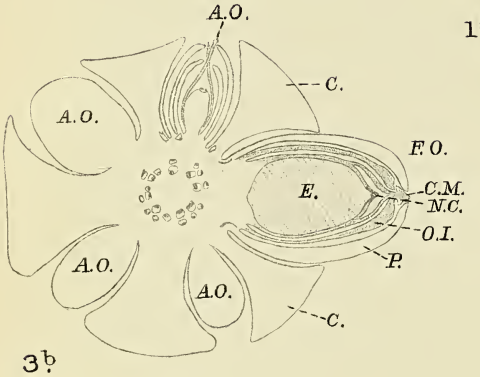

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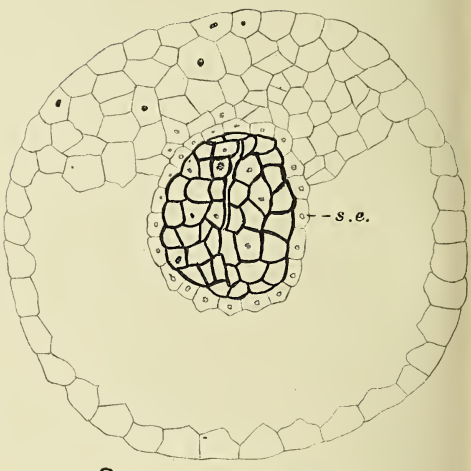

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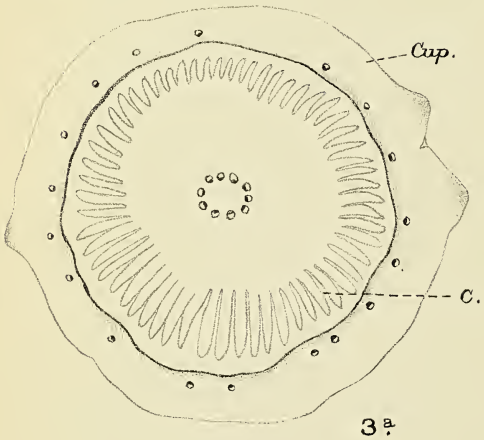

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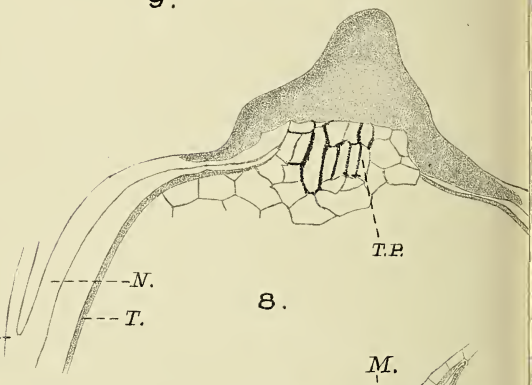

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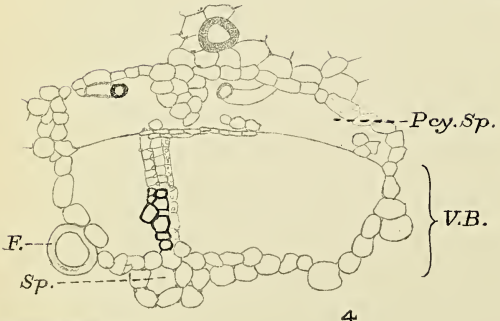

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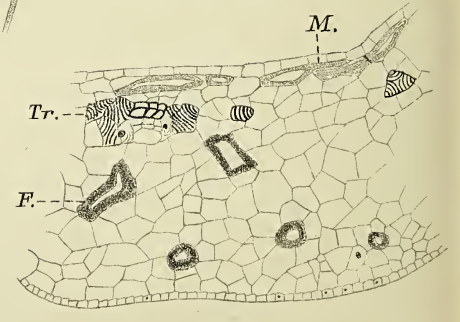

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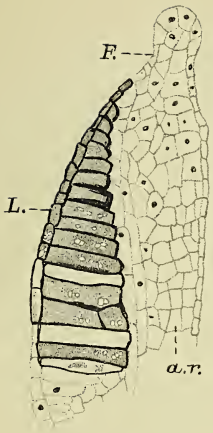
THODAY (SYKES) — GNETUM.



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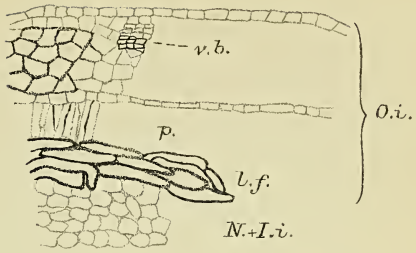

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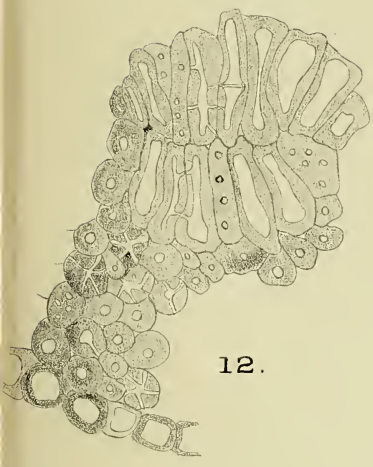

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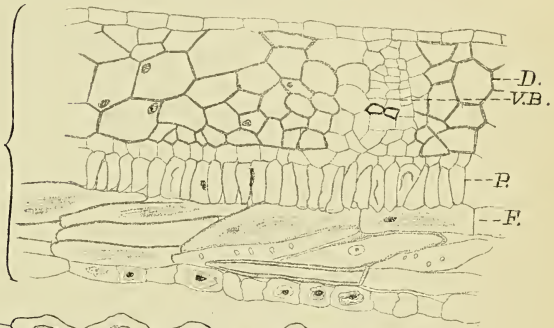

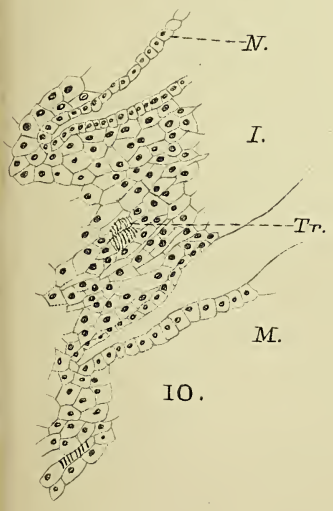

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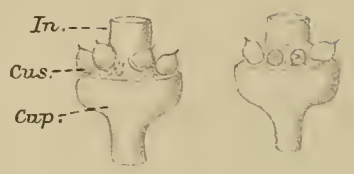

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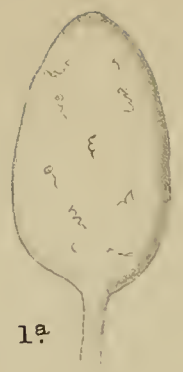
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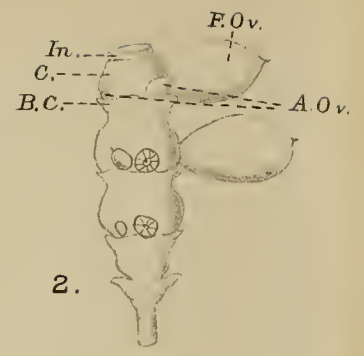

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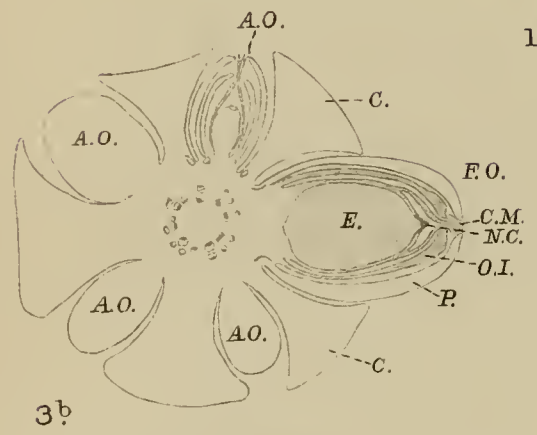

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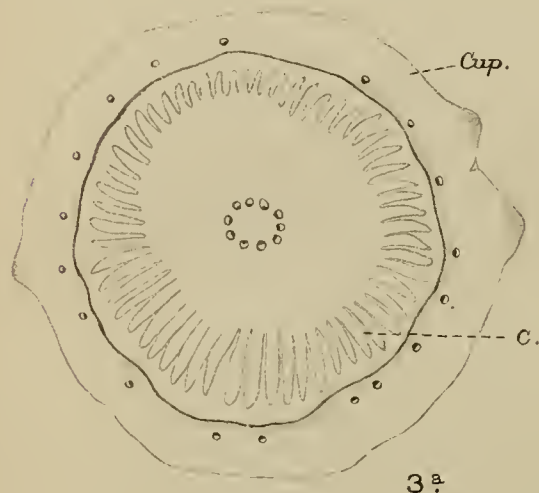

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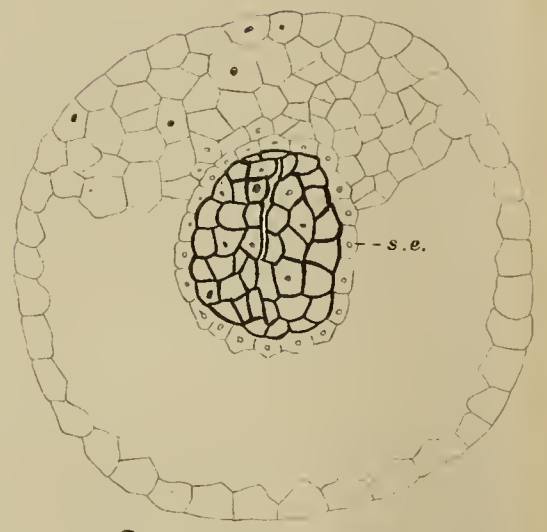

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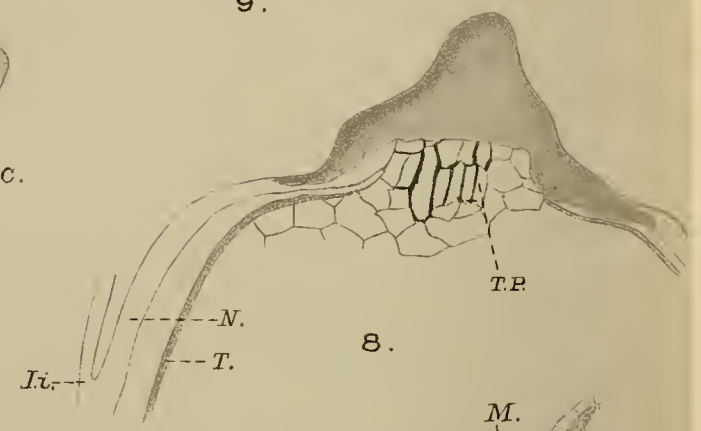

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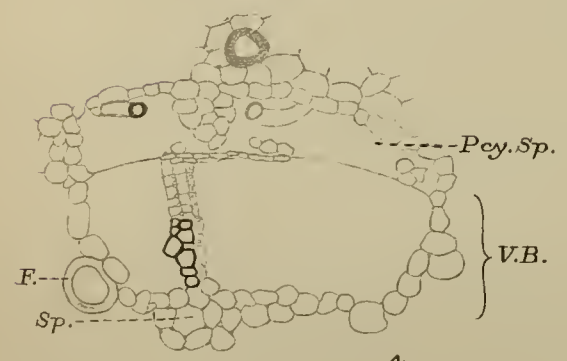

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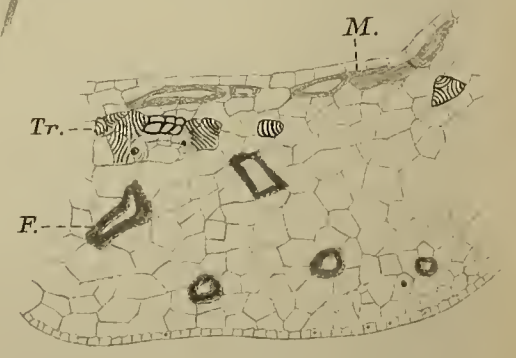

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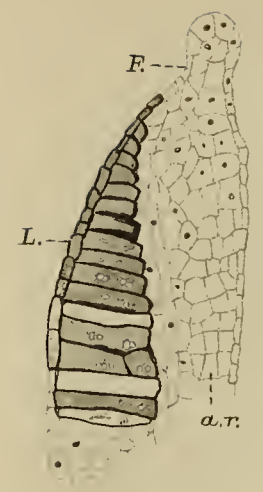

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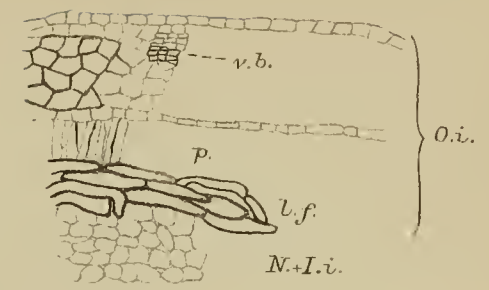

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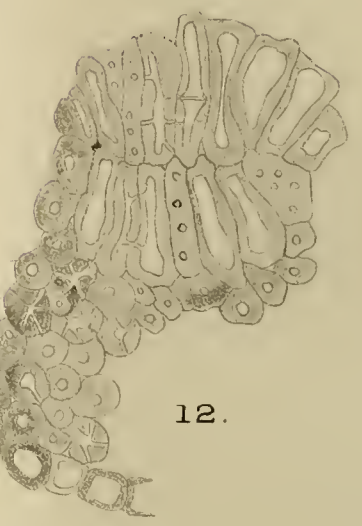
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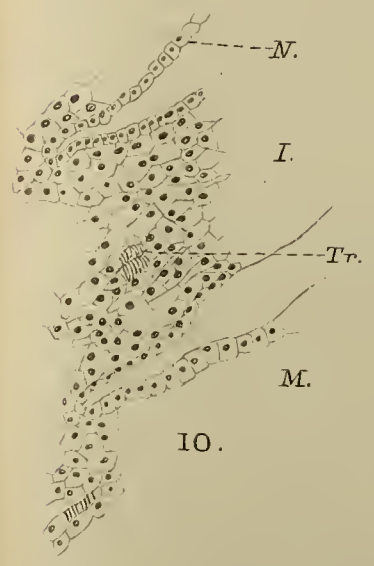

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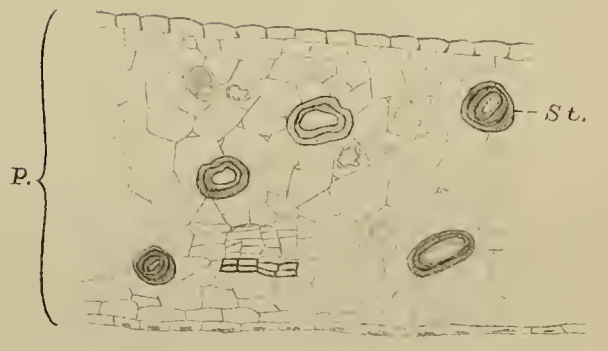
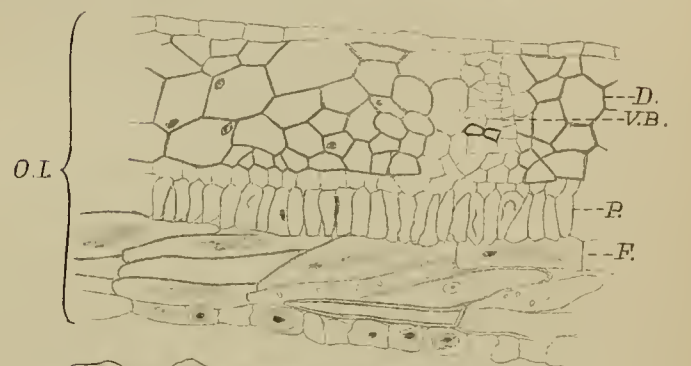


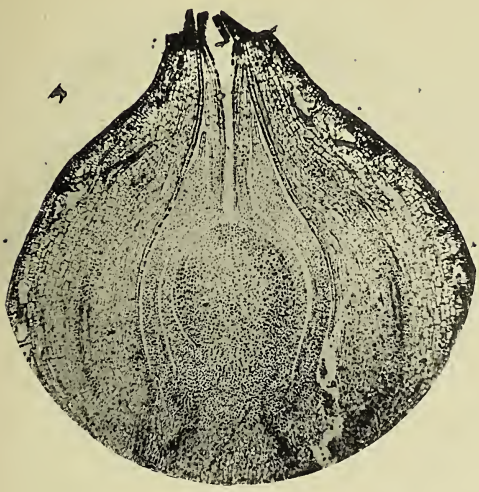


11.

M.G. Thoday (Sykes) del.

Huth, lith et imp.

THODAY (SYKES) — GNETUM.



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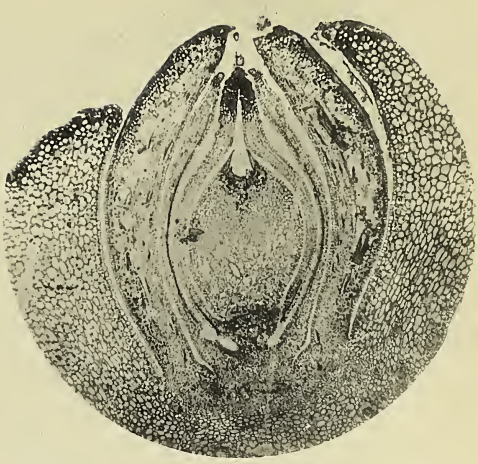

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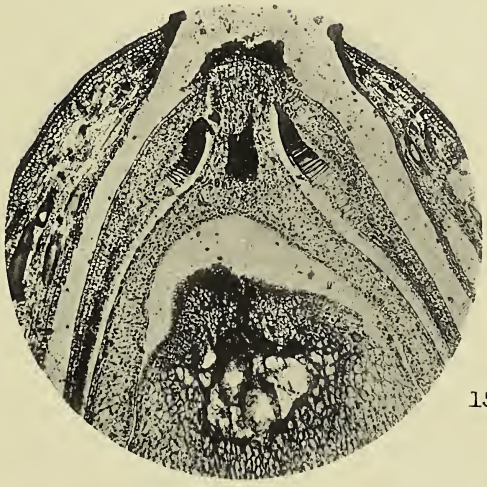

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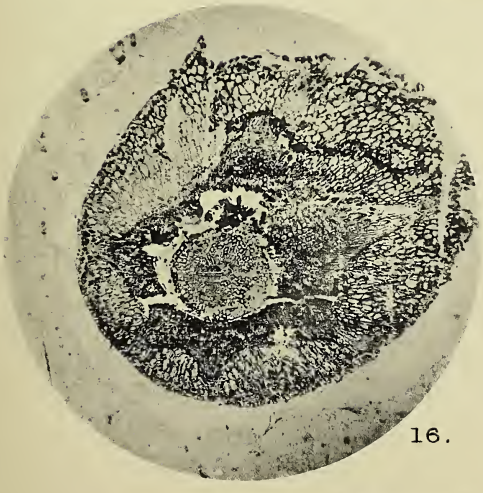

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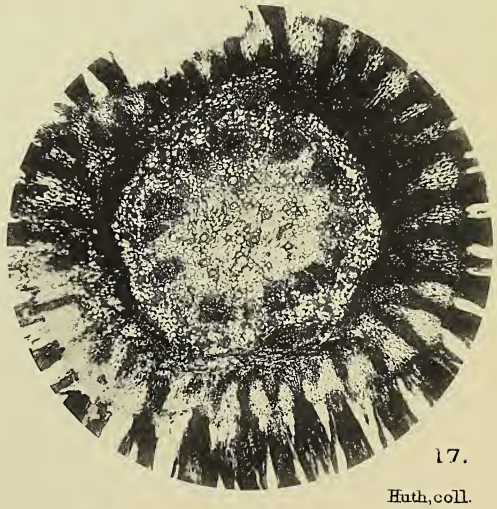

17.

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THODAY (SYKES) — GNETUM.

On the Occurrence of Lens-cells in the Epidermis of Mesembryanthemum pseudotruncatellum.

BY

F. SUMMERS, M.Sc.

Hartley Botanical Laboratories, University of Liverpool.

With ten Figures in the Text.

SOME of the means of protection against excessive insolation, possessed by certain species of *Mesembryanthemum* belonging to the section Sphaeroidea, have already been drawn attention to by R. Marloth (7).

Among the species described by this author were *M. opticum*, Marloth, *M. truncatellum*, Haworth, and *M. Hookeri*, Berger. Further, Marloth has instanced a case of so-called mimicry on the part of *M. calcareum*, Marloth, the aerial portion of which bears a striking resemblance to the limestone pebbles and fragments of the Karroo (8).

For *M. truncatum*, Thiselton-Dyer pointed out that the plant has not only assumed the spheroidal form and the accompanying minimal transpiring surface, but, in order to escape the attentions of animals, it has imitated the form of a water-worn pebble (11).

To the aerial portion of *M. opticum*, Marloth has applied the descriptive term 'corpusculum', which applies also to the other two species mentioned above.

Each 'corpusculum' is from spheroidal to conical in form, and is composed of two similarly shaped leaves which in outward appearance in no wise resemble these organs. They are thick and fleshy, and grow together as far as their upper surfaces, where they are separated by a slit of greater or lesser extent. In the middle arise the two leaves of the next year's shoot. Through the growth of this young leaf-pair the two older leaves are forced apart. They decline with the vegetative season, finally remaining as wrinkled withered sheaths clothing the base of the succeeding corpusculum (Figs. 1 and 2). The upper surface of a corpusculum is more or less convex, and varies in appearance with the different species. It may be smooth, furrowed, wrinkled, or warty; or, as in the case of *M. truncatum*, figured by Thiselton-Dyer (11), it may possess a marbled and mottled appearance.

[Annals of Botany, Vol. XXV. No. C. October, 1911.]

Several other species are figured by Berger in his handbook on 'Mesembrianthemen und Portulaceen' (2).

The assimilation tissue is situated under the epidermis of the side of the corpusculum and forms a hollow cylinder. There is none of this tissue under the upper epidermis.

The above-mentioned species have been placed by Marloth in a class of plants which he describes as being provided with window-leaves ('Fenster-Blätter'). According to him the leaves remain for the greater part buried in the ground, so that only the upper surfaces of these are visible. As under the upper epidermis water-storage tissue is present, while chlorophyll-containing tissue is completely wanting, the light, after entering through the cells of the epidermis, is dispersed and reaches the assimilatory tissue at the sides in a diffuse condition. 'Jedes Blatt hat also ein Fenster, durch welches es sein Licht erhält' (7).

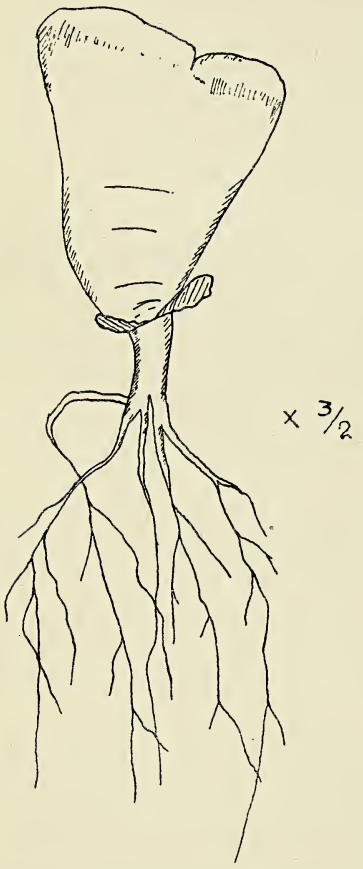

FIG. 1. *Mesembryanthemum pseudo-truncatellum*: two-year-old plant.

In *M. truncatellum*, Haworth, Marloth found that the brown lines of the upper surface were due to cystolith-like structures under the epidermis. These were filled with a strongly refractive substance containing much tannin, which assisted effectually in dispersing the light entering from above.

I have lately had the opportunity of examining some of these peculiar *Mesembryanthemum* species through the kindness of Professor Schinz, of the University of Zürich, who placed at my disposal material collected in Cape Colony and preserved in alcohol. In the case of *M. pseudotruncatellum*, Berger, I received in addition a quantity of living material raised from seed in the culture-houses of the Zürich Botanical Gardens.

A two-year-old plant of this species is usually 2·5–3 cm. in height; in shape it is an inverted truncated cone 2–2·5 cm. in diameter at the upper end. Here, also, the two leaves grow completely together as far as the upper surface, where they are separated by a deep slit (Fig. 1).

In a median longitudinal section perpendicular to the vertical plane containing this slit the following features are seen (Fig. 2). The two older leaves surround a fleshy column composed of the two next youngest leaves, which are at this time almost completely united. The dividing slit is at right angles to the older one, the leaves being therefore decussate. The two older leaves form a cap, which fits closely over the young shoot, the slit forming an aperture in the roof (Fig. 2).

In both the older and younger leaves occur the cystolith structures described by Marloth and referred to above. In *M. pseudotruncatellum* these are almost spherical, and occur at intervals as a single layer and separated from the upper epidermis by two layers of hypoderm. From one another they are separated by the simply perforated cells of the water-storage parenchyma. When young their walls are thin and the tannin content poor. Later, an increase of thickness is seen in the walls, while the tannin content becomes considerable, so that an opaque sphere results.

The assimilatory tissue, which is shaded in Fig. 2, forms a band under and concentric with the side epidermis. It dies out near the upper surface, where the tannin-containing structures appear. In this band the two outer cell layers contain chlorophyll only, but the next eighteen or twenty layers are crowded with both simple and compound starch grains.

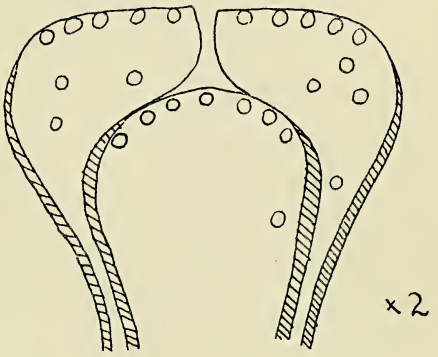

FIG. 2. Longitudinal section through the 'corpusculum'.

The whole of the exterior is strongly encrusted with calcium oxalate, the outer walls of the epidermis being impregnated with it. It is often excreted in considerable quantity on the upper surface, where it is perceptible as white streaks. Over the assimilatory tissue the quantity of calcium oxalate is considerably less. It is easily removable with the help of dilute hydrochloric acid. The stomata are sunken and the depression is free from encrustation.

The most characteristic feature is exhibited by the epidermis itself. Here two extreme types of cell are present, together with transitional forms between them. In section all were found to possess the form of lens-cells, and the possession of the lens function was afterwards confirmed as described below.

The cells of the upper epidermis are in surface view regularly hexagonal, the side walls being strongly cutinized. The basal walls are flat, thin, and consist of pure cellulose, while the upper walls are slightly cutinized. On

their edges the side walls are wedge-like, and as the angles of the wedges are 120°, where three walls meet they fit exactly.

From angle to angle the diameter of an average cell is 72 μ, the side walls being 42 μ in height (Fig. 4).

In the middle the upper wall is slightly raised to form a papilla-like projection, the height here being 45 μ. The wall here is thinner but more strongly cutinized, so that after treatment with reagents a circular area is to be made out in surface view. Over this the deposit of calcium oxalate is almost absent, but towards the margin of the cell it increases in thickness. Over the middle of each side wall very little is deposited, however, so that there is a central clear area, with a surrounding almost opaque area, which

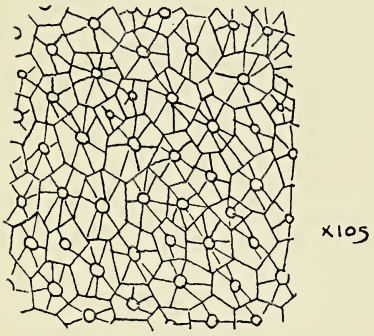

Fig. 3. Cells from the upper epidermis in surface view.

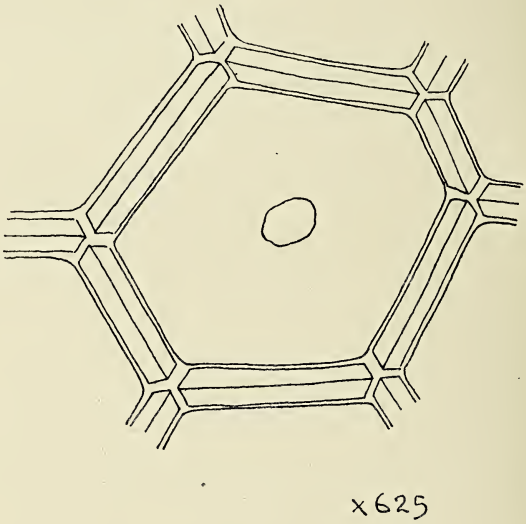

Fig. 4. Cell from upper epidermis after removal of encrustation.

is in turn bounded by a six-sided clear polygon. Darker radial lines connect the two brighter portions (Figs. 3 and 4).

It has already been mentioned that the cells of the side epidermis over the assimilatory tissue possess much less calcium oxalate than those just described. A difference in shape also characterizes them (Fig. 5). The regularity in surface view of the first-mentioned cells is absent in the latter.

In longitudinal section the side walls are seen to be thinner, while in the majority of cases the wedge-joints are absent. The basal wall is thinner and concave. From this basal wall the cell rises as a papilla-shaped structure, the average diameter being 25·5 μ, while the average height is 32 μ. The upper wall is pushed out slightly and a circular central refractive area is developed (Fig. 7).

The epidermis is easily removable from all parts of the plant. A moist chamber was constructed according to the instructions of Haberlandt, and the epidermis was examined by means of the 'Linsenversuch' of this author.

As an object, a cross made of black card was used. Before treating with HCl the cells in all cases formed faint images of the cross a very small distance beyond the basal wall. In the living condition this wall is lined by a thin layer of protoplasm, the nucleus occupying a central position in it. This is, therefore, fully illuminated, a darker area surrounding it.

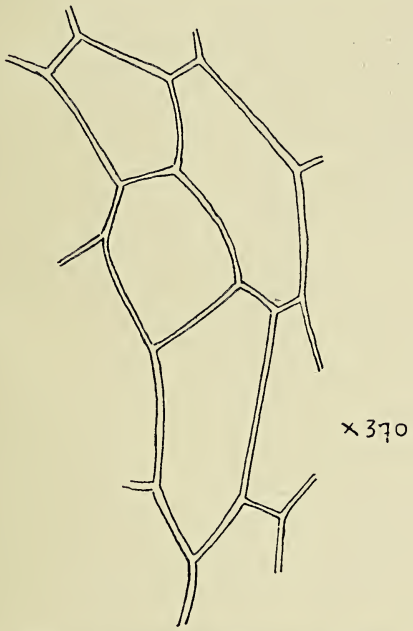

FIG. 5. Cells from epidermis of sides.

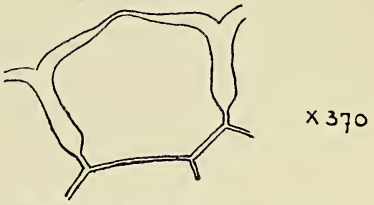

FIG. 6. Cell from upper epidermis in longitudinal section.

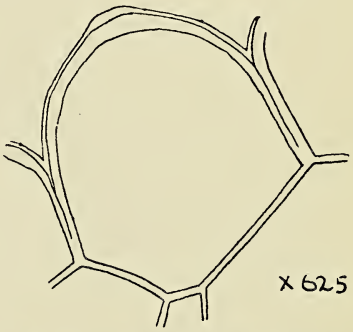

FIG. 7. Cell from side epidermis in longitudinal section.

After treatment with HCl brilliant and sharp images were obtained, while the whole of the cell floor became illuminated.

In a similar experiment with the epidermis of the sides it was found that the image formed before removal of the calcium oxalate encrustation, although still faint and blurred, was very much better developed than in the case of the cells of the upper epidermis. The conclusion is, therefore, that in structure the epidermal cells are all well-developed lens-cells and capable of forming as definite images as those described by Haberlandt, v. Guttenberg, and other authors.

Further, owing to their structure, the cells of the side epidermis are much more efficient lens-cells than those of the upper epidermis. In both

cases there is a tendency for the lens function to be shut out by an encrustation, but this is the case to a greater extent with the cells of the upper epidermis. A blurred image, therefore, results.

The two inner and younger leaves also are provided with the encrustation, but not to such a large extent as is the case with the older ones. Consequently, before removal of the calcium oxalate, the lens function is more efficient than in the cells of the upper epidermis of the older leaves.

In studying the plants raised from seed an examination was first made of seedlings aged three and ten days respectively. In none of these were lens-cells developed, there being no sign of papilla-like structures. The

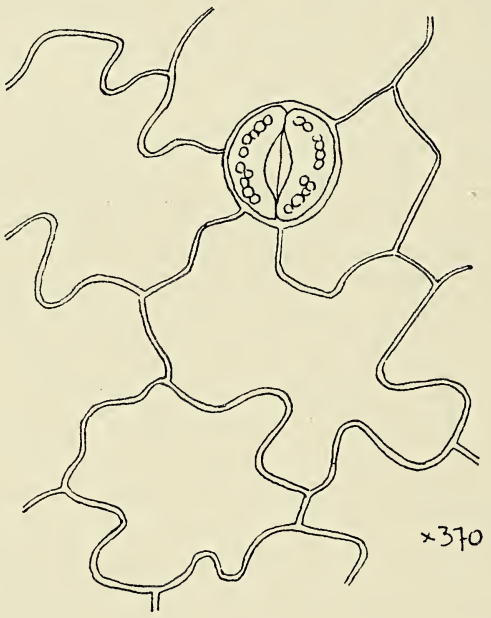

FIG. 8. Cells from upper epidermis of a seedling 20 days old.

cells of the upper epidermis were wavy in outline, as in ordinary epidermal cells from the under side of a bifacial leaf; the stomata were not sunken, and there was no encrustation. The whole of the ground-tissue elements were chlorophyll-containing (Fig. 8).

When the 'Linsenversuch' was carried out with strips of the epidermis of such seedlings it was impossible to obtain images through their cells.

The first traces of an encrustation were detected in the upper epidermis of plants forty days old. It had not as yet developed in the side epidermis, while those of the upper surface still retained their wavy outline and exhibited no sign of a papilla-like structure.

Seedlings sixty-five days old showed a greater increase in the intensity

of the encrustation of the upper epidermis, but showed no sign of papilla-like structure. The wavy outline of the epidermal cell-walls was exhibited to a less extent and the hexagonal shape more approximated to.

The question of the function of papilla-like cells in the epidermis of leaves has been discussed by Haberlandt (5), Albrecht (1), Kniep (6), Stahl (10), Nordhausen (9), and Wager (12). Other workers have contributed to the discussion, which has resulted in a considerably less part being assigned to these cells in the carrying out of heliotropic responses than was formerly the case.

Very few cases of aphotometric species have, however, been examined as to the occurrence thereon of lens-cells.

Wager has described the efficient lens-cells which are developed in the stem and leaf of *Mesembryanthemum cordifolium*. Though here local lens-cells are present in equal numbers on both sides of the leaves, these 'always place themselves with the upper surface exposed to, and more or less at right angles to, the rays of light' (12).

In *M. pseudotruncatellum* we have a plant which in its native habitat is subject to uniform, strong, solar illumination. The illumination of the upper surface is of this character, and is, in addition, direct. The illumination of the side cells is also uniform, but diffuse and weaker. The latter cells are better qualified as perceptive organs than those of the upper surface, and experiments were made to see whether the plant as a whole would show any heliotropic response when subject to strong one-sided illumination. Plants of different ages, between one and three years, were brought into the heliotropic chamber in succession and placed so that the middle of the plant experimented upon came opposite the window of the chamber. The experiments were performed in a hothouse, where the heliotropic chamber was placed with its window facing south. Control plants of different ages were also used. Plants were placed alternately with the surface slit directed towards the window and at right angles to this direction.

During three weeks not the slightest sign of heliotropic response was seen.

In a similar experiment seedlings ten days old showed a decided curvature towards the window after four hours of direct side illumination. On the third day they were all strongly curved, with their upper surfaces inclined almost perpendicularly to the incident rays (Figs. 9 and 10).

At 11 a.m. on the third day the seedlings were turned through an angle of 180°. By 4 p.m. they had lost the greater part of their curvature, while the upper surface had become horizontal. By noon of the following day they had all curved strongly in the direction of the light. As mentioned above, seedlings of this age possess no lens-cells.

From the age of ten days onwards the seedlings continued to exhibit heliotropic curvatures under illumination from the side. Seedlings forty

days old, which already showed traces of an encrustation, responded to heliotropic stimuli but slowly, while the power of response was evidently lost altogether by seedlings sixty-five days old.

In *M. pseudotruncatellum* we have to do with a plant the leaves of which possess lens-cells which are quite efficient in structure, but whose function as such is almost wholly shut out by the encrustation of calcium oxalate found in the epidermis. As the plant in its native habitat is subject to strong solar illumination, the encrustation is a protection when this becomes too strong for the plant. The image formed on the basal wall of each epidermal cell is a duller, shaded and toned-down copy of the one which would be formed there were the encrustation absent. This is borne out by the 'Linsenversuch' with treated and natural pieces of epidermis.

Marloth considers the chief function of this encrustation to be the protection of the plant against excessive insolation, and when insolation and

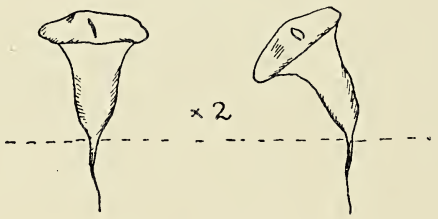

Fig. 9. Seedling 20 days old.
Fig. 10. Seedling 20 days old exhibiting heliotropic curvature.

illumination are considered together the combination of encrustation and lens-cell will be seen to be peculiarly effective and advantageous.

An encrustation of uniform thickness and colour would be a better protection against insolation, but in that case the interior of the plant would not be illuminated at all.

With the development of the papilla $\frac{1}{30}$–$\frac{1}{20}$ of the upper surface of each epidermal cell is available for the collection of light rays, while the remainder is under a protective cover. The protection is extended to the papillae, which are covered with a thin encrustation, but as a compensation are more highly developed as light-ray collectors.

The best developed lens-cells are in the side epidermis over the assimilating tissue, and these are the most weakly illuminated cells. There appears here, therefore, a correlation between the development of lens-cells and assimilating tissue on the one hand and the decrease of illumination on the other. The direct insolation and illumination of the upper surface evidently exercise a repellent influence on the development of chloroplasts in the cells immediately underneath, even when the lens function is almost excluded from the epidermal cells. Lens-cells of slightly better construc-

tion secure the illumination of the chloroplasts developed in those parts of the plant not subject to intense illumination.

It can be definitely said that, for this species of *Mesembryanthemum*, the epidermal cells do not function as organs for the perception of the direction of the incident light-rays, neither have they any connexion with heliotropic phenomena, although their construction, the position of the image formed, and the arrangement of the nucleus and basal plasma-layer make them appear at first sight well qualified for this function.

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A Research into the Amyloclastic Secretory Capacities of the Embryo and Aleurone Layer of Hordeum with Special Reference to the Question of the Vitality and Auto-depletion of the Endosperm.

PART II.

BY

F. STOWARD.

Contents of Part II.

V. On the Partial Suppression of the Amyloclastic Secretory Function of the Aleurone Layer.

In the foregoing sections the methods of eliminating the functional influence of the aleurone layer rest on its actual removal (by filing or cutting it off), or on the annihilation of the secretory mechanism by the action of anaesthetic reagents which by prolonged contact eventually cause the death of this tissue.

There remains yet another method of suppressing wholly, or at least partially, its secretory functions, namely, by conducting culture experiments

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under partial anaerobic conditions, with simultaneous partial removal of the aleurone layer itself.

In the following experiments the dorsal portion of the layer was completely removed before the establishment of the cultures. The objects (Experiment 1) were cultivated in the customary manner under aerobic conditions; in the remaining numbers of the series, under anaerobic conditions, the objects were embedded in the lowest part of a column of agar medium, having a uniform depth of 10 cm., and contained in test-tubes, the mouths of which were closed with loosely fitting cotton-wool plugs.

TABLE XXI.

Cultures of Endosperms deprived of the Dorsal Portion of their Aleurone Layers on 0·8 % Agar, with and without Mineral Salts.

Chilian barley. Seeds, 48 hours absolute alcohol, 48 hours water. Culture period, 68 days.

Exp.	*Objects.*	*Culture medium.*	*Conditions of experiment.*	*Amylase per 20 objects. (equivalent to mg. of Cu.)*		*Reducing sugars in medium.*[2]
				Direct.[1]	*Papain.*[1]	
1.	10 endosperms deprived of dorsal aleurone layer (whole)	0·8 % agar-mineral salts	aerobic	—	—	432
2.	10 endosperms deprived of dorsal aleurone layer (halved)[3]	0·8 % agar-mineral salts	anaerobic	168	4220	216
3.	10 endosperms deprived of dorsal aleurone layer (halved)[3]	0·8 % agar-mineral salts	anaerobic	168	4810	89
4.	10 endosperms deprived of dorsal aleurone layer (halved)[3]	0·8 % agar	anaerobic	168	2194	134
5.	10 endosperms deprived of dorsal aleurone layer (halved)[3]	0·8 % agar	anaerobic	337	3544	113

In Experiment 1 at its termination there remained in the majority of instances the ventral portion of the aleurone layer only, the starch contents and cell-structures of the inner endosperm having almost completely disappeared.

Those in which a starchy residue of inner endosperm still persisted showed on microscopical examination the phenomena customarily presented by endosperms undergoing digestion, namely, deeply and extensively eroded starch grains, and totally disorganized and disintegrated cell-walls and cytoplasmic débris.

On desiccation for a few hours at 30° C. the resistant aleurone-layer tissue only remained, and therefore no estimation of its amylolytic activity was undertaken.

This experiment is of interest because it shows distinctly that the ventral portion of the aleurone layer resembles the dorsal portion (provided the experiment is sufficiently prolonged) in possessing an intense power of depleting the inner endosperm. But unquestionably, during both natural germination and under the artificial conditions described in these experiments, the secretory function of the aleurone layer appears to be more

[1] Amylase in objects determined by direct and papain digestion methods.

[2] Reducing sugars in medium estimated directly.

[3] Objects in Experiments 2–5 halved longitudinally to increase extent of diffusion surface.

readily induced and to exhibit its powers more actively in its dorsal portion.

In the anaerobic series of experiments (Experiments 2, 3, &c.), the early suppression and eventual annihilation of the aleurone-layer secretory mechanism is obvious from a consideration of the lower amounts of reducing sugars present in the media in these experiments; in fact, comparison of the amounts of reducing sugars is a far more accurate criterion and measure of the relative depletive capacities possessed by two tissues, than is comparison of their amylolytic augmentative capacities.

As already insisted, the demonstration of this latter capacity, unless it can be shown that the amylases derived from the aleurone layer and inner endosperm are of equal value in their power of attack upon barley starch under normal conditions of germination, loses much of its importance and value.

The objects in these anaerobic experiments retained their original mass and form, and there was, as shown by a searching macro- and microscopical examination, an almost entire absence of evidence indicative of digestive changes. In all, as was to be anticipated, there were examples of eroded starch grains, produced no doubt prior to the complete inhibition of the secretory functions of the aleurone layer. The characteristic brown colour developed invariably in the functioning aleurone layer, especially at its exposed margins, was wanting.

Lastly, although direct digestion of the objects shows the existence of small amounts of free amylase, thus pointing to its inactivation or destruction as the result of its activity, yet papain-digestion indicates the presence of very considerable amounts of 'latent' amylase. The inference to be derived from these facts is that the reducing sugars found result from the more active aleurone-layer secretion at an early epoch in the course of the experiments.

These series of experiments afford, *on the one hand*, convincing evidence of the effectiveness of the method of sterilization employed; not only did the cultures during this lengthy period of experiment remain absolutely sterile, but the employment of alcohol in place of $CuSO_4$, as a sterilizing agent, obviates the troublesome source of confusion in experiments with endosperms to which reference will be made later. *On the other hand* they present an array of experimental data which renders it extremely difficult to avoid the conclusion that, during the germination process, starch dissolution and digestion (and, as we shall see later, cellulose digestion) are largely dominated by exercise by the aleurone-layer cells of their undoubted secretory functions.

VI. The Behaviour of the Inner Endosperm Tissue towards Anaesthetic Reagents: the Probable Non-possession of Vitality by this Tissue.

The views regarding the possession of vitality by the inner endosperm of barley are diametrically opposed.

Brown and Morris (loc. cit., Part I, p. 800) claim that this tissue consists of non-living cells; Hansteen, and also Puriewitsch, contends that each amyliferous cell in the endosperm of the Gramineae is to be regarded as a living functionary unit. Bruschi (loc. cit.) states that the cells situated at the extreme periphery of the inner endosperm, and immediately subjacent to the aleurone layer, are probably living units.

Hansteen and Puriewitsch's claim rests, as we have seen, on an inadmissible method of proof. Bruschi's statement rests on a sounder basis, namely, cytological examination of the nuclei of the more peripherally situated amyliferous cells and the application of plasmolytic tests.

Evidence derived from a study of the respiratory capacity of this tissue (Stoward, loc. cit., Part I, p. 821) does not permit of the clear definition of this function being due solely to either vital or enzymatic activity.

When an undoubtedly living organism, organ, or tissue is subjected to the action of an anaesthetic reagent for a sufficient length of time, ultimate annihilation of its functions ensues.

Experimental evidence has already been adduced showing that when isolated embryos and aleurone layers are anaesthetized, and the action of the anaesthetic is prolonged, the amyloclastic secretory function of the embryo is totally suppressed, and the same function of the aleurone layer never attains its original level—the appearance of enzyme in the external substratum in this latter case being attributed to the passive outward diffusion of pre-existent enzyme in the tissue, and to that formed during the progress of anaesthesia.

Demonstration of the manifestation of enzymatic activity by a given tissue furnishes, it is true, no criterion of the possession of vitality by the tissue.

The question assumes another aspect, however, in the case about to be treated, where the points to be discussed refer to the behaviour of the embryo, aleurone layer, endosperm, and inner endosperm, when exposed to the continued action of certain anaesthetic reagents. We have seen that the effect of anaesthesia on the aleurone layer is distinctly to diminish and lower its secretory capacity. Suppose endosperms and inner endosperms are *similarly anaesthetized*, and that it is found, after cultivation on gelatine medium and subsequent investigation of the medium and objects for amylase, that the amount of enzyme found in the case of anaesthetized endosperms is distinctly *lower* than that found in similarly constituted culture experiments with unanaesthetized endosperms, while the amount

of amylase found in the case of anaesthetized inner endosperms is *practically the same* as that found in similarly constituted cultures with unanaesthetized inner endosperms, how are these results to be interpreted with regard to the question of the possession or otherwise of vitality by the inner endosperm?

I. *Cultures of endosperms and inner endosperms on gelatine media.*

In the following experiments, which furnish concrete examples of the foregoing proposition, either nitrobenzene or chloroform was plenteously mingled with the medium *prior* to placing the objects in it. It was necessary, on account of the volatility of chloroform, to make fresh additions of this reagent every twenty-four hours: nitrobenzene, being less volatile, proved to be an excellent reagent in experiments of this kind.

TABLE XXII.

Cultures of Endosperms and Inner Endosperms on 5% Gelatine-Mineral Salts with Varying Concentrations of KH_2PO_4, in Presence of Nitrobenzene.

Chilian barley seeds. (1) Absolute alcohol, 48 hours; (2) water, 48 hours. Culture period 18 days.

Exp.	*Objects.*	*Concentration of KH_2PO_4 in*	*Amylase per 20 objects per hour in:—*			*Reducing sugars per 20 objects in:—*	
			Medium.	*Objects.*	*Total.*	*Medium.*	*Objects.*
			(equivalent to mg. of Cu.)				
1.	5 endosperms	P_3	2559	—	—	25	21
2.	5 inner endosperms	P_3	2349	—	—	0·00	0·00
3.	,, ,,	P_1	2066	337	2403	—	—
4.	,, ,,	P_2	2215	337	2552	—	—

The amounts of amylase found in the culture media in the above series of experiments, it will be observed, are of practically the same order of magnitude. The fact is significant, because it shows that the direct result of the action of these *anaesthetics is to suppress almost completely the secretory powers of the aleurone layer* (*Experiment* 1), *and to reduce the endosperm*, considered as a generator of amylase, to the level of the inner endosperm.

TABLE XXIII.

Cultures of Endosperms and Inner Endosperms on 5% Gelatine-Mineral Salts (P_3) in Presence of Chloroform.

Culture period, 12 days. Chilian barley—objects prepared as below.

Exp.	*Objects.*	*Amylase per 20 objects per hour in:—*			*Reducing sugars per 20 objects in:—*	
		Medium.	*Objects.*	*Total.*	*Medium.*	*Objects.*
		(equivalent to mg. of Cu.)				
1.	5 inner endosperms	2489	527	3016	—	0·00
2.	,, ,,	2489	295	2784	—	0·00
3.	5 endosperms	886	950	1836	85	0·00

Experiment 1. Inner endosperms prepared from *endosperms* steeped successively in (1) absolute alcohol, (2) water. 48 hours in each reagent.

Experiment 2. Inner endosperms prepared from air-dried seeds & steeped as in Experiment 1.

Experiment 3. Endosperms from air-dried seeds steeped as in Experiment 1.

Comparison of the results shown in Tables XXII and XXIII above, with those of Tables XII, XIV, and XVI, demonstrates clearly that the augmentative capacity of anaesthetized inner endosperms is *just as great, if not greater* than that of inner endosperms not exposed to the action of the reagents mentioned. The rather higher results afforded by the chloroform experiments appear to suggest that this agent exercises a stimulative action.

A similar comparison made with reference to *endosperms* (see Tables XII, XIV, and XVI), on the other hand, shows that the effect of anaesthetizing these objects is to *lower* their amyloclastic augmentative capacities.

The results of experiments with anaesthetized inner endosperms offer a distinct contrast to those yielded by the similarly treated embryos and aleurone layers in the experiments described in Section IV, while those derived from experiments with anaesthetized endosperms coincide with the experimental results referred to, in that there is a distinct lowering of the output of amylase.

The behaviour of the embryo, aleurone layer, and endosperm under the conditions described, which induce ultimate disorganization of the vital functions of the secretory tissues, is that of a living organism or tissue ; that of the inner endosperm, on the contrary, can hardly be ascribed to a tissue which consists indubitably of living units.

The view advanced by the author, based on these experiments, is that the inner endosperm of barley represents for the most part at least a non-living tissue.

The amounts of amylase found in the culture media of both the above series of experiments, it will be observed, are practically of the same order of magnitude if we exclude Experiment 3, Table XXIII.

This fact is significant because it shows that the direct and ultimate result of the action of these anaesthetics is to suppress completely the secretory powers of the aleurone layer, and to reduce the amyloclastic augmentative capacity of the endosperm to the level of that possessed by the inner endosperm. Accompanying this is the low production of reducing sugars in medium and objects (see Tables XXII, XXIII, and XV), and further, the important fact must be noted that in the endosperm experiments the *starch grains of the endosperms*, just as in the case of inner endosperms, *show absolutely no signs of erosion, which, as we have repeatedly seen when the aleurone-layer secretory function* is not interfered with, is the constant and invariable accompaniment of its activity.

The aleurone layer, moreover, is only removable from the subjacent tissue in these cases (endosperms) with difficulty, and its margins do not exhibit the conspicuous brown colour, both of which features afford fairly reliable means of deciding whether or not this tissue has been actively secreting.

In view of this evidence it is difficult to avoid the conclusion that *it is the amylase secreted by the aleurone layer*, and this *enzyme alone, which in experiments with endosperms produces corrosion* of the mature starch grains stored in the amyliferous cells.

The suggestion which has been made that other substances or bodies derived from the aleurone layer, not of an enzymatic nature, are capable of transforming the amylase resident in and augmented by the inner endosperm into an enzyme endowed with the attributes and properties of the 'secretion' amylase elaborated by the living scutellar epithelium and aleurone layer, can hardly be regarded with favour; for under the conditions of experiment described above, death of the aleurone layer does not preclude the free diffusion of such substances as those suggested into the subjacent amyliferous tissue, and if these were capable of effecting any such transformation as that suggested, we should expect to find as one of the first indications some evidence of starch-erosion. Since this is not the case, there remains only to reiterate the statement, that it is the *amylase* secreted by the aleurone layer which produces the phenomenon observed, and dominates endospermic depletion (as exhibited by the isolated endosperm), and to repeat that the processes which occur in the isolated inner endosperm are distinctly and for the most part of an auto-digestive nature, such as those which a dead enzyme-containing tissue may be capable of inducing in its contents.

II. *Cultures of endosperms and inner endosperms on moist calcium sulphate.*

Similarly constituted experiments were carried out with endosperms and inner endosperms on moist calcium sulphate, the antiseptic now being nitrobenzene, and the temperature of these 'culture' experiments being 24° C.

The results are shown in Table XXIV below, and the conclusions to be derived from them are in no way opposed to those furnished by the gelatine cultures.

There is an entire absence of eroded starch grains in Experiment 3 (a phenomenon which is invariably in evidence when the aleurone-layer functions are not interfered with), and the total reducing sugars found in the medium and objects belong to the same order of magnitude as in Experiments 1 and 2.

TABLE XXIV.

Cultures of Endosperms and Inner Endosperms on Moist Calcium Sulphate saturated with Nitrobenzene.

Exp.	*Objects.*	*Reducing sugars per 20 objects in :—*		*Amylase per 20 objects per hour in :—*	*Weight of 5 objects at termination of culture experiment.*
		Medium.	*Objects.*	*Objects.*	
		(equivalent to mg. of Cu.)			
1.	5 inner endosperms	42	29	—	135
2.	,, ,,	73	—	168	175
3.	5 endosperms	95	29	—	212

Experiment 1. Inner endosperms prepared from air-dried seeds by filing off aleurone layer; objects steeped successively in (1) absolute alcohol, (2) water. 48 hours in each reagent.

Experiment 2. Intact seeds steeped in (1) absolute alcohol, (2) water. 48 hours in each reagent, and aleurone layer then removed with razor.

Comparison of the results of Experiments 1 and 2, Table XXIV with Experiment 3, Table XX, shows that amylohydrolytic action proceeds to practically the same extent in anaesthetized inner endosperms as in those where no anaesthetic agent is employed, i. e. the inner endosperm behaves like a lifeless tissue.

Comparison of the results of Experiment 3, Table XXIV with Experiments 1 and 2, Table XX, shows a very striking difference; not only is the total amount of the reducing sugars significantly lower, but the weight of tissue remaining at the termination of the culture experiment is only feebly reduced.

The results, again, are obviously due to *suppression* of the secretory function of the living aleurone layer, and they afford strong confirmatory evidence of the results already derived from the more direct method of investigating this function described in Section IV, Table X.

VII. Starch-liquefying and Saccharifying Properties of (1) Embryo and Aleurone-layer Secretions, (2) Extracts of Endosperms and Inner Endosperms.

The methods of culture employed permit of the separate collection and investigation of the secretions of the embryos and aleurone layer comparatively free from substances likely to exert any very marked influence on their intrinsic properties. Similar advantages accrue in the case of endosperms and inner endosperms cultivated separately on moist calcium sulphate substrata. In this latter case the examination amounts to a comparison of the behaviour, on the one hand (endosperms), of a mixture of residual amylase and that derived from the aleurone layer; on the other (inner endosperms), to the residual amylase reinforced by that 'liberated' during culture.

Since it was inconvenient and impracticable to prepare the secretions of the embryo and aleurone layer as required, the following method was devised :—

A number of cultures of these objects on 0·55 % asparagin-mineral salt medium were prepared in advance, and when ready, after ascertaining that they were perfectly sterile, the objects were removed, and the amylase-containing medium was withdrawn by means of previously sterilized pipettes which were filled as completely as possible and at once sealed off.

Thus conserved out of free contact with air and protected from the light, the enzyme retains its primitive activity for a considerable time.

Endosperms and inner endosperms, at the termination of the culture period on moist calcium sulphate, were first desiccated at 30° C. for twenty-four hours, and were then transferred to an ordinary desiccator containing H_2SO_4 as desiccating agent.

Determinations of starch-liquefying and saccharifying power of embryo and aleurone-layer secretions, and of aqueous extracts of endosperms and inner endosperms after cultivation on moist calcium sulphate.

After a number of preliminary trials of methods of Lintner Sollied [1] and Pollak,[2] the latter method as modified by Chraszcz [3] was adopted throughout the course of these experiments.

The modification introduced by Chraszcz merely consists in the employment of potato in place of arrow-root starch, and substituting other temperatures for that indicated by Pollak, viz. 37·6° C.

Briefly described the details of this method are as follows:—

Into each of a series of test tubes of uniform size and diameter 10 c.c. of a 4 % potato-starch suspension are pipetted, and the tubes and their contents are then rapidly placed in a boiling water-bath and their contents rapidly gelatinized at a temperature of 65° C. They are then cooled to about 60° C. and transferred to a thermostat the temperature of which is regulated at 55° C. When the contents of the tubes have acquired this temperature a definite but gradually increasing volume of the amylase-containing solution to be investigated is added successively to each tube.

Immediately after the addition of the enzyme solution the tube is closed with a rubber cork; it is then inverted and thoroughly shaken to ensure thorough mixing of its contents, and at once returned to the bath. Each succeeding tube is similarly manipulated, and after the lapse of a chosen time interval, of the same duration for each tube, they are successively removed and at once examined as follows:—

Two to three drops of strong alkali (NaOH) solution are delivered from a pipette directly into the digestion liquid, and the manner in which they descend through the liquid is carefully noted. If the drops, during their descent, retain their spherical form, complete liquefaction has not taken place; if, on the other hand, the drops tend to lose their sphericity and

[1] Zeitschr. f. das gesamte Brauwesen, xxxvi, 1903, p. 329.
[2] Wochenschr. f. Brauerei, xx, 1903, p. 595.
[3] Pierozek, Wochenschr. f. Brauerei, xxvii, 16, 1910, p. 186.

become diffuse, then liquefaction is assumed to be either partial or complete. This point may be even more sharply ascertained by adding two to three drops of an indicator such as alizarin or phenol-phthalein, and again sharply inverting the tube. In those tubes in which liquefaction is incomplete the coloration produced by the indicator distributes itself in streaks, whereas in those in which complete liquefaction has taken place the distribution and intensity of the coloration is uniform throughout the mass. A second experiment with volumes of enzyme solution, chosen so as to include those comprehending and those lying on either side of the limits of incomplete and complete liquefaction indicated by the first experiment, enables the operator to hit off the volume of enzyme solution capable of producing complete liquefaction with a considerable degree of sharpness.

After the acquisition of sufficient preliminary experience the method is capable of affording quite reliable comparative results.

In the following tables are given the results of preliminary experiments with (1) secretions of the embryo and aleurone layers, and (2) aqueous extracts of endosperms, the secretions and extracts employed having equal saccharifying powers as ascertained by previously standardizing them by digestion with soluble starch.

TABLE XXV.

Relative Starch-liquefying and Saccharifying Powers of Secretions and Extracts.

4 % potato-starch paste. Temperature 55° C.

	Volume of solution affording complete liquefaction.	*Time required.*	*Reducing power in mg. Cu.*
Embryo secretion	0·55 c.c.	15 mins.	15·8
,, ,,	0·5 ,,	16 ,,	15·0
Aleurone-layer secretion	0·5 ,,	90 ,,	116
,, ,, ,,	0·54 ,,	90 ,,	121
,, ,, ,,	0·5 ,,	92 ,,	118
Endosperm aqueous extract [1]	0·52 ,,	15 ,,	13
,, ,, ,, [2]	0·5 ,,	15 ,,	15·7

These results show approximately (the determination of liquefying power cannot be regarded as other than approximate, since the personal equation is invariably introduced) that when standardized solutions of embryo and aleurone-layer secretions prepared in the manner described, of equal saccharifying value with regard to soluble starch, are investigated as above the latter (aleurone-layer secretion) appears to possess feebler liquefying powers than that of embryo secretion but approximately the same saccharifying power.

Similarly standardized aqueous extracts of the endosperm, which are virtually extracts of the aleurone-layer secretion, are practically identical with those of embryo secretion.

[1] From culture of distal halves of endosperms.

[2] From culture of whole endosperms, steeped as such.

The point to be considered in these comparative experiments is not so much the velocity of liquefaction as the point to which saccharification of starch paste may proceed.

Another series of experiments was then carried out on a larger scale as follows. Neither secretions nor extracts were standardized in this case, the object being to carry out prolonged digestions and then to determine the more important constants of these.

The following procedure was adopted in this and subsequent series:—

Two grammes of starch (potato) and 50 c.c. of water were placed in each of a series of 100 c.c. flasks, and the starch suspension, gelatinized at 65° C. and afterwards cooled to about 55° C., transferred to a thermostat retained at the latter temperature. Separate digestions were then carried out with embryo and aleurone-layer secretions and aqueous extracts of endosperms and inner endosperms at the various temperatures and times given in the table which follows. Toluene served as the antiseptic, and the flasks were securely corked during and at the termination of each experiment. Amylolytic action was arrested by plunging the flasks into a boiling water-bath for 15–20 minutes. The contents of each, after cooling to 15° C., were then brought to 100 c.c., filtered, and the different determinations recorded made in duplicate on the clear filtrate.

In each instance the values are adjusted by applying a correction for the copper-reducing substances found in the starch and added enzyme-containing solution.

TABLE XXVI.

CONVERSION OF 4 % POTATO STARCH WITH EMBRYO AND ALEURONE-LAYER SECRETIONS AND AQUEOUS EXTRACTS OF ENDOSPERMS AND INNER ENDOSPERMS.

Exp.	*Source of amylase.*	[α] *D.* 3·93	*R.* 3·93	*Final*[3] *coloration with iodine.*	*Temperature of conversion.* 55° C. (*Hours*)	30° C. (*Hours*)
1.	Embryo secretion	148·2°	63·29	No coloration	1½	14
2.	Aleurone-layer secretion	145·5°	64·5	Faintest violet	1½	14
3.	Endosperm extract [1]	149·2°	70·0	No coloration	1½	14
4.	,, ,, [1]	149·8°	70·6	,,	1½	14
5.	,, ,, [2]	140·0°	69·5	,,	3	—
6.	Inner endosperm extract	155·9°	7·66	Blue	8	14

The blue coloration (Experiment 6) persisted throughout the whole course of this experiment.

It is to be noted that the colour reaction given by the aleurone-layer secretion (Experiment 2) was of the faintest violet. The progress of liquefaction in Experiments 3, 4, and 5 was practically at the same rate, as judged by inspection of the limpidity and mobility of digestion liquid.

[1] Culture of distal halves of endosperms, 7 days, calcium sulphate. Seeds steeped 48 hours in absolute alcohol, 48 hours in water.

[2] Endosperms of air-dried seeds steeped as in [1].

[3] Colour reactions with iodine recorded in table noted at termination of digestion at 55° C.

There is a rough general agreement in constants for Experiments 1–5, indicating that the stage to which conversion had finally advanced in each instance was practically of the same order. In particular the colour reactions in these experiments with iodine were identical.

On the contrary the results given by the inner endosperm extract (Experiment 6) notably differentiate the general character of the conversion in this case; not only are the constants of a different order of magnitude, but the reaction with iodine throughout the entire period of experiment was persistently *blue*.

The enzyme present was that of the inner endosperm—the residual amyloclastic enzyme of the ungerminated grain of Brown and Morris—together with a certain proportion of an enzyme of the same type resulting from the liberation of enzyme existent in this tissue which Ford and Guthrie refer to as 'latent' amylase.

It is to be understood that the author does not consider the 'blue' coloration stage as final in this experiment; the probable reason why the ultimate 'violet' stage was not attained must be ascribed to the relative proportions of aqueous inner-endosperm extract and starch paste employed.

Invariably in these culture experiments with inner endosperms on calcium sulphate, whether established with objects prepared from steeped intact seeds (the objection to which is the possible diffusion of enzyme from the embryo and aleurone layer, which we have seen may be regarded as of small moment when steeping operation is confined to time and temperature limits recorded in this paper) or whether inner endosperms are prepared from air-dried material and are then steeped, there is a continual draining away of the amylase pre-existent initially in the tissue and of the amylase which results from the liberation of 'occluded' or 'latent' enzyme, the net result of which is that as the culture experiment under these conditions is sufficiently prolonged this tissue eventually becomes denuded of its amylolytic enzyme content.

The calcium sulphate stratum undoubtedly contains this enzyme during the progress and at the termination of the experiment, but the difficulty of estimating its amount lies in the fact that experimental difficulties are met with; the distribution of enzymes in the semi-solid medium is not uniform, and hence equal division of the substratum by mechanical division into two identical halves is impracticable. Similarly in the extraction of the substratum with water it is impossible to eliminate completely the possible adsorption of enzyme by solid $CaSO_4$. Finally, as already insisted, a far more accurate estimate of the relative depletive power of an amylolytic enzyme contained in or generated by a given tissue is gained by determining the amounts of reducing sugars in the substratum than by comparing its relative amylase content.

In the former we measure the amount of a substance which is

a definite chemical individual, in the latter a body about which we know comparatively little either as regards its constitution or properties.

The object of the foregoing experiments has been to show that wherever the influence of the aleurone layer has been operative there is produced an enzyme which possesses properties which in all respects are comparable, if not identical with those of the enzyme secreted by the columnar epithelium.

The introduction of Experiment 6 in the series had for its object the demonstration of the fact that wherever the functions of the aleurone layer have been eliminated or rendered inoperative the enzyme present does not possess either the attributes of that of the embryo or aleurone-layer secretion.

The means of differentiation between the enzymes from these different sources hitherto relied upon were confined to observations (1) on the mode of attack on intact starch grains, (2) on the quantitative demonstration of the superior depletive activity possessed by the enzymes secreted by the embryo and aleurone layer, and now they are still further emphasized by direct experimental evidence afforded by starch-paste conversions.

The following experiments were carried out with inner endosperms which had been placed on calcium sulphate substrata for ten days. A digestion with soluble starch by the 'direct' method of digestion showed that the amount of 'free' amylase present was minimal, and it was therefore of interest to ascertain whether by the predigestion procedure with papain or neutral salt the 'latent' enzyme liberated by these methods would acquire any of the properties (as judged by their action on starch paste) of the more powerful amyloclastic enzymes derived from the embryo and aleurone layer.

TABLE XXVII.

Starch Conversions with Inner Endosperms. 4 % Starch Paste. 50 c.c.

					Temp. and Time.	
			R. 3·93	[α] *D.* 3·93	55° *C.*	30 °*C.*
1.	10 inner endosperms predigested 40 hrs. with 25 c.c. 0·5 % papain at 30° C.	10 c.c. filtered extract	1·47	166°	12 hrs.	27 hrs.
2.	10 inner endosperms predigested 40 hrs. with 25 c.c. 0·5 % papain at 30° C.	10 c.c. filtered extract, faintly acidified with dilute acetic acid	1·47	173·2°	,,	,,
3.	10 inner endosperms predigested with 5 % KCl. 40 hours at 30° C.	10 c.c. filtered extract	1·70 [1]	94·77° [1]	,,	,,
4.	10 inner endosperms extracted 4 hours with 25 c.c. water at 30° C.	,,	1·85	—	,,	,,
5.	10 inner endosperms finely ground and added directly to starch paste.	,,	2·69	179·7°	,,	,,

[1] Values not adjusted for influence of neutral salt.

Slow but complete liquefaction accompanied by the customary 'break' or separation of a flocculent material occurred in Experiments 1, 2, 3, and 5, but in Experiment 4 these features were entirely absent.

The iodine reaction was persistently *blue* throughout the course of the conversion. The method of predigestion failed to induce in the 'latent' enzyme the acquisition of those specific properties which differentiate 'secretion' amylase as a more or less distinct type of amylolytic enzyme.

These starch-conversion experiments were not regarded as satisfactorily settling this point, and similarly conducted experiments, with the exception that the concentration of starch was lower, were undertaken in order to ensure the action of residual amylase attaining those final stages which are marked by the appearance of a persistent violet coloration with iodine.

It has been definitely shown by Baker[1] that in starch-paste conversions with the amylase of ungerminated barley the reaction never goes beyond the violet coloration, and further that the conversion products formed by its action on starch paste are different from those formed by malt amylase in otherwise similarly conducted conversions.

The view has been recently advanced by Ling[2] that digestive transformations of storage contents and organized structures (cell walls and cytoplasmic contents) of the inner endosperm are conditioned solely by enzymes residual in this tissue. In particular does this refer to digestive changes in the starch-storage reserves. The amylase, both 'free' and 'occluded' or 'latent', resident in the inner endosperm—according to Ling—undergoes transformation into that of malted corn, i.e. the amylase of the resting grain (as the germinative process progresses) virtually acquires the properties of malt amylase, and it is further assumed that the process of transformation in the germinating seed under malting conditions is closely paralleled by that which takes place when resting barley is subjected to auto- or papain-digestion.

In view of this suggestion it will be appropriate in this section to note the following experiment:—

0·5 gramme of finely ground barley was predigested with 25 c.c. of 1 % papain for twenty-four hours at 30° C., a small quantity of toluene being added as antiseptic. The extract thus prepared was filtered and 20 c.c. of the clear filtrate were added to 50 c.c. of 4 % starch paste and digested successively for two and a half hours at 55° C., twenty hours at 30° C., three hours at 55° C., and finally twenty-four hours at 30° C.

The colour reactions with iodine at the termination of each successive digestion interval were respectively intense blue, intense violet, less intense violet, i.e. at the termination of the first interval blue, and a persistent intense violet during the remaining ones; the colour test being carried out

[1] Baker: Journ. Chem. Soc., lxxxi, 1902, p. 1177. [2] Ling: Journ. Inst. Brewing, xiv, 1908.

under identical conditions by removing two drops of the digestion mixture, diluting it by the addition of 5 c.c. of distilled water, and then adding two drops of iodine solution. These results definitely show that the amylase of ungerminated barley, even after predigestion with papain and subsequent lengthy digestion with starch paste, is incapable of advancing the conversion beyond that stage which gives a violet coloration with iodine. That is to say, the experiments so far considered afford presumptive evidence of the fact that the amylase of ungerminated grain does not undergo transformation into or acquire one of the most specific properties ascribed to the amylase found in the malted corn, viz. action on starch paste to the no coloration phase with iodine.

After arrest of amylolytic action by boiling, cooling, and diluting to 100 c.c. and then filtering, the determinations made on the clear filtrate yielded the following constants: R. 3·93, 58·29; [α] D. 3·93, 137·2°—values which no doubt arise as result of series of direct and reversionary actions.

The experimental evidence so far adduced shows that the amylase demonstrable in inner endosperms cultivated on calcium sulphate is differentiated from that contributed by the embryo and aleurone layer by the inferiority of its capacity to attack barley starch in the condition in which it exists in the amyliferous cells, by its mode of attack on starch grains, and further by its incapacity to carry the conversion of starch paste beyond the violet coloration phase, as well as by its inferior starch-liquefying power.

It remains to add that the relatively large share attributed to the residual amylase in the digestion of the starch reserves of the inner endosperm during germination of the grain, as suggested in the more recent contributions to the subject, has been chiefly due to a tendency to confuse enzyme augmentation with enzyme activity and to regard the former phenomenon as an index of the latter. It has been frequently shown in the present inquiry that, although the inner endosperm is undoubtedly capable of augmenting its amylase content, yet this phenomenon is unaccompanied by any marked increase in the digestion of its starch reserves. The question has been further obscured by the apparent failure to recognize that the activity of amylolytic enzyme as measured by its action on soluble starch or starch paste does not necessarily parallel the activity of the same enzyme as measured by progress of its action on barley starch as it exists *in situ* either under the conditions of experiment described in this paper or under normal conditions of germination.

If we are to gain information regarding the complex phenomena met with in the germinative processes we must as far as possible study the mode of action of the enzymes which induce the changes observed not only *in vitro* but also *in situ*.

VIII. The Influence of Various Carbohydrates on the Secretion of Amylase by the Embryo and Aleurone Layer.

The relative nutrient value to the embryo of certain carbohydrates was investigated by Brown and Morris (loc. cit., p. 483). As a source of readily assimilable carbon they found cane sugar to have the highest value of any of the various sugars examined. The important and interesting fact was also elicited that this carbohydrate inhibits the amyloclastic secretionary function of the columnar epithelial cells.

When, for example, cane sugar was included in the starch-gelatine culture medium used by these authors, upon which embryos alone were placed, or when embryos with the proximal halves of their endosperms attached were planted in loosely packed glass wool saturated with 3·5 % cane sugar solution, no obvious signs of disintegration of starch granules were evident. This phenomenon, they state, is not due to inactivation of the secreted amylase (diastase) by the sugar present, but to the fact that 'under these particular conditions the epithelial cells of the scutellum do not secrete any amylo-hydrolytic enzyme'. The secretion of active amylase by the columnar epithelium, they conclude, is to be regarded to some extent as a starvation phenomenon.[1]

The observations of Brown and Morris regarding the selective exercise by the embryo of its secretory functions is probably a widespread phenomenon. The secretion of various enzymes, more especially amylase, by different Bacteria and Fungi, as shown by the researches of Wortmann,[2] Duclaux,[3] Busgen,[4] Fermi,[5] Pfeffer,[6] Katz,[7] and Saito,[8] is influenced by the composition and constitution of the nutrient or experimental substrate.

In view of the important aspect of the regulative control which the embryo exercises over the secretory functions of the scutellum a number of culture experiments were carried out with sterilized embryos on media (liquid and semi-solid) containing either cane sugar, invert sugar, or dextrose, with the express object of studying the influence which each of

[1] Quite recently Wohl and Glimm (Biochem. Zeitschr., xxvii, 1910, pp. 349–75), in an investigation of the extent to which the saccharification of starch paste by amylase is inhibited by addition of various sugars, find that cane sugar in 20 % concentration does not produce any inhibition.

The above statement of Brown and Morris is, therefore, well founded; the epithelial cells under the conditions cited and under my own experimental conditions do not exercise their secretory functions.

[2] Wortmann: Botan. Zeitung, 1890, p. 581; Zeitschr. f. physiol. Chem., vi, 1882, pp. 287–329.

[3] Duclaux: Chimie biologique, pp. 193, 195, and 210.

[4] Busgen: Ber. d. Deutsch. Botan. Gesellsch., Bd. cxi.

[5] Fermi: Centralbl. f. Bakt. u. Parasitenkunde, x, pp. 401–8.

[6] Pfeffer: Pflanzenphysiologie, Bd. i, 1897, pp. 361, 506.

[7] Katz: Jahrb. f. wiss. Botanik, xxi, 1898, pp. 599–618.

[8] Saito: Wochenschr. f. Brauerei, xxvii, 16, 1910, p. 181.

these carbohydrates exercises on the phenomenon of regulative secretion by the isolated embryo.

In the following table examples are given of the regulative control the embryo exercises over the amyloclastic secretory function of the columnar epithelium :—

TABLE XXVIII.

Influence of Cane and Invert Sugar on the Amyloclastic Secretory Function of the Embryo.

African barley. Seeds steeped in 10 % $CuSO_4$[1] 24 hours, water 24 hours.

Exp.	*Culture medium.*	*Duration of cultur .*	*Digestion period.*	*Amylase per 20 objects per hour in medium (equivalent to mg. Cu).*
1.	M.S. + 5 % cane sugar	8 days	20 hours	2·0
2.	0·55 % asparagin-M.S. + 5 % cane sugar	4 ,,	2½ ,,	0.00
3.	0·55 % asparagin-M.S. + 5 % invert sugar	7 ,,	1 hour	156·7

There can be little doubt that in presence of cane sugar, in close agreement with the observations of Brown and Morris, the embryo restricts its amyloclastic secretory powers in a very marked degree. Invert sugar, on the contrary, as Experiment 3 shows, is almost without influence on the exercise of this function of the embryo, amyloclastic enzyme being secreted just as rapidly and freely as when it is not included in the culture medium. The embryo, as we shall see, when the medium contains dextrose, exercises its regulative powers and reins in the activity of the secretory epithelial cells, but not to the same extent as in the case of cane sugar.

As the cultural experiments of Katz (loc. cit.) indicate, the secretory functions of *Penicilium glaucum*, *Aspergillus niger*, and *Bacillus megatherium* are influenced by different carbohydrates in varying degree, depending on the particular carbohydrate and its concentration.

The foregoing experiments and those which follow similarly demonstrate that the barley embryo adjusts or regulates its secretory powers; these vary with the carbohydrate employed, and, no doubt, with its concentration, although this aspect of the subject has not been experimentally investigated.

The data in Table XXIX below relate to cultures of embryos from seeds (Chilian barley) steeped successively in absolute alcohol and water under the customary conditions.

Two contemporaneous cultures of forty embryos each, the one on 5 % gelatine-mineral salts (control), the other on the same kind of medium

[1] The steeping of material in this reagent does not seriously interfere with the subsequent growth and secretory activity of the embryo (see Section XIII : Inadmissibility of $CuSO_4$ as Sterilizing Agent).

with the addition of cane sugar (5 grms. per 100 c.c. of medium), were continued for thirty-three days; the objects were then dissected into scutella, rootlets, and plumules, and the dissected material (after desiccation) and media were then investigated separately.

TABLE XXIX.

Culture of Embryos on Gelatine Medium with and without Cane Sugar.

Exp.	*Culture medium.*	*Amylase per 20 objects per hour in medium, scutella, rootlets and plumules (equivalent to mg. Cu).*		
1.	Gelatine-M.S. + 5 % cane sugar	3·0	0·00	30
2.	Gelatine-M.S. alone (control)	4·0	37·0	11

Throughout this long period of culture the embryos in Experiment 1 apparently did not secrete any amylase; practically none was found in either scutella or medium. The comparatively small amounts found in the control (Experiment 2) are no doubt due to partial destruction of amylase in the culture medium, owing to the unsuitable conditions which the medium offers for its conservation. External factors, atmospheric oxygen and light (although in all these experiments the cultures were shielded from the light as far as possible), also probably conspire to enfeeble and destroy the secreted enzyme

The embryo derives little or no nutriment from the gelatine. Throughout the many series of experiments in which gelatine media have been employed, no single instance of liquefaction by either the embryo or aleurone layer has been observed. The seedling under these conditions, in the absence of both available carbon and nitrogen, simply drains the scutellum of the valuable reserves stored initially in its tissues, reserves which we have assumed also serve in part for the manufacture of enzymes. The experiment is intended to demonstrate that when an ample supply of cane sugar is placed at the disposal of the embryo, it regulates and restricts its secretory activity even, as in the above case, when the culture period is markedly prolonged.

The results comprised in Tables XXX and XXXI refer to contemporaneous cultural experiments with embryos on the asparagin-mineral salt medium with and without dextrose.

TABLE XXX.

Influence of Dextrose on the Amyloclastic Secretory Function of the Barley Embryo.

Chilian barley. Seeds steeped successively in (1) $CuSO_4$ 10 %, (2) water; 24 hours in each reagent; 40 embryos each culture. Culture period, 10 days.

Exp.	*Medium.*	*Amylase per 20 embryos per hour in:—* *Medium.* (*equivalent to mg. of Cu.*)	*Objects.*	*Weight of 40 embryos.*[1] *mg.*
1.	0·55 % asparagin-M.S. (control)	219	22	—
2.	0·55 % asparagin-M.S. + 1 % dextrose	81	14	—
3.	1·1 % asparagin-M.S. (control)	205	29	60
4.	1·1 % asparagin-M.S. + 1 % dextrose	92	14	185

Dextrose in the concentration employed in the foregoing experiments obviously diminishes the secretion of amylase by the embryo; not only, as Experiments 2 and 4 very clearly show, is the amount of enzyme found in the culture medium reduced, but in the objects themselves this also holds. There is, apparently, in these instances where either cane sugar or dextrose is available, no need for internal digestion, and hence the secretion of enzyme is absent or greatly diminished. The embryo, therefore, regulates its secretory mechanism largely according to the nature and constitution of the supply of external nutriment.[2]

Luxuriant growth of the embryo invariably occurs on both solid and liquid media with the addition of either of the carbohydrates mentioned. Comparison of the weights of the embryos (Experiments 3 and 4) affords typical examples of the increased assimilation and growth which take place in these circumstances.

This latter observation at one time suggested that possibly active secretion took place in these cells, but was wholly intracellular, the rapidly absorbed cane sugar undergoing transformation and condensation in the epithelial cell, and the starch thus formed being subsequently hydrolysed into readily diffusible sugar and translocated to the actively growing parts of the young plantlet.

The absorption of nutriment by the isolated embryo under the artificial cultural conditions described is extremely rapid, as shown by the turgid appearance presented by the scutellum. Unless assimilation keeps pace with this enhanced absorption, the osmotic forces within this tissue reach such a high value that the organ ruptures. This frequently occurs in the case of *Zea* (maize) seedlings, the scutellum being split right across, a deep

[1] Desiccated 6 hours at 30° C.

[2] Sections through the scutellum of embryos nourished with these carbohydrates singly show on treatment with iodine the presence of starch in quantity in the scutellar tissues. Similar microtomed fixed and stained sections, especially those prepared from embryos removed during the first 48 hours of culture on cane sugar media, show starch granules in the columnar epithelial cells.

rift traversing it from apex to base. The above experimental results, however, show that inclusion of dextrose in the culture medium inhibits the secretion of amylase in the tissues of the plantlet, for, as the above results indicate, the amount of amyloclastic enzyme in the embryos themselves is distinctly lower than in the controls.

The following table furnishes data of physiological interest, the experiments, as already stated, being contemporaneous with those of Table XXX:

TABLE XXXI.

Cultures of Embryos on Asparagin-Mineral Salt Medium with Dextrose.

Details of preparation the same as given in Table XXX.

Exp.	*Nitrogen initially present in culture medium.*	*Residual sugar in medium.*	*Nitrogen assimilated by plantlets.*	*N% in dry plantlets after culture.*	*Dry weight of 40 embryos.*
1. 0.55 % asparagin-M.S. 1 % dextrose	14 mg.	0.00	14.8 mg.	10.05	147 mg.
2. 1.1 % asparagin-M.S. 1 % dextrose	28 ,,	0.00	20.5 ,,	11.68	175 ,,

The results furnish a rough idea of the manner in which assimilable nitrogen and carbon are removed from the culture medium. In Experiment 1, for example, both the total available nitrogen and carbon were absorbed, and in Experiment 2 a small residue of nitrogen only remained. The possible protective function to the secreted amylase, which has been ascribed by the author to asparagin, if exercised in these experiments and those comprised in Section III, evidently does not cover a very lengthy period of time. The complete removal of dextrose from the culture medium in these experiments leaves the question open whether the employment of higher concentrations of this sugar would stimulate the embryo to exercise its secretory restrictive powers as fully as it does when cane sugar replaces dextrose.

The foregoing results show that cane sugar and dextrose distinctly influence the amyloclastic secretory activity of the epithelial cells of the scutellum; the presence of the former leads to the almost complete cessation of extra-cellular secretion, while the latter very considerably diminishes this phenomenon. Invert sugar, on the contrary, appears to be quite inert, secretion occurring in its presence in the particular concentration used, equally and to practically the same extent as when it is not included in the culture medium.

Similarly conducted experiments were also carried out with dorsal fragments of aleurone layers prepared from seeds steeped in copper sulphate.

These are summarized in the table which follows.

TABLE XXXII.

Influence of Cane and Invert Sugar on the Amyloclastic Secretory Activity of the Aleurone Layer.

African barley. Seeds steeped in (1) $CuSO_4$ [1] 10 %, 24 hours, (2) water; 24 hours.

Exp.	*Medium.*	*Duration of culture.*	*Amylase per 20 objects per hour, equivalent to mg. Cu in culture medium.*
1.	0·55 asparagin-M.S.	4 days	365 mg.
2.	0·55 asparagin-M.S. + 5 % cane sugar	5 ,,	215 ,,
3.	0·55 asparagin-M.S. + 5 % invert sugar	4 ,,	200 ,,

Since the material used in these experiments was prepared under identical conditions, the results given above are comparable among themselves. Apparently these sugars exercise little or no influence on the secretion of amylase by the aleurone layer. It must not, however, be regarded as demonstrated that the cells of this tissue do not exercise that regulative control over the secretory function which they undoubtedly possess. The apparent indifference of the aleurone layer to changes in the nutrient substrate is due to the fact, as already insisted, that we are dealing with an isolated fragment of a tissue, and not with a complete highly organized organism. When in contact with its natural substratum, the inner endosperm, it is highly probable that, just as in the case of the epithelium of the scutellum, the aleurone layer regulates its secretory activities according to the special demands of the moment.

IX. Investigation of the Amylase Content of the Scutella of Barley Seedlings.

In dealing with the amyloclastic secretory capacities of the embryo and aleurone layer (Sections III and IV) it has been suggested that the output of amylase by these objects as measured by the quantitative examination of the culture medium is probably inferior to that which obtains under natural conditions of germination.

It will be generally accepted that the embryo and aleurone layer function best when, as in the germinating seed, they are in close relationship with the inner endosperm. There is, as we have seen, more especially (because more easily demonstrable) in the case of the embryo (see Sections III and VIII), a marked tendency to modify the extent and character of the activity of secretory mechanism localized in its scutellar epithelium according to the composition of the substratum on which it is placed. The exercise of a similar regulative control by the aleurone-layer fragment is not so clearly shown, the reason as already suggested being largely

[1] Special reference will be made to the influence the copper salt has on the secretory activity of the aleurone layer, see Section XIII, p. 1181.

dependent on the fact that instead of a complete organism we are dealing with an isolated fragment of tissue which possibly, during the later phases of cultivation on an artificial substratum, lapses into a semi-pathological condition.

In order to gain some idea of the probable order of the secretory capacity of the embryo under ordinary germination conditions, the inquiry was restricted to the examination of the amyloclastic powers of the scutellum, for it will be obvious that if the examination were extended so as to comprise the plumule, radicle, and rootlets the data obtained would necessarily show but one feature, namely, that of progressive increase due to the formation of new tissue and the laying down in each new cell of a small amount of amylase for the internal economies of the newly-formed units. It is, therefore, the capacity of the scutellum, the cells of which do not undergo multiplication,[1] which is of interest in the comparison of the relative secretory powers of the embryo on its natural nutrient medium and on artificial substrata.

The material for investigation was derived from seeds, which after the customary steeping in absolute alcohol and water were germinated at laboratory temperatures (15°–18° C.) for periods varying from 3 to 8 days.

The embryos after removal from the germinated seeds were dissected into scutella and radicles and plumules, dried for 4–10 hours at 30°, and subsequently the scutella investigated for amylase as already described, the finely ground desiccated material being directly added and well mixed with the starch solution.

TABLE XXXIII.

Amylase Content of Scutella of Barley Seedlings at Various Stages of Germination.

Duration of germination.	*Amylase per 20 objects*[2] *per hour in mg. Cu.*	*Weight of 10 radicles and plumules. mg.*
3 days	263	—
	223	—
4 days	357	46
	—	—
5 days	158	52
	153	47
7 days	158	78
	248	70
8 days	59	119
	59	124
	69	94
	69	74
8 days	84	—
	94	—

[1] Examination of extensive series of serial microtome sections of the scutella of embryos from seeds after 0–21 days' germination failed to reveal any evidence of cell division in any of its phases.

[2] 10 objects were used in each experimental and control digestion.

The above results show on the one hand that the secretory capacity of the scutellum falls as germination proceeds, on the other, that the amount of amylase found in the scutellum during the period 0–4 days [1] is considerable, and if we assume, as we may reasonably do, that a very large proportion of this is destined for external use in the inner endosperm, then the amount of enzyme found in the culture media of embryos on artificial culture is far from over-estimated. The period 0–7 days appears to represent the limits of great secretory activity; from the eighth day onward the level of amylase production falls considerably, and the functions of the scutellum are in all probability during this period principally absorptive.

Beyond the period 0–4 days the cytological changes are more difficult to follow, although distinct nuclear changes are very evident, showing, as do the data in the foregoing table, that the cells are actively functioning.

In short, the evidence here adduced relative to the amyloclastic capacity of the scutellum indicates that the capacity of the embryo is much superior when attached to its natural endosperm than when cultivated even on the most suitable of the artificial media so far essayed. A similar conclusion with regard to the capacity of the aleurone layer may be suggested by a comparison of its amylase content under similarly defined conditions of comparison (Section XIV).

X. The Secretion of Cytase by the Embryo and Aleurone Layer.

The existence of a cellulose-dissolving ferment in germinating barley was noted by Brown and Morris (loc. cit.), and they state that the disintegration and dissolution of the cell walls of the amyliferous cells which commence at the onset of and continue during the progress of germination are due to a specific enzyme, cytase.

The seat of the production of the enzyme, they state, is the columnar (absorptive) epithelium of the embryo; the later work of Brown and Escombe (loc. cit.) shows that the principal seat of elaboration of this cytoclastic enzyme during germination is probably the aleurone layer.

[1] Cytological study of microtomed stained sections of the scutellum during the period 0–4 days affords evidence which in certain essentials is in no way opposed to the biochemical data given above. During the period 0–4 days, the solubilization of the conspicuous 'aleurone' and other grains with which the cytoplasm is crowded initially proceeds most actively; commencing in the sub-epithelial tissue it progresses gradually to the deeper-lying portions of the scutellar tissue. Within the period mentioned the great mass of these grains are rendered soluble and disappear from view. There is undoubtedly some correlation between this disappearance of storage substance and the production of amylase. The period does not represent one of remarkable growth. These substances do not serve purely for purposes of general nutrition, but in all probability largely for the more important work with which the scutellum is occupied at this phase, viz. enzyme elaboration, which proceeds in the specialized epithelial layer.

The specificity of the enzyme has been challenged by Grüss, who suggests that the manifestation of its activity is merely a function of ordinary 'diastase'.

The study of cellulose-dissolving enzymes has been exhaustively carried out by Newcombe,[1] and the conclusion is arrived at that barley-malt extract contains a specific cytoclastic enzyme.

Apart from the question of endeavouring to ascertain whether cytase really represents a distinct enzyme it appeared desirable to ascertain whether, as asserted by Brown and his co-workers, the secretion of a cytoclastic enzyme is one of the several functions of the embryo and aleurone layer.

The preparation of sterilized experimental material appeared to afford exceptional opportunities of testing the cytoclastic secretory powers of these objects over prolonged periods of time and of thus giving them full facilities for the display of their presumed cytoclastic functions under conditions which absolutely precluded the possible interference of disturbing influences, such as micro-organisms, &c.

After the customary series of orientation experiments the following method of experiment was adopted :—Embryos and fragments of aleurone layers were prepared from seeds sterilized by steeping them successively in alcohol and water under the usual conditions, and placed upon thin transverse sections taken from various parts of the endosperms of similarly sterilized seeds.

In each of a series of Petri dishes was placed a triangular glass tripod, and on it were laid several microscopical slides and on each of the latter three microscopical coverslips were placed; the dishes were finally sterilized by heating to 160° C., and afterwards when sufficiently cooled 2–3 drops of 5 % gelatine-mineral salt medium were delivered with a sterilized pipette and a single *thin* endosperm section embedded in each little mass of medium. Prior to the setting of the medium either an embryo or aleurone-layer fragment was laid on the section with its scutellar or inner surface respectively downwards and in close contact with the section. Similarly prepared miniature 'cultures' containing endospermic sections without the superposition of the objects mentioned served as controls. A small quantity of sterilized water was then run into the dish in order to prevent undue desiccation of the droplet of culture medium.

Under these conditions it was possible to carry out experiments of long duration and to examine the section without disarranging its component parts, a point of very considerable importance when, as we shall see in these experiments, often the cytoplasmic network alone remains.

The object of these experiments was not to examine the progress of

[1] Ann. Bot., xiii, 1899, p. 79.

amylo- and cytoclastic action so much as to note what ultimately happens to the walls and to the starch storage contents of the amyliferous cells during periods ranging from 1 to 4 weeks.

Under the conditions described, the enzymes in play having free access to the starch granules in those parts where the plane of section literally divided the endospermic cells into halves and thus exposed their starch contents, solution of starch invariably appeared to precede, in point of time, action on the cell walls; the latter, however, were distinctly swollen at this juncture and difficult to stain with either Congo red or Bismarck brown.

The embryo and aleurone layer do not attack the material at their disposal at equal rates—the action of the former on both starch and cell walls being at all stages of the experiment earlier, the reason being, as already insisted in the discussion of various experiments with fragments of the aleurone layer, that an isolated non-growing [1] fragment of secretory tissue does not, as anticipation would lead one to expect, comport itself in any way comparable with that manifested by an organism like the embryo.

At an early stage, 2–3 days, examination of the sections placed under embryos invariably shows that marked corrosion of starch granules characteristic of the action of the amylase which it secretes, and simultaneously the cell walls of the section acquire a swollen, hyaline appearance. Later, 4–7 days, the section may be practically depleted of its starch contents and the cell walls broken up into spindle-shaped fragments of varying size. A little later these are digested, and then, if care is taken not to disturb the section, after cautious warming of the gelatine and allowing it to reset the section is literally reduced to a diagrammatic representation of the cytoplasmic network of each cell divested of its original cell walls and inclusions. The parts of the diagram previously filled in by the cell walls are now clear spaces. Gentle flooding of the preparation with Congo red or Bismarck brown, both of which admittedly stain cellulosic structures, and subsequent cautious removal of the excess of stain merely result in the staining and rendering more easily visible of the meagre cytoplasmic network; that portion of the section formerly occupied by the cell walls remains unstained, showing that the appearances described are not due to optical properties of any presumed residual matter. At a still later epoch, 7–14 days or longer, the network disappears, portions undergoing disintegration presenting a beaded appearance, and the organized structure noted during the period immediately preceding this phase gradually disappears; the network is reduced to a structureless mass of débris.

Finally it is to be noted that the aleurone layer included in the section entirely resists disintegration.

[1] Repeated examination of the microtomed stained section of the aleurone layer prepared from germinating seeds at various stages fails to reveal any distinct signs of nuclear or cell division.

The evidence afforded by these experiments, positive in character and extremely well defined, leaves little doubt that the embryo secretes an active cellulose-dissolving enzyme.

When we turn to the control sections, these experiments being conducted contemporaneously and under identical conditions, the contrast is remarkable; absolutely no visible sign of change is demonstrable in them. In order to more closely examine the cell walls, many of the sections were divested of their starch by treatment with diluted saliva; microscopical examinations of these sections and similarly treated sections, for purposes of control, taken from endosperms of seeds which had been simply steeped, failed to show any evident sign of alteration, the two series presenting identical appearances.

These results show with reasonable clearness that the changes observed were induced by the embryo.

Similar series of changes to the foregoing are induced by the aleurone layer, but there is, as already pointed out, this important difference: they are more long drawn out, i. e. the action is slower. The important fact to be noted, already to some extent foreshadowed by the evidence afforded by the experiments described in Section IV *a*, is that, *if the miniature 'culture' experiment is sufficiently prolonged* (10–21 *days*), *corrosion of starch granules identical in every recognizable respect with that induced by and hitherto attributed solely to the amylase secreted by the columnar epithelium takes place.*

The type of starch erosion observed by Brown and Escombe (loc. cit., p. 13), which they designate 'sub-aleuronic', represents, so far as my observations go, one of the earlier phases of the phenomenon. During the final stages of the experiments (21–28 days or longer) the phenomenon becomes quite general. The cytohydrolysis produced by the aleurone-layer section presents during its various stages features which closely parallel those described for the embryo.

During the course of the work described in this section the idea frequently suggested itself that the dissolution and digestion of both cell walls and starch granules were induced *not* by the direct action of the cytoclastic enzymes secreted by the epithelial and aleurone-layer cells, but possibly *indirectly* by the action of substances other than secretions, i. e. substances, not enzymatic in nature, on the pre-existent enzymes in the tissues, which pass during the prolonged course of these experiments from these secretory tissues into the subjacent inner endosperm tissue, and possibly activate the pre-existent enzymes present in them.

The experimental evidence adduced in Section VI did not favour this suggestion. The control experiments in those just described were not regarded as quite conclusive, for the reason that no means had been taken to annihilate the enzymes pre-existent in the thin section of endosperm employed; it must, however, be admitted that the controls as thus con-

stituted show that the isolated section of the endosperm was unable to induce autonomously in any degree the changes just described.

In order to test this important point, experiments similar to those described in mode of preparation and duration were instituted with embryos and aleurone-layer fragments, placed upon endosperm sections prepared from seeds which had been previously *put in water at* 90° *C. and retained at this temperature for half an hour.* Many of these burst their integuments, and the starch contents of the sections selected showed distinct evidence of partial gelatinization. It is inferred that the pre-existent enzymes of the ungerminated grain are largely localized, as Ford and Guthrie's investigations show, and my own confirm, in *the peripheral portions* of the endosperm.[1]

The results were in no way opposed to those recorded above; dissolution and digestion of the amyliferous cell walls and starch content proceeded to the same extent as in experiments with unboiled sections, but the processes were obviously retarded, possibly by either physical or chemical changes in the endospermic tissue or both, induced by the agency of heat.[2]

These experiments naturally raise the question of what constitutes a 'control'; strictly speaking, in the above experiments what is wanted is a section of an endosperm with its enzymes destroyed. Heating, if sufficiently prolonged, certainly accomplishes this, but the possibility of introducing new and unfavourable conditions is not precluded, and these may seriously inhibit the action of enzymes, such as amylase, cytase, &c.

The possibility of finding some means whereby self-digestion of the inner endosperm may be accomplished without the aid of the active secretions of the embryo and aleurone layer is granted, but, so far at least, the suggestion rests on a basis which is purely presumptive. The suggestion of Ling that the pre-existent amylase of the resting grain during germination is transformed into the amylase of malt, although the possibilities of such a transformation are conceded, is unsupported by experimental evidence, and for the present at least belongs exclusively to the domain of theory.

It is of interest in this present section to refer to certain results yielded by earlier attempts to demonstrate the cytoclastic secretory powers of the embryo and aleurone layer.

A series of small Petri dishes, 4 cm. in diameter, each containing the gelatine-mineral salt medium, similar to that employed in the 'miniature

[1] Finely ground endospermic substance of barley, placed in dry test-tubes heated for half an hour in an ordinary Koch's steamer, annihilates effectively the pre-existent amylase of the ungerminated grain.

[2] Further light on this subject is afforded by the experimental evidence given in Section XII (Inactivation of Amylase).

cultures' described, to a depth of 10 mm., were prepared and transverse sections of unboiled endosperms were placed in these; before the complete setting of the medium some of the sections sank to the bottom, others remained at the surface; on the latter were placed, on separate plates, embryos and fragments of aleurone layers.

Starch dissolution as usual occurred in the sections at the surface, and also finally (after 2–3 weeks) in those at the bottom of the medium, but little action was noticeable on the cell walls.

The experiments showed very clearly that amylase of secretion had gradually diffused throughout the medium. The comparatively small amount of action on the cell walls, in the light of the experimental results yielded by the miniature cultures, must be taken to mean that the cytase, owing to the immensely large volume of culture medium, was so greatly diluted that its action was rendered almost inappreciable.

In fine, the results recorded in the present section furnish supplementary evidence of the possession by the embryo and aleurone layer of both amylo- and cytoclastic secretory powers. It must be added, in view of the fact that disintegration and apparent digestion of the cytoplasm take place under the conditions of experiment described, that the possession of a proteolytic function is to be ascribed to the embryo and aleurone layer. They further support the view that the inner endosperm is incapable of inducing the depletion of its starch contents.

Reference has frequently been made, in dealing with cultures of endosperms, to the corrosion of starch grains, which is a constant feature in these experiments, and to the invariable absence of this feature in inner endosperms, under similar experimental conditions, the inference being that the amylase secreted by the aleurone layer attacks and erodes starch grains in a manner which it is impossible to differentiate from that induced by the amylase secreted by the columnar epithelium. In the present section direct evidence has been rendered available by the actual placing of aleurone-layer fragments on barley starch grains, and subsequently demonstrating the characteristic features and progress of the action. It has also been shown that when starch-paste conversions are carried out with the aleurone-layer secretion the constants obtained do not differ greatly from those furnished by similarly conducted conversions with embryo secretions, and in either case the conversion proceeds to the stage which no longer gives a coloration with iodine. These facts are regarded as evidence that the cells of the columnar epithelium and aleurone layer are, physiologically speaking, identical.

The phenomenon termed 'double fertilization' or 'triple fusion' has been known for a long time, and the possibility of its widespread occurrence has received general acceptance from the majority of botanists. This phenomenon, although it is claimed that it occurs in *Zea* (maize), has not, so

far as the writer is aware, been demonstrated in the case of *Hordeum* (barley). The fact that the aleurone layer possesses similar physiological attributes to those of the columnar epithelium suggests that the endosperm of *Hordeum* may possibly represent an abortive or undifferentiated embryo, in which case the aleurone layer may on the evidence adduced be regarded as the analogue of the columnar epithelium.

XI. Influence of the Embryo on the Augmentation of Amylase by the Inner Endosperm.

There can be little doubt that, apart from its secretory functions, the embryo by the exercise of its absorptive function modifies the rate of augmentation of amylase in the inner endosperm. Removal of the products of amyloclastic activity is a primary essential if the gradual transformation of the starch reserves is to proceed without interruption. If, for example, the accumulation of starch transformation products exceeds certain limits, then there the progress of endospermic depletion may be markedly retarded.

It appeared to be of interest to study the possible influence the embryo may exercise on the capacity the inner endosperm possesses of augmenting its amylase content. With this object in view sterilized steeped seeds were completely divested of their aleurone layers, and plate cultures were then prepared of inner endosperms with their embryos attached. The objects were embedded in 5 % gelatine-M.S. medium, and the cultures thus established continued for periods of four and six days. Media and objects were then separately investigated for amylase by the methods already described.

The results of these experiments are summarized in the following table:—

TABLE XXXIV.

Cultures of Inner Endosperms with their Embryos attached.

Exp.		*Culture period.*	*Amylase per 20 objects per hour in :— Medium.*	*Inner endosperms.*	*Scutella.*
1.	5 embryos with inner endosperms attached	4 days	3174	952	75
2.	5 embryos with inner endosperms attached	6 days	3055	873	105

The results are of interest in showing that the augmentation of enzyme in the inner endoserm proceeds more rapidly under these conditions than in the isolated inner endosperm under similar conditions of cultivation.

The relatively larger total amounts of enzyme, however, are partly to be accounted for by the secretory activity of the embryo, and probably also by the more prompt removal of the products of amyloclastic activity, leading to the more rapid release of the residual 'latent' amylase of the inner endosperm.

The embryo in these circumstances probably secretes more actively than under the artificial conditions described in an earlier section of this

paper. Unfortunately, if the experiment is continued beyond about four days, there is a decided tendency for the embryo to become detached from its inner endosperm, and this tends to limit the period of experiment.

The inner endosperms in these experiments were carefully examined for starch grains exhibiting signs of erosive action. Repeated examination of specimens of the endospermic starch grains taken from various parts of the inner endosperm fail to reveal any eroded starch grains except in that part of the inner endosperm with which the exterior surface of the scutellum is in close contact.

It is clear, therefore, as in experiments with isolated inner endosperms, so in these experiments, that there is an entire absence of the amylase resident in the amyliferous cells corroding the starch grains at its disposal, this mode of attack being, as already stated, confined to the amyloclastic secretions of the embryo and aleurone layer.

XII. The Inactivation of the Amylase of Ungerminated Barley.

The suggestion has frequently been put forward that either the active secretions or substances other than secretions which pass by diffusion from the epithelial and aleurone-layer cells during the germination process may possibly exercise an activating influence on the amylase pre-existent in the inner endosperm either in the condition of 'free' or 'latent' enzyme.

It therefore appears desirable to subject the question to experimental inquiry, more for the purpose of ascertaining what would happen than with the hope of acquiring any very definite indications of the nature of the changes induced.

In the following essays the influence exercised by the embryo and aleurone-layer secretions on the amylase of resting barley has been made the subject of inquiry. The enzyme solutions used were derived from separate cultivations of embryos and aleurone layers on asparagin-mineral salt solution. The culture liquids containing the active amylase were removed aseptically with sterilized pipettes, which were at once sealed and afterwards conserved in the dark for 2–3 months prior to their use.

TABLE XXXV.

Influence of Embryo Secretion on the Amylase of Ungerminated Barley Substance.

Digestion period, 1 hour at 30° C. Starch solution containing 58 grms. starch solids per 100 c.c. of solution.

Exp.		*Amylase equivalent to mg. Cu.*
1.	5 c.c. embryo secretion + 30 c.c. starch solution	208
2.	5 c.c. water + 30 cc. starch solution + 0·2 grm. barley	343
3.	5 c.c. embryo secretion + 30 c.c. starch solution + 0·2 grm. barley	556
4.	5 c.c. embryo secretion + 30 c.c. starch solution + 0·2 grm. barley (boiled)	159
5.	5 c.c. embryo secretion (boiled) + 30 c.c. starch solution + 0·2 grm. barley	310

The barley used in these experiments and those which follow was finely ground in a Seck mill and afterwards passed three times through a finely-meshed horsehair sieve in order to secure uniformity of sample. The bulk sample prepared as described was conserved during the progress of these and the experiments which follow in a desiccator over H_2SO_4 and carefully shielded from the light.

The values given in the above and following tables have been corrected by deducting the copper-reducing values of similarly conducted control digestions with boiled preparations of secretions and barley substance.

Experiments 1 and 2 give the separate amyloclastic powers of the embryo secretion and barley substance respectively. Experiment 3 shows clearly that the combined activities of the embryo secretion and barley substance roughly correspond to the sum of their separate values; each enzyme apparently retains its individuality and behaves as an independent unit. There is no evidence in this experiment of either activation or inactivation.

Where, as in Experiments 4 and 5, the influence of boiled barley substance or boiled embryo secretion on the embryo secretion and barley substance respectively is investigated, there is positive evidence of inactivation. The amyloclastic power of the embryo falls from 208 mg. to 159 mg., while that of the barley substance decreases from 343 mg. to 310 mg., and the results so far considered negative distinctly the suggestion that activation of the amylase of the resting grain is produced by the amyloclastic secretion of the embryo.

Consideration of Experiments 3, 4, and 5 once more raises the question of what constitutes a control. If, for example, the sum of the values of Experiments 4 and 5, regarded for present purposes as of the nature of control experiments, viz. 159 + 310 = 469 mg., be compared with the value furnished by Experiment 3, which equals 556 mg., then it would appear, in the absence of the results of Experiments 1 and 2, as though the amylase of secretion and barley amylase had mutually activated each other. Similar examples will be met with in the other series of experiments described in this section; these will serve to emphasize the fact that experiments devised to show whether inactivation or activation of a given enzyme does or does not occur require special circumspection in the matter of design.

Below are given the results of similarly conducted experiments with the aleurone-layer secretion and barley amylase.

TABLE XXXVI.

Influence of Aleurone-layer Secretion on the Amylase of Ungerminated Barley.

Digestion period, 1 hour at 30° C. Soluble starch solution containing 5 grms. starch solids per 100 c.c. of solution.

Exp.				*Amylase equivalent to mg. Cu.*
1.	5 c.c. secretion	+ 30 c.c. starch solution		150
2.	5 c.c. water	+ 30 c.c. ,, ,,	+ 0·2 grm. barley	343
3.	5 c.c. secretion	+ 30 c.c. ,, ,,	+ 0·2 ,, ,,	505
4.	5 c.c. ,,	+ 30 c.c. ,, ,,	+ 0·2 ,, ,, (boiled)	127
5.	5 c.c. ,, (boiled)	+ 30 c.c. ,, ,,	+ 0·2 ,, ,,	279

The results parallel those given in the preceding table in that they afford no evidence of activation of barley amylase either by the aleurone-layer secretion or by substances other than the amyloclastic enzyme contained by the secretion.

Experiments were then made to ascertain whether boiled barley substance or the same subjected to prolonged heating in the air-dry condition with and without free access of air exercises a retarding effect on the amylase of the resting grain.

The barley substance in these experiments was either (1) mingled with 5 c.c. water and boiled for ten minutes, or (2) the air-dried substance alone heated for three or more hours at 100° C., or (3) the air-dried substance treated as in (2) and subsequently 5 c.c. water added and the mixture then boiled for ten minutes.

TABLE XXXVII.

Influence of Barley Substance on Amylase of Resting Grain.

Digestion period, 1 hour at 30° C. Soluble starch solution containing 6 grms. starch solids per 100 c.c. of solution.

Exp.				*Amylase equivalent to mg. Cu.*
1.	0·2 grm. barley	+ 25 c.c. starch solution	+ 5 c.c. water	290
2.	0·2 ,, ,,	+ 25 c.c. ,, ,,	+ 0·2 grm. barley (boiled)	148
3.	0·2 ,, ,,	+ 25 c.c. ,, ,,	+ 0·4 ,, ,, ,,	158
4.	0·2 ,, ,,	+ 25 c.c. ,, ,,	+ 0·2 ,, ,, (heated air-dried condition 3 hours at 100° C.)	300
5.	0·2 ,, ,,	+ 25 c.c. ,, ,,	+ 0·2 ,, ,, (heated air-dried condition 3 hours at 100° C., then boiled with 5 c.c. water for 10 minutes)	285

These experiments taken as a whole show that (1) boiled barley substance exercises a retarding influence on the amylase of the resting grain (compare Experiments 1, 2, and 3); (2) barley substance which has been subjected to the treatment indicated in Experiments 4 and 5 apparently

does not exercise any influence on the amylase present in the resting grain (compare Experiments 1, 4, and 5).

At first it appeared to be probable that the inactivation produced by boiled barley substance in the above and preceding experiments might be due to adsorption of enzyme. This suggestion, however, is dispelled by the results of Experiments 4 and 5, and, moreover, Experiment 3, in which double the quantity of boiled barley substance was used, fails to lend support to such a surmise. The reaction of the boiled barley substance was approximately neutral to such indicators as alizarin and methyl-orange; it is therefore difficult to ascribe the results observed to change in the reaction of the digestion medium.

The interpretation of these results is a matter of difficulty; their general trend suggests the possible presence of an anti-enzyme, an anti-amylase, or of a phytotoxin, either of which might possibly be unmasked by boiling the finely-ground barley substance.

Further experiments were then instituted, the barley substance being subjected to one or other of the treatments set forth in the following table:—

Exp.		*Amylase equivalent to mg. Cu.*
1.	0·2 grm. barley + 25 c.c. 6 % starch solution + 5 c.c. water	316
2.	0·2 ,, ,, + 25 c.c. ,, ,, + 5 c.c. ,, 0·2 grm. air-dried barley heated 5 hours at 100° C. in sealed tube	321
3.	0·2 ,, ,, + 25 c.c. ,, ,, + 5 c.c. ,, 0·2 grm. barley heated in sealed tube, 5 hours at 100° C.	190
4.	0·2 ,, ,, + 25 c.c. ,, ,, + 5 c.c. ,, 0·2 grm. barley heated under mercury seal 5 hours at 100° C.	200
5.	0·2 ,, ,, + 25 c.c. ,, ,, + 5 c.c. ,, 0·2 grm. barley heated 5 hours at 100° C. under open-air condenser	205

These results do not differ materially from those given in the preceding table, with the exception of Experiments 3, 4, and 5, which show that more prolonged heating of barley substance in presence of water tends to reduce its retarding effect. As before, when barley substance is heated in the air-dried condition (Experiment 2) the retarding influence which it previously exercised is entirely annulled.

Experiment 4 of the preceding and Experiment 2 of the above series, in which the air-dried substance was freely exposed to air and sealed respectively, seem to show that the inhibitory agent is not a volatile body.

The following series of experiments were essayed with the object of ascertaining whether the amylase which accumulates on predigestion with papain and is presumed to be due chiefly to the transformation of 'latent' into 'free' enzyme would in any way modify the character of the results previously obtained.

Exp.	*Amylase equivalent to mg. Cu.*
1. 0·2 grm. barley + 25 c.c. 6 % soluble starch + 5 c.c. water digested 1 hour at 30° C.	316
2. (0·2 grm. barley + 5 c.c. 0·5 % papain solution, predigested 40 hours at 30° C., then boiled) and afterwards 0·2 grm. barley + 25 c.c. 6 % soluble starch added and digestion continued for 1 hour at 30° C.	205
3. (0·2 grm. barley + 5 c.c. 0·5 % papain solution, boiled) then predigested for 40 hours at 30° C., after which 0·2 grm. barley + 25 c.c. 6 % soluble starch added and digestion continued for 1 hour at 30° C.	216
4. 5 c.c. 0·5 % papain solution, boiled, and predigested for 40 hours at 30° C., after which 0·2 grm. barley added and digestion continued for 1 hour at 30° C.	316

The foregoing results, however, do not modify the results obtained in previous experiments, the retarding effect of boiled barley substance being still in evidence (Experiments 2 and 3).

Finally, in view of the possible presence of substances (or bodies) capable of rendering the final reaction of the digestion mixture faintly alkaline or acid, the following digestions were carried out in presence of varying amounts of KH_2PO_4, the barley substance being boiled before and after the addition of the salt in Experiment 3 and Experiments 2, 4, 5 respectively.

Exp.	*Amylase equivalent to mg. Cu.*
1. 0·2 grm. barley + 25 c.c. 6 % starch solution + 5 c.c. water	316
2. (5 c.c. KH_2PO_4 solution + 0·2 grm. barley, boiled) + 0·2 grm. barley + 25 c.c. 6 % starch solution	221
3. (5 c.c. water + 0·2 grm. barley, boiled) then phosphate added + 0·2 grm. barley + 25 c.c. 6 % starch solution	211
4. (5 c.c. KH_2PO_4 solution + 0·2 grm. barley, boiled) + 0·2 grm. barley + 25 c.c. 6 % starch solution	200
5. (5 c.c. KH_2PO_4 solution + 0·2 grm. barley, boiled) + 0·2 grm. barley + 25 c.c. 6 % starch solution	201

The final percentages of phosphate in Experiments 2, 3, 4, 5 were 0·25 %, 0·25 %, 0·5 %, and 1 % respectively.

The inclusion of boiled barley substance in the digestion mixture again leads to retardation of the same order of magnitude as in the preceding series of experiments. The results obtained in presence of varying amounts of phosphate fail to lend support to the assumption that the final reaction of the digestion medium may have been contributory to the retardation effects observed in the earlier series of experiments. The possibility that the concentrations essayed were too high, it must be understood, is not excluded.

The experimental evidence brought forward in the present section tends on the whole to negative the view that the secretions of the embryo and aleurone layer in any way activate the amylase of the resting grain. The peculiar retardative influence exercised by boiled barley substance on barley amylase is a subject which calls for further investigation.

XIII. The Inadmissibility of Copper Sulphate as a Sterilizing Agent.

Copper sulphate, used in the concentration indicated and even in more dilute strengths, is unquestionably a most efficient reagent for effecting the sterilization of barley seeds, under the conditions described in this paper. It was repeatedly demonstrated, by means of several series of steeping experiments and subsequent bacteriological examination, that moulds were the only organisms which survived after the seeds had been subjected to twenty-four hours' immersion in 10 % copper sulphate solution ; the survival of these organisms, it is to be noted, occurred solely in a few instances, the various types of bacterial flora commonly present on the surfaces of cereal seeds being completely annihilated.

This reagent, however, offers certain disadvantages in an investigation like the present one, the chief of them being the difficulty of ensuring the complete removal of the last traces of the salt from the seeds by washing in water and the consequent risk of thereby introducing a disturbing factor in the subsequent culture and digestion experiments, the copper salt, even in minute traces, being extremely toxic towards amylase.

In the initial phases of the work embodied in this paper the culture experiments were confined to embryos. In view of the sensitiveness of amylase towards copper salts a series of parallel experiments were carried out with embryos steeped either in (1) 10 % copper sulphate or (2) absolute alcohol. As the secretory capacities of the embryos in these experiments were of the same order of magnitude (such differences as were observed being attributable solely to the individualities of the organisms themselves), it was concluded that either the salt had been completely removed or its amount reduced to such small limits that it could no longer be regarded as a disturbing factor.

When, however, similar experiments were made with isolated aleurone layers considerable divergence in their secretory capacities were met with ; aleurone layers removed from seeds which had been steeped in the copper salt solution showed invariably a much lower capacity than aleurone layers from seeds which had been steeped successively in absolute alcohol and water. This divergence in the results was still more pronounced in endosperms and inner endosperms. In certain experiments with these objects, in spite of very thorough washing, the measure of their amyloclastic capacities fell from $\frac{1}{2}$ to $\frac{1}{10}$ of that yielded by similar objects in similarly conducted experiments prepared from seeds steeped in absolute alcohol.

To test the efficiency of attempts to wash out the copper salts, seeds were steeped in 10 % copper sulphate for twenty-four hours, then washed with sterilized tap water (six re-washings being undertaken), and finally

re-steeped in water for twenty-four hours. This steep water on concentration to small bulk acquired a faint but obvious blue colour, and confirmatory evidence of the presence of copper was obtained by testing the faintly acidified solution with potassium ferrocyanide.

It is evident, therefore, that in spite of what appeared to be very thorough washing, complete removal of the copper salt, or reduction of its amount to such small limits that its toxicity may be regarded as negligible, is not accomplished with any degree of certainty. It seems to be highly probable that copper sulphate interacts with a cellulosic or pectinous constituent in the seed coverings, forming a loose compound which possibly slowly dissociates in the culture medium. Although the salt probably exercises its toxic action on the amylase which diffuses from the objects into the culture medium during the progress of the culture experiment, the demonstration of such action is only rendered evident when the medium is digested with soluble starch.

The uncertainty associated with the use of copper sulphate as a steep reagent leads to its abandonment in favour of absolute alcohol. When air-dried barley seeds are steeped in this reagent for 24–48 hours, abstraction of water and certain extractions from the seed coverings take place, but there is no evidence of penetration of the reagent into the interior of the seed, nor does the steeping of barley seeds in this reagent obviously impair their germinative capacities.

XIV. Investigation of Material by the Auto- and Papain-digestion Methods.

The recent work of Ford and Guthrie[1] shows that the amylolytic activity of ungerminated barley varies considerably according to the method of preparing the extract. Their results indicate that the extract of a given barley prepared by the auto- or papain-digestion methods described in their paper exhibits an increase of amylolytic activity over and beyond that yielded by the same barley when the method of preparation adopted is the customary or direct one usually employed. Thus, when a definite weight of ground barley substance is predigested under definite time and temperature conditions, either with plain distilled water, aqueous solutions of certain neutral inorganic salts, certain organic substances (notably amino acids), or with solutions of active (boiled) or passive (unboiled) papain, and the filtered extracts derived from these predigestions are digested with soluble starch under otherwise identical experimental conditions, they exhibit a much higher amylolytic power than do aqueous extracts prepared from the same barley by the customary method of short extraction.[2]

[1] Journ. Inst. Brewing, 1908, xiv, p. 61.

[2] The authors state that these various substances do not directly stimulate amylolytic activity

In ungerminated barley they assume that there exists a certain but variable quantity of amylase, which probably exists both in an insoluble and 'latent' condition. The notable augmentation of amylolytic activity (or more probably of amylolytic enzyme) which follows on predigestion, notably with active papain, they consider is due to the liberation and solubilization of this 'latent' enzyme, i. e. enzyme, it may be, occluded by or associated with a protein complex, which, especially in the presence of *active* papain, by virtue of its proteolytic function, is hydrolysed, and thus the enzyme assumed to be in the 'latent' condition undergoes liberation. Since passive papain leads to an augmentation of enzyme, and further, since the solution of this body possesses amphoteric properties, its functions are regarded as threefold; it (1) proteolyses and (2) solubilizes the latent enzyme, and on liberation and solubilization (3) protects it by ensuring the neutrality of the reaction medium. Aqueous predigestion, and particularly predigestion with aqueous solutions of certain neutral salts, also leads to augmentation of the enzyme, apparently in the main by enhancing its solubility.

Whatever may be the actual condition in which the great mass of the amylase of the resting grain exists, and the real nature of the mechanism operative in its release which ultimately leads to results similar to those described by Ford and Guthrie, there can be little doubt that when the amylolytic capacity of the ungerminated barley substance is investigated under conditions which closely parallel those employed by these authors, there ensues as a final result a very marked increase in the amount of amylase in the material as compared with that furnished by the ordinary method of determination. This notable increase in the amylase of the resting grain has naturally redirected attention to the subject of the causation of endospermic depletion. The questions which present themselves are, does not the residual amylase undergo similar augmentation during the progress of the germinative process, and does it not play a more important rôle in starch digestion than that attributed to it by Brown and Morris? Prima facie considerations would naturally suggest that it does or may do so, but experimental evidence, at least for the time being, does not wholly support this hypothesis.

The pre-existent amylase of the resting grain was regarded, prior to the investigations of Ford and Guthrie, and justifiably so, as a small fraction only of that found in the inner endosperm of the seed after three to four days under conditions favourable to germination.

(Journ. Soc. Chem. Ind., 1904, xxiii, p. 414; and Journ. Chem. Soc., 1906, lxxxix, p. 76); they consider that the increases of activity observed are not due to specific augmentation of the activity of the enzyme.

In short, the general conclusion to be gathered from their contribution is that the results recorded are due to the actual increase of amylolytic enzyme.

Earlier researches, notably those of Rechyler,[1] bear the suggestion of the possibility of autonomous enzyme augmentation by the inner endosperm, and associated with it is the idea that starch digestion must necessarily be the concomitant.

The positive experimental results adduced in the preceding sections amply confirm the first part of this suggestion. The isolated endosperm does possess this augmentative capacity; the phenomenon occurs under each set of experimental conditions exploited, and is quite independent of enzymatic or other possible influential substances which originate or are elaborated in either the aleurone layer or integuments.

In brief, under the conditions described the pre-existent amylase of the inner endosperm undergoes reinforcement, and the point is capable of experimental demonstration.

When we turn to similarly conducted experiments with the isolated endosperm, not only is augmentation of amylase of a superior order of magnitude a persistent feature, but accompanying it there is marked evidence, both microscopical and biochemical, of starch digestion and depletion, features which are conspicuous by their absence or marked reduction in magnitude in the experiments with the inner endosperm. Were it not for the independent proof of the *secretory* activity of the aleurone layer, it might be claimed that the auto-digestive phenomena presented by the isolated endosperms were entirely due to some indirect influence exercised by the former tissue, e. g. by the inward diffusion of salts or salt-like bodies or substances with amphoteric properties which respectively aid solubilization of the latent enzyme and afford the liberated enzyme adequate protection.

It is conceded that influences of the kind enumerated may, and probably do, play a rôle similar to that indicated in the general assemblage of processes which comprise the phenomena of starch digestion, but the view advanced here is that these influences are purely of an *adjunctive* type, and are overwhelmingly overshadowed by the rôle enacted by the enzyme which arises as the result of the operation of the secretory mechanism of the living aleurone-layer tissue. The evidence for this view lies in the very different results which attend the suppression of this secretory mechanism, and which are put forward in a later section.

The means there adopted, while they annihilate the glandular functions of the aleurone layer, do not preclude the diffusion of salt-like combination from this tissue into the inner endosperm. Why then, if digestion of the inner endosperm starch contents is independent of the *secretions* of the aleurone layer, does not the process, if it is conditioned by agents other than the actual secretions derived from this tissue, proceed in the same

[1] Ber. d. d. Chem. Ges., 1899, xxii, p. 414.

manner and to the same extent as when the glandular tissue exercises its full functional powers? Why does not the amylase of the resting grain, as suggested by Ling (l. c.), undergo transformation into the amylase of the malted grain and acquire the properties of the latter as we know them? Evidence will be adduced showing that the amylase derived from the aleurone layer possesses marked powers of attack on barley starch grains, the grains undergoing distinct and visible erosion. On the other hand, the amylase of the inner endosperm possesses but feeble powers of attack on the mature starch grains, and the erosive features are entirely absent. As we shall see when we come to deal with the experiments relating to papain-digestion, which lead apparently to the release of considerable quantities of the amylase resident in the inner endosperm, the fact, important as regards endospermic depletion, is brought to light that here also, in spite of the amount of diastase in action, the digestion of barley starch placed at its disposal under the condition of experiment is negligible. Still other important facts, relative to the nature of this enzyme and the condition in which it exists in the inner endosperm, will be advanced when we come to consider the results of the investigation of the structural parts of *Tropaeolum*, in which presumably the amylase present is wholly represented by the 'translocation' variety.

It appeared desirable, therefore, in view of the importance and stimulative interest possessed by the valuable contribution of Ford and Guthrie, to investigate, along lines closely parallel to those devised by them, the Chilian barley which has served in the majority of the experiments described, and further to extend the inquiry to the investigation of *germinated* material.

Investigation of air-dried and steeped material.

Materials derived from air-dried and steeped seeds were first investigated by the ordinary direct method of digestion with soluble starch, i.e. the finely-ground material was directly added to the starch solution at 30° C., and the digestion continued at this temperature for a definite time-period, the object of these experiments being to ascertain whether the amylase content undergoes diminution after steeping, and also the influence exercised by the husks or certain of their constituents (organic and inorganic) on the result.

The results of the first series of experiments are given in the following table :—

TABLE XXXVIII.

MATERIAL FROM AIR-DRIED SEEDS: Amylase Content of Endosperms digested *with* and *without* Husks; of Husks and Aleurone Layers, Chilian Barley. Direct digestion method.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	5 endosperms + 5 husks	1378
2.	5 „ + 5 „	1280
3.	5 endosperms	1595
4.	5 „	1610
5.	5 husks	20
6.	5 aleurone layers	215

5 objects in each experimental and control digestion.

These results are extremely clear; the order of magnitude of the amylase content of endosperms[1] in Experiments 1–4 is the same, and in the barley in question the digestions with husks, instead of being higher, are lower than those without husks, a result which does not coincide with Ford and Guthrie's experiments. That certain barleys may exhibit this behaviour is possible. The husks themselves contain but little amylase, as is shown by the results of Experiment 5. The aleurone layers removed by filing off this tissue contain approximately $\frac{1}{6}$–$\frac{1}{7}$ of that found in the entire endosperm.

There appears to be a widespread idea that steeping may lead to very considerable reduction of the amylase content of a given barley. The fundamental correctness of this statement is no doubt perfectly sound, but there is considerable probability that the influence exercised by the manner of steeping and the conduct of the operation on different barleys is subject to very wide limits of variation. Ford and Guthrie (l. c., p. 67) suggest that the diminutions of amylolytic activity observed are due to the diffusion away of substances, both organic and inorganic, mainly from the husks during the operation.

The results summarized in the following table are derived from experiments instituted for the purpose of ascertaining in what degree the steeping conditions indicated influence the amyloclastic content of the endosperm.

[1] In these and the experiments which follow, each experimental and control digestion was established with a definite number of objects (just as heretofore), and each object was divided longitudinally into its two component halves, one series of 10 halves serving as the experimental and the other 10 halves as the control digestion set. In this manner it was hoped that divergences due in the main to the *individuality* possessed by the objects under investigation might be in some measure reduced.

TABLE XXXIX.

ALCOHOL AND ALCOHOL WATER STEEPED MATERIAL : Amylase Content of Endosperms from (*a*) Dehusked Seeds steeped in Absolute Alcohol for forty-eight hours, (*b*) Dehusked Seeds steeped successively in (1) Absolute Alcohol, (2) Water : forty-eight hours in each reagent.

Chilian barley. Direct digestion method.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	5 endosperms (*a*)	1435
2.	5 ,, (*a*)	1397
3.	5 ,, (*b*)	1242
4.	5 ,, (*b*)	1164

The values afforded by Experiments 3 and 4 certainly indicate the occurrence of reduction, but it is not enormous, and whether it is due in the major part to diminution of salt-like substances in the seed coats or testa (since the husks were removed before steeping in water in Experiments 3 and 4), or to actual existence of smaller amounts of amylase in the objects, remains an open question. In short, the barley employed is not one which exhibits the feature in a marked degree under the conditions of experiment selected. Moreover, the explanation of the phenomenon of reduction is probably much more complex, since the entry of water into the inner tissues of seed in all probability leads to a readjustment of the entire chemical, biochemical, and physical conditions of the complex reaction system which the endosperm, regarded as the sphere of action, represents.

Investigation of air-dried and steeped material by the auto-digestion method.

The amylase content of air-dried and steeped material was next investigated comparatively by the two methods of predigestion, (1) auto- and (2) papain-digestion, originated by Ford and Guthrie.

As usual, in place of using an aqueous or papain extract of the material these digestions were carried out in the presence of the finely-ground substance.

The uniform method adopted in these experiments, and also in those with material from germinated seed, to be described later, was that given below ; the principal modifications being the reduction in the number of objects taken and increase in the volume and concentration of soluble starch solution used in each digestion experiment, changes necessitated by reason of the increase in the amylase content of certain of the structural parts of the seed under the new conditions of determination.

Each experimental and control predigestion and the subsequent digestion with soluble starch was carried out with 25 objects (intact seeds and structural parts) ; the seeds after removal from the steep or germinator being

at once dissected or left intact as occasion demanded, and the dissected and non-dissected material subsequently dried for 4–10 hours at 30° C. The mixture of desiccated material and sand was placed in small 50 c.c. conical previously steamed Jena flasks, and to each was added 10 c.c. of either (1) plain distilled water (auto-digestion), or (2) solution of neutral salt (auto-digestion), or (3) active or passive papain (papain-digestion), and, finally, a drop or two drops of an antiseptic, nitrobenzene or toluene. The flasks, securely corked, were then transferred to the thermostat at 30° C., and there remained for 20 hours. After the contents of the flasks intended to serve as controls[1] had been slightly but distinctly alkalinized by addition of NaOH, the experimental and control digestions were commenced by the pipetting into each flask of 40 c.c. of soluble starch solution (equivalent to 50 c.c., containing 4 grms. of starch solids per 100 c.c. of solution), and continued for half an hour at 30° C. The experimental digests were then arrested by the addition of alkali.

The table which follows summarizes the results of auto-digestion of the endosperms of air-dried seeds, and affords ample evidence of the capacity possessed by the endosperm under the conditions selected to augment its amylase content. Auto-digestion with husks, however, does not influence the result.

TABLE XL.

AIR-DRIED MATERIAL: Amylase Content of Endosperms from Air-dried Seeds.

Chilian barley. Auto-digestion period, 20 hours. Antiseptic, nitrobenzene.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	5 endosperms + 5 husks	2127
2.	5 ,, + 5 ,,	2366
3.	5 ,, + (without husks)	2183
4.	5 ,, + ,,	2308
5.	2·5 ,, + ,,	2211

Experiments 1–4, 5 objects used in each experimental and control digestion.
Experiment 5, 2·5 ,, ,, ,, ,, ,, ,, ,,

Investigation of ungerminated barley substance by papain-digestion method.

Material prepared from air-dried and steeped barley was subjected to predigestion with papain, under the conditions selected and described in the preceding section.

A series of preliminary trials, instituted for the purpose of gaining some idea of the order of magnitude of the change in the enzymatic capacity of

[1] Arrest of control and experimental digestions was also carried out by boiling the digestion mixture in many instances.

the structural parts of the seed induced by papain-digestions, was undertaken by the method of procedure already formulated. The trials showed definitely that a period of predigestion of the material with a 0·5 per cent. papain solution [1] for 20 hours at 30° C. was ample for the establishment of a condition of equilibrium.

The general characters presented by the results embodied in the tabular statements which follow show clearly that predigestion of material derived from air-dried and steeped seeds (endosperms, &c.) with papain in the manner indicated invariably leads to a marked augmentation of its amylolytic activity or amylase content as compared with that observed when similarly prepared material is investigated by the direct digestion method employed elsewhere in this inquiry.

TABLE XLI.

Air-dried Dehusked Seeds: Amylase Content of Endosperms.

Chilian barley. 0·5 % papain, 20 hours. Antiseptic, nitrobenzene.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	2·5 endosperms	11050
2.	2·5 ,,	10800
3.	2·5 ,,	8693

The results of papain-digestion of inner endosperms and aleurone layers from air-dried seeds are shown in the following table:—

TABLE XLII.

Air-dried Dehusked Seeds: Amylase Content of Inner Endosperms and Aleurone Layers.

Chilian barley. 0·5 % papain, 20 hours. Antiseptic, nitrobenzene.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	2·5 inner endosperms	6414
2.	2·5 ,, ,,	8103
3.	2·5 ,, ,,	7752
4.	5 aleurone-layer filings	2152

The aleurone-layer filings represent 19 % of the total weight of 5 air-dried dehusked endosperms

Experimental inquiry was next directed to ascertain whether the concentration (0·5 per cent.) of papain employed in experiments so far cited was the most favourable, the determination being confined to the examina-

[1] The single specimen of papain used exclusively in these papain-digestions was procured from Messrs. Griffin, London.

tion of seeds which had been steeped successively in absolute alcohol or water.

The papain solutions were prepared by weighing separately the requisite amounts of papain, and adding the necessary volumes of water to furnish the desired concentrations. The preparation of the papain extract was made, as customary in all these experiments described in this and other sections, by maintaining this solution at 30° C. for 3–7 hours in a tightly-corked flask, the risk of micro-organism development being eliminated by addition of one drop of nitrobenzene. To aid the progress of extraction the contents of the flasks were agitated at intervals; finally, the undissolved residue was filtered off, and each experimental and control predigestion carried out with 10 c.c. of the clear filtrate.

TABLE XLIII.

Amylase Content of Dehusked Seeds after steeping successively in (1) Absolute Alcohol, (2) Water; 48 hours in each reagent.

Chilian barley. Varying concentrations of papain, 20 hours. Antiseptic, nitrobenzene.

Exp.	*Objects.*	*Concentration of papain.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	2·5 seeds	0·5 %	10240
2.	2·5 ,,	,,	9001
3.	2·5 ,,	1·0 %	9700
4.	2·5 ,,	,,	8924
5.	2·5 ,,	1·5 %	7039
6.	2·5 ,,	,,	7386

The values furnished by this essay require but little comment; concentrations ranging between 0·5–1·0 % papain evidently do not greatly influence the order of magnitude of the results, the observed difference being regarded as being principally conditioned by the individuality of the objects examined. The employment of a higher concentration (1·5 %), however, obviously does appear to be responsible for a diminution of the amylase content.

On the other hand, rough comparison of these results with those of Table XLI, p. 1189 (endosperms of air-dried seeds), appears to indicate that steeping has led to reduction of the amyloclastic enzyme content of the objects under notice. The interpretation of the influence of steeping is, however, by no means simple, and its further discussion may well be deferred until the results of further experiments, relating to the relative distribution of enzyme in the structural parts of the steeped seed, are adduced.

These are shown in the following table :—

TABLE XLIV.

Amylase Content of Endosperms, Inner Endosperms, and Aleurone Layers for Dehusked Steeped Seeds.

Chilian barley. Seeds steeped successively in (1) absolute alcohol, (2) water; 48 hours in each reagent. 0·5 % papain, 20 hours. Antiseptic, nitrobenzene.

Exp.	*Objects.*	*Amylase per 20 objects per hour (equivalent to mg. Cu).*
1.	2·5 endosperms	8070
2.	2·5 ,,	7985
3.	2·5 inner endosperms	4888
4.	2·5 ,,	5548
5.	2·5 aleurone layers	3414
6.	2·5 ,,	3181

There is an obvious reduction of amylase in the endosperm (compare Table XLI) and inner endosperm (compare Table XLII) and an increase in that of the aleurone layer. The reduction of enzyme apparently falls more heavily on that of the inner endosperm; it does not follow, however, that some reduction does not occur in that of the aleurone layer, and if this be so, then the *increase* in the enzyme content of this tissue due to papain-digestion is actually greater than the value determined. The results observed may be conditioned to some extent by the mere separate digestion of these tissues (aleurone layer and inner endosperm), but the digestion of the endosperm (in which these structural parts are mixed) also shows reduction and therefore fails to lend support to this suggestion. A partial explanation may be suggested by the application of Ford and Guthrie's hypothesis, namely, the diffusion away, during steeping, of salts or salt-like substances and others, some of which, by virtue of their amphoteric properties, protect the released amylase, but bodies of the same type are, according to this hypothesis, contributed by papain.

The explanation, however, is more probably associated with the differences in the biological attributes of the two tissues.

There is good reason to believe that we are dealing with tissues which differ in this important respect. The aleurone layer is distinctly living; the inner endosperm, on the contrary, if it possesses vitality at all, can hardly be regarded without ample qualification as a living tissue. The inner endosperm, whether it forms an integral part of the seed or whether it is isolated, comports itself like a mass of dead tissue; the processes induced in it, viz. the augmentation of enzyme and the limited digestion changes, under cultural conditions and also by different digestion methods, are auto-digestive in character and more or less finite in extent. During steeping of the seed, water enters freely into the inner endosperm tissue, and what happens it is wellnigh impossible to outline. The net result is apparently a reduction in its enzyme content as shown by subsequent digestion, but, on the other hand, during steeping of the intact seed the secretory

mechanism of the aleurone layer is at once set in motion. Its activity during these apparently and outwardly passive phases of activity are, however, distinctly intracellular, i. e. little or no enzyme is excreted into the subjacent tissue, and consequently a heaping up of enzyme and its possible precursors takes place within its cells as shown by predigestion of papain at this stage.

The experimental results, in short, bear out the work of Ford and Guthrie, and show that predigestion with papain under the modified conditions here employed results in an enhancement of the amyloclastic capacity of the material under investigation.

The absolute amylase content or amyloclastic activity of a given plant or seed tissue or organ cannot of course be determined, yet it must be conceded that its value must be finite. The method of papain-digestion described as applied *exclusively*[1] to the investigation of the amyloclastic capacity of ungerminated barley furnishes greatly enhanced values as compared with those afforded by other methods so far examined, and apparently these values are of the nature of *limiting values.*

As such, they may be legitimately utilized as a tentative basis for the approximate calculation of the amount of amylase initially present as pre-existent or 'free' and 'latent' amylase respectively, and the amounts of amylase which are contributed by the 'secretory' epithelium and aleurone layer at certain stages of germination.

Investigation of material from germinated seeds by direct, auto-, and papain-digestion methods.

One of the principal features so far presented is the very considerable *divergence* in the value of the amylase content of the structural parts of the endosperm of the ungerminated seed as separately determined by these methods of determination.

Does this differential feature still hold when similar structural parts from seeds after varying periods of germination are similarly examined?

The question can be efficiently considered only by an appeal to direct experiment.

Throughout, the experimental work described in this subsection refers to the examination of material prepared from seeds which, after the customary preliminary sterilization, were germinated in moist sand in Coldewe germinators at the temperature of the laboratory (15° C.–18° C.).

The seeds after the removal from the germinators were at once dissected into embryos, aleurone layers, and inner endosperms, and afterwards desiccated at 30° C. for ten to twenty hours. The separate examinations of the amyloclastic capacities of the material by the three methods, the

[1] Papain-digestion does not, as we shall see, influence the amyloclastic capacity of the growing embryo of barley nor that of *Tropacolum* or its structural parts.

subsequent determinations of the copper-reducing values, and the method of expressing the results, are precisely identical with those already cited.

In the following table are shown the results derived from the estimation of intact seeds after three and a half to six days' germination :—

TABLE XLV.

Amylase Content of Intact Germinated Seeds.

Digestion method.	*Germination period.* $3\frac{1}{2}$ *days.*	6 *days.*
	Amylase per 20 *objects per hour (equivalent to mg. Cu).*	
Papain	7682	9001
Auto	5664	10161
Direct	5044	8148

There is, as the above values show, a decided tendency as germination progresses for the values of the amylase content of the seed taken as a whole to *converge*; at the end of three and a half days the *divergence* as exhibited by material from air-dried or steeped seeds is very considerably diminished; at the end of six days the values are of practically the same order of magnitude, such divergence as is still existent being almost certainly due to the individuality of the seeds taken for examination.

The results comprised in the table which follows throw some light on the relative distribution of amylase in the structural parts of the seed, and moreover indicate where the divergence mainly exists, namely, in the inner endosperm tissue.

The interesting fact, exemplified not only here but in the table which follows, is brought to light, i. e. that the amyloclastic capacity of the embryo is greatest as measured by the *direct* method of digestion; the values afforded by the aleurone layer tend to approximate, and those for the whole seed, whether determined by direct experiment or calculation, also show a similar tendency.

TABLE XLVI.

Amylase Content of (*a*) Structural Parts from Germinated Seeds, (*b*) Intact Germinated Seeds.

Germination period, 4 days.

Digestion method.	*Embryos.*	*Aleurone layers.*	*Inner endosperms.*	*Total.*	*Intact seeds.*
	Amylase per 20 *objects per hour (equivalent to mg. Cu).*				*Amylase per* 20 *objects per hour (equivalent to mg. Cu).*
Papain	232	2405	8924	11561	10550
Auto	310	2560	5742	8612	8846
Direct	465	1552	4190	6207	6751

A repetition of these characteristic features is shown by the experimental data embodied in the table which follows :—

TABLE XLVII.

Amylase Content of (*a*) Structural Parts of Germinated Seeds, (*b*) Intact Germinated Seeds.

Germination period, 7 days.[1]

Digestion method.	*Embryos.*	*Aleurone layers.*	*Inner endosperms.*	*Total.*
	Amylase per 20 objects per hour (equivalent to mg. Cu).			
Papain	838	2075	9820	12733
Auto	788	2874	8143	11805
Direct	2857	3849	5793	12499

The inverse order of the magnitude of the results in the case of embryos and aleurone layers, and the convergence of results as regards the inner endosperm, are again demonstrated.

The general conclusion to be deduced from these essays is that the *marked divergence* in the amyloclastic capacity possessed by air-dried and steeped seeds, and material derived from them, following the method of digestion employed, tends to disappear, as shown by parallel investigations of similarly prepared germinated seed material from them.

The other outstanding fact of significance is that papain-digestion is responsible for lower results than direct digestion in the case of embryos and aleurone layers.

A short survey of the results furnished by the data as determined by papain-digestion, comprised in the foregoing tables, relative to the amyloclastic capacity of the intact seed, whether determined by experiment or arrived at by calculation, will suffice to show the few deductions of importance concerning the question of the sources of the amylase present or of the relative amounts of amylase in the inner endosperm.

When, however, we turn to the data derived from the separate examination of the amyloclastic capacity of the anatomical parts of the *endosperm* taken from the seed at various stages, during the progress of germination, important conclusions may, if certain assumptions are made, be put forward.

We have seen that papain-digestion, if it does not afford a means of arriving at the maximal amyloclastic capacity of the inner endosperm, at least furnishes what appears to be the 'limiting' values for that capacity, and as such we may with justification employ it tentatively as a basis in an

[1] Investigation of the inner endosperm tissue beyond this stage is rendered irregular and uncertain by reason of the loss of a considerable portion of substance during desiccation on absorbent paper. This applies to circumstances in which the progress of the germinative processes has reduced the residual tissue to a semi-fluid mass. Moreover, at this point considerable diminution of material arises through exercise of the absorptive functions of the embryo.

attempt to evaluate the amounts of amylase which are contributed by the embryo and aleurone layer during the phases of germination cited in the preceding tables.

The inner endosperm during the progress of germination is regarded as the recipient of amyloclastic enzymes which arise as secretions of the embryo (scutellar epithelium) and aleurone layer, both of which tissues have been shown by direct experimental evidence to be glandular in function.

The inner endosperm possesses the capacity of augmenting its amylase content. The tissue is probably non-living, and is unable to deplete itself. This phenomenon occurs indubitably when the aleurone layer forms an integral part of the endosperm, but does not when the *vital* activity of the aleurone layer is annulled, and these supplementary facts are regarded as supporting the assertion that the aleurone layer is living and secretes the enzyme which principally conditions depletive change. Other experimental evidence points to the same conclusion. The assumption, therefore, that part of the amylase found in the inner endosperm is secreted and excreted by the aleurone layer receives justification, and similarly it can be demonstrated that the embryo also contributes a share.

If we assume that the papain-digestion value for the inner endosperm, after removal from the steeped seed, furnishes the *limiting* value of its amyloclastic capacity, and further assume that it represents its finite augmentative capacity (an assumption justified by the evidence adduced for regarding it as not representing a *living* tissue), then we can calculate approximately, from the increase of amylase which takes place in this tissue during certain phases of the germinative process, the relative amounts of amylase derived from the combined secretory activities of the embryo and aleurone layer. These are shown in the following table :—

TABLE XLVIII.

Relative Amounts and Sources of Origin of Amylases present in Inner Endosperms of Germinated Seed.

Papain-digestion.

Amylase initially present in inner endosperm from steeped seed.	*Amylase present in inner endosperm after days of germination.*	*Amylase derived from embryo and aleurone layer.*
Amylase per 20 objects (equivalent to mg. Cu).		
(1)	(2)	(3)
5000	4 days 8900	4900
5500	7 days 9800	4300

The values given under columns (1) and (2) represent the amounts of enzyme, as determined by papain-digestion, present in the inner endosperm initially, and after four and seven days' germination respectively. The

amount of enzyme in the inner endosperm of the germinated seed is taken as the sum of that furnished by this tissue, plus that derived from the embryo and aleurone layer. The values given in the final column (3) are the differences obtained by subtracting the values of column (1) from column (2). The amounts of amylase contributed by the aleurone layer and embryo calculated on this basis are by no means insignificant, for they amount to roughly 45–55 % of the amylase present in the inner endosperm taken at the periods of germination indicated.

The assumption has been tacitly made, however, that the amylase of the inner endosperm and that derived from the aleurone layer and embryo are identical in their nature and amyloclastic attributes, and it has been further assumed that the amylase which originates in the inner endosperm undergoes no reduction in amount as the result of its activity. This cannot, however, be regarded as correct in the light of the experimental evidence afforded by the study of the action *in situ* of the enzyme, which is pre-existent and accumulates in the inner endosperm, or of their behaviour towards gelatinized starch. In short, the inner endosperm appears to be an exchequer which receives enzymatic contributions from the active secretory tissues, and also possesses the power of augmenting its enzyme content autonomously. But the currency it receives is of more value, especially as far as its internal transactions are concerned, than that which it originates itself, and for this reason estimations of the amount of amylase a tissue (such as the inner endosperm) is capable of augmenting are of subordinate significance in relation to the question of endospermic depletion, unless it can be definitely shown that the enzyme possesses a capacity of attacking the starch reserves placed at its disposal, equal or superior to that possessed by the 'secretion' variety elaborated by the embryo and aleurone layer.

The estimates given of the contributory share derived from the aleurone layer and embryo are by no means over-estimated, for there is evidence to show that the amylase of the inner endosperm, when it does manifest its comparatively feeble powers of attack on endospermic starch grains, probably undergoes either inactivation or destruction. And if this phenomenon also takes place in the inner endosperm during the march of the germinative processes, then the amounts of enzyme contributed by the veritable secretory tissues (the scutellar epithelium and aleurone layer) are probably greater than those given in the above table; in other words, the values given tend to under- rather than over-estimate the amount of amylase from these sources.

XV. Investigation of Amylase Content of the Tissues of Tropaeolum by Direct, Auto-, and Papain-digestion Methods.

Determinations of the amyloclastic capacity of embryos (seedlings) and aleurone layers from barley at certain stages of germination having shown, contrary to the experience with ungerminated barley, that auto- and papain-digestions of these objects invariably gave lower values than by the direct method of digestion, at once raised the question whether the amylase present in the tissues of a typical phanerogamous plant, such as *Tropaeolum*, and presumed to consist wholly of the 'translocation' variety, would comport itself in a similar fashion. If so, then certain considerations might with justification be advanced regarding the possible nature and conditions of existence of the pre-existent amylase or its possible antecedent in the inner endosperm of the grain of barley.

A quantity of freshly gathered plants of *Tropaeolum* were procured, dissected into petals, stems, and roots,[1] desiccated for forty-eight hours at 30° C., and afterwards separately investigated for amylase by the direct, auto-, and papain-digestion methods.

Experimental and corresponding control digestions were carried out as follows:—

The desiccated material was first finely pulverized in a mortar, then passed through a finely-meshed horsehair sieve, and quantities of material taken from the bulk of 0·3 grm. were placed in each of a series of conical Jena flasks and afterwards 10 c.c. of either plain distilled water (auto-digestion) or of a 0·5 % or 1 % solution of papain (papain-digestion) were pipetted into each flask.

The flasks and their contents were then placed in a thermostat at 30° C., and after the addition of an antiseptic and tightly corking, the predigestion was continued for twenty hours.

The contents of the control predigestions were then arrested by boiling, cooled, brought to the temperature of the thermostat (30° C.), a fresh addition of antiseptic made, and 15 c.c. of soluble starch solution (equivalent to 25 c.c. at 3 grm. starch solids per 100 c.c. of solution) were added to both experimental and control flasks, and the starch digestions thus established were continued for twenty hours at 30° C.

A similarly constituted series of experimental and control digestions (direct digestions) were carried out simultaneously with the above by adding directly 25 c.c. of 3 % starch solution to each 0·3 grm. of finely-

[1] Brown and Morris: Chemistry and Physiology of Foliage Leaves. Journ. Chem. Soc., 1893, vol. 63, p. 634. After desiccation the stock material was conserved in a desiccator over H_2SO_4.

ground tissue ; the time and temperature of digestion being identical with those of the preceding series, viz. twenty hours at 30° C.

Finally, experimental digestions in all the series were arrested by boiling their contents, care being taken subsequently, in those in which chloroform served as the antiseptic, to expel this reagent. After cooling, diluting to 100 c.c., and filtering, duplicate copper reductions were carried out on the filtrates in the usual manner.

The following table comprises the results yielded by digestions of the leaves of the plant :—

TABLE XLIX.

Amylase Content of Petals of Tropaeolum.

Digestion method. *Antiseptic.*	0·5 % *papain.*	1 % *papain.*	*Auto.*	*Direct.*	
	Amylase per grm. of tissue per 20 hours (equivalent to mg. of Cu).			(*a*)	(*b*)
Chloroform	446	384	446	1314	1261
Toluene	446	396	471	1339	1389
Nitrobenzene	434	396	496	1314	1289

The experimental conditions, as the above table indicates, were varied by the employment of three different antiseptics and two different concentrations of papain. Direct-digestion results were so markedly different in order of magnitude from those of the other methods that a second series (*b*) was carried out in order to be doubly sure of the result.

Obviously the amylase in an actively metabolizing tissue like that of the plant examined does not presumably exist in the latent condition. The behaviour of the enzyme under the conditions introduced by digestion with papain appears to parallel those found in the case of the seedling and the aleurone layer of barley seed during the advance of the germinative processes.

The greatest divergence occurs in the case of the leaf tissues, as the following results, carried out under experimental conditions identical with those in the preceding experiments, show.

Whether, after the tissue has passed the heyday of its existence, papain-digestion would induce enzymatic augmentation, as it apparently does in the case of the endospermic substance of ungerminated barley, or whether the conditions provided by the leaf tissues *per se* as in direct digestion are superior to those provided by papain, must remain a question for future research to determine.

TABLE L.

Amylase Content of Mixed Root, Stem, and Leaf-Structures of Tropaeolum.

Digestion method.	*Antiseptic.*	*Amylase per grm. of tissue per 20 hours (equivalent to mg. of Cu).*	
Papain, 0·5 %	Chloroform	529	
Auto-digestion	,,	628	
Direct digestion	,,	712	
	Amylase content of Tropaeolum stems.		
		(*a*)	(*b*)
Papain 0·5 %	Chloroform	501	448
Direct digestion	,,	817	975

The primary deduction to be drawn from these results is that the condition of existence of the amylase, especially in the leaf-tissue of *Tropaeolum*, which we may justifiably assume is the 'translocation' variety, and that in the reserve system of the barley grain are materially different. Papain-digestion of the tissues of *Tropaeolum*, unlike papain-digestion of the inner endosperm of the air-dried or steeped seed of barley, does not lead to an increase but to a decrease of amylase, and if we assume that the special function of papain is to release the 'latent' amylase existent in a tissue, then it appears to be permissible to conclude that in actively metabolizing tissues, such as those of *Tropaeolum*, the amylase present exists entirely in the 'free' condition, the term 'free' implying that practically the bulk of the enzyme present is unmasked by direct digestion, and that papain-digestion of such a tissue fails to afford higher results than those furnished by direct digestion.

The term 'latent' enzyme, as employed by Ford and Guthrie, implies that a certain proportion of the enzyme existent in the mature inner endosperm cells of barley presumably becomes associated with a protein complex from which apparently it must be dissociated before it can manifest its active powers.

During the course of the present investigation, other points of view have presented themselves regarding the nature of the 'latent' enzyme of the inner endosperm. The chief function of the amylase existent in the endosperm of barley during the development and maturation of the grain relates to the translocation of starch from one cell of the endosperm to another. When the grain reaches maturity the enzymes and reserves are immobilized, and a considerable proportion of the amylase then probably undergoes a series of changes of which 'latent' amylase represents only a phase.

It seems probable, judging from the comparatively feeble capacity the amylase of the inner endosperm has of attacking any other than the small immature starch grains, the feeble auto-depletive powers of the

inner endosperm, the dead nature of this tissue, and its finite enzyme-augmentative capacity, &c., that the 'latent' enzyme of the barley-endosperm may actually represent an enzyme undergoing step by step a series of complex retrograde changes, the final terms of which may be simply the more or less complex protein reserves of the inner endosperm. If such a view be correct, then the 'latent' amylase of this tissue represents an enzyme which has undergone partial retrogradation, i. e. a complex, one portion of which consists of unchanged amylase, the other of amylase which has undergone transformation into protein reserve substances.

The view propounded merely attempts to suggest a possible explanation of the results which follow on predigestion of the inner endosperm with papain, and to hint at the possible mode of origin of the 'latent' amylase of the resting grain of the inner endosperm.

The results recorded in this section show, at least, that papain-digestion as a means of determining the higher limiting value of the amylase content of a given tissue is not of universal application.

The results are in no way equivocal; direct digestion in all cases yields results which are superior in order of magnitude to those furnished by papain-digestion.

XVI. Summary of Conclusions.

The whole of the experimental work described in this paper, comprising an investigation of

(1) the separate amyloclastic secretory capacities of the embryo and aleurone layer;

(2) the amylase augmentative and depletive capacities of the endosperm and inner-endosperm; and

(3) the demonstration of the possession of a cytoclastic secretory capacity for the embryo and aleurone layer of the seed of *Hordeum*,

has been carried out with the sterilized objects and under rigidly aseptic conditions. Throughout, the aim has been to measure quantitatively the amyloclastic capacities of the three principal anatomical parts of the seed (embryo, aleurone layer, and inner endosperm), in order to ascertain as far as possible the (1) sources of origin of the amylases which accumulate in the inner endosperm during the progress of the germinative processes, (2) their relative magnitudes, and (3) the shares the amylases which are elaborated or generated in these tissues take in the digestion of the starch reserves stored in the inner endosperm.

The following conclusions appear to be justified by a general survey of the results furnished by this investigation:—

1. Direct experimental investigation of the amyloclastic secretory

capacities of isolated embryos, aleurone layers, and endosperms, and also of the amyloclastic generative capacity of the inner endosperm of *Hordeum*, by means of cultures of these objects on variously constituted media and substrata, and subsequent determination of the amounts of amylase in the culture medium and objects or of the products of amyloclastic action, shows—

(*a*) Both embryos and aleurone layers possess an amylo- and a cytoclastic secretory function.

(*b*) These secretory functions are completely arrested and annulled when these objects are subjected to the action of anaesthetic reagents such as chloroform and nitrobenzene. This result, in the case of the aleurone layer, is regarded as evidence that this tissue possesses vitality.

(*c*) The inner endosperm, under varying conditions of experiment described, possesses the capacity of augmenting its amylase content. This augmentative capacity, however, does not represent a process of veritable secretion (the amyliferous cells are not secretory in function), and, in contradistinction to the secretory capacities of the embryo and aleurone layer, is not arrested or diminished by the action of chloroform or nitrobenzene. This latter fact, viz. the capacity of a tissue to augment its enzyme content as readily and to the same (or a greater) extent both in the presence and absence of reagents which are acknowledged to be fatal to living protoplasm, is taken to signify that (1) the inner endosperm represents a mass of non-living tissue, and that (2) its behaviour under the conditions described is such as a dead mass of enzyme-containing tissue would exhibit.

2. Comparative experiments with isolated endosperms and inner endosperms under identical conditions of experiment, ample provision being afforded for the ready outward diffusion of the products of digestive action, show that—

(*a*) Isolated endosperms possess the capacity of inducing the complete auto-digestion and auto-depletion of their storage contents, the phenomena observed being the gradual reduction of the mass of the inner endosperm, the appearance of reducing carbohydrates in the experimental substratum, dissolution of cell walls and cytoplasm of the amyliferous cells, ready separation of the aleurone layer from the sub-aleuronic tissue, and characteristic erosive dissolution of the starch grains.

(*b*) Isolated inner endosperms are incapable of inducing auto-digestive and auto-depletive processes in any way comparable with those observed in the case of endosperms, the phenomena observed

resulting in a very small reduction in the mass of the inner endosperm and the appearance of smaller amounts of reducing sugars in the culture substratum. There is, moreover, an entire absence of any obvious evidence of cell-wall or cytoplasmic disintegration, and the dissolution of starch, which appears to be confined to the more minute grains, is unaccompanied by any signs of erosive dissolution.

Although augmentation of amylase invariably occurs in both types of objects, that of the endosperm is superior to that of the inner endosperm; yet in the former complete depletion occurs, while in the latter it only advances to a stage which falls very far short of complete depletion. In other words, no proportionality or apparent relationship exists between the enzyme-augmentation capacity of the inner endosperm and the amount of self-digestion it is capable of inducing in its starch reserves as they exist *in situ*.

The capacity which a tissue such as the inner endosperm may possess of augmenting enzyme in its cells is of subordinate importance for the question of endospermic depletion, unless it can be shown that the enzyme it generates is capable of attacking the starch reserves as they exist *in situ* in the inner endosperm of the steeped seed. The fact that the amylase of the resting grain or of the inner endosperm of the steeped seed (which are regarded as being identical) readily liquefies and hydrolyses starch paste cannot be accepted as a criterion of the possible rôle this enzyme may play in endospermic depletion.

3. Comparative experiments with endosperms and inner endosperms subjected during the entire period of culture to the action of chloroform or nitrobenzene, and comparison of the results with those of similarly conducted non-anaesthetized objects (cf. 2), demonstrate very clearly that—

(*a*) the augmentation of amylase by the endosperm undergoes marked reduction, being reduced to the same order of magnitude as that of the inner endosperm;

(*b*) the augmentation of amylase by the inner endosperm is unaffected, i.e. it is of the same order of magnitude as that of non-anaesthetized inner endosperms;

(*c*) anaesthetized endosperms, unlike non-anaesthetized endosperms, are incapable of inducing complete auto-depletion of their reserve storage materials. There is an entire absence of erosive dissolution of starch grains, of cytoclastic action; the aleurone layer remains closely adherent to the underlying tissue, and the amounts of reducing sugars found in substrate and objects are of the same order of magnitude as in experiments with anaesthetized and non-anaesthetized inner endosperms.

The above results are interpreted as being directly due to the

suppression of the secretory functions of the aleurone layer, and are regarded as confirmatory of the experimental evidence furnished by direct examination of the secretory functions of the isolated aleurone layer.

In short, endospermic depletion, as it occurs in the intact seed, is principally conditioned by the action of enzymes secreted by the aleurone layer and embryo; in the isolated endosperm, by the aleurone-layer secretions, and in both cases the principal rôle is due to the aleurone layer; the amyloclastic enzyme pre-existent in the inner endosperm and that which it generates is adjunctive.

4. The amyloclastic enzymes secreted by the embryo and aleurone layer are identical, and are distinct from that existent and generated by the inner endosperm.

The columnar epithelial and aleurone-layer cells, although morphologically different, possess similar physiological functions. The view, based on this fact, is advanced:—the aleurone layer is the analogue of the columnar epithelium, and this analogy suggests that the endosperm of *Hordeum* represents an abortive or undifferentiated embryo, and consequently, on biochemical grounds, the phenomenon of 'double fertilization' or 'triple fusion' possibly occurs in *Hordeum*.

5. Both auto- and papain-digestion of ungerminated barley substance lead to considerable augmentation of its amylase content or its amylolytic activity over and beyond that which follows on direct digestion.

Similarly conducted comparative digestion experiments with material from intact seeds and with material from seeds placed under germination conditions show that—

(1) The values of the amylase content of intact seeds as determined by the three methods of digestion tend to converge as the period of germination advances.

(2) The values of the amylase content of parts of the seed as determined by the three methods of digestion vary. Thus:—

(*a*) the embryo throughout gives higher values by direct than by either papain- or auto-digestion;

(*b*) the aleurone layer at first gives lower and afterwards higher values by the direct method of digestion;

(*c*) the inner endosperm throughout gives lower values by the direct method, but the divergence of results tends to diminish as germination proceeds.

Examination of the amylase content of the leaf and other organs of *Tropaeolum* by the three methods of digestion shows that the direct method invariably yields higher results than either the papain- or auto-digestion method. This result implies that in actively functionary tissues as opposed to the reserve storage organ of *Hordeum*, the amylase is present wholly in the 'active' or 'free' condition, and further indicates that papain-digestion,

as a means of determining the amylase content of a plant organ or tissue, is probably not of wide application.

6. Cane sugar inhibits the secretion of amylase by the embryo, dextrose diminishes it, but invert sugar has apparently no influence on the phenomenon.

7. The suggestion that the amylase which arises as secretions of the embryo and aleurone layer may activate that of the inner endosperm is not supported by experimental evidence.

In conclusion, I desire to express my deep thanks to Professor Adrian J. Brown, F.R.S., for his generous and helpful advice, guidance, and criticism, and also for the many facilities he has afforded me during the conduct of this investigation. My best thanks are also due to Messrs. T. H. Pope, B.Sc., and Ch. Evans, B.Sc., who have been good enough from time to time to offer many valuable suggestions and criticisms.

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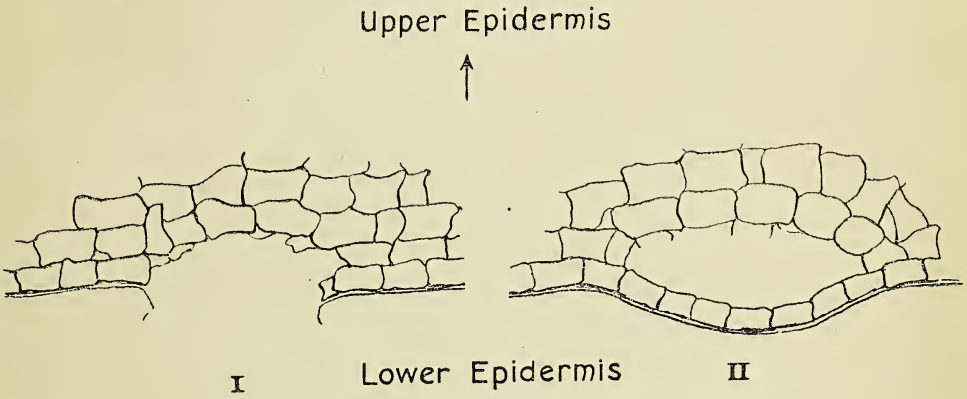
NOTES.

GRAPE SUGAR AS AN EXCRETION IN PLATYCERIUM.—Nectarial tissues are by no means common among the Vascular Cryptogams, their presence having been only observed in the genera *Cyathea*, *Hemitelia*, *Angiopteris*, and *Pteridium*;[1] their significance remains as yet obscure.

Specimens of *Platycerium grande* in the Tropical Ferneries at Kew afford another instance of nectar-excretory tissues among the Ferns, where in the early hours of the morning (6–9 a.m.) the lower surfaces of the young barren fronds or 'mantle leaves' are copiously studded towards their tips with drops of liquid, which disappear later on in the day, and subsequently wholly at the maturation of the fronds.

In *Platycerium alcicorne*, the Stag's-horn Fern, and *P. biforme*, these exudations occur to a lesser extent, and occasionally on the fertile fronds as well.



These drops have a sweetish taste, and on testing with Fehling's solution yield the brick-red precipitate characteristic of grape sugar; the presence of ants in their neighbourhood primarily suggested the test.

A microscopic examination of the lower surface of the 'leaves' reveals the existence of stellate hairs with pointed ray cells, and others similarly branched with obtuse ray cells, which latter appear glandular. Between these hairs, slight rounded elevations correlated with internal lacunae, which show no definite epithelial layer, but contain a liquid excretion presumably, are visible.

Occasionally these lacunae are below the epithelial layers, but it sometimes happens that the epidermal cells are ruptured, when the lacunae are exposed (Figs. I

[1] Engler and Prantl: Pflanzenfamilien Filicales I, iv, 1899, p. 66.

and II); in the former instance the excretion is apparently able to pass through the cell-walls, as in the case of *Datura*.[1]

Although these sugary excretions are to be regarded primarily as of the nature of waste products, nevertheless they may have a subsidiary function to perform in attracting insects whose presence may be of benefit to the plant.

The varied instances of 'Myrmecophily' have been ably expounded by different authors, and Ridley[2] has alluded to *Platycerium biforme* in this respect. Its 'mantle leaves', with their concomitant of débris, constitute an ideal home for ants in their native habitat, and these sugary excretions, presuming they occur in nature, only regard as an adjunct to an interesting case of mutualism.

R. DÜMMER.

Royal Botanic Gardens,
Kew.

[1] Strassburger, Textbook, Engl. Trans., p. 191, 1903.

[2] Ann. Bot., vol. xxiv, 469, 1910.

CORRECTION BY PROFESSOR BOWER.—There is a sentence in my paper 'On Medullation in the Pteridophyta' which I desire to amend. It is on p. 573, line 17 of the present volume, and it runs as follows:—'The fact of solenostelic structure in such a Fern as *Pteris aquilina* can have no direct bearing upon questions of their medullation, &c.'—that is, the medullation of seed plants. This sentence as it stands may be a stumbling-block to purists in description. It will better stand thus:—'The fact that solenostelic structure exists in the Pterideae, and that a modified derivative of that structure is seen in *Pteris aquilina*, &c.' The argument is unaffected by the change. What is intended is to show that such a type as *Pteris aquilina*, or indeed any of the Pterideae, can be no guide directly to the phyletic origin of the structure seen in seed plants unless it can be shown that the Pterideae had a common ancestry with the seed plants compared, and that that ancestry was already medullated and solenostelic before the two phyla were segregated. Without such evidence, comparison can bring to light nothing more than distant analogies of structure. It is obvious that the argument remains the same whether the Pterideae are quoted as a whole, or a specific case, such as *Pteris aquilina*.

It may be added that phyletically the Pterideae are not a very primitive type of Ferns, while in anatomical as well as in certain other characters *Pteris aquilina* is itself an advanced representative of the family.

Glasgow,
August, 1911.

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.
LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

The general plan of the work may be gathered from Part I, which was published in 1908 as a royal 4to volume of 211 pages. In it are described the following twelve types of floral structure, selected from familiar garden flowers that bloom in the early part of the year (January–April):—

Helleborus niger . .	Christmas Rose.	*Viola odorata*	Sweet Violet.
Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant.
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

The description of each type is illustrated by a full-page coloured plate, giving an accurate longitudinal section of the flower, and by a black-and-white plate giving the inflorescence, the floral diagram, and other structural details. As each subsidiary species has also a coloured plate allotted to it, the volume contains no less than forty coloured and fourteen uncoloured plates, in addition to a large number of figures, chiefly developmental, included in the text. It can be obtained at the original price of £1 1*s.* net by subscribers to Part II.

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