ANNALS OF BOTANY

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The Formation of the Capillitium in Certain Myxomycetes.

BY

R. A. HARPER AND B. O. DODGE.

With Plates I and II.

THE nature of the capillitium as an intraprotoplasmic secretion was first recognized by Strasburger (43), who noted the fundamental differences between the method of its formation and that of the structurally and functionally similar elaters of the Liverworts, &c. Strasburger described the capillitium of *Trichia fallax* as originating in vacuolar spaces in the cytoplasm which elongate and take on the tubular form of the young capillitial threads. He described the formation of the wall and spiral thickenings as due to the deposition of granules, which he characterized as microsomes of the membranogenous type, such as he believed are also found in the formation of the cell-plate in cell-division in the higher plants. The essential point in his description is that the spirally thickened capillitial threads are not elongated cells, but are intracytoplasmic secretions or depositions, and in this he has been confirmed by subsequent observers so far as they have considered the point. Strasburger does not figure the earlier stages in the appearance of these capillitial vacuoles nor their elongation to form tubular threads.

The examination of material prepared for the study of nuclear division and spore formation led to the discovery of well-marked aster-like figures at certain stages in the formation of the capillitium, which seem to throw light on the general nature and functions of fibrillar structures in the cytoplasm. Such figures were first observed by Miss A. L. Dean, working in the botanical laboratory of the University of Wisconsin, and a preliminary report on the evidence that these figures are due to cytoplasmic streaming, and are to be associated with other fibrillar structures due to streaming, such as are found in the storage cells of *Pyronema*, &c., was made at the meeting of the Botanical Society of America in 1907 (20). These figures were described as aster-like radiations about the circular cross-section of the forming threads.

Kränzlin (28), on the basis of studies on *Oligonema nitens* and *Arcyria*, has described the capillitial threads as arising from centrosomes, and has

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endeavoured to find an analogy between the function of the centrosome here and its function as a blepharoplast in the formation of the cilia of motile male cells, &c., or as the centre of a system of astral rays in the formation of the bounding membrane of the ascospore. According to Kränzlin certain nuclei start to divide but form only a unipolar (heteropolar) spindle. The centrosome of this heteropolar spindle becomes an 'elateroplast' and marks the starting-point of a capillitial vacuole, and becomes thus the initial organ in the formation of a capillitial thread. The rays tend to disappear with the full development of the vacuole, and are probably to be considered only as the 'visible expression of the chemical relations between the expanding vacuole and the cytoplasm which surrounds it'. Miss Kränzlin's conception is that the capillitial threads are to be phylogenetically derived from flagella.

Our studies have been extended to a number of forms, with the result that everywhere these radial fibrillar systems have been found at a definite stage in the development of the capillitium.

The figures in *Hemiarcyria clavata* and *Trichia* fix readily and stain sharply, and may be taken as typical for forms having a thread-like hollow capillitium with more or less spiral or annular thickenings. Our material was fixed in Flemming's weaker solution and stained with the familiar triple combination, safranin, gentian violet, and orange G, and with iron-haematoxylin.

The creeping out and development of the sporanges of *Hemiarcyria* is only a matter of a night or even a few hours under favourable conditions. Our material was largely obtained from specimens grown on rotten logs kept moist in a Wardian case in the greenhouse, so that material of known age could be fixed at all stages of development. It is not altogether easy to get complete series of stages from single collections of material in the field.

It is also of the greatest importance that the material be fixed at once without unnecessary jarring or disturbance. Jarring causes the most palpable disorganization of the protoplasm, and the resulting figures, while not easy to characterize in a word, leave no question as to their abnormality. This is especially true in the case of the developing sporanges of *Stemonitis*.

The formation of the capillitium begins practically as soon as the sporange has reached its full size. It precedes spore formation, and there is no evidence of the initiation of progressive cleavage before the capillitium is completely formed.

Prior to the formation of the capillitium the cytoplasm is rather homogeneous, the nuclei are evenly distributed, and there are no large vacuoles, though the sponge-like network of the cytoplasm may be more open towards the centre of the sporange. In *Trichia* and *Lycogala* at this stage we have observed threads and fibrils of considerable length irregularly distributed in the cytoplasm (Pl. II, Fig. 6). In some radial sections of quite young sporanges of *Hemiarcyria*, these fibrils form a series running from the base and centre of the sporange outwards towards its periphery as if they marked the path of currents flowing into the sporange from the plasmodium below.

As Strasburger (43) described, the formation of the capillitium begins with the appearance of vacuoles of oval or irregular outline, which early become connected together to form series and anastomosing systems of rather angular openings in the cytoplasm. Such stages are shown in Figs. 8, 9, 10, 11, and 12. Fig. 11 shows eight such connecting vacuolar openings which are irregularly three-cornered in outline, and are connected by more or less narrow tubular branches. The cytoplasm immediately surrounding these vacuoles becomes very dense, with many fibrils more or less radiately arranged. The nuclei, as noted, are at first rather evenly distributed through the cytoplasm, but simultaneously with the appearance of the vacuoles they begin to take up a characteristic position which persists through the whole process of the differentiation of the capillitial threads. They at first move away from the vacuoles to a rather constant distance, equal to about three or four times their diameter and just at the surface of the denser layer of cytoplasm. For a time their distribution further out in the intervacuolar spaces seems to remain unchanged, especially in *Hemiarcyria* (Figs. 13–15). Later these outer nuclei seem to migrate also, moving inward toward the vacuoles, so that they are all finally gathered in quite a definite layer around the forming capillitial threads (Figs. 16–19). Between this nuclear zone and the vacuoles the cytoplasm is quite dense; beyond the nuclei it is much more openly reticulated in its appearance (Figs. 8–10).

The nuclei have evidently gathered about the vacuoles in which the capillitial threads are to form, but appear also to be crowded back in some degree out of the denser plasm which immediately surrounds the vacuoles. As noted, this characteristic relation between the distribution of the nuclei and the position of the forming capillitial threads persists till the threads have attained their normal form and diameter. The vacuoles are at first rather angular and connected by narrow anastomosing extensions into series which wind tortuously through the cytoplasm in the fashion of the future capillitial threads (Figs. 7 and 8). If the mature capillitium is to consist of simple unbranched threads, as in *Trichia*, the vacuoles form single series, or possibly a single vacuole may elongate to form a thread; if the capillitium is to form a reticulated net the connexions of the vacuoles are formed accordingly, i. e. a single vacuole may become connected with three others instead of with two only (Figs. 9–11). The vacuolar membranes show no special thickening or differentiation at this time. They are apparently ordinary tonoplastic boundaries enclosing non-stainable sap like that in ordinary vacuoles. In section the arrangement and relations of the vacuoles suggest somewhat vaguely that of the cleavage vacuoles in the sporanges of *Pilobolus* or *Rhizopus*. In reality, however, their relations are quite different. The cleavage vacuoles become connected by extended knife-edge cleavage furrows, while these capillitial vacuoles anastomose, as noted by tubular extensions, which soon come to have the constant diameter of the future thread. The pathway of the threads through the cytoplasm and their cavities may be said to be already determined at this stage. The series of connected vacuoles form in fact an extremely nodular thread, the vacuoles constituting a series of vesicular expansions on a continuous vacuolar opening whose course through the cytoplasm is roughly that of the future thread (Fig. 11). At this stage the system of anastomosing vacuoles may be compared in its form to the mature capillitium of *Physarum*, *Badhamia*, and *Tilmadoche*, and the evidence is strong that the latter represent a more primitive stage through which the capillitium of *Trichia* and *Hemiarcyria* passes in its development. The stages immediately following involve what may be characterized as the smoothing up of this series of vesicular expansions into the tubular capillitial thread of practically constant diameter throughout its whole extent. All stages in this process of equalizing the diameter of the capillitial tube can be found in great abundance. It consists for the main part apparently in the reduction of the transverse diameter of the vesicles with a corresponding increase in their length. This lengthening of the vacuoles in the axis of their connecting anastomoses and the consequent increase of the total length of the series lead thus to the still more tortuous windings of the ripe capillitial threads. The narrow connexions of the original vacuoles soon reach the size of the capillitial thread, and the greater part of the change in form of the original series of vacuoles seems to go directly to increase their length by reducing their transverse diameters. The capillitial thread is nodular and uneven in diameter for a considerable period, as is shown in Figs. 8, 9, 10, and 11. As it gains its permanent form as a contorted tubular opening through the cytoplasm, its membrane becomes more distinctly differentiated as the wall of the future capillitial thread. From an early stage deeply staining granules of various forms are abundant in the region of the vacuoles (Fig. 1). They are especially abundant in *Hemiarcyria*. They may be on the surface of the thread or some distance from it, and may be single or in groups or series (Figs. 1–4). They tend to take the safranin stain quite characteristically. It is possible that the process of fixation disturbs the position of these granules, and that normally they all lie directly on the wall of the capillitial thread. These granules should perhaps be identified with those described by Strasburger, but it seems more probable that the idea of membranogenous corpuscles, on which he lays such emphasis, was at least intended to refer to something much more minute and more evenly distributed than these relatively large and conspicuously differentiated bodies.

The most striking and perhaps the most characteristic feature in the whole process of the formation of the capillitium are the fibrillar asters which appear about the capillitial vacuoles. They consist of delicate fibrillar strands, extending from the forming capillitium in all directions through the surrounding cytoplasm. These strands are quite sharp, clean-cut filaments which under low magnifications seem in general to extend radially outwards from the forming capillitial tube. Closer observation shows, however, that they are really oriented on the above-mentioned granules (Pl. I, Figs. 1–4). When these granules are distributed evenly on the walls of the tube, the fibrils appear most nearly radial with reference to the forming thread (Fig. 2). Cases are found, however, in which one or several fibres are centred on a granule at some distance from the tube (Fig. 1). It is such cases as these, probably, which led Kränzlin (28) to regard these radial systems as actual astrospheres about genuine centrosomes. As a matter of fact the fibres are quite commonly not oriented on the centre of the capillitial thread. In many cases their course is almost or quite tangential to the surface of the capillitial tube (Figs. 3 and 4). This is due, as noted, to the fact that they are really oriented on the granules. A granule on the surface of the capillitial tube may be the centre for rays that run in several directions from it, only one of which is radial to the tube.

We have not seen cases in which the rays form a complete aster running in all directions from a granule, nor cases in which they are oriented on a granule lying at the centre of the cross-section of a capillitial thread such as are represented by Kränzlin (28) (Figs. 14–16, Taf. IV, and Text-fig. VII, 4). In our material the capillitial thread in cross-section is a clear circular vacuolar cavity. It may and generally does contain granular material, but this regularly lies free in a clear unstained vacuolar cavity. The threads never extend through the membrane of the capillitial thread, and the latter is at these stages clean-cut and circular.

In longitudinal sections the orientation of the fibrils with reference to the thread is shown with especial clearness. It is very evident here that they are centred on the granules instead of on the general outline of the capillitial thread (Fig. 2). They appear as irregularly distributed groups along the sides of the capillitial thread, a group of fibres for each granule. In general form they suggest the cytoasters figured by Mead and Morgan (36, 37) as the results of treatment with salt solutions in the cytoplasm of Echinoderm and other eggs. With low magnifications the cross-sections of the capillitial threads at these stages suggest strongly the appearance of astrospheres as conceived by Strasburger (43). The circular section of the capillitial thread represents the centrosphere, and there are frequently granules in the cavity of the thread which may represent one or several centrosomes. These granules are, however, rarely so placed in the centre of the thread section as to really represent a centrosome or microcentrum. With low powers, however, the fact that the radiating fibrils are centred on granules on the walls of the capillitial thread rather than on the general outline of the thread itself is not conspicuous, and the superficial resemblance of the whole structure to the polar view of a karyokinetic figure is very striking.

The fibres of one system may extend through the cytoplasm until they reach and mingle with those from the next adjacent system (Fig. 1). In the region outside the nuclear zone the fibres are less numerous, but those which do extend so far are generally quite thick and conspicuous. They may even appear to taper and thin out as they pass into the denser cytoplasm inside the nuclear zone. The direction of the fibres is apparently not influenced in any way by the nuclei. They run past and among the nuclei without showing any evidence of connexion with or origin from them. The nuclei show no conspicuous centrosomes at this stage, and there is no possibility of confusing the appearance of the capillitial asters at this stage with any such phenomenon as ‘ heteropolar nuclear division ’ or anything of that sort. In the triple stain the fibrils perhaps tend slightly to take a violet colour. As noted, the fibres, centred on granules as they are, may run quite tangentially to the surface of the capillitial thread. Sometimes these tangential fibres all diverge from the radial in the same direction and produce an appearance as if the capillitial thread had twisted on its long axis. The differentiation of the membrane of the capillitial thread goes on rapidly during this period, while the radiating fibrils are conspicuous and the nuclei maintain their zonal distribution.

The conditions suggest rather strongly that the fibrils represent cytoplasmic streams which are bringing in material for the formation of the capillitial wall and its thickenings. It would seem in this case that the above-mentioned granules on which the rays are centred ought to be distributed quite regularly on the boundary of the forming capillitial thread. This, however, is not the case, and the granules cannot be regarded as at all fully representing the material brought in, even in case the fibrils are interpreted as having their origin as streams of cytoplasm.

The nuclei maintain their peculiar zonal distribution about the capillitial threads until the spiral thickenings of the latter are well outlined. The spirals when first formed are markedly granular. They plainly constitute thickenings formed on the membrane of the capillitial tube, which is faintly recognizable before they make their appearance. Stages can be found when the spirals are mere rows of granules seen only with the greatest difficulty and under the most favourable optical conditions. They seem to form simultaneously along the thread rather than intermittently or progressively. Even after the spirals have become quite distinct their edges can be seen on careful focusing to be made up of rows of granules. The material of the spiral certainly seems to be deposited as granules rather than as laminae. The walls of the young thread and the spirals when well enough developed to show characteristically their affinity for stains take up the orange in the triple stain, as do the young cell-walls in root-tips, pollen grains, &c.

In *Hemiarcyria clavata* there are regularly four left-handed spirals on the capillitial thread (Fig. 5). Just why there should be this number and why the granular material should be distributed in spirals on the membranes of the thread is not obvious from any visible arrangement of the elements involved in their formation. Neither the position nor the arrangement of the nuclei or fibrillar rays suggests any explanation.

The granular material in the interior of the capillitial thread (Figs. 1 and 2) becomes less as the wall thickens and the spirals appear, and as the thread matures it practically disappears (Figs. 3 and 4). There is, of course, no evidence that granular material as such passes from the interior of the thread into the forming spirals. We are inclined to suspect that the stainable granules in the interior of the thread are precipitation products formed in fixation, and that in the living condition the capillitial cavity contains only materials in solution in the cell-sap. These materials may be used up in the formation of the capillitial wall and spirals so that in late stages no such precipitation products are formed.

At the stage shown in Fig. 5 the fibres have disappeared, the nuclei have lost their zonal distribution, and are scattered irregularly in the cytoplasm as they were before the process of forming the capillitium began. The coarsely staining granules on which the fibrils were centred in the earlier stages have also disappeared, and it seems not improbable that their material has gone to the formation of the capillitial wall and the beginnings of the spiral thickening. At this stage the nuclei appear commonly to be of two types which are conspicuously differentiated from each other by their relative size and the density of their stainable constituents. The larger nuclei are similar in all respects in their appearance to the resting nuclei of earlier stages. The smaller nuclei are dense, show no nucleoles, and appear somewhat like daughter nuclei in early telophase stages (Fig. 5). These smaller nuclei are doubtless those which Kränzlin in 1907 (28) and Jahn in 1907 (25) described as having failed to fuse, the larger nuclei corresponding to Jahn's fusion nuclei. In view of his later observation of nuclear fusions in the first pairs of fusing swarm spores in the formation of the plasmodium, Jahn (27) has withdrawn his earlier interpretation of the presence of nuclei of two sizes at this stage in the development in the sporange. We have seen no division figures at just this stage. A little later, when cleavage is about to begin, they are common. The smaller nuclei here may well be young daughter nuclei as suggested. The appearance of some of them, however, really suggests that they are degenerating. They are also not conspicuously distributed in pairs, as they might be expected to be if they had just been formed by division, and they are more variable in size than might be expected if they represent the same telophase stage.

In their mature condition the spirals are somewhat more strongly developed than they are at the stage shown in Fig. 5, but the main difference is due to the shrinkage which has taken place in the ripening and drying out of the whole sporange. As a result of this drying the lumen of the capillitial tube is quite small, so that the structure of the thread appears to be almost rope-like. The spores are at first uninucleated and thin walled. With the full development of the thickened and sculptured spore-wall the fixation and staining of the nuclei becomes more difficult.

The formation of the capillitium in the Slime-moulds is in many respects a unique process. As noted in their shape and surface markings the capillitial threads of many forms suggest the elaters of the Liverworts. They are, however, in no sense modified cells—the spiral and other types of thickening are on their external surfaces. The nearest analogy to the conditions under which they are formed is perhaps to be found in the relation of the periplasm to the formation of the epispore in the Oomycetes. In both cases we have to do with a multinucleated protoplast which produces in some way a symmetrically sculptured wall about another centre than its own. Little is known as to the real nature of this process by which the epispore is laid down, further than that, as was first shown by de Bary and Strasburger (42), the process is centrifugal from the oospore as a centre just as it is in the case of the formation of the perinium in the Water Ferns. The periplasm disorganizes in building the epispore, while the spore-plasm of the Slime-moulds proceeds at once to cleavage and spore formation.

The deposit of materials in the vacuoles from which the capillitial thread is formed may properly be called a process of intraprotoplasmic secretion, and is no doubt similar to that by which other soluble materials are deposited in the cell-sap of vacuoles, and may then crystallize out if the solution becomes sufficiently concentrated. Such crystals are regularly formed in the vesicular nodes of the capillitium of *Tilmadoche*, *Physarum*, and other types, as noted above. It is hard, however, to think of the formation of the spirally sculptured capillitial thread as caused simply by crystallization. We have here an adaptive morphogenetic process of the same type in these respects as we have in the formation of specially thickened and sculptured cell-walls. There seems to be no question also that the wall of the thread is built by appositional growth, though it shows no laminated or stratified structure.

In the fact that a multinucleated mass produces in its interior symmetrically developed and oriented secretion products, the conditions here suggest those in the formation of the siliceous framework of the Radiolarian skeleton as analysed by Haecker (19) and others. It is interesting to note that Haecker is inclined to conclude that the formation and orientation of the Radiolarian endo-skeleton are controlled by so-called 'regulating centres', which may have arisen by multiple central body formation. Such a conception is in striking agreement with Kränzlin's (28) account of the origin of capillitium initials from centrosomes. As noted, however, our observations indicate that the radial fibrillar systems about the capillitial vacuoles are not oriented as Kränzlin described, and do not arise as spindle-poles and asters. As intraprotoplasmic secretion products the capillitial threads certainly have the closest possible analogy with the protozoan endo-skeleton, but the capillitium shows no such specialized orientation as is found in the case of the endo-skeleton.

The appearance of figures so closely imitating the polar asters of the mitotic figure in connexion with a purely vegetative process of secretion, and in no direct connexion with the processes of nuclear or cell-division, affords an opportunity for the examination of their functions and the conditions involved in their formation, free from the assumptions associated with their activities in connexion with the movements of chromosomes, &c. We have referred to the evidence that these figures are due to streaming (20). The fact that the formation of the capillitium implies the deposition of materials in the vacuolar spaces first formed naturally suggests that these more or less radially placed lines mark the pathways by which materials are brought in from the surrounding cytoplasm. The accumulation of the nuclei in a quite definite zone at a rather constant distance from the forming thread may be taken as further evidence of their relation to the metabolic factors in the growth and morphogenetic processes here involved, and suggests that they may in this case be concerned in some way in the production of the material to be used in the formation of the capillitium.

It is clear, as we have shown, that the aggregation of the nuclei in this zone involves the migration of some of them towards the capillitial vacuoles, as well as the crowding back of others out of the dense cytoplasm immediately surrounding the vacuoles. Our preparations give no suggestion as to just how these nuclear movements are brought about. But that the nuclei do come to lie in a definite zone is a most conspicuous fact, and must certainly be conceived as related in some way to the formative processes going on in the capillitial vacuole. It is to be remembered, however, that the radial fibrils in many cases extend between and beyond the nuclei, and hence cannot merely mark lines of flow from the nuclei to the forming threads.

The formation of the capillitial thread membrane with its sculpturings may be conceived as comparable to the ordinary processes of cell-wall formation. The spiral thickenings should really be conceived as forming centripetally with reference to the source of their material, just as in the case of the spiral thickenings in the wall of a duct or elater. Radial fibrillar strands are, however, not present so far as known in connexion with the accumulation of material for the formation of the secondary thickenings of ordinary cell-walls. Recent workers (42, 44) have been inclined to associate the functions of the central spindle with the formation of the cell-plate and new plasma membranes rather than the cellulose wall, but the two processes may both be involved. If the cellulose wall is a dissociation product of the proteid materials of the membrane, the accumulation of materials for both structures should be one and the same process.

The relations of the nuclei to the fibrillar strands and the forming membrane of the capillitial threads, both as to position and as to the processes going on, are strikingly similar to those in cell-division figures of the higher plants, especially at the stage when the central spindle is widening out to fill the whole diameter of the cell and the new fibrils for this increase in its dimensions are being formed. The most conspicuous difference in the two cases is in the fact noted, that the fibrils in the formation of the capillitium extend between and beyond the nuclei instead of ending upon them, as in the case of the central spindle. That the nuclei, on the one hand, and the region of deposition of membrane material, on the other, are in both cases more or less definitely connected by conspicuous fibrillar elements in the cytoplasm must be taken as strongly suggesting that these fibrils represent paths for the transportation of material.

In the case of free cell-formation in the ascus we have excellent evidence that the astral rays in their metamorphosis to form the plasma membrane of the ascospore grow in length by a process of flowage outwards from the centrosome. The figures in *Erysiphie communis* show clearly enough that the whole astral system must be conceived as increasing its extent by the addition of material from the region of the centrosome, and that the latter, with its direct connexion with the chromatin through the nuclear beak, may well be characterized as a point of interaction between the nucleus and the cytoplasm, at which kinoplasmic material is formed (21). It is quite clear that the kinoplasmic material must increase in amount if it is to form a complete boundary for the spore, and the blue-stained boundary layer is at first thicker round the centrosome, and only gradually thins out by the flow of its material over the opposite pole of the spore.

Fraser (13) accepts the conception of the centrosome as the point of origin for the material which forms the boundary of the spore, and characterizes the rays as direct currents set up in the cytoplasm about the centrosome. Later, Fraser and Welsford (14) advance the conception that the material formed at the centrosome is of the nature of an enzyme. Fraser rejects the comparison of the rays in the ascus to cilia, but there is certainly a striking similarity between the astral rays which are formed by the flow of a differentiated substance from the centrosome outwards through the cytoplasm and the fibrillar cilia formed from a blepharoplast on the surface of a cell. In the case of the antherozoids of Ferns and Cycads it has been shown beyond question that the cilia first push out within the cytoplasm, and continue their growth through the plasma membrane into the surrounding medium. That kinoplasmic fibrils may be formed as streaming material, and later become contractile elements, is not inconsistent with any known facts, and seems to be clearly suggested by the mass of data which has accumulated as to the relations of centrosomes, blepharoplasts, and the fibrillar elements developed about them. It seems possible now to include in the category of central spindle fibres, astral rays as seen in free cell-formation and cilia as the outgrowths of blepharoplasts, the further type of radial fibrils about the capillitial vacuoles, for which the evidence, both from their appearance and their probable functions, so strongly favours the view that they represent streams of specially active formative materials.

Faull (10, 11) seems to have failed to understand the account of the movements of the astral rays in the formation of the ascospore just referred to, for the description given (21) is in no way susceptible of the interpretation which he puts upon it. Nothing is said of a simultaneous movement of all the rays of the aster, in which each would keep its relative position to all the others. The figures give no evidence for such a movement. On the other hand, it is distinctly specified that the folding-over movement begins with the rays which extend in the line of the axis of the nuclear beak, and that these rays thus come to crowd upon those next to them. The cone-shaped opening in the aster shows that the movement begins in this way, and that the crowding and fusing which result from this movement accumulate the material for a continuous plasma membrane. Faull (11) admits that the plasma membrane of the spore forms from the centrosome outwards. Admitting this, his own figures give good evidence against his words that the recurved rays and not an imaginary limiting layer, the precursor of the spore membrane, determine the location of and form the boundary for the spore. The relative positions of the rays in his Figs. 62 and 63, Pl. XL, certainly suggest that at least some of them have moved.

A further type of fibrillar structures which may also be due to cytoplasmic streaming are Němec's (38) longitudinal fibrillar systems in the cells of root-tips, to which he assigns the function of transmitting stimuli from the perceptive to the motor region. Haberlandt (18) has attempted to show that such fibrillar structures are due to streaming on the basis of their general resemblance to the appearances, many times described, for streaming protoplasm, and the fact that he was able to observe streaming movement in the central protoplasmic strand of root-tip cells. Unpublished observations by Marquette, which we are allowed to report, show that in the living root-tip cells numerous delicate protoplasmic streams can be seen flowing lengthwise of the cells curving out around the nuclei, &c., their distribution thus corresponding closely to that figured by Němec (38) for his fibrillar systems in the cells of the root-tips of *Allium*, certain Ferns, and other types.

It seems probable that the fibrillar structures described by the Bouins (4) and others are in the same way evidence of the movement of food-stuffs in definite directions in the embryo sac. M. and P. Bouin (3) regard the ergastoplasm as identical with the mitochondria of animal eggs, without changing their opinion as to its fibrillar structure and its relation to the metabolic processes going on in the embryo sac. They accept as a basis of their interpretation of the ergastoplasmic structures in the embryo sacs Garnier's (15) interpretation of the fibrillar elements in the serous gland-cells of animals, which are apparently quite similar in their appearance. It is to be noted, however, that Orman (39), on what appears to be not very adequate evidence, concludes that the ergastoplasm consists of lamellae and not fibrillae.

The barrel-shaped systems of fibrils, which are present in the storage cells from which the paraphyses arise in *Pyronema* (22), afford a case in quite a different connexion in which the suggestion is very strong that the fibrils represent the lines of transportation of food materials from one cell to the next.

The evidence that these radial fibrillar systems are due to streaming seems quite convincing, and that cytoplasmic fibrillar structures in general arise by streaming seems by no means impossible. Bütschli (5, 6) has advanced two conceptions as to the astral figure. First, it may be due to diffusion streams, and, second, it may be due to tensions set up about a region of contraction. The possibility that such figures as we find about the capillitial vacuoles may originate as a result of the pull about a condensation centre must certainly be considered. Such radial systems seem, in fact, to illustrate especially well Bütschli's conception that the visible elements of an aster are due entirely to a rearrangement of the cytoplasmic elements, which results from centrifugal tensions set up about the centrosome. There is no question that the rays in these Slime-moulds are actual fibrils, and when cut transversely appear as points. It is not so certain, however, that such an appearance is inconsistent with the view that they at least frequently lie at the angles of intersection of meshes and alveolar membranes. The fibrils are certainly not entirely free in a homogeneous ground substance. The structure of the cytoplasm in these Slime-moulds is conspicuously reticulated and spongy rather than alveolar alone, but just how the radial fibrils are related to the remaining elements of the spongy colloidal mass is not easy to determine optically.

Bütschli's radial figures, which are formed about air bubbles in cooling gelatine films by the contraction of the air and its absorption in the surrounding medium, suggest a very interesting possible parallel to the conditions and chemical changes taking place in the case of the forming capillitium. The materials of the capillitial wall are presumptively dissociation products of proteid, whether they are conceived as set free by secretion or by direct transformation. If the chemical changes resulting in the production of the solid elastic and hygroscopic capillitial thread out of the watery colloidal cytoplasmic mass involve a reduction in volume we should have tensions set up similar to those produced about the contracting air bubble in the gelatine film. The formation of vacuoles as the initial step in the development of the thread may be regarded as conclusive proof that the production of the solid materials of the wall of the thread involves the setting free of water which at first may contain considerable quantities of dissolved transition products. As noted, these dissolved materials are apparently used up finally in the process of building the thread.

Our knowledge of the chemical character of the cell-walls in the Slime-moulds is quite fragmentary. Wigand and de Bary (2) reported the common tests for cellulose in the case of the inner layer of the wall of the sporanges of *Trichia varia*, *furcata*, and *pyriformis*, and in the spore-walls of *Arcyria cinerea*, *punicea*, and *nutans* and *Lycogala epidendron.* More recent work on the colour reactions of chitin and its derivatives with iodine leaves these results uncertain.

The work of Wisselingh (45) and still earlier authors gives sufficient proof that chitin is a widely occurring constituent of fungus cell-walls, but that cellulose is also characteristically present in certain cases. In the Slime-moulds Wisselingh (45) reports cellulose as present in the spore-walls of *Didymium squamulosum*, chitin in the spore-walls of *Plasmodiophora brassicae*, and neither in the spore-walls and membranes of *Fuligo septica.* Scholl (41) has made careful studies of the methods of isolating chitin from *Boletus edulis*, and there can be no question that the plant and animal material are essentially similar in their chemical characteristics.

Wisselingh (45) believes that chitin and cellulose do not occur together, and gives evidence that other unknown elements may be associated with chitin in fungus cell-walls. Not enough is known as to the actual chemical constitution of chitin to give a basis for a conclusion as to the method of its formation in the capillitial vacuoles, even in case it is found to be an essential constituent of the thread. The available evidence (40), (12), (4 *a*) indicates that fundamental similarities between the method of its deposition and that of cellulose are to be expected. Czapek (8) suggests that glyco-proteids are perhaps to be assumed as the sources of cellulose. As noted, our own observations show that the young capillitial threads have the same selective affinity for orange G as is observable in the young cellulose walls of the higher plants. Conclusions based on microchemical tests cannot be regarded as very convincing. The stages of formation of the cell-walls in the spores, and much more the membrane of the capillitial thread, are not easy to study as compared with the walls of pollen grains, bast fibres, &c. There are, however, at least the three clearly established facts just discussed, which bear on the chemistry of the processes involved in the formation of the capillitial thread.

1. The process is initiated by the liberation of water and the formation of vacuoles.

2. The vacuolar sap at first contains materials in solution, which are apparently precipitated in fixation, and which later disappear and probably furnish material for the capillitial wall and spirals.

3. The spirals are laid down as organized material, in a definite form on the outside of the thread next to the vacuolar membrane, although functionally, as we know from the elaters of the Liverworts, the position of the spiral on the inside of the thread would be also hygroscopically effective.

The possibility that the chemical changes which lie back of these processes may produce volume changes and radial tensions such as Bütschli assumes is certainly to be considered. The question as to whether the polar aster and the whole karyokinetic spindle figure may be regarded as due to streaming, and the further question whether the streams are directed towards or away from the centrosome, were considered by the earliest students of nuclear division (35), and the evidence on both sides has been many times discussed. The most recent papers on nuclear and cell-division throw little further light on the matter. Lundergårdh (32–4) regards with scepticism all fibrillar structures, in the prophases and polar caps especially, as possible artifacts due to poor fixation, since they are not recognizable at once in the living cells. He finds that poor fixation gives more fibres than good fixation, but his elaborately worked out and vague conception of the directing and correlating influence of metabolic changes on the structures and movements involved in nuclear and cell-division is, as he freely confesses, as yet by no means illuminating.

Lawson's (29) conception that the spindle fibres are due to tensions set up in the cytoplasm as a result of the shrinkage of the nuclear membrane and that the fibres have nothing to do with the movements of the chromosomes has been adequately criticized by Farmer (9) and Lundegårdh (34). In the essential point of his conception of the origin of radial systems as a result of tensions developed about a region of shrinkage, Lawson (29), though not referring to him, follows the lines laid down by Bütschli. While the possibility that such an explanation may apply in the case of the aster about the forming capillitial thread must be carefully considered, it alone is plainly inadequate to account for the development of the bipolar spindle, and the author does not attempt to apply it to the movement of the chromosomes.

The more definite form which Hartog (24) has given to the much discussed resemblance of the astral rays and karyokinetic figure to a magnetic field of force has received support in Lillie's (30, 31) contention that by centrifuging the spindle figure may be moved in the cell without distortion of its elements—may in fact change its substance with the displacement of the fields of force without any indication of drift in the material under the influence of the assumed chains of force. It is, of course, not clear in this case just how centrifuging could influence the fields of force. The magnetic analogy is perhaps still to be reckoned with for the bipolar systems of the karyokinetic figure, but the conception of a field of force would certainly not be thought of in connexion with the systems of rays oriented on independent granules, quite irregularly placed as they are in the radial systems distributed along the forming capillitial thread. The nature of the fibrillar systems must of course be interpreted in connexion with their appearance in longitudinal (Fig. 2) as well as in transverse (Figs. 3 and 4) sections of the thread.

Such movements of fibres as occur in the transformation of the radial into the felted zone stage in the pollen mother-cells of *Larix* as described by Allen (1) and others, and even such an extreme case as the folding over of the astral rays, described by Grégoire and Berghs (17) for *Pellia*, to form the spindle, must not, it seems to us, be regarded as inconsistent with the origin of the fibres as streams. The movement of the fibres like cilia and their growth by a flow of kinoplasm from the centre outwards are both well illustrated in the metamorphosis of the astral system into a spore membrane in the ascus.

INDEX TO LITERATURE.

1. ALLEN, C. E. : The Early Stages of Spindle Formation in the Pollen Mother-cells of *Larix*. Ann. Bot., vol. xvii, March, 1903, pp. 281–312, Pl. XIV, XV.
2. BARY, A. DE : Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien. i–xvi + 1–558 pp., Figs. 1–198. Leipzig, 1884.
3. BOUIN, M., et BOUIN, P.: Ergastoplasme et mitochondrie dans les cellules glandulaires séreuses. Compt. Rend. Soc. Biol., vol. lviii, 1905, pp. 916–17.
4. ——————————: Sur la présence de filaments particuliers dans le protoplasme de la cellule-mère du sac embryonnaire des Liliacées. Bibliographie Anatomique, vol. vi, 1898, pp. 1–10, Figs. 1–5.

4 *a*. BRACH, H : Untersuchungen über den chemischen Aufbau des Chitins. Biochem. Zeitzschr., vol. xxxiii, 1912, pp. 468–91.

5. BÜTSCHLI, O. : Ueber die künstliche Nachahmung der karyokinetischen Figur. Verh. nat.-med. Vereins Heidelberg, N.F., vol. v, 1893, pp. 28–41.
6. ——————: Untersuchungen über mikroskopische Schäume und das Protoplasma. i–iv + 1–234 pp., Pl. I–VI + Figs. 1–23. Leipzig, 1892.

7. Chamberlain, C. J.: Mitosis in *Pellia.* Bot. Gaz., vol. xxxvi, July 16, 1903, pp. 28–51, Pl. XII–XIV.
8. Czapek, F.: Biochemie der Pflanzen, 2nd ed., vol. i, p. 708. Jena, 1913.
9. Farmer, J. B.: 'Nuclear Osmosis' and its Assumed Relation to Nuclear Division. New Phytologist, vol. xi, Apr. 1912, pp. 139–44.
10. Faull, J. H.: Development of Ascus and Spore Formation in Ascomycetes. Proc. Boston Soc. Nat. Hist., vol. xxxii, June, 1905, pp. 77–113, Pl. VII–XI.
11. ——————: The Cytology of *Laboulbenia chaetophora* and *L. Gyrinidarum.* Ann. Bot., vol. xxvi, Apr. 1912, pp. 325–55, Pl. XXXVII–XL.
12. Fränkel, S., und Kelly, A.: Beiträge zur Constitution des Chitins. Monatsh. Chemie, vol. xxiii, 1902, pp. 123–32.
13. Fraser, H. C. I.: Contributions to the Cytology of *Humaria rutilans,* Fries. Ann. Bot., vol. xxii, Jan. 1908, pp. 35–55, Pl. IV, V.
14. —————— and Welsford, E. J.: Further Contributions to the Cytology of the Ascomycetes. Ann. Bot., vol. xxii, July, 1908, pp. 465–77, Pl. XXVI, XXVII.
15. Garnier, C.: Contribution à l'étude de la structure et du fonctionnement des cellules glandulaires séreuses. Du rôle de l'ergastoplasme dans la sécrétion. Journ. Anat. et Physiol., vol. xxxvi, 1900, pp. 22–98, Pl. I–III.
16. ——————: Les filaments basaux des cellules glandulaires. Bibliographie Anatomique, vol. v, 1897, pp. 278–89, Fig. 1–13.
17. Grégoire, V., et Berghs, J.: La figure achromatique dans le *Pellia epiphylla.* La Cellule, vol. xxi, 1904, pp. 193–239, Pl. I, II.
18. Haberlandt, G.: Ueber Reizleitung im Pflanzenreich. Biol. Centralbl., vol. xxi, June 15, 1901, pp. 369–79.
19. Haecker, V.: Tiefsee-Radiolarien. Wiss. Ergebn. Deutsch. Tiefsee-Exped. a. d. Dampfer 'Valdivia', vol. xiv, 1908.
20. Harper, R. A.: Figures produced by Protoplasmic Streaming in Fungi and Slime-moulds. Science, II, vol. xxv, Feb. 22, 1907, p. 282.
21. ——————: Kernteilung und freie Zellbildung im Ascus. Jahrb. wiss. Bot., vol. xxx, 1897, pp. 249–84, Pl. XI, XII.
22. ——————: Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp. Ann. Bot., vol. xxiv, Sept. 1900, pp. 321–400, Pl. XIX–XXI.
23. ——————: Sexual Reproduction and Organization of the Nucleus in certain Mildews. Carnegie Inst., Washington, Publ., vol. xxxvii, Sept. 1905, pp. 1–91, Pl. I–VII.
24. Hartog, M.: The Dual Force of the Dividing Cell. Part I: The Achromatic Spindle Figure illustrated by Magnetic Chains of Force. Proc. Roy. Soc. London, vol. B. lxxvi, 1905, pp. 548–67, Pl. IX–XI.
25. Jahn, E.: Myxomycetenstudien. 6. Kernverschmelzungen und Reduktionsteilungen. Ber. deutsch. bot. Gesellsch., vol. xxv, Feb. 21, 1907, pp. 23–6.
26. ——————: Myxomycetenstudien. 7. *Ceratiomyxa.* Ber. deutsch. bot. Gesellsch., vol. xxvi, 1908, pp. 342–52, Figs. 1, 2.
27. ——————: Myxomycetenstudien. 8. Der Sexualakt. Ber. deutsch. bot. Gesellsch., vol. xxix, 1911, pp. 231–47, Pl. I.
28. Kränzlin, H.: Zur Entwicklungsgeschichte der Sporangien bei den Trichien und Arcyrien. Archiv Protistenkunde, vol. ix, 1907, pp. 170–94, Pl. IV + Figs. 1–7.
29. Lawson, A. A.: Nuclear Osmosis as a Factor in Mitosis. Trans. Roy. Soc. Edinburgh, vol. xlviii, Nov. 7, 1911, pp. 137–61, Pl. I–IV.
30. Lillie, F. R.: A Contribution towards an Experimental Analysis of the Karyokinetic Figure. Science, II, vol. xxvii, June, 1908, pp. 907–8.
31. ——————: On the Specific Gravity of the Constituent Parts of the Egg of *Chaetopterus,* and the Effect of Centrifuging on the Polarity of the Nucleus. Science, II, vol. xxvii, June 12, 1908, pp. 905–7.
32. Lundegårdh, H.: Die Kernteilung bei höheren Organismen nach Untersuchungen an lebendem Material. Jahrb. wiss. Bot., vol. li, 1902, pp. 236–82, Pl. II + Figs. 1–8.
33. ——————: Chromosomen, Nukleolen und die Veränderungen im Protoplasma bei der Karyokinese. Beitr. Biol. Pflanzen, vol. xi, 1912, pp. 373–592, Pl. XI–XIV.
34. ——————: Das Caryotin im Ruhekern und sein Verhalten bei der Bildung und

Auflösung der Chromosomen. Archiv für Zellforschung, vol. ix, 1912, pp. 205–330, Pl. XVII–XIX + Figs. 1–9. And Archiv für Zellforschung, vol. viii, Apr. 23, 1912, pp. 198, 199.

35. MARK, E. L.: Maturation, Fecundation, and Segmentation of *Limax campestris*, Binney. Bull. Mus. Comp. Zool. Harvard, vol. vi, 1881, pp. 173–625, Pl. I–V.
36. MEAD, A. D.: The Origin of the Egg Centrosomes. Journ. Morph., vol. xii, Feb. 1897, pp. 391–4, Fig. 1–3.
37. MORGAN, T. H.: The Action of Salt Solution on the Unfertilized and Fertilized Eggs of *Arbacia* and other Animals. Archiv Entwickelungsmech., vol. viii, June 27, 1899, pp. 448–539, Pl. VII–X.
38. NĚMEC, B.: Die Reizleitung und die reizleitenden Strukturen bei den Pflanzen. 1–153 pp., Pl. I–III + Figs. 1–10. Jena, 1901.
39. ORMAN, E.: Recherches sur les différenciations cytoplasmiques (ergastoplasme et chondriosomes) dans les végétaux. 1. Le sac embryonnaire des Liliacées. La Cellule, vol. xxviii, 1912, pp. 365–443.
40. SCHMIEDEBERG, O.: Ueber die chemische Zusammensetzung des Knorpels (p. 395). Archiv exp. Pathol. u. Pharmakol., vol. xxviii, 1891, pp. 355–404.
41. SCHOLL, E.: Die Reindarstellung des Chitins aus *Boletus edulis*. Monatsh. Chemie, vol. xxix, 1909, pp. 1023–36.
42. STRASBURGER, E.: Ueber den Bau und das Wachstum der Zellhäute. i–xv + 1–264 pp., Pl. I–VIII. Jena, 1882.
43. ——————: Zur Entwickelungsgeschichte der Sporangien von *Trichia fallax*. Bot. Zeitung, vol. xlii, May 16, 1884, pp. 305–16, Pl. III; May 23, 1884, pp. 321–3.
44. TIMBERLAKE, H. G.: The Development and Function of the Cell Plate in Higher Plants. Bot. Gaz., vol. xxx, Aug. 1900, pp. 73–99, Pl. VIII, IX; Sept. 1900, pp. 154–70.
45. WISSELINGH, C. V.: Mikrochemische Untersuchungen über die Zellwände der Fungi. Jahrb. wiss. Bot., vol. xxxi, 1898, pp. 619–87. Pl. XVII, XVIII.
46. ZOPF, W.: Die Pilzthiere oder Schleimpilze, i–vi + 1–174 pp., Figs. 1–51. Breslau, 1885.

EXPLANATION OF PLATES I AND II.

Illustrating Messrs. Harper and Dodge's paper on Formation of the Capillitium in Certain Myxomycetes.

The figures in Plate I were drawn with the aid of the camera lucida, from preparations stained with the triple stain—Figs. 1–4 with Zeiss apochromatic obj. 2 mm. n. ap. 4, ocular 8; Fig. 5 with obj. 3 mm. n. ap. 1·3, ocular 12.

The microphotographs in Plate II were made with the Zeiss apochromatic objectives 3 mm. n. ap. 1·3, and 8 mm., and the compensating oculars Nos. 4, 6, and 8. Figs. 8, 14, and 15 are from preparations stained with iron-haematoxylin. The other figures are from preparations with the triple stain. In the latter case a green ray filter was used.

PLATE I.

Fig. 1. *Hemiarcyria clavata.* Shows fibrils from adjacent systems crossing. Granules in series and quite separate from the capillitial vacuoles. The latter with very dense content so that their outlines are scarcely discernible.

Fig. 2. *Trichia* sp. Shows distribution of granules and fibrillar systems in longitudinal section of capillitial thread.

Fig. 3. *Hemi. clavata.* Shows long fibrils extending out between the nuclei.

Fig. 4. *Hemi. rubiformis.* Capillitial vacuole larger than final diameter of the thread. Fibrils very numerous and delicate. Those fibrils which appear to anastomose in this and the other drawings really lie in quite different focal planes.

Fig. 5. *Hemi. clavata.* Capillitium well differentiated, spiral bands running in the direction of the thread of a left-handed screw. Another capillitial thread which bends down into

the plane of the drawing was cut so that this section contained only its lower surface. The nuclei are evenly distributed throughout the sporangium. Three of the smaller deeply staining nuclei present at this stage are shown.

PLATE II.

We have not been able to bring out adequately in our microphotographs the delicate radial fibrillar structures shown in the drawings in Plate I. The microphotographs do, however, show clearly the distribution and relations of the capillitial vacuoles, the denser cytoplasm around them, and the zoned arrangement of the nuclei. Figs. 6, 8–15, 16, 17, and 19, *Hemi. clavata.* Figs. 7 and 18, *Trichia.*

Fig. 6. Section of spore-plasm before the formation of the capillitium. The cytoplasm is in general spongy in structure, but numerous fibrils are seen running in all directions among the nuclei. × 300.

Fig. 7. Section of entire sporange, stipe, and portion of substratum. Convoluted appearance of the spore-plasm due to zoned distribution of the nuclei about the denser plasm in which the capillitial threads are forming. × 50.

Fig. 8. More highly magnified section of sporange at a little earlier stage than that shown in Fig. 7, showing the distribution of the nuclei about the anastomosing capillitial vacuoles and forming threads. × 100.

Figs. 9, 10, and 11. More highly magnified sections showing groups of anastomosing vacuoles with the nuclei in a zone just outside the denser plasm. Figs. 9 and 10 × 230; Fig. 11 × 160.

Fig. 12. Three capillitial vacuoles with series of granules on the surface of the tubular connexions between them. × 370.

Figs. 13, 14, and 15. *Hemi. clavata.* Show more highly magnified views of cross-sections of the forming thread. The radial fibrillar strands show very faintly in the photographs. The nuclei have withdrawn from the region around the thread but are still rather irregularly scattered. These photographs represent in general the same stages as the drawings Nos. 2, 3, and 4 in Plate I. Figs. 13 and 14 × 370; Fig. 15 × 490.

Figs. 16 and 17 represent slightly later stages stained with the triple stain. In Fig. 17 a granule lies in the centre of the capillitial thread section, thus imitating the appearance of a centrosome surrounded by a centrosphere and aster. Fig. 16 × 270; Fig. 17 × 370.

Fig. 18. *Trichia.* The nuclei tend notably to lie in a circle about the section of the capillitial thread. The figure also shows faintly the radial fibrillae extending between and beyond the nuclei. × 490.

Fig. 19. *Hemi. clavata.* Stage corresponding to that in Fig. 18. The nuclei tend to lie in a single series about the section of the thread. × about 160.

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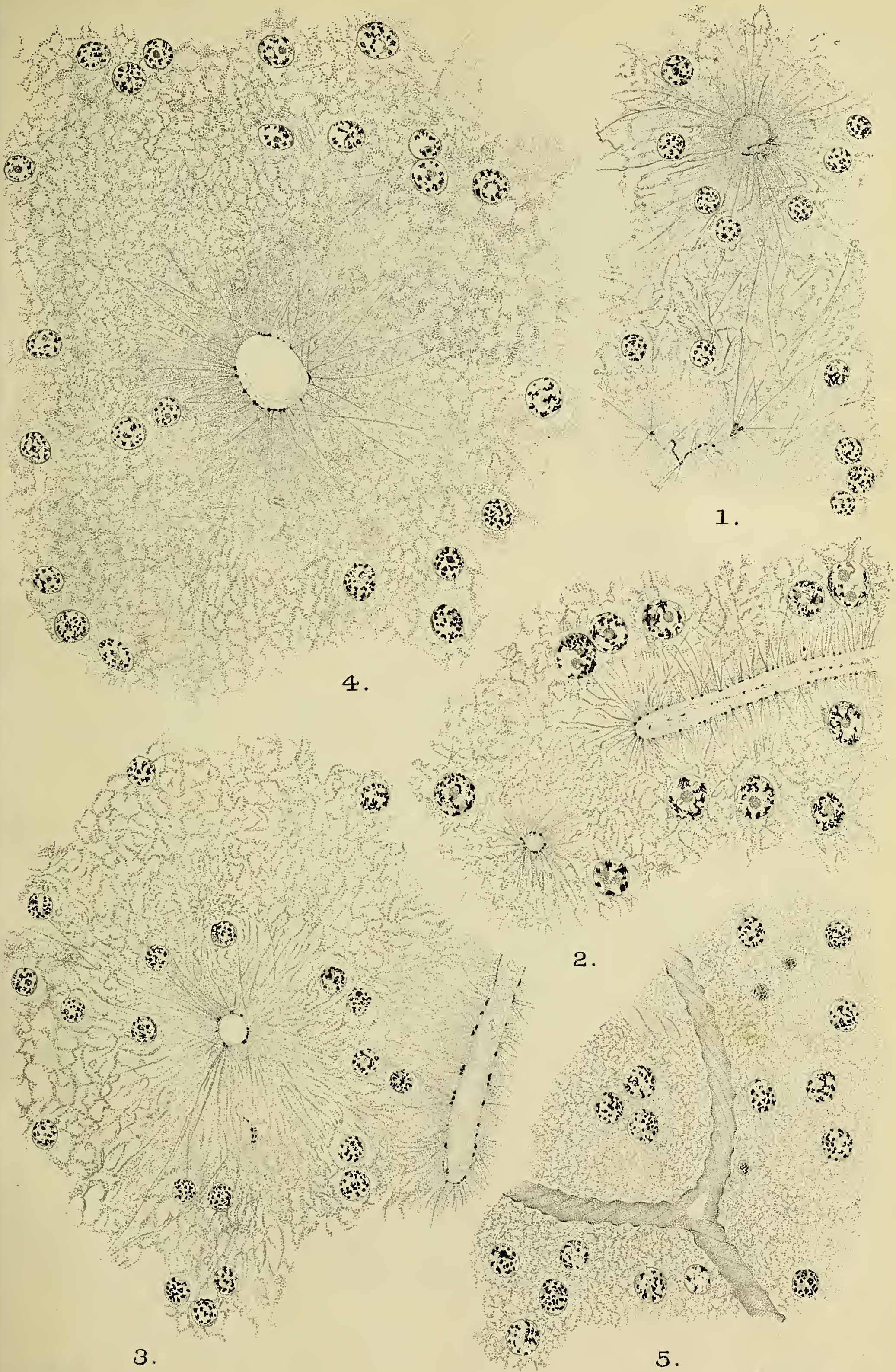

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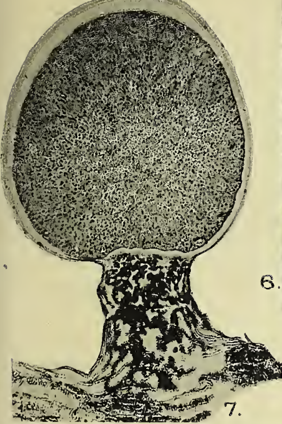
HARPER & DODGE —— MYXOMYCETE CAPILLITIUM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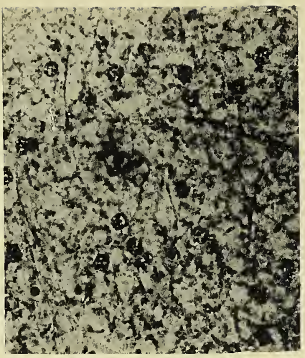
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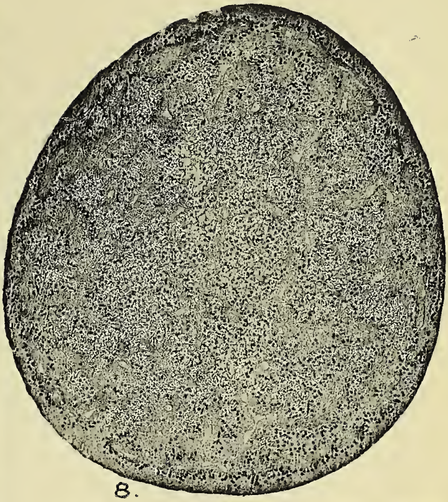
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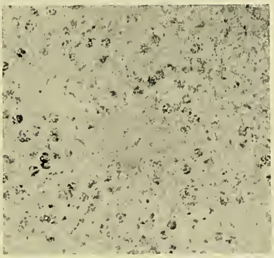
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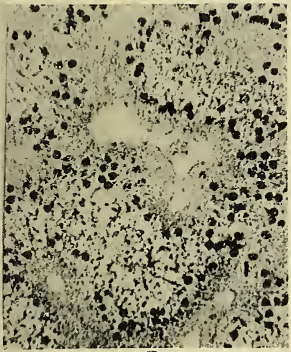
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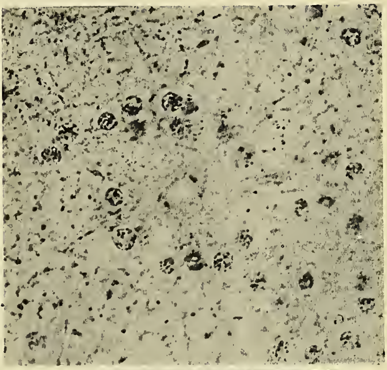
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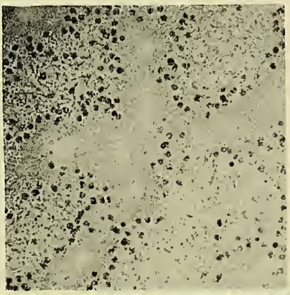
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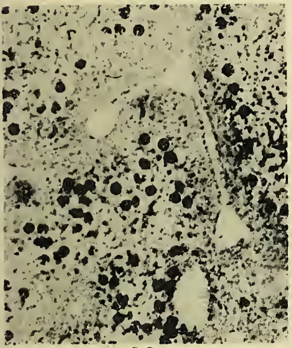
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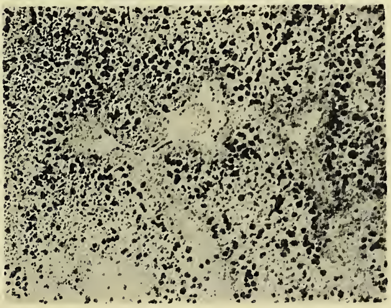
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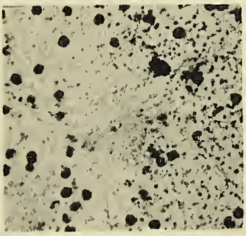
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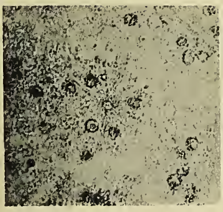
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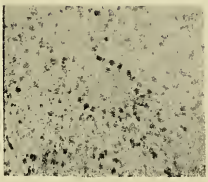
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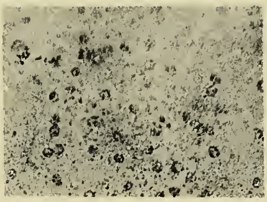
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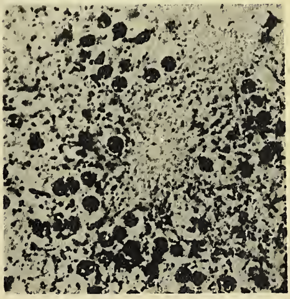
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HARPER & DODGE — MYXOMYCETE CAPILLITIUM.

Studies in the Morphology and Anatomy of the Ophioglossaceae.

II. On the Embryo of Helminthostachys.

BY

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With Plate III and nine Figures in the Text.

THE embryology of the Ophioglossaceae[1] is of peculiar interest on account of the variety in type of embryo within the group. The developmental story is pretty fully known for *Botrychium lunaria* and *B. virginianum*, in which the embryo has no suspensor, and the salient facts are recorded for *B. obliquum*, in which a suspensor was discovered by Mr. Lyon. For *Ophioglossum* the embryology is known in *O. vulgatum*, *O. moluccanum*, and *O. pendulum*. The embryos of these species, while agreeing in having no suspensor, exhibit considerable differences in detail. The present paper is concerned with the embryology in the third genus of Ophioglossaceae, and is thus supplementary to a paper published in this Journal in 1902, which dealt with the prothallus and young plants of *Helminthostachys zeylanica*.

In my earlier paper no attempt was made to deal with the embryogeny since the material seemed so inadequate. One section of a mature embryo was figured and briefly described, and it was remarked with regard to the earlier stages, 'A number of archegonia had apparently been simultaneously fertilized on a few of the prothalli which were attached to young plants. The preservation of the arrested embryos, which were present in addition to the plant, was not good enough to make a study of the segmentation possible. It was, however, clear that, as in *Botrychium*, the embryo soon becomes deeply seated.'[2] The similarity of these young arrested embryos to the young embryos of *Botrychium obliquum* figured by Bower[3] from Mr. Lyon's preparations suggested that the embryo of *Helminthostachys*

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[1] The recent full summaries and discussions of the facts by Prof. Bower (Land Flora, 1908) and Prof. Campbell (The Eusporangiatae, 1911) make detailed reference to the literature unnecessary.

[2] Lang, Ann. of Bot., xvi, 1902, p. 40.

[3] Land Flora, Fig. 266, p. 472.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.]

also might have a suspensor. The study of a number of series of sections through young plants attached to prothalli established the existence of a well-developed suspensor in *Helminthostachys*; this was recorded in a brief preliminary note.[1] These additional series also provided two embryos of intermediate age which were present alongside successful plants.

It is obviously desirable that the embryogeny of *Helminthostachys* should be fully worked out on normal and adequate material. My material was collected in the Barrawa Forest Reserve in Ceylon in the month of March, after the annual inundation of the forest had subsided. All the female prothalli found bore young plants or mature embryos. Since Campbell's experience in the same locality in February, 1906, was similar, no unfertilized prothalli being found, his inference [2] that there is an annual periodicity in the development of the prothallus of *Helminthostachys* seems highly probable. The prothalli probably grow from the spores shed in the previous season, and are fertilized at maturity, the young plants developing forthwith and the prothalli perishing. Under the peculiar conditions that obtain in this locality the collection of a full series of prothalli and embryos may be a matter of great difficulty, and it seems advisable to publish the results obtained from the study of the imperfect material at my disposal. The main features of the embryogeny will therefore be described in this paper, and the form of the young plant followed until the horizontally growing rhizome is established. The consideration of the vascular anatomy will be deferred until the next number of these studies.

Before considering the early stages in the embryogeny it may be well to point out that in the advanced embryo three tiers or regions can be distinguished. The tier next the archegonial neck will be referred to as the upper tier of the suspensor; it often remains as a large undivided cell. The next tier also takes no part in the formation of the embryo proper and will be spoken of as the second tier of the suspensor. The third tier gives rise to the organs of the embryo plant and is comparable to the whole embryo in those Ophioglossaceae that have no suspensor. The embryo proper can be distinguished into a hypobasal half adjoining the suspensor and an epibasal half occupying originally the end of the embryo furthest from the archegonium. It will be shown that the large foot is formed from the hypobasal half, while the stem-apex, first leaf, and probably also the first root, all arise from the epibasal half. The two tiers of the suspensor (s^1, s^2) in relation to the young plant or embryo are seen in Pl. III, Photos. 10 and 12. This anticipation of the organization of the mature embryo will make the significance of the younger stages clearer as they are described in order.

The very young embryos were in almost all cases more or less contracted or broken down; the limits of the cavity, in which the embryo

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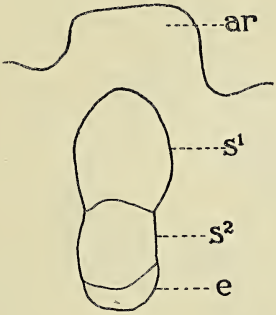
[1] Ann. of Bot., xxiv, 1910, p. 611.

[2] loc. cit., p. 54.

was contained, served however to indicate its shape and its relation to the archegonial venter in which the fertilized egg originally lay. Such a young arrested embryo as is shown in Pl. III, Photo. 1 may be compared with Bower's figures of the young embryo of *Botrychium obliquum*. The wider end next the surface of the prothallus marks the position of the enlarged archegonial venter; the still undivided embryo has broken through the lower limit of this and is extending deeper into the prothallus. The embryo shown in Photo. 2 was somewhat older when its growth was arrested; its position in the prothallus is seen in Photo. 4 at e^3. In this case also the position of the archegonial venter is recognizable above, but the embryo had extended more deeply into the prothallus. The tissues of the embryo are broken down, but the mass in the lower end of the cavity showed the presence of a number of nuclei, and represented the embryo proper. Segmentation had thus considerably advanced before this embryo perished. The walls separating the three tiers of the embryo (s^1, s^2, e) were not preserved. They are shown, however, in Text-fig. 1, which represents an embryo intermediate in age between those in Photos. 1 and 2. It is unnecessary to go further into the features of these very young arrested embryos, a considerable number of which were studied. They showed the similarity of the early stage of development to *Botrychium obliquum*, and they indicate that the region of the suspensor is distinguished from the rest of the embryo at an early period, but they do not justify inferences as to the early stages of segmentation of the embryo proper.



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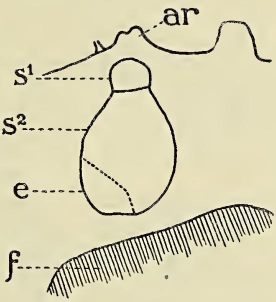

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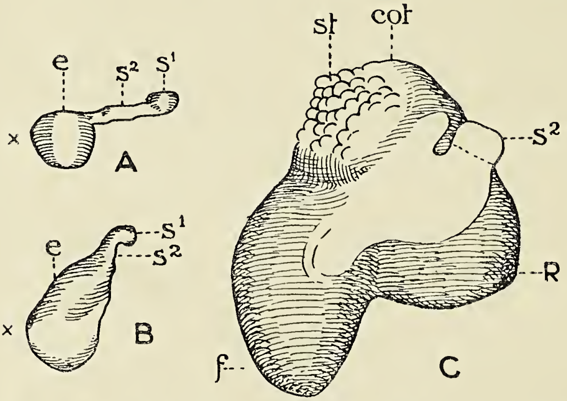
TEXT-FIG. 1. Young arrested embryo consisting of three cells in longitudinal section. *ar*, archegonial neck; s^1, upper tier of suspensor; s^2, second tier of suspensor; *e*, the embryo proper. × 200.

TEXT-FIG. 2. Older arrested embryo cut horizontally. *f*, foot of the plant borne on the same prothallus; *ar*, archegonial neck above the embryo; s^1, upper tier of suspensor; s^2, lower tier; *e*, embryo proper. The broken-down tissues of the embryo are not indicated. × 50.

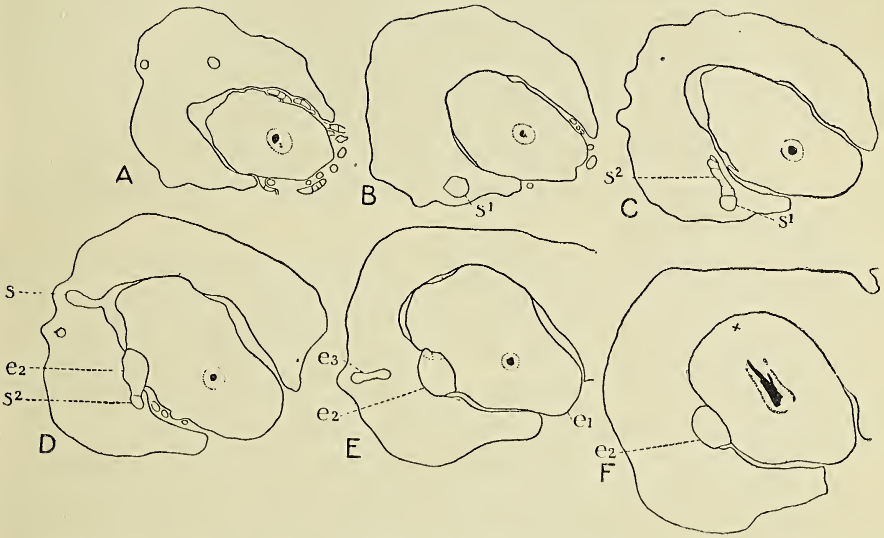
The older arrested embryo, a horizontal section through which is represented in Text-fig. 2, showed the upper tier of the suspensor marked off by a thick wall from the second tier, while the limit between the terminal tier destined to develop into the embryo proper and the second tier of the suspensor was evident though not so strongly marked; the terminal tier was more developed in the next lower section which missed the upper tier of the suspensor. The preservation of the tissues of this embryo was not sufficiently good to allow of detailed representation, but the outline is given because it affords a truer idea of the shape of the embryo at this stage than does the slightly more advanced embryo next to be described.

The first of the two well-preserved embryos of intermediate age had an abnormally long second tier to its suspensor. This had carried the embryo proper into the cavity in the prothallus occupied by the base of the successful plant. The transverse sections of the prothallus cut the embryo horizontally, and, since the latter extended downwards and inwards into the prothallus, an idea of its shape and construction as a whole could only be obtained by combining the information obtained from the successive sections. Text-fig. 3, A shows the shape of the embryo as given by a model constructed to scale, the embryo being seen from the side, while outline figures of a number of the sections of the region of the prothallus containing the embryo are given in Text-fig. 4. Two of the sections are also represented in Pl. III, Photos. 3 and 4. As the latter photograph shows, the prothallus bore a well-developed plant (e^1), the embryo under consideration (e^2), and the younger arrested embryo already described (e^3). The embryo of intermediate age is seen in Text-fig. 4, B–F. In B the large cell forming the upper tier of the suspensor (s^1) is seen cut obliquely, but the level of the section is still above the rest of the embryo. In C the lower portion of this cell is still seen (s^1), and continuing obliquely downwards and inwards from it is a row of cells corresponding to the elongated second tier of the



TEXT-FIG. 3. Outline figures of reconstructions of three embryos. × about 30 diameters. A, the smaller intermediate embryo seen from the side. B, the larger intermediate embryo seen from the side. s^1, upper tier of suspensor; s^2, lower tier; *e*, embryo proper. The cross is opposite the apex of the shoot. C, mature embryo seen from the side on which the suspensor is attached. s^2, suspensor; *f*, foot; *r*, root; *cot*, first leaf; *st*, position of apex of stem covered by hairs.

suspensor (s^2). In D the lower end of the suspensor (s^2) is seen to be continuous with an oval mass of cells belonging to the embryo proper (*e* 2), and the following sections only show this, their level being below that of the suspensor. At the level passed through in E the embryo proper is seen to consist of two tiers, the limit between which is indicated by the dotted line. Still lower sections (F) miss the terminal or epibasal tier of the embryo and indicate that the hypobasal tier bulges downwards. If the figure of the reconstructed embryo (Text-fig. 3, A) is considered in the light of the separate sections it will be clear that it consists of the upper tier of the suspensor (s^1), composed of a single large cell; of the second tier of the suspensor (s^2), which in this specimen is much elongated and composed for the most part of a row of cells; and of the globular embryo itself (*e*), which is enlarged downwards where the foot is developing. The epibasal tier with the apical region is opposite the cross, and it will be evident that the embryo is as yet straight in that the epibasal tier is not directed upwards.



TEXT-FIG. 4. Outline figures of a number of transverse sections of a prothallus bearing a young plant (*e* 1); an embryo of intermediate age (*e* 2); and a young arrested embryo (*e* 3). s^1, s^2, first and second tiers of the intermediate embryo; *e* 2, the embryo proper. *s*, the cavity once occupied by the suspensor of the plant. × in F, position of root apex of the plant.

Pl. III, Photo. 3 shows the appearance of the elongated suspensor (s^1, s^2), while Photo. 4 is of a section passing through the embryo proper (e^2) at a level which traverses both the hypobasal and epibasal tiers. A similar section of the embryo is seen more highly magnified in Photo. 5. As this shows, the lower or hypobasal tier (*hyp.*) is composed of larger cells not presenting a meristematic appearance, while the small terminal tier (*ep.*)

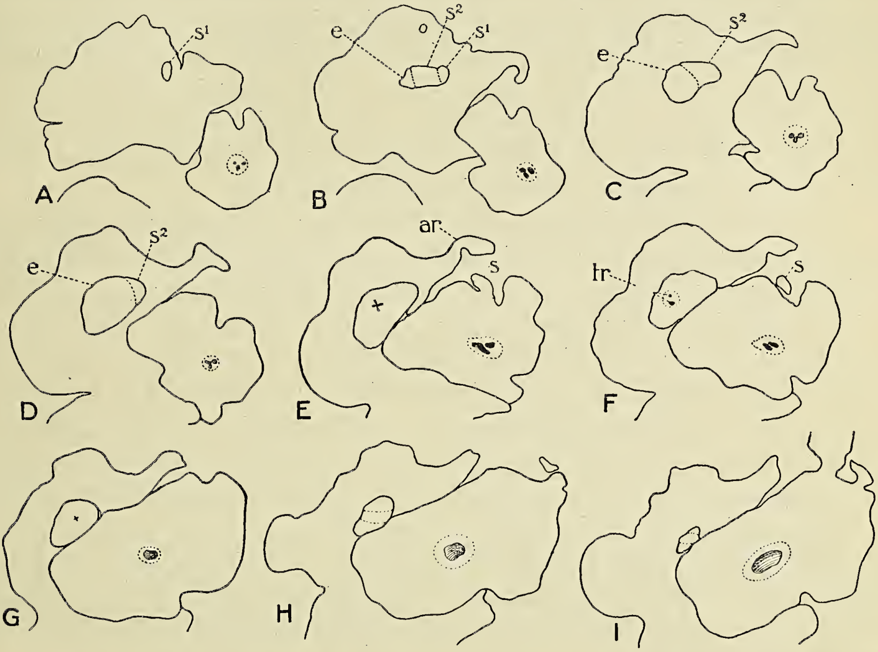
contrasts with it in the meristematic character of its densely stained cells. Although the initial cell of the stem could not be distinguished with certainty, it is clear that the embryo of *Helminthostachys* conforms to and supports Bower's generalization that the apex of the shoot is initiated close to the centre of the epibasal pole.

From this single specimen it is not possible to speak with certainty as to the segmentation of the embryo, but careful study suggests the following interpretation, which is consistent with the appearances presented by both younger and older embryos. The first transverse wall evidently separated the cell forming the upper tier of the suspensor from the rest of the embryo. The next wall was also transverse and separated the second tier of the suspensor from a terminal cell giving rise to the embryo proper. The embryo proper has increased markedly in size as compared with the suspensor, and has undergone differentiation into two tiers *which are comparable to the epibasal and hypobasal tiers of other embryos* whether provided with suspensors or not. It is not clear whether the separation of these two tiers was effected by the first transverse wall in the terminal cell or was deferred until after longitudinal division.

There is no reason to doubt that the foot is developed from the hypobasal tier, and the shoot including the stem-apex and first leaf from the epibasal tier. Reasons will be given later for believing that the first root is also derived from the epibasal tier. The root is certainly not differentiated at this stage, but the stem-apex is commencing to be depressed and the first leaf *may* be defined, though it was not distinguishable.

The second embryo of intermediate age was considerably larger than the one just described, and showed the first appearance of vascular tissue. Its shape as reconstructed is represented in Text-fig. 3, B; the upper tier of the suspensor (s^1) consisted of a single large cell, the second tier (s^2) was not unduly elongated as in the preceding example, and widened gradually towards the embryo proper. Both tiers of the latter had contributed to the enlargement of the embryo, but the apex is still not directed upwards, its position being indicated by the cross.

The position of the embryo in the prothallus, which also bore a well-developed plant, and the plane in which the embryo was cut will be evident from Text-fig. 5. The plane of section in this case was practically at right angles to the long axis of the embryo. Starting from the back of the embryo below the remains of the archegonial neck, the series first showed the large cell forming the upper tier of the suspensor (Text-fig. 5, A, s^1). This joined on at the lower end to the second tier of the suspensor (s^2), which was composed of a tissue of rather large cells and widened gradually to its junction with the base of the embryo proper (*e*). The second tier of the suspensor is seen in B joining on to the lower end of the upper tier; in C the second tier of the suspensor is seen above, while below it the section passes through the posterior portion of the embryo proper. Still further forward (D) only the anterior and upper edge of the suspensor is cut by the section, the main part of which passes through the hypobasal tier of the embryo. The section in E traverses the anterior portion of the hypobasal tier, the cells bounding the lower surface of which had the characteristic appearance of the absorbent epithelium of the foot. The arrangement of the cells in the region marked with a cross suggested that the section here passes through the apex of the primary root. A section a little further forward (F) shows (immediately above the presumable position of the root-apex) a desmogen strand, in which a few short tracheides are lignified. Still further forward (G) the apical region of the shoot marked with a cross is cut through. The portion of the next two sections marked off by the dotted lines corresponds to the young cotyledon or first leaf bent closely over the stem-apex.



TEXT-FIG. 5. Outline figures of a number of longitudinal sections of the upper portion of a prothallus bearing a young plant and an embryo of intermediate age. The suspensor of the plant is seen at *s* in E, while the corresponding archegonium is seen at *ar*. s^1, first tier of suspensor of the intermediate embryo; s^2, second tier of suspensor; *e*, base of embryo proper; × in E marks position of the apex of the root; *tr* in F the tracheides developed in the stele; × in G the position of the apex of the stem.

The detailed structure of four sections of this embryo is shown in Photos. 6–9. It would have been difficult to make detailed drawings of this poorly stained embryo without either over-emphasizing the indications of segmentation which it affords, or on the other hand missing these indications, so that in this case photographs afford the best representation possible. The section in Photo. 6 passes through the second tier of the suspensor (s^2) and the hindmost portion of the hypobasal tier of the embryo (*e*); the distinction between the two regions is brought out by the difference in size and appearance of the cells composing them. The section in Photo. 7 passes through the hypobasal tier of the embryo proper. It seems clear that the lower half of this (i. e. the two downwardly directed quadrants) has developed more than the upper half. The cells of the lower half have the characteristic appearance of cells of the foot. The foot thus appears to be developed from the hypobasal tier of the embryo proper and mainly from the two downwardly directed quadrants. The section in Photo. 8 passes through the base of the axial stele a little further forward than the root-apex; the short tracheides (*tr.*) are seen in the stele. The section in Photo. 9 passes through the region of the stem-apex. The meristematic tissues show indications of having been derived by the segmentation of a three-sided initial cell, the position of which is indicated by a cross.

It will be noted that the cells composing the portions of this last section above and below the meristematic region (and the same holds for the portions above and below the stele in Photo. 8) present the same appearance as the cells of the hypobasal region or foot. Thus the whole young shoot appears as if sunken in the basal region of the embryo. This may indicate that the epibasal region is partially enveloped by the hypobasal as the growth of the embryo proceeds. It is possible, however, that the cotyledon is not derived from a portion of the epibasal tier independently of the stem-apex, but that the apical cell of the stem is first established and the cotyledon derived from the first segment of this. In this case the tissue surrounding the apical region of the shoot would be derived from the epibasal tier. One or both of these explanations are required to account for the way in which the stem-apex and cotyledon appear sunk in the basal portion of the embryo.

Four prothalli[1] were obtained with large normal embryos ready to grow further into young plants. These prothalli were cut into series of longitudinal sections, but this did not result in cutting the embryos in any definite corresponding plane. The embryos were all cut more or less obliquely, so that their interpretation from isolated sections was difficult. The most readily understood specimen was that of which a section is figured in my earlier paper.[2] This section, longitudinal as regards the embryo proper, was approximately median for the young shoot which was directed upwards. It passed through the stem-apex, which was overhung by the first leaf. Below the first leaf was the bulge of the young root, while on the other side the large foot bulged into the prothallus. The relative

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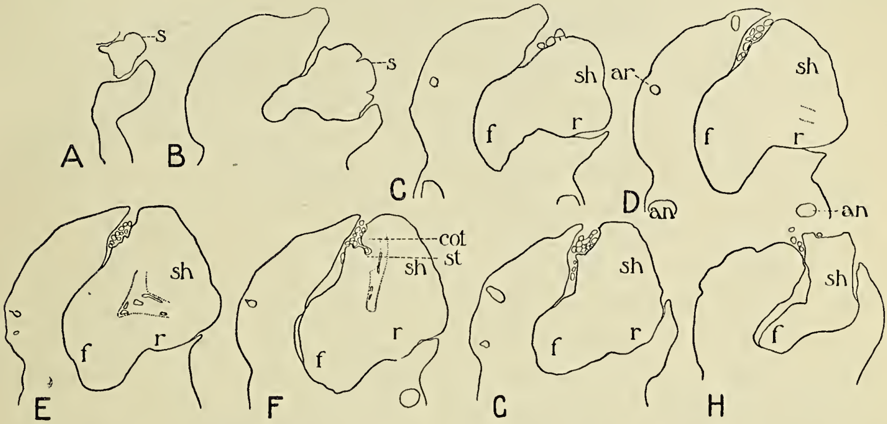
[1] Cf. Ann. of Bot., vol. xvi, Pl. II, Figs. 35–37.

[2] Ibid., Fig. 59.

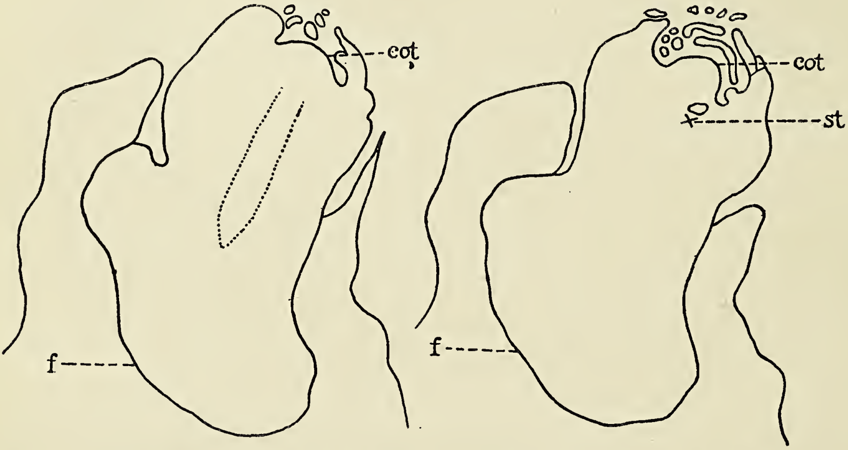
positions of these organs is also seen in the accompanying outline figure (Text-fig. 6, F). While, as stated in my earlier paper, the relative position of the primary organs of the embryo appears to be the same as is described for the embryo of *Botrychium virginianum*, an essential difference comes out when all the sections of the series are considered, and especially when the embryo is reconstructed. A number of sections of the series are represented in Text-fig. 6. The plane of section had passed at right angles to the insertion of the suspensor, which was somewhat flattened by the expansion of the embryo. The suspensor, probably the lower tier, is seen in Text-fig. 6, A and B at *s*. From it the embryo widened out suddenly, and the other sections figured allow of the foot (*f*), root (*r*), and shoot (*sh*) being followed through the series. The apex of the root is in the section D, while the cotyledon (*cot*) and the apex of the stem (*st*) are distinguishable in F, which is median for the upwardly directed shoot. The position of the vascular strand in the shoot and root can be gathered from the sections in D, E, and F.



TEXT-FIG. 6. Outline figures of a number of longitudinal sections of the upper portion of a prothallus bearing a mature embryo. The prothallus, as usual, bore antheridia (*an*) as well as archegonia (*ar*). The embryo is cut in the longitudinal plane of the erect shoot and the figures commence at the side where the suspensor is attached. *s*, suspensor; *f*, foot; *r*, root; *sh*, shoot; *cot*, first leaf; *st*, stem. The central vascular system is indicated in D, E, and F.

The reconstructed embryo seen from the suspensor side is represented in Text-fig. 3, C. It shows the suspensor (*s*) and the result of development of the embryo proper. The great difference from the smaller embryos described is that the shoot with further growth has become vertical. The position of the first leaf (*cot*) and of the stem-apex (*st*) are indicated, as well as the development of hairs over the apical region. The root (*r*) has grown downwards and towards the side away from the mass of the prothallus, while the large foot (*f*) has extended into the latter. Comparison of the three embryos in Text-fig. 3 will show what a considerable readjustment must have taken place, as the embryo not only grew but changed the direction of growth of the shoot. This readjustment has led to the similarity in position of the organs to the embryo of *Botrychium virginianum*, though the latter is inverted from the commencement of development and has no suspensor.

The reconstruction not only explained the organization of this embryo, but enabled the other series through embryos of corresponding age to be interpreted. Thus the two sections figured in outline in Text-fig. 7 are from an approximately longitudinal series in a plane at right angles to that of the series in Text-fig. 6. The first section includes the stele and the trace to the first leaf or cotyledon, the second shows the tip of the cotyledon bent over the stem-apex. These sections are figured, because they afford a further illustration of the way in which the shoot-apex, including the cotyledon, is sunk in the summit of the embryo.[1] The surrounding rim of tissue bears numerous multicellular hairs.



TEXT-FIG. 7. Outline figures of two sections through another mature embryo. *f*, foot; *cot*, first leaf; *st*, apex of stem. The apex of the shoot is surrounded by a rim of tissue-bearing hairs.

The section represented in Pl. III, Photo. 10 was from another series, which cut the embryo in an obliquely transverse plane. Its interest lies in the fact that it cut the perfectly preserved suspensor longitudinally. A photograph on a larger scale of the suspensor is shown in Plate III, Photo. 11. The suspensor is seen to consist of the upper tier (s^1), composed of a large cell, in which, however, a few divisions had taken place, and of the lower multicellular tier (s^2), which widened gradually towards the embryo proper. The two tiers of the suspensor are separated by a thick wall.

The change from mature embryos, such as those just described, to the young plants with an expanded green leaf, such as those figured in my earlier paper, is simply due to further growth of the root and shoot. The

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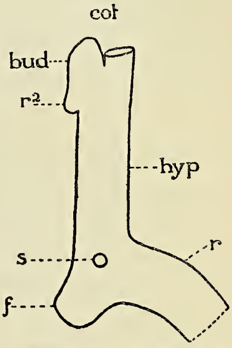
[1] Cf. p. 26.

primary or first root grows out and turns downwards into the soil, while the hypocotyl lengthens, carrying up the cotyledon and the terminal bud. The relations of the basal part of the plant to the prothallus remain unchanged, though the suspensor may be more or less completely broken down. An example of this is afforded by Text-fig. 4, D, where the suspensor of the plant is unrecognizable, although the cavity which it occupied (*s*) is evident, leading down from the archegonial neck. Pl. III, Photo. 12 shows a fairly well preserved suspensor in relation to the base of a young plant. The large upper cell of the suspensor was undivided, while the second tier of the suspensor was divided up into cells. The suspensor figured in Pl. III, Photo. 13 is from another young plant. It is of interest in that it shows the remains of the archegonial neck above (*ar*) and also because in this case both tiers of the suspensor have become multicellular. The plant in Text-fig. 5 which was cut longitudinally shows the suspensor (*s*) in E and F.



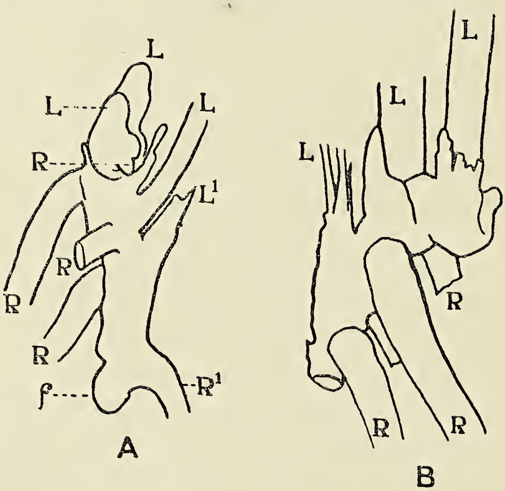
TEXT-FIG. 8. Basal region of a young plant detached from the prothallus and viewed from the suspensor side. *s*, suspensor; *f*, foot; *r*, primary root; *hyp*, hypocotyl; *cot*, first leaf; *bud*, apical bud; r^2, second root.

The reconstructed embryo (Text-fig. 3, C), the young plants described, and a number of others studied agree in the orientation of the organs of the embryo in relation to the suspensor and present the usual type of this. It is shown also in Text-fig. 8, which represents a small plant dissected free from the prothallus. The position of the suspensor was recognizable at *s*, the foot (*f*) bulged out to one side, while the primary root (*r*) had grown out on the opposite side. The plant differed from the mature embryo in the marked elongation of the hypocotyl (*hyp*), but the first leaf (*cot*) is seen to stand above the first root, while the enlarged apical bud is enclosed by the sheath of the first leaf. Below the bud the second root (r^2) is seen.

The general appearance and external morphology of the young plants have already been described and figured in my earlier paper and also by Campbell. In all the young plants observed by Campbell the first leaf or cotyledon remained rudimentary, and did not come above ground or expand its lamina. I have also met with examples of this behaviour, but in the majority of the young plants I have studied I find that, as described in my earlier paper, the first leaf became fully developed and expanded above ground. I am thus unable to accept Campbell's generalization[1] that 'it is probable that the cotyledon in *Helminthostachys*, as in *Botrychium lunaria* and *Ophioglossum vulgatum*, is always a rudimentary organ and never appears above ground'. The fact is that it may be either fully developed or more or less arrested, and presumably the former condition is the more primitive.

[1] loc. cit., p. 67.

In the further growth of the young plant the originally vertical direction of the axis appears to be maintained for a short period, although as regards the position of the leaves on one side of the stem and the roots on the other dorsiventrality is established at once. The dorsiventrality is probably related to the position of the three-sided initial cell. After a few more leaves and roots have been developed, however, the apex changes its direction of growth and bends over; the further growth of the rhizome proceeds horizontally. The change is shown in the outline figure of the larger plant in the accompanying figure (Text-fig. 9, B). In the other plant (Text-fig. 9, A) the axis, so far as it is well developed, had grown vertically, but the tip was curved over and is directed towards the spectator. It will be evident from this specimen that the origin of the plant from an embryo is shown not only by the presence of the foot, but by the different position occupied by the first root to that of all the succeeding ones. The later roots all spring from the ventral side of the rhizome, while the first root stands below the first leaf and apparently on the dorsal side of the rhizome. The significance of this will be discussed later. The second specimen, which shows the change in direction of growth of the rhizome better, also had the foot and first root at its base, but this had unfortunately got broken off before it was drawn.



Text-fig. 9. A, young plant still showing the foot (*f*) and the primary root (r^1) at the base. The shoot has grown erect for a time but is now extending horizontally towards the spectator. B, similar plant from the side, showing the change from the vertical to the horizontal rhizome. The foot and primary root have been broken off.

Discussion of Results.

The preceding account of the embryo of *Helminthostachys*, though incomplete, provides the main facts. It is clear that the first stage of development of the fertilized egg-cell exhibits a definite polarity with the apical pole directed inwards, i. e. away from the archegonial neck. Following on this come divisions at right angles to the long axis of the embryo, resulting in a stage in which the embryo consists of a row or filament of three cells. Further divisions may take place in all three cells, but the large cell next the archegonial neck often remains undivided; it forms the upper tier of the suspensor. The next cell undergoes division and usually forms the short and wide second tier of the suspensor; it may, however, take the form of an elongated row of cells. The embryo proper is derived from the third or terminal cell. The divisions in this result in the distinction of a hypobasal half from which the foot is mainly developed, and of an epibasal half in which the apex of the shoot is initiated close to the central point. By further growth of the epibasal half all the primary members of the young plant are laid down, the first leaf developing close to the apex of the stem, while the primary root probably arises endogenously and originally points backwards towards the hypobasal half and the suspensor. The vascular system of the plant is initiated in the axial line between the apex of the shoot and the primary root.

Up to this stage the growth of the embryo would appear to be determined by its position in the prothallus with relation to the archegonial neck, and the axis of the embryo is approximately straight. The further growth to the condition in the mature embryo involves a marked curvature. This is not a simple bending, for the whole embryo is growing at once. It results in the enlarging shoot becoming vertical. The facts do not allow of the full mechanism of this being understood, but two factors must be recognized, both of which probably come into play. On the one hand, there is the great and unequal enlargement of the hypobasal tier giving rise to the foot, which bulges towards and into the prothallus from which nutriment has to be obtained. On the other hand, and possibly more important, is the change in irritability of the growing shoot in relation to gravity; the shoot ceases to grow in the original line of the primary filament of the embryo and grows vertically. Both these factors probably contribute to the changed configuration of the mature embryo. The erect position of the shoot is not attained passively by the enlargement of the foot, but by active growth. On the other hand, the apparently lateral position of the first root is probably the result of the great increase of the hypobasal region on one side in forming the foot.[1]

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[1] The distinction between two stages in the development of the embryo, the first in which the form and structure is dependent on internal factors and on the correlations between the embryo and the surrounding tissues, and the second in which the direction of growth of the organs is dependent on their reactions to such external stimuli as gravity, is possibly an important one. It may be pointed out that it is seen in the Spermaphytes in (1) the growth of the embryo in the embryo-sac of the developing seed, and (2) the growth of the embryo on the start of germination. In this case the two stages appear separated by the period of rest. In such a case as the embryo of *Helminthostachys* the two stages are recognizable, but the development goes on without a break. The interesting

The nature of this complete readjustment of the organs of the embryo of *Helminthostachys* during the curvature must be borne in mind in comparing it with embryos (such as that of *Danaea*) in which the axis remains straight throughout development, and the foot shows no marked enlargement.

If the embryo of *Helminthostachys* is compared with what we know of the embryo of *Botrychium obliquum*[1] it will be clear that here also the first direction of development was in a line directly inwards from the archegonial neck, and that later a change in direction of the growth of the embryo proper led to the shoot growing vertically upwards and the root vertically downwards. The root and shoot of the fully grown embryo are in the same straight line, and it is noteworthy that no well-developed foot is described in this plant. With our present imperfect knowledge it must remain an open question whether the suspensor as figured by Lyon has two tiers, and corresponds exactly to the suspensor of *Helminthostachys*, or whether the second tier in his figure corresponds to the poorly developed hypobasal half of the embryo proper which in *Helminthostachys* forms the foot.

In the other species of *Botrychium* and in *Ophioglossum* there is no suspensor, the embryo is inverted from the first, and the primary distinction is into the hypobasal half directed downwards into the prothallus and forming the more or less marked foot, and the epibasal half directed towards the archegonial neck and forming all the organs of the plant. It is not necessary to enter into detailed comparisons with these forms, and reference may be made to the works of Bower and Campbell cited.

It is difficult to discuss any one peculiar pteridophytic embryo without being led into a general survey of the embryogeny in the phylum. To avoid this the comparative discussion in Chapter XLII of Bower's 'Land Flora' may be assumed as a starting-point. This is the more justifiable, as this chapter may be regarded as the first critical comparative statement on the morphology of the embryo of Pteridophyta.

Professor Bower regards the relation of the apex of the axis to the primary segmentation of the embryo as constant, and the apex of the stem as occupying as nearly as possible the centre of the epibasal hemisphere. Whatever subsequent change in the direction of the apex occurs, the polarity has been determined before, and is indicated by, the first segmentation of the embryo. All further knowledge of particular embryos since the date when Professor Bower made this generalization seems to confirm it, and it appears to state a primary fact.

Within the same phylum, as Professor Bower further points out, examples of embryos with suspensors and embryos without suspensors are

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physiological problem thus raised is when and how the internal correlative influences between the embryo and the prothallus (or seed) are superseded by this irritability of the parts of the embryo to gravity.

[1] Cf. the figures in the Land Flora, pp. 471, 472.

met with. The apical pole of the embryo with a suspensor always points away from the archegonial neck, but the position of embryos without suspensors exhibits differences. The position of the embryo (as compared with embryos with suspensors) may be completely inverted, the epibasal pole being directed towards the archegonial neck instead of away from it. This is the position of the embryo of *Equisetum*, which has no suspensor. It is found in *Isoetes* in contrast to the embryos of other Lycopodiales where suspensors are present. In the Ophioglossaceae similarly, and even in the single genus *Botrychium*, embryos with and without suspensors are met with, and inversion of the polarity of the embryo is associated with the absence of a suspensor. From the evidence afforded by the Lycopodiales and Ophioglossaceae, Bower regards the type of embryo with a suspensor as the more primitive and as having been associated with a bulky prothallus, often subterranean and saprophytic. It appears to be implied, though not actually stated, that the suspensor is here biologically useful in carrying the embryo deeper into the bulky prothallus. The non-development of the suspensor and the associated inversion of the embryo are regarded in both of these groups as having been an advantage in avoiding an 'awkward curvature' and simplifying 'an awkward and inconvenient process'. The type of embryo without a suspensor is regarded as 'characteristic of stocks with less bulky prothalli, usually above ground and self-nourishing'. Whether a similar progression from embryos with to those without suspensors has taken place in the Filicales (excluding the Ophioglossaceae) and the Equisetales is left an open question by Bower, but the progress from more bulky to less bulky prothalli in the Filicales is regarded as consistent with such an assumption.

Thus we have as further generalizations (in addition to the primary one of the constant position of the apex of the shoot at the centre of the epibasal pole): (*a*) the probability that the type with suspensor is relatively primitive; (*b*) that the presence of a suspensor is correlated with more bulky prothalli; (*c*) that the loss of the suspensor on inversion of the embryo may be regarded as a biological advantage.

Since the date of publication of the 'Land Flora' our knowledge of the embryology of the Filicales has been increased by a fuller account of the embryos of Marattiaceae (including the embryo of *Danaea*,[1] which is provided with a suspensor) and by the main facts for *Helminthostachys* described in the preceding pages. The existence of forms with and without suspensors in two comparatively narrow circles of affinity, such as the Ophioglossaceae and the Marattiaceae, is of great interest. The interest is increased by the probability that both groups are relatively primitive among the Filicales. It thus appears most profitable to confine this discussion to the Filicales, and to critically consider the generalizations reached by Bower as applied to this phylum. In doing so it will of course be clear that all the facts for

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[1] Campbell, loc. cit., pp. 142-6.

the Filicales we now possess were not before Professor Bower when his views were expressed.

As already indicated, all the additional facts for the Marattiaceae and for *Helminthostachys* support Bower's primary generalization as to the early determination and constant position of the stem-apex. Some facts also strongly suggest, though proof is wanting, that the first leaf or cotyledon may be derived in some cases from the further growth of the apex of the stem, and not be laid down as a primary member independently of the apical cell of the stem. If this is so the primary importance of the axis would be increased.

The discovery of a suspensor in a second genus of Ophioglossaceae, and also in *Danaea* among the Marattiaceae, is in favour of Bower's further generalization that the type of embryo with a suspensor may be regarded as relatively primitive. It would seem probable that, while the suspensor had been suppressed in certain Pteridophytes, it was originally present, at least in the Filicales and Lycopodiales.

Critical consideration of the available facts does not, however, appear to me to support the view that the presence or absence of the suspensor is correlated with the bulk of the prothallus, or that it is to be explained on biological grounds. Thus, within the Marattiaceae the prothallus is always fairly massive and green.[1] The prothallus is least massive in *Danaea*, and it is in this genus, as Campbell has shown, that the first division in the embryo separates a suspensor from the embryo proper. This is not the case in the other genera where the fertilized ovum gives rise directly to the embryo proper. There is no change in orientation, the axis of the embryo remaining straight, with the shoot-pole turned away from the archegonial neck. On further growth the shoot bursts upwards through the prothallus and the root vertically downwards into the soil. Thus in the Marattiaceae the suspensor seems to be of no particular use, and, on the other hand, the change from an embryo with a suspensor to one without seems to involve no biological advantage and not to stand in any obvious relation to the bulk of the prothallus.

The same result follows if the Ophioglossaceae are considered. Here in all cases the prothalli are relatively bulky and saprophytic. Though, owing to the conformation of the prothallus and the direction of the archegonia, the embryo cannot grow straight upwards through the prothallus as in the Marattiaceae, the available evidence is against regarding the curvature of the embryo as involving any serious disadvantage. It has been shown that it does not prevent the successful growth of the plant in *Helminthostachys*. The suppression of the suspensor thus does not seem well explained on biological grounds, though it certainly leaves the way

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[1] The fact that the prothallus contains an endophytic fungus does not appear to be of primary importance as regards the embryogeny.

open for a new arrangement of the primary polarity of the embryo which may be more satisfactory. A similar rearrangement of polarity is found in the Leptosporangiate Ferns where a suspensor is wanting and the initial polarity of the embryo is different.

Thus, while accepting fully Bower's conclusions as to the importance of the primary polarity of the embryo, and as to the probability of the type of embryo with a suspensor being relatively primitive, it does not seem to me clear, from a consideration of the Marattiaceae and Ophioglossaceae, that the suspensor is to be explained as having originated on account of its biological advantage, and it is also not clear that the curvature of the embryo entails any effective biological disadvantage. I am inclined to think that the presence of a suspensor and also the simplification of the embryo by the omission of a suspensor may be better explained on morphological than on biological lines.

The alternative view, to which I incline, may be briefly stated. The presence of a suspensor of one or two tiers appears to be a fact of organization in a number of forms which are relatively primitive. Its presence may be looked upon as the last indication of the construction of the plant body from a filament or row of cells, i. e. as a juvenile stage in the development rapidly passed over and often suppressed. This juvenile stage may have suited the conditions of development and been retained and elaborated, as appears to have been the case in the line of descent leading to the Spermaphyta. On the other hand, the non-development of the suspensor may have allowed of useful changes in the primary orientation of the embryo, but its morphological significance appears to lie in the suppression of the last indication of the juvenile filamentous stage.

I do not propose to enter fully at present into the extended application of this way of regarding the suspensor. It may be pointed out, however, that it can be supported by analogies from the germination of spores of Algae, Bryophyta, and Pteridophyta, where a filamentous stage is often hurried over or suppressed. It also allows of application to the somewhat similar case of the presence of a cell taking no part in the further development, at the base of the sporogonium of the Jungermanniaceae, and its absence in the more direct development of the sporogonium of other Hepaticae.

One other feature of the construction of the embryo in the Filicales may be referred to in conclusion. This is the position of the primary or first root. Regarding this, Bower remarks : 'It is notable that though the root in Seed-Plants directly faces the suspensor, this is not the case in any Pteridophyte ; in them it is always a lateral appendage, however nearly it may sometimes approach the centre of the hypobasal hemisphere. Accordingly it cannot be held to be itself the continuation of the primitive axis.' I do not propose to enter into the question as to how far this statement holds for the other phyla, but it does not seem to apply without considerable modification to the Filicales. It seems clear from Campbell's figures that the primary root is endogenous and practically median in the Marattiaceae even when, as in *Danaea*, a suspensor is present. What we know as to the initiation of the primary root in *Helminthostachys* suggests that the same holds here also, though the root subsequently appears lateral owing to the great development of the foot and the change in direction of the axis of the shoot. The direct continuation of the axes of the shoot and primary root is very striking in Lyon's figure of *Botrychium obliquum*.

It has been further pointed out above that the first root in *Helminthostachys* occupies a different position, relatively to the dorsiventral axis, from that occupied by the later roots, and this difference may have its explanation in the original polar position of this root as the continuation backwards of the primary axis of the plant. In this respect the relations in *Helminthostachys* appear closely comparable to those shown for the dorsiventral rhizome of the young plant of *Polypodium vulgare* in a figure by Professor Bower.[1] There is thus some evidence for regarding the first root in the Filicales as different from the succeeding roots and potentially a main or tap-root. It would be comparable in not giving rise to the whole root-system to the primary root of a Monocotyledon. The position of the primary root-apex relatively to the suspensor in such a plant as *Danaea* is comparable to what is the rule in Gymnosperms, Dicotyledons, and Monocotyledons.

That such resemblances in the primary arrangement of the organs should be traceable between the Spermaphyta and the more primitive Filicales is of some interest and importance. It seems possible that the presence of a suspensor and the relative position of the organs of the embryo (including the terminal root with its apex directed towards the suspensor) may be characteristics of the higher plants that have persisted from the condition present in a filicineous ancestry. Proof is impossible in such a question, but it seems justifiable to state this view without asserting it to be the only or the complete explanation of the facts.

SUMMARY.

1. The embryo of *Helminthostachys* extends down into the prothallus before segmentation takes place. The first two walls are transverse. Of the row of three cells, the one next the archegonial neck becomes the upper tier of the suspensor; it may remain undivided or become divided up into smaller cells. The next cell forms the second tier of the suspensor and becomes divided up into cells. The embryo proper is formed from the terminal cell of the filament.

2. The embryo proper is at first straight, continuing the line of growth

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[1] Land Flora, p. 214, Fig. 110.

of the suspensor. It becomes differentiated into a hypobasal half from which the foot later arises, and an epibasal half from which the stem-apex, first leaf, and probably the first root, are formed. The apex arises near the centre of the epibasal tier.

3. With further growth the shoot becomes vertical and the axis of the embryo curved. Further development of the shoot gives rise to the hypocotyl bearing the first leaf and apex of the stem. The shoot is at first vertical, though dorsiventral in construction. Later its apex bends over and growth proceeds horizontally.

4. The relative positions of suspensor and embryo, and of the organs of the embryo, are compared for Marattiaceae, Ophioglossaceae, and Seed-Plants, and it is suggested that the suspensor represents the last trace of the filamentous juvenile stage in development of the plant, and may have persisted in the Seed-Plants from their filicineous ancestry.

DESCRIPTION OF FIGURES IN PLATE III.

Illustrating Prof. Lang's paper on *Helminthostachys.*

All the figures are from untouched photographs.

Photo. 1. Young embryo (*e*) still unsegmented but extending down into the prothallus; *f*, foot of plant on the same prothallus. × 140.

Photo. 2. Older arrested embryo showing the upper tier of the suspensor (s^1), the second tier of the suspensor (s^2), and the embryo proper (*e*). The segment walls of the embryo are broken down, but a number of nuclei are visible. × 158.

Photos. 3, 4. Two sections of a prothallus bearing a plant (e^1), the younger embryo of intermediate age (e^2), and the young arrested embryo (e^3) figured in Photo. 2. s^1, upper tier of suspensor of the intermediate embryo; s^2, second tier of suspensor. × 40.

Photo. 5. Horizontal section of the embryo proper of e^2 more highly magnified. *hyp*, the hypobasal tier; *ep*, the meristematic epibasal tier. × 158.

Photos. 6–9. Four sections of the second intermediate embryo shown in Text-fig. 3, B. The junction of the second tier of the suspensor (s^2), and the embryo proper (*e*) is seen in Photo. 6; Photo. 7 shows the hypobasal half of the embryo proper giving rise to the foot. In Photo. 8 the base of the vascular cylinder is seen with the first tracheides (*tr*). Photo. 9 passes through the apex of the stem which is marked with a cross. × 158.

Photo. 10. Obliquely transverse section of a mature embryo showing the well-preserved suspensor (s^1, s^2). × 40.

Photo. 11. More highly magnified figure of this suspensor. s^1, upper tier; s^2, lower tier. × 158.

Photo. 12. Transverse section of a prothallus bearing a young plant attached by the suspensor, the two tiers of which are seen at s^1 and s^2. × 40.

Photo. 13. Section of the suspensor of another young plant in relation to the prothallus. Both tiers of the suspensor are multicellular. *ar*, archegonial neck; s^1, upper tier of suspensor; s^2, lower tier. × 158.

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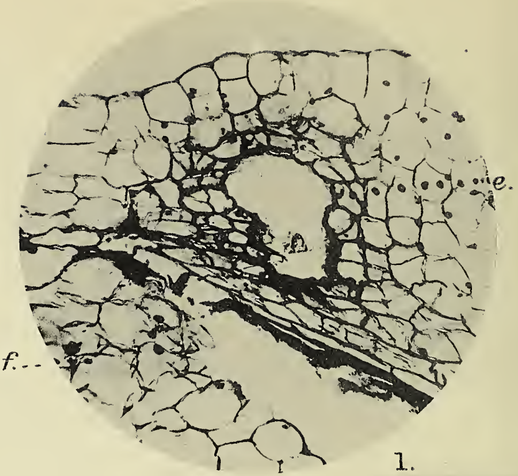

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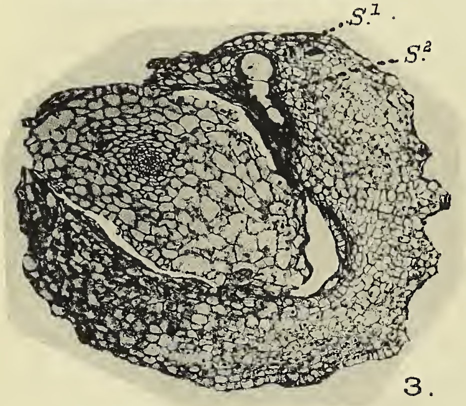

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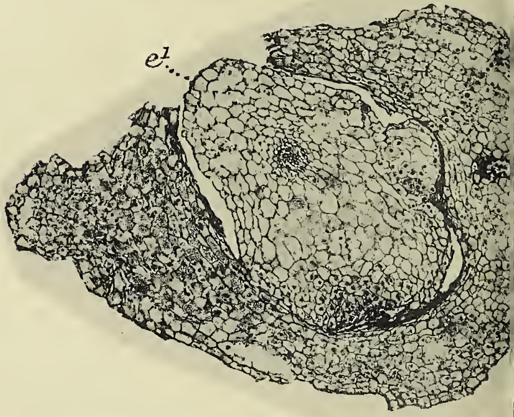

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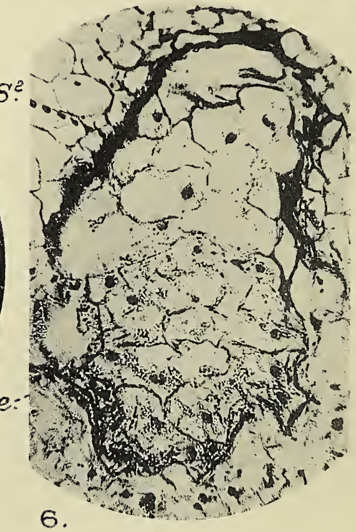

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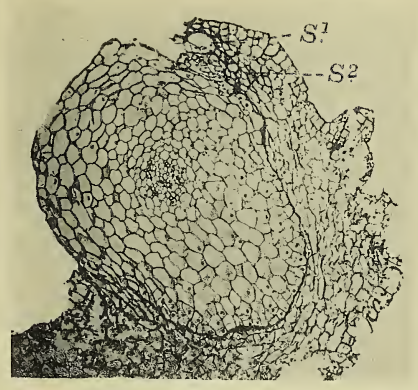
LANG—EMBRYO OF HELMINTHOSTACHYS.



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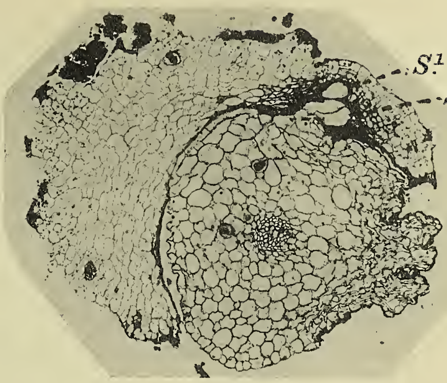

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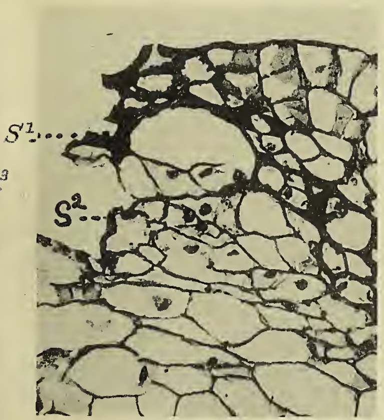

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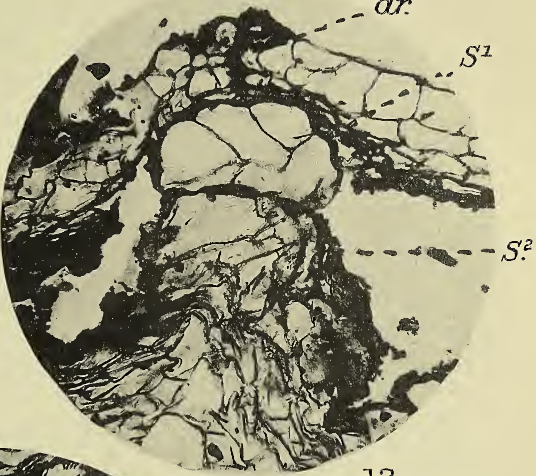

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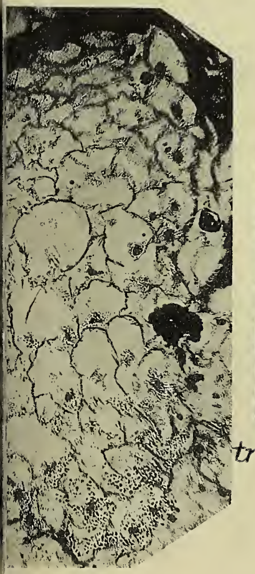

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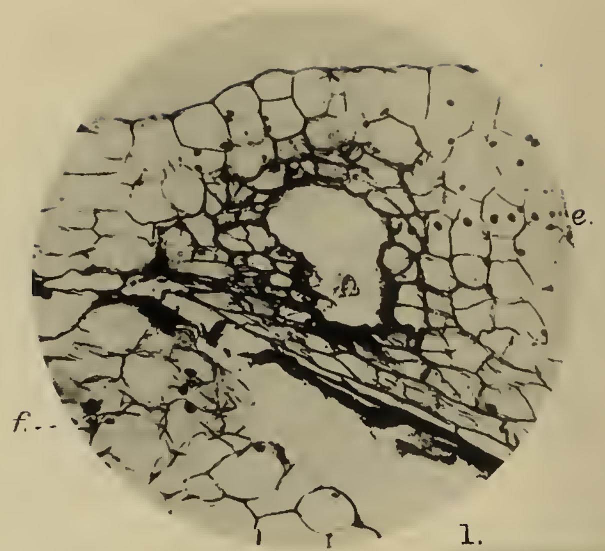

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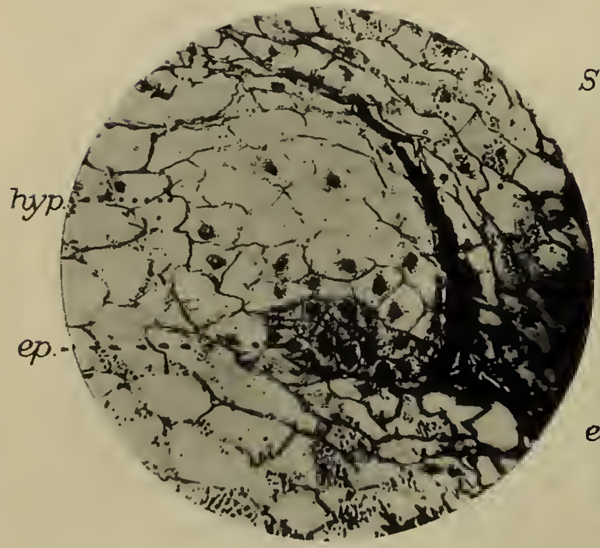

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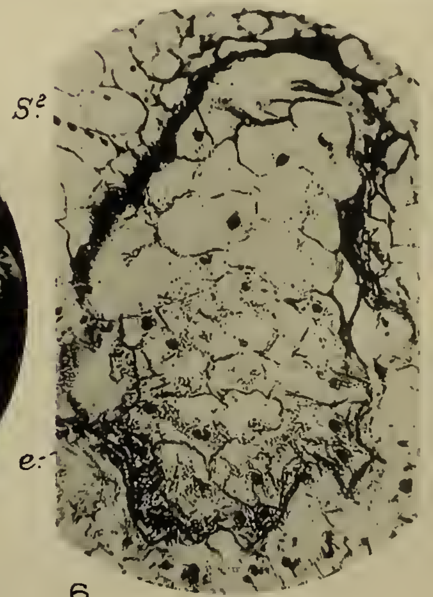

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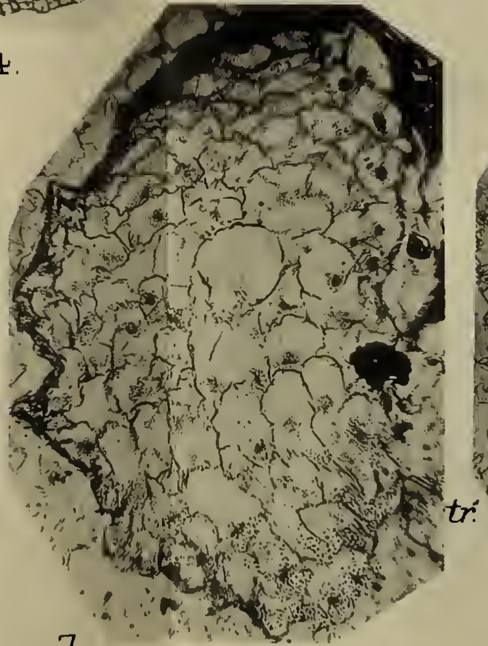

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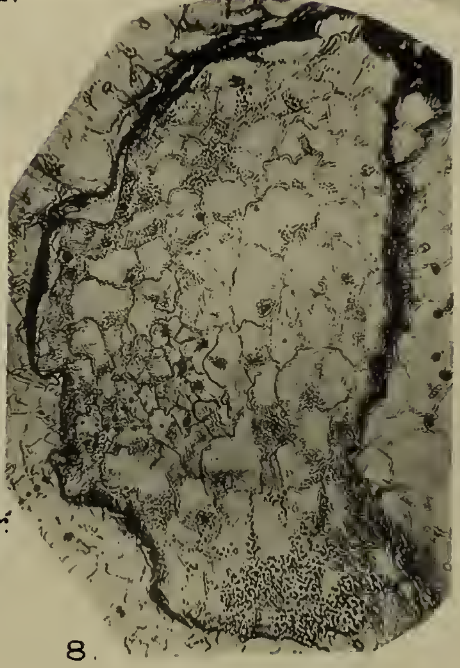

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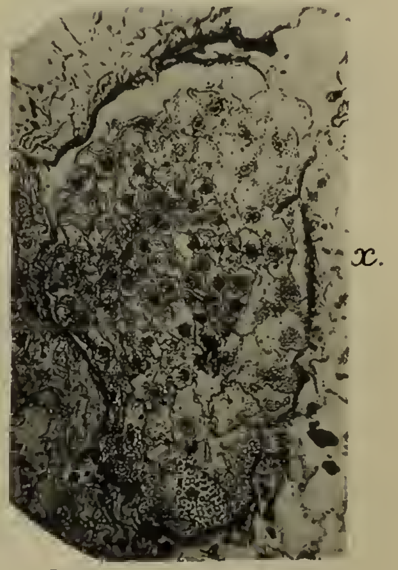

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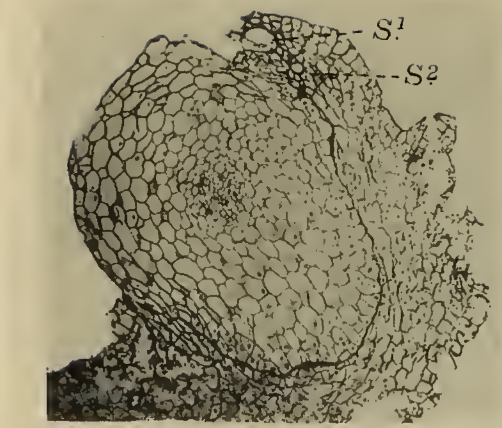

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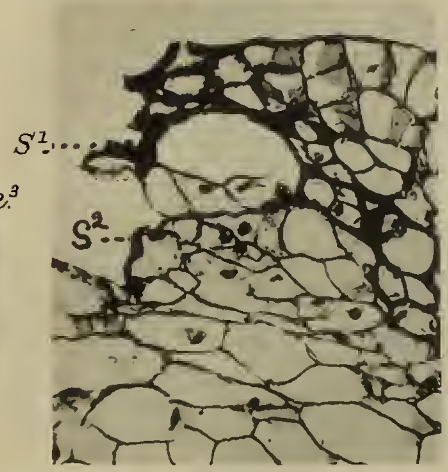

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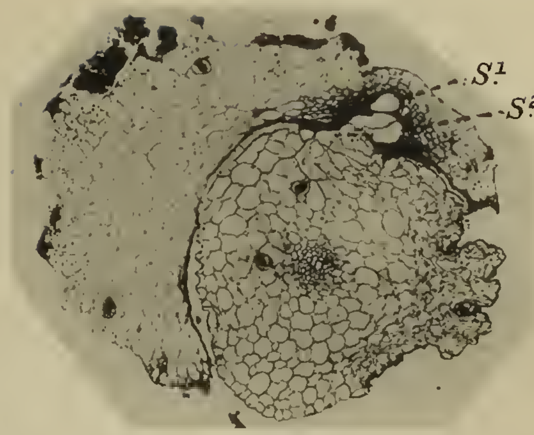

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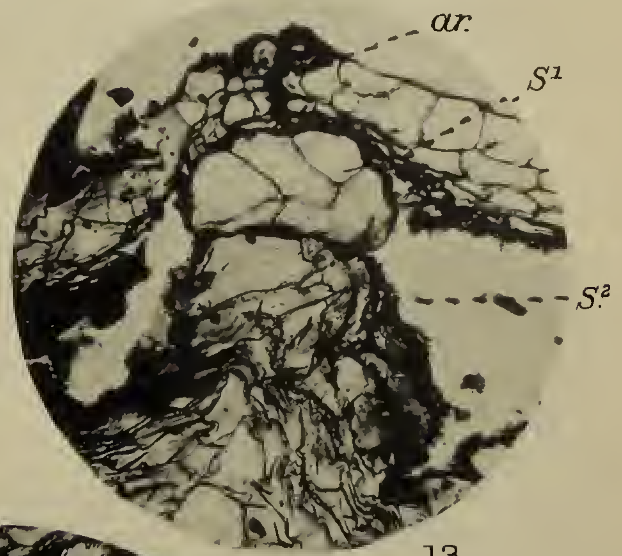

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W.H.L. photo.

LANG — EMBRYO OF HELMINTHOSTACHYS.

Huth coll.

On the Structure and Relationships of Trigonocarpus Shorensis, sp. nov.

A New Seed from the Palaeozoic Rocks.[1]

BY

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With Plates IV and V and eight Figures in the Text.

CONTENTS.

I. INTRODUCTION.

THE bulk of the material which forms the basis of this communication was placed in my hands for investigation by Prof. F. W. Oliver; and I gladly take this opportunity of expressing my deep indebtedness to him for much helpful advice and criticism, and also for putting at my disposal such slides of the University College Collection as I have had occasion to consult.

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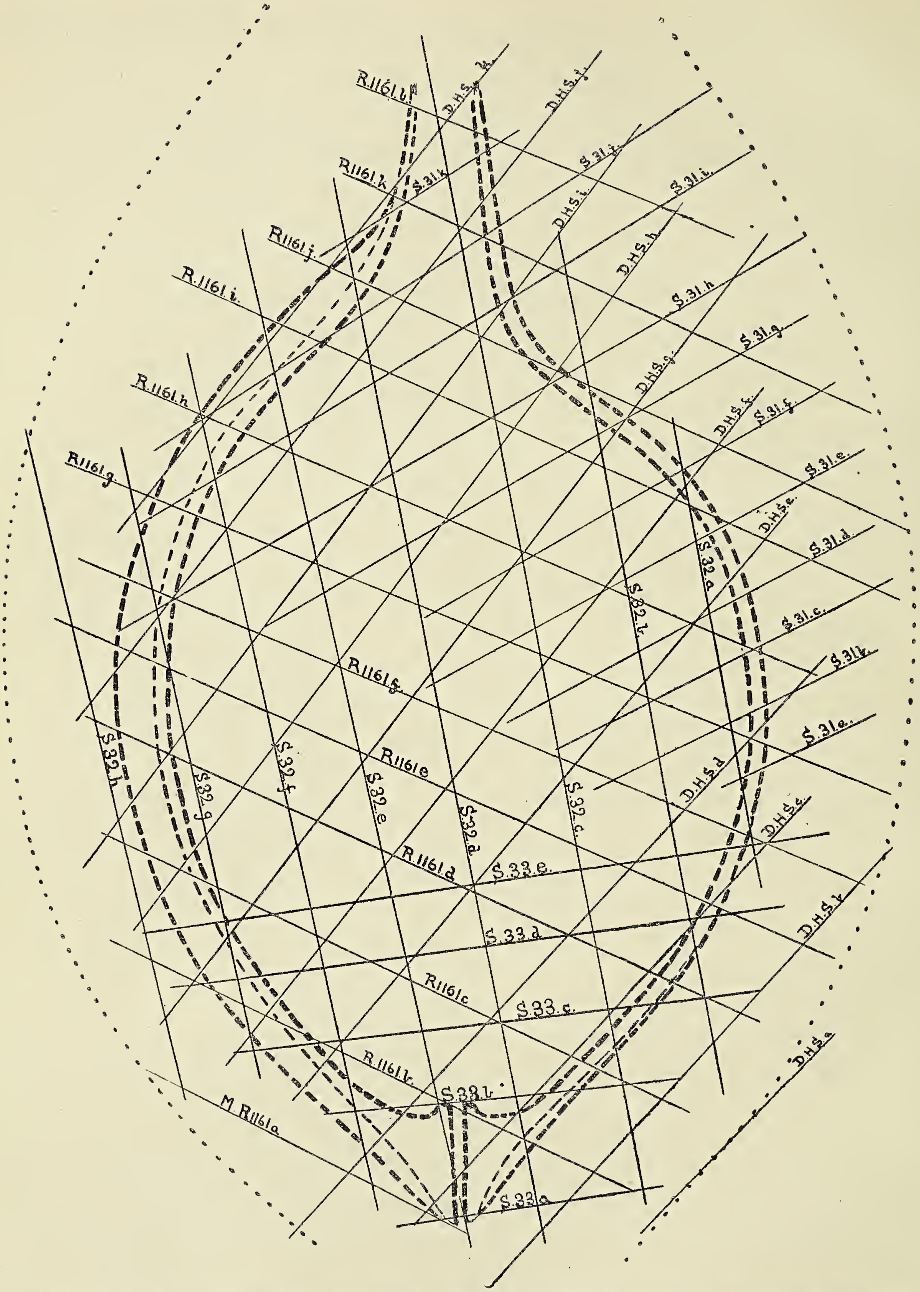
[1] Thesis approved for the degree of Doctor of Science in the University of London.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.]



TEXT-FIG. 1. Plottings on an ideal median longitudinal section showing the planes of all the preparations. D.H.S. = Dr. Scott's series; R. = Owens College, Manchester, series; S. = University College, London, Collection.

My thanks are also due to Dr. D. H. Scott and to Prof. F. E. Weiss for the loan of preparations. To Dr. Scott I am also indebted for permitting me to examine the more important sections in his unique collection of *Trigonocarpus Parkinsoni*, without which an adequate comparison between the two seeds would not have been possible.

The same methods have been employed in the reconstruction of the present seed as were used in the investigation of *Conostoma oblongum* and *C. anglo-germanicum*.[1] These have been recently described in a separate article[2] and need not be recapitulated here.

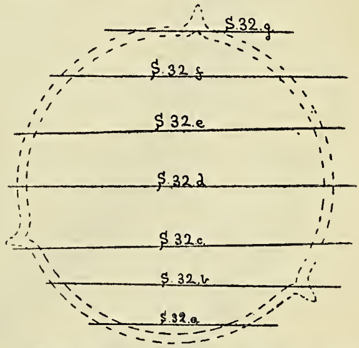
The sections that furnish data for the following account are all cut from seam nodules obtained from the well-known locality at Shore Littleborough, re-opened through the generosity of the late Mr. Sutcliffe. To mark its origin it has been thought appropriate to designate this seed by the specific name of *Shorensis*.

II. GENERAL FEATURES.

The seed with which the present investigation deals was of large size, approximately elliptical in form, and circular in transverse section. The exact limits at either end cannot with certainty be determined, but the total length was probably considerably over four centimetres. In breadth the seed attained a maximum diameter, about half-way up, of nearly two and a half centimetres.

Although specimens showing the actual attachment of the seed are wanting, the chalazal end certainly tapered towards its insertion and, judging from the general direction of the surface curvature, followed a more gentle curve inwards to the apex.

The testa comprised three or perhaps four layers, the two outer of which constituted a broad sarcotesta remarkable for the presence of a number of scattered secretory sacs and representing nearly one-third of the total width of the seed.



TEXT-FIG. 2. Plottings on the transverse section of the S. 32 series.

Within the sarcotesta was a hard sclerized shell bearing three salient ridges which extended from the base to the apex. These ridges were symmetrically placed around the body of the seed, and from the chalazal end to about a third the height of the sclerotestal shell were present three more ribs much less pronounced than the former, and occupying positions intermediate between them.

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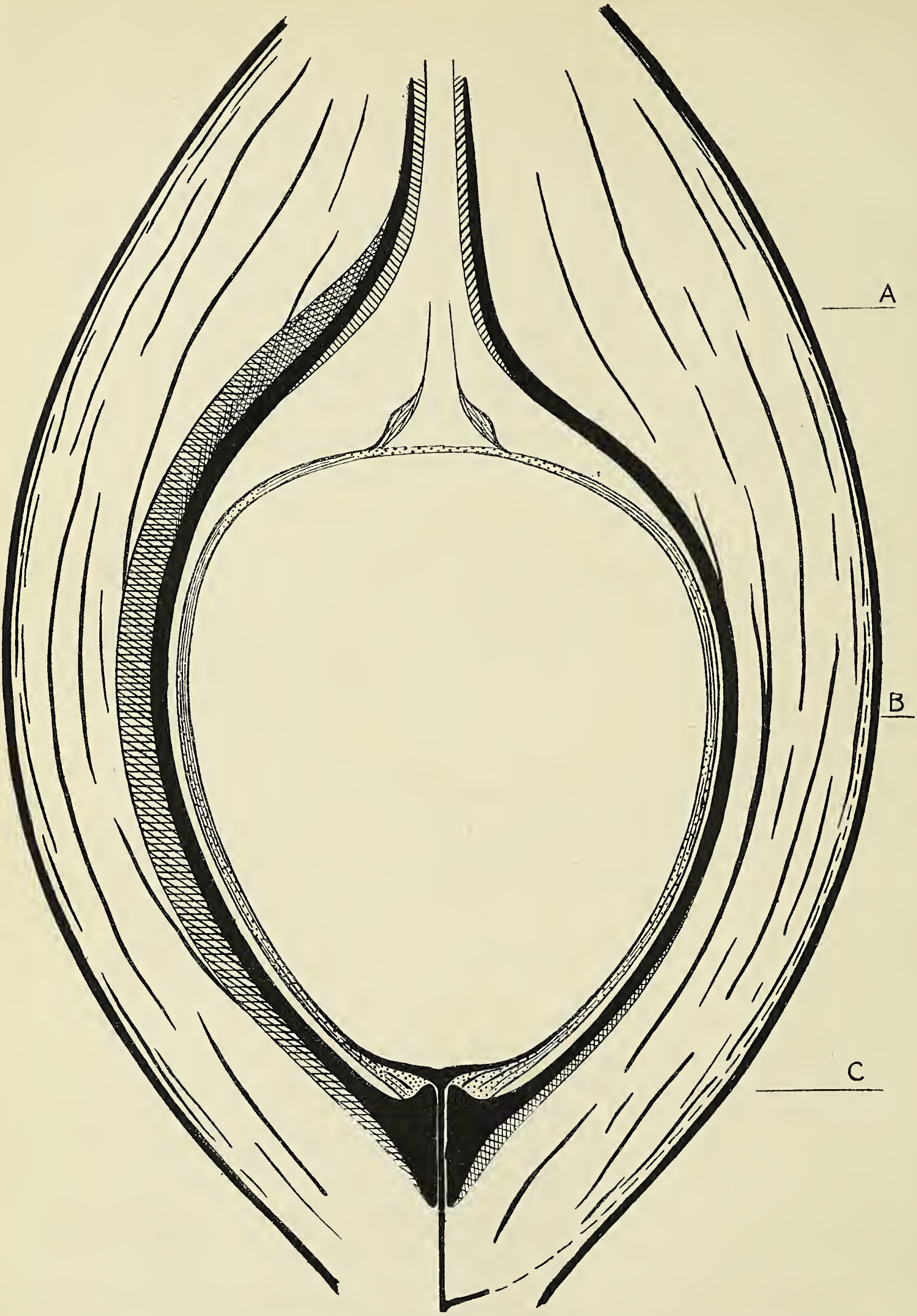
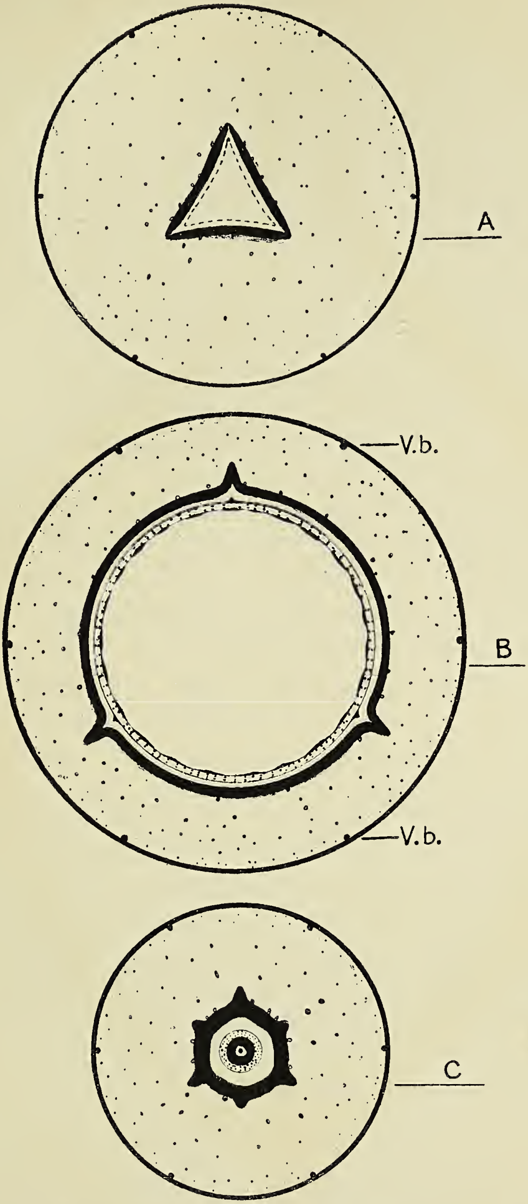
[1] Oliver and Salisbury: On the Structure and Affinities of the Palaeozoic Seeds of the *Conostoma* group. Ann. Bot., vol. xxv, 1911.

[2] Ann. Bot., vol. xxvii, No. cvi, 1913.



Text-fig. 3. Diagrammatic restoration of an ideal median longitudinal section of *Trigonocarpus Shorensis* passing through a primary and secondary rib. The sclerotesta and limiting layers of the sarcotesta are shown in black; the ribs are cross-hatched, and the inner flesh, the extent of which is hypothetical, is represented by diagonal shading. The branching of the chalazal bundle is based on analogy with *T. Parkinsoni*, and the direction of one of the sarcotestal strands is also shown as a dotted line, though not actually present in the plane of section. Nucellar tissue dotted, secretory sacs black.



TEXT-FIG. 4. Transverse restorations of *Trigonocarpus Shorensis* at the levels A, B, C in Fig. 3. Tissues represented as in the previous diagram. The six sarcotestal strands are represented by large black dots, and the secretory sacs by smaller.

In the chalazal region there were thus six ribs, of which three soon die out, whilst the remaining three persist.

In general form the sclerotesta was ovoid, the pointed end corresponding to the chalazal region, whilst the blunt end was surmounted by a triangular micropylar tube, the angles being continuations of the ribs lower down, into which they pass by a hollow curve.

Within the sclerotic tissue was a fourth layer, probably confined to the micropylar region and representing the 'inner flesh' of related seeds.

The megaspore cavity was much less pointed at the base than was the hard shell around, and through this divergence of the two surfaces a chalazal cushion of sclerotic tissue was formed from which arose a nucellus, that stood up erect and free within the seed cavity, surmounted at its apex by a large pollen-chamber.

The nucellus was bounded at the exterior by a well-differentiated epidermis, and within was a tracheal sheath forming the upward continuation of a single vascular strand which pierced the chalazal cushion.

The vascular organisation was essentially similar to that of other seeds of this group, and consisted of a sarcotestal system of bundles without and a nucellar system within.

Neither pollen nor prothallus are preserved in any of our sections, which may indicate that all our specimens were abortive ovules, though the presence of numerous fungal spores within the megaspore cavity may indicate its former occupation by an organized tissue which served as nourishment to the saprophytic organisms.

III. THE TESTA.

1. The Sclerotesta.

The obovoid sclerotesta had an average thickness of about 0·5 mm. and bore, as we have seen, six longitudinal ribs, of which the three major persisted as far as the triangular beak. At the apex these ribs pass gradually into the three angles of the micropyle, and, except at the base, are commissured. The major ribs from the base of the micropyle to within some 6 mm. of the chalazal extremity have about the same radial extent, but exhibit varying development in the different specimens (0·75 mm. from surface in S. 33, *a–e*, to 1·4 mm. in S. 31, *a–k*). The three minor ribs alternated with the three major, were without commissures, and attained their greatest extent (0·4 mm.) about 2 mm. from the base, above which point they gradually diminished and finally died out some 8 mm. from the chalaza. At the base of the seed the sclerotesta formed a solid inverted cone about 3·5 mm. high, perforated by a narrow canal through which the single vascular bundle passed to the floor of the nucellus. The base of this cone projected upwards as a dome-shaped papilla about 0·5 mm. high and 1·5 mm. in diameter, and its margin served as the place of attachment for the nucellar epidermis. As the ribs, both major and minor, approached the chalazal end they became gradually smaller till at the extremity all indication of ribbing had disappeared. The cavity within the sclerotesta was, owing to the sclerotic papilla, much blunter than the external form, and from the base of the micropylar canal measured some 19 mm. with a diameter in its broadest part of slightly over 14 mm.

Sections through the micropyle (Pl. IV, Fig. 2) show that the sclerized sides of the triangular canal about half-way up, formed of four layers of longitudinally directed fibres, were from 0·13 to 0·14 mm. in thickness and distinctly concave, the internal diameter of the canal measured from the apex of an angle to the middle of one of the sides being just over 2 mm. In the highest of our sections (Pl. IV, Fig. 5), viz. Man. Coll. R. 1161, *b*, the limit between sclerotesta and sarcotesta is indefinite and the diameter of the micropyle is reduced to 1·4 mm. On that side of this oblique section which is at the higher level the sclerized layer is seen to be even less developed than on the lower, and the concavity of the flanks has resolved itself into a slight double convexity (thickness between the angles 0·10 mm. and at the convexities 0·16 mm.) (Pl. IV, Fig. 8, *m*, *c*).

Although this section is broken there is evidence that the sclerotesta towards the apex segregated into three distinct portions separated at the angles by intervening soft tissue and each itself composed of two parts fused in the median plane.

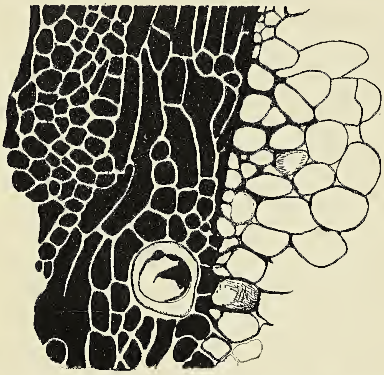
Except for the slight local thickening, both here and in the preceding section, there is a thinning down of the sclerotic beak which, if maintained at the same rate, must have resulted in its complete elimination at a level not far above that at which our section was cut.

From these facts it would appear that the beak was comparatively short (probably not more than 8 mm. in length), although the extensive development of the sarcotesta at this level and the curve which successive sections show its surface to have followed indicate a much further persistence of that tissue. So that in this respect our seed resembled most closely the condition that obtains in present-day Cycads.

Histology of the Sclerotesta. The sclerotesta was formed of from 14 to 15 layers of thickened fibrous elements, of which the inner, and most of those forming the ribs, were longitudinally directed. In the best-preserved specimens the innermost layer is seen to have been composed of radially flattened fibres which form a smooth internal surface, and negative the suggestion of a more interior tissue. In transverse sections the fibres appear rounded or polygonal (28–42 μ) with dark contents separated by a thin membrane (? middle lamella), on either side of which is a translucent yellow zone about 3·5 μ in width perhaps representing the cell-wall. The fibres consisted of superposed cells which can be traced for over 1·5 mm.

The sclerotic fibres, 5–9 deep, forming the outer layers behave as aggregates or bands, each of from one to two elements in the radial direction and of a very variable number in the tangential (Text-fig. 5). For a time each band follows the longitude of the seed, and then almost abruptly all the fibres bend, some to the right and others to the left, or all of them in the same direction, so that their course is at a very narrow angle with the horizontal plane, and in transverse sections they appear cut obliquely or almost longitudinally for some distance. Where the members of a band are in one layer they appear to all bend in a similar manner, but where the band is more than one element in thickness those of each layer probably bend in opposing directions, but it cannot be definitely ascertained whether or no they again take up a longitudinal direction.

The extreme irregularity of the course of the fibres is shown by the inconstant numerical relation between those cut obliquely and transversely, and, to still further complicate the structure, the bands do not always remain in the same vertical plane.



TEXT-FIG. 5. A portion of the sclerotesta as seen in transverse section, showing the vertically directed fibres at the interior and the almost horizontal direction at the periphery; a single secretory sac is also present.

Owing to this complex interlacing of the outer elements of the sclerotesta, it presents a plait-like appearance when cut by sections tangential to the surface, as is seen in Pl. IV, Fig. 3.

Eventually the thickened elements of the sclerotesta pass obliquely outwards by a well-marked transition into the sarcotesta.

In the outer part of the sclerotesta, and there only, elongated secretory elements partially or entirely immersed in the sclerotic tissue are present, which pass out into the sarcotesta at one point and occasionally into the sclerotesta again at another (Pl. IV, Fig. 6, *m.s.*). In all respects these secretory elements resemble those present in the sarcotesta.

For convenience of description the sclerotesta has been treated as consisting of two parts, the plane at which the different structure is assumed being variable. There is, in fact, little doubt that the outer zone represents the terminations of a large portion and perhaps even all of the longitudinally directed fibres, and cannot possibly be regarded as the region of fusion of two morphological surfaces, as has been suggested for the stony layer of the Cycadean ovule.[1]

2. The Sarcotesta.

(*a*) *General Structure.* The sarcotesta in the living state must have been the most pronounced feature of this seed, for even as petrified it invests the sclerotic shell in the middle region to a thickness of over 4 mm.

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[1] Stopes: The Double Nature of the Cycadean Integument. Ann. Bot., vol. xix, 1905.

At the base this decreased to under 2 mm., but the almost sudden concavity of the sclerotesta at the micropyle gives the sarcotesta in that region a maximum thickness of nearly 6 mm. Although our highest sections pass not far below the apex no flattening is exhibited, but the sarcotesta curves inwards equally on all sides. This is in conformity with the impressions of seeds attached to *Neuropteris heterophylla* and *Neuropteris obliqua* described by Kidston [1] and Kidston and Jongmans.[2]

The junction of the sclerotesta with the sarcotesta, composed as the latter was of thin-walled cells, appears superficially as a sudden change, but the innermost cells of the sarcotesta can be recognized as direct continuations of the rows of sclerized elements. It is only in tangential or longitudinal sections that this transition can be properly seen, since the innermost sarcotestal cells, which are elongated and tubular (0·15 mm.–0·25 mm. × 0·033–0·049 mm.), are directed obliquely outwards and upwards (Pl. IV, Fig. 5). Owing to this the transition, which was of equal gradation throughout, appears much more sudden in transverse sections through the middle region than in those through the base or apex, where the surface is cut obliquely. This may perhaps be true also of the similar observations of Scott and Maslen [3] for *T. Parkinsoni.*

Very frequently cells comprising the innermost layer of the sarcotesta are seen cut longitudinally, also in tangential sections (Pl. IV, Fig. 5, *st. c.*) these cells alter their direction in a similar sinuous manner to those of the outer sclerotesta. Here and there slight thickening of the walls can be detected, and the intercellular spaces are either very minute or completely absent. It is clear, then, that the structure of the cells and architecture of the innermost tissue of the sarcotesta were essentially the same as in the outer layers of the sclerotesta—a continuity of structure which can only be reconciled with a homogeneity of origin. As we pass further outwards the intercellular spaces become more and more pronounced, and the constituent cells, at first tubular, very quickly become more rounded, and *pari passu*, as we pass to the exterior, develop irregularities in the form of projections which connect on to those of adjacent cells and give an almost stellate structure to the tissue (Text-fig. 6).

Owing to the rounded character of the middle sarcotestal cells, the intercellular spaces between them were of more or less equal dimensions in every direction. At a very little distance out, however, the cells were slightly flattened, and tend, especially at the periphery, to form short vertical series separated from one another by elongated lacunae. Sections in this plane also show that the arms or projections of successive cells were often superposed, so that each large lacuna is frequently without trabeculae,

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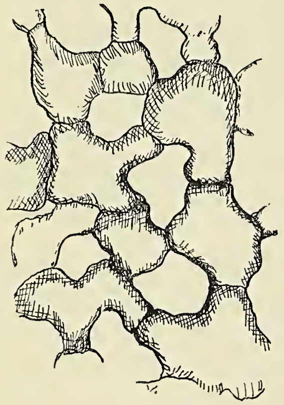
[1] On the Fructification of *N. heterophylla.* Proc. Roy. Soc. London, vol. cxcvii, 1904, p. 1.

[2] Archives néerlandaises d. sci. exactes et naturelles, sér. iii, B, tome i, 1911, p. 25.

[3] Ann. Bot., vol. xxi, No. lxxxi, 1907.

and the projections themselves often appear as narrow vertical series partially or completely separating adjacent intercellular spaces (Pl. IV, Fig. 10). Although the above fundamentally expresses the arrangement of the cells in this region, it was subject to great irregularity.

In the living condition this tissue must therefore have been remarkably light and spongy, and if these seeds were shed into water would render them of great buoyancy. Several seeds of this affinity, e. g. *Trigonocarpus Parkinsoni*,[1] *Pachytesta*,[2] and *Aethiotesta elliptica*,[3] exhibit a lacunar sarcotesta, and the suggestion put forward by Renault for *Aethiotesta* that this served as a mechanism for dispersal by water may well have been true also in the present instance, all the more that the method of occurrence of fossilized vegetable remains favours the view that they were in part components of water-borne drift.



TEXT-FIG. 6. A small portion of the outer sarcotesta, showing the large intercellular spaces.

(*b*) *The Peripheral Zone.* At the exterior the sarcotesta was bounded by a very ill-preserved layer of thin-walled cells ($1{\cdot}5\,\mu$–$3\,\mu$ tangential $\times\ 2\,\mu$ radial). Beneath this epidermis there were numerous sclerized fibres between which a soft-walled parenchymatous tissue was most likely present originally, though all except a few remnants of walls have become disintegrated.

The total width of this peripheral zone where there is no evidence of crushing or contraction is about 0·3 mm. In most cases the sclerized elements appear irregularly scattered, probably due to post-mortem changes, as in several places, where this zone has almost retained what was probably its original width, they are seen to be grouped together to form somewhat irregular radial plates (Pl. V, Fig. 19). Each plate was formed of from 2 to 6 elements, the higher number probably being the more usual, whilst tangentially the groups generally form either a single or a double row.

Not infrequently there were considerable local aggregations tangential to the surface, forming broad bands which may well have arisen through the lateral fusion of a number of the sclerotic plates. In outline each broad band formed a continuous hypodermal layer of sclerotic elements, with occasional projections inwards. Owing to crushing and contraction, many of the sclerotic bands have come to occupy an oblique or even tangential direction, causing irregularity and superposition of the originally radial plates

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[1] Scott and Maslin, loc. cit., p. 101.

[2] Renault: Bassin houiller et permien d'Autun et d'Épinac, vol. iv, p. 390, and Pl. LXXXIII, Fig. 10.

[3] Renault: Mém. Soc. d. Sci. nat. d. Saône-et-Loire, p. 1, 1887.

The individual sclerotic fibres were roughly rectangular, with a slight flattening in the radial direction, and usually about $2 \cdot 5 \times 4 \cdot 5\,\mu$. The wall was much thickened (about $0 \cdot 8\,\mu$) and formed of an outer transparent, yellowish layer and an inner and slightly thicker brown layer, both of which show clearly defined lamellation.

The longitudinal sections do not show the outer region of the sarcotesta preserved. The maximum longitudinal dimension in oblique sections is a quarter of a millimetre, and no doubt the real length was much greater.

The width of the intervals between successive plates is usually fairly regular, but rarely they are seen in very close proximity even where there is no evidence of displacement, and these are, perhaps, like the larger aggregations, to be interpreted as due to anastomoses.

Irregularly interspersed amongst the sclerotic cells are numerous secretory sacs, some $7 \cdot 5\,\mu$ in diameter, which were no doubt situated in the soft parenchyma between the sclerotic plates, and become very numerous just beneath this zone.

Here and there the secretory sacs occur two or three together, arranged radially, and it is possible that these too, as in the nucellus, formed rows alternating with those of the sclerized elements.

Where cut obliquely, the secretory cells show fine longitudinal striation of the external surface of their walls; in one section, which is especially well preserved, this wall is seen cut transversely as a series of dark beads separated by clear spaces of about equal width. Surrounding the carbonized contents is a clear space which probably represents a thick sclerotic wall: the elements, in fact, were almost identical in appearance with the thick-walled mucilage sacs found in the sporophylls and testa of present-day Cycads.

In favourable cases, four or five connexions at more or less regular intervals can be seen between the dark central mass and the thin external wall. They probably represent radial pittings of the cell-wall, but must not be confused with the numerous and much finer and fainter radial striations, which are doubtless a matrix effect.

Occasionally the secretory sacs were situated at one or other end of the sclerotic plates, or even in the middle, replacing the sclerotic elements themselves.[1]

The presence of the numerous hard plates in the peripheral zone of the sarcotesta must have given to that region considerable rigidity, and the dark layer often seen between it and the soft underlying tissue is no doubt to be interpreted as originating through the compression of the outer cells of this latter.

(*c*) *The Secretory System.* The sarcotesta is not only distinguished from that of other seeds by its extreme development, but also by the

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[1] Cf. Petiole of *Medullosa.* Scott's Studies in Fossil Botany, Fig. 176.

numerous secretory sacs which permeate it throughout (Pl. IV, Fig. 2, *m.s.*). In all respects the secretory elements of the interior appear to agree with those of the periphery.

Each secretory sac is sheathed by a continuous layer of parenchymatous cells, which separate it off from the surrounding lacunar tissue. Component cells of this sheath are seen in tangential sections to have been vertically elongated and to have reached a length of some five times their width.

TEXT-FIG. 7. Plottings of the secretory sacs in three successive sections. In order to make the corresponding sacs more clear, they have been connected up by means of dotted lines.

By carefully mapping the distribution of the secretory sacs between the same pair of ribs in successive sections of a series, one can recognize, from the relative positions which they occupy to one another, that the same sacs often extend for a considerable distance (Text-fig. 7).

In the subjoined table an analysis of the results obtained from S. 31, *b–i*, is given; the vertical series show the number of sacs in each section which can be traced back as far as that of which the designating letter heads the column. In Section *f* the sarcotesta is much broken, hence the figure for *g* is probably double its real value. If this be the case, then the number of additional sacs appearing at any given level is about twenty-one, or sixty-three for the whole circumference. The whole interval included between S. 31, *b* and S. 31, *i* is about 14 mm., and out of the total of 195 separate secretory elements encountered, not more than eight persist throughout. A few are only present in one section, whilst the greater number extend into the next (an interval between sections of about 2 mm.) with a falling off in each successive section.

S. 31.		*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*Total.*
Section S. 31.	*c*	46	23							69
,,	*d*	45	15	26						86
,,	*e*	35	12	24	22					93
,,	*f*	?	?	?	?	10 +				?
,,	*g*	23	6	2	8	10	37			86
,,	*h*	20	5	2	7	6	32	11		83
,,	*i*	8	4	2	6	6	13	5	20	64

The level of *k* in the Manchester series is the base of the micropyle, and of *c* that at which the nucellus is attached to the chalazal cushion. The number of secretory sacs in each case represents approximately one-third of the total at that level.

Man. Coll.	*c*	34	Man. Coll.	*h*	101
,,	*d*	46	,,	*i*	92
,,	*e*	65	,,	*j*	93
,,	*f*	82	,,	*k*	109
,,	*g*	98			

A study of the two series shows that, in spite of irregularities, there was a decrease in number both towards the apex and base. From a comparison of the same sacs cut at different levels, they are seen to have tapered gradually both above and below, so that where cut across near their terminations they are of very small size and, where the surrounding wall is disclosed, very closely resemble developmental stages in the formation of secretory canals, the surrounding sheath simulating an epithelium, which may perhaps be the explanation of the similar appearances described by Professor Seward in the medullosean petiole *Rachiopteris Williamsoni*.[1]

A single secretory element, as seen in one section, is in several instances replaced by two in intimate contact in the succeeding section (Pl. V, Fig. 12); the fact that both are usually of nearly the same cross-section seems to favour the view that this is due to branching.

The normal course followed by the secretory elements was slightly sinuous and approximately parallel to the direction of the surface, with an outward tendency exhibited by the more interior. The distribution was irregular, with a marked increase—especially of the smaller—towards the periphery; whilst the largest (0·124–0·166 mm.) are found midway between the sclerotesta and the external surface (cf. Text-fig. 3, p. 42).

[1] Ann. Bot., vol. viii, No. xxx, 1894.

3. The Inner Flesh.

All those sections which pass through the body of the seed, even where the preservation of soft tissues is excellent, show no indications of an inner flesh. It is certainly true that in most of the preparations the internal surface of the sclerotesta exhibits an irregular outline, but in the transverse series S. 33, *a*–S. 33, *d*, in which the internal preservation is almost perfect, the inner margin of the sclerotesta in some places forms a perfectly even surface. This latter affords positive evidence of far more value than the negative evidence of a ragged surface, and still more so since we find in the badly preserved material that the sclerotesta does show signs of superficial disintegration.

In dealing with the nucellus we shall find that it was possessed of a highly differentiated and probably strongly cuticularized epidermis, and there is evidence that it was in close contact with the sclerotic tissue.

Having due regard to all these facts, one is forced to the conclusion that in this species of *Trigonocarpus* an inner flesh was not present in the middle part of the seed cavity.

When we come, however, to about the level of the insertion of the micropyle, the zone of sclerization begins as it were to shift slightly outwards, and we thus find within the sclerotesta several layers of cells which, though possessing thick walls, have not the dark brown contents of the sclerotic fibres themselves, and, moreover, become thinner-walled as we pass inwards. This tissue reached its maximum thickness, of some four layers, midway between the angles, and on its inner margin exhibits a broken surface that here might well indicate a greater extent of probably more parenchymatous elements (Pl. IV, Fig. 8, *i.f.*).

Even then, if we regard this micropylar lining as homologous with the inner flesh of other Trigonocarpeae, our seed is as extreme a member on the one hand, as *Pachytesta* on the other.

IV. The Vascular Organization.

(*a*) *The Chalazal Bundle and Nucellar System.*

The lowest of our transverse sections yields us no information as to the vascular structure, but from S. 33, *b*, Man. R. 1161, *b*, and the longitudinal section S. 32, *e*, we find that a single vascular bundle entered the chalazal end of the seed. This passed up to the base of the nucellus, at which level it is cut by the section S. 33, *b* (Pl. V, Fig. 16). The bundle is there seen to have been a solid mass of xylem very slightly triangular in outline, the angles corresponding in position with the three main ribs of the seed. It was formed of from sixty to seventy elements, and attained a diameter of about 0·18 mm. The larger tracheae are situated mostly at the periphery

(Pl. V, Fig. 16), and in their radial dimension (30–40 μ) considerably exceed their width (75μ–0·025 mm., average about 0·015 mm.). The central elements are polygonal in form and from 0·015 mm. to 0·02 mm. in diameter. The bundle therefore has the appearance in transverse section of a central core surrounded by a single layer of radially flattened elements.

The smallest tracheae, measuring some 0·8μ in diameter, lie just within this outer layer and appear to form six not very clearly defined groups, two corresponding to each of the three angles. That these are the protoxylem elements is rendered the more probable by an examination of the single longitudinal section passing through the chalazal bundle (S. 32, *e*). From this we find that the majority of the elements were scalariform or densely spiral (Pl. V, Fig. 18), whilst separated by one such element from the periphery of the bundle there appear to be one or two narrow elements with distant spiral thickenings.

The xylem is completely surrounded by a ring of parenchymatous tissue (Pl. V, Fig. 16, *p.s.*) composed of three to five layers of thin-walled elements which together attain a width of about 0·05 mm. In the longitudinal section this tissue is seen to have consisted of narrow, vertically elongated components, perhaps undifferentiated phloem. The whole bundle, including this delicate sheath, was surrounded by larger-celled and thin-walled nucellar tissue in which were numerous secretory sacs. One element exhibits parallel markings, and may be a transfusion tracheide comparable perhaps to those found by Scott and Maslen between the bundles of the sarcotesta of *T. Parkinsoni* (loc. cit., p. 114 and Pl. XIV, Fig. 25, *st.*).

The single bundle passed through the base of the nucellus, where it expanded in a cup-like manner and formed a tracheal investment (1–4 elements) to the megaspore cavity. This is well seen in section D of Dr. Scott's series, which passes obliquely through the base of the megaspore cavity, and shows the continuous and several-layered tracheal cup around its base.

As seen in transverse section, the tracheal mantle appears to be built up of broad, laterally fused, bands traversing the longitudinal direction which are thickest in the middle and gradually diminish towards the sides. As can be seen from the plottings (Fig. 1), the section S. 33, *c* is slightly oblique to the axis of the seed, and it is on the higher side of the section that this grouping of the tracheides is the more marked.

Presumably, then, the vascular tissue between the level of this section and the base of the megaspore cavity consisted of a uniform zone of tracheides, which on the upper side became incompletely segregated into bands. The number of these latter cannot be ascertained with certainty, owing to the oblique direction of our most nearly transverse sections, but if we assume the slight indications of thinning on the lower side to mark the lateral limits of vascular aggregations, then there were probably twelve such bands, the width of the more clearly delimited varying between 1 mm. and 1·3 mm.

The internal diameter of the megaspore cavity, as seen in section S. 33, *c*, from which the above data were obtained, is 4·4 mm., which represents a periphery of approximately 13·8 mm. If we assume each band to have been of equal width, this gives us 1·15 mm. as the measurement of each if their number were twelve. This figure is sufficiently close to the actual measurements to support the belief that this was the actual total of the bands. Unfortunately, none of our other preparations furnish any corroborative data, but it is worthy of note that the repeated hexamerous character of the seed-structure again recurs in this figure.

The next section (S. 33, *d*), though ill preserved, shows the tracheides were completely segregated into lenticular strands which were much narrower (0·25 mm.–0·5 mm.) and more numerous than at the preceding level (probably over twenty).

These strands were probably mesarch in structure, of from four to five elements in the radial plane, diminishing both in number and size on either side. In some cases, tracheides at the edge of the xylem bands can be seen passing horizontally, as is the case in *Trigonocarpus Parkinsoni*,[1] but actual connexions were not observed.

Tangential sections show that the individual tracheides were often oblique or curved, and the appearance of horizontally directed elements, in the slightly oblique transverse sections, might easily be accounted for in this way. In spite of the excellent preservation, no indication of phloem has been observed in relation to the nucellar strands.

The final section of the transverse series only contributes confirmatory evidence that the bands of xylem were disconnected, and that their smallest elements were centrally placed.

For the further course of the bundles only longitudinal and oblique sections are available. In these, the bands of tracheides can be traced to what must have been a level close to the floor of the pollen chamber. Only two sections, viz. S. 32, *c* and Section I of Dr. Scott's series, actually pass through this structure; neither of these shows any tracheides within, but the internal preservation of the latter section is very poor, whilst the former has a broken and ill-preserved floor.

The vascular elements of the nucellar system appear to be very uniform in structure (0·022–0·03 mm.), and exhibit scalariform thickenings which occasionally show anastomoses between adjacent horizontal bars. (Interval between bars, 0·0015–0·003 mm.)

From the above description it will be seen that the internal vascular system is almost identical with that described by Scott and Maslen for *Trigonocarpus Parkinsoni* (loc. cit., pp. 120–1), and it may be of some

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[1] Scott and Maslen, loc. cit., Pl. XIV, Fig. 13.

significance that in neither species has the nucellar system been traced beyond the plane of insertion of the pollen chamber.

(*b*) *The Sarcotestal System.*

In several sections of the University College series S. 31 *a*–S. 31, *k*, and perhaps in section R. 1161, *h* of the Manchester series, bundles in various conditions of preservation are to be found at the extreme periphery of the sarcotesta proper, where the outermost lacunar tissue abuts upon the limiting layers containing the radial sclerotic plates. The only parallel amongst nearly allied seeds to so external a position is to be found in *Pachytesta*,[1] where, however, if we regard the exotesta as representing sarcotesta and sclerotesta combined,[2] the bundles are only far out if considered in relation to the testa as a whole.

Owing to the incomplete preservation of the periphery, it is hardly surprising that in no slide are more than three sarcotestal bundles present at one and the same time. In several of the University College series, S. 31, *a*–S. 31, *k*, two bundles are cut in successive sections, and both of these are fortunately situated between the same pair of major ribs, and also occupy the same positions relative to them. Altogether, four distinct bundles can be distinguished in this series, and two possible bundles are also recognizable in one of the Manchester sections (R. 1161, *h*). In every case these sarcotestal vascular strands are situated in the radial plane midway between a major and a minor rib, and though the bundles are not present, in any one of our specimens, on all three faces, yet the occurrence of two, and two only, in several successive sections between the one pair of major ribs seems to point conclusively to the total number as being six, symmetrically disposed around the circumference of the seed.

The sarcotestal system was then identical as to the number of its components, and almost so as to their arrangement, with that which has been described for *Trigonocarpus Parkinsoni* and *Polylophospermum*,[3] only differing in the latter respect with regard to the proximity of the bundles to the sclerotic shell.

The structure of an individual strand is shown in S. 31, *i* (Pl. V, Fig. 17), where, owing to the curvature of the surface, the section which is oblique to the axis of the seed passes transversely through a sarcotestal bundle exhibiting extraordinarily perfect preservation. As can be seen from the figure, the bundle is slightly more extended in the tangential than in the radial direction (0·29 mm. x 0·17 mm.) and consists of some seventy xylem elements, of which the smallest occupy the central region (Pl. V, Fig. 17, *pt. x*). The latter, which probably represent the protoxylem, comprise some

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[1] Renault, loc. cit.

[2] Oliver: On Gymnospermous Seeds, New Phytologist, vol. i, No. 7, 1902, p. 148.

[3] Oliver, Ann. Bot., vol. xxi, No. lxxxii, 1907, pp. 303-4.

four tracheides (0·0075 mm. x from 0·0075 mm.–0·015 mm.) with thicker walls than those around. From the mesarch protoxylem there extends in the centrifugal direction an irregular fan-shaped group (Pl. V, Fig. 17, *cf.x.*), the elements of which, though larger than those of the protoxylem, are distinctly smaller than both those which lie on their flanks and those on the inner side of the bundle.

The centripetal xylem (Pl. V, Fig. 17, *cp.x.*) exhibits a further distinction in that the elements here are mostly cut more or less obliquely, so that the scalariform or reticulate sculpturing of their walls can be seen. A single layer of xylem elements on the centripetal side of the bundle immediately next the protoxylem do not show this character, although they exhibit the increased dimension.

The bulk of the centripetal wood evidently then consisted of short tracheides, such as are frequently present in centripetal xylem that is becoming obsolete, as is exemplified in *Lepidodendron vasculare*[1] or *Osmundites Kolbei.*[2]

(*c*) *Additional Facts as to the Vascular System of Trigonocarpus Parkinsoni.*

The course of the sarcotestal bundles at the base of the seed of *Trigonocarpus Shorensis* cannot be traced, as the peripheral portion is lacking in the sections which pass through that region.

Two sections probably belonging to *Trigonocarpus Parkinsoni*, viz. S. 34, *a* and S. 34, *b* of the University College collection, have however recently come to hand, which Prof. Oliver has also placed at my disposal for description, and which, owing to their excellent preservation, add considerably to our knowledge of the vascular structure in this region. The absence of similar data regarding our own seed, and the essential similarity between the vascular systems of the two species, are sufficient justification for inclusion here.

The first section, viz. S. 34, *a*, just passes through the lower limit of the sclerotesta, and the second, parallel to the first, obliquely through the seed base, but without traversing the megaspore cavity. In this latter section eight ribs can be distinguished, of which three are doubtless the primary ribs, and from the distribution of the remaining five it seems likely that the seed was twelve-angled, the missing ribs having become obliterated either by obliquity or in consequence of the lower level at which the seed is cut on one side. A further point of importance is that this seed was evidently blunt at the base and not tapering,

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[1] Hovelacque, M.: Recherches sur le *Lepidodendron selaginoides*, Sternb. Mém. Soc. Linn. Normandie, vol. xvii.

[2] Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae, Pt. IV. Trans. Roy. Soc. Edinburgh, 1910.

as shown by the great difference in the area of sclerotesta sectioned at the two successive levels. These facts, taken together with the position of the sarcotestal bundles, seem to justify the assumption that the seed was *Trigonocarpus Parkinsoni*.

In both of the sections a few secretory sacs can be seen. Dr. Scott has permitted me to examine his best preparations of *T. Parkinsoni* with the sarcotesta preserved, and particularly Wild's section S. 1952, figured by Scott and Maslen,[1] in which there are present some dark bodies surrounded by a clear space 0·045 mm.–0·052 mm. in diameter. Neither for these nor for similar bodies in the other preparations can one definitely assign a secretory nature, but a comparison with ill-preserved secretory sacs of *T. Shorensis* discloses a striking similarity between them. Probably, then, secretory sacs were present in *T. Parkinsoni*, both at the base and apex of the sarcotesta, but in very much smaller numbers than in *T. Shorensis*.

The central region of the lowest of the two sections cuts the single main chalazal bundle obliquely, at the level where the sclerotesta begins to be differentiated.

The general outline of the vascular bundle was very slightly triangular, with a diameter of about 0·3 mm., the angles corresponding in position to the secondary ribs. From each of the two upper of these angles (Pl. V, Fig. 14, *v.b.*) a strand is seen in process of being given off into the sarcotesta.

The main bundle is surrounded by a thin-walled parenchymatous tissue (*par.*) consisting of vertically elongated elements. In the centre is a solid mass of xylem, throughout which are scattered short tracheides horizontally directed, exhibiting scalariform or pitted thickenings. The section is sufficiently oblique to show that the longitudinally directed tracheides were scalariform.

In the centre of the xylem a single secretory cell can be detected (Pl. V, Fig. 14, *m.s.*).

The periphery of the bundle is formed of a band of short tracheides 1–2 elements in width, which for the most part are separated from the central xylem mass by what appears to have been parenchymatous tissue similar to that surrounding the bundle as a whole. But this tissue is interrupted at several points by junctions between the outer and inner tracheides. The smallest xylem elements lie mostly at the periphery of the central core, but others are situated more interiorly.

From an examination of the angle which is cut at the highest level, it can be seen that from this peripheral zone pass out the xylem elements of the sarcotestal bundles. In this particular case, the portion has partially separated off from the central mass, and the corresponding bundle, owing to the double obliquity of the section, is again cut further

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[1] Loc. cit., Pl. XII, Fig. 11.

out in the sarcotesta. What was doubtless the second half of this strand is seen at the edge of the bundle cavity, and clearly shows that the course of the two halves was divergent.

In the next section, viz. S. 34, *b*, which is nearly parallel to the former, five sarcotestal bundles can be distinctly recognized, whilst the position of a sixth is marked by a lacuna.

Where the bundles are cut on the lower side of this section, the level is only about one-third of a millimetre above that at which the central bundle is cut in the previous slide, so that the distance is scarcely sufficient for other bundles to have been given off.

It would appear then, from these two preparations, that three bands of xylem in continuity with the peripheral part of the chalazal strand were given off simultaneously, each of which then almost immediately underwent bifurcation into two divergent halves.

The soft parenchymatous tissue which surrounded the bundle consisted of cells which, when cut transversely, appear more or less polygonal and isodiametric : where cut longitudinally they measure about 0·12 mm. × 0·022 mm., and are separated by slightly oblique transverse walls. This tissue may perhaps have served the purpose of phloem, though apparently quite undifferentiated; that it had some definite function is supported by its sharp delimitation from the surrounding tissue, even where this too was parenchymatous in nature. A similar sheath accompanied each of the six sarcotestal strands in this, the basal region of the seed.

As seen in S. 34, *b* (Pl. V, Fig. 15) the central part of each bundle was occupied by a strand of elongated scalariform tracheides (*n.tr.*), and around this was a complete ring of much shorter and stouter spiral or slightly reticulate elements (*s.tr.*). Separating the outer zone from the central core, was a narrow space from which the bulk of the tissue has perished, but here and there can be seen patches of elongated parenchymatous elements similar to those forming the bundle-sheath, and no doubt they originally constituted a complete ring.

Each sarcotestal bundle was then essentially similar to that supplying the chalaza. An examination of the bundles of Dr. Scott's section 626 of *T. Parkinsoni* through the same region as S. 34, *b*, though exhibiting far less perfect preservation, seems to agree with the description here given.

Before leaving these sections it should be said that, though the type of preservation is the same as that of the bulk of the specimens of *T. Shorensis*, in none of the secretory elements present was I able to find the longitudinal striation characteristic of the wall in that species, though this feature is exhibited by well-preserved ducts in the stems of *Medullosa anglica.*

V. THE NUCELLUS.

The total length of the free nucellus, from the point of junction with the testa to the base of the pollen chamber, was about 18 mm., and its diameter in the widest region about 11 mm. As the cavity in this part was some 15 mm. in width, there is left a space of 2 mm. all round for which to account. The question of an inner flesh has already been discussed, but in any case these dimensions for the nucellus are probably much too small, owing to post-mortem contraction.

The general outline of the nucellus conformed closely to that of the cavity within which it stood, following the same lines until near the base of the micropyle, where the nucellar tissue curved sharply inwards to the insertion of the pollen chamber.

Disregarding this latter structure, the conformation was that of a sharply pointed egg with its narrower end directed downwards and attached to the sclerotesta, whilst the blunt free end was surmounted by the pollen chamber.

The nucellar tissue is in most cases either lacking altogether or ill preserved, but fortunately, in the transverse series S. 33, *a* to S. 33, *d*, the structure is beautifully shown, though considerably contracted. In the uppermost of the series the nucellus occupies a one-sided position which is in part due to the slight obliquity, but no doubt in the main to displacement through asymmetrical shrinkage.

In the second of this series the nucellar tissue is seen surrounding the chalazal bundle. As already described, this consists of parenchymatous tissue, of which the cells are thin-walled and vary in size from about 0·01 to 0·050 mm., interspersed with secretory ducts, the largest of which are at the periphery and measure about 0·07 mm. in diameter. This ring of tissue is only 0·2 mm. in width, but from its ragged edge was evidently much more extensive in life.

Owing to the shrinkage of the soft underlying tissue, the much less contractable epidermis is thrown into numerous folds, but the projections thus formed do not, as in *Trigonocarpus Parkinsoni*, correspond to underlying vascular strands,[1] but to radial files of secretory elements (Pl. V, Fig. 20, *m.s.* and *n.r.*). Taking the distance from the edge of the megaspore cavity to the summit of these ridges as the basis of our estimate, the thickness of the nucellar tissue must have been, near the chalazal end of the seed, at least 0·3 mm., though above this level it diminished slightly and then remained of constant width up to the base of the pollen chamber. At the outside there was a very strongly defined epidermis, (S. 33, *c*, Pl. V, Fig. 20, *n.e.*), the cells of which (0·05 mm. radial × 0·03 mm. tangential) stand out all the more clearly since their contents are preserved as dark carbonaceous masses that have contracted away from the cell walls. Where, owing to the contortions

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[1] Scott and Maslen, loc. cit., p. 119.

of the surface, the longitudinal dimension can be estimated, it is about three times as great as the width (0·09 mm.). As seen in this view, the cells are more or less oblong in shape, whilst cut transversely they appear distinctly convex on the external face, a feature which seems to point to the absence of close contact with an inner flesh.

Under the best conditions of preservation, the epidermal cells resemble very closely the secretory sacs and, like them, have the appearance of possessing a thickened wall. In several places the outer layer of the exterior walls, together with part of the middle lamellae from between each pair of the component cells, has split away, so that in transverse section it appears as a thin membrane with pegs projecting inwards. Evidently this outer layer was of a durable character, as it can occasionally be recognized even where the interior tissue has decayed away, and is much less subject to contraction, suggesting that it may probably have been a cuticularized layer such as we find with similar pegs amongst modern plants.

Such a specialized epidermis we should hardly expect to find in a completely enclosed structure like the nucellus, if it were in close contact with a soft inner flesh.

Between the epidermis and the tracheal sheath there intervened a zone of soft parenchymatous elements in which numerous secretory cells were present. The ground-tissue was without intercellular spaces and formed of polygonal cells with very thin walls (0·03 mm. to 0·08 mm. × 0·15 mm.). The secretory cells were embedded in this ground-tissue, and where they did not occur singly, formed radial plates of varying extent and usually consisting of a single row of secretory elements, though more rarely at the base of the seed of two such rows. The greatest number of secretory cells which were present in any one radial plane appears to have been five. The proximity of the radial plates and the number of elements in each decreased as the apex of the seed was approached, so that some two-thirds from the base of the pollen chamber the plates were about a third of a millimetre apart and reduced to one, two, or at the most three elements. The secretory sacs themselves (0·06–0·09 mm.) exhibit much the same structure as those of the sarcotesta, but with thinner walls. In the longitudinal direction the carbonized contents show segmentation into short lengths, but the septation thus produced probably bore no relation to transverse walls.

In two of the seeds (viz. S. 31, U. C. Coll., and Man. R. 1161), besides the longitudinal ridges already referred to, others more pronounced are present, occupying positions opposite to the commissural ribs, and bear no relation to contained radial files of secretory elements. In the series S. 33, U. C. Coll., with its excellently preserved nucellus, no such ridges are however to be seen, which may be correlated with the fact that this seed was probably in an immature condition, judging from its relatively small size, the thinness of the testa, and the slight development of the ribs. In S. 31, *g*, such ridges, corresponding to both the major ribs preserved, can be distinguished. Such commissural ridges,[1] of which an example is figured in Pl. V, Fig. 13, *n.f.*, from section S. 31, *e*, furnish the strongest evidence that the nucellar surface was in the mature condition in close contact with the sclerotic testa, a point which the well-developed cuticle further emphasizes.

VI. THE POLLEN CHAMBER.

Our information respecting the structure of the pollen chamber is extremely meagre, as only two sections furnish data of any importance. The general form of the pollen chamber, as seen in S. 32, *c*, is rectangular, with longer sides forming the roof and floor (3 mm. wide × 1·26 mm. high). As will be seen from the diagram, the direction of the section passes tangentially through the pollen chamber in a plane slightly oblique to that of a minor rib.

The central part of the roof in this section shows a triangular, blunt-ended, upward projection of the epidermis, which may be the base of the micropylar tube.

The epidermis of the pollen chamber is continuous with that of the nucellus, and the component cells up to about half its height are of the same form in both. Beyond this point they become much larger (0·066 mm. radial by 0·04 to 0·08 mm. vertical), with thinner walls, and do not possess the black carbonaceous contents present in the lower part. The increased size is especially noticeable where the sides curve inwards to form the roof; these shoulders project somewhat laterally, partly owing to the larger size of the epidermal cells and partly to the slightly greater internal diameter (Pl. V, Fig. 11). The blunt apex of the angular projection is formed of cells which are much narrower in the tangential direction, 0·005 mm. Interiorly, the sides of the pollen chamber were occupied by a soft parenchymatous tissue, consisting of elongated cells with tapering ends. This is most pronounced in the shoulders, and the appearance presented very closely resembles that described and figured for other seeds of this affinity, as for example *T. Parkinsoni*,[2] *T. pusillus*,[3] *Aethiotesta*,[4] and *Stephanospermum*.[5]

In S. 32, *h*, the pollen chamber exhibits a prominent angle corresponding in position to a major rib, and also in Section I of Dr. Scott's series the shoulders appear double-angled, the lower of the four angles being the true

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[1] Cf. Renault: Angling of Nucellus in *Trigonocarpus pusillus*. Flore fossile d'Autun et d'Épinac, pt. 2, p. 398.

[2] Scott and Maslen, loc. cit., p. 121.

[3] Oliver: New Phyt., vol. iii, 1904, Pl. II, Fig. 3.

[4] Renault: Mém. Soc. d. Sci. Nat. de Saône-et-Loire, 1887, Pl. XVI, Fig. 3.

[5] Oliver: Trans. Roy. Soc. Lond., p. 370, Pl. XLII, Figs. 11 and 18.

shoulders, and the upper again coinciding with the positions of major ribs. There seems little doubt, then, that the pollen chamber was three-angled in correspondence with the projections of the nucellus lower down.

VII. COMPARISON WITH OTHER TYPES.

1. *Trigonocarpus Parkinsoni*, Brongniart.

It is hardly necessary to recapitulate in detail the many points of general resemblance which *Trigonocarpus Parkinsoni* and *Trigonocarpus Shorensis* have in common. The main structural plan in both seeds is essentially the same, but this only serves to throw into greater prominence the many differences of their more minute structure.

We note the entire absence in *Trigonocarpus Shorensis* of tertiary ribs subtending the vascular bundles, and though the behaviour of the primary sutured ribs is alike in each, the beak into which they pass is short in *T. Shorensis* and long in *T. Parkinsoni.*

The secondary ribs of the latter seed are subject to considerable variation, sometimes being almost absent; but usually they persist to near the apex, where they die out before the micropylar beak is reached, an earlier disappearance of the secondary ridges which is still more pronounced in *T. Shorensis.* Scott and Maslen give the thickness of the stony layer in *T. Parkinsoni* as from 1 to 1·5 mm. (loc. cit., p. 106), which is two to three times the corresponding dimension for our seed. Probably this added thickness and the increased number of ribs are to be correlated with the small extent of the sarcotesta, as compared with that of *T. Shorensis*, necessitating greater mechanical strength in the layer beneath; or perhaps, put more correctly, the broad sarcotesta in the latter species, with its peripheral sclerotic system, had not involved the necessity for development of such mechanical strength in the sclerotesta.

The most interesting comparison between these two seeds is afforded by the sarcotestal structure. This tissue was in *T. Parkinsoni* bordered at the exterior by a narrow epidermis, followed by a thickened palisade-like hypodermis (Scott and Maslen, loc. cit., p. 102). As we have seen, the limiting layers in the present seed were much more complex, and formed a, probably anastomosing, complex of radial plates, accompanied by numerous secretory elements present also further in, which, though represented, were extremely few in *T. Parkinsoni.* Now the occurrence of radial sclerotic plates at the periphery, and of secretory elements both within these and interiorly, are characteristic features of the vegetative organs of Medulloseae, and especially of their petiolar structure,[1] so that *T. Shorensis* in this respect would appear to be far more primitive than its congener. The absence of the prolonged

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[1] Scott, D. H.: On *Medullosa anglica.* Phil. Trans., B, vol. cxci, p. 101, and Pl. VIII, Fig. 18, 1899.

sclerotic beak and secondary ridges opposite the bundles also point to a lesser degree of specialization.

A further striking difference is the tapering insertion of the seed of *T. Shorensis* as compared with its abrupt insertion in *T. Parkinsoni*; the former is probably a relatively older type than the latter, just as the horizontal departure of the leaf-trace characterizes the modern plant, in contradistinction to the oblique insertion, as found in the more ancient.

Analogy with *Physostoma elegans*[1] would seem to suggest that the presence of a secretory system in the nucellar tissue is a more primitive feature than its absence. If this has any significance, and the close relationship between the Trigonocarpeae and Lagenostomales supports such an assumption, then in this respect *T. Shorensis* stands in the same relation to *T. Parkinsoni* as does *Physostoma* to the other known members of the latter group.

We have shown good reason for believing that *T. Shorensis* was without a definite inner flesh, except in so far as we can apply that term to the lining of the micropylar canal. Such a tissue would, however, appear to have been present in *T. Parkinsoni.* Perhaps at first sight this might seem to be a pronounced distinction between the two, but the extreme peripheral situation of the sclerized layer in *Pachytesta,* surrounding an extensive inner flesh, coupled with the undoubtedly close relationship of the two genera, indicate that the position taken up by the region of sclerization was subject to considerable fluctuation in the group as a whole, and therefore of no great significance when exhibited in a smaller degree by members of the same genus.

The well-defined nucellar epidermis, even more pronounced than that of *T. Parkinsoni*, indicates a stage less far removed from the condition in which the nucellus was a naked sporangium unprotected by a surrounding integument; and probably the production of an inner flesh is likewise correlated with a phylogenetically more prolonged contact between the two surfaces, which functionally have become internal.

As previously indicated, the vascular organization of the two seeds was essentially similar, perhaps the most outstanding distinction between them being the much more peripheral position occupied by the sarcotestal strands in *Trigonocarpus Shorensis.* On the whole, the internal vascular system of our seed probably shows a somewhat more pronounced tracheal investment at the base of the nucellus, whilst the separate strands into which this passed were broader, though this may well be an outcome of the larger nucellus they supplied. If, as may have been the case, the Trigonocarpeae were derived from fern-like plants having sporangia with a complete internal tracheal investment, then it would appear from Scott and Maslen's description that in this respect *Trigonocarpus Parkinsoni* was more primitive than

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[1] Oliver: Ann. Bot., vol. xxiii, p. 73.

T. Shorensis, for in the latter anastomoses, if they existed, were probably of infrequent occurrence.

2. *Trigonocarpus Oliveri; its systematic position.*

This seed was described and figured by Scott and Maslen in 1907,[1] from a series of four sections through a single seed, and the diagnosis there given is as follows:

> 'Length nearly 2 cm., diameter about 0·9 cm., characteristically coffin-shaped in vertical sections. Base flattened. Sclerotesta produced around the base of the seed in the form of a circular ridge enclosing the stalk of the seed. Longitudinal ridges of the sclerotesta acute-angled, not rounded as in *Trigonocarpus Parkinsoni*.'

The number of longitudinal ridges which the seed bore is not explicitly stated, but, presumably from the description given, was assumed to have been six.

The writer has carefully examined the preparations and employed for their interpretation the methods recently described.[2] The conclusions arrived at differ essentially from those of Scott and Maslen, who evidently did not fully recognize the marked effects of obliquity in this seed, which are so clearly brought out by the modelling method.

As can be seen, the first section (S. 28, *d*)[3] is tangential to the surface of the seed and passes at unequal depths through two ribs, the divergent axes of which, towards the upper part, indicate that the plane was below the middle region, sloping away from the chalaza. The next section (S. 28, *c*) shows the more deeply cut of these ribs sectioned nearer the axis, and consequently represented by two angular projections, one at the apex and the other at the base. This section cuts the seed so far in that two lateral ribs, one on either side, are also encountered; where these are cut near the apex they appear as angular projections, whilst near the base they exhibit a curious truncated outline, but are unequal in size, owing to the obliquity which was also manifested in the previous section. It is these two lateral ridges at the base which have been interpreted as a circular ridge, though the true character where sectioned near the apex was recognized. In order to explain the peculiar form of the lower projections, the assumption was made that they were incomplete. The objections against such an interpretation furnished by this section alone are, that if a chalazal ring were present, it is highly improbable that the central rib would extend below it and the lateral ribs be in no way represented; whilst this difficulty cannot be overcome, since the interpretation of the median ridge as a part of the stalk is inadmissible, owing to the plane of section.

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[1] The Structure of the Palaeozoic Seeds *Trigonocarpus Parkinsoni* and *Trigonocarpus Oliveri.* Ann. Bot., vol. xxi, No. lxxxi, 1907.

[2] Salisbury: Methods of Palaeobotanical Reconstruction. Ann. Bot., April, 1913.

[3] Loc. cit., Pl. XIII.

On the interpretation here put forward no difficulties are involved, since converging ribs cut in a plane at a narrow angle with that which they themselves follow would necessarily acquire in section the square-ended form which they actually present.

In the section just described, one notes that the region of attachment of the nucellus is cut through, so that since in the next preparation the nucellus shows as a complete oval membrane it is clear that this section passes right across the axis of the seed, cutting through a rib on either side almost vertically, and it is the slight angling of the cavity corresponding to these which, as in similar sections of *Conostoma oblongum*, results in the coffin-shaped appearance.

At the apex of S. 28, *b*, we see the three ribs represented in S. 28, *c*, and at the lower end three other ribs sectioned almost transverse to their direction, so that the triangular form is preserved. If, on the other hand, they were really a chalazal ring, a section in this plane should render them convergent, not divergent, and furthermore, their size on that view is incompatible with the projections in the previous section, especially if the latter are to be regarded as incomplete.

T. Oliveri was then an eight-angled seed, and, until further specimens are obtained with better-preserved internal tissues, its systematic position, except for inclusion in the vague group of the Radiospermeae, must for the present remain uncertain. The removal of this seed from the genus *Trigonocarpus* makes it therefore unnecessary to institute any comparison between it and *Trigonocarpus Shorensis.*

3. **Fructifications of *Neuropteris heterophylla* and *Neuropteris obliqua.***

Attention has already been called to the tapering and the abrupt insertion found within the genus *Trigonocarpus*, both of which are represented in the fructifications found attached to Neuropteridian foliage. Three specimens of *Neuropteris heterophylla* have been described by Dr. Kidston with attached seeds,[1] in which the abrupt insertion of the *Parkinsoni* type is clearly exhibited. More recently, Kidston and Jongmans[2] have described fructifications attached to the fronds of *Neuropteris obliqua*, in which the seed tapers towards its insertion on a bifurcated axis, thus conforming to the second type as represented by *T. Shorensis.* Owing to the incomplete preservation of the latter at the apex, its longitudinal extent cannot be exactly estimated, but nevertheless the comparison of the approximate dimensions of the two types of impressions and petrifactions given below shows that broadly the resemblances of insertion are accompanied by an approximately similar ratio in size.

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[1] Phil. Trans. Roy. Soc., ser. B, vol. cxcvii, 1904, p. 1.

[2] Archives néerlandaises d. sci. exactes et nat., sér. iii, B, tome i, 1911, p. 25.

Species.	*Length.*	*Breadth.*
T. Parkinsoni	4–5 cm.	2 cm.
N. heterophylla	3 cm.	1·1–1·4 cm.
T. Shorensis	Over 4 cm.	2·4 cm.
N. obliqua	About 6 cm.	2·25 cm.

A further feature that the fructifications of *N. obliqua* and *T. Shorensis* have in common is found in the marked striation exhibited by the surface of the former, and attributed by the authors cited above to the presence of a large number of sclerenchymatous strands of tissue near the surface of the seed. These are no doubt identical with the numerous radial sclerotic plates which we have described as a prominent feature in the peripheral layers of the sarcotesta in *T. Shorensis*.

In view of the occurrence of the seeds of *N. obliqua* in pairs on a bifurcated axis, it may be of some significance that in one of our series a portion of a second seed is present, having approximately the same orientation as the more complete specimen close to which it lies.

These considerations render it likely that our seed was itself borne on a plant possessing foliage of the *Neuropteris* type, and almost certainly belonged to the same subsection of the genus as Kidston and Jongman's specimens.

4. *Trigonocarpus corrugatus.*

Amongst the casts of Trigonocarpean seeds, none approach so closely to *Trigonocarpus Shorensis* as that described by Renault under the name *Trigonocarpus corrugatus*. The following is the diagnosis given by that author:

'Graines trigones, dilatées un peu au-dessus du milieu de leur hauteur, longues de 22 millimètres et larges dans la partie renflée de 12 millimètres, marquées de trois côtes saillantes qui vont jusqu'au sommet de la graine sans produire de point. Entre ces trois côtes on remarque trois plissements en relief qui s'étendent à peu près jusqu'à mi-hauteur.'[1]

From the above we see that the dimensions of the seed, though smaller than those of our own species, bear very nearly the same ratio for the corresponding parts of the sclerotesta.

Species	*Width* (*max.*)	*Length.*	*Ratio* *Length, Width.*
T. corrugatus	12 mm.	22 mm.	1·83
T. Shorensis	15 mm.	28 mm.	1·86

The presence of the three secondary ribs extending for only a part of the total length of the hard shell, though persisting for a greater distance than in *T. Shorensis*, also the absence of a beak (a description that might easily in this type of preservation be consistent with the presence of a very short structure of such a character), and the tapering base, still further strengthen the resemblance between the two seeds.

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[1] B. Renault, Bassin houiller et permien d'Autun et d'Épinac, p. 399.

5. Comparison with Cycads.

Recent work on the Cycadean ovule,[1] and the additional facts regarding the structure of *Trigonocarpus* here brought forward, serve to emphasize the unmistakable relationship between the two groups. In the organization of the integument, differentiated into three layers, the agreement is extremely close, even as regards the broad structure of the stony layer which in Cycads as well as in *Trigonocarpus* is formed of longitudinally directed fibres on the inside, which at the outside become interwoven with horizontally directed elements.[2] Also, in the vascular organization and the structure of the pollen chamber[3] the resemblances are particularly evident.

The chief interest of the present work in this connexion results from the recognition of the short Cycadean type of sclerotic beak within the genus *Trigonocarpus*, and the presence of secretory elements situated in the sarcotesta of the latter, and resembling very closely the mucilage sacs of the modern group.

The tendency for the non-vascular ribs to die out, of which an early stage is shown by *T. Shorensis*, reaches its culmination in the Cycadean family, where, too, the development of ribs in relation to the sarcotestal bundles, as found in *T. Parkinsoni*, likewise constitutes a prominent feature of certain genera.

The absence of a nucellar system in the Lagenostomales is probably correlated with the fusion between testa and nucellus. How, then, are we to account for the retention of this same system under similar conditions in the Cycadean ovule? The explanation seems to be found in the isolation of the nucellar and integumental systems from one another by the intervening sclerotesta, which thus prevents the latter from performing the functions of both.

The suggestion advanced by Worsdell[4] that the fused integument and nucellus was brought about by a congenital fusion rather than that it arose as an intercalated zone of growth, as suggested by F. W. Oliver,[5] seems the more probable, both on the grounds there adduced and in view of the absence of any evidence in Cycads, comparable to that found in the Lagenostomales, of such having taken place.

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[1] Kershaw: Structure and Development of the Ovule of *Bowenia spectabilis*. Ann. Bot., vol. xxvi, No. 103, 1912.

[2] Stopes: On the Double Nature of the Cycadean Integument. Ann. Bot., vol. xix, 1905, p. 564; Chamberlain: The Ovule and Female Gametophyte of *Dioon*. Bot. Gaz., vol. xlii, 1906, p. 332.

[3] Kershaw, loc. cit., p. 643.

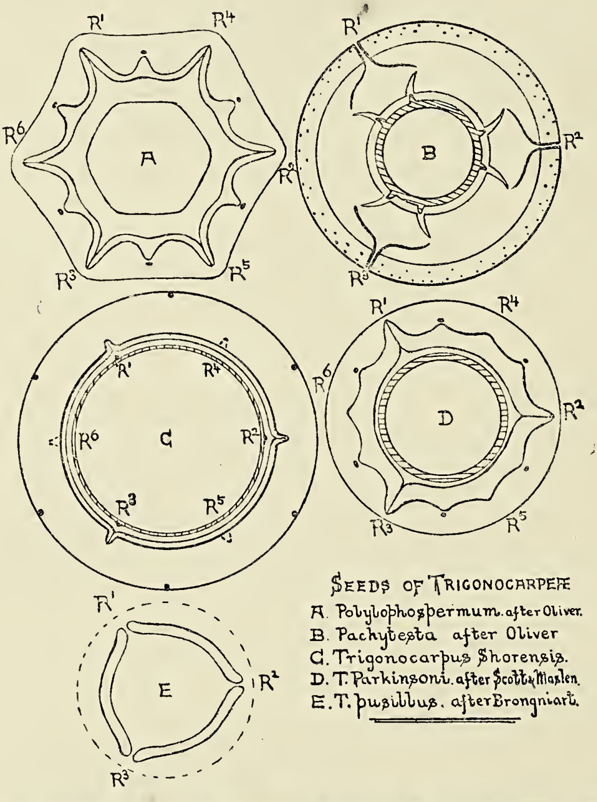
[4] Fasciation, its Meaning and Origin. New Phyt., vol. iv, 1905, p. 38.

[5] The Ovules of the Older Gymnosperms. Ann. Bot., vol. xvii, 1903.

VIII. General Discussion of the Testa.

1. The Multiple Origin of the Integument in the Trigonocarpeae.

The general facts supporting the theory of a multiple origin for the integument of the Lagenostomales are well known,[1] and in the Trigonocarpean series the integument is also composed of several equivalent units, which may well have had a similar origin. We shall therefore briefly review the chief testal features which the members of this group exhibit.



Text-fig. 8. Transverse sections of the seeds of various Trigonocarpeae. Corresponding ribs are in each case marked R^1, R^2, &c.

In the genus *Trigonocarpus* itself *T. Parkinsoni* had twelve ribs, three primary and fissured, three secondary and non-fissured, and six tertiary, opposite which the sarcotestal bundles were situated (Text-fig. 8, D).

In *T. corrugatus*[2] and *T. Shorensis* six ribs only were present, of which the three non-commissured died out, about half-way up in the former seed and one-third in the latter (Text-fig. 8, C).

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[1] Oliver and Salisbury: Palaeozoic Seeds of *Conostoma*. Ann. Bot., vol. xxv, 1911, p. 41.

[2] Renault, loc. cit.

In *T. pusillus*[1] the ribs had almost become obsolete and the sclerotesta was nearly circular in outline, with three commissured ribs only very slightly projecting (Text-fig. 8, E).

Polylophospermum[2] possessed twelve ribs, of which six were fissured, the alternating and non-fissured ribs corresponding to the radial planes of the six sarcotestal bundles (Text-fig. 8, A).

Ptychotesta and *Hexapterospermum*.[3] Six-angled seeds with very prominent ribs, all of which in the case of the former were fissured (perhaps also in the latter) and enlarged at the ends, owing to the separation of the two parts in that region.

Polypterospermum.[4] An hexagonal seed with six acute ribs at the angles of the testa, and alternating with them six others which were short and blunt.

Pachytesta.[5] A circular seed, but exhibiting, like *T. pusillus*, three commissures. The bundles had undergone considerable branching, so that not only are they numerous tangentially, but also form two concentric series, a complexity of vascular organization that goes hand in hand with an elaborate internal structure (Text-fig. 8, B).

Stephanospermum.[6] The two species were circular, and all vestiges of ribbing have disappeared. The sarcotesta is usually only represented by the tissue occupying the apical cup, so that its vascular structure is unknown.

The species of *Trigonocarpus*, we see, thus form a consecutive series involving the gradual elimination of the secondary ribs.

The production of ribs in relation to bundles is a phenomenon of widespread occurrence, met with elsewhere in the seeds of Dicotyledons, Gnetales, and Cycadales, as well as in the vegetative organs of many plants. They are probably an expression of mechanical utility, and, except from the taxonomic standpoint, the absence of such ribs from the seeds of *T. Shorensis* and *T. corrugatus* is a point of little importance.

Disregarding the vascular ribs, the general sclerotestal structure in *Trigonocarpus Shorensis* and *T. Parkinsoni* is identical. Scott and Maslen[7] remarked on the variability in development of the secondary ribs in the latter species, which always die out before the primary, and in some cases are so slightly developed as to be practically absent. The two species *T. corrugatus* and *T. Shorensis* constitute further stages in the reduction series, for in the former these ribs only extend for half the length of the seed, and in the latter are mere chalazal vestiges. In *T. Noeggerathi* only the primary ribs are present. And, finally, in species such as *T. pusillus* and *T. elongatus* all indication of the secondary ribs is lost, which applies almost equally to the primary ones also. It is evident that the secondary ridges in the genus *Trigonocarpus* were in an obsolescent condition, which would fully account for the absence of commissures if such did, as the writer believes, originally exist.

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[1] F. W. Oliver, New Phyt., vol. iii, 1904, pp. 96–104.

[2] F. W. Oliver, Ann. Bot., vol. xxi, 1907, pp. 303–4.

[3] Brongniart, Comptes rendus, tome lxxviii, 1874, pp. 15a, 16.

[4] Brongniart, loc. cit., p. 16.

[5] F. W. Oliver, New Phyt., vol. i, No. 7, 1902.

[6] Oliver, Trans. Linn. Soc. Bot., vol. vi, 1904.

[7] Loc. cit., p. 107.

F. W. Oliver has called attention to the remarkable resemblance between the plans of *T. Parkinsoni* and *Polylophospermum*,[1] one which is rendered complete if we assume that the secondary ribs of the former were the representatives of originally commissured structures. The conclusion, then, seems warranted that the six ribs of *T. Shorensis* and the non-vascular ribs of *T. Parkinsoni* are homologous with the six commissured ribs of *Polylophospermum* (in other respects, of course, this seed is specialized). It is, moreover, probable that the sutures themselves represent the planes of lateral fusion between six originally free members—a view which is further supported by the recognition of six component units in the micropylar region of *T. Shorensis.*

Newberry[2] has figured a cast showing the apex of a *Trigonocarpus* with its sarcotesta preserved, and in which the micropylar canal is seen as a star-shaped opening surrounded *by six small but free sarcotestal lobes.*

We have noted how, in the genus *Trigonocarpus*, ribs have become completely eliminated and each pair of units fused laterally, so that three sutures only remain. This condition obtains in the genus *Pachytesta*, and further reduction might well result in the production of a non-commissured seed, as in *Stephanospermum.*

The remaining genera cited above are either six- or twelve-ribbed seeds, and in the latter case the additional members may well correspond to the six vascular ribs of *Polylophospermum.*

Other genera there are, belonging to the Radiospermeae, which nevertheless possess ribs that in number are some multiple of two, e. g. *Eriotesta*, *Codonospermum.* Our review has, however, shown that there existed a group of seeds agreeing in their internal structure and consistent with our hypothesis in their sclerotestal plan, moreover including all those forms in which an undoubtedly Trigonocarpean organization has been established.

The possible origin of dimerous from trimerous forms is considered in the sequel, but the Radiospermeae is doubtless an artificial aggregate of which the Trigonocarpeae is one of the included natural groups; a statement that is borne out by the constant association with these seeds of the Neuropteridian type of foliage.[3]

None of these genera militate, then, against the theory that the integument originated as a whorl of six free members, each with a single vascular bundle, which subsequently became laterally fused. Such an interpretation necessitates the recognition of the sarcotesta and sclerotesta as constituting a phylogenetically homogenous structure, and in the next section will be given data that amply warrant that conclusion.

A striking feature of the Trigonocarpeae is not only the occurrence

[1] Ann. Bot., vol. xxi, 1907, pp. 303–4.

[2] Rep. Geol. Survey of Ohio, vol. i, Pt. 2, 1873, p. 336 and Pl. XLII, Fig. 5.

[3] See M. Grand' Eury, Comptes rendus, vol. cxxxix, 1904, p. 3.

throughout of the hexamerous type, but also the absence of variation in the individual species. This stereotyped character suggests analogy with present-day Monocotyledons, and is in marked contrast to the variability exhibited by the Lagenostomales, not only as between different genera and species, but also as between individuals. For example, in *Physostoma*[1] nearly 50 per cent. of the seeds had ten ribs, but the remainder exhibited from nine to twelve. In *Conostoma* the number was six or eight according to the species, with one recorded variant of *Conostoma oblongum* possessing seven.[2] In *Lagenostoma Lomaxi* the normal number was nine,[3] and for *L. ovoides* eight, with variation from six to nine.[4] Finally, in *Gnetopsis elliptica* the number of ribs was four, with perhaps two others vestigial.[5] In this series we see that there is almost every variation from four up to twelve, and whilst a multiple of three is by no means infrequent—a reminiscence perhaps of relationship to the Trigonocarpeae—the more normal feature is some multiple of two.

Our knowledge of the structure of *Conostoma* and *Gnetopsis* has shown how narrow is the dividing line between radiospermy and platyspermy.[5] Also the recent discoveries of *Aneimites fertilis*[6] and *Pecopteris Plukeneti*,[7] together with the obvious relationships between Pteridosperms and the Cordaiteae, render it necessary to consider the possibility of deriving bilateral forms from a trigonous group. The fact that the fructification *Pecopteris Plukeneti* was borne on a Medullosean type of foliage certainly indicates such a change, so that the analogy afforded by examples from the carpellary structures of the present-day Flora may not be without value. The *Carices* are represented in the British Flora by about fifty species, of which over thirty possess three stigmas associated with a triangular nutlet; the remaining species mostly have two stigmas, and the nut is either bilaterally symmetrical or plano-convex. In a few species with two stigmas the nut is slightly trigonous, and in *Carex paludosa*, Good., the stigmas vary from three to two, accompanying which the nut is either trigonous or lenticular.

As an example from the Dicotyledonous series, the Polygonaceae furnish us with a group in which triangular fruits are the rule. In part of the genus *Polygonum*, and in the genus *Rumex*, a triangular nut is associated with three stigmas. In the section *Persicaria* of the genus *Polygonum*, and in *Oxyria*, the fruit is lenticular and composed of only two carpels, as shown by the pair of stigmas.

To come much nearer, the fructifications of *Ginkgo biloba* have been

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[1] Oliver: Ann. Bot., 1909.

[2] Oliver and Salisbury: Ann. Bot., vol. xxv, 1911.

[3] Oliver and Scott: Phil. Trans. Roy. Soc., vol. v, 1903, p. 197.

[4] Prankerd, T. L.: Jour. Linn. Soc. Bot., vol. xl, No. 278, p. 463.

[5] Oliver and Salisbury, loc. cit.

[6] Dr. David White: The Seeds of *Aneimites*. Smithsonian Misc. Coll., vol. xlvii, pt. 3.

[7] M. Grand' Eury, Comptes rendus, vol. cxl, p. 920.

found with three ribs in place of two, a variation that may even be a reversion.

Such examples suffice to show that the assumption of an originally hexamerous integument (later becoming trimerous in some forms) for the Trigonocarpeae does not preclude the origin of closely allied genera possessing bilaterally symmetrical structures. (The position of the tetramerous Radiosperms awaits details of their internal structure.)

In view of the frequent association in the monocotyledonous series of trimerous flowers with a triangular stem structure, an expression probably of similar mechanical relations, it is of interest to note that the general outline of the stems both of *Medullosa anglica* and *Sutcliffia insignis* was broadly triangular,[1] though the character does not of course hold for other species of the former genus.

Before leaving this subject it is of interest to note that Drs. Stopes and Fuji regarded *Yezostrobus Oliveri* as more nearly approaching to *Trigonocarpus* than any other known fossil or recent group,[2] and therefore, on the foregoing hypothesis, the triangular outline which this seed exhibits in transverse section[3] may have a phylogenetic significance.

2. The Question of a homogeneous or dual Phylogeny.

The question at once arises in relation to the integument: was this structure of uniform origin or was it dual, as suggested by Stopes for Cycads?[4] The close agreement of the two groups makes the assumption of a double nature for the one almost necessitate its acceptance for the other. The main grounds on which this theory is based are, briefly, the presence of two series of bundles, both supposed to be integumental, and the resemblances that exist between *Lagenostoma* with its cupule on the one hand, and the Cycadean ovule with its sarcotesta on the other. The recent work of Miss Kershaw[5] on *Bowenia spectabilis* has shown that in this species at least the inner vascular system is nucellar. On the other hand, Dr. Stopes traced some members of the inner vascular system of Cycads into the integument beyond the free part of the nucellus, and unless future work should show that it is only the accessory branches from the integumental system that behave in this manner, the evidence as to the nature of the inner bundles must remain in its present contradictory state. But in view of the diverse data at present available, we are not warranted in attaching any great importance to the occurrence of two systems, even should they prove to be both integumental in certain cases. The branched

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[1] Scott, Phil. Trans. Roy. Soc., B, vol. cxci, 1899; and Scott: On *Sutcliffia insignis*. Trans. Linn. Soc. Bot., vol. iii, 1906, Pt. 4, ser. 2.

[2] Phil. Trans. Roy. Soc., ser. B, vol. cci, 1909. More recently, however, Prof. Fuji has come to regard the nearest living relatives of this plant as being the Araucarieae.

[3] Loc. cit., Fig. 14.

[4] On the Double Nature of the Cycadean Integument. Ann. Bot., 1905.

[5] Ann. Bot., vol. xxvi, No. 103, 1912, p. 636.

and double system of integumental strands in *Pachytesta* evinces a tendency that might well have subsequently developed, and its origin in consequence have gradually become obscured.

With regard to the second argument in relation to *Lagenostoma*, recent work has shown that, in spite of the free nucellus exhibited by the Trigonocarpeae, they agree both in the structure of their fructifications and stem anatomy much more closely with the modern group than do the Lagenostomales.[1]

When we turn to *Trigonocarpus Shorensis* we find that the evidence for a homogeneous origin for the whole integument is abundantly clear. Such inner flesh as is present is but the unsclerized internal lining of the hard shell, and this latter, though broadly composed of an outer and inner part, is formed from one and the same tissue by the different course which its elements pursue.

The sarcotesta on its inner periphery shows a complete transition from the outer sclerotestal cells, so there is no zone to which we can point as possibly representing the fusion plane of two morphological units. And if further evidence were necessary it is furnished by the course of the secretory elements which pass from sclerotesta to sarcotesta irrespective of the differences of texture which, in the mature fruit, these regions present.

IX. THE ORIGIN OF THE INTEGUMENT IN THE TRIGONOCARPEAE AND THE LAGENOSTOMALES.

The addition of yet another Trigonocarpean seed, showing a well-defined nucellar epidermis, adds to the certainty that the group was characterized by the possession of an extensive free region of the nucellus, to which part the megaspore cavity was almost entirely confined. This would seem to be a fundamental difference separating them off from the other members of the Pteridospermeae.

The considerable resemblances which the Trigonocarpeae bear to the Lagenostomales, both as regards the general organization of their seeds and the broad features of anatomy, in the few stems which have been allocated to fructifications, indicate that the two must have had a closely allied ancestry, either in some semi-Pteridospermic group which possessed a generalized type of fructification, giving rise to the characters of both; or in two parallel developments arising independently from the Pteridophytic stock.

The theory of intercalated growth put forward by F. W. Oliver in a similar connexion[2] to homologize modern with fossil forms, applied in the manner indicated below, seems to offer the best explanation of both the resemblances and differences which these two groups exhibit.

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[1] Kershaw, loc. cit.; and de Fraine: On *Sutcliffia insignis*. Ann. Bot., vol. xxvi, 1912.

[2] The Ovules of the Older Gymnosperms. Ann. Bot., vol. xvii, 1903.

In *Physostoma*, the most archaic type of the Lagenostomales, the megaspore cavity projects into the free portion of the nucellus, the plinth being only slightly developed.

In the more advanced *Lagenostoma*, the plinth exhibits intercalary growth, so that there is a greater extent of free nucellus in the older than in the younger phase, whilst in *Conostoma* the plinth reaches its greatest extent.

These facts seem to indicate that the free apical portion of the nucellus was in this series a phylogenetically late development, resulting from a zone of intercalated growth.

On various grounds *Physostoma* is regarded as the most primitive seed yet known,[1] therefore it is of greater significance as being the only member of the Lagenostomales exhibiting a free apex of the nucellus into which the megaspore cavity projects. We may therefore suppose that the potentiality for the development of a free nucellus was possessed by the ancestors of *Physostoma*; but was only exhibited by the majority of the group in the intercalated growth of the region *above* the contained megaspore, resulting in the elaboration of the plinth.

Probably, then, the common ancestors which gave rise to the Lagenostomales on the one hand and the Trigonocarpeae on the other possessed a nucellus unenclosed at the apex, and round the base of which were fused a whorl of members with free apical portions, affording protection to the sporangium, in which the prothallus was now retained. With the inception of the seed habit came an increase in the size of the megaspore cavity, accompanying enlargement of the nutritive prothallus. Here is where the divergence in the two lines of descent arose. In the one case there was an upward extension of the megaspore cavity and the surrounding free pro-integumental lobes, resulting in the production of a free nucellus which may either have had its origin in an already vascularized sporangium,[2] or the nucellar system may have arisen in relation to the greater demands upon the water-supply now created.

Along the line of the Lagenostomales the increased dimensions were brought about by a similar phylogenetic intercalation of growth, which took place, however, in the lower part where the whorl of protective members constituting the pro-integument was laterally fused to form a ring around the base of the sporangium. The close proximity of the integumental bundles would account either for the suppression or non-development of a special nucellar system. Such a view would not only explain the intermediate characters exhibited by the archaic *Physostoma*, but it further accords with the broad features of the testa as seen in the two groups.

A marked characteristic throughout the Lagenostomales is that at the

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[1] Oliver: Ann. Bot., 1909.

[2] Oliver: A Vascular Sporangium. New Phytologist, vol. i, 1902, p. 60.

apex of the seed the multiple nature of the integument exhibits itself with almost sudden clearness after it becomes free from the nucellus. In the seeds of the Trigonocarpeae, on the other hand, the component units of the testa are in general equally distinct at the base as at the apex, or even throughout their length. These two conditions seem only explicable on the hypothesis that in the one group lateral fusion was simultaneous throughout, representing in fact the elongation of the free parts, and in the other took place in the 'canopy' and body of the seed at phylogenetically distinct periods.

Our investigation of the present seed has shown us that the external periphery of the integument was circular in outline throughout; in other words, the lobing is only shown superficially at the internal periphery, where it extends from near the base to the apex, though in the body of the seed the six lobes manifest near the chalaza, and reappearing at the micropyle, had become reduced to three.

What is true of this species was, judging from impressions with sarcotesta preserved, true also for its congeners, and may well have been a group character. The Trigonocarpeae were then characterized by an internal lobing almost to the base of the seed, whereas in the Lagenostomales such internal lobing is only to be found in the apical region, so that superficially as well as internally the major part of the integument in the one is to be homologized with the distal extremity in the other.

We have assumed that the ancestral fructification of the two groups possessed a whorl of surrounding members fused at the base between themselves. In both *Trigonocarpus Shorensis* and *Trigonocarpus Parkinsoni* the sutures of the primary ribs disappear a little before the floor of the seed cavity is reached.

The structural facts therefore justify the assumption that in both groups there was a phylogenetically earlier lateral fusion of the pro-integumental members at the base, followed later by their fusion at the apex.

The congenital fusion of the integument with the contained nucellus was but a matter of time, naturally evinced first in the basal region or zone of earlier lateral fusion. This latter, in the Trigonocarpeae, is wellnigh vestigial, so that a free nucellus resulted, whilst in the Lagenostomales it represents the greater part of the seed body.

Later on in the history of the Trigonocarpeae and their descendants, congenital fusion followed in the upper part, giving us the condition in modern Cycads; though here, as in the more recent Lagenostomales, the apical region has remained permanently free in relation to its specialized structure and functions.

X. RELATION TO VEGETATIVE ORGANS.

In two of our series of sections there occur, associated with the seed structures, Medullosean petioles of which portions of as many as three are present in one and the same preparation. This fact would, in the light of present knowledge,[1] appear of itself significant, and, added to this, numerous secretory elements are present in these petioles which agree in structure with those found in the sarcotestal tissue of *Trigonocarpus Shorensis*. Each is enclosed by a sheath-like layer of somewhat flattened cells, and the wall surrounding the dark carbonaceous mass in the centre exhibits externally the characteristic longitudinal striations. Both of these features are also exhibited in the stems and petioles of *Medullosa anglica*.

The individual bundles are collateral in structure, the phloem being represented by an empty space upon which the smallest protoxylem elements abut. The xylem is surrounded by a band of sclerotic fibres in close contact with the tracheides. The xylem exhibits no admixture of parenchymatous elements, so that in all respects the bundles are distinct from those of *Rachiopteris Williamsoni*,[2] but agree very closely with those of *Myeloxylon*.[3] If, then, the petioles above described really belonged to the plant which bore our seed, the petrifaction known as *Myeloxylon* probably represents an aggregate of petiolar structures corresponding to at least two species of Trigonocarpean fruits, and possibly in both cases having as their stem a structure of the *Medullosa anglica* type. In any case, the evidence at present available is against any suggestion that *Trigonocarpus Shorensis* was the fructification of *Sutcliffia insignis*, though the above association may be entirely without significance.

The presence of numerous secretory ducts is a feature shared by both the known British Medullosean stems. In the structure of the chalazal bundle, whilst the protoxylems of *Trigonocarpus Shorensis* appear to be grouped in pairs (a feature of *Sutcliffia insignis*), in the presence of internal parenchyma and a secretory duct within the xylem our sections of *Trigonocarpus Parkinsoni* show a closer agreement with that stem.

XI. DIAGNOSIS.

Trigonocarpus Shorensis, sp. nov.

Locality: Shore Littleborough.
Horizon: Lower Coal Measures.

A radially symmetrical obovoid seed tapering to its insertion and circular in transverse section. Length over 4 cm., width 2·4 cm.

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[1] Scott, Progressus Rei Botanicae, vol. i, p. 206.

[2] Seward, Ann. Bot., vol. viii, No. 30, 1894, p. 208.

[3] Ibid., vol. vii, 1893, p. 1.

Testa differentiated into two parts:

(*a*) **Sclerotesta** (length 2·8 cm., width 1·5 cm.) produced at the apex into a short triangular beak and bearing six ribs, viz. three principal and commissured extending throughout, and three minor and non-sutured reaching to some 8 mm. from the base. (*b*) **Sarcotesta** about 4 mm. in thickness, lacunar in structure with numerous secretory sacs, and six peripherally situated vascular bundles alternating in position with the ribs. Limiting layers containing sclerotic strands. **Nucellus** free, containing vertically directed secretory sacs, and limited within by vascular tissue forming a continuous lining at the base but above passing into separate bundles.

XII. SUMMARY.

In the foregoing pages a detailed description is given of the Palaeozoic seed *Trigonocarpus Shorensis*, of which the chief diagnostic features are summarized in the preceding section.

The sclerotesta differed principally from that of the well-known species in its obovoid form and tapering insertion, the absence of ridges in relation to the vascular bundles, and in the relatively short beak. In the early disappearance of the secondary ribs it resembled closely the external cast of *Trigonocarpus corrugatus*.

Both sarcotesta and sclerotesta showed considerable complexity, with nevertheless a well-marked transition, such as can only be reconciled with differentiation of the same organic unit. The sclerotesta was built up of fibrous elements longitudinally directed within and without, intertwined in a complex manner. The sarcotesta was unusually broad, lacunar in structure, and contained numerous secretory sacs comparable to those of Cycads.

The limiting layers were formed of a ground-tissue of parenchyma containing a system of probably anastomosing and tangentially flattened strands of fibres, with secretory sacs like those of the interior. The whole structure in this region approached much more nearly to that of the peripheral layers of a Medullosean petiole than in *Trigonocarpus Parkinsoni*.

The sarcotesta exhibited no flattening, but was circular in form throughout, whilst an inner flesh was probably only present as an inconspicuous layer within the micropyle.

The vascular system comprised two parts, viz. a sarcotestal and a nucellar.

The most notable feature of the former is the extreme peripheral position occupied by the six mesarch vascular bundles.

The single chalazal bundle had the protoxylem groups just within the periphery, and expanded at the base of the nucellus into a tracheal cup, which was probably formed of twelve laterally fused bands that gradually increased in number to form numerous mesarch lenticular bundles lining the inner limit of the nucellus.

The nucellus was attached at the base to a sclerotic papilla, but was otherwise free from the testa, and its outstanding features were—a well-developed and thick-walled epidermal layer; the occurrence of three longitudinal flanges, corresponding with the commissures; and the presence of numerous secretory sacs in the ground-tissue, arranged in radial files.

Two sections of *Trigonocarpus Parkinsoni* are described which indicate that the chalazal bundle gave off three branches simultaneously, which almost immediately bifurcated to form the six sarcotestal strands.

A comparison with Cycads and allied types leads to the conclusion that *Trigonocarpus Shorensis* is in several respects a relatively more primitive type than its congeners. In this connexion *Trigonocarpus Oliveri* is shown to have been an eight-angled seed, and is therefore probably to be excluded from the Trigonocarpeae.

In the general discussion on the testa, the evidence for its homogenous origin is set forth, and the theory is advanced that it had its inception in the lateral fusion of a whorl of six originally free members. The testal structure of the various genera is discussed in the light of this theory.

The resemblances and differences between the Trigonocarpeae and Lagenostomales are shown to be explicable on the hypothesis of intercalated growth, followed by subsequent congenital fusion between the nucellus and integument.

Medullosean petioles associated in the coal-balls with *Trigonocarpus Shorensis* are briefly described, and resemble *Myeloxylon.*

EXPLANATION OF PLATES.

Illustrating Mr. Salisbury's paper on *Trigonocarpus Shorensis.*

R = Owens College, Manchester, Collection; S = University College Collection; D.H.S. = Dr. Scott's Collection.

PLATE IV.

Figs. 1–10, photomicrographs.

Fig. 1. Nearly transverse section through the middle of the seed, showing the three commissural ridges (R^1, R^2, R^3) and the extensive sarcotesta (*st.*). The nucellus is seen in part on the right with a ridge near the commissure slightly displaced (*n.f.*). The black dots in the sarcotesta are the carbonaceous contents of the mucilage sacs. R. 1161, *h.* × about 4.

Fig. 2. Slightly oblique section through the micropyle, showing sarcotesta with secretory sacs (*m.s.*). Within the triangular sclerotesta the inner flesh can be faintly seen. R. 1161, *k.* × 2·5.

Fig. 3. Tangential section through the outer sclerotesta. The band-like aggregates of fibrous cells are seen overlapping n a plait-like manner (*f.b.*) and passing in different directions. S. 32, *g.* × 60.

Fig. 4. Slightly oblique transverse section near base of seed; on one side a small projection is seen, representing the termination of a minor rib (r^1). Within is the contracted nucellus (*nu.*) with secretory sacs in radial files, over which the contracted epidermis has formed ridges. S. 33, *c.* × 2.

Fig. 5. A tangential section through the zone of transition between the sclerotesta (*scl.*) and the sarcotesta (*st. c.*). The slightly thickened walls, the elongated form, and the sinuous course of the inner sarcotestal cells are here seen. S. 32, *g.* × 90.

Fig. 6. An oblique section through the base of the micropyle, showing the extensive unflattened sarcotesta with limiting layers at *l.l.*, and secretory sacs in outer sclerotesta (*m.s.*). S. 31, *i.* × 5.

Fig. 7. Obliquely longitudinal section through chalazal end of the seed, showing vascular bundle (*v.b.*), chalazal papilla (*c.p.*), and the tapering base of seed. S. 32, *e.* × 2½.

Fig. 8. Section through extreme apex of sclerotic beak. The sclerotesta is seen to be thinning out rapidly, and at the higher level on the right is only some three elements in width. The sides show a median constriction (*m.c.*) resulting in a six-lobed structure (*m.l.*). The inner flesh (*i.f.*) shows a gradual transition from the sclerotic tissue. R. 1161, *e.* × 28.

Fig. 9. Oblique section through the base of the seed, passing through all six ribs (R^1, R^2, R^3, and r^1, r^2, r^3). At *c.p.* the chalazal papilla is seen perforated by the single vascular bundle (*v.b.*). R. 1161, *b.* × 4.

Fig. 10. Tangential section through the periphery of the sarcotesta, showing the lacunae (*lac.*) separated in places by the superposed peg-like projections of the stellate cells (*p.*), and elsewhere by the cells proper. S. 32, *h.* × 90.

PLATE V.

Figs. 11–20, photomicrographs.

Fig. 11. Vertical section through the pollen-chamber wall (*p.w.*), from which the cuticle has become separated (*cu.*). The shoulder is occupied by thin-walled parenchymatous tissue (*sh.*). The oval bodies are fungal spores (*sp.*). S. 32, *c.* × 50.

Fig. 12. Transverse section through the sarcotesta with two secretory elements, probably derived by branching, in close contact (*m.s.*). S. 31. × 60.

Fig. 13. A portion of a transverse section to show the nucellar flange, consisting of parenchymatous tissue (*n.f.*) and corresponding in position to a commissural rib (*com.*). A lenticular nucellar strand is seen on the left (*v.b.*). S. 31, *e.* × 30.

Trigonocarpus Parkinsoni.

Fig. 14. Transverse section through the chalazal bundle of *Trigonocarpus Parkinsoni* at the level at which the sarcotestal strands (*v.b.*) are given off. At the top one of these has branched into two. The central xylem mass (*c. xy.*) is surrounded by a discontinuous sheath of parenchyma (*par.*), exterior to which more tracheides (*tr.*) are seen. A single secretory element (*m.s.*) occupies a position within the xylem mass. In the space between the bundle and the sclerotesta several more secretory elements are seen and remains of parenchymatous tissue. S. 34, *a.* × 90.

Fig. 15. A single sarcotestal bundle from near the base of *Trigonocarpus Parkinsoni*, showing the dual nature of the xylem. The centre of the strand is occupied by narrow tracheides (*n.tr.*) surrounded by a parenchyma sheath (*par.*), and this again is followed by a zone of short broad tracheides (*s.tr.*). S. 34, *b.* × 90.

Trigonocarpus Shorensis.

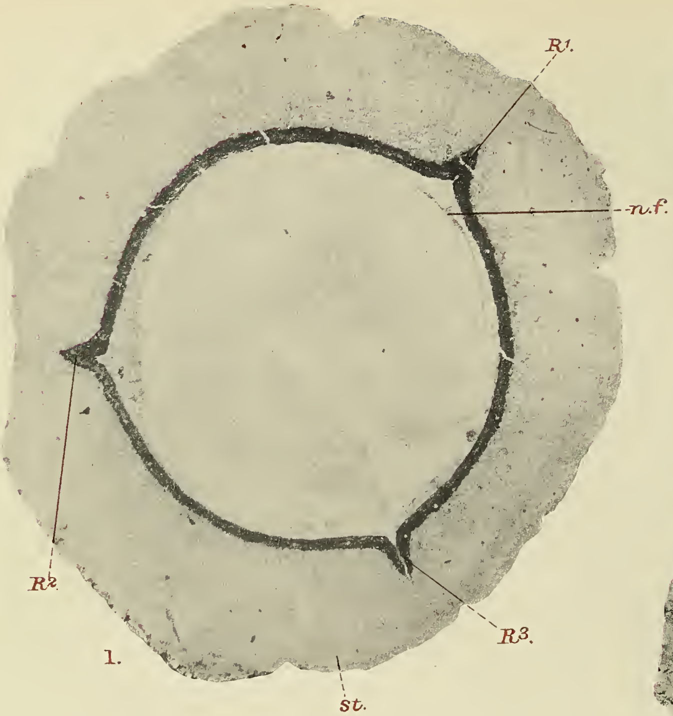
Fig. 16. Transverse section through the chalazal bundle of *T. Shorensis*. The parenchyma sheath (*p.s.*) around the bundle and the radially extended tracheides of the outer zone are clearly seen. The protoxylems are seen occupying a position just within these latter. *m.s.*, mucilage sacs. S. 33, *b.* × 100.

Fig. 17. Transverse section through a sarcotestal bundle, showing the thick-walled protoxylem elements (*pt.x.*), the small elements of the centrifugal xylem (*cf.x.*), and the large short tracheides comprising the centripetal (*cp.x.*). Touching the bundle on the right is a sclerotic strand of the limiting layers. *m.s.*, mucilage sac. S. 31, *i.* × 100.

Fig. 18. Oblique section through the chalazal bundle, from the same preparation as Fig. 7, showing the scalariform thickenings of the tracheides (*tr.*). *scl.*, sclerotesta. S. 32, *e.* × 80.

Fig. 19. Transverse section through the limiting layers of the sarcotesta, occupied by several radially extended sclerotic strands (*scl.p.*) partially displaced by contraction. The sclerotic plate on the extreme left is interrupted by a medianly placed mucilage sac, and both here and in the other here present (*m.s.*) the thickened wall stands out clearly as a transparent zone around the dark contents. S. 31, *i.* × 100.

Fig. 20. Transverse section through the nucellus from the same preparation as Fig. 4. The radial files of mucilage sacs (*m.s.*) are clearly seen, causing corresponding ridges (*n.r.*) upon the surface of the contracted nucellar epidermis (*n.e.*). The ground-tissue is occupied by thin-walled parenchymatous tissue (*par.*), and the inner limit is marked by a zone of tracheides (*tr.*). S. 33, *c.* × 120.



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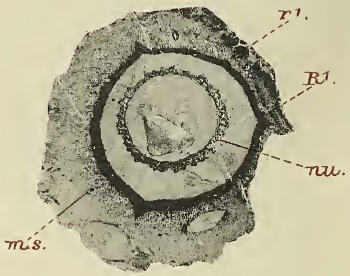

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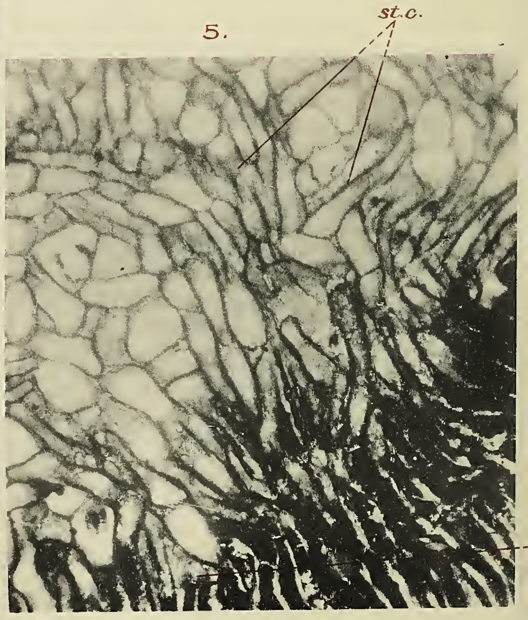

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SALISBURY—TRIGONOCARPUS SHORENSIS.

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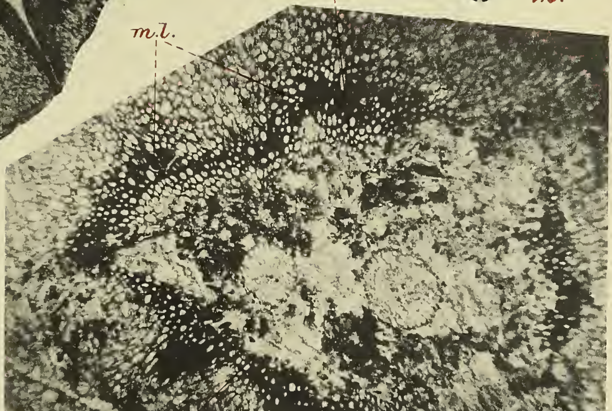

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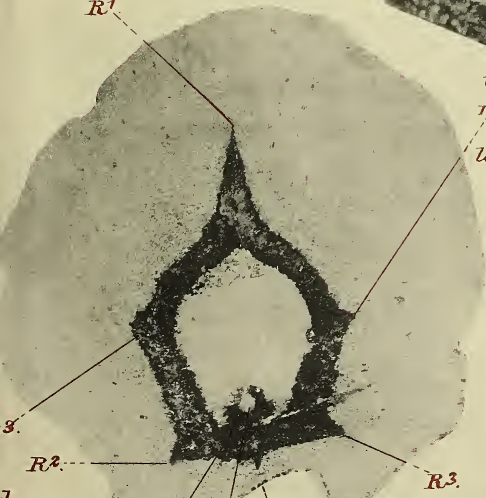

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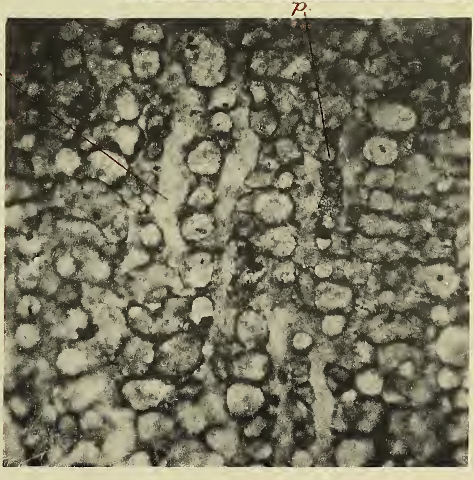

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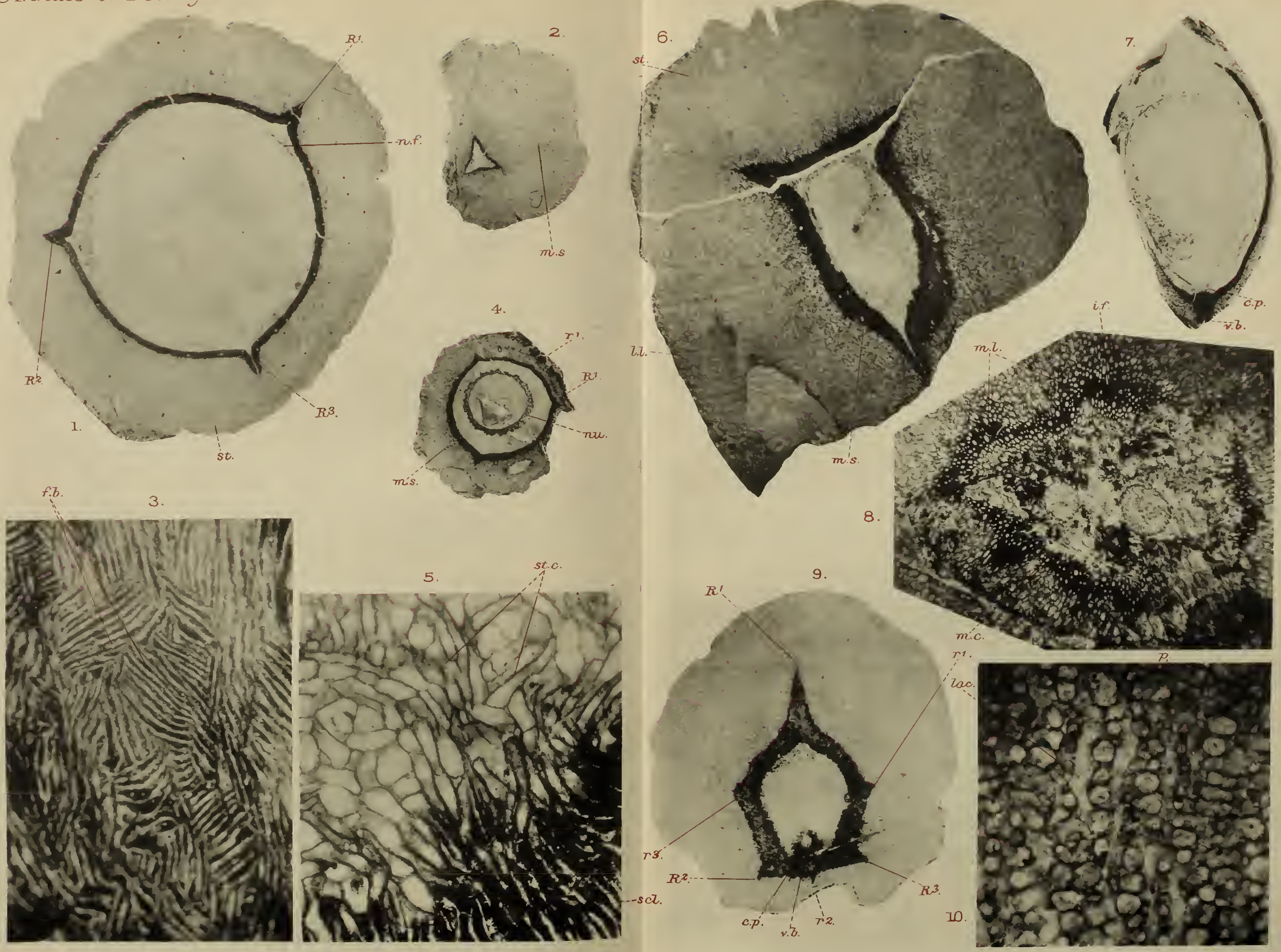

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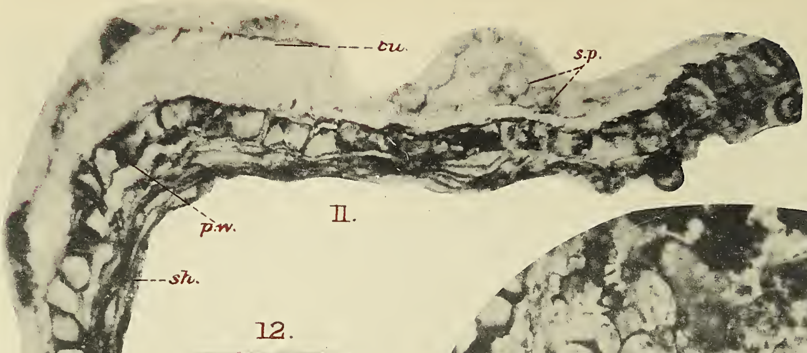
SALISBURY—TRIGONOCARPUS SHORENSIS.



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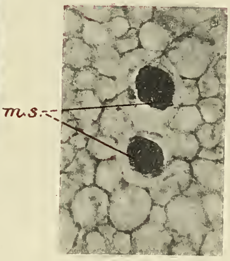

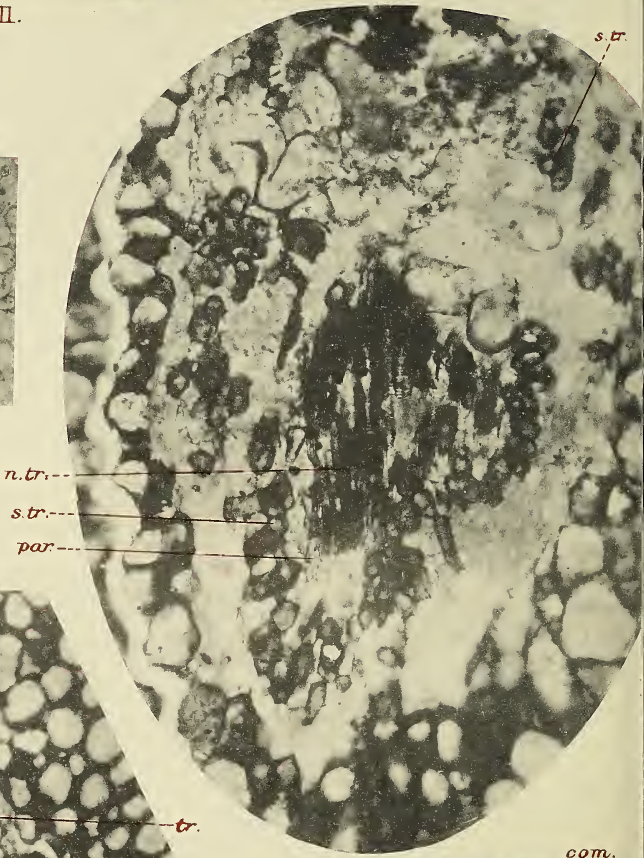

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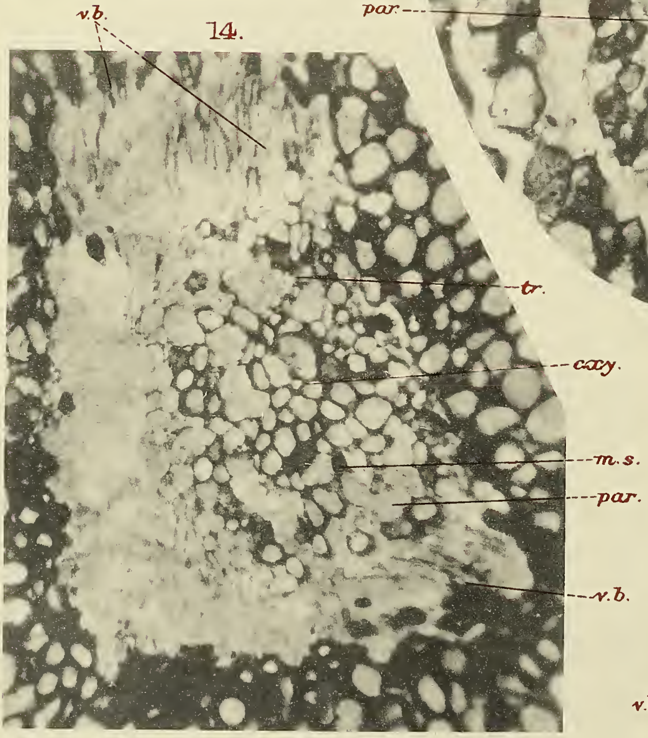

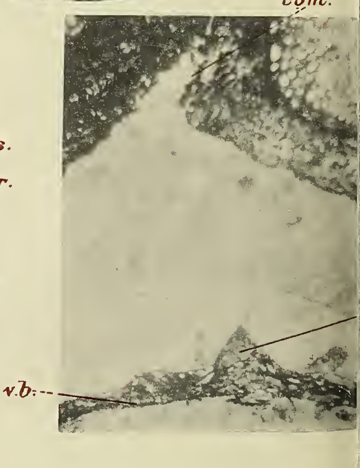

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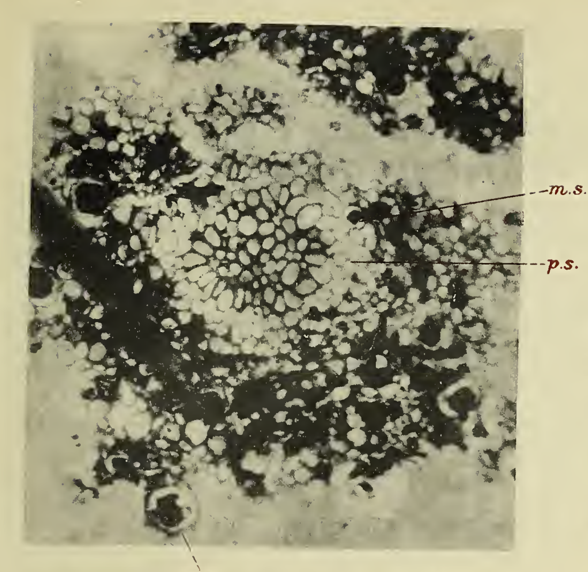

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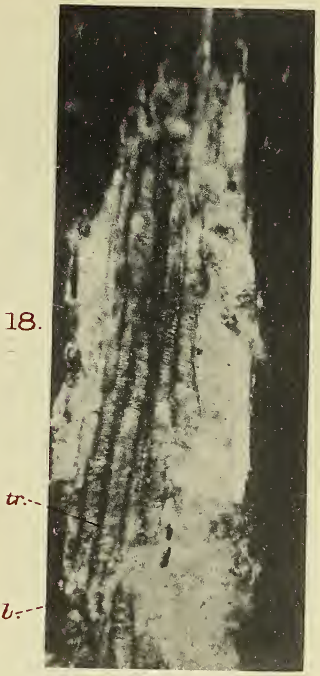

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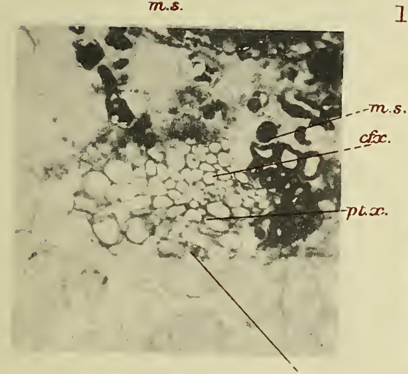

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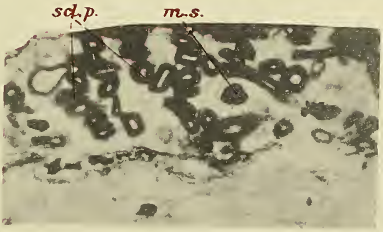

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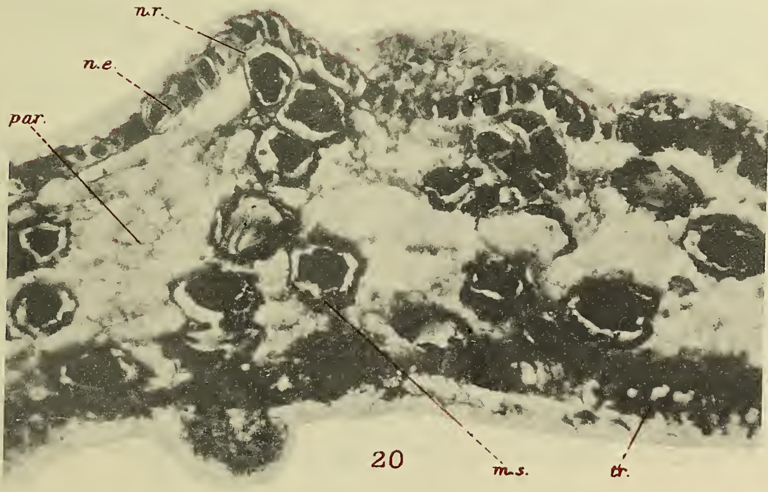

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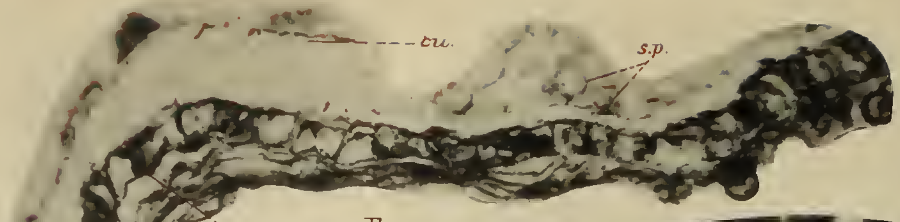

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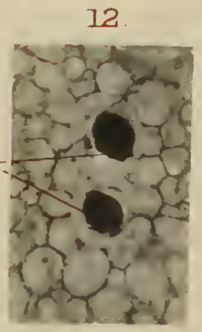

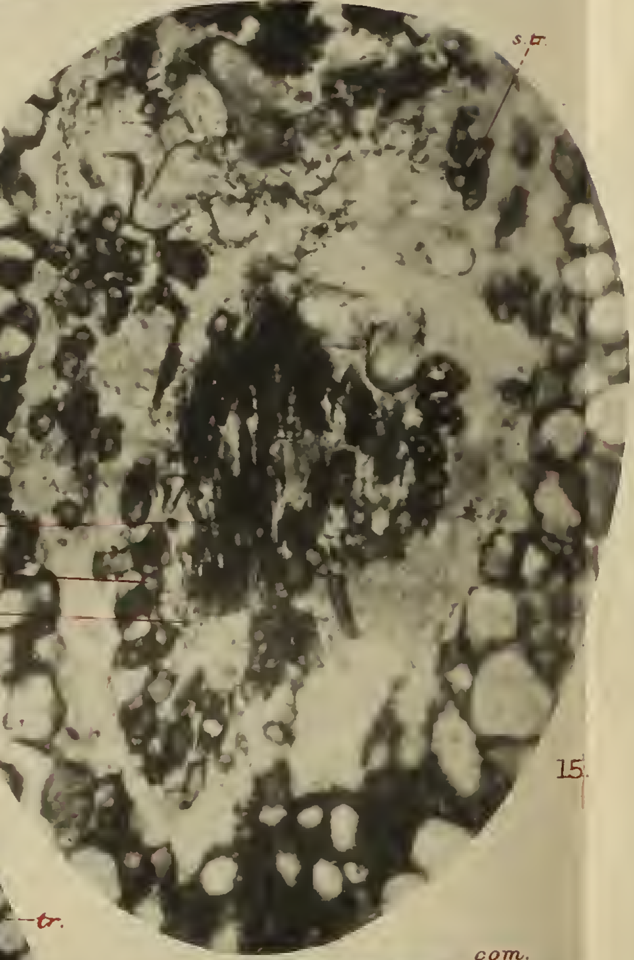

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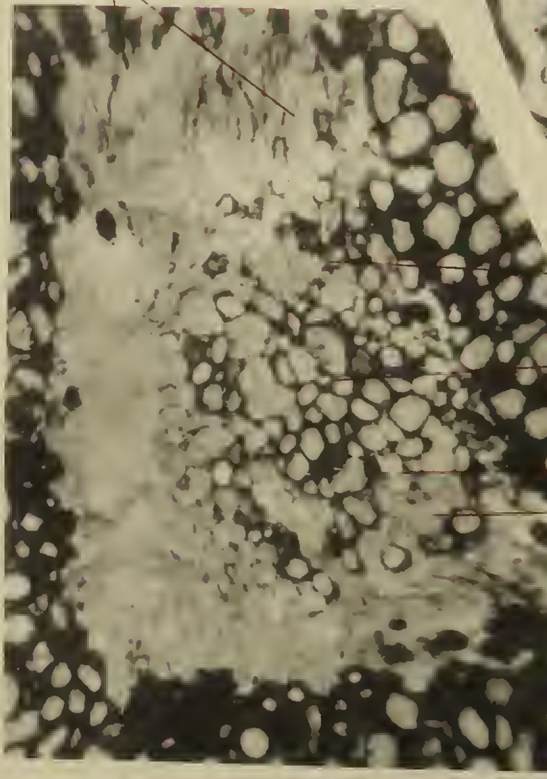

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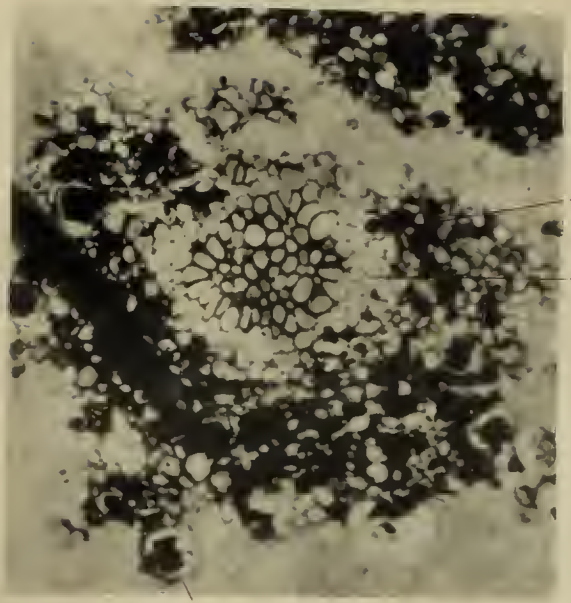

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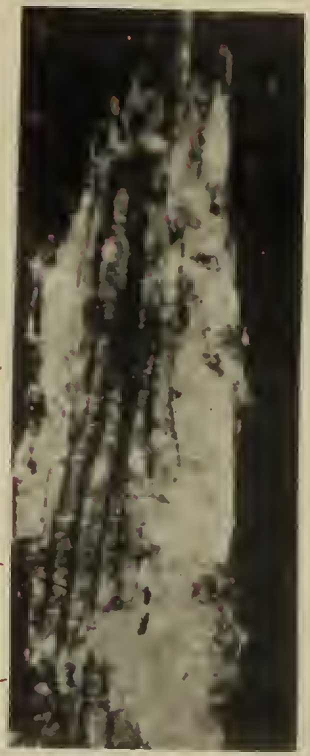

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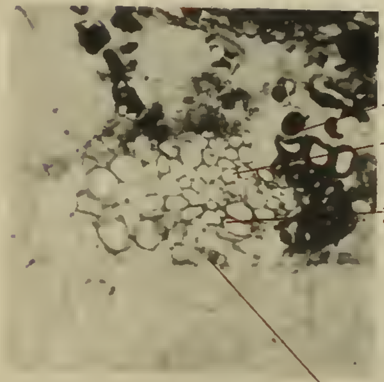

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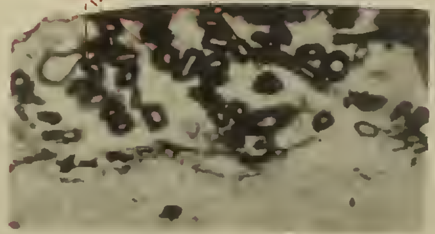

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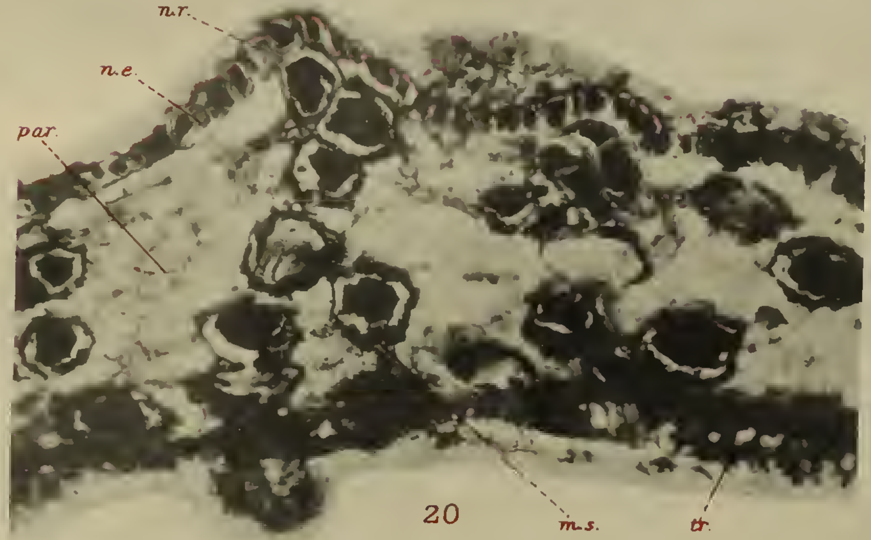

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SALISBURY — TRIGONOCARPUS SHORENSIS.

Huth coll.

A Revision of the Seed Impressions of the British Coal Measures.

BY

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With Plates VI–VIII and eight Figures in the Text.

THE occurrence of a variety of impressions or casts of seed-like bodies in the British Coal Measures has been known since the days of John Woodward[1] (1729), yet at the present time there is probably no set of Coal Measure fossils which stands in greater need of systematic revision than these. The most recent list of such objects, published by Dr. Kidston[2] in 1894, includes only five genera with nineteen species from the whole of the British Coal Measures. Of these genera one, *Carpolithus*, containing nearly one-third of the total number of species, is nondescript, and amounts to little more than the word 'seed'. It is a useful term under certain circumstances, but should be regarded only as a temporary expedient. There is little doubt, I imagine, that the Coal Measure seeds which have been referred to this genus are of several fundamentally distinct types.

When determining Coal Measure fossils, one frequently finds that, while there is no hesitation as to the specific assignment of a particular seed, there is often doubt as to whether such and such a species really belongs to the genus to which it has commonly been assigned, and especially whether it possesses any real morphological affinity to the other species contained therein. In my own case, however, I have found it necessary to defer any opinion on such questions until an opportunity should occur for re-investigating the grouping of Coal Measure seeds in general.

In attempting this difficult task here, I am well aware that the principles on which a nomenclature should be founded are to some extent matters of opinion, and that in some cases differences of opinion are likely to arise as to matters of fact. The botanical interpretation of the features exhibited by a particular impression is sometimes by no means an easy

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[1] Woodward (1729), p. 53.

[2] Kidston ('94).

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task, yet it is on these grounds that a classification should be based. In my opinion one of the most fundamental distinctions among Coal Measure seeds should be founded on the symmetry. We know from the study of petrified specimens that most of these fossils belong to two distinct types, the Radiosperms and the Platysperms, as F. W. Oliver[1] pointed out in 1903. The former are symmetrical in more than two planes, the latter in only two or in a single plane. At the same time a classification based solely on symmetry is not ideal. Certain seeds, such as *Trigonocarpus Parkinsoni*, are radiospermic from one point of view, and platyspermic from another. Although the number of such cases is few at present, an intermediate class may have to be recognized eventually.[2] The main difficulty as regards the recognition of the type of symmetry among impressions arises, of course, from the compression which many seeds have undergone before or during fossilization. From an isolated example it may be sometimes impossible to determine whether the seed in question is radiospermic or platyspermic, especially if it be a new or rare type. In such circumstances it is best referred to the genus *Carpolithus*. But it will be found that, when a considerable number of specimens of the same seed from different localities are compared, it is usually fairly easy to settle this question, at least provisionally. It is extraordinary how, in many cases, both radiospermic and platyspermic seeds have escaped compression, or have been flattened only to a slight degree. Even such seeds as *Cordaicarpus*, the nucellus of which in the natural state was probably narrowly elliptical in transverse section, and which, one imagines, would only require comparatively slight pressure perpendicular to the major axis to become quite flattened, have often escaped with but little distortion of the original form.

At the present time there is considerable confusion as to the limits of the generic terms in common use. This arises partly because the subject has not undergone revision for more than half a century, and partly because of the extremely unfortunate practice of including impressions in genera founded on structure material, or *vice versa*, without at the same time affording satisfactory proof of the correlation of the impression with the petrifaction or *vice versa*. This is quite unjustifiable. It was perhaps less glaring a decade ago, when the diversity in anatomical structure to be met with in Palaeozoic seeds had not been so fully recognized. New types of seed structure should receive temporary names as they are discovered, until the time arrives when a definite proof can be afforded of their generic correlation with impressions, the names of which have been in existence for a century. Further, a diagnosis of a genus such as *Cardiocarpus* should take into account the external form of the seed in addition to the details of its anatomical structure, for this name has been in use for impressions since 1828.

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[1] Oliver ('03), p. 453.

[2] Oliver and Salisbury ('11), p. 44.

I do not propose in the present instance to deal at all with structure specimens, still less with the difficult correlation of such petrifactions with impressions. My present task is merely to sort out and to reclassify the impressions.

From the point of view of the study of impressions the testa and its characters are all important. I assume that very few radiospermic seeds, as Oliver[1] has pointed out, and as experience with impressions confirms, have a testa differentiated into a fleshy outer sarcotesta and a hard inner sclerotesta. The converse, however, does not hold good. Some Platysperms, such as *Cardiocarpus*, as here restricted, *Cornuspermum* and *Cordaicarpus*, show no signs of a differentiated testa. In others, however, such as *Samaropsis* and *Samarospermum*, the differentiation of the testa is very obvious. In the latter type, it is convenient to speak of the central portion of the seed as the 'nucule', an ancient term implying the nucellus surrounded by the hard sclerotesta. The soft sarcotesta as viewed in impressions is nearly always flattened over the nucule, and the rim of sarcotesta surrounding the nucule is conveniently described as the 'wing'.

I regret that it has been found necessary here to institute a large number of new generic terms, partly because a considerable number of new species are described for the first time, but more so because there is a very great variety among seed impressions,—a variety which must find adequate expression unless these fossils are to remain bunched indiscriminately into such nondescript genera as *Carpolithus*. On the other hand, I am well aware that it may eventually be found that more than one generic type has here been included in a single genus, especially in the case of the new genus *Radiospermum*. But this is a defect which only time and further evidence can remedy. The total number of genera here recognized is 14, and 37 species are described. The latter part of the paper is devoted to an attempt to define these genera and species more exactly than has hitherto been possible. Of the 14 genera here recognized, 9 are new, the remainder have been already employed for continental specimens. On the other hand, of Dr. Kidston's 18 species, all but 4 are recognized, though the great majority of them are referred to other genera. Ten new species are described here, and twenty-seven others are recognized which have either been previously recorded by Dr. Kidston, or have been unknown from Britain though known from the Continent. A figure of every British species is included.

It is hardly necessary to point out perhaps that the detached seed impressions, here under consideration, belong to at least two very diverse groups, the Pteridospermeae and the Cordaitales. At present we are unable to discriminate between the seeds of these groups. All that we can

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[1] Oliver ('03), p. 453.

say is that *some* species of *Samaropsis* and *Cordaicarpus* belong to the Cordaitales, and that, of the other genera, *some or all* species may belong to the Pteridosperms. The platyspermic seeds appear to be the more varied type, nearly double the number of genera and species of these seeds, as compared with the Radiosperms, being recognized here.

I will begin with *Trigonocarpus*, the best known Coal Measure seed, both as an impression and as a petrifaction. This is a happy case in which it has been possible to correlate both types of preservation very exactly. Though *Trigonocarpus Parkinsoni*, Brongn., stands secure, the other types which have been referred to this genus have caused trouble and confusion, and may continue to do so for some time yet.

Trigonocarpus, Brongn.

T. Parkinsoni, Brongn. This species has been so thoroughly investigated by Scott and Maslen[1] that a bare mention of it here is all that is necessary. As an impression this seed occurs in three states of preservation.

Forma α. This shows the external surface of the sarcotesta, the true outer surface of the seed. It is the ancient *Carpolithes alata* of Lindley and Hutton, and is one of the most unmistakable of Coal Measure fossils.

Forma β (Pl. VI, Fig. 2) shows only the sclerotesta and micropyle. In impressions the latter is frequently covered up by the rocky matrix, and in this condition it is sometimes difficult to recognize this state. The surface of the sclerotesta, in my experience, is smooth, as in Fig. 2, and a difficulty also arises here, as Scott and Maslen have pointed out,[2] for in the petrified specimens the outer surface of the sclerotesta possesses twelve longitudinal ridges[3] of unequal prominence. I am unable to throw any light on this matter. This state was originally described as *Rhabdocarpus Bochschianus* by Berger.[4]

Forma γ (Pl. VI, Figs. 3–5), the well-known, tri-ridged casts of the nucellus or seed cavity. This is the state to which both the specific and generic names were first applied in 1828. So far as I am aware these casts never show any sign of splitting at the pointed end, or of a definite foramen at the rounded basal end. The latter is sometimes quite smooth (Fig. 4, lower seed), or sometimes there is a small, projecting, oval umbilicus, as in Figs. 4 (upper seed) and 5, which Scott and Maslen[5] regard as probably indicating 'the edge of the tracheal disc from which the free nucellus sprang'. I have, however, never seen any definite foramen disclosing an inner cast, and this, as we shall see, is important in relation to some other casts, which have so far been referred to the same genus.

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[1] Scott and Maslen ('07), p. 96.
[2] Ibid., p. 130.
[3] Ibid., p. 106.
[4] Berger ('48), p. 21, Pl. I, Figs. 13, 14.
[5] Scott and Maslen ('07), p. 128.

We have next to inquire whether any other species of this genus are known from the British Coal Measures as impressions. After a careful study of the subject, I have arrived at the somewhat startling conclusion that there are only three other species known from this country, and none of them so completely as *T. Parkinsoni*, Brongn. These are:

Trigonocarpus Moyseyi, sp. nova. Forma α.
Trigonocarpus Dawesi, L. & H. Forma γ.
Trigonocarpus clavatus (Sternb.). Formae α and β.

Trigonocarpus Moyseyi, sp. nova (Pl. VI, Fig. 1). I originally figured[1] this fine specimen in Dr. Moysey's collection as *Trigonocarpus* sp., with the suggestion that perhaps the so-called *T. Noeggerathi* of Sternberg might be the forma γ of this seed. At that time I was under the impression that the above species was a state of a true *Trigonocarpus*. A more careful study of the subject, however, has led me to remove *T. Noeggerathi*, Sternb., from the Platysperms, at least provisionally, for the reasons stated below (p. 86). I now regard Dr. Moysey's specimen as the type of a new species, which I name *T. Moyseyi*, sp. nova, in honour of one who has done so much for the palaeontological investigation of the Nottingham coalfield.

T. Moyseyi differs from *T. Parkinsoni* chiefly in size and shape, as will be realized if Pl. VI, Fig. 1 be compared with the specimen figured by Scott and Maslen on Pl. XII, Fig. 17 of their paper. Both figures are magnified equally. *T. Moyseyi* is much broader in proportion to its length, and has a shorter micropyle. The formae β and γ of this seed are unknown.

Trigonocarpus Dawesi, L. and H. (Pl. VI, Fig. 8). This seed is only known as forma γ casts, which correspond almost exactly to *T. Parkinsoni*, forma γ except in size. It is twice as large as the latter. It is a rare type.

Trigonocarpus clavatus (Sternb.) (Text-fig. 1, p. 95). The obscure and rare types, the *Carpolites clavatus* and *C. langenarius* of Sternberg, which Geinitz and others have referred to *Rhabdocarpus*, are in my opinion probably a small *Trigonocarpus*, formae α and β. The general shape of the seed, the micropyle, and the fleshy sarcotesta support this conclusion. The fine longitudinal striation of the testa, on which stress has been laid as an indication of generic affinity, is in reality common to several genera of Palaeozoic seeds. This is the smallest British example of the genus, and may possibly be only an immature condition of another species, though this is by no means certainly the case.

These are the only British representatives of the genus which I am inclined to recognize. There are, however, several other species, usually placed in this genus, which may be briefly discussed. A knowledge of the forma γ casts of *Trigonocarpus Parkinsoni* has had a very profound influence on the nomenclature of other Palaeozoic seeds. Any egg-shaped cast,

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[1] Arber ('10), p. 150, Pl. XVIII, Fig. 1, Pl. XIX, Fig. 3, and Arber ('09), p. 42.

showing three prominent ribs, has sometimes been referred to the genus *Trigonocarpus* without hesitation. For some years past I have had a strong suspicion that a cause of error may have arisen here. I am now convinced that several tri-ridged casts represent, not forma γ casts of *Trigonocarpus*, but the external surfaces of radiospermic seeds, though it will no doubt be a matter of dispute for some time yet as to whether I am correct or not in this conclusion.

The most difficult case in this connexion is the seed which I here term—

Schizospermum Noeggerathi, Sternb.

This is a seed (Pl. VIII, Figs. 48–50) similar in some respects to the forma γ casts of *T. Parkinsoni*, and of nearly equal abundance in the Coal Measures, which was originally described by Sternberg[1] in 1826 as *Palmacites Noeggerathi*, and regarded by him as the seed of a palm. Brongniart[2] in 1828 transferred this fossil to his *Trigonocarpum*, and this view has been adopted by Berger[3] and Zeiller,[4] who have since figured specimens of the same seed. So far the synonymy has remained pure, and so far as one can judge all the specimens figured are identical. Of the two specimens more recently figured by Dr. Kidston, that from Radstock[5] may be Sternberg's plant, though the figure does not show the characteristic features of this species. I am convinced, however, that the figure of the Lancashire specimen[6] represents *Trigonocarpus Dawesi*, L. and H.

There are a large number of specimens of Sternberg's species in the Sedgwick Museum, from the Middle and Upper Coal Measures of many localities. A careful examination of these shows that they possess two well-marked features, which I have never observed in *T. Parkinsoni*, forma γ. At one end of the cast, the outer coat of the seed splits into three valves (Pl. VIII, Fig. 49). This is almost universally the case, and is not due to compression. At the other end of the seed, there is a foramen, a fairly large round or oval hole, disclosing another cast within (Pl. VIII, Fig. 50). These facts are exactly as depicted in Zeiller's figures (see above). From the apex of each of the three valves a fairly well-marked longitudinal ridge proceeds nearly to the base, hence the attribution to *Trigonocarpus*. These three ridges alternate with the splitting lines, which are themselves ridges, so that there are six ridges in all.

It appears to me to be quite impossible to believe that these casts are casts of the internal surface of the sclerotesta of a *Trigonocarpus*. If they are, how are the valves explained? They are certainly not accidental nor confined to a few specimens. What is the foramen, and the cast internal to

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[1] Sternberg ('20), Heft iv, p. xxxv, Pl. LV, Figs. 6, 7.

[2] Brongniart ('28), p. 137.

[3] Berger ('48), pp. 15, 18, Pl. I, Figs. 1, 2.

[4] Zeiller ('86), p. 649, Pl. XCIV, Figs. 8–11; ('00), p. 223, Fig. 149.

[5] Kidston ('88), p. 403, Pl. XXIII, Fig. 3.

[6] Ibid. ('89), p. 414, Pl. II, Fig. 4.

the valves? It seems to me to be much more likely that we are dealing here with the true external surface of a *radiospermic* seed. In a very large number of examples no trace of any fleshy sarcotesta is to be seen, and therefore this is presumably not a forma β cast. My view is that it is the external surface of a seed, the integument of which when ripe opened by three valves exposing the nucellus at the apex of the seed; that the foramen represents the point of attachment of the stalk, and that the cast seen within is a cast of the nucellus. I therefore propose to transfer this species to the Radiosperms, as a member of a new genus *Schizospermum*, namely *S. Noeggerathi* (Sternb.). The possession of three longitudinal ridges is common to several other Radiosperms, as will be shown here, and the foramen and inner cast are also seen in some of these. Further, in a unique but obscure specimen, at present referred to the new genus *Radiospermum* as *R. problematicum* (Pl. VII, Fig. 37), there is also evidence of splitting at one extremity of the seed. At present, however, it is not known whether this was a natural feature or not.

Radiospermum ovatum (L. and H.) (Pl. VII, Fig. 32). There is a large and very rare seed in the Middle Coal Measures of Coalbrookdale and Yorkshire, which was first figured by Lindley and Hutton[1] as *Trigonocarpum ovatum*, and by Morris[2] as *Carpolithes zamioides*. The cast is cylindrical; there are three well-marked longitudinal ridges, as in many other radiospermic seeds, and a foramen occurs at the base, disclosing an inner cast. It differs from *Schizospermum Noeggerathi* in the fact that the integument of the seed does not separate into three valves, and also in size and shape. This also I am inclined to regard provisionally as the external surface of a radiospermic seed, and not as a cast of the seed cavity of a γ form of *Trigonocarpus*. It seems to me to fall within the new genus *Radiospermum* as defined below (p. 92).

Platyspermum, gen. nova., and *Rhabdocarpus*, Berg.

This completes the list of British Coal Measure plants which have been referred to the genus *Trigonocarpus*. We now turn to the platyspermic seeds, and especially to those hitherto referred to the genus *Rhabdocarpus*. This name appears to be used in a somewhat loose sense at the present time.[3] In my opinion it should be reserved for unsymmetrical seeds, similar to *Rhabdocarpus tunicatus*, Berger,[4] as the type of the genus, and *R. subtunicatus*, Zeiller.[5] These seeds are Platysperms, with a large

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[1] Lindley and Hutton ('31), vol. ii, Pl. CXLII a.

[2] Morris ('40), p. 489, Pl. XXXVIII, Fig. 4.

[3] Berger, the founder of the genus, himself included several different types under this name. His paper is, however, merely a student's thesis for a degree, and it is doubtful whether such dissertations should be recognized.

[4] Berger ('48), p. 20, Pl. I, Fig. 8 (right side).

[5] Zeiller ('92), p. 93, Pl. XV, Fig. 11; the *R. tunicatus* of Grand' Eury ('77), pp. 206, 313, Pl. XV, Fig. 12; and Renault ('90), p. 638, Pl. LXXII, Fig. 19.

unsymmetrical nucule, enclosed in a large unsymmetrical wing or sarcotesta, the seed being apparently symmetrical only in one plane. The fact that the sarcotesta is ornamented with close, parallel, longitudinal striae is probably of no systematic importance, for the same feature occurs in the case of other platyspermic and also among radiospermic seeds. My conclusion is that this Stephanian and Permian type of seed has been hitherto unknown from the British Coal Measures, and that all the British species so far referred to it are symmetrical in two planes, and are not obviously winged. I therefore propose to transfer them to a new genus, *Platyspermum*. *P. sulcatum*, Sternb. (Pl. VI, Fig. 11), and *P. elongatum*, Kidst. (Pl. VI, Fig. 12), belong here, and so does the true *P. multistriatum* of Sternberg. The specimen from the Radstock coalfield, figured by Dr. Kidston[1] under this name (Pl. VI, Fig. 9), I believe to be a new species, and not Sternberg's seed. I have included it here as *P. Kidstoni*, sp. nova, and have also figured what I take to be the true *P. multistriatum* of Sternberg (Pl. VI, Fig. 10). *P. Kidstoni* appears to me to lie nearer to *P. sulcatum*, Sternb., than to *P. multistriatum*, Sternb., in that the ridges approximate at the base and apex. I, however, only know Dr. Kidston's specimen from the published drawing. Another new species, *P. rugosum*, with coarse ribbing, is also described here (Plate VI, Fig. 13).

While the above species, here referred to the new genus *Platyspermum*, are in my view morphologically distinct from *Rhabdocarpus tunicatus*, Berg., and *R. subtunicatus*, Zeill., there is, however, one seed known to me which does appear to be a true member of the latter genus. Two examples of this are figured on Plate VII, Fig. 21. It is apparently a new species which I propose to call *Rhabdocarpus Lillieanus*, sp. nova.

The genus *Platyspermum* is not intended to include all platyspermic seeds of Coal Measure age, but only those which are not winged and in which the testa possesses many close, parallel ribs or striations. There are several other types, among which are:

Cornucarpus, gen. nova.

This new term is proposed for a well-known platyspermic seed, symmetrical in two planes, which is very distinct from all other seeds. It was first described by Lindley and Hutton[2] in 1833 as *Cardiocarpon acutum* (Pl. VI, Fig. 14, and Text-fig. 2, p. 97). There is considerable evidence for believing it to be the seed of *Eremopteris artemisiaefolia*, Sternb., but it has not yet been possible to demonstrate actual continuity between this seed and the fertile fronds of that species. Some years ago Dr. Kidston showed me prepared specimens of this seed rendered transparent, so that the pollen

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[1] Kidston ('88), p. 404, Pl. XXIII, Fig. 4.

[2] Lindley and Hutton ('31), vol. i, p. 209, Pl. LXXVI.

chamber could be seen, and there is thus no doubt as to the seed nature of this fossil.

Cornucarpum acutum (L. and H.) is easily distinguished by the more or less triangular shape and the broad apex, with a fairly long horn at each corner. There is also a median ridge. It is thus quite distinct from *Cardiocarpus* as here defined.

Cardiocarpus, Brongn.

This name was first instituted by Brongniart[1] in 1828 for seeds with the following characters: 'fruits comprimés, lenticulaires, cordiformes ou réniformes, terminés par une pointe peu aiguë.' In recent years there has been some difference of opinion as to whether this genus should be made to include winged seeds. Brongniart's diagnosis says nothing about wings. I am inclined to reserve it for those seeds which apparently did not possess a broad sarcotesta, but in which the testa was heart-shaped or reniform. The following are the British species: *Cardiocarpus congruens*,[2] Grand' Eury (Text-fig. 3, p. 97), and *C. Gutbieri*, Gein. (Pl. VI, Fig. 15). The species referred to this genus by Artis as *C. marginatus* (Pl. VIII, Fig. 46) is still a type of very uncertain affinities. It may be a radiospermic seed. It must also be remembered that the nucule of badly preserved *Samaropsis* seeds, in which the wing is not preserved, may simulate *Cardiocarpus*. In some species of *Cardiocarpus* there appears to be a slight rim or wing, but I am inclined to think that this is wholly due to the effects of pressure.

Samaropsis, Goepp.

The genus *Samaropsis*, Goepp., is maintained here as used by continental authorities in more recent years, though in a slightly restricted sense. In these seeds there is a distinct nucule in the centre, surrounded by a broad rim called the wing. The nucule is heart-shaped or oval, and the whole wing circular, or oval, or heart-shaped. I would restrict this genus to seeds which are not much longer than broad, or broader than long, separating those forms with a very long and narrow wing as a distinct genus *Samarospermum*. *Samaropsis* differs from *Cardiocarpus* in the clear distinction between the nucule and the wing. In other words, in *Samaropsis* there is a well-developed sarcotesta; little, if any, in *Cardiocarpus*. The nucule is of course the sclerotesta.

I propose to refer all Platysperms, with a triangular or heart-shaped nucule, surrounded by a more or less circular wing, to Goeppert's genus *Samaropsis*, founded in 1864. The British species of *Samaropsis* are *S. fluitans*, Dawson (Pl. VI, Fig. 18), *S. crassa*, Lesq. (Text-fig. 4), *S.*

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[1] Brongniart ('28), p. 87.

[2] This may be the *Carpolites corculum* or *C. contractus* of Sternberg (Vers. Darstell. Flora d. Vorwelt, Heft 5-6, p. xl, Heft 1, Pl. VII, Figs. 6 and 7, 1820-33), but Sternberg's figures appear to be too indefinite to permit of any certain correlation.

emarginata, Berg. (Text-fig. 5), *S. Meachemi*, Kidst. (Pl. VI, Figs. 16, 17), and *S. subacuta*, Grand' Eury (Text-fig. 6).

Samarospermum, gen. nova.

I propose here to restrict the genus *Samaropsis* to those seeds in which the wing is of nearly equal length and breadth. There is, however, another type, confined until quite recently to the Stephanian and Permian of Europe, and represented by but a few species. This was originally referred by Helmhacker[1] to Fiedler's[2] genus *Jordania*, as *J. moravica*, Helmh., which was first figured by Geinitz.[3] It has, however, been pointed out that this fossil does not agree generically with the species of *Jordania* figured by Fiedler, and thus Zeiller, Renault, and others have more recently referred it to the genus *Samaropsis*. Zeiller[4] has refigured Helmhacker's type specimens. It appears, however, to me to be a perfectly distinct type, worthy of generic distinction, and, since the genus *Jordania*, Fiedler, is unsuitable for its reception, I here propose the new generic name *Samarospermum*. This species *Samarospermum moravicum* (Helmh.) (Pl. VI, Figs. 19, 20) has recently been found in the Middle Coal Measures of the Kent coalfield. The chief peculiarity of this genus is the great elongation of the seed, which is very narrow in proportion to its length, and possesses only a small nucule.

The specimens figured by Weiss[5] in 1879, under the name *Jordania moravica*, appear to be specifically distinct from the plant first figured by Geinitz.

Microspermum, gen. nova.

So far as I am aware, no seed impression has as yet been described from Britain which appears to be only symmetrical in one plane. As far as one can judge, without any knowledge of the anatomy, all the British Platysperms, hitherto described, appear to be symmetrical in two planes. We now come to a small seed, if indeed it be a seed, which has several curious features, and which like *Rhabdocarpus Lillieanus*, sp. nova (see p. 88), is only symmetrical in a single plane. I have only seen this seed from the Middle Coal Measures of the Nottinghamshire coalfield, and I am indebted to my friend Dr. Moysey for the loan of a fine suite of specimens of it. It is here placed in a new genus *Microspermum*, as it is quite distinct from any other type of seed known to me.

The seed in question is a small, ovate, or pyriform body from 5–10 mm. long, one extremity being rounded, the other contracted acutely. One side is keeled, the other grooved, and a large foramen occurs near the broader end and on the grooved surface.

The British specimens figured on Pl. VII, Figs. 22–27 agree fairly well with

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[1] Helmhacker ('71), p. 81.

[2] Fiedler ('57), p. 283.

[3] Geinitz, E. ('75), p. 11, Pl. I, Figs. 10, 11.

[4] Zeiller ('92), p. 95, Pl. XV, Figs. 9, 10.

[5] Weiss ('79), p. 36, Pl. III, Figs. 17–19.

the specimen figured by M. l'Abbé Carpentier[1] as *Carpolithes ? samaroides*, Carp., from the Westphalian of the Nord de France coalfield. The seeds agree in size, though the British specimens are somewhat broader as a rule. M. Carpentier regards his specimens as winged, which is certainly not the case in the English examples, and he does not mention the foramen, which, however, appears to be clearly seen at the broader end of his seed. In his figured example, which only shows one side of the seed, the median groove is much broader than in the specimens figured here. Judging chiefly by his figure, I am, however, inclined for the present to include the British examples in the French species.

The present specimens differ from other seeds in certain important respects, chiefly in their unsymmetrical form. The foramen is, however, a feature met with in certain species of *Radiospermum* and *Schizospermum*, where, however, it is terminal. The fossils are solid bodies, and are certainly not foliar in nature. Neither do they appear to be sporangial. They may thus be regarded, at least provisionally, as seminal structures.

Cordaicarpus, Geinitz.

Next we have the genus *Cordaicarpus*, Geinitz, 1862, which in common with Geinitz,[2] Grand' Eury,[3] and Zeiller[4] I am convinced belongs to a member of the *Cordaitales.* There are several species of impressions, of which *Cordaicarpus Cordai*, Gein. (Pl. VII, Fig. 29), may be regarded as the central type. These are fairly small seeds, oval or orbicular in form, with a smooth testa. Two other well-marked species occur in Britain, *C. areolatus* (Boul.) (Text-fig. 7) and *C. ovoideus*, Berg. (Pl. VII, Fig. 30). These are undoubtedly Platysperms. They commonly occur compressed quite flat, in which state there is usually a narrow rim bounding the seed (Pl. VII, Fig. 29). They are, however, frequently also found in an almost unflattened condition, and are then elliptical in section (see Pl. VII, Fig. 30), resembling *Diplotesta*, which Bertrand[5] has shown to be a Cordaitean seed.

Megalospermum, gen. nova.

To this genus I ascribe a certain very large seed, without any indication of a wing or a nucule, which has been described by Dr. Kidston as *Carpolithus Wildii*, the testa of which is ornamented by numerous fine and close thread-like striae (Pl. VII, Fig. 28). There may be some doubt at present whether this seed is Radiospermic or Platyspermic, for only a single example is known from Britain. Others, believed to be generically similar,

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[1] Carpentier ('11), p. 7, Pl. XIV, Fig. 3; Ibid. ('10), p. 599.

[2] Geinitz, H. B. ('61), p. 150.

[3] Grand' Eury ('77), p. 233, Pls. XXV, XXVI, Fig. 9.

[4] Zeiller ('86), pp. 637, 641.

[5] Bertrand ('11).

have been described on the Continent, and these appear to be undoubtedly Platyspermic.

Certain other species of this genus have been included by Grand' Eury in the genus *Pachytesta*, Brongn., a structure genus. At present, however, the identity of *Megalospermum* and *Pachytesta* has not been proved, and I agree with Dr. Kidston that, under these circumstances, the impressions should not be placed in the structure genus. This type of seed impression is, however, very distinct, and is worthy of generic separation and removal from the nondescript genus *Carpolithus*. It is very rare in the Westphalian rocks of England, being more frequent on higher horizons on the Continent.

The radiospermic seeds recorded from the Coal Measures are fewer than the platyspermic types.

Radiospermum, gen. nova.

This name is proposed to include all the British subcylindrical seeds at present known, except *Schizospermum* and *Neurospermum*, the latter being distinguished by the close ribbing or striation of the testa. In *Radiospermum*, a few distant ribs and discontinuous, small, longitudinal striae may occur, or the testa may be without either type of ornament. *Radiospermum* includes the small seeds which, when the structure is preserved, are known as *Lagenostoma*, *Physostoma*, and *Conostoma*.[1] It is however rarely, if ever, possible to recognize the distinguishing characters of these three genera in such minute seeds, when preserved as impressions. Such impressions are best grouped together under *Radiospermum perpusillum*, Lesq. (Pl. VII, Fig. 31). In some cases these seeds occur in cupules.[2] Cupules are also known in the case of *R. Sinclairi* (Arber)[3] (Pl. VII, Figs. 33, 34), but in none of the other species at present. The *R. perpusillum* of Lesquereux may be identical with the earlier published *Carpolithes ellipticus* of Sternberg, but the figure given of the latter is so indefinite that this name is best ignored.

Two other species, *R. inflatum*, Lesq. (Text-fig. 8), *R. Kidstoni* (Arber)[4] (Pl. VII, Figs. 35, 36), have already been recorded, and four new species, *R. problematicum*, sp. nova (Pl. VII, Fig. 37), *R. grande*, sp. nova (Pl. VIII, Figs. 44, 45), *R. ornatum*, *R. elongatum*, are added here. *R. grande* approximates to the *Carpolithes insignis* of Karl Feistmantel,[5] a species which Dr. Kidston[6] has already doubtfully recorded from the Upper Coal Measures. The British examples are, however, much smaller, and, I think, specifically distinct. Another new seed, *R. ornatum* (Pl. VII, Figs. 38–41), is a very distinct type, triangular in section with twelve longitudinal ribs,

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[1] Cf. Oliver and Salisbury ('11), pp. 37–8.

[2] Carpentier ('11), Pl. XII.

[3] Arber ('05).

[4] Ibid.

[5] Feistmantel, K. ('81), p. 99, Pl. VII, Figs. 4, 5.

[6] Kidston ('94), p. 251.

and a peculiarly ornamented testa. The fourth new type, *R. elongatum* (Pl. VII, Figs. 42, 43), is cylindrical in form, with faintly marked, rather distant ribs.

I have also doubtfully referred to *Radiospermum* the *Carpolithes marginatus* of Artis (Pl. VIII, Fig. 46), a very obscure and little known type, the testa of which is quite destitute of any characters which can be cited as distinctive. There may be room for difference of opinion as to whether this seed was radiospermic or platyspermic. The few specimens which I have seen are all compressed, but I think it probable that this is due to pressure, and that the seed was originally radiospermic. The narrow marginal rim seen in some examples is probably also a pressure effect.

Neurospermum, gen. nova.

This type of seed, which is undoubtedly radiospermic, has in two cases been shown by Dr. Kidston[1] to belong to Medullosas with the *Neuropteris* type of foliage. The seed of *Neuropteris heterophylla*, which has not been named by Dr. Kidston, though it has been compared with certain species of *Rhabdocarpus*, a platyspermic seed, should, I think, be determined, for it more often occurs in the detached state than in continuity with *N. heterophylla*. As it is obviously impossible to apply the name *heterophylla* to it, I therefore propose to give it the name *Neurospermum Kidstoni* (Pl. VIII, Fig. 47) and to regard it as the type of a new genus, in which the testa of the oval or subcylindrical seed possesses close, prominent, parallel, longitudinal striae.

The seed of *Neuropteris obliqua*[2] has similar characters, but this seed has not yet been recorded in Britain.

Pterospermum, gen. nova.

There is a single specimen (V. 1183) in the Geological Department of the British Museum (Nat. Hist.) of a most remarkable seed (Pl. VIII, Figs. 51, 52), in an ironstone nodule from the Middle Coal Measures of Coseley, South Staffordshire, which is the only example known from this country. The ironstone nodule has been broken into three pieces, each corresponding to the spaces between the giant wings of this seed. The whole can thus be fitted together, and the general form of the seed studied. Although there is no cross-section of the whole nodule, I deduce that the nucellus was probably of very small transverse area, and triangular in form, each angle being produced into a large flap-like wing, of equal length with the rest of the seed. The nodule has broken naturally in planes parallel to each wing, the planes of least resistance, and I do not

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[1] Kidston ('04).

[2] Kidston and Jongmans ('11).

think that more than three wings occur, though this conclusion is at present provisional.

The only seed with which I can compare the British Museum specimen[1] is Grand' Eury's[2] *Carpolithes oblongus*, from the Stephanian of the Loire. Here, however, the seed is only half the size of the British example. I believe that both these seeds belong to the same genus, for it will be remembered that Grand' Eury also concluded that his seed had three wings. I think this type is certainly worthy of removal from the nondescript genus *Carpolithus*, and to be placed in a genus of its own, for which I propose the name *Pterospermum* ('winged seed'). As the British example appears to be of so very different dimensions to the French, I propose to distinguish the former as *Pterospermum anglicum*, sp. nova.

SYSTEMATIC LIST OF BRITISH COAL MEASURE SEED IMPRESSIONS.

GENUS TRIGONOCARPUS, Brongniart, 1828.

Pleomorphic impressions or casts of seeds, of medium or large size, elongately oval, usually longer than broad, unsymmetrically winged, the apex being produced into a micropyle. Testa differentiated into a soft outer sarcotesta and a hard inner sclerotesta. Inner surface of sclerotesta, with three well-marked grooves, appearing as three longitudinal ridges on the casts of this surface, more or less equidistant.

(1) *Trigonocarpus Parkinsoni*, Brongn.

T. Parkinsoni, forma α. Impressions of the external surface of the seed. Type *Carpolithes alata*, Lindley and Hutton, 'Fossil Flora,' Vol. ii, Pl. LXXXVII, Figs. 1–3, 1833. Recorded from the Transition, Middle, and Lower Coal Measures of many of the Midland, Pennine, North of England, and Scottish coalfields.

Seed elongate, oval, 3·5–5·5 cm. long, and 1·5–2 cm. across at greatest width. Micropyle about 1·5–2·5 cm. long, sarcotesta smooth, sclerotesta oval, about 1·8 cm. long and 1·2 cm. broad at its greatest width.

T. Parkinsoni, forma β (Pl. VI, Fig. 2). Impressions of the sclerotesta and micropyle. Type *Rhabdocarpus Bochschianus*, Berger, 'De Fruct. et Semin. Form. Lith.,' p. 21, Pl. I, Figs. 13, 14, 1848. Records as above. Sclerotesta oval, smooth, not ridged, 1·8 cm. long and 1·2 cm. at greatest width. Micropyle 1·5–2 cm. long.

T. Parkinsoni, forma γ (Pl. VI, Figs. 3–5). Casts of the inner surface of the sclerotesta. Type *Trigonocarpum Nöggerathi*, Lindley and Hutton, 'Foss. Flora,' Vol. ii, Pl. CXLII, Figs. C 1–3, 1833–35. Records as above.

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[1] This specimen has for many years past been labelled with a MS. name of Dr. Kidston's.

[2] Grand' Eury ('77), p. 187, Pl. XV, Fig. 9.

Cast ovoid, with three sharp, equidistant longitudinal ridges. Apex pointed, base often rounded, sometimes with a slight umbilicus, but no foramen. Length of cast from 1·8–2·8 cm., greatest breadth usually less than 1·5 cm.

(2) *Trigonocarpus Moyseyi*, sp. nova.

T. Moyseyi, forma α (Pl. I, Fig. 1). Impressions of the external surface of the seed. Type *Trigonocarpus*, sp., Arber, 'Fossil Plants,' Fig. on p. 42, 1909; Arber, Proc. Yorks. Geol. Soc., vol. xvii, pt. ii, p. 150 Pl. XVIII, Fig. 1, Pl. XIX, Fig. 3, 1910. Recorded from the Middle Coal Measures of the Notts. and Derby coalfield.

Seed broadly elliptical, 4·2 cm. long and 2·5 cm. across, broadly rounded at each end. Micropyle more than 1 cm. long, nucule ovate, 1·8 cm. long and 1·5 cm. broad at its greatest width, pointed at the apex, broadest at the base. Wing broad, especially at the apex, 2·3 cm. broad at the lower termination of the micropyle, ? irregularly plicated. Testa smooth.

T. Moyseyi, forma β } unknown.
T. Moyseyi, forma γ }

(3) *Trigonocarpus Dawesi*, Lindley and Hutton.

T. Dawesi, forma α } unknown.
T. Dawesi, forma β }

T. Dawesi, forma γ (Pl. VI, Fig. 8), Type, *Trigonocarpum Dawesii*, Lindley and Hutton, 'Fossil Flora,' vol. iii, Pl. CCXXI, Figs. 1 and 2, 1837. Recorded only from the Middle Coal Measures of South Lancashire. Cast similar in every respect to *T. Parkinsoni*, forma γ, except in dimensions. Cast about 4·5 cm. long and 2·5 cm. across.

(4) *Trigonocarpus clavatus* (Sternberg) (Text-fig. 1). Types, *Carpolites clavatus* and *C. lagenarius*, Sternberg, 'Vers. Darstell. Flora d. Vorwelt,' Heft 1, Pl. VII, Figs. 14 a, 14 b, 16, 1820; Heft 5–6, pp. xl–xli, 1833. Recorded from the Transition Coal Measures of the South Lancashire coalfield.

TEXT-FIG. 1. *Trigonocarpus clavatus* (Sternb.). After Sternberg.

Seed small, 2 cm. long and 1 cm. or more across, flask-shaped, nucule pestle-shaped, micropyle short, sarcotestal wing narrow, apex truncated, testa striated with fine, parallel striae.

GENUS PLATYSPERMUM, gen. nova.

Seeds of medium size, oval or elongately oval, not winged, testa ornamented with many ribs, approximated or not approximated at the base and apex, with or without fine parallel striations or ribs.

(1) *Platyspermum sulcatum* (Presl) (Pl. VI, Fig. 11).

Type *Carpolites sulcatus*, Presl in Sternberg, 'Vers. Darstell. Flora d. Vorwelt,' vol. ii, Heft 7, p. 208, Pl. X, Fig. 8, 1838. Recorded from the Transition Coal Measures of South Staffordshire, and from the Middle Coal Measures of Warwickshire and Yorkshire.

Seeds of medium size, oval or elliptical, broadly rounded at both extremities, about 2·5–3 cm. long, and 1·3–1·8 cm. across, with about nine, rather prominent and somewhat distant, longitudinal ribs on each side, approximated at base and apex, (?) sometimes confluent.

(2) *Platyspermum elongatum*, Kidston (Pl. VI, Fig. 12).

Type *Rhabdocarpus elongatus*, Kidston, 'Trans. Geol. Soc. Glasgow,' vol. viii, p. 70, Pl. III, Fig. 6, 1886. Recorded from the Middle and Lower Coal Measures of various coalfields in the Midlands and in Scotland.

Seeds small, very narrow, oblong, up to 2·3 cm. long, and 3·5–5 mm. wide, rounded at the apex, gradually contracted towards the base. The testa has four or more longitudinal ridges on each side.

(3) *Platyspermum Kidstoni*, sp. nova (Pl. VI, Fig. 9).

Type *Rhabdocarpus multistriatus*, Kidston, 'Trans. Roy. Soc. Edinb.,' vol. xxxiii, p. 404, Pl. XXIII, Fig. 4, 1888. Recorded from the Upper Coal Measures of Radstock.

Seed oval, 3·5 cm. long, and 2·1 cm. broad, with about eight longitudinal ridges on each side. Ridges approximated at the apex and base. Between the ridges numerous fine, parallel striae occur.

(4) *Platyspermum multistriatum* (Presl.) *non* Kidston (Pl. VI, Fig. 10).

Type *Carpolites multistriatus*, Presl. in Sternberg, 'Vers. Darstell. Flora d. Vorwelt,' vol. ii, Heft 7, p. 208, Pl. XXXIX, Figs. 1–2, 1838. From the Middle Coal Measures of North Staffordshire.

Seed ovate, very broad at one end, somewhat more pointed at the other, 3–4 cm. long, 2·2 cm. across at its broadest part. Ribs about nine on each surface, not approximated at apex nor base.

(5) *Platyspermum rugosum*, sp. nova (Pl. VI, Fig. 13).

Types Nos. 1655, 2337, Carbon. Plant Coll., Sedg. Mus., Camb. From the Upper Coal Measures of Radstock, Somerset, and the Middle Coal Measures of the Mattice Hill Boring, Kent, at 1,484 feet.

Seed elongately elliptical, bluntly pointed at both ends, up to 4 cm. or more in length, and about 2 cm. across at broadest part. Ribs broad, prominent, separated by wide, shallow grooves, three to five on each side of seed, ribs approximated at base and apex. Testa smooth or very finely striated.

GENUS CORNUCARPUS, gen. nova.

Seed small, not winged, triangular in shape, the apex of the triangle being directed downwards and ending in a short stalk, one side of the triangle forming the broad apex of the seed. Two well-marked horns project from the angles at the apex. A median ridge may occur.

Cornucarpus acutus (Lind. & Hutt.) (Pl. VI, Fig. 14 and Text-fig. 2).

Type *Cardiocarpus acutus*, Lindley and Hutton, 'Foss. Flora,' vol. i, p. 209, Pl. LXXVI, 1833. Recorded from the Transition Coal Measures of Kent, the Middle Coal Measures of Leicestershire, and the Lower Coal Measures of the Newcastle, Ayrshire, and Lanarkshire coalfields.

TEXT-FIG. 2. *Cornucarpus acutus* (L. & H.). × 2.

Seed triangular, about 6–10 mm. long, with a broad apex, and two well-marked horns at the angles of the apex. A median ridge extending from apex to base is prominent on most specimens. Testa smooth.

GENUS CARDIOCARPUS, Brongniart, 1828.

Seeds small, heart-shaped or reniform, rounded or emarginate at base, more or less acute at apex, not winged, with or without a narrow, marginal rim.

(1) *Cardiocarpus congruens*, Grand' Eury (Text-fig. 3).

TEXT-FIG. 3. *Cardiocarpus congruens*, G.' E. After Grand' Eury.

Type *Cordaicarpus congruens*, Grand' Eury, 'Flore Carbon. Loire,' p. 236, Pl. XXVI, Fig. 21, 1877. Recorded from the Middle Coal Measures of Warwickshire.

Seed small, 6–10 mm. long, apex obtuse. Testa smooth.

(2) *Cardiocarpus Gutbieri*, Gein. (Pl. VI, Fig. 15).

Type *Cardiocarpon Gutbieri*, Geinitz, 'Vers. Steinkohlenf. Sachs.,' p. 39, Pl. XXI, Figs. 23–25, 1855. Recorded from the Upper Coal Measures of Radstock, and the Middle Coal Measures of Yorkshire and North Staffordshire.

Seed of medium size, as broad as long or longer than broad, from 1–2 cm. across, heart-shaped, bluntly pointed at the apex, with or without a very narrow, marginal rim.

GENUS SAMAROPSIS, Goeppert, 1864.

Platyspermic seeds, small or of medium size, more or less circular, not longer than broad, with a sclerotesta, and a well-developed sarcotesta forming a well-marked wing. Sclerotesta usually heart-shaped or ovate. Sarcotesta subcircular or heart-shaped, symmetrical or asymmetrical around the sclerotesta. Micropyle usually fairly long.

(1) *Samaropsis crassa* (Lesq.) (Text-fig. 4).

TEXT-FIG. 4. *Samaropsis crassus* (Lesq.). After Lesquereux.

Type *Cardiocarpus crassus*, Lesquereux, 'Coal Flora Pennsylv.,' vol. iii, p. P. 812, Pl. CIX, Fig. 12, Pl. CX, Figs. 6–9, 1884. Recorded from the Middle Coal Measures of the Leicestershire, and the Lower Coal Measures of the Lanarkshire coalfields.

Seeds small, about 1 cm. long, oval or subcircular. Nucule large, ovate. Wing narrow, nearly equally symmetrical all round the nucule. Micropylar region pointed.

(2) *Samaropsis emarginata*, Berger (Text-fig. 5).

Type *Cardiocarpon emarginatum*, Berger, 'De Fruct. et Semin.,' p. 24, Pl. III, Fig. 35, 1848. Recorded from the Lower Coal Measures of Ayrshire.

TEXT-FIG. 5. *Samaropsis emarginatus* (Berg.).

Seeds of fair size, circular, 1·3–2 cm. in diameter. Nucule large, cordate, pointed above. Wing very broad, but narrower at the base than elsewhere. Micropylar region obtuse.

(3) *Samaropsis fluitans* (Daws.) (Pl. VI, Fig. 18).

Type *Cardiocarpum fluitans*, Dawson, 'Quart. Journ. Geol. Soc.,' vol. xxii, p. 165, Pl. XII, Fig. 74, 1866. Recorded from the Upper, Transition, and Middle Coal Measures of the Southern, Midland, and Pennine coalfields.

Seeds oval, 8–15 mm. long, nucule ovate, 4–5 mm. long and 4–6 mm. across. Wing often narrow at the base, produced above the nucule for 2–4 mm. around the micropyle. Micropylar region acute.

(4) *Samaropsis Meachemi* (Kidst.) (Pl. VI, Figs. 16 and 17).

Type *Cardiocarpus Meachemii*, Kidston, 'Trans. Roy. Soc. Edinburgh,' vol. xxxvi, Pl. II, p. 330, Pl. — , Figs. 5–7, 1891. Recorded from the Middle Coal Measures of the Kent and South Staffordshire coalfields.

Seed cordate, about 13 mm. long and broad, base emarginate, apex acute. Nucule cordate, central, with a median ridge extending from apex to centre of base. Wing about 4 mm. broad, and of nearly equal breadth round the nucule. Micropylar region very rounded.

(5) *Samaropsis subacuta*, Grand' Eury (Text-fig. 6).

Type *Samaropsis subacuta*, Grand' Eury, 'Flore Carbon. Loire,' p. 281, Pl. XXXIII, Fig. 5, 1877. Recorded from the Middle Coal Measures of Yorkshire.

TEXT-FIG. 6. *Samaropsis subacuta*, G.' E. After Grand' Eury.

Seed small, ovate or oblong, 1 cm. long, 7 mm. across. Nucule ovate, keeled, large, apex acute. Wing small, very narrow or absent at the base, broadest at the apex.

GENUS SAMAROSPERMUM, gen. nova.

Winged seeds very elongate and narrow, usually rounded at both ends, and narrowly elliptical in form. Nucule small, elliptical, situated midway between the apex and the base. Sarcotesta with numerous, fine, parallel, longitudinal striae.

Samarospermum moravicum (Helmhacker) (Pl. VI, Figs. 19 and 20).

Type *Jordania moravica*, E. Geinitz, 'Neues Jahrb. Mineral.,' year 1875, p. 11, Pl. I, Figs. 10, 11; *Samaropsis moravica*, Zeiller, 'Flore Foss. Bass. Houill. et Perm. Brive,' p. 95, Pl. XV, Figs. 9, 10. Recorded from the Middle Coal Measures of the Kent coalfield.

Seed narrowly elliptical, from 2–3 cm. long and 5–8 mm. broad, broadly round at both ends. Nucule central, small, about 6 mm. long and 1·5–3 mm. across, elliptical. Sarcotesta with numerous, fine, longitudinal striations.

GENUS RHABDOCARPUS, Berger, 1848.

Seeds large, oval, only symmetrical in one plane, winged, more or less pointed at one extremity, rounded at the other. Nucule large, unsymmetrically oval or semicircular. Wing broad, unsymmetrical. Testa with close, thread-like, longitudinal striae.

Rhabdocarpus Lillieanus, sp. nova (Pl. VII, Fig. 21).

Type No. 2034, Carbon. Plant Coll., Sedgwick Mus., Camb. (Lillie Collection). Upper Coal Measures (Farrington Series), Coal Pit Heath Colliery, Bristol coalfield.

Seed broadly oval, exceeding 4 cm. in length and 3 cm. in width at its greatest breadth, somewhat contracted towards the bluntly pointed apex. Base unknown. Central portion of seed rugose, with irregular wrinkles or ridges, transverse or oblique, and a faint outline of large nucule. Testa smooth, striae fine and thread-like.

GENUS MICROSPERMUM, gen. nova.

Seed small, symmetrical only in a single plane, with a large foramen. Seed flattened, the two sides being dissimilar.

Microspermum samaroides (Carpent.) (Pl. VII, Figs. 22–27).

Type *Carpolithes ? samaroides*, Carpentier, ' Comp. Rend. Assoc. franç. Avanc. Scienc.,' Lille, 1909 ; Notes et Mém., p. 599, 1910 ; and ' Rev. Gén. Bot.,' vol. xxiii, p. 7, Pl. XIV, Fig. 3, 1911. From the Lower Coal Measures of Trowell Colliery (Horizon of the Kilburn Coal), Nottingham.

Seed small, ovate or pyriform in shape, 5–12 mm. long and 2·5–6 mm. across at its greatest width. One extremity broadly rounded, the other acute. Seed flattened, unsymmetrical ; one side with a strong keel extending from the pointed end nearly to the opposite extremity ; the other side more or less convex, with a slight median groove, on either side of which are several faint longitudinal striations, converging towards the pointed end. A large foramen occurs at the broader end on the grooved surface, and more rarely a small foramen is also seen at the pointed extremity.

GENUS CORDAICARPUS, Geinitz, 1862.

Seeds rather small, oval or orbicular. Testa smooth, not ribbed or striated longitudinally.

(1) *Cordaicarpus areolatus* (Boul.) (Text-fig. 7).

Type *Cardiocarpus* (*Cordiacarpus*) *areolatus*, Boulay, ' Rech. Pal. Végét. Terr. Houill. Nord France,' p. 34. Recorded from the Middle Coal Measures of Warwickshire.

TEXT-FIG. 7. *Cordaicarpus areolatus* (Boul.). × 2.

Seed small, orbicular, 4–6 mm. in diameter, the testa ornamented with a network of large, polygonal meshes, not very prominent.

(2) *Cordaicarpus Cordai*, H. B. Geinitz (Pl. VII, Fig. 29).

Type, *Carpolithus Cordai*, Geinitz, ' Vers. Steinkohlenf. Sachsen,' p. 41, Pl. XXI, Figs. 7–16. Recorded from the Middle Coal Measures of several coalfields in England.

Seed of fair size, orbicular, 8–20 mm. in diameter. Testa smooth.

(3) *Cordaicarpus ovoideus* (Berg.) (Pl. VII, Fig. 30).

Type *Rhabdocarpos ovoideus*, Berger, ' De Fruct. et Semin.,' p. 22, Pl. I, Fig. 17. Recorded from the Upper Coal Measures of Radstock, and the Middle Coal Measures of South Staffordshire and Yorkshire.

Seed small, oval or elliptical or almost orbicular, between 6–10 mm. along the greatest diameter, both extremities rounded or more or less pointed.

GENUS MEGALOSPERMUM, gen. nova.

Seeds very large, 6–10 cm. long, subcircular or elliptical, blunt at both ends, without longitudinal ridges. Testa ornamented by a series of close, fine, thread-like striae.

Megalospermum Wildi (Kidst.) (Pl. VII, Fig. 28).

Type *Carpolithus Wildii*, Kidston, 'Trans. Manchester Geol. Soc.,' vol. xxi, p. 408, Fig. on p. 408. Recorded from the Middle Coal Measures of the South Lancashire coalfield.

Seed oblong, 6 cm. long and 3 cm. broad, rounded at the apex, very slightly narrowed at the base, stalked.

GENUS RADIOSPERMUM, gen. nova.

Seeds cylindrical or ellipsoidal in form, longer than broad, not winged, apex with or without notches or lobes. Testa smooth, with or without a few distant longitudinal ridges but not striated longitudinally. Seeds with or without a cupule.

(1) *Radiospermum elongatum*, sp. nova (Pl. VII, Figs. 42–43).

Types Nos. 2177 and 2990, Carbon. Plant Coll., Sedgwick Museum, Camb. From the Upper Coal Measures of Radstock and Bishop Sutton, Somersetshire.

Seed oval or elongately elliptical, 3·5–4·5 cm. long and 1·3–1·5 cm. across, slightly rounded at both ends. Testa smooth, with very slight, distant longitudinal ribs, about 2 mm. apart at a point midway between base and apex.

(2) *Radiospermum grande*, sp. nova (Pl. VIII, Figs. 44, 45).

Type No. 1668, Carbon. Plant Coll., Sedgwick Museum, Camb. Known from the Upper Coal Measures of the Radstock and Forest of Dean coalfields, and the Middle Coal Measures of South Lancashire (No. 52584 in the Brit. Mus. Nat. Hist.).

Seed large, cylindrical, broadly rounded at each end, 3–5 cm. long, 2·5–3·5 cm. broad. Testa smooth, with three longitudinal, rounded ribs, and short, discontinuous, microscopic grooves.

(3) *Radiospermum inflatum* (Lesq.) (Text-fig. 8).

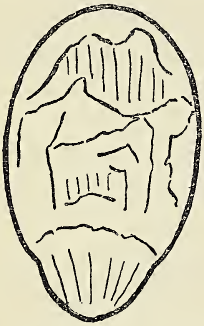
Type *Rhabdocarpus inflatus*, Lesquereux, 'Coal Flora Pennsylv.,' vol. iii, p. P. 815, Pl. CX, Fig. 36, 1884. Recorded from the Middle Coal Measures of Yorkshire and South Lancashire.

Seed large, up to 4 cm. long, ovoid, broadly rounded at one end and obtusely pointed at the other. Testa smooth, wrinkled, without ribs or striae.

(4) *Radiospermum Kidstoni* (Arber) (Plate II, Figs. 35, 36).

Type *Lagenostoma Kidstoni*, Arber, 'Proc. Roy. Soc. London,' vol. B. 76, p. 247, Pl. I, Figs. 1–4; Pl. II, Figs. 1–6. Recorded from the Lower Coal Measures of the Lanarkshire coalfield.

Seeds small, elliptical, 6 mm. long, 2·5–3 mm. at greatest breadth. Testa smooth, slightly ridged longitudinally. Apex with several, up to six, well-marked lobes. No cupule.



TEXT-FIG. 8. *Radiospermum inflatum* (Lesq.). After Lesquereux.

(5) ? *Radiospermum marginatum* (Art.) (Pl. VIII, Fig. 46).

Type *Carpolithus marginatus*, Artis, 'Antedil. Phytol.,' Pl. XXII, B & C, 1825. Recorded from the Middle Coal Measures of the Wyre Forest and Yorkshire coalfields.

Seed fairly large, ? radiospermic, subcircular, 2–2·5 cm. across, with a very narrow marginal rim. Testa smooth, unornamented.

(6) *Radiospermum ornatum*, sp. nova (Pl. VII, Figs. 38–41).

Types No. 1890, 1894, and 2111, Carbon. Plant Coll., Sedgwick Museum, Camb. From the Middle Coal Measures, above the Top Hard Coal, Shipley Clay Pits, Derbyshire.

Seed rather small, elongately triangular, tapered at both ends, rounded at one extremity, rather pointed at the other, 12–15 mm. long, each side of triangle about 5–6 mm. broad. Testa with twelve longitudinal ridges, three main ridges at the angles, and three minor, slightly less prominent ridges between each pair of major ridges. All ridges approximated at both extremities. Testa ornamented with conspicuous, approximated, transverse striations.

(7) *Radiospermum ovatum* (L. and H.) (Pl. VII, Fig. 32).

Type *Trigonocarpum ovatum*, Lindley and Hutton, 'Foss. Flora,' vol. ii, Pl. CXLII A. Recorded from the Middle Coal Measures of Coalbrookdale and Yorkshire.

Casts elongately ovoid, 4·5–6 cm. long and up to 3·5 cm. broad, rounded at one end, and slightly narrowed at the other, at which there is a foramen through which an inner cast may or may not project. Testa smooth, with three equidistant, blunt, longitudinal ridges.

(8) *Radiospermum perpusillum* (Lesquereux) (Pl. VII, Fig. 31).

Type *Carpolithes perpusillus*, Lesquereux, 'Coal Flora Pennsylv.,' vol. iii, p. P. 825, Pl. CXI, Figs. 22–24. Recorded from the Middle Coal Measures of the Midland, Pennine, and Welsh Borderland coalfields, and from the Lower Coal Measures of the Scottish coalfields.

Seeds minute, cylindrical, about 3 mm. long. Testa smooth, with at least one fairly prominent ridge, slightly tapered at one extremity, rounded at the other.

(9) *Radiospermum problematicum*, sp. nova (Pl. VII, Fig. 37).

Type No. 2669, Carbon. Plant Coll., Sedgwick Museum, Camb. From the Middle Coal Measures of the Wyre Forest coalfield.

Seed ovoid, about 4 cm. long and 2 cm. across at its greatest breadth, broadly pointed at one end and rounded at the other, at which it splits up into several valves. Testa smooth, with very fine and close discontinuous striae, and more than five (? ten) not very prominent, and somewhat distant longitudinal ridges.

(10) *Radiospermum Sinclairi* (Arber) (Pl. VII, Figs. 33–34).

Type *Lagenostoma Sinclairi*, Arber, 'Proc. Roy. Soc. London,' vol. B. 76, p. 251, Pl. II, Figs. 7–11. Recorded from the Lower Coal Measures of the Ayrshire coalfield.

Seed small, elliptical-oblong in shape, 4–5·5 mm. long and 1·5–3 mm. broad. Testa smooth, slightly ridged longitudinally. Apex notched or fluted. Cupule 8–9·5 mm. long, sac-like in form, prominently ridged longitudinally, and divided at the apex into a number of lanceolate, erect lobes.

GENUS NEUROSPERMUM, gen. nova.

Seeds large, oval or subcylindrical, not winged. Testa with numerous fine, parallel, longitudinal striations.

Neurospermum Kidstoni, sp. nova (Pl. VIII, Fig. 47).

Type, cf. *Rhabdocarpus*, sp. Kidston, 'Phil. Trans. Roy. Soc.,' Ser. B, vol. cxcvii, p. 1, Pl. I, 1904. Recorded from the Middle Coal Measures of the South Staffordshire coalfield.

Seed cylindrical, about 2·8 cm. long and 1–1·4 cm. across, contracted towards the apex into a somewhat sharp point.

GENUS SCHIZOSPERMUM, gen. nova.

Seeds of medium size, ovately triangular, splitting at the apex into three valves along the angles of the triangle, each valve having usually a median longitudinal ridge. At the base a foramen occurs, through which an inner cast is seen.

Schizospermum Noeggerathi (Sternb.) (Pl. VIII, Figs. 48–50).

Type *Palmacites Noeggerathi*, Sternberg, 'Vers. Darstell. Flora Vorwelt,' Heft 4, p. xxxv, Pl. LV, Figs. 6, 7, 1826. Recorded from the Upper, Transition, and Middle Coal Measures of various coalfields in the South of England, South Wales, and the Pennine fields.

Seeds short, ovoidly triangular, 2·5–4 cm. long, 1·2–3 cm. broad, rounded at the base, obtusely pointed at the apex, splitting into three valves along the angles of the triangle, each valve having a less well-marked median ridge. Foramen at base large.

Genus Pterospermum, gen. nov.

Seeds large, elongate, angular in section, the angles being produced into flap-like, broad wings, nearly as long as the main portion of the seed.

Pterospermum anglicum, sp. nova (Pl. VIII, Figs. 51–52).

Type V. 1183, Geol. Dept., British Museum (Nat. Hist.), from the Middle Coal Measures of South Staffordshire. The only known example.

Seed large, (?) triangular in section, which is of small area, elongate, 5·5–6 cm. long and 2·3–2·6 cm. across between the wings. Three (? more) large, flap-like wings arise one from each angle, 5 cm. long and 2 cm. or more in breadth. Testa smooth.

BIBLIOGRAPHY.

Arber, E. A. N. ('05): On some New Species of *Lagenostoma*, a type of Pteridospermous Seed from the Coal Measures. Proc. Roy. Soc., Ser. B, vol. lxxvi, 1905, p. 245.

——— ('09): Fossil Plants. Gowan and Gray's Nature Series, No. 21, Glasgow, 1909.

——— ('10): On the Fossil Flora of the Southern Portion of the Yorkshire Coalfield in North Derbyshire and Nottinghamshire. Proc. York. Geol. Soc., vol. xvii, pt. ii, 1910, p. 132.

Berger, R. ('48): De Fructibus et Seminibus ex Formatione Lithanthracum. Vratislaviae, 1848.

Bertrand, C. E. ('11): Le bourgeon femelle des Cordaïtes d'après les préparations de Bernard Renault. Bull. soc. sci., Nancy, 1911.

Brongniart, A. ('28): Prodrome d'une histoire des végétaux fossiles, 1828.

Carpentier, A. ('10): Note sur quelques végétaux fossiles du bassin houiller du Nord. Compt. Rend. Assoc. franc. Avanc. Sci., Lille, 1909; Notes et Mém., 1910, p. 599.

——— ('11): Sur quelques fructifications et inflorescences du Westphalien du Nord de la France. Rev. génér. bot., vol. xxiii, 1911, p. 1.

Feistmantel, K. ('81): Der Hangendflötzzug im Schlan-Rakonitzer Steinkohlenbecken. Arch. naturwiss. Landesdurchf. Böhm., Prag, vol. iv, 1881, No. 6, Geolog. Teil, p. 1.

Fiedler, H. ('57): Die fossilen Früchte der Steinkohlen-Formation. Verhandl. K. Leop.-Carol. Akad. Naturf., vol. xxvi, 1857, p. 241.

Geinitz, E. ('75): Über neue Aufschlüsse im Brandschiefer der unteren Dyas von Weissig, bei Pillnitz in Sachsen. Neues Jahrb. Mineral., 1875, p. 1.

Geinitz, H. B. ('61): Dyas oder die Zechsteinformation und das Rothliegende. Leipzig, 1861–2.

Grand' Eury, C. ('77): Flore carbonifère du département de la Loire et du Centre de la France. Mém. sav. étrang. Acad. Sci., vol. xxiv, 1877, No. 1.

Helmhacker, R. ('71): Über einige neue Vorkommnisse von böhmischen Mineralien und Petrefakten. Sitzungsb. K. Böhm. Gesellsch. Wissensch., Prag, 1871, p. 77.

Kidston, R. ('88): On the Fossil Flora of the Radstock Series of the Somerset and Bristol Coalfield (Upper Coal Measures). Trans. Roy. Soc., Edinb., vol. xxxiii, 1888, part II, p. 335.

——— ('89:) On the Fossil Plants in the Ravenhead Collection in the Free Library and Museum, Liverpool. Trans. Roy. Soc., Edinb., vol. xxxv, 1889, pt. II, p. 391.

——— ('94): On the Various Divisions of British Carboniferous Rocks as determined by their Fossil Flora. Proc. Roy. Phys. Soc., Edinb., vol. xii, 1894, p. 183.

——— ('04): On the Fructification of *Neuropteris heterophylla*, Brongniart. Phil. Trans. Roy. Soc., Ser. B, vol. cxcvii, 1904, p. 1.

Kidston, R., and Jongmans, W. J. ('11): Sur la fructification de *Neuropteris obliqua*, Bgt. Arch. Néerland. sci. exact. et nat., 3^{e} sér., B., vol. i, 1911, p. 25.

LINDLEY, J., and HUTTON, W. ('31) : The Fossil Flora of Great Britain. Vols. i–iii, London, 1831–7.

MORRIS, J. ('40) : In Prestwich, On the Geology of the Coalfield of Coalbrook Dale. Trans. Geol. Soc., Ser. 2, vol. v, 1840, p. 488.

OLIVER, F. W. ('03): The Ovules of the Older Gymnosperms. Ann. Bot., vol. xvii, 1903, p. 451.

OLIVER, F. W., and SALISBURY, E. J. ('11): On the Structure and Affinities of the Palaeozoic Seeds of the *Conostoma* Group. Ann. Bot., vol. xxv, 1911, p. 1.

RENAULT, B. ('90) : Études sur le terrain houiller de Commentry. Livr. ii, Flore Fossile, 2^e^ partie, Saint-Étienne, 1890.

SCOTT, D. H., and MASLEN, A. J. ('07) : The Structure of the Palaeozoic Seeds, *Trigonocarpus Parkinsoni*, Brongniart, and *Trigonocarpus Oliveri*, sp. nova. Part I. Ann. Bot., vol. xxi, 1907, p. 89.

STERNBERG, K. ('20) : Versuch einer geognostisch-botanischen Darstellung der Flora der Vorwelt. Leipzig und Prag, 1820–38.

WEISS, C. E. ('79) : Beiträge zur fossilen Flora. II. Die Flora des Rothliegenden von Wünschendorf bei Lauban in Schlesien. Abhandl. Geol. Specialk. Preuss., Thür. Staat., vol. iii, pt. i, 1879.

WOODWARD, J. (1729) : An Attempt towards a Natural History of the Fossils of England, &c. Tome II : A Catalogue of the Additional English Native Fossils, &c. London, 1729.

ZEILLER, R. ('86): Flore fossile du bassin houiller de Valenciennes. (Étud. Gîtes Minér.) Paris, 1886–8.

———— ('92): Flore fossile du bassin houiller et permien du Brive. (Étud. Gîtes Minér.) Paris, 1892.

———— ('00) : Éléments de paléobotanique. Paris, 1900.

EXPLANATION OF THE PLATES

Illustrating Dr. Newell Arber's Revision of the Seed Impressions of the British Coal Measures.

(The photographs are partly by the author, partly by Mr. Tams, Cambridge.)

PLATE VI.

Fig. 1. *Trigonocarpus Moyseyi*, sp. nova, forma α. An almost complete impression of the external surface of the seed. From the Middle Coal Measures of Shipley Clay Pit, Derby. The type specimen in Dr. Moysey's collection. × $\frac{2}{1}$.

Fig. 2. *Trigonocarpus Parkinsoni*, Brongn., forma β. Impression of the sclerotesta and micropyle. From the Middle Coal Measures of Yorkshire. Sedgwick Museum, Cambridge (No. 1184, Carbon. Plant Coll.). Nat. size.

Fig. 3. *Trigonocarpus Parkinsoni*, Brongniart, forma γ. Tri-ridged casts of the inner surface of the sclerotesta. From the Middle Coal Measures of Peel Quarry, Bolton, Lanc. Sedgwick Museum, Cambridge (No. 485, Carbon. Plant Coll.). Nat. size.

Fig. 4. *Trigonocarpus Parkinsoni*, Brongniart, forma γ. The bases of two specimens seen from above. That of the lower specimen is smooth, that of the upper shows an oval umbilicus. From the Middle Coal Measures of Peel Quarry, Bolton. Sedgwick Museum, Cambridge (No. 485, Carbon. Plant Coll.). Nat. size.

Fig. 5. *Trigonocarpus Parkinsoni*, Brongniart, forma γ. The bases of two further specimens, both showing an umbilicus. From the Middle Coal Measures of Peel Quarry, Bolton. Sedgwick Museum, Cambridge (No. 485, Carbon. Plant Coll.). Nat. size.

Fig. 6. *Trigonocarpus Parkinsoni*, Brongn. A petrified example described by Mrs. E. A. N. Arber on p. 195. Median longitudinal section through the seed, showing the micropylar beak of the sclerotesta with great completeness. Binney Coll., Sedgwick Museum, Cambridge, Slide A.B. 11. × 2·5 circa.

Fig. 7. *Trigonocarpus Parkinsoni*, Brongn. A petrified example described by Mrs. E. A. N.

Arber on p. 195. Part of another longitudinal section through the same seed as that represented in the last figure, showing the connexion between the nucellar wall (to the right) and the seed coat (occupying the whole of the lower left-hand portion of the figure). The nucellar tracheides are visible in the lower half of the figure at the extreme right. Binney Coll., Sedgwick Museum, Cambridge, A.B. 12. × 127 circa.

Fig. 8. *Trigonocarpus Dawesi*, L. and H., forma γ. A tri-ridged cast of the inner surface of the sclerotesta. From the Middle Coal Measures of Peel Quarry, Bolton, S. Lanc. Sedgwick Museum, Cambridge (No. 490, Carbon. Plant Coll.). Nat. size.

Fig. 9. *Platyspermum Kidstoni*, sp. nova. From the Upper Coal Measures of Radstock, Som. The type, after Kidston. Nat. size.

Fig. 10. *Platyspermum multistriatum* (Sternb.). From the Transition Coal Measures of Longport, North Staffordshire. In the Sedgwick Museum, Cambridge (No. 2176, Carbon. Plant Coll.). Nat. size.

Fig. 11. *Platyspermum sulcatum* (Presl.). From the Middle Coal Measures of Ravenshore, S. Lanc. In the Sedgwick Museum, Cambridge (No. 2991, Carbon. Plant Coll.). Nat. size.

Fig. 12. *Platyspermum elongatum* (Kidst.). From the Lower Coal Measures of Trowell Colliery, Nottingham. From a specimen in Dr. Moysey's collection. × $\frac{6}{5}$.

Fig. 13. *Platyspermum rugosum*, sp. nova. From the Middle Coal Measures of the Mattice Hill Boring, Kent, at a depth of 1,484 ft. Type in the Sedgwick Museum, Cambridge (No. 2337, Carbon. Plant Coll.). Nat. size.

Fig. 14. *Cornucarpus acutus* (L. and H.). From the Middle Coal Measures of the Goodnestone Boring, Kent, at a depth of 2,000 ft. In the Sedgwick Museum, Cambridge (No. 2230, Carbon. Plant Coll.). Nat. size.

Fig. 15. *Cardiocarpus Gutbieri*, Gein. From the Middle Coal Measures of the Mattice Hill Boring, Kent, at a depth of 1,111 feet. Sedgwick Museum, Cambridge (No. 2375, Carbon. Plant Coll.). Nat. size.

Fig. 16. *Samaropsis Meachemi* (Kidst.). From the Middle Coal Measures of the Woodnesborough Boring, Kent, at a depth of 1,651 ft. Sedgwick Museum, Cambridge (No. 2461, Carbon. Plant Coll.). × 3.

Fig. 17. *Samaropsis Meachemi* (Kidst.). From the Middle Coal Measures of the Woodnesborough Boring, Kent, at a depth of 1,176 ft. Sedgwick Museum, Cambridge (No. 2465, Carbon. Plant Coll.). × $\frac{5}{3}$.

Fig. 18. *Samaropsis fluitans* (Daws.). From the Upper Coal Measures of Camerton, Somerset. Sedgwick Museum, Cambridge (No. 1652, Carbon. Plant Coll.). Nat. size.

Fig. 19. *Samarospermum moravicum* (Helmh.). From the Middle Coal Measures of the Oxney Boring, Kent, at a depth of 2,439 ft. In the Sedgwick Museum, Cambridge (No. 2992, Carbon. Plant Coll.). Nat. size.

Fig. 20. *Samarospermum moravicum* (Helmh.) Enlarged view of the reverse of Fig. 19. × 3.

PLATE VII.

Fig. 21. *Rhabdocarpus Lillieanus*, sp. nova. From the Upper Coal Measures (Farrington Series) of Coal Pit Heath Colliery, Bristol coalfield. Type in the Sedgwick Museum, Cambridge (No. 2034, Carbon. Plant Coll.). Nat. size.

Fig. 22. *Microspermum samaroides* (Carpent.). A specimen showing the grooved surface and the large foramen at the lower end. From the Middle Coal Measures of Shipley Clay Pit, Derby. Type in Dr. Moysey's collection. Nat. size.

Fig. 23. *Microspermum samaroides* (Carpent.). The same specimen as Fig. 22. × $\frac{5}{2}$.

Fig. 24. *Microspermum samaroides* (Carpent.). A specimen showing the keeled surface. From the same locality and in the same collection. × 2.

Fig. 25. *Microspermum samaroides* (Carpent.). A specimen of (?) the keeled surface showing a large 'dimple' below, and some indication of (?) a small foramen at the pointed end. From the same locality and in the same collection. × 2.

Fig. 26. *Microspermum samaroides* (Carpent.). A specimen showing a foramen at the pointed end, but not very like the other specimens of this species here figured, though probably only another form of the same seed. From the same locality and in the same collection. × 2.

Fig. 27. *Microspermum samaroides* (Carpent.). A specimen showing the grooved surface with

the foramen at the broader end, similar to Figs. 22 and 23. From the same locality and in the same collection. × 2.

Fig. 28. *Megalospermum Wildi* (Kidst.). From the Middle Coal Measures of Bardsley, South Lancashire. The counterpart of the type in the Manchester Museum. Nat. size.

Fig. 29. *Cordaicarpus Cordai*, Gein. Several seeds from the Middle Coal Measures of Kinlet Colliery, Wyre Forest, Shropshire. Sedgwick Museum, Cambridge (No. 2676, Carbon. Plant Coll.). Nat. size.

Fig. 30. *Cordaicarpus ovoideus*, Berg. Several seeds from the Upper Coal Measures of Radstock, Somerset. Sedgwick Museum, Cambridge (No. 1228, Carbon. Plant Coll.). × $\frac{9}{7}$.

Fig. 31. *Radiospermum perpusillum* (Lesq.). From the Middle Coal Measures of Billingsley Colliery, Wyre Forest, Shropshire. Sedgwick Museum, Cambridge (No. 2708, Carbon. Plant Coll.). × 3.

Fig. 32. *Radiospermum ovatum* (L. and H.). From the Middle Coal Measures of Barnsley, Yorkshire. In the British Museum Collection (V. 11525). Nat. size.

Fig. 33. *Radiospermum Sinclairi* (Arber). Two seeds enclosed in their cupules and attached to a branched axis. From the Lower Coal Measures of Grange Colliery, Kilmarnock, Ayrshire. In Dr. Kidston's collection. × 3.

Fig. 34. *Radiospermum Sinclairi* (Arber). A drawing of a seed. From the same locality, and in the same collection. × 5.

Fig. 35. *Radiospermum Kidstoni* (Arber). Four seeds attached to an axis. From the Lower Coal Measures of Swinhill Colliery, Stonehouse, Lanarkshire. In the British Museum Collection (V. 6241). × 2.

Fig. 36. *Radiospermum Kidstoni* (Arber). A drawing of a single seed, from the same locality, and in the same collection. × 5.

Fig. 37. *Radiospermum problematicum*, sp. nova. The type from the Middle Coal Measures of Kinlet Colliery, Wyre Forest, Shropshire. Type specimen in Sedgwick Museum, Cambridge (No. 2669, Carbon. Plant Coll.). × 2.

Fig. 38. *Radiospermum ornatum*, sp. nova. A specimen showing the longitudinal ridges, from the Middle Coal Measures of Shipley Clay Pit, Derbyshire. Type specimen in the Sedgwick Museum, Cambridge (No. 2111, Carbon. Plant Coll.). × 3.

Fig. 39. *Radiospermum ornatum*, sp. nova. A specimen showing the longitudinal ridges and the transverse striations. From the same locality. (Type No. 1890 in the same collection.) × 2.

Fig. 40. *Radiospermum ornatum*, sp. nova. The same specimen as Fig. 39 (No. 1890). Nat. size.

Fig. 41. *Radiospermum ornatum*, sp. nova. The same specimen as Fig. 38 (No. 2111). Nat. size.

Fig. 42. *Radiospermum elongatum*, sp. nova. From the Upper Coal Measures of Bishop Sutton Colliery, Somerset. Type specimen in the Sedgwick Museum, Cambridge (No. 2990, Carbon. Plant Coll.). Nat. size.

Fig. 43. *Radiospermum elongatum*, sp. nova. From the Upper Coal Measures of Radstock, Somerset. Type specimen in the Sedgwick Museum, Cambridge (No. 2177, Carbon. Plant Coll.). × $\frac{5}{3}$.

PLATE VIII

Fig. 44. *Radiospermum grande*, sp. nova. From the Middle Coal Measures of Wigan, South Lancashire. Type specimen in the British Museum Collection, No. 52584. Nat. size.

Fig. 45. *Radiospermum grande*, sp. nova. From the Upper Coal Measures of Trafalgar Colliery, Forest of Dean, Gloucestershire. Type specimen in the Sedgwick Museum, Cambridge (No. 1668, Carbon. Plant Coll.). Nat. size.

Fig. 46. ? *Radiospermum marginatum* (Art.). From the Middle Coal Measures of the Alton Boring, Dowles Valley, Wyre Forest, Worcestershire. Sedgwick Museum, Cambridge (No. 2594, Carbon. Plant Coll.). Very slightly enlarged.

Fig. 47. *Neurospermum Kidstoni*, sp. nova. From the Middle Coal Measures of Clays Croft, Coseley, S. Staffordshire. Type specimen in the Sedgwick Museum, Cambridge (No. 2986, Carbon. Plant Coll.). Nat. size.

Fig. 48. *Schizospermum Noeggerathi* (Sternb.). Side view of the seed. From the Upper Coal

Measures (Farrington Series) of Coal Pit Heath Colliery, near Bristol. Sedgwick Museum, Cambridge (No. 2032, Carbon. Plant Coll.). Nat. size.

Fig. 49. *Schizospermum Noeggerathi* (Sternb.). View of the apex of the seed showing the three valves. From the Middle Coal Measures of Darfield Quarry, Barnsley, Yorks. Sedgwick Museum, Cambridge (No. 729, Carbon. Plant Coll.). Nat. size.

Fig. 50. *Schizospermum Noeggerathi* (Sternb.). View of the base of the seed showing the foramen and inner cast. From the (?) Middle Coal Measures. Sedgwick Museum, Cambridge (No. 487, Carbon. Plant Coll.). Nat. size.

Fig. 51. *Pterospermum anglicum*, sp. nova. Part of a specimen showing two of the three wings. From the Middle Coal Measures of Clays Croft, Coseley, S. Staffordshire. Type specimen in the British Museum (Nat. Hist.), No. V. 1183. Nat. size.

Fig. 52. *Pterospermum anglicum*, sp. nova. Part of the same specimen as Fig. 51, showing one of the wings seen in that figure and a third wing. From the same locality and collection. Nat. size.

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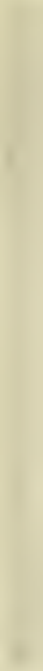

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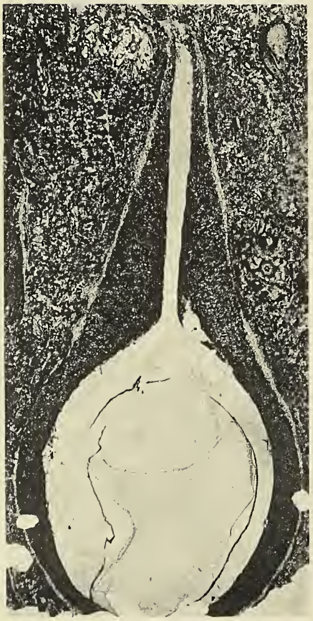

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E.A.N.A.&W.Tams,Photo. ARBER — SEED IMPRESSIONS

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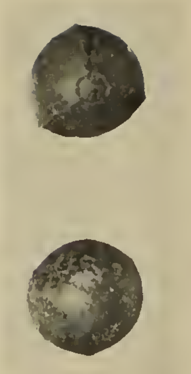
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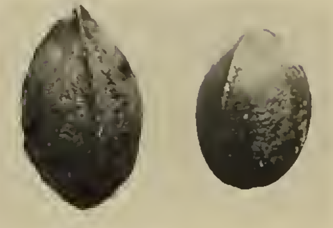
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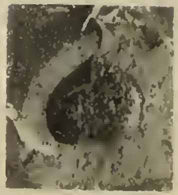
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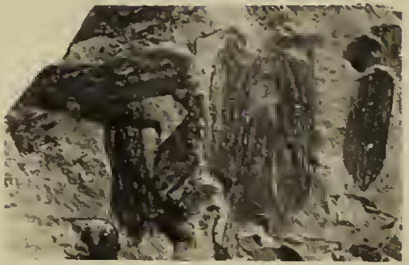
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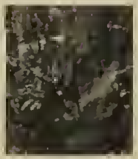
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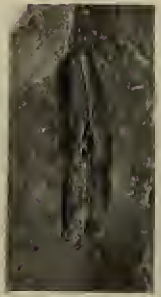
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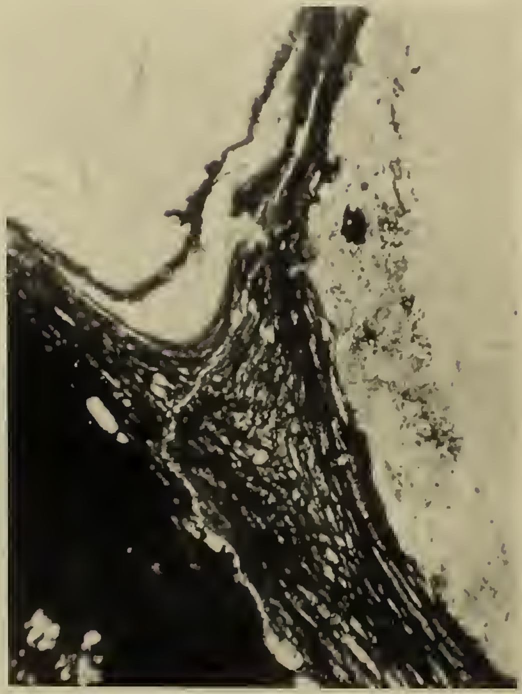
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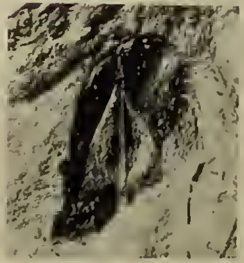
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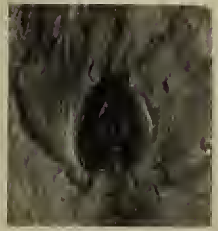
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E.A.N.A. & W. Tams, Photo.

ARBER — SEED IMPRESSIONS

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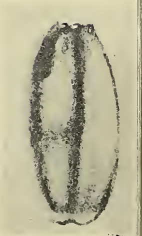

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ARBER — SEED IMPRESSIONS

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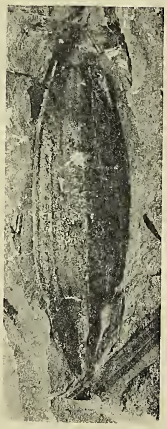

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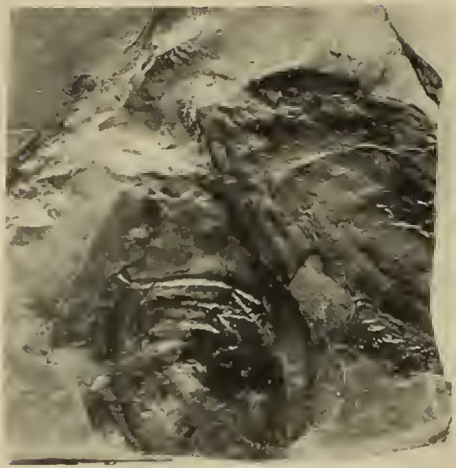
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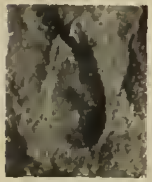
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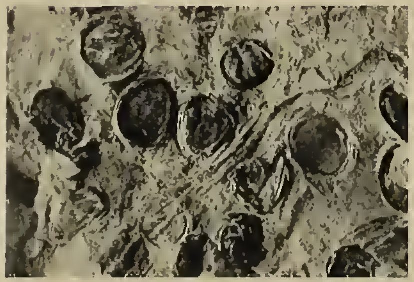
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ARBER — SEED IMPRESSIONS

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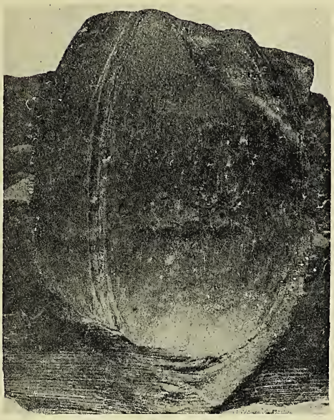

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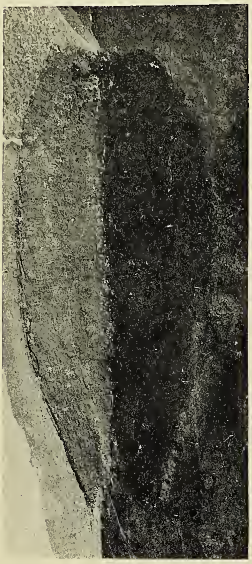

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W. Tams. photo

ARBER — SEED IMPRESSIONS.

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A Recording Transpirometer.

BY

V. H. BLACKMAN, Sc.D., F.R.S.

AND

S. G. PAINE, B.Sc., F.I.C.

With two Figures in the Text.

AN instrument which will automatically record the amount of water lost by a transpiring plant is of great service in many physiological and ecological studies. Of such instruments the most practicable type is that in which the loss in weight is automatically registered by means of a balance provided with some special mechanism. A number of such instruments have been devised, mainly by workers in the United States, but either they have never been put upon the market or else their cost is very high.

The first accurate recording balance which could be satisfactorily used for measuring the transpiration of a potted plant appears to be that described by Anderson,[1] who was the first to use the method of balancing the scale-pans by means of metal balls released at intervals by an electrical device. This balance seems never to have been put upon the market, and obviously would be costly to reproduce.

Woods[2] has invented another type of transpirometer on the plan of a recording hygrometer, which does not seem to have come into use. The best-known type of recording instrument is that of Ganong,[3] which of late years has been somewhat modified, and is described in his book on Plant Physiology. In this instrument, which is very ingenious and convenient, the scale-pans, as in Anderson's balance, are kept in equilibrium by steel balls which are added when required. It has, however, two drawbacks: it is expensive, thus prohibiting the use of a number of instruments in comparative work, and it is adapted to one particular weight only, the steel balls weighing one gramme. The instrument thus only records the time-intervals required for the loss of one gramme of water; an interval which is

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[1] Alex. P. Anderson: On a new Registering Balance. Minnesota Botanical Studies, Bull. 9, Pt. IV, 1894, p. 177.

[2] Bot. Gaz., xx, 1893, p. 473.

[3] Bot. Gaz., xxxix, 1905, p. 141.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.

often too long in the case of slowly transpiring plants. Of course, balls of lighter weight can be used,[1] but they must be of one particular size to suit the valve-mechanism.

The apparatus here described is an attempt to provide an instrument of considerable accuracy at a comparatively low cost, since part of the apparatus should be available in every well-equipped physiological laboratory. It has the further advantage that the water-content of the soil is kept constant automatically, a point of considerable importance, since soil moisture affects the rate of transpiration.

The principle on which the apparatus is constructed is, like that of Anderson and of Ganong, the addition, at intervals, of a weight equivalent to that lost in transpiration; but the novelty lies in the use of *water-drops* instead of steel balls, the water being added directly to the soil. By this means the combined weight of plant and pot and soil is constantly brought back to its original amount, and the soil to its original degree of moisture.

The apparatus consists of four parts: (1) a water-reservoir with a tube supplying drops of water of constant size; (2) an electrical device consisting of two solenoids (hollow magnets), which draw a tube backwards or forwards when energized by currents of a suitable direction; (3) a balance (provided with a 'damper' to prevent undue oscillations), the pans of which bear cups of mercury into which platinum points dip; (4) a revolving drum with recording pen actuated electrically. The arrangement of the various parts is shown in the photograph (Fig. 1), where the apparatus is in duplicate, one recording the loss of water from a transpiring plant, the other the loss of water from a porous-cup evaporimeter.

The pot containing the plant is contained in one of Ganong's aluminium 'shells', roofed over by a sheet of indiarubber which surrounds the stem of the plant and is perforated for a glass tube, which projects free above and is embedded below in the soil.[2] This tube receives water from the dropper when the loss of weight by transpiration has risen to a certain prearranged value. The 'dropper' is a bent tube, provided with a stopcock to regulate the rate of flow of the drops, and connected with a reservoir in which the pressure of water is kept constant by an air tube which dips below the level of the water. The reservoir and tube are supported on a stand adjustable in height.

The tube which serves to catch the water passes, as described above, through two solenoids, and is provided with a funnel at one end, the other end projecting over a reservoir which catches the waste water. When an electric current passes through one solenoid the tube is drawn back;

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[1] Transeau (Bot. Gaz., lii, 1911, p. 54) has made some modifications in this direction and has invented a ball-supplying mechanism to be used separately from the recording apparatus.

[2] This tube can be connected below with a small porous pot, so that the water supplied may be distributed more evenly to the soil.

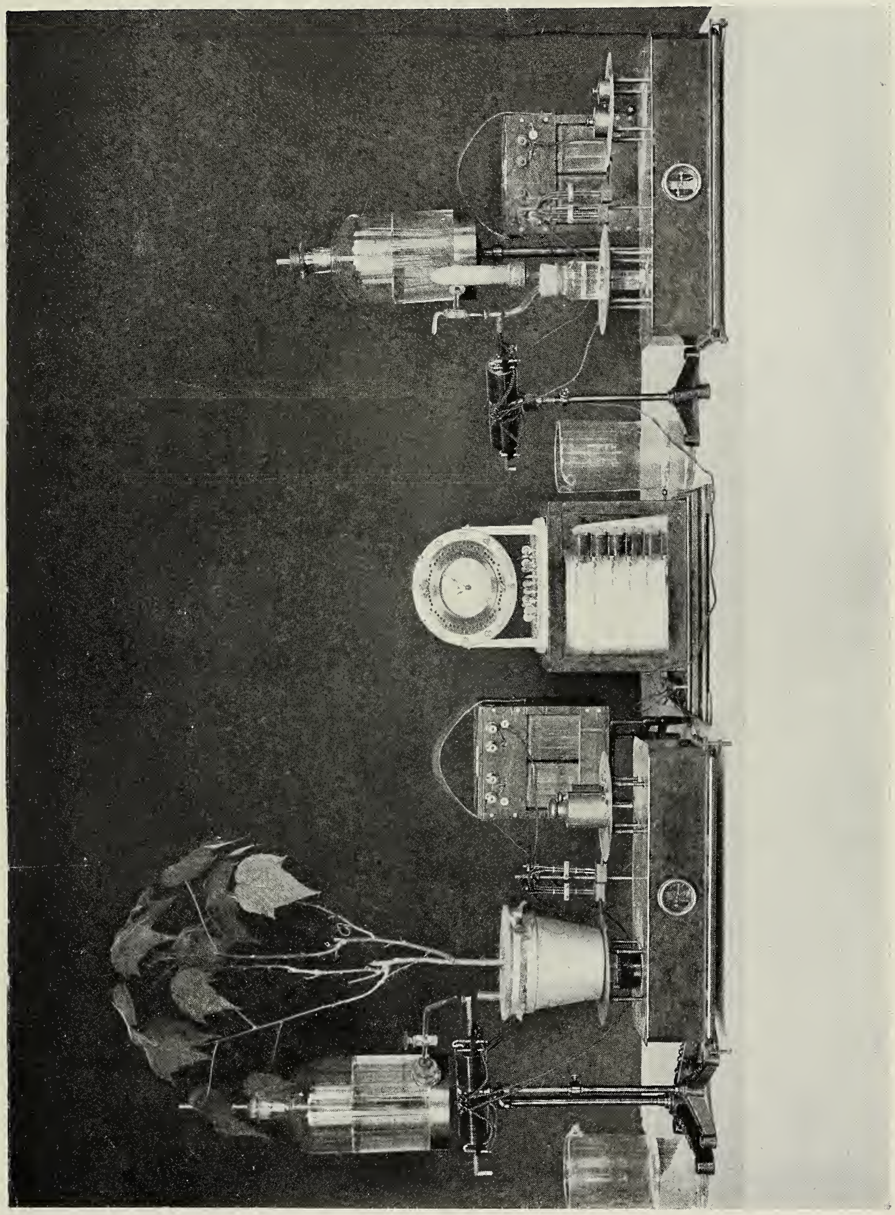

FIG. 1.

when the current passes through the other the tube is brought forward again. The tube is so placed that in its forward position the funnel stands immediately above the glass tube which is fixed in the soil of the pot, and the water-dropper is placed so as to deliver its series of regular drops into the funnel, whence they run to waste through the tube.

A balance of almost any type can be used provided that it is sufficiently sensitive and that it allows sufficient room above the scale-pan for the foliage of the plant. The balance employed in the apparatus photographed is of a simple, familiar type, with open pans supported below. This is sufficiently sensitive for ordinary work, but one with a bigger movement of the scale-pans, such as a large so-called physical balance, or Ganong's special transpiration balance, is more suitable for very accurate work. In any case, the balance must be provided with a 'damper' and an appliance for making contact as the pans rise. The damper is used to prevent the unduly large oscillations which would otherwise result from the fall of water into the pot, and from air-currents acting on the pans or plant. In the form of balance shown in the photograph, this purpose is accomplished in a very simple manner by attaching beneath one of the pans an aluminium disc which moves on a vessel containing some viscous fluid such as treacle or glycerine. In addition to this 'damper' there is attached to each pan a small vulcanite cup holding mercury. Above each cup stand a pair of platinum points, supported on the framework of the balance and in electrical connexion with the battery, the solenoids, and the recording pen.

When the apparatus is in use, the pot is placed on the left-hand scale-pan and is accurately counterbalanced, and the funnel of the solenoid tube and the dropper are placed immediately above the glass collecting-tube in the pot. As the plant transpires it loses weight, and the pan rises so that the left-hand pair of platinum points dip into the mercury in the cup attached to this pan; the electrical circuit is thus closed, with the result that the current passes through one of the solenoids and the metal tube with its funnel is drawn back. The water-drops are now free to fall into the glass tube fixed in the pot and so reach the soil. The addition of water to the pot continues until the increased weight in this pan causes the other pan with its attached mercury cup to rise and close another circuit. As a result, a current is sent through the other solenoid, which by its attraction brings back the tube to its forward position, and the water now runs again to waste. Every time the left-hand pan rises and closes the circuit, a portion of the current passes through the magnet of the recording pen, and so makes a vertical line on the revolving drum.

The platinum points can be raised or lowered by means of a simple screw arrangement, and so the apparatus can be set to respond to a small or a large number of drops. The loss of water from slowly transpiring plants is thus recorded as readily as that of more active ones. The actual weight of water lost corresponding to each mark on the record must of course be obtained by weighing the appropriate number of drops. The solenoids are provided with 'cut-outs', so that the current only passes momentarily at the time the points first make contact with the mercury. The solenoids and recording pen can be readily worked with two 2-volt accumulators in series; the interposition in the circuit of a short piece of manganin wire is useful in adjusting the strength of current, so that the movement of the tubes is not too rapid. The rate at which the drops fall can be controlled by the tap, and should be rather slow, as the 'damped' balance is naturally somewhat sluggish in movement. Distilled or filtered water is preferably used in the reservoir to avoid any sediment partially blocking the tube. There is a certain loss of soil-water by evaporation through the glass tube, but it is very small in amount; if necessary it can be measured and allowed for. A special thick-walled tube, ground flat at

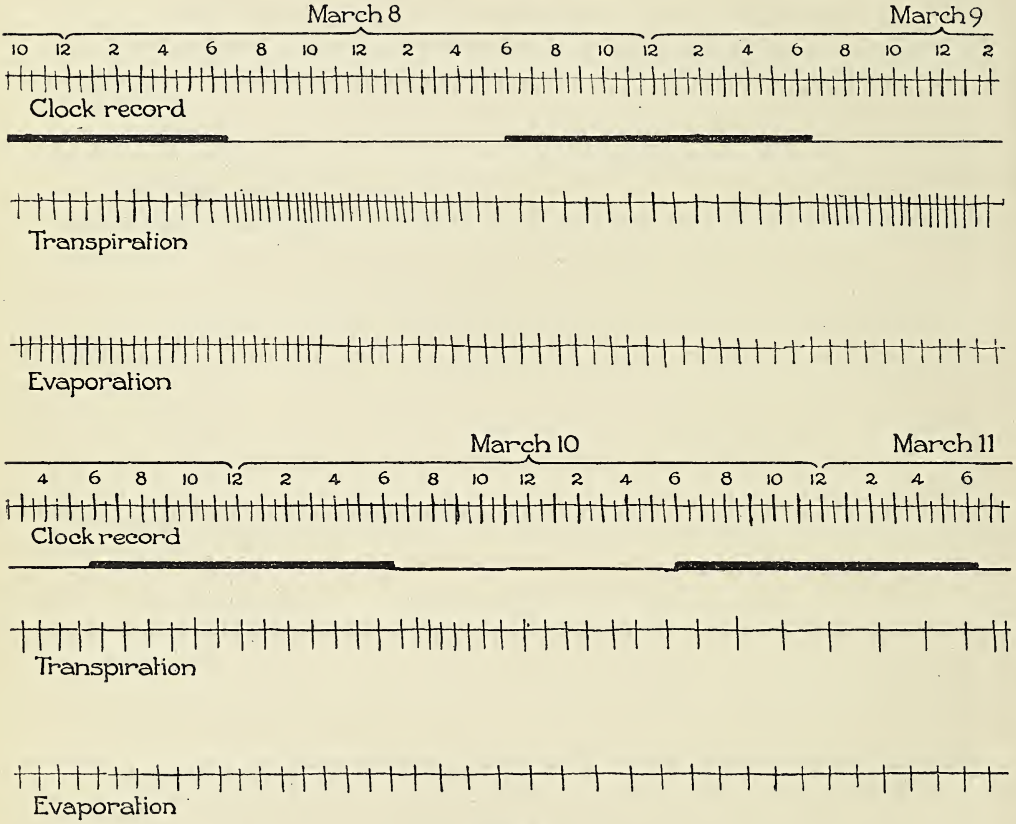

FIG. 2.

(The record has been cut into two and reduced to two-thirds.)

its orifice like a stalagmometer tube, can be used for dropping, but it is hardly necessary.

In the apparatus shown in the photograph, instead of a simple revolving drum, which is sufficient for ordinary purposes, there is a more elaborate drum (seen in the centre beneath the clock) with six pens, so that a number of comparative observations can be made. In this case a transpiring plant is being compared with the evaporation from a porous cup. A comparison of two such tracings enables one to eliminate the effect of such factors as humidity, temperature, air-currents, &c., which act directly on transpiration and evaporation alike.

A record obtained by this means is shown in the text (Fig. 2). The top line shows the time record, made by connecting a special clock (shown in the centre of the photograph (Fig. 1)) with a battery and one of the six pens mentioned above. The thick, long lines represent hours, the thin, short ones half-hours; the hours between sunset and sunrise are indicated by the black lines below them. The second line is the transpiration record, the lowest line that of the porous cup. The effect of light and darkness is brought out very sharply; while the transition from day to night has no effect on simple evaporation from the porous cup, the rate of transpiration is very much greater in the light, as is shown by the greater closeness of the vertical lines when the plant is illuminated. It will be noticed that the record shows that the rate of transpiration fell off as the experiment proceeded. In the absence of a record of simple evaporation under the same conditions, such a reduction might have been put down to some change in the plant. A glance at the lowest record shows that the evaporation from the porous cup is similarly reduced. In fact, while the experiment was started in warm dry weather, it was concluded in cold damp weather.[1]

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[1] By arrangement with the authors the various parts of the apparatus are supplied by Messrs. Baird and Tatlock, Cross Street, Hatton Garden, London, E.C.

Mitosis in the Pollen Mother-cells of Acer negundo, L., and Staphylea trifolia, L.

BY

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With Plates IX and X.

DURING the past three years the writer has been engaged, with more or less interruption, upon the study of the mitotic processes in the microspore mother-cells of species of Aceraceae and of *Staphylea trifolia*, L., of the Staphyleaceae, a family closely related to the Maples. In the native flora of Indiana, *Staphylea trifolia*, L., is the sole representative of the family. This paper will be confined, however, to the writer's observations on the mitotic phenomena in only one species of the genus *Acer* and to *Staphylea trifolia*, L., while the results obtained in other Maples will be reserved for a future publication.

In 1909 Darling published the results of a study on the two divisions in the microspore mother-cells of *Acer negundo*, L., but, as will be seen from what follows, his conclusions differ widely in certain respects from those of the writer.

ACER NEGUNDO, L.

The resting nucleus. The resting nucleus of the pollen mother-cell at the beginning of the growth period reveals the presence of a sparse and delicate network upon which are distributed small chromatin granules, and a very distinct globular nucleolus. The nucleolus may or may not show the presence of vacuoles (Figs. 3, 4). The chromatin granules are distinct, and stain definitely both with the triple stain and iron-alum-haematoxylin. The granules vary somewhat in size, and frequently tend to form small collections, as may be seen in Fig. 4. In fact, the larger granules are usually found to represent aggregates of smaller ones. The majority of these chromatin granules are arranged along the nuclear membrane, but they occur also upon the linin net which traverses the nuclear cavity. Very delicate linin threads frequently extend to the nucleolus, and sometimes

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chromatin granules are clustered about the same, as has been shown in the case of *Lilium* (Mottier, '07), and as probably occurs in spore mother-cells of all plants at the corresponding stage in development. In many cases, however, these threads are so delicate and so few that the nucleolus appears to lie in the centre of a perfectly colourless space. I find no support here for the theory of prochromosomes, as only rarely are pairs of granules seen, and when the same occur they are without doubt the result of chance. It is a matter of common observation in all nuclei of both vegetative and spore-bearing cells that larger granules are frequently found to be collections of smaller ones. The cytoplasm is of a uniform network, with fine granules (Figs. 3, 4, 5).

At the stage of the development of the anther from which Figs. 3, 4, and 5 were taken there is a sharp contrast in appearance between tapetal and spore mother-cells, due to the greater density of the cytoplasm of the former, in which the granules are finer and more closely arranged. The writer desires to emphasize the fact that, at the stage in question, the nuclei of the tapetal cells have precisely the same visible structure as that of the spore-bearing cells, namely, fine chromatin granules scattered upon a delicate linin net and a relatively large nucleolus (Fig. 1), although there may be two nucleoli. One nucleus is present in each tapetal cell at this stage, but later, at about the time of synapsis, this nucleus divides, when each cell contains two. This binucleate condition of the tapetal cells is remarkably constant both here and in *Staphylea*, to be mentioned in a later paragraph (Fig. 2). At the binucleate stage the tapetal cells have enlarged somewhat, and their cytoplasm presents a more coarsely granular appearance.

From the stages of Figs. 3 and 4 the nuclear net becomes more pronounced. There seems to be a greater number of meshes with thicker strands, and the chromatin granules are larger. The nucleolus is unchanged in so far as I have been able to determine. Very soon the stage of Fig. 5 passes into synapsis.

During the period of development described in the foregoing or in the presynaptic stages, Darling asserts ('09, p. 184) that the chromatin content is built up from material budded off from the nucleolus. The nucleolus, according to his view, gives off small spherical droplets, which migrate to the nuclear wall, where they diffuse out upon the linin to build up the spireme. Although small bud-like protuberances of the nucleolus are of frequent occurrence here and in other species of *Acer* examined by me, yet I cannot agree with Darling that this fact indicates that these bud-like protuberances separate and diffuse out into the net to build up the chromatin. However, I do not deny that the nucleolus furnishes material for the chromatin, as I have given expression to this idea in earlier publications. When the nucleolus breaks up at a later stage, to be described below, it shows frequently bud-like protuberances, but this is merely a fragmentation in the disintegrating process of the nucleolus, which has been so often described for other plants. Darling claims (loc. cit., p. 185) also that a spireme thread is well formed before synapsis, citing as evidence his Figs. 9, 10, and 11. In this respect I am unable to accept his interpretation, as I find prior to synapsis no indication of a spireme thread such as he figures. It is my conviction that Darling has in this instance misinterpreted the sequence of steps in the process, an error which is so prevalent in cytological literature dealing with plants. His Figs. 10 and 11 are certainly post-synaptic stages if, as he claims, his Figs. 13, 14, and 15 are post-synaptic. I do not consider the nuclear structure of Figs. 4 and 5 as spiremes, but a network with meshes of varying sizes. The structure shown in Fig. 5 now passes directly into synapsis (Figs. 6 and 7). From Fig. 6 it is seen that the nuclear network tends to accumulate towards one side of the nucleus, and this accumulation may or may not include within itself the nucleolus. In the gradual contraction of the nuclear network the chromatin granules are brought closer together, some of the smaller ones doubtless collecting into larger ones. Likewise, some of the delicate linin threads of the meshes will be united and others severed. The synaptic contraction is the initiation of the spireme, and as the spireme is seen to be present as soon as any indication of a loosening up of the densely contracted mass is evident, it is difficult to understand how a spireme could be formed from a network in any other manner. The most that can be said is that, as the net contracts into the synaptic mass, there is a tendency to form a spireme. This tendency is more pronounced in some nuclei than in others. I find no support for the doctrine of Lawson ('11) that synapsis is brought about merely by an enlargement of the cavity of the nucleus. There is no doubt whatever that we have to do with a real contraction or balling up of the nuclear network.

Figs. 7 and 8 represent the earliest stages of complete synapsis. The granules are densely massed, yet it can be seen that there are distinct granules. In Fig. 10 the contracted mass appears to be made up of larger and coarser lumps. Although this cell is larger than Fig. 8 and the cytoplasm gives evidence of rounding off, yet it is not possible to say that Fig. 10 is a later stage than Fig. 8, for the pollen mother-cells differ considerably in size, and there is considerable variation in the time of the rounding off of the cells. There is a possibility that fixation may be less perfect in cases like Fig. 10, as there may be different degrees of poor or faulty fixation. In Fig. 9 we have a stage in synapsis that is a little more advanced than that of Figs. 7 and 8. One large and one small nucleolus lie near the contracted mass, in which it may be clearly seen that a chromatin thread or spireme is in process of formation. At the stage of Fig. 9 the cell usually shows signs of rounding off at the corners preparatory to separation from the primary cell-wall of the mother cell (Fig. 11). In this, as in many other plants, the writer has found much variation in the time at which the first rounding off of the mother-cell is distinguishable, using the appearance of the synaptic mass as a guide. In the earliest stages of the contraction the cells are invariably polygonal, forming a compact tissue without any intercellular spaces (Figs. 7, 8). With the appearance shown in Fig. 8 the rounding off usually begins, and the same is evident in all cells as soon as the contracted ball has loosened up sufficiently to show clearly the definite spireme character (Fig. 11). Whether the appearance of the first rounding off of the cells is due to a slight shrinkage caused by the reagents cannot be stated with certainty. It is possible, of course, that this may be true to a limited extent, for it not infrequently happens that the cells in one loculus may be noticeably or even badly shrunken, while those of the neighbouring loculus of the same anther will show perfect fixation.

As pointed out for other plants, the loosening of the synaptic mass is first manifested by the extension of loops or turns of the spireme into the nuclear cavity (Fig. 11). These continue to spread out, and soon the entire chromatin cord is distributed throughout the nucleus, forming what is so well known as the hollow spireme. The larger portion of the spireme is probably arranged along the nuclear membrane, yet it also traverses the cavity in various directions, some portions following straight courses through the cavity, others being more or less looped or kinked. Fig. 12 represents the stage of the thick hollow spireme in section, not all of the nucleus being shown. The earlier steps in the formation of this spireme have shown that it is made up of a succession of chromomeres that are held in the linin. No longitudinal fission or double nature of the spireme is seen in this plant, although this fact is not a proof that a longitudinal fission may not have taken place, as is the general rule in plants, and as will be seen in *Staphylea*, to be described below. The fact that the spireme is seen as a single cord makes it easier to trace certain later steps in the mitotic process with much greater certainty than were the confusing feature of the diverging halves of the spireme present in longer or shorter stretches, as frequently happens in the Lilies and in other plants. At this stage the pollen mother-cell has separated from the primary cell-wall and has formed a soft, thick wall of its own. The nucleolus is still present, and it is about as large as in any preceding stage.

The formation of the bivalent chromosomes from the hollow spireme. The next series of steps in the process following the regular, thick, hollow spireme result in the formation of the bivalents from the same. This transition brings about certain changes by which the regularity in the disposition of the turns of the spireme is lost, and which give rise to the phenomenon known as the second contraction. In the second contraction the spireme is so rearranged that a part of it is more or less closely entangled into a knot near the centre of the nuclear cavity, from which extend somewhat radially loops and straight portions. In many cells in *Lilium* this arrangement of radiating loops and straight portions of the spireme from the central knot occurs with almost the regularity of a diagram.

In *Acer negundo* there is present the feature that represents the second contraction, but it does not appear with the regular radiating loops, save, perhaps, in rare cases. During this rearrangement the spireme loses, as a rule, its more regular course and uniform thickness. It shows a tendency in many cells to become relatively thick in some places and attenuated in others. The loops and turns become shorter and so arranged that a picture of the whole nucleus, or a thick section of the same, does not present the regularity of Fig. 12. The most regular appearance of the spireme observed just before the segmentation into the chromosomes is represented in Fig. 13. Here certain parts of the spireme extend in straight stretches from a somewhat centrally located entanglement, while other parts form longer or shorter loops, which may be variously twisted or kinked. The stage following Fig. 13 is shown in Fig. 14. In this we see that the spireme is at least partly segmented. This nucleus shows a much greater regularity in the arrangement of the chromatin than is usual, and I have selected such nuclei as illustrations for the reason that the parts may be clearly and definitely made out. It is evident that the spireme has segmented into pieces that are either straight or in the form of loops. Closely following upon the stage of Fig. 14 is that of complete segmentation shown in Fig. 15. The spireme, which gives no evidence of being double or longitudinally split, has segmented transversely into a number of pieces that were previously joined end to end to form that spireme. These pieces (Figs. 15, 16, 17, and 18) are in the form of straight rods, closed or open rings, or loops. The parallel sides of the loops may be twisted about each other, sometimes tightly and closely, sometimes loosely, forming figure 8's, as has been described for other plants. Each loop, ring, U, or 8 represents a bivalent chromosome made of two somatic chromosomes which have not separated at the extremities representing the bend of the loop. A loop or a ring is, therefore, a continuous piece of the spireme composed of two somatic chromosomes (Figs. 17, 18). Figs. 17 and 18 are tangential views of nuclei, while Figs. 15 and 16 are more nearly radial sections. A ring is formed when the two halves of a loop curve away from each other equally, leaving the free ends closely juxtaposed or touching, or even slightly overlapping, each other. The formation of a U from a piece of the chromatin spireme is self-explanatory. Of course, a ring may not necessarily owe its form to a previously looped portion of the spireme. For example, the large ring in Fig. 17 may have arisen from a loop, the free ends of which have touched end to end by the bending of the two sides, while at the point of bending of the loop the other ends of the two somatic chromosomes have tended to pull apart; or two curved somatic chromosomes may touch at the free extremities. I hold the first alternative as the most probable. In any event, the large ring in Fig. 17 is not formed by the divergence of the halves of a longitudinally split spireme with the free ends adhering, for there is no longitudinal fission evident, and if the fission really exists this fact is not made apparent by the separation of the halves. Furthermore, all other evidence is against the view of the separation of longitudinal halves to make a bivalent. When the sides of a U are twisted about each other, we have the familiar figure 8 form or the hour-glass shape. At one end of the 8 the free ends frequently overlap, while the other end may represent the bend in the loop. A glance at Figs. 15 to 18 shows that all the bivalent chromosomes are not rings, U's, or 8's. Some are composed of two straight or slightly curved rods that may be parallel side by side with or without any twisting about each other; or the two pieces may adhere at one end to form V's, cross each other to form X's, or two of the free extremities may diverge to form Y's. All of these familiar forms may be found in the same nucleus. It sometimes happens also that the two straight somatic chromosomes may lie in a straight line adhering end to end, a phenomenon found in various other plants. Now the bivalents that appear as two straight rods lying in contact side by side, or that form X's, &c., did not necessarily arise from the spireme as loops, but were in all probability derived from those parts of the spireme that were straight, or even from the more closely knotted or entangled parts of the chromatin thread. However, they represent different lengths of the spireme that have approximated side by side, and not the halves of the same piece of the thread that had split lengthwise. It follows, therefore, that, if the spireme were split lengthwise, as is the case in many plants, each member of the bivalent or somatic chromosome would be composed really, if not visibly, of two halves that tend to separate during the anaphase, and that do separate from each other during the telophase. This separation, which is finally and fully brought about during the second division, is the equational division, because the chromosomes separating in the second division represent halves of somatic chromosomes produced by the longitudinal split. The approximation or the so-called pairing of two somatic chromosomes to form bivalents is not understood by the writer as a conjugation. The assumption that there is an exchange of material taking place at this step in the mitotic process is based upon purely hypothetical data. If there is an exchange of material between chromosomes, is there not greater opportunity for such an exchange in the spireme itself, or in synapsis, or even in the resting stage, when the chromatin is in its most finely divided state? A further statement of this point will appear below.

In the entire history of the nucleus from the stage of rest to the formation of the twelve bivalents, nothing is clearer to the writer than the fact that all of the bivalents are derived from the spireme; that no spireme is formed previous to synapsis; that there is no union of two spiremes either before, during, or after synapsis, and that the spireme is composed of the somatic chromosomes placed end to end. In *Acer negundo* a longitudinal fission is not observed with certainty. The writer is unable to accept the origin of the bivalents as described by Darling (loc. cit., pp. 185, 186), who asserts that eight chromosomes come from the spireme, while five bud off bodily and full fledged from the nucleolus. As stated in a foregoing paragraph, Darling claims that the spireme itself is derived from the nucleolus by portions of the same which bud off and diffuse out into the linin, thus forming the chromatin thread, and that later five other bivalents separate from the nucleus by direct budding. I have expressed the view in earlier publications that the nucleolus contributes material to the nourishment of the cell, and it is not denied that the chromatin may receive some of this material, but I do not interpret the small bud-like protuberances of the nucleolus which are frequently observed in earlier and later stages of the first mitosis of *Acer negundo*, and in other species of *Acer* studied by myself, as representing the manner in which the nucleolus contributes material to the cell. In all stages up to that of the multipolar spindle, the nucleolus is present, and sometimes one or two smaller additional ones (Fig. 11). Even after the segmentation of the spireme, when the full number of bivalents is present, and on into the multipolar spindle, the nucleolus is still on hand, and it is about as large, or sometimes larger than at an earlier stage (Fig. 16, 19). During the formation of the spindle, however, the nucleolus fragments usually into smaller bodies, and finally all traces of nucleolar material disappears, save small extra-nuclear nucleoli, which may be seen distributed throughout the cytoplasm. In some cases, just prior to the appearance of the multipolar spindle complex, the nucleolus may be seen to form large bud-like protuberances (Fig. 20 *a*), and this may be reasonably interpreted as the breaking up of the nucleolus. Previous to the formation of the spindle, one or more bivalent chromosomes are not infrequently found lying close to, or in contact with the nucleolus (Fig. 20 *b*), but in no case have I found anything to lead me to believe that any chromosome is formed bodily from the nucleolus.

The formation of the spindle. At the complete segmentation of the spireme into the chromosomes, the latter appear much larger than in succeeding stages. The halves of any given bivalent may be thicker than the spireme from which they have just been formed. This is doubtless due to a shortening which always follows soon after complete segmentation. The bivalents now become distributed throughout the nuclear cavity, where they are seen to be connected with each other and with the nuclear membrane by delicate threads. They now undergo the process of condensation by which their size is greatly reduced, and show a tendency to be distributed along the nuclear membrane. With the continual condensation of the chromosomes and their consequent reduction in size, there is developed about the nucleus the weft of kinoplasmic fibres which form the multipolar spindle complex (Fig. 19). The nuclear membrane fades from view as a sharp line or boundary, and in its place there appear the kinoplasmic fibres. In an earlier publication the writer has expressed the opinion that the nuclear membrane is changed into kinoplasmic fibres, a view he still holds. The nucleus gradually loses its regular globular form, because of the encroachment of the spindle complex, and the straightening out and convergence of fibres to form the several poles. In short, the entire process of spindle formation is identical with that repeatedly described by the writer for other plants.

The condensation of the chromosomes continues until the spindle is fully formed, at which time they have probably reached their smallest size. In the equatorial plate each bivalent appears usually in the form of two thick and somewhat rounded lumps, or they may be somewhat elongated in the form of rods (Fig. 22). As a rule the bivalents appear more nearly like the one in the centre in Fig. 22. They are generally closely crowded, and appear in the form of a disc in polar view. In the judgement of the writer twelve bivalents are present. Even with the relatively small number the form and arrangement make accurate counting difficult, under the most favourable conditions. In Fig. 21 twelve bivalents and a nucleolus are seen. This figure was obtained from a multipolar stage of the spindle in which all the objects could be clearly made out by changing the focus. The chromosomes that lie above others in the focus are made darker. The members of two of the bivalents (*a* and *b*) are not closely applied. If each of these were counted as two, fourteen would be the number. I have not been able to convince myself that the number is thirteen as stated by Darling. It is either twelve or fourteen.

In the mature spindle the poles may be pointed or broad as in Fig. 22.

Anaphase and telophase. The two members of each bivalent (the two somatic chromosomes) now separate in metakinesis, one going to each pole. On their way to the poles each is seen to be split lengthwise (Fig. 23). This split may sometimes be observed in the equatorial plate (Fig. 22), but, as a rule, no indication of the longitudinal fission is noticeable at this stage, and not always is it very evident during the anaphase. Having arrived at the poles the chromosomes are almost always found closely crowded together, giving the appearance of a compact mass of lumps. Later they separate and undergo to some extent the process of reticulation or alveolization by which each may elongate, becoming attenuated in certain places, but remaining thicker in others, and the nuclear membrane is formed. The thicker lumps or fragments are connected with each other by means of delicate threads, while the attenuated parts also appear as connecting threads. The result is that the daughter nucleus consists of a number of larger and smaller lumps, irregular in shape, and connected by anastomosing threads. During the process one or more nucleoli make their appearance in each daughter nucleus, at the same time extra-nuclear nucleoli are still present in the cytoplasm (Fig. 23). Each daughter nucleus consists, therefore, of a lumpy network, together with one or more nucleoli. The chromatin is distributed chiefly along the nuclear membrane (Fig. 24). A more detailed description of the formation of the nuclear membrane and the nuclear cavity or vacuole will be given below in connexion with *Staphylea*, which, on account of the larger size of the cells, is a more favourable object for the study of this phase.

The second mitosis. Whether the process of reticulation, alveolization, or fragmentation of the chromatin described in the foregoing, is carried to the finely divided state that is recognized as the resting condition, I am not able to state with certainty. This is held to be a matter of little theoretical importance, chiefly for the reason that in some plants the chromatin of the daughter nuclei reaches the finely divided state of a resting nucleus, while in others it does not. The daughter nuclei divide simultaneously, the spindles being formed as multipolar complexes which change to the bipolar form as has been described for many other plants (Fig. 25). In this cell, which is typical, the chromosomes have not as yet been brought into the equatorial plate. Each is composed of two lumps, and these pairs of lumps are assumed to be the two pieces formed by the longitudinal splitting of the somatic chromosomes on their way to the poles in the preceding mitosis. In this second mitosis the chromosomes are arranged in a regular compact plate at the mature spindle stage. During the process of spindle formation nucleoli are present, lying either among the spindle fibres or out in the cytoplasm (Fig. 25). The granddaughter nuclei are formed in a manner similar to those of the first mitosis. The four nuclei are connected by systems of connecting fibres, before cell-division takes place as in many other Dicotyledons (Figs. 26 and 27). All four granddaughter nuclei are alike, both as to the nature and amount of chromatin and the presence of nucleoli.

STAPHYLEA TRIFOLIA, L.

In certain important respects, *Staphylea* is a more favourable object for a study of mitotic processes than *Acer negundo* and other species of *Acer* now under investigation, partly because of the larger size of the cells and nuclei, and partly because of the clearness with which other and secondary phenomena are brought to view. I have decided to present the details of each separately, rather than to combine the corresponding steps under common headings. This is done because of the greater ease with which the more essential differences can be followed by those who have not made a study of the subject at first hand, but whose work, though along different lines, requires a rather clear understanding of the essentials concerning which there is much difference of opinion even among those who have been extensively engaged in the study of mitotic phenomena. I have also abstained from the use of numerous new terms that have crept into cytological literature during the past decade, because I believe that much of the new terminology is not only not necessary to a clear statement of the phenomena, but that a number of the new terms have tended more to confuse than to elucidate.

From the resting nucleus to the hollow spireme. The sporogenous cells of *Staphylea*, at the beginning of the growth period, and after the same has fairly begun, form a compact and uniform mass of polygonal cells without any intercellular spaces. Fig. 28 is typical of the cells at this stage. The large nucleus presents a large number of chromatin granules of varying sizes distributed rather uniformly along the periphery, and also within the cavity upon a delicate linin network. I say upon a linin net, because I do not interpret the delicate and colourless threads connecting the distinctly stained granules or collections of granules as merely drawn out threads of chromatin substance. Within the cavity of the nucleus is the very large nucleolus. The nucleoli in these cells are comparatively free from the small bud-like protuberances met with in *A. negundo*, and in other species of Maples. There is not what the writer would regard as the semblance of a spireme or spiremes, but only a *net*. The structure shown in Fig. 28 passes directly into synapsis. This consists in the collection into a compact mass of the network and granules. In Fig. 29 the contraction or balling up is going on, the majority of the granules having collected about the nucleolus, partly enclosing it. In Fig. 30 we have complete synapsis. In this plant the nucleolus is very frequently not included within the mass, but may be found entirely outside, and sometimes removed a short distance from the mass of chromatin. In sections of anthers at the stage of complete synapsis, the large nucleoli lying without the balled-up mass is a very noticeable phenomenon when a section of an entire loculus is viewed with low powers. Figs. 28, 29, and 30 were drawn from the same section of the same loculus, the condition of Fig. 30 prevailing in one end of the anther pocket, and that of Fig. 28 in the other. Between the two ends are found all gradations from Fig. 28 to Fig. 30. Figs. 28 and 29 belong to neighbouring cells.

With further progress the contracted mass begins to loosen up, and it is seen that a spireme has in the meantime been in the process of formation (Fig. 31). As a rule the cell begins to round off when loosening up is initiated, as stated for *A. negundo*, but, as in that plant, there is some variation in the time. In Fig. 31 the cell had not rounded off, but other cells in the same loculus presented a rounding off at the corners. As the spireme in the contracted mass loosens up, it is seen in some cases to be double, or to show the longitudinal split as in the Lilies, but this phenomenon is of less frequent occurrence here. If the longitudinal fission is constant, it is not always recognizable, for in the fully developed and regularly distributed spireme the presence of a longitudinal fission is rather the exception (Figs. 32, 33). Fig. 32 is a slightly older stage than Fig. 31. The cell has well rounded off, and more of the spireme is distributed in the nuclear cavity. Fig. 33 represents the stage in which the spireme has reached its most uniform distribution. The nucleolus is large and presents a uniform structure, although what is usually interpreted as vacuoles may sometimes be seen within it. Small bud-like protuberances of the nucleolus are much less frequent here than in *Acer negundo* and some other species of *Acer*. The cytoplasm of the cell presents a rather uniform appearance. It sometimes happens that the condition of Fig. 33 may by shrinkage appear like that of Fig. 32, and thus be taken for a much earlier step in the process. Care must be taken, therefore, to distinguish between good and faulty fixation in this as well as in other mitotic stages.

As in *A. negundo*, there now takes place the rearrangement of the spireme, or second contraction, which ushers in the cross-segmentation and the formation of the bivalents. Here we have a nearer approach to the loops and straight portions of the spireme extending out or radiating from a more centrally placed knot or close entanglement of the spireme that is found in *Lilium*. The knot or entanglement (Fig. 34) may or may not include the nucleolus, and instead of one such entanglement there may be two or more. This figure represents a thick section of a nucleus, of which the nucleolus lay in a neighbouring section. At this stage there is much twisting and kinking of the thread. As a rule the nucleus presents a much more confused picture than Fig. 34. Transverse segmentation has undoubtedly begun, although certain free ends and short pieces shown in the figure have been made by the knife in sectioning. Because of the tangled condition of the thread and of the number of shorter and longer pieces to be observed, this is one of the most difficult steps in the entire mitotic process in which to obtain a clear view of all details. The spireme, in many cases at least, is more slender than in the stage just preceding, its diameter being much thicker in some parts than in others. It seems also a more sensitive stage, as shrinkage of the cells is frequent. In fact the entangled, twisted, and knotted parts of the thread are so confused that it is extremely difficult to make out clearly and definitely all details.

When segmentation is complete there soon follows a shortening and thickening of the chromosomes. The bivalents separate, becoming more regularly spaced in the nuclear cavity, and the form of each is clearly revealed (Figs. 35, 36, 37). The condition of Fig. 34 is frequently present in one or two loculi of an anther, while in the others that of Figs. 36 and 37 prevails. The different forms of bivalents observed in *Staphylea* are the same as those described for *Acer negundo*, with the exception that they are much larger and more numerous. Fig. 35 represents a median section of a nucleus, while tangential views of nuclei of the same loculus are represented in Figs. 36 and 37. In Fig. 36 the long piece of chromatin seems to indicate delayed segmentation. Such phenomena are frequent at this stage. *a*, *b*, and *c*, Fig. 38, represent three sections that include the entire nucleus. The different sizes and shapes of the bivalents and the presence of the large homogeneous nucleolus are typical. In *a* two bivalents were pushed out of the nucleus in sectioning. Nothing is more striking at this stage, in this as well as in all other plants studied by the writer, than the perceptible differences in the size of the chromosomes, and if such differences have any significance in plants, there is, at the present state of our knowledge, no means of knowing. Since the various forms of the bivalents are the same as those of *A. negundo*, further details in this respect seem unnecessary.

Effort was made to ascertain accurately the number of chromosomes, but, because of the larger number and of their crowding together at the stages favourable for counting, I was unable to convince myself that the exact number was determined beyond question. In my judgement the haploid number is about thirty-six, or three times the haploid number of *Acer negundo*. Thirty-six was also the number ascertained for *Acer rubrum*. In either case it does not seem probable that the number exceeds forty or forty-two. Correlated with this larger number in *Staphylea* and in other species of *Acer*, is the much larger size of the pollen mother-cells than in *Acer negundo* with its twelve or fourteen bivalents.

When the chromosomes are distributed in the nuclear cavity following their formation, they may be connected with each other, with the nuclear membrane, and with the nucleolus, by very delicate threads (Fig. 35). In more densely stained cells these threads are distinct, but if the staining be less dense they appear faintly or not at all.

From spindle to telophase. The chromosomes now undergo a condensation until they are arranged in the equator of the spindle, when they have attained their minimum size (Fig. 39). The process of spindle development is similar to that in other pollen mother-cells. As described for the Lilies and other Dicotyledons, the kinoplasmic fibres may sometimes appear first as a weft running parallel with the nuclear membrane, and at a distance midway between the latter and the cell-wall (Fig. 35). The fully developed spindle is usually sharply pointed at the poles and the various fibres have the usual well-known arrangement. A polar view of this stage shows that the bivalents are arranged in the form of a circular disc (Fig. 40). They are usually more closely crowded together than in this figure. By looking at Fig. 40 it might seem that the task of counting is an easy one, and that accurate results may be obtained without difficulty, but such is by no means the case. The members of the bivalents take the form of short lumps. The rods which are fastened to the spindle fibres at one end may lie parallel with the spindle axis or at right angles to it. A glance at Fig. 39 will show how, in polar view, one member of a bivalent may obscure or partly conceal a neighbouring bivalent, and if, as almost always happens, the two halves of a bivalent do not lie closely applied to each other, each half may appear as a whole chromosome. Likewise the close arrangement of the segments as they pass to the poles is also an element to interfere with strict accuracy in counting. A comparison of Figs. 35 and 37 with Fig. 39 shows that a very marked condensation has taken place in the chromosomes. At the spindle stage the difference in size among chromosomes is less than at previous stages.

A phenomenon worthy of note at the stages in question is seen in the zone of larger granules formed about the nucleus, and which later surrounds the spindle (Figs. 35, 39, and 40). At the stage of the loose, hollow spireme the cytoplasm is uniformly granular, with very small nucleolar-like bodies scattered throughout the cell, and later, as the chromosomes approach the poles, the cytoplasm presents again a more uniform appearance in regard to the distribution of granules upon its network. To these granules may be applied Strasburger's term 'trophoplasm', but in so doing the writer does not intend to convey the idea that trophoplasm is necessarily alveolar in structure. In pollen mother-cells these granules do not represent alveolae at all. They are merely amorphous particles held within the cytoplasmic network. In Fig. 33 we have this somewhat uniformly appearing cytoplasmic network with the granules more evenly distributed, while in Figs. 35 and 39 there is present the same character of network, but the granules are more numerous in circumscribed areas. It may be true that more of these granules are present, but whether the granular matter has increased in quantity, or has merely become collected into the region in question from other parts of the cell, may be left an open question. In the spindle stage there appear also groups of globules in the cytoplasm lying in a plane with the nuclear plate (Figs. 39 and 40). These globules are not extra-nuclear nucleoli, but oil-drops which turn black with osmic acid, and from which the dark colour may be readily removed by hydrogen peroxide. The peculiarity about these globules is the position they occupy in the cell. Later they may disappear entirely (Fig. 41).

The large nucleolus which is present at the stage of complete segmentation of the spireme into the chromosomes (Figs. 35, 38) has disappeared by the time the spindle is mature, and at this stage and during the anaphase the amount of nucleolar material demonstrable as extra-nuclear nucleoli is very meagre. It is perfectly clear that the nucleolar matter is not taken up by the chromosomes, because the latter undergo a very considerable diminution of size, presumably by a process of condensation, during the disappearance of the nucleolus. The nucleolus undoubtedly changes into a substance or substances that do not take chromatin stains, and I am further strengthened in the conviction, expressed in earlier papers, that the nucleolus not only supplies material for the chromatin, but also for other parts of the cell.

On their way to the poles the daughter chromosomes undergo the longitudinal fission, or it may be that they merely reveal the longitudinal split which had taken place in the spireme stage (Fig. 42). As they reach the poles they are closely crowded together, so closely that they often appear as a fused mass. In polar view they are seen to form a rather compact disc.

The construction of the daughter nucleus. The daughter chromosomes on approaching the pole are probably brought into close contact by the convergence of the spindle fibres, for it is very difficult to escape the conclusion that the fibres are the active agents in the disposition and movements of the chromosomes. The assembled chromosomes now come to occupy the position of the spindle poles. When this has been accomplished, they begin to separate gradually from each other. On the side of the mass towards the centre of the cell, the cytoplasm seems to withdraw, leaving a vacuoli-like space free from granules, and at the same time a sharp boundary between the colourless space and the cytoplasm is seen, which is continued around the mass of chromosomes (Fig. 43). This cavity in which the chromatin now lies is the nuclear cavity, and its boundary is the nuclear membrane of the daughter nucleus.

As stated in the foregoing for *Acer negundo*, the chromatin collection separates into pieces irregular in outline, and connected by delicate anastomosing threads (Fig. 44). This figure is a polar view of a stage a little later than Fig. 43. Each large piece of chromatin in this figure represents probably more than one daughter segment, and the larger ones doubtless undergo further fragmentation, or alveolization into smaller pieces. As is well known, the extent of the alveolization or fragmentation varies in different plants, extending in some, as in *Pinus*, to the finely divided state known as the resting nucleus. Fig. 45 represents a longitudinal section of a daughter nucleus from a neighbouring cell in the same section as that from which Fig. 44 was taken. The pieces of chromatin are smoother and more slender. I did not find a continuous chromatin spireme in the daughter nuclei. The chromatin in the daughter nuclei of *Staphylea* does not reach the finely divided state of the resting nucleus.

The details of the second mitosis are the same as those in *Acer negundo.*

SUMMARY AND CONCLUSIONS.

The resting nucleus of both *Acer negundo* and *Staphylea trifolia* consists of a delicate linin network, or reticulum, upon which are distributed chromatin granules or collections of chromatin granules of varying sizes, and a large nucleolus.

Before synapsis there is no chromatin spireme present, nor is there any structure referable to any definite spireme or spiremes.

Synapsis is a normal phenomenon, and consists in the contraction of the nuclear reticulum into a more or less compact mass, which may or may not include the nucleolus.

Within the synaptic mass, and during the loosening up of the same, the definite chromatin spireme is formed. The assembling of the granules of the nuclear network into the contracted mass probably represents the initiatory step or steps in the formation of the spireme. The spireme is formed by the serial arrangement of the chromatin granules or groups of granules into a row of chromomeres, and furthermore by the union of certain meshes of the network along with the severing of others. This seems to be the only way a continuous thread of spireme can be formed from a network.

In *Acer negundo* the spireme appearing immediately on the loosening up of the synaptic contraction does not show a longitudinal split, which, however, may be observed in *Staphylea* at this and at later stages. Taking all of the pollen mother-cells into consideration, the appearance of the longitudinal split is probably the exception rather than the rule even in *Staphylea.* The view accepted here is that the spireme has undergone a longitudinal fission. If the chromomeres do not split lengthwise, they must be formed or assembled into the spireme as a double row instead of as a single row of granules ; for there is no fusion of two spiremes, either before, or during, or after synapsis. In the opinion of the writer, the formation of the spireme is more probably the cause of synapsis than that synapsis is the cause of the formation of the spireme.

In both species there is finally developed the uniform hollow spireme, which is either endless, or, if free ends are present, these are few in number. This spireme consists of the diploid number of chromosomes placed end to end.

In both, there is present the feature known as the second contraction, or rearrangement of the spireme from the more regular to a more complicated form, in which are to be seen knotted and entangled parts, from which may extend a varying number of straight portions or loops. The looping is more frequent in *Staphylea* than in *Acer negundo*, where radiating loops from an entanglement may only rarely occur. There is not the regularity of radiating loops extending from a central entanglement as is characteristic of *Lilium*.

During the complicated rearrangement the cross-segmentation of the spireme begins. Portions of the spireme, more especially those appearing as loops, may segment into pieces equal to two somatic chromosomes or a whole bivalent, but all the pieces or segments which result from the transverse separation do not represent whole bivalents.

The looped portions usually form the rings, U's, or 8-shaped bivalents, while the separated somatic chromosomes make up the X's and the rods lying in contact side by side. In any and all cases where the two members or halves of bivalents lie side by side, these forms are due to the approximation side by side of the respective halves that were *end to end* in the spireme.

All chromosomes are formed from the chromatin spireme. Chromosomes do not bud off bodily and full-fledged from the nucleolus.

The number of bivalents is probably twelve in *Acer negundo*, and thirty-six, or thrice this number, in *Staphylea*. The larger number of chromosomes seems to be correlated with the larger size of the pollen mother-cells in these two plants.

The spindle is developed according to the accepted multipolar type, which becomes later bipolar.

In *Staphylea* especially, a marked zone of granules (trophoplasmic granules) appears about the nucleus during spindle formation and persists until the anaphase, when the entire cytoplasm is again more uniform in appearance. Collections of oil-drops are very frequently present, lying in the plane of the nuclear plate. Later they disappear.

On their way to the poles the daughter segments are seen to be split lengthwise.

During the construction of the daughter nuclei and later the chromosomes undergo a fragmentation, reticulation, or alveolization into lumps or fragments of varying sizes, which are connected with each other by anastomosing threads. The finely divided state of the chromatin characteristic of resting nuclei of similar size was not observed in the daughter nuclei. A continuous, smooth and uniform spireme was not observed in the daughter nuclei prior to their division.

The second mitosis presents nothing unusual for Dicotyledonous plants. There is no unequal distribution of chromatin to the granddaughter nuclei.

The nucleolus represents material which contributes to the building up of the constituents of the cell. It doubtless serves as nutrition for both chromatin and cytoplasm, according to the demand made upon it by the activities of the different parts of the living substance.

PUBLICATIONS REFERRED TO.

As a discussion of the literature would greatly exceed the limits of this paper, only those contributions will be listed to which direct reference is made.

DARLING, CHESTER A. ('09) : Sex in Dioecious Plants. Bull. Torr. Bot. Club, vol. xxxvi, 1909, pp. 177-99.

LAWSON, A. ANSTRUTHER ('11) : The Phase of the Nucleus known as Synapsis. Trans. Roy. Soc. Edin., vol. xlvii, 1911, pp. 591-604.

MOTTIER, D. M. ('97) : Beiträge zur Kenntniss der Kernteilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahrb. f. wiss. Bot., vol. xxx, 1897, pp. 169-204.

———— ('07) : The Development of the Heterotypic Chromosomes in Pollen Mother-cells. Ann. Bot., vol. xxi, 1907, pp. 309-47.

———— ('09) : On the Prophases of the Heterotypic Mitosis in the Embryo-sac Mother-cell of *Lilium*. Ann. Bot., vol. xxiii, 1909, pp. 343-52.

EXPLANATION OF PLATES IX AND X.

Illustrating Professor Mottier's paper on Mitosis in the Pollen Mother-cells of *Acer negundo*, L., and *Staphylea trifolia*, L.

All figures were drawn from sections with the aid of the Abbe camera lucida with Zeiss apochromatic immersion 2 mm., apert. 1·40, and compensating ocular 12. Magnification about × 2,400.

Acer negundo, L.

Fig. 1. Tapetal cell during the early growth period of pollen mother-cells.

Fig. 2. Tapetal cell at a later stage. The nucleus has divided. At the stages of Figs. 1 and 2 the nuclei show a structure identical with that of the young pollen mother-cells.

Figs. 3 and 4. Young pollen mother-cells, showing typical structure of nucleus and cytoplasm.

Fig. 5. Slightly older than Figs. 3 and 4, showing nuclear structure just prior to synapsis.

Fig. 6. The beginning of synapsis.

Figs. 7 and 8. Complete synapsis. In both cases the nucleolus has not been included in the balled-up network.

Fig. 9. The synaptic mass is beginning to loosen up, and a spireme is becoming visible.

Fig. 10. A similar stage, in which the details are much less distinct. The synaptic mass is seen from the edge, or its more indefinite lumpy nature is partly due to the reagents. The cell has just begun to round off.

Fig. 11. A later stage in the loosening up of the synaptic mass. The cell has rounded off, but the new cell-wall was not evident in this case.

Fig. 12. Complete hollow spireme at the stage of its greatest regularity and uniformity. The cell is provided with its new thick and soft wall. No longitudinal split is to be observed in the spireme.

Fig. 13. A step in what corresponds to the stage of the second contraction.

Fig. 14. The spireme is partly segmented.

Fig. 15. Segmentation just complete.

Figs. 16, 17, and 18. Later stages. Fig. 16 represents a thick radial section, while Figs. 17 and 18 are tangential. At this stage the bivalents seem to have attained their maximum size.

Fig. 19. Multipolar spindle. The bivalents have condensed into thick lumps.

Fig. 20, *a*, *b*. Showing manner of fragmentation of nucleolus just previous to, and during formation of, the spindle. At *b* the nucleolus shows a small protuberance which is frequently seen at earlier stages. Near *b* lies a bivalent chromosome, which might easily have been interpreted as a mass budding off from the nucleolus. One or more bivalents are frequently found touching the nucleolus, a phenomenon of frequent occurrence in plants.

Fig. 21. Outline of a cell at the stage of the multipolar spindle: the chromosomes and nucleolus only were drawn. This is interpreted as containing twelve bivalents and the nucleolus. At *a* and *b* the halves of the bivalents are not closely applied. If, however, the two members of *a* and *b* are in themselves bivalents, there are fourteen bivalents. The former is held to be the more probable.

Fig. 22. Stage of the mature spindle, which often has broad poles. The chromosomes are unusually elongated in this cell. The usual form is shown by the chromosome at the middle. The two members of the chromosome at the right are probably at the point of separating, or metakinesis. An indication of the longitudinal fission of each daughter segment is visible.

Fig. 23. Anaphase; the daughter segments are now seen to be split lengthwise.

Fig. 24. Cell showing the two mature daughter nuclei connected by the connecting fibres. Two extra-nuclear nucleoli are present in the cytoplasm.

Fig. 25. The division of the daughter nuclei. The chromosomes have not been brought into the equatorial plate. When the spindle is mature, the chromosomes are arranged close together in a disc as in the first mitosis. Two nucleoli are present, one being derived from each daughter nucleus.

Fig. 26. Telophase of second mitosis. Extra-nuclear nucleoli are present in the cytoplasm.

Fig. 27. The four granddaughter nuclei have been formed, and all are connected by systems of connexion fibres. In respect to chromatin and nucleoli they all appear alike.

Staphylea trifolia.

Fig. 28. Pollen mother-cell showing typical nuclear reticulum with its chromatin granules and the large nucleolus.

Fig. 29. Nucleus going into synapsis. The nucleolus is partly surrounded by the contracted reticulum.

Fig. 30. Synapsis completed. The nucleolus is almost free from the mass. The part of the reticulum extending over the nucleolus indicates the manner of spireme formation from the network or reticulum.

Fig. 31. The synaptic mass has loosened up considerably and the spireme has been formed. In the straighter portions the longitudinal fission is seen; the halves, which are twisted about each other, have separated somewhat in the long stretch crossing the nucleus. At this stage the cell is frequently rounded off at the corners.

Fig. 32. The spireme spreading out in the nuclear cavity. The longitudinal split, if present, is not well marked. This is the usual appearance of the spireme at this and the next stage, Fig. 33. The nucleolus lay in a neighbouring section.

Fig. 33. The complete hollow spireme with large nucleolus. Very small darkly stained bodies are usually present in the cytoplasm. The cytoplasmic reticulum immediately around the nucleus is finer meshed than out near the cell-wall.

Fig. 34. Nucleus showing second contraction. This represents a thick section of the nucleus. The looping is more regular and pronounced than is usually the case.

Fig. 35. All the bivalents have been formed and a large nucleolus is still present. The weft of kinoplasmic fibres running parallel with the contour of the nucleus is present midway between the latter and the cell-wall.

Fig. 36. Tangential section of nucleus showing bivalents. The long piece of the spireme may represent more than one bivalent.

Fig. 37. The same stage in radial section. The form of the bivalents is typical.

Fig. 38, *a*, *b*, *c*. Three sections including an entire nucleus in the same stage as the preceding figure. In *a* two bivalents have been pushed out of the nucleus in the preparation of the section. These three figures show the variation in the size of the bivalents and the different disposition of the two members of each other that may exist in the same nucleus.

Fig. 39. The mature spindle of the first or heterotypic mitosis. Note the zone of coarser granules in the cytoplasm surrounding the spindle and the collections of oil globules in the plane of the equator.

Fig. 40. A polar view of the same stage, showing the thick lumpy form of the chromosomes and their arrangement in a circular disc or plate.

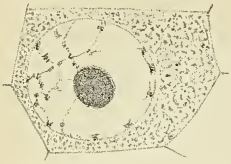
Fig. 41. The close of the anaphase. The longitudinal split in the daughter segments is scarcely visible. Cytoplasmic granules more evenly distributed.

Fig. 42. One pole of the spindle at the close of the anaphase, showing details more distinctly.

Fig. 43. One nucleus in a telophase of first mitosis. The nuclear membrane has just been formed.

Fig. 44. A polar view of a daughter nucleus at a later stage than the preceding. The masses of chromatin of varying sizes are connected by numerous fine anastomosing threads.

Fig. 45. A longitudinal section of a daughter nucleus in about the same stage as Fig. 44.



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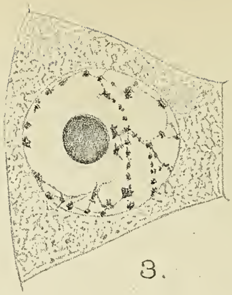

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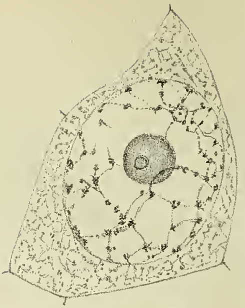

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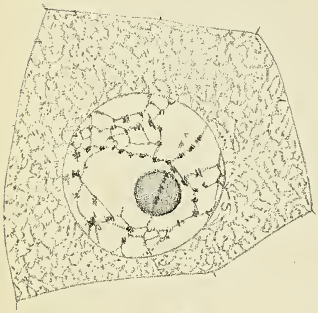

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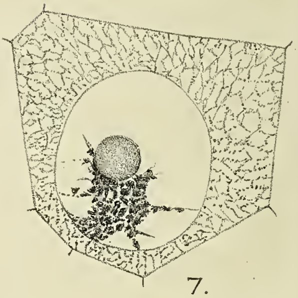

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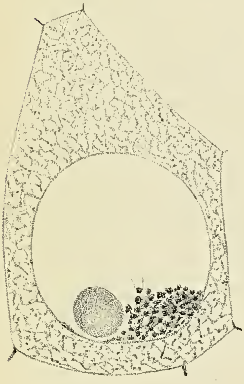

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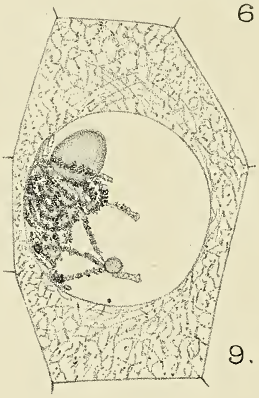

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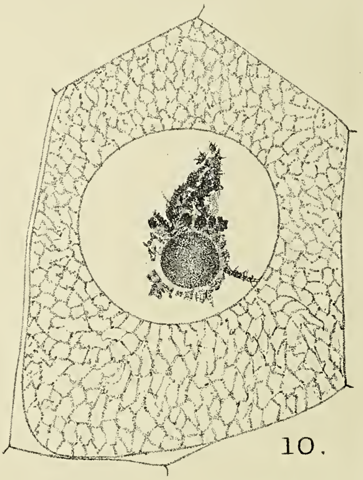

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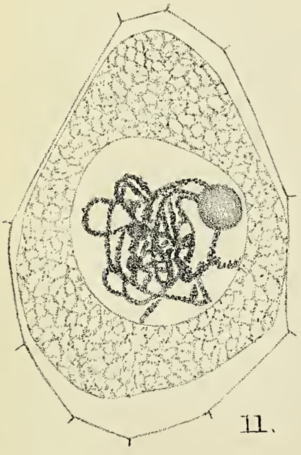

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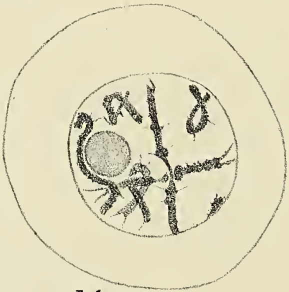

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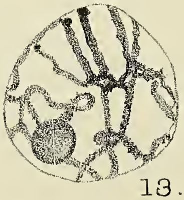

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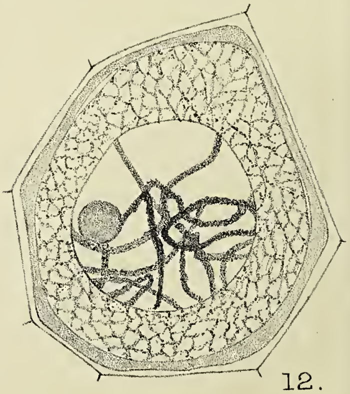

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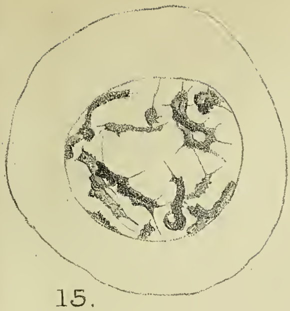
MOTTIER — MITOSIS IN POLLEN MOTHER CELLS.



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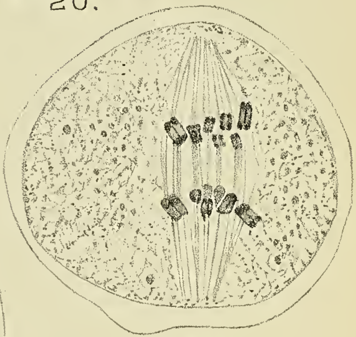

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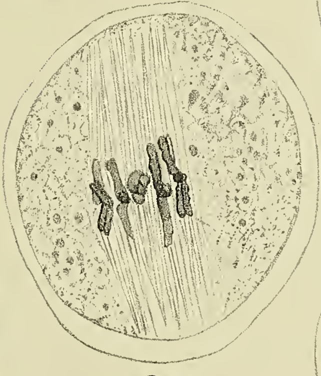

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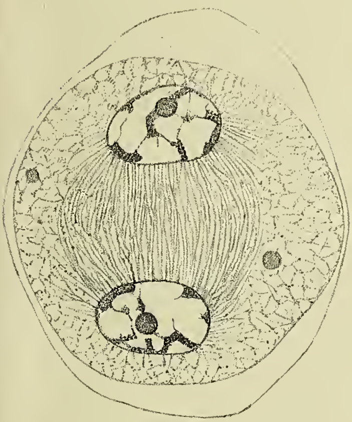

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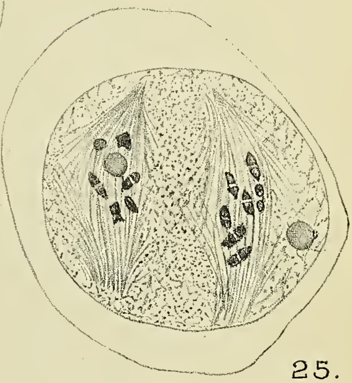

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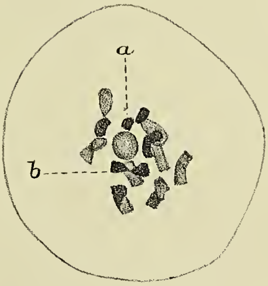

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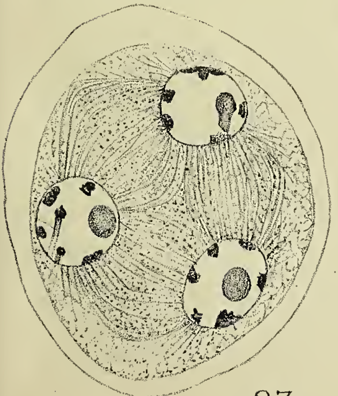

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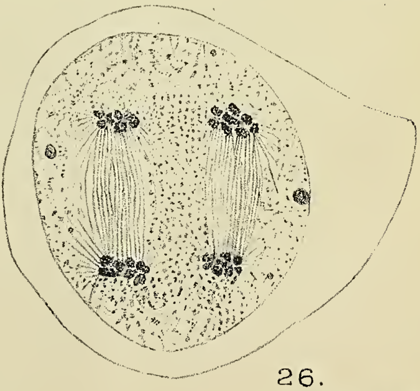

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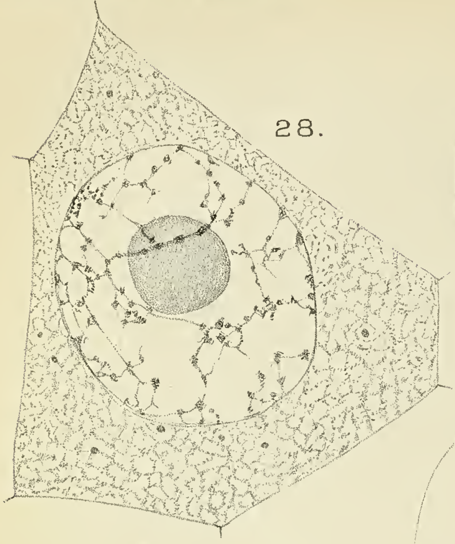

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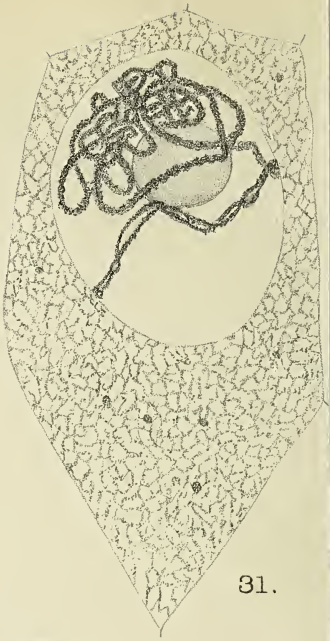

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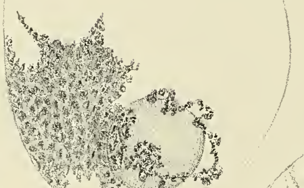

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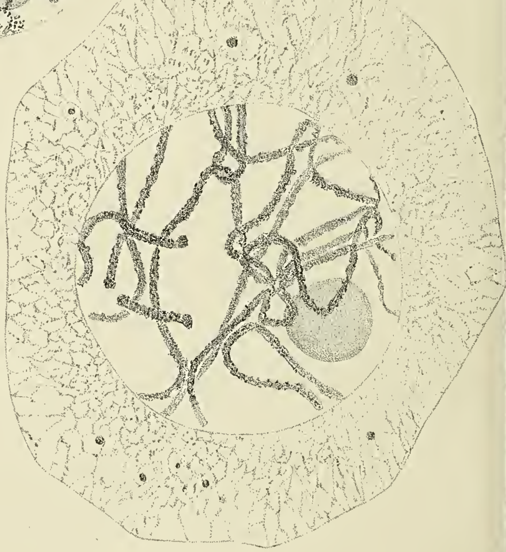

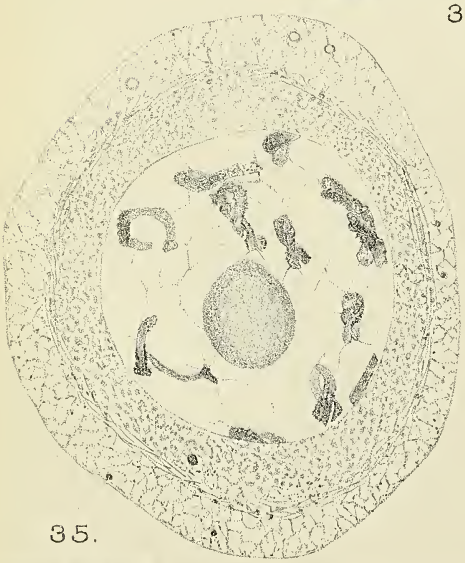

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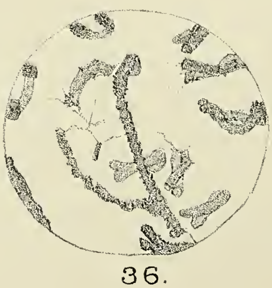

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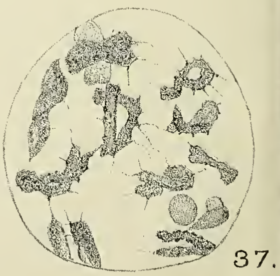

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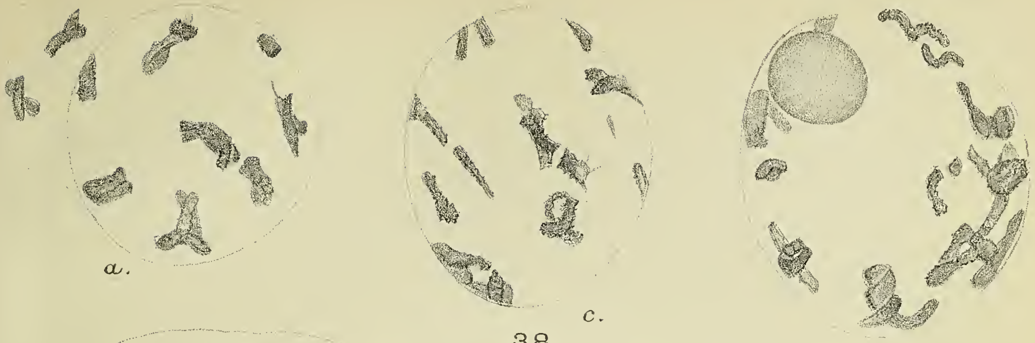
MOTTIER — MITOSIS IN POLLEN MOTHER CELLS.



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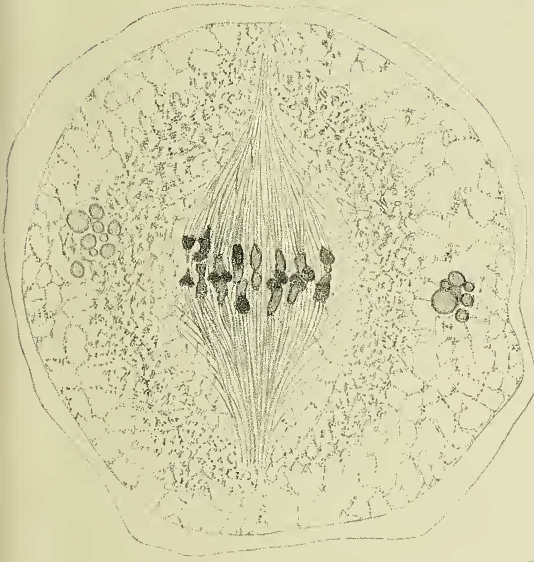

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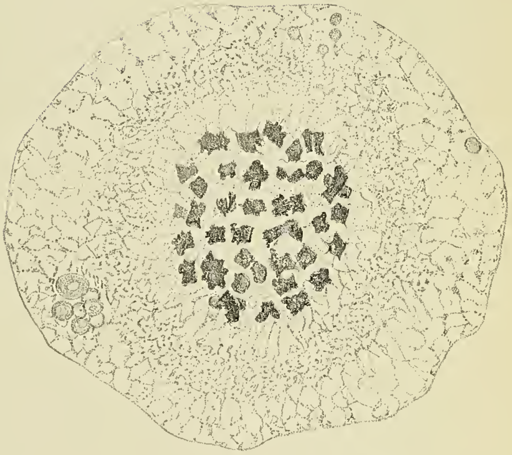

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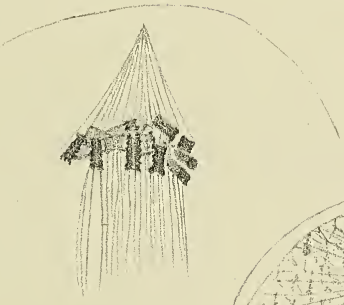

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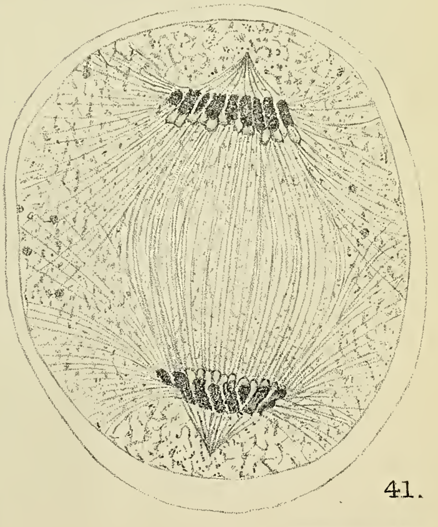

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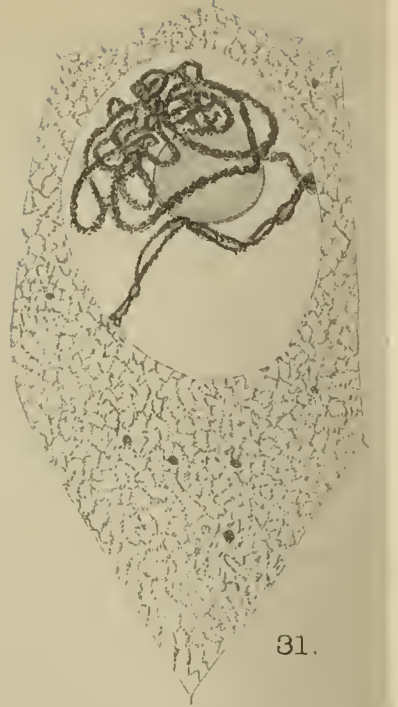

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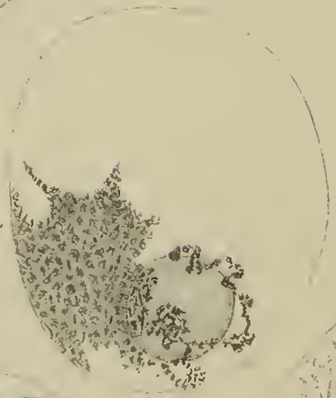

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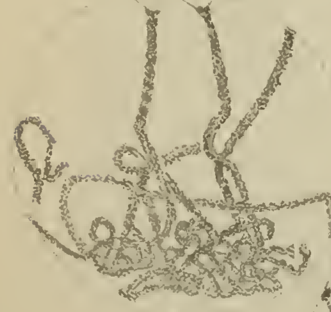

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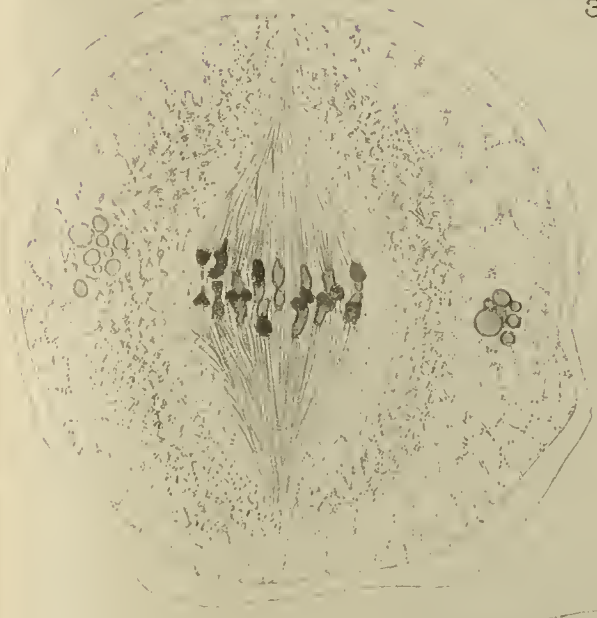

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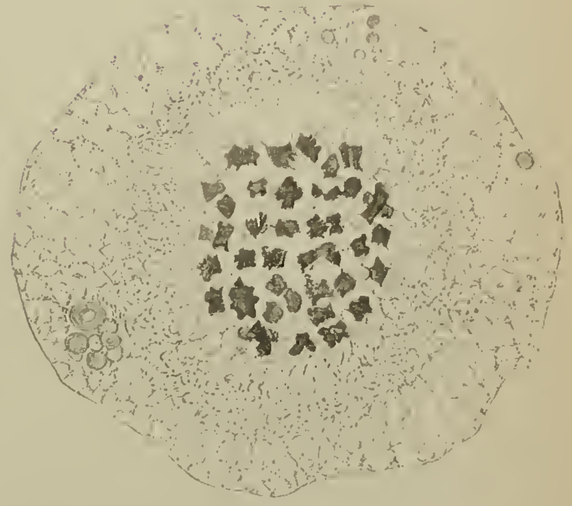

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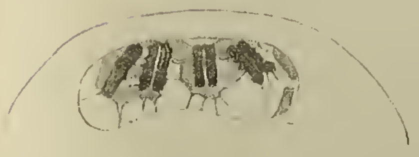

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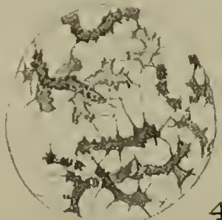

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MOTTIER — MITOSIS IN POLLEN MOTHER CELLS.

The Respiration of Plants under Various Electrical Conditions.

BY

R. C. KNIGHT

AND

J. H. PRIESTLEY,

Botanical Department, University of Leeds.

With six Figures in the Text.

INTRODUCTION.

IN a large number of field experiments carried out during recent years, plants, otherwise under normal conditions, have been subjected to an electric discharge from an overhead system of wires during a considerable portion of their growing period, and, as a result, acceleration of growth and increase in yield have been constantly reported.

It has been pointed out in a *résumé* of these experiments, published by one of us,[1] that this treatment must considerably alter many factors in the plant habitat and in the plant's reaction to this habitat, and consequently it is difficult to ascribe the effect, apparently due to the electric discharge, to any particular physiological cause.

This paper records some of the first results of an attempt to analyse the effect of the discharge upon the plant by an investigation, under laboratory conditions, of the effect produced by such discharge upon one physiological function of the plant.

The particular function first selected for investigation was respiration in so far as this is measured by the output of carbon dioxide, in part because this can be estimated fairly accurately, but more especially because it was considered that the amount of carbon dioxide evolved by the plant would give a good indication of the progress of katabolic processes within the plant. Attention was directed to these katabolic processes because it was thought that an increase of available energy within the plant might explain the general acceleration of growth upon electrification, which has been so characteristic a feature of the reports of the field trials.

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[1] Journal of the Board of Agriculture, vol. xvii, p. 14.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.]

Attention has been for some time directed to the apparent acceleration of growth and maturation reported in field trials, because this phenomenon rendered it impossible to gauge the extent to which increased yield was a direct result of electrification. If a crop matures earlier, and particularly if it is therefore harvested earlier, it may mean that the plants are not exposed to a period of bad weather during which the yield, instead of improving, may actually decrease in amount. If acceleration is a result of treatment, it needs to be considered in all cases where the causes of an increased yield have to be analysed. It therefore seemed desirable to arrive as soon as possible at a decision as to the possible cause of such an acceleration as has been reported in many field trials. The results obtained in these trials have been given at length elsewhere,[1] but in view of the importance of the question of acceleration in relation to the subject-matter of the present paper, it will perhaps be advisable to extract from the previous reports the data which formed the foundation for this research, and to subject them to further criticism in the light of later work.

ANALYSIS OF RESULTS OF FIELD TRIALS.

In the earliest experiments at Bitton, with an inefficient influence machine driven by an oil engine as the source of electrical power, and with the overhead wires close to the plants to be treated, the following results, amongst others, were obtained:

Broad beans. Decrease in yield of 15 per cent., but ready for picking five days earlier than the control.

Spring cabbages. Ready for cutting ten days earlier.

In all the other experiments from which data have been taken, the Lodge-Newman system of electrification, described in previous papers,[2] has been used.

At Evesham in 1906 the Canadian wheat under electrical treatment was ready for cutting some three or four days before that of the control area. In the case of the English wheat, the time of ripening was unchanged by electrification, and it is perhaps significant that the yields in the two cases were as follows:

	Bushels per acre: *Electrified.*	*Non-electrified.*	*Increase.*
Canadian (Red Fife)	35½	25½	39 %
English (White Queen)	40	31	29 %

Results which indicate a greater increase in yield in the crop showing acceleration in rate of growth.

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[1] Journal of the Board of Agriculture, loc. cit., and also vol. xx, p. 582.

[2] Journal of the Board of Agriculture, loc. cit., also Journal of the Royal Horticultural Society, vol. xxxvii, p. 15.

Strawberries at Evesham have on several occasions shown marked acceleration. Thus in 1907:

First complete picking, June 28th, Electrified 56 lb.
,, ,, Control 33 lb.

The size of the area electrified was only 56 per cent. of the size of the control area, so that this relative yield, if maintained, would mean an increase of about 200 per cent. as the result of electrification; but the effect was mainly acceleration, and the final difference in yield between the two plots represented an increase of only 25 per cent.

In 1908 the results with strawberries were as follows:

Pickings, June 20th and 22nd. (Areas as in 1907.)
Electrified 1,318 lb.
Control 1,876 lb.

This yield maintained would represent an increase of about 25 per cent., but the total pickings actually showed 9 per cent. less strawberries from the electrified area.

Similarly with tomatoes at Evesham in 1908; the early pickings up till September 24, when compared with the total yield, show marked acceleration in ripening under electrification.

	Electrified.		*Control.*
	A	*B*	
lb. per plant till Sept. 24th	1·36	0·36	0·10
lb. per plant total yield	4·29	3·5	2·4

From these figures it is evident that the yield from the control plants in time approaches that from the electrified. The reasons for expressing the yields from the electrified area in two sections, *A* and *B*, is that whilst plot *A* was, as far as could be ascertained, exactly comparable with the control, the plot *B* was slightly favoured as regards aspect and also irrigation.

In the Bitton experiments in 1908, where cucumbers under glass were grown under electrification, the yields in the first month seemed to indicate distinct acceleration, and are therefore given in full. They correspond to the time before the plants had grown up behind the supporting wires, which later effectually screened them from the discharge. The pickings were usually made three times a week; the dates are given in the top line of the following table:

		No. of cucumbers gathered on April:										
House No.		*10th*	*13th*	*15th*	*17th*	*20th*	*22nd*	*24th*	*27th*	*29th*	*Total.*	*Total for Season.*
1	Control	—	—	8	12	17	14	27	69	67	214	2,410
2	,,	—	14	34	86	108	82	23	22	24	393	2,477
3	Electrified	8	51	63	124	93	54	10	34	48	485	2,753
4	,,	15	67	64	110	98	55	11	26	41	487	2,710
5	,,	12	55	53	87	95	38	14	33	37	424	2,729

The foregoing data indicate that the acceleration effect occurs sufficiently frequently to be worth analysis, but a further study of the method of carrying out a field experiment has thrown a considerable amount of doubt upon the conclusions they have so far suggested.

When the distribution of the electric discharge from an overhead system of wires is studied by electrical methods, it soon becomes clear that any electrical effect produced will not be restricted to an area of ground just beneath the wires. On the contrary, when the slope of electric potential is mapped over the field, the effect of the discharge is seen to spread a number of yards on all sides of the outer wires; and when a wind is blowing the current is carried a very long distance down the wind.[1] Thus, when the matter is investigated, it may happen that a point on the control, as far removed as possible from the overhead wires, may be receiving about one-tenth the current per unit area that the ground directly beneath the overhead wires is receiving, while a proper control, obtaining only the normal atmospheric discharge, would receive at most a current that was ten thousand times less than that under the wires. Under such conditions it is clear that the control plot fails in its object. An attempt is now being made in the field (at Lincluden Mains, Dumfries) to determine the question as to whether acceleration really takes place under more rigorous experimental conditions, i. e. with a control as carefully screened from additional electric discharge as is practically possible.

It is perhaps worth pointing out that the most consistent reports as to acceleration have come from the Evesham experiments, where the areas have been large and where it is at any rate probable that the control area has to some extent been under normal conditions.[2]

Until some physiological analysis of the effect of electrification upon the katabolic and anabolic processes of the plant has been carried out, it is not possible definitely to attribute the increased yield to direct effect upon any single process in the plant.

The result of an investigation of the effect of electrical stimulation upon carbon dioxide output is given in the following pages, and although, under the conditions obtaining in our experiments, we have been able to record a distinct increase in carbon dioxide output during electrification, we have found that this increase can be wholly attributed to a rise of temperature, which takes place owing to the production of heat during the discharge. Thus we are led to conclude that the explanation of the acceleration phenomena recorded above is not to be found in the response of the respiratory function of the plant to electrification.

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[1] This is especially marked in recent experiments where the overhead wires are from 10 to 15 feet above the ground.

[2] For a fuller discussion of this question see paper in the Journal of the Board of Agriculture, vol. xx, p. 582.

This does not mean that an acceleration process, if present, is inexplicable. It may well be that one result of electrification may be increased transpiration; this alone would account for a more rapid attainment of maturity by the plant. The effect of such electrical conditions upon transpiration is now being investigated by the biophysicists of the Department of Agriculture in Washington.

The effect of electrical discharge upon the important anabolic processes of the plant is at present being further investigated, but it may be pointed out that an increased activity of constructive metabolism has been previously reported by Pollacci, Koltonski, Berthelot, and others.

This work, which has been in progress for several years, has been made possible only through various research grants that have been made from time to time. The work was started at Bristol as the result of a grant from the Colston Research Fund of the University of Bristol, and it has been possible to continue the investigation in the Botanical Department of the University of Leeds, owing to grants received from the special Research Fund placed at the disposal of the Board of Agriculture by the Development Commissioners.

During the progress of the work we have been led into fields very remote from the ordinary path of the botanist, and we have to acknowledge with gratitude the great help received from colleagues in other scientific departments; especially have we to thank Dr. A. M. Tyndall, of the Physics Department, University of Bristol, and the staff of the Physics Department of the University of Leeds for continuous kindness and advice.

EXPERIMENTAL.

In the experimental investigation of the influence of electricity upon the carbon dioxide output, it was considered advisable to employ germinating seeds and young seedlings kept in the dark, so that the reverse process of photosynthesis was eliminated.

The methods of electrification were similar to those which have been tried in the field experiments, and they may be conveniently discussed under two heads, viz.:

1. Experiments with small direct currents at a relatively low voltage.
2. Experiments with electric discharge at high tensions.

Before giving an account of the methods of applying these electrical conditions, it will be advisable to describe the apparatus used in the determination of the carbon dioxide output, after which the alterations necessitated by different methods of electrification can be easily indicated.

The respiration was quantitatively determined by a volumetric method, the amount of carbon dioxide being calculated in grammes. The method was the same in all experiments, and was essentially that advocated by Detmer.[1]

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[1] Practical Plant Physiology, Eng. ed., 1898, p. 264.

A stream of air, free from carbon dioxide, was passed over the seeds or seedlings and afterwards passed through baryta solution to absorb the carbon dioxide which the living material had evolved. Before entering the respiration vessel, the air was passed through a series of wash-bottles containing a concentrated solution of potash, or, as in the later experiments, a long tube packed with soda-lime, in order to free it from carbon dioxide. To test the purity of the air-stream another wash-bottle containing clear baryta solution was inserted before the respiration chamber. After leaving the vessel containing the living material, the air-stream entered one of a series of Pettenkofer's tubes, containing a known volume of a standard solution of barium hydrate, which absorbed the carbon dioxide, precipitating barium carbonate, and finally the air-current passed through a second wash-bottle of clear baryta to ascertain whether or not all the carbon dioxide had been removed (Fig. 1). Various methods of keeping the air-stream in motion were resorted to, including aspirators, filter-pump, and a gas-holder, but the most satisfactory was a water-pressure filter-pump, the water-supply for which was maintained at constant pressure by means of an overflow device. The rate of the air-stream was thus rendered independent of slight variations of the water pressure from the main, which were found very troublesome at first.

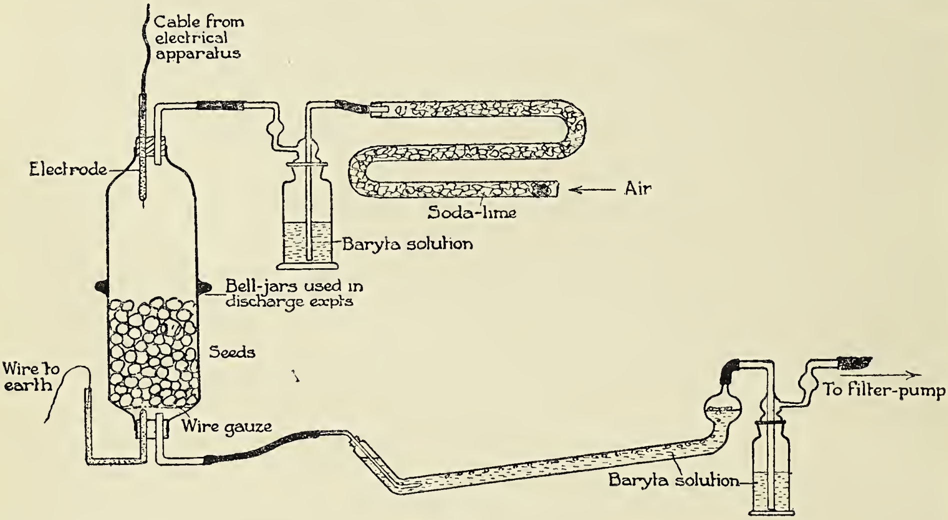

FIG. 1.

When seeds were employed, they were soaked in water for at least twenty-four hours before being experimented upon, and immediately before being introduced into the respiration chamber, the water was drained off and the seeds dried somewhat in a cloth. This removed the excess water from the outside, thus preventing the accumulation of water in the tubes of the apparatus.

The living material having been enclosed in the chamber, the air-stream was drawn through the apparatus for a definite period—half an hour, an hour, or two hours,—depending upon the time required for the evolution of a measurable quantity of carbon dioxide.

The stream was then diverted through a second Pettenkofer tube for a similar period, and so on until the required number of determinations had been made. By means of two seven-way pieces of glass tubing and six Pettenkofer tubes, each capable of being closed separately by pinchcocks and rubber connexions, this transference of the air-stream from one tube to another was rendered rapid and easy.

The titration was carried out with standard oxalic acid solution. The baryta solution in the Pettenkofer was quickly transferred to a flask of about 125 c.c. capacity, so as to introduce as little atmospheric carbon dioxide as possible, corked and shaken.

After a few minutes the precipitate had settled and 10 c.c. of the clear supernatant liquid were pipetted off into another flask and titrated from the acid burette, using phenolphthalein as indicator.

The standard solutions were kept in large bottles provided with siphoning tubes, which were attached to side tubes in the respective burettes. Air was admitted, both to stock bottles and burettes, only after passage through tubes containing sticks of potash, so as to prevent access of atmospheric carbon dioxide. The baryta solution contained approximately 9 grm. per litre, and was standardized by the oxalic acid solution, which was made up to 1·432 grm. of the crystallized acid per litre, 1 c.c. of this solution being equivalent to 0·0005 grm. carbon dioxide. This, coupled with the fact that the volume of baryta in each Pettenkofer tube was exactly 100 c.c., simplified the calculation of results.

Before entering upon the experiments under various electrical conditions, extensive control trials were carried out, in order to ascertain to what extent variations of carbon dioxide output might occur under the conditions of the experiment, apart from the influence of the electric current.

Determinations of the carbon dioxide evolved were made every half-hour from the moment the living material was introduced into the chamber, and continued without intermission for as many as six hours, the material being unelectrified the whole time. The figures obtained were then plotted against time, and a curve was thus obtained which gave an indication of the process of the respiratory function. During the investigation, as will be seen, it was found necessary to employ various types of respiration-chambers, and with each type a series of these control experiments was carried out.

1. EXPERIMENTS WITH SMALL DIRECT CURRENTS AT LOW VOLTAGES.

In these experiments the respiration chamber was a glass tube about 30 cm. long and 3·5 cm. in diameter, with rubber stoppers at both ends fitted with two delivery-tubes. The current was applied at first through unpolarizable electrodes (Detmer, loc. cit., p. 157), but these were later replaced by simple mercury-platinum contact electrodes, as with these it was found that very little progressive reduction in current strength took place. Contact was ensured by shaking the seeds well together before inserting the rubber stoppers. Current strength was measured by a calibrated Paul millivoltmeter, shunted when necessary. In the later experiments a thermometer bulb was introduced through a side tube into the respiration chamber (see Fig. 2).

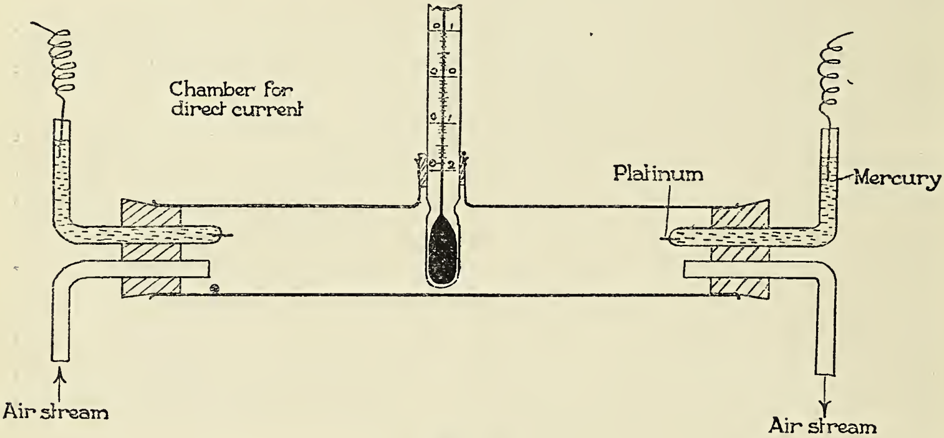

FIG. 2.

In all experiments the temperature was kept constant as far as possible, but slight variations were unavoidable. When these occurred, the carbon dioxide figure was corrected on the basis of the Van 't Hoff law for chemical reactions, a rise of 10° C. being considered to produce an increase of 100 per cent., all values being reduced to the temperature at which the first estimation was made. F. F. Blackman[1] showed that respiration approximately followed this law, and we found that a control-curve obtained under varying temperature conditions, when corrected in this manner, became identical in form with the curve obtained from respiration at constant temperature.

Peas were used in the direct-current experiments, and the control-curve was of the form shown in Fig. 3 (see also Table I).

For the sake of simplicity, a few typical results are introduced into the

[1] Report of British Association, Dublin, 1908, p. 896.

text in each case, and an account of the repetitions of many of the experiments will be found in the Appendix.

In plotting the curves, each figure for carbon dioxide is attributed to the point of time terminating the period for which that value was obtained: thus in No. 113, Fig. 3, the number 0·0100 represents the carbon dioxide output for the second half-hour and is plotted at time one hour.

The 'normal curve' of respiration, as represented by No. 113, Fig. 3, takes a decided downward trend from the beginning, its slope decreasing with time, until, about two hours from the start, the variations are reduced well below 10 per cent. This downward trend was observed in every control experiment carried out with germinating seeds, and is doubtless to be attributed to the fact that moisture is continually evaporating from the surface of the seeds, and owing to this loss of water the vital processes are retarded, the conditions gradually approaching the normal winter state, when respiratory functions are practically at a standstill.

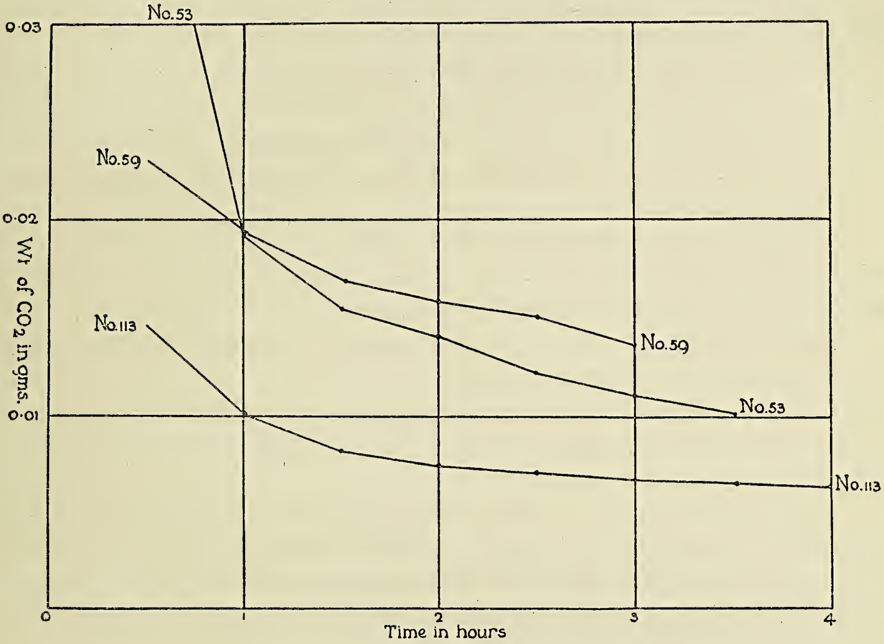

FIG. 3.

It has been observed that slight alterations in the experimental conditions have a corresponding influence upon the shape of the curve, and it is perhaps advisable to enumerate these here.

The position of the first point of course depends primarily upon the number of seeds employed and the extent to which they have germinated.

The initial slope of the curve varies, as shown by a comparison of Nos. 53 and 59, and this is probably due to the variation in the rate of evaporation, which in turn is caused by variations of initial temperature; but no trials have been carried out to substantiate this.

It will be seen that the curve of No. 113 is quite regular, and the same applies to the controls enumerated later, but Nos. 53 and 59 show irregularities, amounting to not more than about 2 or 3 per cent., at the fourth and fifth point respectively. In view of the later experiments, there is no doubt that these irregularities are due to slight changes of temperature, since similar discrepancies appeared in No. 113 and others before the temperature corrections were applied. Unfortunately, in the earlier experiments no record of temperature was obtained.

Having then determined the course of the 'normal curve' of respiration, it was obvious that there were two possible methods to adopt, viz.:

1. To allow the seeds to remain under the conditions of the experiment for, say, three hours, till the variation of normal respiration was negligible, before making any determinations, and then to measure the carbon dioxide output for two successive periods, during the second of which the seeds were electrified.

2. To make determinations of carbon dioxide output from the beginning and so ascertain the slope of the curve for the particular existing conditions, and then to apply the current and observe its effect upon the slope.

The first method was employed in many of the earlier trials, and led to many misinterpretations of results. The chief objection to it is that, with so few points on the curve available, it is impossible to determine the normal slope, and, since the slope is liable to variations, this knowledge is essential.

The second method has the disadvantage of requiring such a large number of determinations before a point of reasonable slope is reached. Consequently, a compromise was finally made, measurements of carbon dioxide being begun from one to two hours—a matter of convenience—after the introduction of the seeds, and generally continued for six or eight successive periods, each of half an hour's duration, the current being applied during the fourth or fifth, whichever seemed more advisable.

Some thirty experiments were carried out in this manner, the current densities employed varying from about 10^{-6} amperes to more than 10^{-4} amperes, the intention being to imitate the current-conditions of the field trials (see Table II). In some of the early determinations it seemed as if the current had, indeed, produced an effect upon the evolution of carbon dioxide, but in the later ones, where temperature changes were accurately recorded, there was no deviation from the normal curve following the application of the current. The apparent effect referred to was evident in only a few cases, and its magnitude and even direction varied, and it is probable that it was due to unrecorded changes of temperature.

When the current density approached the upper limit there was a slight rise in the temperature of the seeds quite independent of the temperature changes of the laboratory, and this rise was found to correspond with that which was to be expected from a calculation from current strength.[1]

This rise of temperature has probably been an important factor in many of the results recorded in the field experiments, more especially where comparatively large direct currents through the soil have been employed. Löwenherz[2] describes a rise of temperature observed in some experiments on the effect of direct currents upon the growth of barley, and suggests that the acceleration of growth which was recorded may have been due to this rise of temperature.

In the course of our direct-current experiments, doubts arose as to what proportions of the current measured actually passed through the peas and their water films respectively, and rough experiments were made to ascertain the relative conductivities of the water and the seeds. The conductivity of the respiration tube filled with water in which seeds had been soaked was first determined, and then seeds were packed in, the interstices between them being filled with water, and the conductivity was again determined. The presence of the seeds lowered the conductivity in the ratio of 2 : 1. Peas packed in a tube such as that used occupy only about one-half its volume, so that by the introduction of the seeds the area of the cross-section of the original conducting medium, water, has been reduced by about one-half, and incidentally the conductivity has been reduced in a similar ratio. From this it appears that the seeds themselves have a very high resistance, and consequently, in the ordinary low tension current experiment, by far the greater part of the measured current must pass through the water films on the seeds.

Thus we were unable to show any alteration of the katabolic processes of germinating seeds when subjected to minute low tension direct currents, but it was not possible to determine the exact current strengths in the seeds themselves. With low tension currents, then, unless the current is sufficiently strong to raise the temperature of the plant or its surroundings, no increase in the plant's respiration is to be expected. With high tension currents, no experiments have been carried out with currents greater than from 10^{-13} to 10^{-11} amp. per sq. cm.; but no direct deductions can be drawn from this fact, as with the higher tension the distribution of the current in the plant may be very different.

2. EXPERIMENTS WITH HIGH TENSION DISCHARGE.

These trials were carried out chiefly with germinating peas, although a number of experiments were made upon seedlings. The method used was

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[1] But this calculation was based upon the formula for the heating effect of a current passing through a conductor. If the seeds behave as an electrolyte to the passage of the currents this calculation will not apply.

[2] Zeitschr. f. Pflanzenkrankheiten, vol. xviii, 1908, p. 336.

identical with that described above, except in the form of the respiration chamber employed, which consisted of two bell-jars with ground flanges fitted together and rendered airtight with a vaseline-rubber composition. Each bell-jar was stoppered with a rubber stopper through which passed a delivery-tube and a platinum-mercury electrode. (See Fig. 1.) The platinum wire of the lower electrode was attached to a disc of wire gauze on which the seeds rested, thereby ensuring good contact, whilst the mercury was 'earthed' by means of a wire connected to a water-pipe. The upper electrode was connected to the positive pole of the discharge apparatus by means of a rubber insulated cable running through quartz tubes borne on porcelain telegraph insulators.

The air-stream entered the chamber through the upper delivery-tube, and left it through the lower.

In the early experiments the source of electricity was a large influence machine kept running by a motor, but later the Lodge-Newman installation was used. This was much more satisfactory, the electrical output of the influence machine being very variable, and to some extent at the mercy of the weather.

It will be convenient to describe first the series of control experiments which were carried out under different conditions, as it is only in the light of these control experiments that experiments with the electric discharge can be interpreted.

1. Seeds were packed into the lower of the two bell-jars, and the curve of respiration determined as in the low tension current experiments, with the result that it was found impossible to depend upon obtaining a smooth normal curve every time. The irregularities were sometimes in one direction and sometimes in another, and it was thought to be due to the fact that the close packing of the seeds prevented efficient aeration, the respiration being thus rendered partially anaerobic (Table III). In view of later observations, however, it seems likely that at least some of the irregularities were due to temperature variations, which were not recorded.

2. The seeds were separated from each other by layers of glass wool to ensure proper aeration, and the normal curve was again determined. The irregularities here were much less marked, and accordingly glass wool was always used in subsequent experiments (Table IV).

3. Electric discharge from a point in air causes the interaction of the gases of the air, with the formation of oxides of nitrogen and ozone, and probably, if water vapour is present, a little hydrogen peroxide.

Owing to misinterpretations of some of our results, coupled with some conclusions arrived at by Hill and Flack,[1] who found that very small quantities of ozone were toxic to animals, it was thought that these gases had a deleterious effect upon the plants, and steps were taken to prevent

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[1] Proc. Roy. Soc., 84 B., 1911, p. 404.

them from reaching the living material. A small vessel of turpentine was introduced into the respiration chamber above the peas, which was found to be effective in absorbing the toxic gases. (See later experiments as to the effect of gases on seedlings.)

Since turpentine was used in many of the experiments, it became necessary to carry out controls with it, to ascertain its effect, if any, upon respiration. Accordingly the respiration curve was determined for peas separated by glass wool as in 2, with a vessel of turpentine in the respiration chamber, discharge not being applied.

The curve was found to coincide with the normal, the small amount of turpentine vapour which was unavoidably present in the atmosphere having no effect upon respiration (Table V).

4. The possibility of the decomposition of the turpentine by the discharge was next considered, and it was realized that if this took place, an evolution of carbon dioxide was a conceivable consequence, this being naturally undesirable, since carbon dioxide output was taken as a criterion of respiration.

To test this, a vessel of turpentine was placed in the respiration chamber, no seeds being present, and the discharge switched on, a stream of air being drawn through the whole apparatus as described above.

Analysis of the baryta solution in the Pettenkofer tube showed that no carbon dioxide had been evolved (Table VI).

Having completed these controls, full results of which will be found in the Appendix, experiments upon the effect of electrification upon the seeds were proceeded with.

When a platinum-mercury contact electrode was used to apply the discharge, it was found that under the conditions of experiment the current produced was very much in excess of that used in the field trials, and since our experiments were intended to be comparable with those trials, it was thought expedient to employ currents of the same order of magnitude. In order to cut down the voltage available with our apparatus, the following device was adopted and found effective. The tube of the electrode (see Fig. 1) was filled with xylol instead of mercury, and the cable from the high tension installation rested in the xylol, which completed the contact with the platinum discharge point. By varying the length of the column of xylol between the ends of cable and platinum points, and thereby varying the resistance, it was possible to vary the current passing from the discharge point.[1]

When, however, this distance was below about 1 centimetre, a new factor became evident in the passing of sparks between the end of the cable and the platinum wire. The heat produced caused the xylol finally to boil, and it became necessary to switch off the discharge. Another disadvantage

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[1] See N. R. Campbell, Phil. Mag., Ser. vi, vol. 22, p. 301, Appendix to paper on Delta Rays.

of the short xylol resistance is the fact that sparks are liable to penetrate the glass tube and pass down the side of the respiration chamber, incidentally allowing the xylol to escape on to the seeds. Although quartz electrodes were tried instead of glass ones, this difficulty was never entirely obviated. A series of experiments was carried out using different current densities, but owing to the obstacle mentioned we were unable to get any current densities above 3×10^{-6} amperes, except that obtained with a mercury-platinum contact electrode, namely about 5 or 6×10^{-6} amperes.

The method of procedure in these discharge trials was the same as that described for the direct-current experiments, i. e. the seeds were introduced into the respiration vessel and maintained under normal conditions for a time, determinations of carbon dioxide output being made every half-hour. These values gave an indication of the trend of the normal curve for the particular set of seeds under observation, and the discharge was switched on usually during the fifth half-hour from the start, two or three determinations being made after the return to the unelectrified condition.

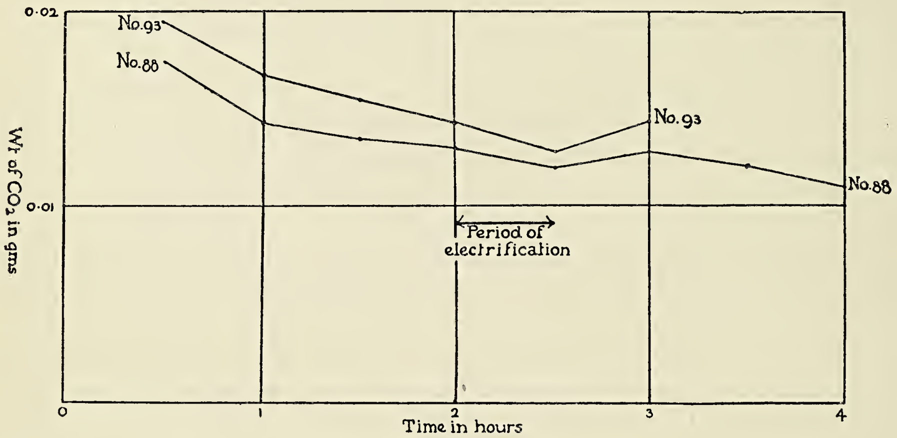

FIG. 4.

The results of these experiments are given in full in the Appendix, and it will be seen that discharge producing a current density of less than about 3×10^{-6} amperes has no effect upon the respiration of the seeds, the normal curve being uninterrupted at any point. When, however, a current density of 3×10^{-6} amperes was reached there was a definite irregularity in the curve (see Fig. 4). The irregularity consists of a rise which takes place, not during the period of electrification, but during the half-hour following it. Sometimes the rise is very slight, and in one case (No. 90) there was no actual rise, but the slope of the curve was diminished, indicating that the carbon dioxide output was nevertheless greater, consequent upon electrification, than it otherwise would have been. The magnitude of this general accelerating effect of the discharge seemed to bear a definite relation to the current strength (Tables VII and VIII).

Having demonstrated that the application of overhead discharge caused an increase in the respiration of the seeds, it became necessary to analyse further the cause of the increase.

The first source investigated was the gases produced by the discharge in air, as it was thought these might have some effect upon the katabolic processes of the plant. This was investigated in the following manner:

Two pairs of bell-jars were fitted up as respiration chambers and connected in series in the apparatus. The first was left empty except for an earthed piece of wire about half-way down, where the upper level of the mass of seeds would ordinarily be. The second chamber contained the seeds and had no electrodes and no turpentine, but merely delivery-tubes.

The discharge now took place in the first chamber, and the gases were led into the second. In this way there was much less chance of ions from the discharge point reaching the peas.

With this method the respiration was found to be unaffected by the discharge, and the curves show no deviation from the normal (Table IX) except in one reading, No. 121. 4, the significance of which is doubtful. (See note *re* temperature corrections, p. 145.)

We concluded, therefore, that the gaseous products of the discharge in air had no effect upon the respiration of seeds, which was rather surprising in view of the results of Hill and Flack working with ozone. The source of the effect produced by the discharge must therefore be sought in another direction.

Experiments were carried out in which the period of electrification was extended from half an hour to three hours (Nos. 96–8), and the usual increase in carbon dioxide output was observed, but on dismantling the apparatus at the conclusion it was noticed that the temperature of the chamber was very much above that of the room; especially was this the case in No. 97, in which the amount of respiration was enormously increased (see Fig. 5). Attention was at once directed to the influence which this rise of temperature might have when the discharge was continued for only half an hour.

Accordingly a trial was proceeded with (No. 98), wherein the temperature of the seeds was taken from time to time by a thermometer inside the chamber. The discharge was switched on for one hour, and determinations of carbon dioxide were made every half-hour. By this means the curve (No. 98) in Figs. 5 and 6 was obtained. This curve was then subjected to two tests to determine to what extent temperature was the active factor in inducing the upward trend. The figures obtained were corrected in the manner indicated for the direct-current experiments (p. 142) and the new curve (Fig. 6) compared with the normal curve and No. 98.

The corrected curve, it will be seen, almost coincides with the normal curve.

A series of experiments was carried out in this manner, the corrected curves showing a similar coincidence with the normal curve (Table XI). It should be noted here, and the same applies to all temperature corrections, that such corrections can only be regarded as approximate owing to the difficulty of making accurate determinations of temperature.

The rate of conduction of heat through peas is very slow, and in some extreme cases it was observed that after electrification there was a difference of temperature between the highest and lowest level of seeds of 6° C. This is liable to increase the possible error of temperature readings, which, in order to be consistent, were always taken with the thermometer bulb just covered with seeds, and readings were taken as often as convenient, viz. at intervals of five or ten minutes.

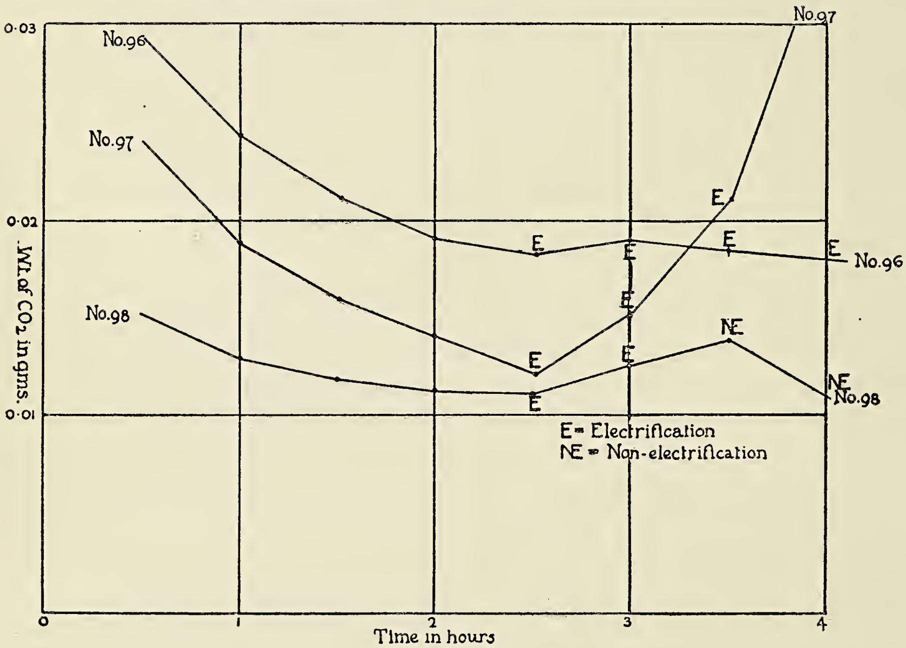

Fig. 5.

This inaccuracy probably accounts for the fact that the corrected curve mentioned above is not quite smooth, as a normal curve should be.

Steps were now taken to ascertain if, as the form of the corrected curve seemed to suggest, when temperature effects were eliminated, the effect of the discharge upon respiration was also eliminated.

This was first attempted in an indirect manner thus:

The respiration chamber was immersed in a large water-bath, and instead of applying the discharge, the temperature was regulated by means of the bath, and was made to vary to the same extent and at the same intervals as in No. 98.

By this means all effects of the discharge, other than those caused by temperature changes, would be eliminated, and any discrepancies between the curve obtained in this case and that in No. 98 would be due to stimuli other than a rise of temperature.

In this experiment (No. 99) the curve obtained was almost exactly parallel to that obtained in No. 98 (see Fig. 6). The only divergence appeared at the last two points, and during these periods the temperature of the water-bath, and consequently that of the seeds, did not correspond exactly to the readings in No. 99. When these two are corrected the discrepancy is even less (Table XII).

Thus the whole effect of the discharge recorded in No. 98 was obtained merely by altering the temperature and in the absence of any other effects of the discharge.

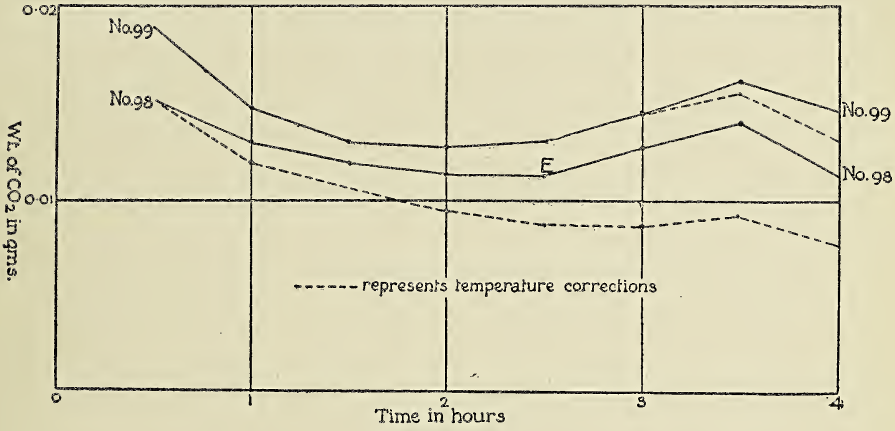

FIG. 6.

Attempts were now made directly to eliminate the effects of temperature by keeping the seeds at a constant temperature during electrification. Three methods were adopted (Table XIII):

1. The respiration chamber was immersed in a water-bath, the temperature of which was lowered as that of the seeds tended to rise under the influence of the discharge. This was found impracticable, owing to the low thermal conductivity of the peas (No. 103).

2. A thick layer of glass wool was superposed upon the seeds to absorb the heat evolved, but this was quite ineffectual (No. 104).

3. The glass-wool layer was supplemented with a layer of peas previously killed by prolonged boiling, but this also failed to keep the temperature of the live peas at a constant level (No. 105).

Thus we were unfortunately unable directly to eliminate the effects of temperature from the experiments, with a discharge producing relatively high currents.

A suggestion presented itself to the effect that the rise of temperature so constantly observed might be the result, and not the cause of the acceleration of the katabolic processes accompanying it, but this explanation was confuted by experiments upon the electrification of dead peas (Table XIV). In this case it was found that there was again a rise of temperature, whereas there was certainly no acceleration of the katabolic processes, the conclusion being that the increased carbon dioxide output in the experiments upon living seeds was indeed due to the temperature rise.

Some experiments were also carried out upon the effect of the electric discharge upon the respiration of seedlings. The plants used included peas, brussels sprouts, wheat, and rye, the two last proving the more satisfactory, on account of their more rapid germination and larger evolution of carbon dioxide per unit space occupied.

The seeds were planted in sand in the lower bell-jar of the apparatus described on pp. 145 and 146, and the delivery-tube for the exit of the air-stream was made to reach the surface of the sand. The second hole through the stopper was left open for drainage purposes whilst the plants were growing, and was later used for the 'earthed' electrode, which merely made contact with the sand which was penetrated by the roots of the plants.

When the plants had grown to a convenient height, the upper bell-jar was waxed on and the whole chamber covered with thick brown paper to prevent access to light, thus precluding the possibility of photosynthesis taking place, and control experiments were carried out under these conditions.

The carbon dioxide output was measured for several consecutive periods without any electrification, and it was found that the respiration, as in the case of the seeds, gradually decreased as the experiment proceeded, but with the seedlings the variation was never more than 5 per cent. after corrections had been made as usual to compensate for temperature changes (Table XV).

Accordingly, instead of finding a normal curve in each experiment, as in the case of seeds, it was considered permissible to measure the carbon dioxide evolved during two successive periods, the discharge being switched on at the beginning of the second. The length of each period was usually one hour, sometimes two, a convenient quantity of carbon dioxide being produced in this time. In the results, differences of less than 5 per cent. were disregarded as insignificant in view of the information obtained from controls.

A series of experiments was carried out under these conditions, employing current densities up to 10^{-6} amperes, which were obtained by means of the xylol resistance previously described. Above this strength no results were obtained, owing to the failure of the xylol to withstand the heat produced (see p. 147).

In no case was it possible to record any significant variation of the amount of carbon dioxide produced consequent upon the application of the discharge, the differences being always less than 5 per cent. (Table XVI).

Experiments with discharge from a mercury-platinum contact electrode, giving a current density of 3 to 6 × 10^{-6} amperes, were attempted, but no quantitative observations were made owing to the tendency of sparks to pass from the discharge wire to the plants, thereby causing them to shrivel.

The effect of the gaseous products of the discharge in air upon seedlings was then tested, the method being identical with that used for similar tests with seeds (p. 149).

The discharge in the first chamber was maintained for periods varying from five minutes to two hours, the products being drawn into the second chamber containing the seedlings.

The result was a rather unexpected one, namely, an apparent increase in the carbon dioxide produced, in one case a matter of 67 per cent. When the discharge was on for only five minutes the increase was inconsiderable (Table XVII).

In addition, when the plants were examined after the experiment, it was found that the chlorophyll in the upper portions had been bleached by the oxidizing agents produced in the atmosphere by the discharge.

If a vessel of turpentine was introduced into the chamber containing the seedlings, the deleterious effect of the gases was obviated, leaving the plants unaffected by continued application of discharge in the first chamber.

Some controls under very rigorous conditions were carried out to ascertain whether the products of the discharge—the oxides of nitrogen in particular—were liable to vitiate the foregoing experiments by neutralizing some of the baryta solution independent of carbon dioxide production. Accordingly the products of discharge in an empty chamber were led (1) directly through baryta solution in a Pettenkofer; (2) through a chamber containing dead peas, and then through baryta solution. The period in each case was thirty minutes, and the current density was approximately 6 × 10^{-5} amperes, the highest employed in any experiments.

The result showed a maximum production of acid equivalent to 0·0011 grm. carbon dioxide in the case where the gases were led directly through the alkali. Where peas intervened—the normal case—the carbon dioxide equivalent was 0·0002 grm. (Table X).

Thus in the experiments upon the effect of the products of discharge upon seedlings the apparent increase in the amount of carbon dioxide evolved was probably due to the acidity of the oxides of nitrogen present, but in the experiments with seeds these gases were absorbed before reaching the baryta solution.

As a result of this work we have reached the conclusion that the electric discharge has no direct effect upon the respiration of germinating seeds and seedlings, but under laboratory conditions, wherein the plants are in a confined space, the heating effect of the discharge may provide a stimulus causing an acceleration of the katabolic processes and a consequent increase in the carbon dioxide production.

The failure of the discharge itself to produce any stimulation of the destructive metabolism of the organism should not, however, be emphasized overmuch when seeking an explanation of the results of the large scale trials. As has been pointed out before, although these results cannot be interpreted in terms of katabolism, other functions of the plants may be accelerated as a consequence of electrification, resulting finally in earlier maturity.

In view of the nature of our conclusions there is little to be said as to their application to field trials.

With the current density employed in the field—10^{-12} to 10^{-10} amperes per square centimetre—the heating effect will certainly be negligible, and the effect upon respiration therefore nil. In addition, were the heating effect, and no other, involved, there would be no economic value in applying electric discharge in order to obtain it, since the same result might be arrived at by much less elaborate methods. This, however, is a question upon which no final judgement can be passed until the reported acceleration effect in the field has been further analysed.

SUMMARY.

1. Field trials upon the effect of electrical conditions upon plant growth have suggested that an increased crop or an earlier ripening may be the result of such treatment, and in the present paper an attempt has been made to analyse this apparent effect, by determining the effect of such electrical conditions upon respiration.

2. Our experiments have shown that direct currents of a density 10^{-6} to 10^{-4} amperes have no effect upon the respiration of peas, other than that due to accompanying changes of temperature. The proportion of these currents actually traversing the peas was, however, probably very small, the majority being taken by the water films on the seeds.

3. Overhead discharge, producing a current of density less than 3×10^{-6} amperes, has no effect upon respiration. When higher currents are employed a definite increase of the carbon dioxide output was observed.

4. This increase can be wholly attributed to the rise of temperature caused by the discharge.

5. In the field, where the currents are too small to produce any appreciable rise of temperature, electrification will have no effect upon respiration, and explanation of the acceleration of growth must be sought in other functions of the plant.

6. The gaseous products of the discharge in air have no effect upon germinating peas, but are deleterious to young seedlings.

APPENDIX.

This portion of the paper consists of a detailed account of some of the experiments carried out, including repetitions. Reference to these has been made in the foregoing pages, and typical experiments have been quoted.

Full discussion has already been entered upon, and therefore only necessary explanations accompany the following figures.

The numbers, unless otherwise stated, represent the weight of carbon dioxide in grammes.

Experiments marked * are those in which corrections have been made for temperature differences, and only the corrected numbers given.

E represents a period of electrification, and a bracketed number following it the current density in amperes. Where none is given, no measurement was made.

TABLE I. CONTROLS WITH CHAMBER USED FOR DIRECT CURRENT EXPERIMENTS (p. 143).

Experiment No. *Estimation No.*	53	59	113*
1	0·0381	0·0229	0·0146
2	0·0191	0·0192	0·0100
3	0·0153	0·0168	0·0081
4	0·0139	0·0157	0·0073
5	0·0121	0·0148	0·0069
6	0·0108	0·0134	0·0066
7	0·0099	—	0·0064
8	—	—	0·0062

N.B.—The number of experiments here is small, but was considered sufficient owing to the fact that the conditions were almost identical with those in the control experiments with the chamber for discharge upon seeds (Table IV).

TABLE II. EFFECT OF DIRECT CURRENT ON PEAS.

Experiment No. *Estimation No.*	54	55	56	57	58
1	0·0402	0·0360	0·0349	0·0494	0·0262
2	0·0283	0·0304	0·0225	0·0288	0·0207
3	0·0236	0·0264	—	0·0156	0·0179
4	0·0220 E (0·3 × 10^{-6})	0·0222 E (0·6 × 10^{-6})	0·0154	0·0119 E (0·5 × 10^{-6})	0·0166 E (0·5 × 10^{-6})
5	0·0171	0·0176	0·0127	0·0090	0·0155
6	0·0154	0·0158	0·0113 E (0·5 × 10^{-6})	0·0092	0·0156
7	0·0135	0·0125	0·0106	—	—
8	—	—	—	—	—

Continued.

Experiment No. *Estimation No.*	65	63	64	62	66
1	0·0350	0·0273	0·0236	0·0344	0·0234
2	0·0227	0·0202	0·0180	0·0290	0·0184
3	0·0182	0·0187	0·0157	0·0252	0·0162
4	0·0143 E (5·8 × 10^{-6})	0·0144 E (6 × 10^{-6})	0·0145 E (6 × 10^{-6})	0·0189 E (6·5 × 10^{-6})	0·0134 E (7·4 × 10^{-6})
5	0·0134	0·0140	0·0113	0·0157	0·0115
6	0·0117	0·0131	0·0119	0·0117	0·0101
7	0·0091	0·0127	—	0·0089	0·0090

TABLE II. *Continued.*

Experiment No. *Estimation No.*	68	67	61	69	70
1	0·0478	0·0504	0·0347	0·0346	0·0347
2	0·0403	0·0415	0·0284	0·0279	0·0266
3	0·0327	0·0362	0·0250	0·0250	0·0222
4	0·0260	0·0316	0·0219 E	0·0230	0·0197
			$(17{\cdot}6 \times 10^{-6})$		
5	0·0212	0·0275 E	0·0186	0·0206 E	0·0181 E
		$(15{\cdot}1 \times 10^{-6})$		$(20{\cdot}4 \times 10^{-6})$	$(20{\cdot}6 \times 10^{-6})$
6	0·0188 E	0·0249	0·0159	0·0178	0·0162
	$(13{\cdot}9 \times 10^{-6})$				
7	0·0170	0·0221	0·0143	—	0·0144
8	0·0143	—	—	0·0168	0·0128

Continued.

Experiment No. *Estimation No.*	60	71	72	73	74
1	0·0250	0·0188	0·0190	0·0375	0·0246
2	0·0202	0·0162	0·0155	0·0258	0·0214
3	0·0177	0·0134	0·0126	0·0214	0·0183
4	0·0159	0·0118	0·0105	0·0186	0·0160
5	0·0148 E	0·0095 E	0·0087 E	0·0164 E	0·0130 E
	(24×10^{-6})	(25×10^{-6})	(30×10^{-6})	$(47{\cdot}3 \times 10^{-6})$	$(50{\cdot}5 \times 10^{-6})$
6	0·0141	0·0085	0·0083	0·0152	0·0119
7	—	0·0078	0·0075	0·0140	0·0101
8	—	0·0070	—	0·0135	0·0094

Continued.

Experiment No. *Estimation No.*	75	76	77	78	79
1	0·0277	0·0145	0·0369	0·0281	0·0264
2	0·0233	0·0105	0·0243	0·0209	0·0188
3	0·0199	0·0094	0·0203	0·0186	0·0161
4	0·0169	0·0088 E	0·0174	0·0160	0·0153
		$(75{\cdot}6 \times 10^{-6})$			
5	0·0151 E	0·0078	0·0143 E	0·0140 E	0·0133 E
	(67×10^{-6})		$(80{\cdot}4 \times 10^{-6})$	$(83{\cdot}1 \times 10^{-6})$	$(96{\cdot}4 \times 10^{-6})$
6	0·0141	0·0072	0·0126	0·0132	0·0132
7	0·0132	0·0070	0·0122	0·0129	0·0121
8	0·0122	—	0·0111	0·0116	0·0103

Continued.

Experiment No. *Estimation No.*	116*	117*	114*	115*
1	0·0149	0·0168	0·0166	0·0115
2	—	0·0129	0·0139	0·0094
3	0·0096	0·0111	0·0112	0·0076
4	0·0088	0·0097	0·0091	0·0070
5	0·0086 E	0·0084 E	0·0091 E	0·0077 E
	$(135{\cdot}8 \times 10^{-6})$	$(145{\cdot}5 \times 10^{-6})$	$(149{\cdot}8 \times 10^{-6})$	$(151{\cdot}2 \times 10^{-6})$
6	0·0078	0·0076	0·0084	0·0084
7	0·0076	0·0068	0·0067	0·0089
8	0·0069	0·0065	0·0063	0·0094

In No. 115 the temperature variations were very considerable, 3° C., and the corrections are therefore probably not to be considered accurate.

TABLE III. CONTROLS WITH CHAMBER FOR DISCHARGE METHODS. PEAS NOT SEPARATED BY GLASS WOOL.

Experiment No. *Estimation No.*	1	2	3	4	5	6
1	0·0369	0·0600	0·0161	0·0226	0·0169	0·0227
2	0·0371	0·0612	0·0281	0·0348	0·0262	0·0316
3	0·0437	0·0683	0·0275	0·0331	0·0261	0·0278
4	0·0332	0·0505	0·0245	0·0259	0·0223	0·0255
5	0·0289	0·0519	0·0238	0·0244	0·0223	0·0247
6	0·0385	—	0·0229	0·0237	0·0226	0·0228

Continued.

Experiment No. *Estimation No.*	7	8	9	10	11	12
1	0·0107	0·0106	0·0226	0·0140	0·0165	0·0086
2	0·0275	0·0114	0·0348	0·0118	0·0215	0·0131
3	0·0182	0·0121	0·0331	0·0105	0·0200	0·0106
4	0·0159	0·0124	0·0259	0·0100	0·0181	0·0107
5	0·0156	0·0103	0·0244	0·0097	0·0174	0·0108
6	0·0178	0·0113	0·0237	0·0094	0·0174	0·0096
7	—	—	—	0·0109	0·0180	0·0096
8	—	—	—	0·0104	0·0162	0·0083
9	—	—	—	—	0·0184	0·0089

TABLE IV. CONTROLS WITH CHAMBER FOR DISCHARGE METHODS. PEAS SEPARATED BY GLASS WOOL. NO ELECTRIFICATION.

Experiment No. *Estimation No.*	13	14	15	16
1	0·0337	0·0347	—(?)	0·0608
2	0·0319	0·0317	—(?)	0·0446
3	0·0307	0·0278	0·0416	0·0363
4	0·0295	0·0237	0·0369	0·0287
5	0·0292	0·0223	0·0349	0·0227
6	0·0281	0·0194	0·0304	0·0208
7	—	0·0179	0·0296	0·0187
8	—	0·0158	0·0279	0·0172
9	—	0·0136	0·0264	0·0155
10	—	—	0·0250	0·0140
11	—	—	0·0241	0·0125
12	—	—	0·0227	—
13	—	—	0·0213	—

TABLE V. CONTROLS WITH CHAMBER FOR DISCHARGE METHODS. VESSEL OF TURPENTINE WITHIN. SEEDS SEPARATED BY GLASS WOOL.

Experiment No. *Estimation No.*	17	18	19	20	136
1	—	0.0423	0·0549	0·0420	0·0299
2	0·0152	0·0389	0·0494	0·0355	0·0276
3	—	0·0331	0·0399	0·0317	0·0239
4	—	0·0275	0·0336	0·0261	0·0195
5	0·0148	0·0239	0·0282	0·0228	0·0165
6	0·0140	0·0215	0·0242	0·0191	0·0138
7	0·0135	0·0195	0·0235	0·0171	0·0126
8	0·0132	0·0191	0·0216	0·0165	—
9	0·0117	0·0185	0·0208	—	—
10	—	0·0181	0·0185	—	—
11	—	0·0177	—	—	—
12	—	0·0173	—	—	—

TABLE VI. CONTROLS. ELECTRICAL DISCHARGE UPON TURPENTINE. NO SEEDS PRESENT.

The figures represent the volume of standard oxalic acid solution required to neutralize 10 c.c. of the baryta solution after the passage of the air current.

Experiment No. *Estimation No.*	23	24	26
1	23·59	23·52	23·09 E
2	23·61 E	23·51 E	23·12
3	23·63	23·57	23·11
4	23·61 E	23·41 E	—

The maximum difference here is 0·16 c.c., equivalent to 0·00008 grm. CO_2, which is within the limits of experimental error.

TABLE VII. EFFECT OF ELECTRICAL DISCHARGE UPON SEEDS.

This table includes preliminary experiments with high current densities. No current measurements were made, but a mercury-platinum electrode was used in each case.

Experiment No. *Estimation No.*	21	22	25	29
1	0·0228	0·0373	0·0248	0·0662
2	0·0180	0·0317	0·0199	0·0441
3	0·0147	0·0263	0·0172	0·0333 E
4	0·0130	0·0250 E	0·0160 E	0·0367
5	0·0191 E	0·0175	0·0153	0·0423
6	0·0181	0·0128	0·0148	—
7	0·0170	—	0·0147	—
8	0·0151	—	0·0158 E	—
9	0·0140 E	—	0·0163	—
10	0·0153	—	0·0166	—
11	0·0125	—	0·0159	—
12	0·0110	—	0·0151	—

Continued.

Experiment No. *Estimation No.*	137	138
1	0·0355	0·0421
2	0·0319	0·0327
3	0·0276	0·0301
4	0·0223 E	0·0282
5	0·0252	0·0230 E
6	0·0220	0·0256
7	—	0·0239

TABLE VIII. CONTINUATION OF TABLE VII.

These results were obtained using a xylol resistance in the electrode (p. 147).

In Nos. 81–7 inclusive the current density was less than 3×10^{-6} amperes.

Experiment No. *Estimation No.*	81	82	83	84	85	86	87
1	0·0240	0·0203	0·0180	0·0230	0·0260	0·0224	0·0174
2	0·0177	0·0167	0·0167	0·0168	0·0197	0·0195	0·0154
3	0·0162	0·0149	0·0156	0·0148	0·0168	0·0175	0·0139
4	0·0148	0·0133	0·0150	0·0139	0·0150	0·0158	0·0132
5	0·0138 E	0·0125 E	0·0153 E	0·0135 E	0·0132 E	0·0147 E	0·0126 E
6	0·0134	0·0121	0·0145	0·0135	0·0125	0·0138	0·0122
7	0·0127	0·0114	0·0144	0·0135	0·0120	0·0129	0·0116
8	0·0120	0·0108	0·0140	—	0·0113	0·0121	0·0112

There is an indication in Experiments 81, 82, 83, 84, and 85 that electrification has caused a modification in the respiration curve, but the change was so slight as to be almost within the limits of experimental error, and was therefore deemed insignificant.

Table VIII. *Continued.*

Experiment No. *Estimation No.*	88	89	90	91	92	93
1	0·0175	0·0154	0·0203	0·0182	0·0230	0·0194
2	0·0144	0·0124	0·0167	0·0153	0·0177	0·0168
3	0·0136	0·0116	0·0156	0·0145	0·0156	0·0155
4	0·0129	0·0111	0·0150	0·0142	0·0145	0·0143
5	0·0120 E	0·0107 E	0·0142 E	0·0139 E	0·0135 E	0·0129 E
	(3×10^{-6})	(3×10^{-6})	(7×10^{-6})	(7×10^{-6})	(10×10^{-6})	(30×10^{-6})
6	0·0129	0·0111	0·0140	0·0148	0·0146	0·0144
7	0·0122	0·0108	0·0130	0·0145	0·0132	0·0127
8	0·0112	0·0102	0·0126	—	0·0123	—

Table IX. Effect of the Products of Discharge upon Peas.

Experiment No. *Estimation No.*	120*	121*	122*	123*	124*
1	0·0304	0·0230	0·0293	0·0249	0·0407
2	0·0254	0·0154	0·0251	0·0204	0·0253
3	0·0208	0·0132	0·0213	0·0183	0·0211
4	0·0170	0·0132 E	0·0177	0·0164	0·0180
5	0·0143 E	0·0118	0·0156 E	0·0152 E	0·0160 E
6	0·0125	0·0105	0·0139	0·0143	0·0145
7	0·0112	—	0·0130	0·0137	0·0133
8	0·0101	—	0·0125	0·0133	0·0127

N.B.—A strip of paper moistened with a solution of starch and potassium iodide indicated the presence of the oxidizing gases in the second chamber almost immediately after switching on the discharge in the first, but none could be detected in the tubes between the peas and the baryta solution.

Table X. Effect of Continued Discharge. Platinum-Mercury Contact Electrode.

Experiment No. *Estimation No.*	96	97
1	0·0293	0·0240
2	0·0243	0·0187
3	0·0212	0·0159
4	0·0191	0·0143
5	0·0183 E	0·0120 E
6	0·0190 E	0·0153 E
7	0·0185 E	0·0210 E
8	0·0181 E	0·0361 E
9	0·0172 E	—
10	0·0168 E	—

No temperature readings were taken, but in No. 97 the final temperature was noticeably very much above room temperature.

Table XI. Continuation of Table X.

In the following experiments temperatures were taken, and on the correction of temperature changes the curves follow a normal course.

The carbon dioxide figures given are those actually measured.

Experiment No.	102		106		118	
Estimation No.	*Carbon Dioxide Measurements.*	*Temp.*	*Carbon Dioxide Measurements.*	*Temp.*	*Carbon Dioxide Measurements.*	*Temp.*
1	0·0209	10·8° C.	0·0140	14·2° C.	0·0316	15·8° C.
2	0·0165	11·2° C.	0·0126	14·2° C.	0·0211	16·5° C.
3	0·0154	11·7° C.	0·0115	13·3° C.	0·0182	17·1° C.
4	0·0148	12·1° C.	0·0108	13·5° C.	0·0172	17·6° C.
5	0·0130 E	12·2° C.	0·0107 E	16·4° C.	0·0159 E	17·8° C.
6	0·0142 E	12·8° C.	0·0101	16·4° C.	0·0149	17·8° C.
7	— E	13·7° C.	0·0094	16·1° C.	0·0142	17·8° C.
8	0·0150 E	14·1° C.	0·0086	15·6° C.	0·0138	17·9° C.

N.B.—In No. 102 the temperature of the upper layers of seeds was higher than those given, which were recorded about the centre of the mass. This explains the fact that in this case the corrected figures all lie slightly above the normal curve.

TABLE XI. *Continued.*

Experiment No.	119		107	
Estimation No.	*Carbon Dioxide Measurements.*	*Temp.*	*Carbon Dioxide Measurements.*	*Temp.*
1	0·0321	14·7° C.	0·0183	12·8° C.
2	0·0187	15·5° C.	0·0142	13·1° C.
3	0·0143	16·5° C.	0·0129	13·8° C.
4	0·0138	16·9° C.	0·0118	14·3° C.
5	?0·0127 E	18·9° C.	0·0116 E	18·5° C.
6	0·0157	20·3° C.	0·0112	17·5° C.
7	0·0137	20·1° C.	0·0096	15·3° C.
8	0·0134	20·2° C.	0·0086	14·7° C.

TABLE XII. EXPERIMENTS 98 AND 99. (See text.)

Experiment No.	98			99		
Estimation No.	*Carbon Dioxide Measurements.*	*Temp.*	*Corrected Values.*	*Carbon Dioxide Measurements.*	*Temp.*	*Corrections to Temps. in No.* 98.
1	0·0152	13·9° C.	0·0152	0·0188	13·9° C.	—
2	0·0129	14·7° C.	0·0119	0·0147	14·7° C.	—
3	0·0118	15·3° C.	0·0104	0·0129	15·3° C.	—
4	0·0112	15·8° C.	0·0094	0·0127	15·8° C.	—
5	0·0112 E	16·8° C.	0·0087	0·0129	16·8° C.	—
6	0·0126 E	18·6° C.	0·0086	0·0143	18·6° C.	—
7	0·0138	19·3° C.	0·0090	0·0160	19·8° C.	0·0152
8	0·0110	18·3° C.	0·0076	0·0145	19·4° C.	0·0130

TABLE XIII. ATTEMPTS TO PREVENT RISE OF TEMPERATURE. (See text.)

Numbers represent mean temperatures for half-hour periods.

Experiment No.	103	104	105
Estimation No.			
1	13·0° C.	13·3° C.	14·2° C.
2	13·3° C.	13·8° C.	14·3° C
3	13·3° C.	14·3° C.	14·7° C.
4	13·9° C.	14·6° C.	15·3° C.
5	14·2° C. E	15·0° C. E	16·4° C. E
6	14·4° C. E	17·2° C.	16·9° C.
7	14·7° C. E	16·9° C.	16·4° C.
8	—	16·6° C.	15·8° C.

103. Chamber in a water-bath. Temperatures of lower layers of seeds recorded. Final temperature of upper layers 18·6° C.

104. Seeds covered with glass wool. Temperatures of upper layers recorded.

105. Glass wool and dead seeds over live seeds. Temperatures of upper layers of living seeds recorded.

TABLE XIV. TEMPERATURE CHANGES OF *DEAD* PEAS UNDER ELECTRICAL DISCHARGE.

100		101		
Time.	*Temp.*	*Time.*	*Room Temp.*	*Temp. of Seed.*
3.20	8·9° C.	9.55	12·2° C.	12·2° C.
3.40	10·0° C.	10.10	12·2° C.	12·2° C.
4.0	11·1° C.	10.25	12·2° C.	12·2° C.
4.15	11·7° C.	10.40	12·8° C.	12·2° C.
4.30	12·2° C.	10.55	12·8° C.	12·8° C.
Electrification continuous.	Thermometer bulb at the bottom of the chamber. Final temp. of top 17·8° C.; of centre 16·7° C.	11.15	12·8° C.	12·8° C.
		11.30	13·3° C.	13·3° C.
		11.40	13·3° C.	13·3° C.
		11.55	13·3° C.	13·9° C.
		12.10	13·9° C.	13·9° C.
		12.30	14·4° C.	13·9° C.
		12.50	14·4° C.	14·4° C.
		Electrification continuous.	Final top temperature 22·2° C.	

TABLE XV. CONTROLS WITH APPARATUS FOR ELECTRICAL DISCHARGE UPON SEEDLINGS. NO ELECTRIFICATION.

Experiment No. *Estimation No.*	108 (*Wheat*)	125 (*Wheat*)*	126 (*Wheat*)*	131 (*Rye*)	132 (*Wheat*)*	
1	—	0·0120	—	0·0087	0·0039	Total 0·0141
2	0·0025	0·0115	—	0·0095	0·0039	
3	0·0026	0·0110	0·0131	0·0092	0·0033	
4	0·0029	0·0110	0·0131	—	0·0030	
5	0·0030	—	0·0125	—	0·0136	
6	0·0029	—	0·0121	—	0·0132	
7	0·0031	—	—	—	—	
8	0·0030	—	—	—	—	

No. 108. 30-minute periods.
Nos. 125 and 126. 60-minute periods.
No. 131. 120-minute periods.
No. 132. 1, 2, 3, and 4, 30-minute periods; 5 and 6, 120-minute periods.

TABLE XVI. ELECTRICAL DISCHARGE UPON SEEDLINGS.

Experiment No. *Estimation No.*	109* (*Wheat*)	110* (*Wheat*)	111* (*Wheat*)	112* (*Peas*)	133 (*Wheat*)	134* (*Wheat*)	135* (*Wheat*)
1	0·0133	0·0117	0·0120	0·0091	0·0137	0·0130	0·0129
2	0·0132 E	0·0116 E	0·0119 E	0·0098 E	0·0139 E	0·0129 E	0·0121 E
	(10^{-6})	(3×10^{-6})	(3×10^{-6})	(10×10^{-6})	(10×10^{-6})	(6×10^{-6})	(10×10^{-6})

120-minute periods in all cases.

No. 112. Discharge applied for ten minutes only on account of the passage of sparks to the plants. This probably caused the increase in carbon dioxide production owing to a large local rise of temperature which would be unrecorded.

TABLE XVII. EFFECT OF THE GASEOUS PRODUCTS OF DISCHARGE UPON WHEAT SEEDLINGS.

Experiment No. *Estimation No.*	127*	128*	129*	130*
1	0·0302	0·0139	0·0100	0·0151
2	0·0261	0·0134	0·0167 E	0·0152 E
3	0·0283 E	0·0150 E	—	—

No. 127. Temperature correction for 2 was large, and would probably account for the difference between 1 and 2.

Nos. 127 and 128. Electrification 30 minutes.
No. 129. Electrification 15 minutes.
No. 130. Electrification 5 minutes.

TABLE XVIII. EFFECT OF THE PRODUCTS OF THE DISCHARGE UPON THE BARYTA SOLUTION.

The figures given are the oxalic acid equivalent of 10 c.c. of the baryta solution after the passage of the air-stream.

Experiment No. *Conditions of Experiment.*	139	140
1. No discharge	29·66	26·39
2. Discharge over dead peas	29·60	26·34
3. Discharge in empty chamber	29·43	26·17

The Structure and Development of the Prothallus of Equisetum debile, Roxb.

BY

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Professor of Botany, Government College, Lahore.

With forty-five Figures in the Text.

INTRODUCTION.

ALL the species of *Equisetum* whose prothalli had been investigated before 1905 are confined to Europe (Goebel, p. 195). The writer is not aware if any extra-European species have been investigated since then. As the prothalli of *Equisetum debile* whose range is given by Baker ('Fern Allies', p. 5) as 'Tropical Asia from the Himalayas and Ceylon eastward through the Malay Isles to Fiji', were found growing in large numbers along the banks of the river Ravi in Lahore, and as they differed in general characters from the prothalli hitherto described, it was thought that a study of the development might bring out some interesting points. The result of this study carried on in the winter of 1912–13 is given in the following pages.

Aitchison and Stewart describe *Equisetum debile* as the only species of *Equisetum* occurring in the Punjab, and certainly this is the only species met with in or near Lahore. It may be mentioned that Baker remarks that this species is doubtfully distinct from *Equisetum ramosissimum*, Desf., which is cosmopolitan in the warm temperate and tropical zones, but nothing is known as regards the prothallus of this latter species.

MATERIAL.

The plant grows in and near Lahore in great abundance along the banks of the river in sandy soil or in the shady and swampy soil of the wood along the river. In the former place it remains tufted and small, being only a foot or two high, but in the wood it may reach a height of 10 to 15 feet, being supported by the neighbouring trees, and attain a thickness of half a centimetre. For general characters Baker's 'Fern Allies' may be consulted. The small spikes are formed at the ends of ordinary branches

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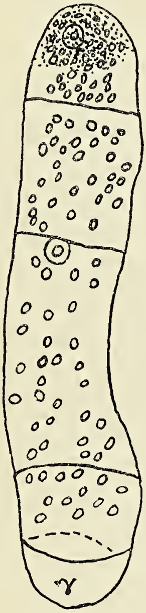
and are ripe in September to October. The spores were often found in bluish-green masses filling the space between the last normal leaf-sheath and the stem below the spike.

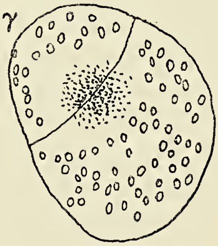
For very early stages the spores were grown in the laboratory in soil brought from the river bank where the prothalli were ordinarily found growing. The soil, which is a mixture of sand and clay, was sterilized by heat before the spores were sown. For the study of the mature prothallus, material was always collected from the river-side.

GERMINATION OF THE SPORE.

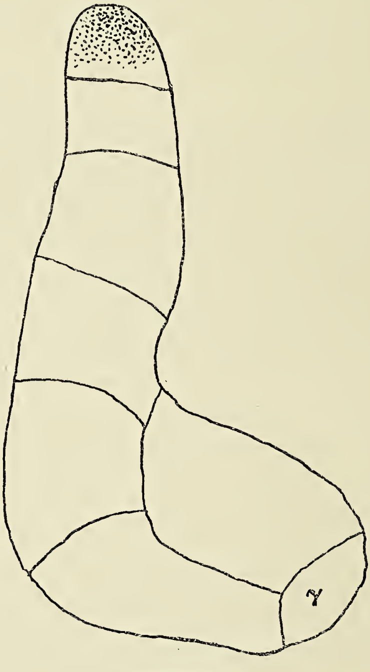
The spores germinate readily. The first wall is curved, and it divides the spore into a small cell containing a few chloroplasts and a larger cell which contains most of the chloroplasts. Later on the chloroplasts disappear from the small cell (Figs. 1 and 2). The next divisions are very variable. The small cell may grow out soon into a rhizoid, or may remain as it is for a much longer time, or it may not grow out at all. The larger cell may grow in length and become divided by transverse walls only, so that it forms a longer or shorter filament (Figs. 2 and 10). In other cases a longitudinal wall may be first formed in the larger cell, and either one or both of the cells thus formed may grow forward or laterally and divide by transverse walls (Fig. 3). In yet other cases the walls in the larger cell may



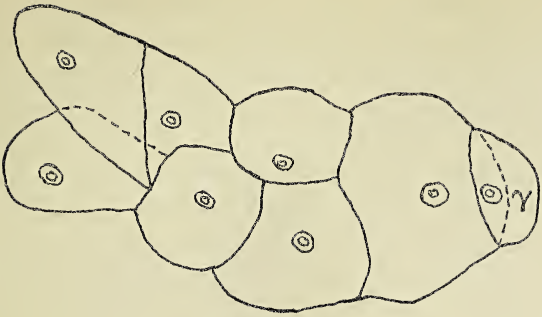
TEXT-FIG. 2.



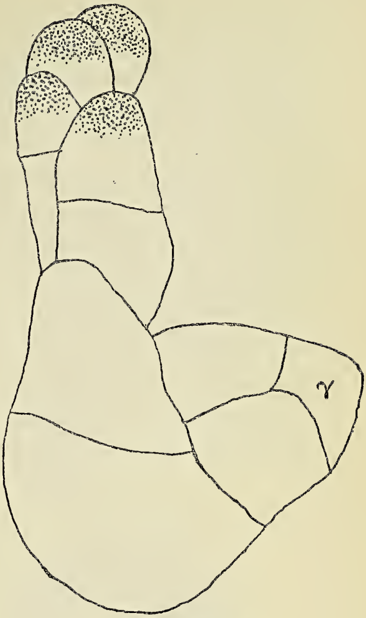
TEXT-FIG. 1.



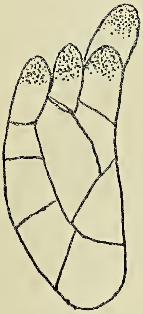
TEXT-FIG. 3.



TEXT-FIG. 4.

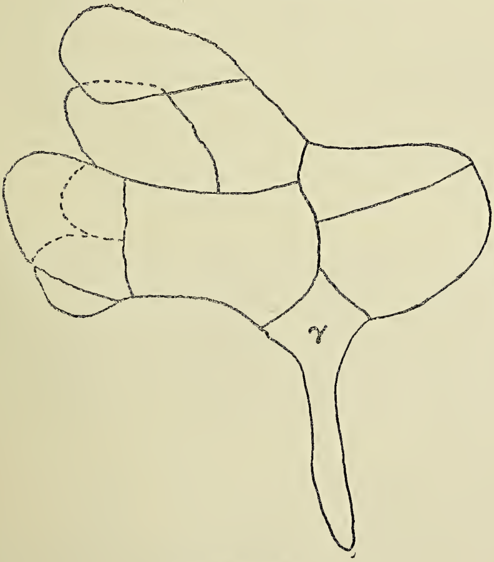


TEXT-FIG. 7.

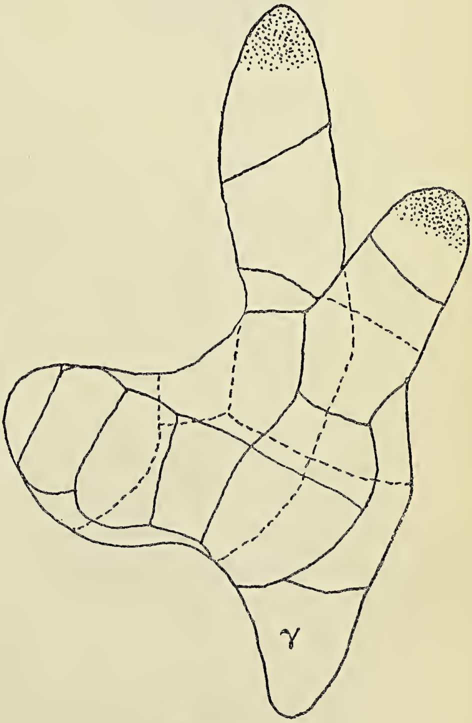


TEXT-FIG. 5.

TEXT-FIG. 6.

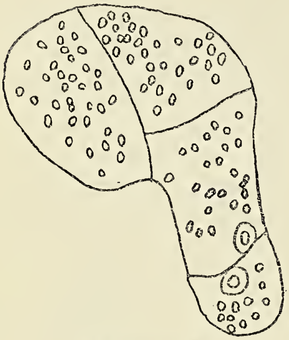


TEXT-FIG. 8.

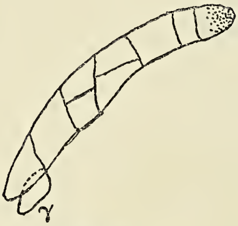


TEXT-FIG. 9.

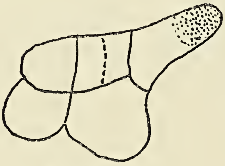
be so formed that a cell-mass results (Figs. 5, 7, 9). After the formation of these cell-walls, cells from the surface may project upwards by increase in length (Figs. 5, 6) and ultimately by further divisions develop into the characteristic lobes; or only a single cell projects outwards from the cell-mass, and this in its turn gives rise to several cells which project upwards and form the characteristic lobes (Fig. 7). Thus when a cell-mass is first formed after the germination of the spore the prothallus body may be formed directly or indirectly through the formation of a 'primary tubercle'. In the meantime rhizoids grow out from the lower surface which fix the erect prothallus to the soil (Figs. 14, 15).


TEXT-FIG. 10.

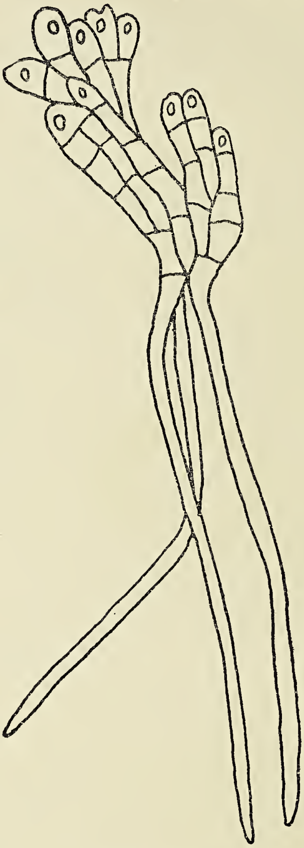

TEXT-FIG. 11.



TEXT-FIG. 12.


TEXT-FIG. 13.

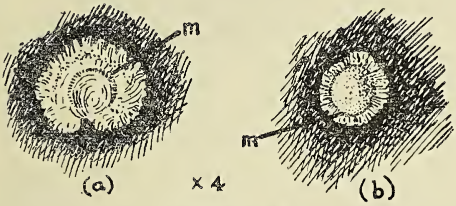

TEXT-FIG. 14.


TEXT-FIG. 15.

MATURE PROTHALLUS.

As described above, the lobes arise from single cells which have increased in length and project above the general level, and are naturally quite small and simple at first. As these cells grow they become divided by transverse and vertical walls, and more cells from the side of these rudimentary lobes begin to grow vigorously and project outwards. Thus the lobes become branched, and although united at their bases to the main body of the prothallus (as yet only the cell-mass more or less changed by this time) and to each other, they are quite free above (Figs. 14, 15). Soon the cells at the base of all the lobes begin to divide so that they contribute to the tissue of the cell-mass below and form new lobes above. Thus the structure of the mature prothallus is established.

Numerous prothalli were found growing wild on the river bank, less than one millimetre in diameter, looking like green or red pin-heads. Even at this stage they have the same essential structure as the large prothalli of a diameter of 3 centimetres. They are minute hemispherical cushions consisting of compact parenchymatous tissue in their lower half, and of perfectly erect lobes, with narrow spaces between them, in their upper half. A very good idea of their structure can be obtained by saying that they resemble miniature cauliflowers without the surrounding leaves. The prothallus is firmly fixed to the soil by numerous rhizoids. The rhizoids are so numerous and so firmly attached to the mud that it is very difficult to wash it away, and usually most of the rhizoids are removed during the operation. The rhizoids are smooth long hyaline unicellular structures and do not present any peculiarity.



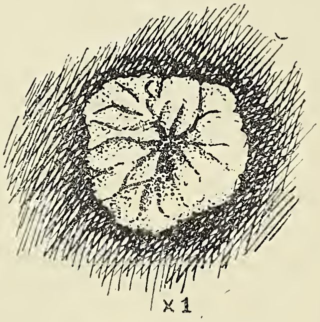
TEXT-FIG. 16.

TEXT-FIG. 17.

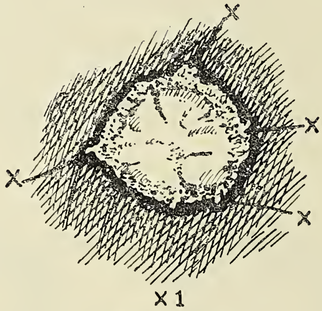
At a stage when the diameter of the hemispherical prothallus is about $\frac{1}{8}$ to $\frac{1}{4}$ of an inch (3 to 6 millimetres) it is usually of a circular outline with an entire margin (Fig. 16 *b*). Often, however, the outline is cordate or reniform with a notch on the *posterior* end, probably due to the manner of cell-division in the early stages. When a cell-mass has given rise to the prothallus directly, the outline naturally would be circular, as the meristem is developed uniformly on all sides. If the prothallus is formed after the formation of filaments or a 'primary tubercle', the meristem arises late on the *posterior* end and has a reniform or cordate outline (Figs. 16 *a*, 17). There is, however, no trace of the 'primary tubercle' in the older stages.

The lower part of the prothallus, as stated above, consists of parenchymatous cells without any air spaces between them. The cells are usually all similar, but sometimes some cells are elongated in the radial direction, and all are thin walled. All the cells in this part are full of starch grains, the chloroplasts which were present in the whole body in the early stages having been transformed into leucoplasts. This transition is gradual and the upper layers of the compact part are green like the lobes.

The meristem is situated all along the margin (Figs. 16, 17) on the under surface, and it produces the parenchymatous cells of the compact portion and the lobes of the upper spongy portion. The ventral position of the meristem explains the fact that the under surface of the prothallus is almost always concave. The upper spongy portion consists, as stated above, of lobes. Each lobe arises from the single projecting cell of the meristem, which divides at first by transverse walls and then by other walls in various directions. Later, some cells from the side of the lobe grow out and behave like the cells of the meristem, and the lobe thus becomes branched. The lobe at its base is several cells thick, but higher up it becomes thinner and the ultimate part is only a single layer of cells in thickness. The terminal cells in all cases are very turgid and either spherical or more or less deeply lobed and form a tuft (Figs. 23–27).



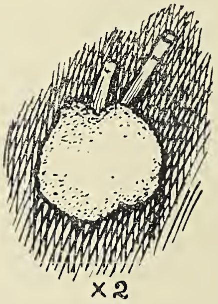
Text-fig. 18.



Text-fig. 19.

Text-fig. 20.

Text-fig. 21.



Text-fig. 22.

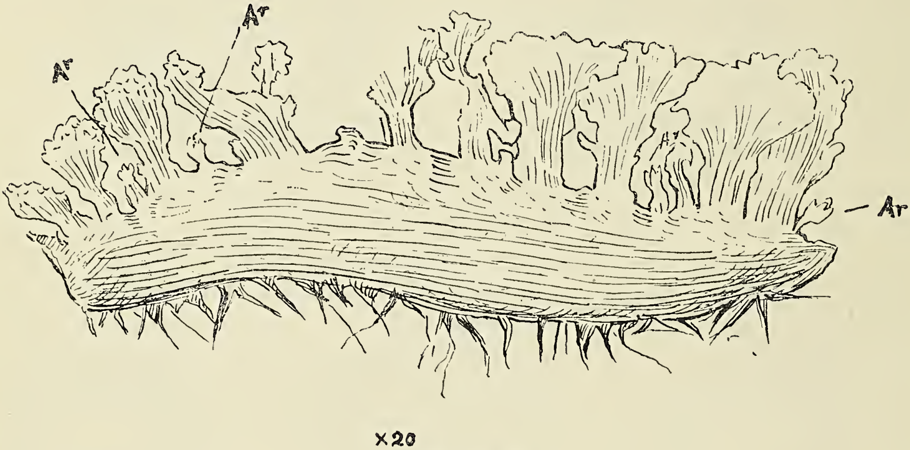
On account of the ventral position of the meristem the young lobes are directed obliquely forwards and upwards, but are soon carried upwards and become perfectly erect.

The upper surface of a comparatively small prothallus has the form shown in Fig. 22; it is quite smooth and slopes down gradually towards the margin, but in large prothalli furrows and ridges appear on the surface (Fig. 18) partly owing to the unequal height of the lobes and partly to the inequalities of the soil. These furrows are, however, quite superficial.

The margin of the prothallus is quite entire in small prothalli (Figs. 16 *a* and 16 *b*), but as the prothallus increases in size the whole body of the prothallus becomes lobed. This is chiefly due to mechanical reasons, as the prothallus in its advance comes into contact with foreign bodies which interfere with the growth. Moss plants were often found in the fissures between these lobes of the prothallus; also a compact form of *Riccia crystallina*, which is extremely common on the river-side, and similar bodies. The prothallus may have an inherent tendency to divide, but it cannot be definitely stated as regards this species. According to Campbell dichotomy of the archegonial meristem does occur in prothalli of other species ('Mosses and Ferns,' p. 453). Although the first cleft was found in many cases in the median line in many heart-shaped prothalli, this was not always the case. Two such fissures are shown in Fig. 17. The margin of the prothallus on the two sides of the fissure are in intimate contact with each other, so that there is hardly any space between them. The existence of these fissures cannot be detected from an examination of the prothallus from above; in most cases it is necessary to wash away the mud from the ventral surface to demonstrate their presence.

Looked at from above the prothallus appears merely to be spongy, and one would not suspect the existence of separate lobes inside, so closely together are they situated. This compactness, however, varies to some extent with external conditions. The colour of the prothallus also varies similarly. If the prothallus grows in an exposed place directly exposed to intense light, it is invariably compact and red in colour. (If one part of the prothallus is exposed to direct sunlight and the other shaded—e. g. if the prothallus happens to be growing in a pit obliquely—the two colours may be seen in the same prothallus.) There is absolutely no trace of the lobes from the surface. The red colour is due to the presence of a pigment in the numerous chloroplasts of the upper cells of the lobes in addition to chlorophyll. The red colour easily comes out if the prothallus is placed in absolute alcohol, while chlorophyll remains in the chloroplasts for a much longer time. The pigment is probably a decomposition-product of chlorophyll. If the prothallus grows in a shaded place under some tree or in some small pit, or if it is kept in the laboratory, it is always of a bright green colour and less compact. If a compact prothallus of a red colour is brought to the laboratory and kept inside the room in very dim light with plenty of water, the new parts formed are yellowish green in colour and much more open than the older parts. The lobes also are longer, less broad, and more branched (Figs. 19, 23, 24, 26). This condition may be compared to etiolation in Flowering plants.

A well-grown prothallus that has been growing for some months may be as much as 3 centimetres in diameter; usually it is only 2 to 3 millimetres in height, including the lobes. The smaller prothalli also are of about the same height, so that the height does not vary so much as the diameter. Usually a little less than one-half of the height is formed by the lower compact part of the prothallus, and it consists of 6 to 12 layers of cells. The rest of the height is formed by the projecting lobes (Fig. 23).

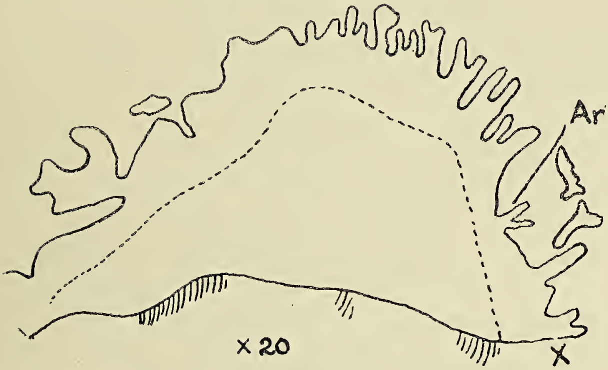


Text-fig. 23.

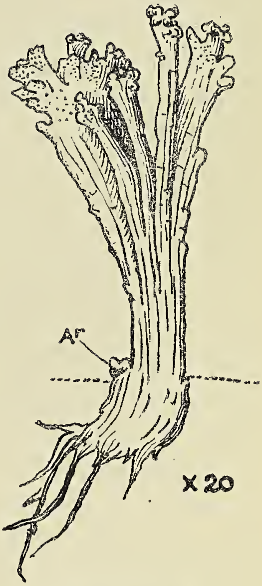
In some cases, however, the lobes were found to be very small and the lower part of the usual thickness or much thicker (Fig. 25). In Fig. 25 the thickest part of the lower half below the lobes consisted of about twenty layers of cells. In very large prothalli the central part may die, and if the prothallus is lobed the lobes may become more or less separated and continue to grow independently for some time. It reminds one of the similar process in the Liverworts.

Lastly, it may be mentioned that the prothallus is very often attacked by a fungus, often found in the upper cells of the lobes, though the cells at their base also may be sometimes affected. The fungus does not appear to do much harm: on the contrary, the prothalli seem to be flourishing vigorously. The contents of the affected cells are transformed into small and large brown globules, stained black with osmic acid. When stained with fuchsin-iodine green mixture, they take the green colour in preference to the fuchsin. The fungus multiplies by minute oval conidia formed in chains on erect conidiophores which have come out of the cells. It is probably a case of parasitism, and as the prothallus is so large it is not very injuriously affected. Occasionally prothalli were found showing the destruction of a large patch of the terminal cells of the lobes, while the rest of the prothallus was growing normally. Infection of prothalli of other species by Fungi also occurs (Tubeuf).

Text-fig. 24.

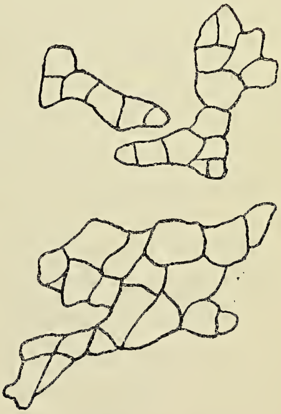


Text-fig. 25.



Text-fig. 26.

The small spaces between the lobes serve to attract water by capillarity. If a prothallus is watered plentifully the water can be seen rising in various parts of the prothallus, and the whole may become quite wet, if the amount of water was sufficiently large, by absorption of water from below.



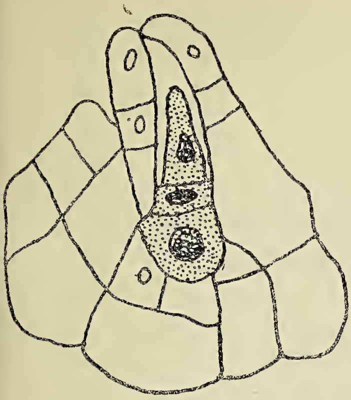
Text-fig. 27.

SEXUAL ORGANS.

Archegonia. The prothallus invariably forms archegonia first, the antheridia being formed only on older female prothalli. Out of several hundred prothalli of all ages which were examined not one was a pure male, but most were female and others bore antheridia in addition to archegonia. The antheridia, moreover, are formed very late in the year.

Archegonia were found on prothalli less than 1 millimetre in diameter. They are formed as usual at the base of the lobes, but all the lobes may not bear them. They are formed in very large numbers and may be 200 or more in vigorous prothalli. The early stages are difficult to follow satisfactorily, as the development is very rapid and the cells in young archegonia do not differ from the ordinary cells of the prothallus. In the mature archegonium the neck projects freely and consists of four rows of cells, each row in the free part consisting of two or three cells. The terminal cells are very long, and on maturity become brownish and bend strongly outward after separating from each other. The most interesting feature in the archegonium, however, is that there is a single neck-canal cell, and not two cells as is the case in other species of *Equisetum.* The ventral canal-cell is small with only a little cytoplasm round the nucleus. The egg at this stage is concave or flat above. Later the canal-cells become disorganized, and if the archegonium is not fertilized the egg also shares their fate and becomes brown, but if fertilized the egg becomes enlarged and rounded. No embryo was found on any prothallus before the end of December. Thus all the archegonia produced before that time were never fertilized and the prothalli continued to increase in size. The fact that all these archegonia were barren is explained when it is mentioned that no antheridia were found before December. Figs. 28–30 show the structure of the archegonium.

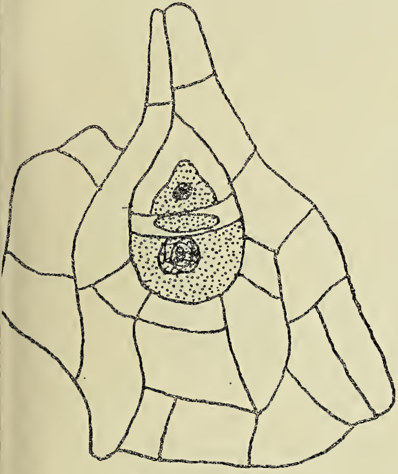
Antheridia. The antheridia are usually formed on prothalli which have been growing for months and are therefore large. Occasionally, however, they occur on small prothalli which have produced comparatively few archegonia, but in no case antheridia alone were found. They are produced at the margin by the meristem and are always embedded. They are not formed along the whole of the margin continuously (Figs. 20, 21). On account of the greater lateral development as compared with radial growth when antheridia are being formed the margins generally become folded. As a rule the formation of lobes is stopped, but this is not invariably the case. Fig. 33 shows an antheridium at the base of a lobe. Even when no large lobes are formed between the antheridia, they are represented by short filaments as shown in Fig. 32. The position, general arrangement of antheridia, and the presence of 'paraphyses' are points which strikingly recall the figures of the same objects in the prothallus of *Lycopodium clavatum* as given by Lang and Bruchmann. The paraphyses contain chloroplasts.



TEXT-FIG. 28.

TEXT-FIG. 31.

TEXT-FIG. 32.



TEXT-FIG. 29.

TEXT-FIG. 30.

TEXT-FIG. 33.

It is very easy to distinguish the prothalli bearing antheridia. The folded margins, their red colour (deeper when the whole prothallus is red), their smoother surface, all indicate their presence.

It is known that in other species of *Equisetum* it is possible to convert female prothalli into male by starving them, and as a rule the smaller and poorly nourished prothalli are male. What are the conditions which induce the formation of the antheridia when the prothalli of *E. debile* have been forming archegonia for some time was not determined experimentally. Other considerations, however, make it probable that temperature and age are the determining factors. In the first place only a few *small* prothalli were found even in December bearing antheridia, although small prothalli are very common at that time. In the latter part of December many small prothalli were seen with one or two plants growing out of them. As regards temperature, it will be seen from the following table that the temperature gradually falls from September onwards, and the lowest mean as well as the lowest mean minimum temperature is reached in December and January. I have to thank the officials of the Lahore Meteorological Observatory for the following figures:

Temperature in Shade (Fahrenheit) at Lahore for 1912–13.

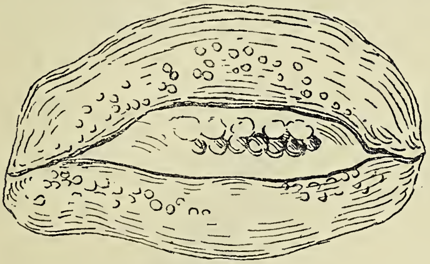
Diurnal range.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Mean maximum . .	100·2	96·3	80·7	71·9	73·1	70·4	78·0
Mean minimum . .	71·0	60·5	49·2	42·7	42·4	48·7	52·0
Mean	85·6	78·4	64·9	57·3	57·8	59·6	65·0
Average monthly .	29·2	35·8	31·6	29·3	30·7	21·8	25·9
Absolute maximum	103·3	103·4	89·6	77·6	78·4	76·3	94·6
Absolute minimum .	62·5	55·1	38·6	37·2	36·1	39·7	45·2

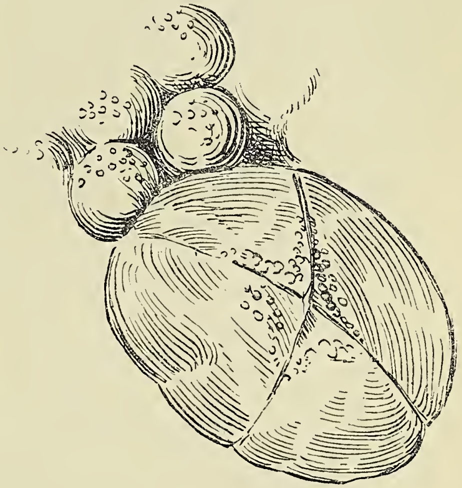
Development of the Antheridium.

It is easy to follow the development, as antheridia in various stages of development are often found in the same vertical radial section (Figs. 31, 32). Each antheridium is developed from a single superficial cell which divides by a periclinal wall ; the upper cell contains a large number of chloroplasts in which the red pigment mentioned above is associated with the chlorophyll. These chloroplasts here as in other cells of the prothallus are often concentrically arranged round the nucleus. The lower cell gives rise to the spermatogenous cells. It first divides by a periclinal wall, and later on periclinal and anticlinal walls are formed with considerable regularity. The spermatogenous cells have the same structure as described by Sharp, but plastids are not generally met with. One or more nucleoli are present in the nucleus as in all the cells of the prothallus. The outer cell divides once or twice, rarely oftener, by anticlinal walls and forms a roof of 2–4, or rarely more, cells (Figs. 34, 35). Dehiscence takes place by the separation of these cells (Fig. 34), which also bend outwards. The mature antheridia are separated from each other by at least two layers of cells, one layer forming the lateral wall of each. In the ripe state, however, the cells of these layers become compressed, and sometimes it is not possible to distinguish them. In the young state they may be separated by a single layer of cells (Fig. 32).

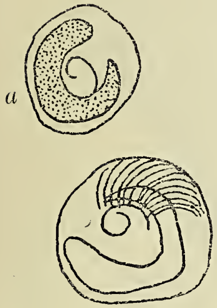
The chromatin in the spermatogenous cells in the early stages of antheridial development is deeply stained with gentian violet in preference to safranin when treated with the triple stain, as is stated by Chamberlain.



TEXT-FIG. 34.



TEXT-FIG. 35.



TEXT-FIG. 36.

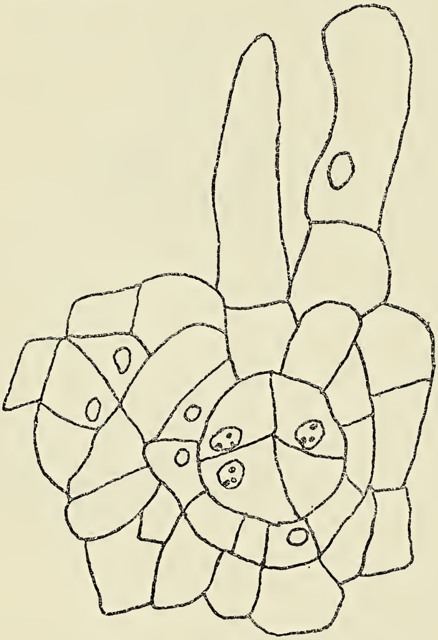
TEXT-FIG. 37.

Later on the chromatin is stained more deeply with safranin. The blepharoplast was seen in sections when the nucleus had assumed the parietal position and had begun to curve (Fig. 36). About this stage the spermatozoid mother-cells, which are already separate from each other, begin to develop a mucilaginous degeneration of their wall, and in sections or teased-out cells mucilage processes are seen spreading out in all directions from the wall, giving the cell a stellate appearance.

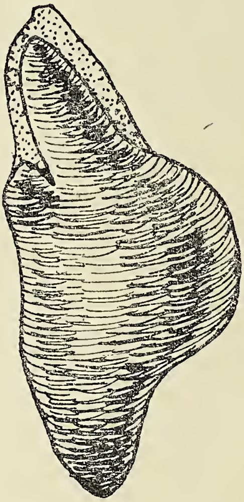
The mature spermatozoid (Fig. 37) is spirally coiled and flattened throughout the greater part of its body. The anterior end is rounded and bears numerous cilia. Quite a large part of the body is formed by chromatin and the blepharoplast. The length of the spermatozoid exclusive of cilia is about 20 μ. It is practically the same as the length of a spermatozoid of *E. arvense*, as given by Sharp, i. e. 19·7 μ.

EMBRYO.

The first wall in the fertilized egg is at right angles to the long axis of the archegonium. The second wall is at right angles to this and produces the quadrants. The octant stage is shown in Fig. 38, where the embryo is cut transversely and obliquely and shows four cells. The hypobasal half produced by the first wall gives rise to the foot and the root, and the epibasal half to the stem and leaf. The foot can be easily recognized by the larger size of its cells. It is at first quite small, but in about a week becomes large (Fig. 39). The young embryo is surrounded by the tissue of the prothallus and the neck also has closed above in the meantime. The first leaves are usually three in number, but two or four are sometimes present as is the case in other species (Campbell). The number of bundles in the stem below the first leaf-whorl corresponds to the number of leaves. The first shoot shows no cavity in the centre, but all the later branches are hollow. The first lateral branch is given off very early and always below the first leaf-whorl. It is given off on the side away from the foot and comes out by passing under the prothallus. The structure of the hypocotyl at this point is still that of the root. Figs. 40–43 show the gradual transition from root to stem structure. The figures were drawn from a series of sections of the same plant. After the branch-stele has left the main stele

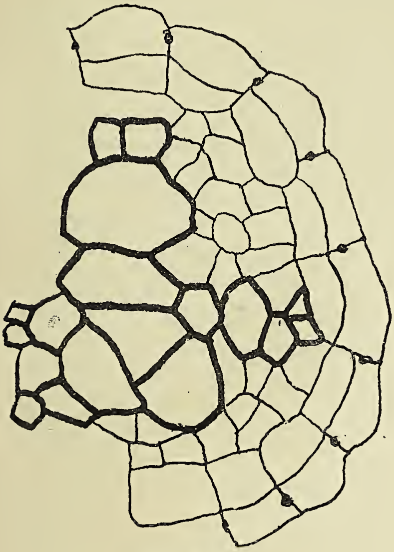


TEXT-FIG. 38.

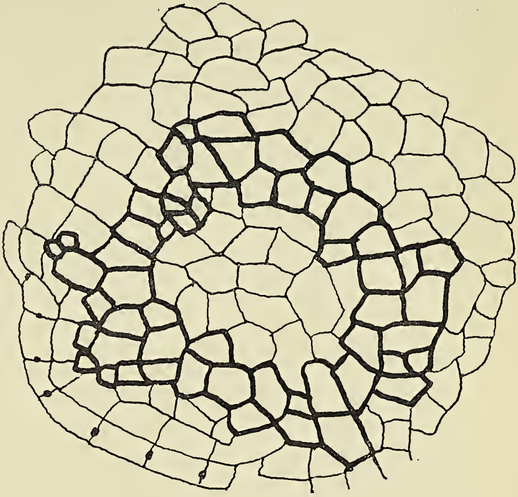


TEXT-FIG. 39.

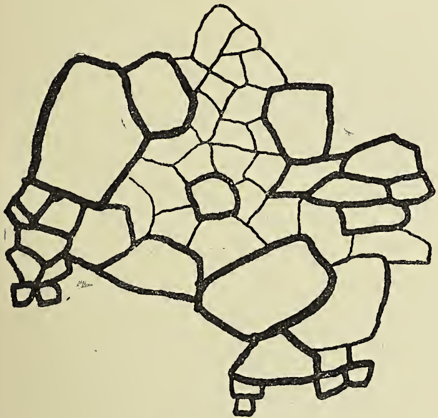
a gap appears in the latter (Fig. 41) and parenchyma passes into the middle of the stele. The two protoxylem groups bend towards each other and later turn completely inwards. The course of the third protoxylem group



TEXT-FIG. 40.



TEXT-FIG. 42.

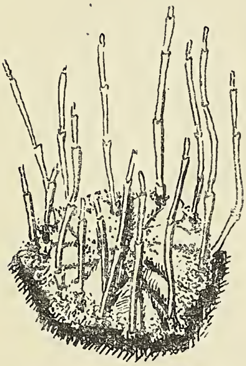


TEXT-FIG. 41.

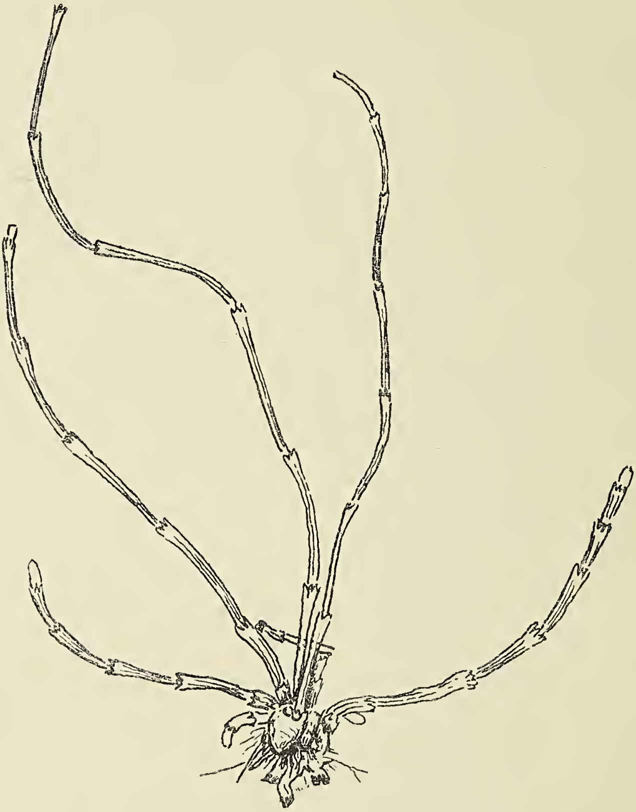
TEXT-FIG. 43.

is not clear. A little higher the stele assumes the form of a hollow cylinder (Fig. 42), and still higher the bundles of the stem become separate from each other (Fig. 43). These changes are similar to those described by Jeffrey (as reported by Campbell) for *Equisetum hiemale*.

A small prothallus may bear a single plant, but eight or ten plants on a prothallus are not unusual. Fig. 44 shows a prothallus bearing fifteen plants. The prothallus was brought into the laboratory without any plants on it, and all of them were produced on that part of the prothallus which was formed while in the laboratory. The proximity of several male plants facilitated fertilization. It may also be mentioned that it is rare to find a prothallus with antheridia and a young plant growing out of it. Self-fertilization, therefore, does not take place, as the archegonia which were produced on the bisexual prothallus would be shrivelled before the antheridia are ripe and no new archegonia can be produced, it appears, along with the antheridia. In the very few cases where a plant was found growing out of an antheridium-bearing prothallus the archegonium may have been fertilized from another prothallus. Fig. 45 shows a single plant coming out of a small prothallus. The plant here has already produced branches of the second and third order.



TEXT-FIG. 44.



TEXT-FIG. 45.

CONCLUDING REMARKS.

The prothallus of *Equisetum debile* is remarkable in the following features:

1. The great diversity in the methods of the formation of the walls in the early stages. Particularly interesting is the occasional occurrence of a 'primary tubercle', comparable to that of *Lycopodium cernuum* (Lotsy—after Treub—figures on p. 415 of volume ii) (Figs. 3, 7).

2. The lobes are always erect and very close together in prothalli found in nature. Even when the amount of light is very small, as inside a room in a dark corner, the lobes are still erect. It has been remarked (Campbell, p. 446) that the 'more or less upright position assumed by the prothallus of *Equisetum* is due to the amount of light'. This does not hold good for the species under consideration.

3. The radial symmetry of the prothallus from the earliest stages (Figs. 5, 6, 16 *b*, 18, &c.). Jeffrey has already compared the prothallus of *Equisetum* to that of *Lycopodium cernuum* (Campbell, p. 446); but Goebel has remarked that there is this fundamental difference that the prothallus of *Equisetum* is not radial but dorsiventral ('Organography,' Part II, p. 195) This objection, however, is not applicable to the prothallus of *Equisetum debile.* Even in the older prothalli the radial symmetry is very well seen, as in Fig. 44, where the plants are formed by the prothallus all along the circular margin. The chief difference which strikes the writer is that in the prothallus under discussion the lower compact portion is very small compared with the same portion in the prothallus of *L. cernuum.* When we consider the great diversity of form in the prothalli of various species of *Lycopodium* (Lang, Bruchmann) the difference does not seem to be very great. At the same time we must remember that the prothallus of *Lycopodium cernuum* is mycorrhizal and the great development of the compact portion is connected with the action of the external agent.

4. The large size of the mature prothallus. The latter is much larger than the largest specimens figured by Buchtien and Goebel.

5. The changes which the prothallus exhibits in the structure and colour of the upper half in relation to light, being very compact and red in bright light (direct sunlight) and spongy and green in shade.

6. The absence of purely male prothalli.

7. The protogynous condition of the prothallus, helping in cross fertilization. All female prothalli, however, do not produce antheridia.

8. In position, general structure, and possession of 'paraphyses' the antheridia resemble those of *Lycopodium* (cf. Figs. 20, 21, 31–33 of this paper, with Figs. 3 and 9 of Lang's paper, and Figs. 14 and 28 of Bruchmann's paper).

9. The archegonium has a single neck-canal cell, which is another point of resemblance with the prothallus of *Lycopodium cernuum*. It must be pointed out in this connexion that although the prothallus of *L. cernuum* is generally accepted to be of a primitive type (Lang), still it has only a single neck-canal cell. This suggests that the larger number of the neck-canal cells in other species of *Lycopodium* is a secondary feature.

The features pointed out above indicate in most cases a clear affinity with the prothallus of *Lycopodium cernuum*, and when we remember the great difference exhibited by the sporophyte generations of *Equisetum* and *Lycopodium* in their external characters and internal anatomy, the resemblance in the prothalli is at least striking.

My cordial thanks are due to Professor A. C. Seward, Cambridge, who kindly corrected the first proofs. I have seen the final proofs myself, and am alone responsible for everything contained in this paper.

LITERATURE CITED.

AITCHISON, J. E. T. : A Catalogue of the Plants of the Punjab and Sindh. 1869.
BAKER, J. G. : Handbook of the Fern-allies. 1887.
BRUCHMANN, H. : Flora. 1910.
BUCHTIEN, O. : Entwickelungsgeschichte des Prothallium von *Equisetum*. 1887.
CHAMBERLAIN, C. J. : Methods in Plant Histology.
GOEBEL, K. : Organography of Plants, Part II. English translation. 1905.
LANG, W. H. : The Prothallus of *Lycopodium clavatum*. Ann. Bot., June, 1899.
LOTSY, J. P. : Vorträge über botanische Stammesgeschichte. 1909.
SHARP, LESTER W. : Spermatogenesis in *Equisetum*. Bot. Gaz., 1912.
STEWART, J. L. : Punjab Plants. 1869.
TUBEUF, — : Plant Diseases. English translation by W. G. Smith. 1897.

EXPLANATION OF FIGURES IN THE TEXT.

Fig. 1. First wall in the spore. × 300.

Figs. 2–13. Early stages in the development of the prothallus. Figs. 2, 3, 4, 7, 8, 9, 10 × 300. Fig. 13 × 125. Figs. 6, 11, 12 × 70. Figs. 3, 7, and 13 show the 'primary tubercle'. *r* in all cases indicates the rhizoid cell.

Figs. 14, 15. Prothalli, three weeks old. × 70.

Fig. 16 (*a* and *b*). Two older prothalli from the ventral side. Rhizoids removed. The posterior notch is seen in *a*; none in *b*. *m* = meristem. × 4. From a shady place.

Fig. 17. A prothallus rather older than Fig. 16. Ventral view. × 2. The fissures showing the division of the prothallus are seen. *m* = meristem.

Fig. 18. A well-grown prothallus growing on the soil. Nat. size. From a shady place.

Fig. 19. Another compact prothallus kept inside a room and watered plentifully for about one month. The lobes formed during that time are long and loose. Nat. size. Four young plants are seen at places marked ×.

Fig. 20. A female prothallus which has begun to form antheridia. × 2.

Fig. 21. Another and larger female prothallus with antheridia on the margins. × 2.

Fig. 22. A female prothallus with a plant growing out of it near the margin. The first lateral branch of the main shoot was already two inches in length. The main shoot was still longer. × 2. From a shady place.

Fig. 23. A vertical section through a well-grown prothallus, showing arrangement and general outline of lobes. *Ar* = archegonia. × 20.

Fig. 24. One of the lobes. × about 70.

Fig. 25. Vertical section through the marginal part of a prothallus, showing large compact portion and small lobe. Roughly, the part above the line is green, that below it is full of starch, but contains no chloroplasts. × 20. *Ar* = archegonia; x = meristem.

Fig. 26. A lobe from the margin of Fig. 19. *Ar* = archegonium. The line shows the upper level of the compact part of the prothallus. × 20.

Fig. 27. Horizontal sections of a few lobes at various heights.

Figs. 28, 29. Mature archegonia in longitudinal section. × 300.

Fig. 30. Transverse section of the neck of an archegonium.

Fig. 31. A part of the prothallus in vertical section, showing antheridia. × 20.

Fig. 32. Three antheridia in various stages of development. Vertical section. *Pa* = paraphysis. × 300. The spermatogenous cells in this and the two following figures are shaded.

Fig. 33. A ripe antheridium at the base of a lobe, in vertical section. × 70.

Fig. 34. An open antheridium seen from above. × 300.

Fig. 35. A ripe but still closed antheridium seen from above. × 300.

Fig. 36. Sperm mother-cells showing curved nucleus and blepharoplast. × 1250. *a*, from a complete teased-out cell fixed by osmic acid.

Fig. 37. Sperm fixed over osmic acid fumes, while swimming. × 1250. The part derived from the chromatin and the blepharoplast is shaded.

Fig. 38. Embryo in longitudinal section. × 300.

Fig. 39. Complete embryo about a week old. × 40.

Figs. 40–43. Transverse section of a young plant from the root upwards through the hypocotyl to the shoot.

Fig. 40. Transverse section of root.

Fig. 41. Formation of a gap and pith.

Fig. 42. Closed ring of the stele with pith in the centre.

Fig. 43. Stem structure with separate vascular bundles. The typical endarch structure has not yet been established.

Fig. 44. A large prothallus with fifteen plants growing out of it. Nat. size.

Fig. 45. A small prothallus with a single, much-branched plant attached to it. The roots have been cut short. Nat. size.

Observations on the Centripetal and Centrifugal Xylems in the Petioles of Cycads.[1]

BY

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With Plate XI and one Figure in the Text.

THE Cycads possess a number of characters which may be considered as sign-posts pointing out possible paths of evolution. A noteworthy example is the peculiar structure of the foliar bundle with its double xylem arrangement, one portion centripetal, the other centrifugal (Pl. XI, Figs. 7–10).

The significance of this unusual type of structure was first appreciated and carefully investigated by Mettenius.[2] Most of the observers who followed him have interpreted the facts in relation to some special line of evolution from Palaeozoic plants. Hence it is that while facts are generally agreed upon, the interpretations differ according to the theories the facts are supposed to prove, corroborate, or favour.

We consequently find a class of writers considering the foliar bundle of Cycads as strictly mesarch, agreeing closely with the *Lyginopteris* (*Lyginodendron*), to which genus the Cycads are usually considered to be related.[3]

Another school opposes this view on the ground that it is based on a mere appearance: the bundle is apparently and not strictly mesarch. It is due to an inversion of the primary normal xylem. 'Pour certains auteurs, et ce sont les plus nombreux,' says Bertrand, 'le bois centripète n'est autre chose que le bois primaire normal retourné.'[4] Some authors, e. g. Chodat, would derive the centrifugal xylem from the centripetal xylem. 'Le bois centrifuge serait, d'après Chodat, du métaxylème détaché latéralement.'[5]

Bertrand's own opinion, in his collaboration with Renault, is summarized in these words: 'Un faisceau unipolaire diploxylé est donc un faisceau unipolaire normal dans la partie supérieure duquel le bois primaire s'est

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[1] A summary of this paper was given before Section K at the British Association, Birmingham, 1913.

[2] G. Mettenius ('61).

[3] W. C. Williamson and D. H. Scott ('96).

[4] C. Eg. Bertrand et B. Renault ('86).

[5] A. Sprecher ('07), p. 34. Cf. also R. Chodat, Sur quelques fossiles.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.]

réduit alors qu'il s'y est développé un tissu ligneux *supplémentaire* centripète . . .'[1] He then refers Cycads to the Poroxylon type.

The difficulty of deciding among these various interpretations led Chodat to suggest a reconsideration of the whole problem.[2] I would not have ventured to undertake a part of this difficult task if Professor Seward had not invited me to do so and encouraged me during the investigation. I wish to take this opportunity of expressing to him my hearty thanks for his kind interest and advice during this work. The material, with the exception of a frond for which I am indebted to the Director of the Royal Gardens, Kew, was obtained from the Botanical Garden, Cambridge, and generously supplied by Mr. Lynch, the Curator, to whom also many thanks are due.

Five genera of Cycads, including seven species, and as far as possible of different ages, have been examined: *Cycas circinalis* and *C. revoluta*, *Stangeria paradoxa*, *Dioon edule*, *Ceratozamia mexicana*, *Encephalartos horridus* and *E. villosus*.

The observations recorded in this communication were limited to the Cycadean leaf, and are restricted to the relation of the centripetal and centrifugal xylems to one another, and the relation of both to other tissues, these relations form the crucial point of the whole problem.

It was not until a preliminary account of my investigations had been written that I discovered a paper much on the same lines by Dr. Carano, of Rome. This paper, which is very detailed, has not perhaps received all the attention it deserves.[3]

Observations.

My observations lead me to conclude that the sketches and plates published do not usually represent the structure of a vascular bundle such as is found at *the very base* of a Cycad. This is, however, of great importance, as it is there that the most interesting changes occur; the transition from the stem-type of xylem into the petiolar arrangement of that tissue is seen along a few centimetres of petiole which immediately follow the stem.

The shoot of a fully developed petiole of a Cycas is first described as a basis for subsequent discussion. A few bundles pass into the leaf at the junction of petiole and stem. Their number varies much, but there are at least two main bundles to which the secondary ones seem to be related. These secondary bundles sometimes reunite with the main one, forming loops, before the latter begins to branch rapidly and forms part of the well-known large ω set of bundles which will run up the petiole. This happens, say, at 1 cm. above the junction with the stem.

From our point of view the main bundles are the important ones, for they remain fairly constant for a distance of 2 cm. or 3 cm., even while they

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[1] C. Eg. Bertrand ('86). [2] R. Chodat ('08). [3] E. Carano ('03).

give rise to other bundles which go to take their relative places in the general disposition of the foliar strands. Their forms vary considerably: they may be simple and collateral (Fig. 2), or concentric[1] with parenchyma in the centre and medullary rays running radially all round, thus reproducing the structure of the stem or even of some peduncles (Fig. 3). A bundle may also be a compound structure, combining more or less completely the characters of the two first types mentioned (Fig. 1). Intermediate types, as one would expect from such a variety of structures, are not infrequent.

But in this variety of forms there is a distribution of elements which remains constant at the base of the petiole. The xylem is entirely endarch or centrifugal (Figs. 1–3). An internal protoxylem (*px*) is succeeded by numerous rows of lignified cells continued by cambium and phloem cells, with medullary rays running across the xylem, cambium, and phloem layers. This structure persists, say, for a distance of 2 cm. from the base of the petiole, and then gradually changes in the course of the next 3–4 cm. The cells in connexion with the protoxylem are no longer strictly centrifugal, but seem to run in a lateral direction. Only the central rows remain properly centrifugal (normal to the pith), undergoing, however, a process of reduction (Fig. 4).

This reduction is more and more apparent as we proceed up the petiole; the lateral rows pass behind the protoxylem, new independent groups of scattered lignified cells appear, and the centripetal xylem is initiated (Figs. 4 and 9).

At a higher level the centripetal xylem is more abundant than the centrifugal; the gap which we observed behind, between the two lateral segments, is entirely closed; a complete ring is formed which soon breaks up on both sides (Figs. 7 and 8). There remains only a few centrifugal cells scattered in small groups and without any visible connexion with the centripetal xylem, except through a parenchymatous tissue of thin-walled cells in which it is embedded (Fig. 10). This is the ultimate form of structure met with practically throughout the petiole, apart from the 5 cm. or 6 cm. we have described. A last stage may be reached in which no trace of centrifugal xylem is left. This, however, is rare, and I have met with it only in the smaller bundles of the leaflets of *Encephalartos horridus*.

The above is a general survey of the natural and normal distribution of xylem elements in the petioles of Cycads at an adult stage.

Wound structures.—Other structures may be artificially induced. A young petiole of *Cycas circinalis*, 15 cm. long, was wounded at the end of April; one of the wounds was transverse and not far from the base, the other wound was longitudinal and higher in the petiole. The leaf still grew well, though less luxuriantly than its companions of the same age.

On July 12, when it was about 1 metre long, it was cut and examined.

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[1] See also H. Matte ('07), Pl. I, Fig. 7.

The transverse wound was about 6 cm. from the base; it had cut through some two-thirds or three-quarters of the area of the petiole, thus breaking the continuity of most of the foliar bundles. The sections prepared from the portions situated below the wound showed no abnormality, except a reduction of growth in the inactive and useless bundles as compared with their companions which had to carry out the whole work. This result is what might be expected in view of the fact that the injury cut off the supply of food from the leaflets. But just above the wound a remarkable phenomenon was observed. We must remember that at this height and even much below the wound the xylem was mainly centripetal xylem, disconnected from the centrifugal xylem. The cambium looked very active; it passed all round the bundle, producing xylem towards the centre and phloem on the outside (Fig. 12). There was secondary xylem formed even behind the centripetal xylem. Such a bundle is difficult to define; it looks concentric rather than mesarch. As we look further from the wound this peculiar structure gradually disappears till we finally reach a normal distribution of elements in the bundle, perhaps some 3 cm. from the wound. It may be noted that this phenomenon occurred in a great number of the severed bundles. In connexion with these a new structure was induced. A large bundle built up of stout lignified cells, having much the appearance of a transfusion tissue, brought all the severed bundles into communication with the unwounded bundles, and allowed a transport of material to and from the channels interrupted by the wound, like a short loop-line connecting the ends of a section of rails under repair (Fig. 13).

The longitudinal cut was narrow but long, its distance from the base varying between 10 cm. and 20 cm. It was situated near the first leaflet and higher in the petiole. The leaf reacted against the injury by repairing the damage done and filling up the gap with new tissue, disposed in regular rows quite distinct from the rest of the petiole. A large bundle, giving rise to this tissue, originated in the internal margin of the wound. Its cells are pitted and run somewhat irregularly, showing a tendency to imitate the transfusion tracheides met with lower down just above the transverse cut. The bundles next to the wound were also considerably affected; they became closed and concentric, showing new regions of growth. This influence is less marked further away from the wound. The meaning of these traumatic structures is considered later. Bertrand and Renault tested the effect of longitudinal wounds, but they considered the results as accidental and of no significance.

Interpretation.

The disposition of the vascular bundles at the very base of the petiole, as we have seen, offers a striking appearance. The various forms (Figs. 1–3) they assume are, I believe, of no phylogenetic importance, but serve to show the facility with which bundles or steles can adapt themselves to either morphological or physiological requirements. Their main significance is that they illustrate the need of caution in basing phylogenetic relationships on morphological structures which may be the result of long and progressive efforts of the plant to adapt itself to different needs.

More worthy of notice is the special and constant arrangement of the elements themselves, with their regular rows of xylem, cambium, and phloem, separated by distinct medullary rays (Figs. 1 and 5). Such a structure would naturally suggest a secondary growth, an assumption made by several writers with less definite or clear sections before them, at least judging from the sketches they have published.[1] I was too ready to make the same assumption when Professor Lang, of Manchester, warned me that there are similar occurrences in primary structures. A section across a petiole of *Ginkgo*, for instance, would show it clearly.[2] Such a structure may be a predetermined arrangement and not the result of cambial activity. This objection is a most serious one, attacking the very foundation of the argument and, consequently, the conclusion itself. We must therefore recast the whole proof or give up the assertion.

It is a difficult problem to work out critically owing to the impossibility of studying the same individual bundle at different ages; on the other hand, the structure of the bundle varies considerably and rapidly with the distance from the stem or even at the same level on the petiole.

There is, however, a possible solution. Taking petioles of different ages from the same species and the same stem, which have had the same chances and the same opportunities for growth, the only difference being that of age, we may count the number of cells contained in a row passing straight from the cambium to the first protoxylem cell. To be fair we must take for comparison the highest number of cells found in a row at different ages in different petioles, or we might also use averages; the former method was followed.

The present account is based mainly on *Cycas revoluta* and *Cycas circinalis*. The youngest petioles used were about 10 cm. long. Dr. Carano gives a sketch of a still younger specimen.[3] His drawing would seem to lend a strong support to my argument, but it is safer not to use it as we do not know the age of the stem from which the petiole was taken.

The oldest petiole (probably four years) of *Cycas circinalis* examined showed straight rows of 22 lignified cells, an average of 16–20 being of common occurrence (Fig. 1). A specimen two years old numbered 16 cells. Other petioles of the present year showed a decrease corresponding with their age, the maximum being about 12 in a specimen 40 cm. long. A young leaf of *Cycas revoluta*, 10 cm. long, possessed rows

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[1] E. Carano ('03), and C. Eg. Bertrand et B. Renault ('86).

[2] A. Sprecher ('07), p. 64.

[3] E. Carano ('03), Tav. ix, Fig. 9.

of 10 cells (Fig. 6), as compared with 16 or 18 in a leaf two years old belonging to the same plant (Fig. 2).

Apart from numerical results, the mere look of the sections would force one to conclude that secondary growth occurs in the centrifugal xylem at the base of the petioles. This view is confirmed by the examination of the cells near the cambium in a moderately old petiole over 1 metre long; the cells at the ends of the rows are evidently undergoing a process of lignification, for they are thinner than their neighbours, stain less deeply, giving, in fact, all the transitional shades specially visible in sections stained with gentian violet and orange G.

It seems therefore proved that the centrifugal xylem at the base of the petiole is a secondary growth, at least in its main bulk, and we have to disagree with Dr. Scott, who holds that 'the centrifugal part of the wood must be regarded as a primary structure, though in certain cases it may receive subsequent addition from a cambial layer'.[1] This small allowance for secondary growth is at least too limited.

Following the bundle still higher, we have seen the ring of xylem breaking up altogether; the two xylems are completely separated by a layer of thin-walled cells; the protoxylem is in contact with the centripetal xylem, but not, generally speaking, with the centrifugal xylem (Fig. 10). No centrifugal xylem is found in young petioles at this level where the centripetal xylem is well developed. It is produced later than the centripetal xylem, and appears to be in continuity with the radial rows of meristem cells. There is at least no definite line between the large amount of undoubted secondary centrifugal xylem at the base of the petiole and the much smaller amount found at the higher level, and we are inclined to agree with Dr. Carano, who regards the latter as also of secondary origin: 'd'origine secondaria s'intende bene, ultimi avanzi del legno centrifugo.'[2]

From these observations I am led to inquire into the relations between the centripetal xylem and centrifugal xylem. I shall deal with them only in the light of my own observations.

The gradual bending backwards of the lateral centrifugal xylem as one passes away from the base would seem to favour the inversion theory (Figs. 4 and 9). There is also a tendency in the phloem to follow this motion, though, in fact, it lags a good deal behind. But perhaps this inversion is more apparent than real. The theory is based upon an assumption which needs proof—namely, that it is the same xylem which takes up these different positions.

Not only is proof lacking, but the facts themselves seem to contradict the hypothesis. We have seen that the centrifugal xylem at the base of the petiole is a secondary growth, at least in its main bulk, or, with the

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[1] W. C. Williamson and D. H. Scott ('96).

[2] E. Carano ('03).

exception of the protoxylem, perhaps entirely secondary, for it is not easy to see where to draw a limit in rows of cells which look so uniform. On the other hand, it would be easy to show that the centripetal xylem is a primary structure, the result of the division of the meridesmogen strand; there is no sign of cambium activity, the cells are distributed in their proper place a long time before they are gradually lignified. In fact, the primary nature of the centripetal xylem is now generally admitted.

But, if this is the case, it is difficult to regard a primary structure as the continuation of a secondary one. The continuity theory breaks down here altogether: to be in succession or in contact is a different thing from being continuous; to be in succession may mean only juxtaposition; to be continuous implies, moreover, community of origin or derivation. Such structures could not therefore be called homologous, and still less identical with each other. It is much like the Poroxylon described by Bertrand.[1] The difference in the nature of the xylem cells could also be quoted in favour of this statement; but this proof is of little value, as the nature of the centripetal xylem varies so much and so rapidly with the distance from the protoxylem, giving in succession spirally thickened, reticulate, annular, scalariform, pitted, multiseriate pitted tracheides (Fig. 11).

There is a remarkable resemblance between the vascular tissue of the Cycad petiole and that of a leaf of *Cordaites*, as described by Dr. Marie Stopes.[2] The cells forming the inner sheath are very like the reduced centrifugal xylem of Cycads both in longitudinal and transverse sections, the only visible difference being the position. In the *Cordaites* described they are in close relation to the bundle-sheath, but in many leaves of this genus a large amount of phloem separates the centrifugal xylem from the bundle-sheath. There seems to be no adequate reason for regarding the inner sheath as other than the centrifugal xylem.

Dr. Carano insists strongly on the fact that the centrifugal xylem is in continuation with the centripetal xylem or even the protoxylem. But this relation is more apparent than real, if we admit that the centrifugal xylem and centripetal xylem are juxtaposed rather than continuous. It is only at the very base that the xylems join hands and that the protoxylem becomes common to both.

The facts seem to bring out the independence of the xylems; they are independent in origin and nature but are juxtaposed to carry out their functions; in fact, in places they are brought into communication with one another by transfusion cells, or by the laterally stretched wings of the centripetal xylem. They are continuous physiologically but not morphologically. This interpretation has much in common with the view supported by Bertrand and Renault. The centrifugal xylem is the continuation of the

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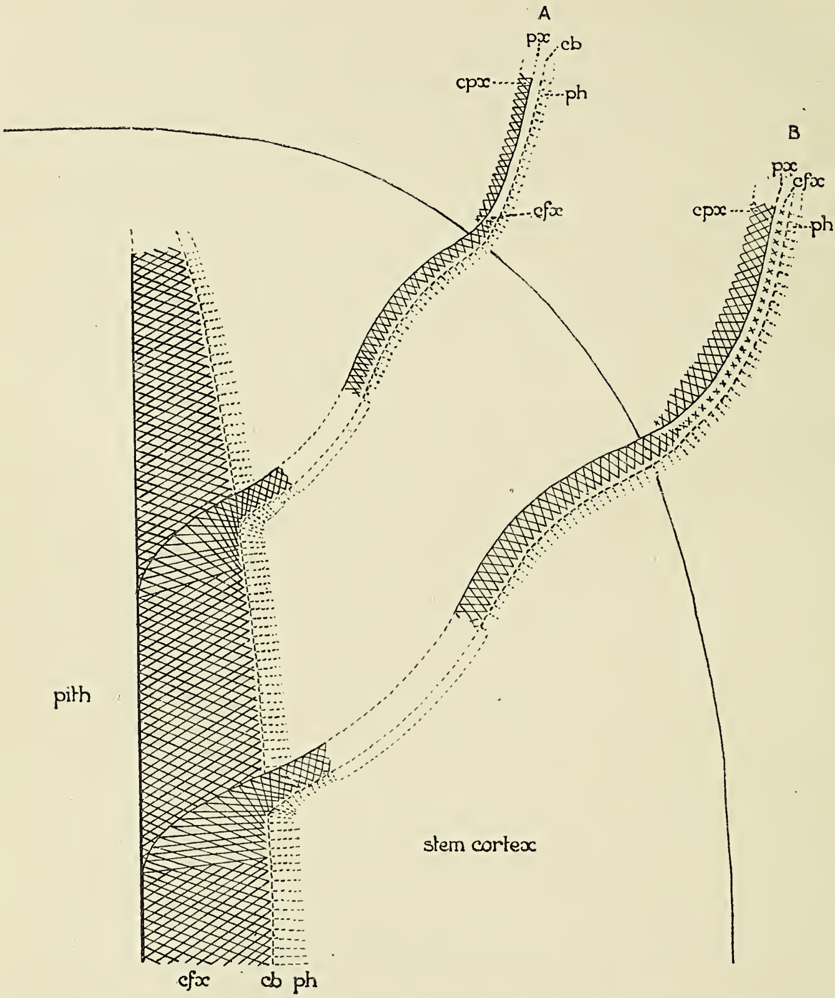
[1] C. Eg. Bertrand et B. Renault ('86). Cf. also D. H. Scott, Studies in Fossil Botany, 1909.

[2] Marie C. Stopes ('03).

normal centrifugal xylem of the stele and the girdles; the centripetal xylem is an independent tissue, probably the remnant of an ancient and more developed structure—a conclusion supported or suggested by the structure



TEXT-FIG. Longitudinal section of apex of stem and of petiole, showing relation between centripetal and centrifugal xylem. *cpx*, centripetal xylem; *px*, protoxylem; *cb*, cambium; *cfx*, centrifugal xylem; *ph*, phloem. A, younger petiole without centrifugal xylem except at base; B, older petiole with centrifugal xylem throughout, but not in connexion with the protoxylem except at the base before the centripetal xylem appears.

of several Palaeozoic types.[1] It is worthy of remark that the first centripetal xylem elements to be found at the base are often scattered and unconnected with the centrifugal xylem. The text-figure shows the relation between the two xylems.

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[1] D. H. Scott, Ann. Bot., 1912, p. 1014. Cf. also New Phyt., 1902, pp. 25–30.

Bertrand bases his assertion on the fact that the centrifugal xylem comes out of the stem undistorted; but this is hardly an argument, as the centrifugal xylem might well be inverted higher up in the petiole, as it apparently is. His other argument is based on the idea that the protoxylem attached to the centrifugal xylem gradually disappears. There is much to be said for such a view, only that it is not easy to show that this protoxylem is disconnected and different from the protoxylem which accompanies the centripetal xylem.[1]

It would seem that at least the centripetal xylem has been reduced at one of its extremities; the few blind xylem elements scattered at the base of the petiole indicate that they formerly extended lower down, perhaps even into the stem; 'it has only survived as long as it has because of the conservatism of the foliar bundles;'[2] while the centrifugal xylem might possibly have been secondary all along its course, approaching some types of Palaeozoic fossils, e.g. Poroxylon.

The terminology used by different observers reflects accurately the theories these observers have in mind. According to Dr. Scott, the term mesarch 'implies that the development begins in the middle of the strand of wood'.[3] It is difficult to find such a bundle in the petiole of Cycads where the protoxylem is connected (*a*) either with a xylem which is entirely centrifugal, or (*b*) with a xylem which is centripetal, in which case the protoxylem and the centrifugal xylem of secondary origin are usually disconnected.

The French school, on the contrary, constantly employ the word 'diploxylic'—'diploxylé'—because 'they oppose the two parts to one another as "bois centripète" and "bois centrifuge", and regard them as distinctly different things' (Solms-Laubach).[4] Then Solms-Laubach adds that if the word 'mesarch' had been invented before the French authors published their results, 'we might perhaps have been spared this misconception.' This remark is tantamount to saying that a hypothesis may be derived from a coined word rather than a definition or word be made, created if necessary, to fit in with a theory.

Wound-structures.

Do the structures artificially induced throw any light on this problem? There is now a tendency to consider such structures as primitive. In this view the anatomical modifications brought about in the petiole of *Cycas* by cuts would be most instructive. The bundles much influenced looked concentric; it would then follow, according to this theory, that the foliar bundles were originally concentric, that they have lost this character and passed through all the stages met with in passing away from a wound.

[1] C. Eg. Bertrand et Renault ('86).

[2] D. H. Scott, New Phyt., 1902, p. 29.

[3] W. C. Williamson and D. H. Scott ('96).

[4] Solms-Laubach ('91), pp. 256–7.

Also these wound-induced tissues imitate structures often met with at the base of a petiole. It would be reasonable to suppose that all the phases in the evolution of the vascular bundle can be traced in a living petiole of a Cycad. Unfortunately the principle at the base of this argument is not proved in its generality, and to quote the *Cycas* wounds as an instance would obviously be a vicious circle.

It is, perhaps, more likely that such structures are developed in response to a physiological need. As a general outlook no one can help seeing that the anatomy of plants corresponds to a physiological function or purpose. Through some mechanism of life they must have been developed in relation to such a need. In this instance the increased activity of the cambium in the bundles tends to answer such a purpose by increasing the area of the bundles as their number is reduced. Also the new bundles of transfusion cells are evidently adapted to fulfil a function, a need. Why this is so could not, perhaps, be answered by Experimental Botany, which is more concerned with the question how it is done.

Thus, reduced to a physiological interpretation, these structures artificially induced are without any direct bearing on the phylogenetic interpretation of the bundle. Our final conclusions are therefore:

1. At the very base of the petiole the structure of the vascular bundles of Cycads is entirely centrifugal, and assumes different forms, concentric, collateral, or the combination of both.

2. The centrifugal xylem at the base is, at least in its main bulk, a secondary growth. This is indicated by the regular rows of xylem, cambium, and phloem, separated by medullary rays, and proved by the study of petioles at different ages.

3. The centripetal xylem is a primary structure laid down at an early age, but only gradually lignified.

4. The centrifugal xylem and centripetal xylem are probably distinct in origin, juxtaposed in response to physiological demands but morphologically discontinuous.

5. During most of the course along the petiole the two xylems remain distinct, therefore the bundle is more properly called pseudo-mesarch[1] or diploxylic.

6. The two xylems overlap at their ends. The remains of centripetal xylem scattered at the base might point to a time when it ran further down, perhaps into the stem.

[1] A word suggested by Prof. A. C. Seward.

LITERATURE CITED.

1. BERTRAND, C. EG., et RENAULT, B.: Remarques sur les faisceaux foliaires des Cycadées actuelles. Arch. Bot. du N. de la France, 1886.
2. CARANO, E.: Contribuzione alla conoscenza della morfologia e dello sviluppo del fascio vascolare delle foglie delle 'Cicadacee'. Annali di Botanica, Roma, 1903.
3. CHODAT, R.: (*a*) Sur quelques fossiles végétaux. Bulletin de l'Herbier Boissier, 2^e^ série, tome vii; (*b*) Archives des Sciences physiques, tome xxvi, 1908.
4. MATTE, H.: Recherches sur l'appareil libéro-ligneux des Cycadacées. Mémoires de la Société Linnéenne de Normandie, vol. xxii, Caen, 1907.
5. METTENIUS, G. H.: Beiträge zur Anatomie der Cycadeen. Abh. K. Sächs. Gesellsch. Wiss., vol. vii, 1861, pp. 565–608.
6. SCOTT, D. H.: (*a*) Studies in Fossil Botany. London, 1909; (*b*) Annals of Botany, 1912; (*c*) New Phytologist, Feb., 1902.
7. SOLMS-LAUBACH, H., GRAF: Fossil Botany. English translation, 1891.
8. SPRECHER, A.: Le *Ginkgo Biloba*. Genève, 1907.
9. STOPES, DR. MARIE C.: New Phytologist, 1903, pp. 91–8.
10. WILLIAMSON, W. C., and SCOTT, D. H.: Further Observations on the Plants of the Coal Measures. Phil. Trans. Roy. Soc., London, vol. B. clxxxvi, 1896, pp. 703–79.

EXPLANATION OF PLATE XI.

Illustrating Dr. Le Goc's paper on Centripetal and Centrifugal Xylem in the Petioles of Cycads.

Fig. 1. *Cycas circinalis*, four years old, base of petiole, a compound structure. *clb* = collateral bundle; *ccb* = concentric bundle; *px* = protoxylem; *cfx* = centrifugal xylem; *ph* = phloem; *mr* = medullary ray.

Fig. 2. *C. revoluta*, two years old, base of petiole. *clb* = collateral bundle.

Fig. 3. *C. revoluta*, base of petiole. *ccb* = concentric bundle.

Fig. 4. *C. circinalis*, near base. *cpx* = centripetal xylem.

Fig. 5. *C. circinalis*, present year, base of petiole 40 cm. long.

Fig. 6. *C. revoluta*, present year, base of petiole 10 cm. long.

Fig. 7. *Encephalartos horridus*, near base; *cpx* and *cfx* separate.

Fig. 8. *Stangeria paradoxa*, near base; *cpx* and *cfx* separate.

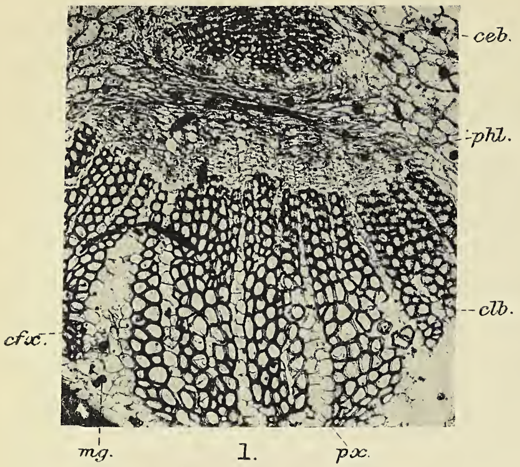
Fig. 9. *Cycas circinalis*, near base; *cpx* independent.

Fig. 10. *C. circinalis*, far from base; *cfx* separate from *px* and *cfx*.

Fig. 11. *C. circinalis*, far from base. Longitudinal section showing different forms of tracheides.

Fig. 12. *C. circinalis*, wound structure, secondary *cpx*.

Fig. 13. Large bundle of transfusion tissue (*tfb*).



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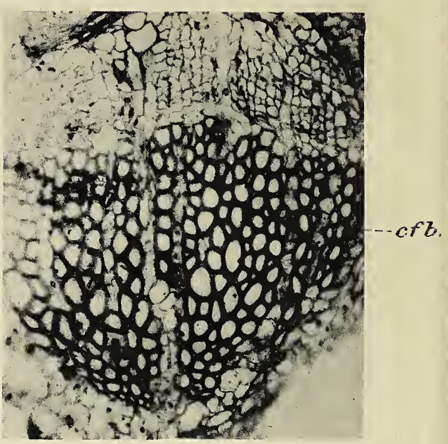

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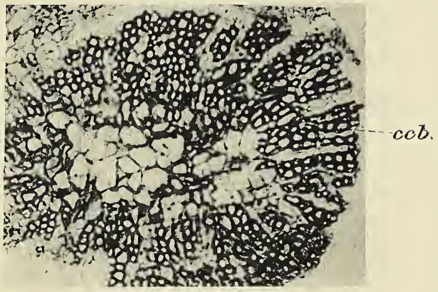

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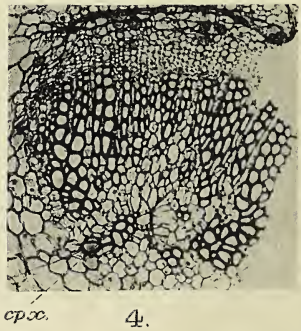

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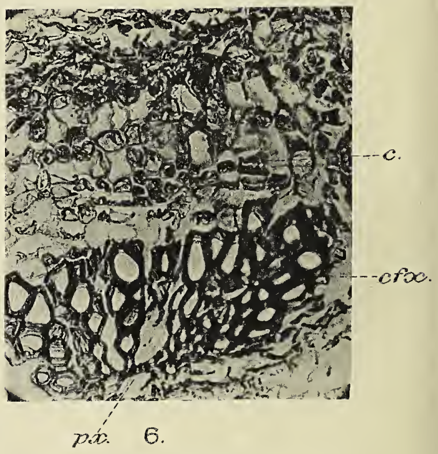

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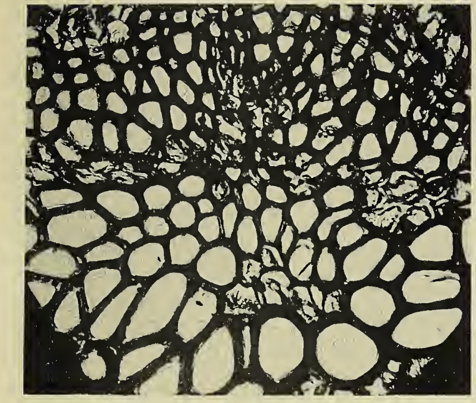
M.J. Le Goc. photo.

LE GOC — CYCADEAN PETIOLES.



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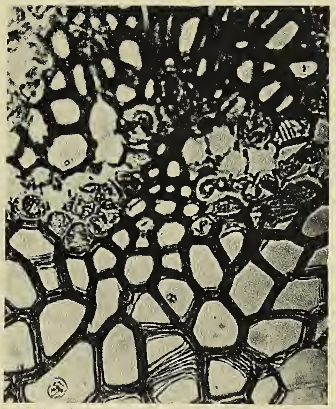

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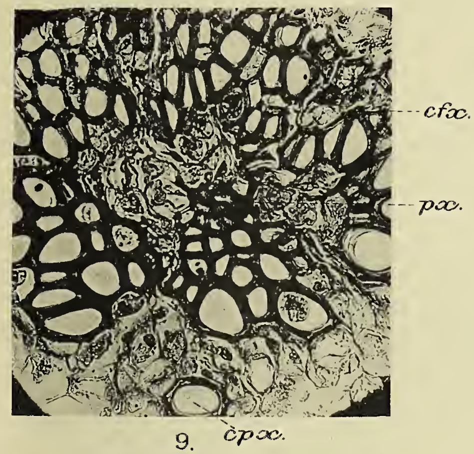

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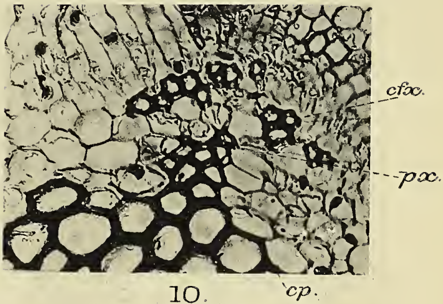

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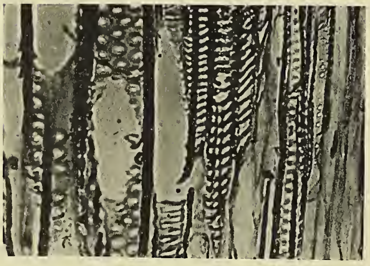

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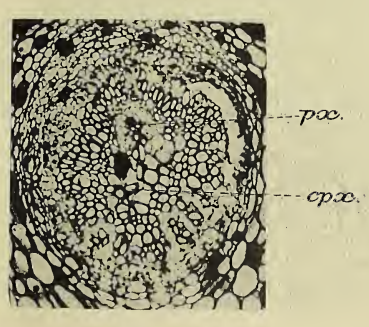

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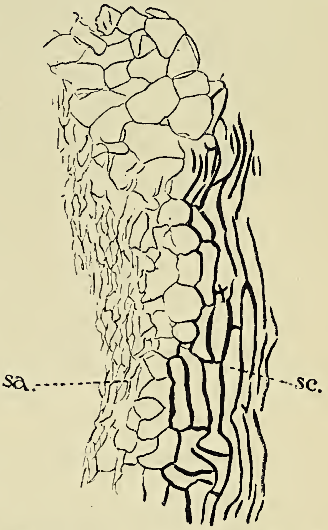
NOTE.

A NOTE ON TRIGONOCARPUS.—The object of the present note is to draw attention to one or two points in the structure of a specimen of *Trigonocarpus*, which has been lately brought to light on cutting sections of one of the blocks in the Binney Collection, Sedgwick Museum, Cambridge. This block formed part of a 'coal-ball', but the locality from which it came is unknown. It is not improbable that it was derived from the same source as the seeds of *Trigonocarpus* described by Hooker and Binney,[1] which were 'found imbedded in nodules of limestone, enclosed in a thin seam of bituminous coal not above 6 inches thick, in the lower part of the Lancashire coal-field'.

Three longitudinal sections[2] of the new specimen of *Trigonocarpus* have been cut, one of which[3] is practically median (Pl. VI,[4] Fig. 6). The chief interest of the specimen lies in the fact that the sclerenchyma of the micropylar beak is preserved as far as its extreme apex, this region being shown more completely than in any other longitudinal section at present known. The text-figure represents one side of the micropyle; the sarcotesta (*sa.*) is not complete, though it is prolonged for a little distance beyond the sclerotesta (*sc.*).



TEXT-FIG. *Trigonocarpus* sp. Top of left-hand side of the micropyle shown in longitudinal section in Pl. VI,[4] Fig. 6. × 47. *sa.* = sarcotesta; *sc.* = sclerotesta.

Another point on which the present specimen throws some light is the question of the connexion of nucellus and integument. The appearance of the tissues, at the level at which the nucellus leaves the integument, distinctly suggests that, in life, the nucellus was actually free from the integument almost to the base of the seed, as Scott and Maslen[5] are inclined to believe. Pl. VI,[4] Fig. 7, represents the junction of nucellar wall and seed coat. Even if an 'inner flesh' was present, there is no reason to suppose that it filled the entire space between nucellus and sclerotesta. An analogy may be found in the seed of *Phyllocladus*.[6] Here the seed-coat consists of a sclerotesta which is both clothed

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[1] Hooker, J. D., and Binney, E. W.: On the Structure of certain Limestone Nodules enclosed in Seams of Bituminous Coal, with a description of some Trigonocarpons contained in them. Phil. Trans. Roy. Soc., London, vol. 145, 1855, p. 149.

[2] A.B., 10, 11, 12. Binney Collection.

[3] A.B. 11.

[4] This plate accompanies the paper by Dr. E. A. N. Arber, 'A Revision of the Seed Impressions of the British Coal Measures,' p. 81.

[5] Scott, D. H., and Maslen, A. J.: The Structure of the Palaeozoic Seeds, *Trigonocarpus Parkinsoni*, Brongniart, and *Trigonocarpus Oliveri*, sp. nov. Part I. Ann. Bot., vol. xxi, 1907, p. 89.

[6] Robertson, A.: Some Points in the Morphology of *Phyllocladus alpinus*, Hook. Ann. Bot., vol. xx, 1906, p. 261, Pl. XVII, Fig. 11.

[Annals of Botany, Vol. XXVIII. No. CIX. January, 1914.]

externally and lined internally with soft tissue, while the nucellus stands freely up into the seed cavity.

Scott and Maslen ascribe most of the British petrifactions of this genus to *Trigonocarpus Parkinsoni*, Brongniart. They include under this species the seeds described by Hooker and Binney without a specific name, and by Williamson [1] under the name of *Trigonocarpon olivaeforme*, L. and H. A comparison of the seed, which is the subject of the present note, with typical examples of *T. Parkinsoni*, with especial reference to the measurements and figures in Scott and Maslen's paper, leads to the conclusion that, though our specimen shows a general similarity to *T. Parkinsoni*, and is of about the diameter of a large specimen of this species, yet its seed body is distinctly shorter. The form is thus more rounded and less oval. The sclerotesta at the base of the micropylar canal shows, also, a greater increase in thickness than is usually to be noticed in *T. Parkinsoni*. The new specimen, on the other hand, bears a very close resemblance in its dimensions to one of the petrifactions figured by Hooker and Binney.[2] An 'impression' of *Carpolithes alata* (*Trigonocarpus Parkinsoni*?) from Jarrow, described by Scott and Maslen,[3] though somewhat larger than the specimen under discussion, resembles it closely in proportions. It is probable that this impression, together with Hooker and Binney's section to which reference has just been made, and the petrifaction described in the present note, may prove eventually to belong to a distinct species.

AGNES ARBER.

SEDGWICK MUSEUM, CAMBRIDGE.

---

[1] Williamson, W. C.: On the Organization of the Fossil Plants of the Coal-measures. Part VIII. Phil. Trans. Roy. Soc. London, vol. 167, 1877, pp. 248, &c.

[2] Hooker, J. D., and Binney, E. W.: loc. cit., Pl. IV, Fig. 7. The figured slide is preserved in the British Museum, Nat. Hist. Gen. Coll., S. 3529.

[3] Scott, D. H., and Maslen, A. J.: loc. cit., p. 130, and Pl. XIII, Fig. 18.

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.

LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

The general plan of the work may be gathered from Part I, which was published in 1908 as a royal 4to volume of 211 pages. In it are described the following twelve types of floral structure, selected from familiar garden flowers that bloom in the early part of the year (January–April):—

Helleborus niger . .	Christmas Rose.	*Viola odorata*	Sweet Violet.
Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant.
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

The description of each type is illustrated by a full-page coloured plate, giving an accurate longitudinal section of the flower, and by a black-and-white plate giving the inflorescence, the floral diagram, and other structural details. As each subsidiary species has also a coloured plate allotted to it, the volume contains no less than forty coloured and fourteen uncoloured plates, in addition to a large number of figures, chiefly developmental, included in the text. It can be obtained at the original price of £1 1*s*. net by subscribers to Part II.

It was hoped that the reception of so striking a volume as Part I would have been such as to justify the Delegates of the Press in proceeding forthwith to publish Part II, the material for which is in readiness. Inasmuch as this anticipation has unfortunately not been realized so far, the Delegates are not disposed to undertake the publication of Part II without some assurance that the necessarily large expenditure involved will meet with the general support of those who, in one way or another, are interested in flowers. But the University Press has received such warm commendations of the work from Botanists who desire to push on the study of Botany in the English-speaking countries that they desire, if possible, to continue publication. They propose, therefore, to ask for subscriptions for copies of Part II at One Guinea each, on the understanding that Part II will, like Part I, consist of descriptions of twelve types of flowers, with allied forms, and be similarly illustrated, though it may be found necessary to reduce somewhat the number of coloured plates. Any copies not subscribed for will not be sold at less than thirty shillings each.

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EDITED FOR THE

BRITISH ECOLOGICAL SOCIETY

BY

FRANK CAVERS

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The Journal of Ecology, being largely a Review, is indispensable to those desiring to keep abreast of the extensive and widely scattered literature of Ecology, of which it will take a comprehensive view, including in its scope not only work dealing with general principles, methods, surveys, and other ecological studies, but also publications dealing with Geography, Geology, Meteorology, Climatology, Plant Physiology and Anatomy, the science of soils (soil physics, chemistry, and the investigation of soil floras and faunas), and other branches of Natural Science, so far as these have a direct bearing upon Ecology—the relationships of plants to their environment in the widest sense, including the inter-relationships between plants and animals. In brief, it aims at furnishing a comprehensive review of progress in the entire field of Ecology, touching, as this field does, kindred branches of science on various sides.

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Some Studies on Yeast.

BY

HORACE T. BROWN, F.R.S.

With eight Figures in the Text.

PART I.

THE RELATION OF CELL-REPRODUCTION TO THE SUPPLY OF FREE OXYGEN.

THE rate of increase of living cells undergoing division in an environment suited to their growth is influenced by so many factors that any attempt to analyse them, and to demonstrate the limitations which they severally impose on cell-increase, becomes exceedingly difficult if attention is confined to multicellular organisms.

The problem can be much simplified if we consider a unicellular organism such as ordinary yeast, for in such a case we have not only the power of controlling the external conditions with comparative ease, but also a ready means of estimating the actual cell-increase by direct counting of the cells. Moreover, there is the further advantage that the technical applications of yeast in the fermentation industries have stimulated inquiry to so large an extent that we have a considerable accumulation of facts which can be utilized in any such investigation.

When active yeast-cells are suspended in a well aerated nutrient liquid, such as a malt-wort of suitable concentration, and containing an excess of all the organic and mineral substances requisite for their complete nutrition, we should expect a uniform rate of reproduction to be maintained as long as the temperature is invariable and the cells are so sparsely distributed as to avoid mutual interference by crowding; always provided that no restrictive influence is exerted by the products of growth or fermentation. Under these ideal conditions, if, at stated intervals of time, a census were taken of the number of yeast-cells present in unit volume of the liquid we should expect to find, on plotting the 'density of population' against the time, that the resulting curve was a logarithmic one, and any departure from such an experimental rate of increase would necessarily connote the existence of some factor or factors tending to restrict the free reproduction of the cells.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

When such a suitable nutrient liquid, saturated with atmospheric oxygen, is seeded with a small amount of yeast anywhere in the neighbourhood of 100 c.c. of pressed yeast per 100 cc. (an amount equivalent to about 1 cell per unit volume of $\frac{1}{4000}$ cubic mm.[1]) and cell-counts are made in the haemocytometer at intervals of a few hours, the cell-reproduction expressed as a function of the time always progresses at a much slower rate than is demanded by the exponential law. This is due, as was first pointed out by Adrian Brown, to the reproduction being restricted and limited by the amount of oxygen dissolved in the nutrient liquid.

Quite recently, however, it has been shown by A. Slator (Biochem. Journ., vii (1913), p. 197) that it is possible to realize the logarithmic rate of increase by inoculating the nutrient liquid with very small amounts of yeast, and slowly agitating the containing tubes in a thermostat. The seedings of yeast employed corresponded to from 1,360 to 90,100 cells per c.c., which are equivalent respectively to 0·0003 and 0·0225 cells per unit volume of $\frac{1}{4000}$ c.mm.

These are conditions which might be expected to give the theoretical exponential rate of increase, since, owing to the wide distribution of the cells and the constant stirring, there must have been within the range of the experiments a practically unrestricted supply of oxygen, and consequently an absence of competition for this essential factor of cell-reproduction.

The rate of reproduction of yeast-cells under the ordinary conditions of seeding a nutrient liquid in flasks with limited access of oxygen has been investigated by Adrian Brown (Trans. Laboratory Club, vol. iii (1890); and Journ. Chem. Soc., Trans., 1905, p. 1395).

In two series of experiments in which the nutrient liquid was on the one hand a solution of dextrose with yeast-water, and on the other a malt-wort of sp. gr. 1·053, flasks containing equal volumes of the solutions were seeded with yeast in amounts varying from 0·145 to 15·75 cells per unit volume of $\frac{1}{4000}$ c.mm., this initial rate of seeding being denoted by *A*. At the end of eighteen hours, the temperature remaining constant, a further count was made in each case; this is denoted by *B*. And the 'rate of cell-reproduction'[2] is given by $\frac{B}{A}$. It was found that 'cell-reproduction proceeds at the maximum rate when the number of cells present is at a minimum, and that the rate falls with a *decreasing* velocity as the number of cells in the experiments is increased'. Later on the author suggests that

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[1] One cell per unit volume of $\frac{1}{4000}$ c.mm. is equivalent to 4 million cells per cubic centimetre. This rate of seeding is closely approximated when 0·1 gramme of ordinary brewers' yeast (top fermentation) which has been washed and pressed between folds of blotting-paper is mixed with 100 c.c. of liquid.

[2] The 'rate of cell reproduction', $\frac{B}{A}$, really represents the average number of cells derived from a single cell during the experiment.

the 'rate of reproduction' $\frac{B}{A}$ is in inverse proportion to the square root of the number of cells of the initial seed-yeast A.

If this were strictly correct it follows that the value $\frac{\sqrt{A} \times B}{A} = \frac{B}{\sqrt{A}}$ should be constant for all the experiments in any one series.[1] On making the calculations it was found that the value for $\frac{B}{\sqrt{A}}$, 'although not constant for each series, nevertheless exhibited a general similarity, which is very remarkable when it is considered that the experiments from which they originate are concerned with the complex functions of living organisms', and 'the relatively close agreement among so many experiments renders it very improbable that such a result has been obtained by mere chance'.

Whilst my own work on this subject fully confirms that of Adrian Brown as regards the limitation of yeast-reproduction by deficient supply of free oxygen, I have found that under similar experimental conditions cell-multiplication stands in a much more simple relation to the time interval than is here stated, and this relation may be expressed as follows:—

During the period of active reproduction of yeast in a suitable medium in which access of oxygen is limited to that initially present in solution in the liquid, the number of cells present at any moment is directly proportional to the time.

If, for example, we denote the number of cells of the seed-yeast per unit volume by n, and the cell-count at any time during active reproduction by N, then when either N or $N-n$ is plotted against the time, the result is a straight line, and the 'curve' only begins to depart from the rectilinear direction as the point of maximal cell-reproduction is reached.

This fact is illustrated in the graphs of Fig. 1, in which Curve A represents the results of one of my own experiments in which one cell per unit volume[2] of seed yeast was employed. Curve B shows the plotted results of a somewhat similar experiment described by Adrian Brown (Trans. Laboratory Club, 1890) in which the initial seeding was at the rate of 0·65 cell per unit volume. Both sets of results illustrate the well-marked rectilinear character of the curves during the main period of cell-reproduction, and the comparatively sudden change in their direction as the point of maximal cell-increase is approached. How far these curves depart from the logarithmic rate of increase is shown by the dotted line C, which gives the logarithmic curve of cell-increase for Experiment A, on the

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[1] In the original paper (Journ. Chem. Soc., Trans., 1905, p. 1398) the above value, owing to a misprint, appears as $\frac{\sqrt{A \times B}}{A}$.

[2] The unit volume referred to here and throughout the paper is $\frac{1}{4000}$ c.mm.

assumption that the successive doublings of the cell-counts had occupied equal intervals of time.

There can be no doubt that the departure from the logarithmic rate in experiments of this kind indicates the existence of some restricting agency which exercises an influence even in the very earliest stages of cell-growth, but it must be one of an exceptional and peculiar nature to account for the rate of cell-reproduction being so consistently proportional to the time. That mere overcrowding of the cells suffices to account for the result is extremely improbable from the following considerations.

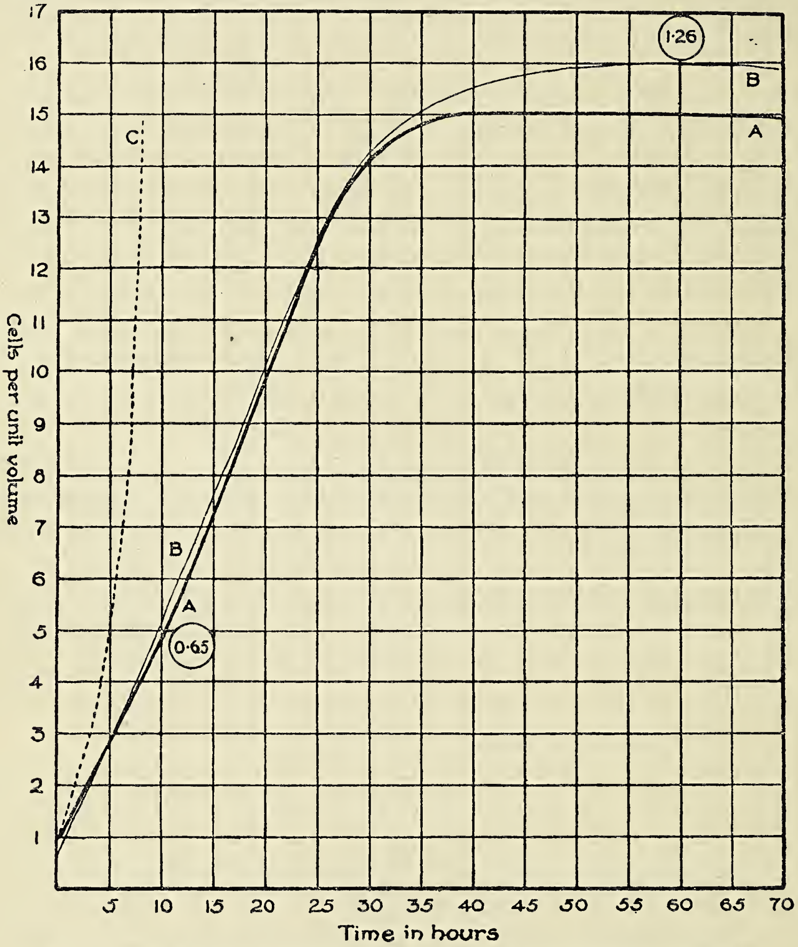

FIG. 1. The figures in small circles denote alcohol percentages for Curve B.

In Experiment A (Fig. 1) the seed-yeast amounted to one cell per unit volume of $\frac{1}{4000}$ c.mm., the average diameter of a yeast-cell (assumed to be spherical) being 0·008 mm. If we imagine the unit volume of liquid and its contained yeast-cell to be magnified a little over 1,587 diameters the unit volume will be represented by a cubic decimetre, and the yeast-cell by a sphere of 12·6 mm. diameter. The apparent volume of the magnified cell will be 1·06 c.c., or about 0·1 per cent. of the total volume of the liquid in which it is suspended. With this distribution the average distance apart of the cells of the yeast-seed would be about eight diameters, and even when the cells have increased to eight cells per unit volume their average distance apart will not be less than four diameters.

When these facts are taken into consideration, and also that the individual cells are bathed in a nutrient liquid of a concentration hundreds of times greater than that requisite to supply all their demands by the process of diffusion, it must be evident that distributions or packings of the cells of such orders of magnitude as we are now considering cannot be responsible for the great slowing down of the cell-increase which is implied by the progression of such increases being an arithmetical and not a geometrical one. Were this the case there would be some indications of it in the earlier stages by an upward trend of the curve, whereas the more carefully the experiments are made the more strictly rectilinear is the 'curve' found to be, subject to a slight departure at the commencement, marking a short incubation period before active growth commences.

We are therefore obliged to seek for an explanation of the linear progress of yeast reproduction in some other direction than that suggested by overcrowding and mutual interference of the cells in obtaining their food material from the nutrient liquid containing them.

The two most potent factors in controlling the reproduction of yeast-cells are undoubtedly the amount of free oxygen at the disposal of the cell prior to the commencement of reproduction, and the limitation and partial inhibition of growth brought about by the gradually increasing amount of alcohol produced by the fermentative action of the yeast. These facts were thoroughly well established by Adrian Brown (loc. cit.), who also showed that neither the carbon dioxide[1] nor the non-volatile products of fermentation had any appreciable effect. The final conclusions at which he arrived were that in a suitable nutrient liquid yeast reproduction is conditioned and governed mainly by the amount of oxygen originally at the disposal of the cell prior to the commencement of reproduction, and that it is the exhaustion of the stimulating influence of this oxygen which becomes the limiting factor in arresting the reproductive functions, and not the alcohol produced, since the sharp arrest of cell-growth is often noticed when the percentage of alcohol falls below that necessary to produce any appreciable effect. The finite amount of oxygen initially present in the liquid was also

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[1] A. Slator (Biochem. Journ., vii (1913), p. 201) refers incidentally to the influence of carbon dioxide in retarding yeast-growth. I am unaware of any experiments which show this influence of CO_2 apart from its effect of preventing access of oxygen. Even should it be shown that a distinct retarding effect can be produced by CO_2, this would not have any material bearing on the experiments here recorded, since such effect would speedily attain a maximum when the liquid was saturated with CO_2, i. e. in the earliest stage of the experiment.

regarded, and no doubt correctly, as explaining the remarkable fact that under fixed conditions the maximal cell reproduction is independent of the number of cells of seed-yeast per unit volume. A satisfactory explanation was also afforded of the observation that if more cells are added in the form of seed-yeast than correspond to the maximal reproduction then no appreciable cell-increase takes place, the limited amount of oxygen at the disposal of each cell being insufficient to stimulate its reproductive functions.

A few years ago when investigating the assimilation of nitrogen by yeast I had occasion to examine somewhat critically these conclusions of Adrian Brown, and during the course of the inquiry I carried out a considerable number of experiments under varying conditions, especially with a view to determining the quantitative relation of available free oxygen to cell-increase. My results were entirely confirmatory of the proposition that the first limiting factor is the exhaustion of the stimulus given by a relatively very small supply of oxygen, and they further led to an approximation of the absolute amount of oxygen required by a yeast-cell to complete its process of subdivision.

The experiments were for the most part conducted on malt-worts of a sp. gr. varying from 1·050 to 1·055, which contain an excess of all the nutrient materials required for yeast reproduction. Before seeding with a definite amount of yeast the nutrient liquids were fully aerated by strong agitation in the presence of air at the temperature at which the experiment was to be conducted, and immediately after seeding the air in the flasks over the liquid was displaced with CO_2. In this manner the free oxygen concerned in the subsequent process was confined to that which was originally present in solution, and the absolute values representing this oxygen were deducible from the known coefficient of solubility of atmospheric oxygen in the liquid.[1]

After seeding the yeast, a series of cell-countings was made in the haemocytometer, thus forming a datum line to which subsequent cell-countings could be referred. A close approximation to any required density of seeding can always be obtained in the first instance by adding definite weights of the freshly grown and washed yeast, after pressing between folds of filter paper. One gramme of an ordinary top-fermentation yeast prepared in this manner and evenly distributed in 100 c.c. of liquid will give a cell-count approximating very closely to 10 cells per unit volume of $\frac{1}{4000}$ c.mm.

The actual cultivations were carried on as nearly as possible at a uniform temperature, and successive cell-countings were made at fixed intervals of time. In some cases the progression of cell-reproductions was obtained

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[1] An objection might be raised to this procedure on the ground that immediately on replacing the air in the flask with CO_2 the nutrient liquid must begin to lose its free oxygen by a process of diffusion into the oxygen-free atmosphere above it. Practically this objection has no weight since, as we shall see later, the dissolved oxygen is very rapidly fixed by the seed-yeast when this amounts to anything like one cell per unit volume.

from separate flasks maintained under exactly similar conditions, but in most cases the samples for examination were derived from one flask of several litres capacity, so arranged that uniform samples could be forced out by pressure of CO_2.[1]

Occasions sometimes arise when it is inconvenient or even impossible to determine the progress of cell-reproduction by direct counting, and we have to fall back on an indirect method which gives equally good results.

As I have fully described this method elsewhere (see Journ. Institute of Brewing, 1909, p. 232), I need only refer to it briefly. It is based on the fact that freshly cultivated yeast when seeded in a malt-wort maintains a constancy in both the average dimensions of its constituent cells and in their nitrogen content.

Let us assume that we have determined by the Kjeldahl method the percentage of nitrogen in a sample of washed and pressed yeast which we are about to use for seeding a wort, and that we have also determined the number of cells per unit volume of $\frac{1}{4000}$ cm., which are given by mixing a definite weight of this same yeast with water, and making up the volume to 100 c.c. From these data we can calculate the actual amount of yeast-nitrogen in 100 c.c. if the distribution of the cells corresponded to exactly one cell per unit volume of $\frac{1}{4000}$ cm. This value I have termed the 'nitrogen coefficient' of the yeast, and since the 'nitrogen coefficient' under the experimental conditions of growth in malt-wort remains constant, the cell-reproduction which occurs between any two intervals of time is a measure of the actual amount of nitrogen assimilated from the liquid; and conversely, if the loss of nitrogen per 100 c.c. has been determined for the same period by two successive nitrogen determinations in the malt-worts filtered from the yeast, this nitrogen loss can be used as a measure of cell-reproduction. As an example, let us suppose that a particular sample of pressed yeast contains in its moist condition 1·98 per cent. of nitrogen, and that 1 grm. of this yeast, corresponding to 0·0198 grm. of nitrogen when mixed with water up to 100 c.c., gives a cell-count in the haemocytometer of 10·39 cells per unit volume. Then the 'nitrogen coefficient' of the yeast (that is to say, the total nitrogen in the 100 c.c. if the distribution of the cells were exactly one cell per unit volume) would be represented by $\frac{0{\cdot}0198}{10{\cdot}39} = 0{\cdot}00191$. This value can now be used as a measure of the cell-increase between any two intervals of time, if the loss of nitrogen in the filtered liquid has been determined: we then have

$$\text{cell-increase per unit volume} = \frac{\text{Loss of nitrogen per 100 c.c.}}{\text{Nitrogen coefficient of yeast}}.$$

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[1] Certain obvious precautions have to be taken in such cases to ensure that a sample drawn off in this way is truly representative of the bulk.

This alternative method of determining the cell-increase gives results which are quite comparable in accuracy with those obtained by direct counting.

Before proceeding to the consideration of the quantitative relations between cell-reproduction and available oxygen, we must discuss the second factor which may, under certain conditions, have an important influence in restricting growth—viz. the alcohol which the yeast produces in the course of an experiment.

Adrian Brown found that additions of alcohol at the commencement had but little effect on the final cell-reproduction if these additions did not exceed about 3 per cent., but that above this amount cell-increase fell off rapidly, although it was not completely inhibited by the addition of 8·4 per cent.

Since the yeast was producing alcohol during its period of increase, the amount of alcohol capable of producing any distinct inhibiting effect must have exceeded 3 per cent.

Instead of merely noting the influence of definite amounts of alcohol on maximal yeast-increase, I have repeated these experiments in such a manner as to be able to follow the whole course of cell-reproduction, taking care that the conditions of oxygenation of the liquid were the same in each case, and that all other conditions were as far as possible identical. The results of these experiments are given in the series of curves of Fig. 2, the amounts of alcohol added at the commencement being respectively 0·5, 1·0, 3·0, 4·0, 6·0, and 8·0 grm. per 100 c.c. The figures in small circles at various points of the curves indicate the actual amounts of alcohol present—that is to say, the amount added *plus* that produced by fermentation. For purposes of comparison there is included the curve of an experiment made under identical conditions but without the previous addition of any alcohol. The general results indicate that when oxygen supply is limited to that afforded by complete aeration of the liquid at the commencement, a sensible effect on the cell-reproduction of the particular class of yeast used[1] begins to be noticeable when the alcoholic content reaches about 2·5 per cent., and that amounts of about 8·0 per cent. almost entirely inhibit reproduction under these conditions.

If we turn once more to Fig. 1, where the alcohol percentages are indicated on Curve B by the figures within small circles, we see that the sudden falling off of cell-reproduction, as indicated by the sharp bend of the curve, cannot have been due to the inhibitory effect of the alcohol produced, since the quantities are too small to have had any such effect. But, although under these special conditions of limited supply of oxygen the gradually increasing alcohol is not a factor in producing, at a certain point, the rapid diminution of cell-reproduction which is so characteristic of all such

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[1] The yeast used was an English high-fermentation yeast.

experiments, it may well become the paramount factor under another set of conditions. If, for instance, a rapid stream of air is urged through the culture liquid, and due regard is paid to keeping up the necessary food supply, then cell-reproduction is essentially controlled and limited by the alcohol factor.

In order to investigate more fully the relation between oxygen supply and cell-increase, a further series of experiments was instituted on an entirely different plan. The general idea underlying these was to cultivate the yeast in a medium containing variable but known amounts of oxygen, and to express the final reproduction in each case as a function of the available free oxygen.

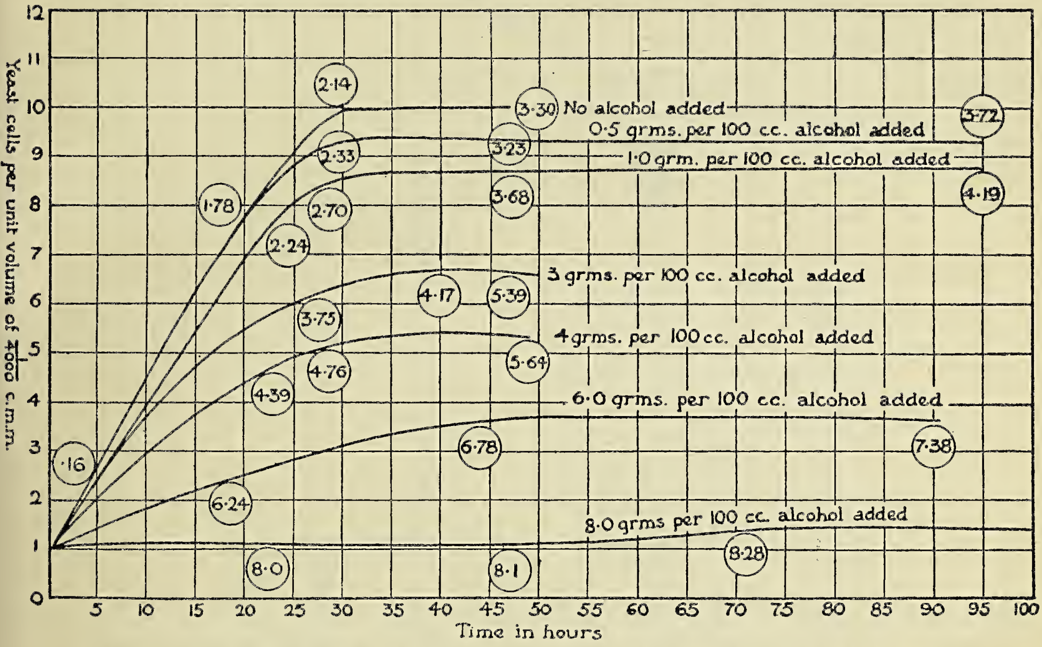

FIG. 2. Yeast cultivations with limited oxygen supply and varying amounts of added alcohol. Temperatures 27°–30° C. Seed-yeast 1 c.cm. per unit volume (= 0·1 gramme pressed yeast per 100 c.c.) N.B. The figures in small circles denote total percentage of alcohol.

Malt-wort was again the medium chosen for the cultivations. A flask of about four litres capacity was fitted up as shown in Fig. 3. This was filled to about two-thirds of its capacity with malt-wort, which was then boiled rapidly for an hour or an hour and a half while a constant stream of hydrogen was passed into the flask through tube A, escaping through C, the pinchcock B being at first closed. At the close of the boiling process some of the wort was forced into tube B by closing C. B was then again closed and C reopened, the flask and its contents being allowed to cool whilst the stream of hydrogen was still passing. This de-oxygenated wort could then be passed over by hydrogen pressure into a series of cultivation flasks, one of which is shown at F;[1] these were of about 225 c.c. capacity, and were fitted in such a manner that the gas-delivery tube did not extend below the rubber stopper, in order that at the final operation the flasks could be completely filled with the wort, which was also driven round the bend E.

Some of the de-aerated wort was then withdrawn from the large flask, and was fully saturated with air by strong agitation, the temperature being noted. A measured quantity of this fully aerated wort, along with a weighed

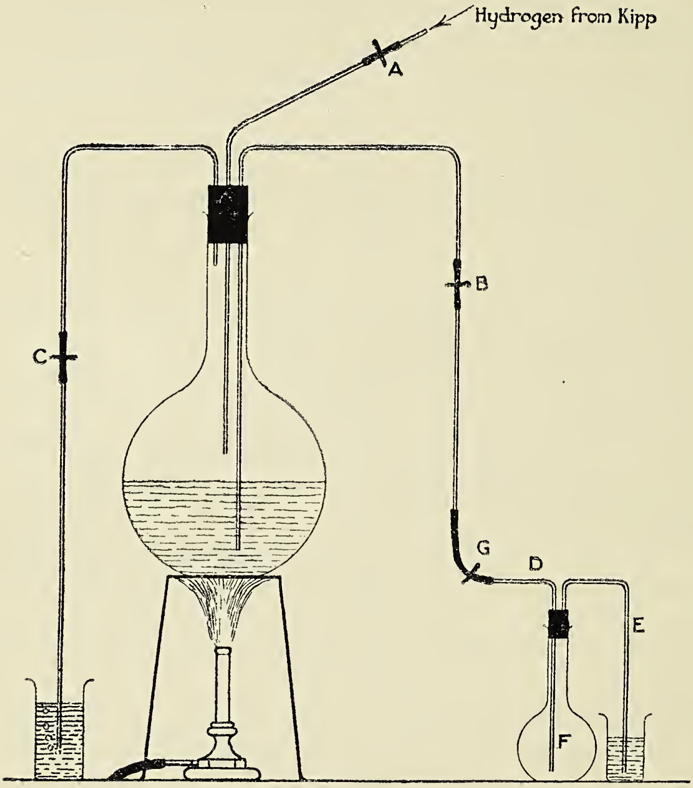

FIG. 3.

amount of freshly cultivated and pressed yeast, generally amounting to 0·2 grm., was transferred to one of the small flasks F, which was then rapidly and completely filled up with de-aerated wort through the tube D. The pinchcock G was then closed and the rubber connexion severed just behind it.

In this manner a series of flasks was obtained containing known proportions of fully aerated and de-aerated wort with definite amounts of yeast. A complete series consisted of nine such flasks ranging from fully aerated

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[1] Before inserting the plug of F the supply tube B, G, D must be completely filled with the de-aerated wort.

to completely de-aerated wort, the intermediate members of the series being mixtures of the two in known proportions.

In filling the flask with completely de-aerated liquid special precautions had to be taken in introducing the seed-yeast. This was spread on a thin cover-slip and dropped into the empty flask, a current of hydrogen being then passed through the flask for some time before it was filled with the oxygen-free wort.

All the flasks were then allowed to ferment slowly at the same temperature for ten days or so with their outlet tubes dipping under mercury.

Since the flasks were quite full it is manifest that as fermentation proceeded there would be a slight loss of liquid from the outlet tubes and the upper part of the flasks in the first stages of fermentation. It was therefore more convenient to determine the cell-reproduction by the indirect method of nitrogen loss than by cell-counting. The nitrogen coefficient of the seed-yeast was therefore ascertained, and also the number of cells per unit volume given by mixing a definite weight of it with water and making up to 100 c.c. The estimation of the nitrogen in the wort before and after fermentation then completed all the necessary data.

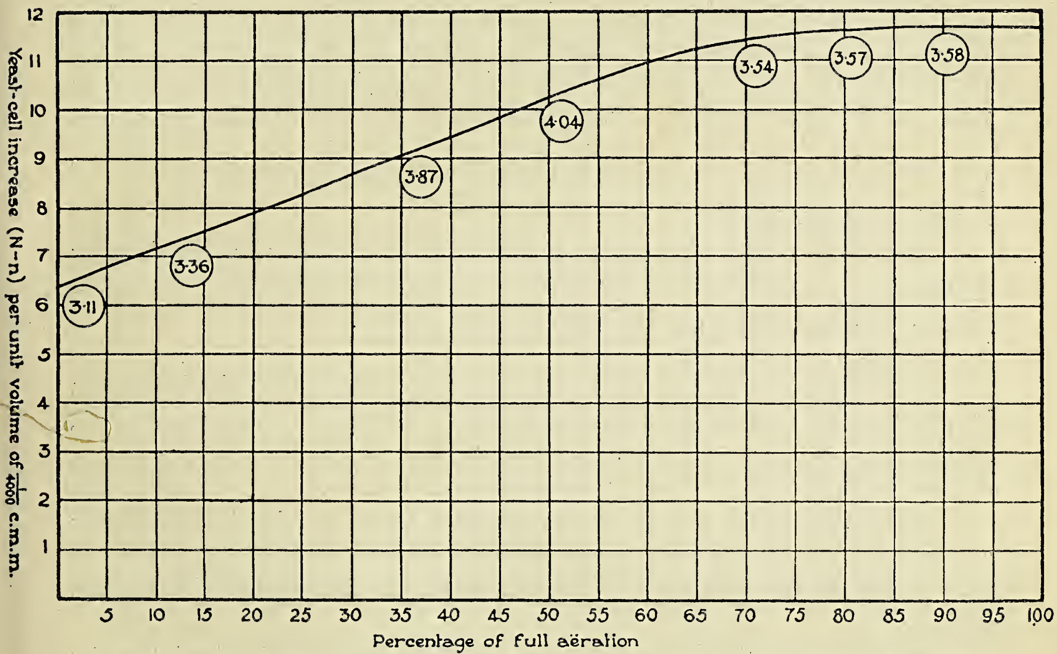

FIG. 4. Curve showing influence on yeast-cell reproduction of varying amounts of dissolved oxygen in the culture-liquid (malt-wort). The cultures were made with definite mixtures of fully aerated worts and of wort fully deprived of oxygen. Seed-yeast = 1 cell per unit volume. The figures in small circles denote percentages of alcohol found at the termination of each experiment.

Fig. 4 shows the result of plotting the maximal cell-increases attained in nine such experiments with the same wort and the same seed-yeast, the conditions of cultivation in all cases being maintained constant with the exception of the degree of aeration, which is the only variable. The cell-increases per unit volume are indicated on the line of ordinates, whilst the intervals on the abscissa line show the percentages of fully aerated wort contained in the mixture.

It will be noted that up to 65 or 70 per cent. of complete aeration the cell-increase is directly proportional to the available oxygen present in the culture medium at the commencement of growth, and that beyond this point further increments of oxygen produce a smaller and ultimately vanishing effect.

In order to carry the argument further and to obtain the absolute values of the free oxygen involved in these experiments, we must know the ratio of the coefficient of solubility of atmospheric oxygen in malt-wort to that in water at the same temperature. If we denote the former value by c_1 and the latter by c, then the ratio $\frac{c_1}{c}$ as given by Pasteur is about 0·86 for a wort of sp. gr. 1·060.

In a series of experiments on worts of sp. gr. 1·050, using the Adeney apparatus for pumping out the dissolved gases after complete saturation with air, I have found the ratio $\frac{c_1}{c} = 0{\cdot}82$ at 16° C.

According to Roscoe and Lunt, the solubility of atmospheric oxygen in water at 16° C. and 760 mm. pressure is 0·682 c.c. per 100 c.c. of water, so that a malt-wort of sp. gr. 1·050 when fully saturated with air should contain $0{\cdot}682 \times 0{\cdot}82 = 0{\cdot}559$ c.c. of oxygen per 100 c.c.

From the curve of Fig. 4 we see that its course is almost exactly rectilinear up to 60 per cent. of oxygen saturation of the wort, that is to say that within these limits cell-increase is a linear function of the available free oxygen.

This 60 per cent. of complete saturation is represented in absolute units by $0{\cdot}60 \times 0{\cdot}559 = 0{\cdot}335$ c.c. of oxygen per 100 c.c. The consumption of this amount of oxygen has resulted in the production of $11{\cdot}0 - 6{\cdot}4 = 4{\cdot}6$ cells per unit volume of $\frac{1}{4000}$ c.mm., so that for the production of one cell per unit volume there has been used up per 100 c.c. an amount of oxygen represented by $\frac{0{\cdot}335}{4{\cdot}6} = 0{\cdot}07$ c.c. But one cell per unit volume of $\frac{1}{4000}$ c.mm. is equivalent to 400 millions of cells per 100 c.c., so that each single cell of this aggregate has on an average required for its production the expenditure of $\frac{7 \times 10^{-2}}{4 \times 10^{8}} = 1{\cdot}7 \times 10^{-10}$ c.c. of oxygen.

Now the average volume of a yeast-cell, assuming it to be a sphere of 8 μ in diameter, is $2{\cdot}68 \times 10^{-10}$ c.c., so that the above-mentioned volume of oxygen requisite for the subdivision of one yeast-cell into two, when expressed as a fraction of the volume of the original cell, is $1{\cdot}7 \times 10^{-10} \div 2{\cdot}68 \times 10^{-10} = 0{\cdot}63$.

It will be noted from the curve of Fig. 4 that even after complete de-oxygenation of the culture medium there has been a cell-increase equivalent to about 6·5 cells from each single cell of the seed yeast. It might be supposed that this is due to a small amount of oxygen left in the liquid, or to its subsequent introduction by faulty manipulation. It is impossible, however, to accept this explanation, since similar results are obtained when extreme care is taken to eliminate these sources of error. It is no doubt a fact that the yeast-cell has a limited power of reproduction under strictly anaerobic conditions, owing, as we shall see later, to its power of storing up oxygen prior to the seeding process.

We may now turn to the effects which variations in the amount of seed-yeast exert on cell-reproduction when all other conditions remain the same.

Adrian Brown came to the conclusion that when the supply of free oxygen is constant, the cells increase up to a certain maximal amount per unit volume which remains constant even when the amount of seed-yeast varies considerably.

With certain limitations this conclusion is no doubt approximately correct, especially when we compare the true values of the cell-increases $N-n$ instead of the final counts N, and care is taken that the available oxygen is strictly identical in the various experiments. In my own experiments this was ensured by arranging, in the manner already described, that the available free oxygen was confined to that dissolved in the culture-liquid, and that no further access of oxygen was possible at any subsequent stage.

Under these conditions, and provided the seed-yeast does not fall below one cell per unit volume, not only is the maximal reproduction $N-n$ constant, or nearly so, for considerable variations in the seeding, but during the reproductive stage the number of new cells formed in a given interval of time is also constant and independent of the rate of seeding.

This is shown in Figs. 5 and 6. In Fig. 5 the results are given of three of my own experiments in which the seed-yeast amounted to 0·93, 1·87, and 2·8 cells per unit volume.

Fig. 6 gives the curves which I have deduced from a series of Adrian Brown's experiments, in which the cells of seed-yeast per unit volume initially amounted to 1·57, 2·35, and 3·14 respectively.

The general parallelism of these curves bears out what has been said as to the progressive and final reproduction being independent of the original seeding. They are phenomena which might have been deduced from the fundamental fact that there is, within certain limits, a quantitative relation between cell-multiplication and the supply of free oxygen.

I have stated that the above relations do not hold good when the amount of seed-yeast falls much below 1 cell per unit volume. If the initial seeding is as low as 0·1 cell per unit volume, the departure from the above rule is very great. The explanation of this is to be found in the behaviour of the seed-yeast to the dissolved oxygen at the commencement. As we shall see a little later, the first action of the yeast, long before there are signs of reproduction, is to absorb and fix the dissolved oxygen. This absorption is very rapid with a 'density of population' of yeast-cells equivalent to 1 cell or more per unit volume, but with anything less than this the rate of absorption falls off rapidly, and with anything like 0·1 cell per unit volume the absorption is so slow that sufficient time elapses to allow a more or less complete diffusion of the dissolved oxygen into the inert gas which replaces the air above the liquid. Under these conditions reproduction is relatively

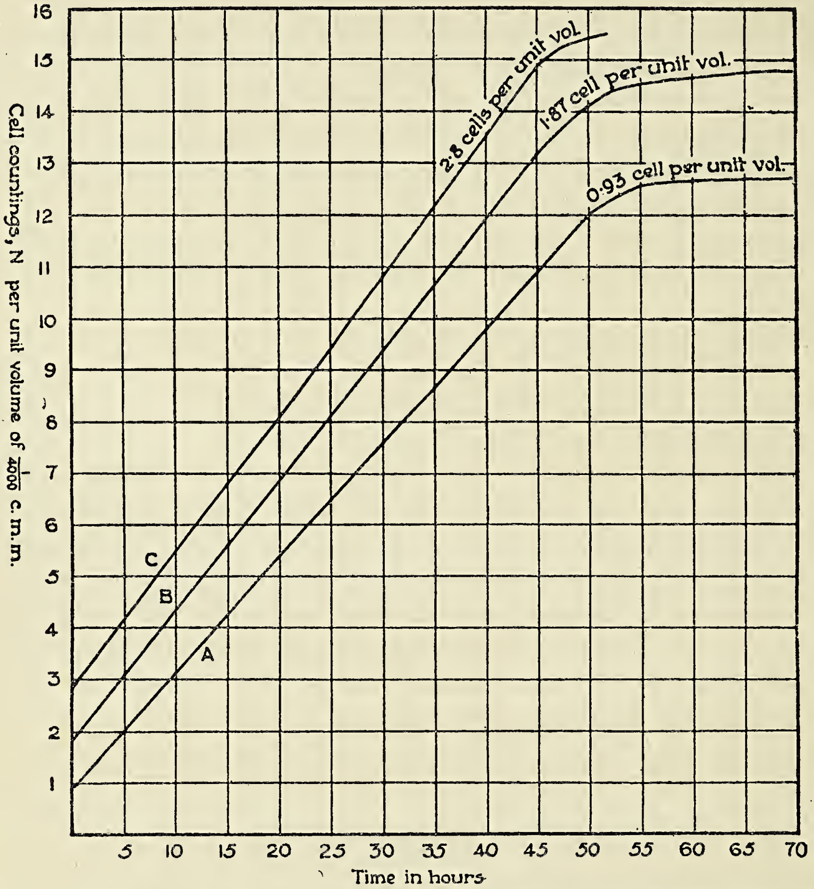

FIG. 5. Fermentations with limited supply of oxygen, and with varying initial amounts of seed-yeast, 17·0° C. A. Seed-yeast 0·93 cell per unit volume. B. 1·87 cells per unit volume. C. 2·8 cells per unit volume.

slowed down and is no longer comparable with that observed for higher rates of seeding.

Pasteur, in his 'Études sur la bière', describes certain experiments which he made to determine the rate at which free oxygen dissolved in malt-worts is absorbed by yeast, making use of the Schützenberger method for oxygen estimation. He gives the particulars of an experiment made in a brewery in which the free oxygen dissolved in a malt-wort was determined hour by hour after the addition of the yeast, the temperature being 6° C.

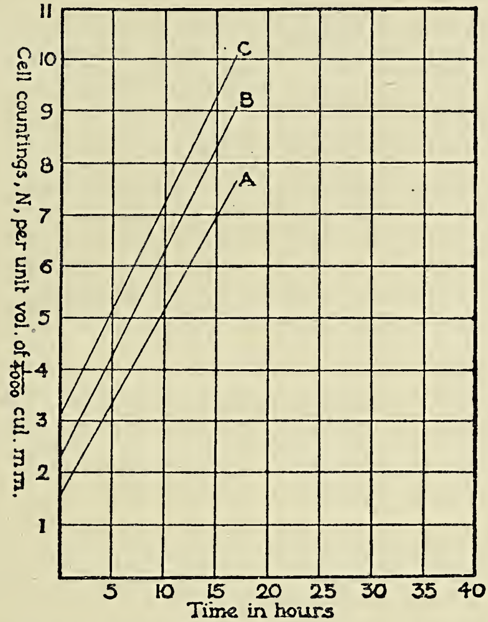

FIG. 6. Plottings of three experiments of Adrian Brown (see Journ. Chem. Soc., 1905, p. 1398) showing final countings of yeast-cells after cultivation for seventeen hours, the initial seed-yeast being varied in amount. Curve A. Seed-yeast 1·57 cells per unit volume. Curve B. Seed-yeast 2·35 cells per unit volume. Curve C. Seed-yeast 3·14 cells per unit volume.

From the particulars given of the amount of yeast used, it must have been equivalent to about 1 cell per unit volume of $\frac{1}{4000}$ c.mm. The results which were plotted out show that within six hours the residual oxygenation had only about 25 per cent. of the initial value, and that at the end of twelve hours from the commencement all the dissolved oxygen had disappeared. With double the amount of yeast the free oxygen disappeared in less than half the time. Pasteur further states that when this free oxygen had been removed from the wort the yeast-cells still showed no signs of reproduction,[1] but they had assumed a younger and fuller appearance, and he draws the

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[1] It must be remembered that the yeast used in this experiment of Pasteur was a 'bottom yeast' which was functioning at a low temperature; hence oxygen absorption and initial rate of reproduction were relatively slow.

conclusion that the oxygen had been stored up in them, and functioned as a *primum movens* of their subsequent life and nutrition. If for ‘life and nutrition’ we read ‘power of reproduction’, this is a striking instance of the prophetic insight of genius, for we have seen that this *primum movens* is not only the essential determinant of cell-reproduction, but that the stored-up oxygen exerts a ‘potential’ which is quantitatively related to the amount of subsequent reproduction.

The limited growth which, as we have seen, yeast exhibits when seeded in a liquid from which oxygen has been wholly excluded (see p. 209), so far from being in opposition to this view is really in its favour, for owing to the very nature of the experiments the cells of the seed-yeast in such a case must have carried with them an ‘oxygen charge’ which enabled them to reproduce to a certain limited extent even under anaerobic conditions.[1]

I have further investigated this remarkable power of yeast to store up oxygen for further use by submitting it to long-continued aeration before it was used for seeding, and in some cases I have passed oxygen through a suspension of the yeast in water. Such treatment in all cases failed to give a yeast which, after separation, possessed increased potentialities of growth, and in some cases the power of reproduction was actually diminished, especially when it was treated with pure oxygen. It seems to be impossible to give a yeast a higher effective charge of oxygen than it has ordinarily acquired by processes of washing and filtration in contact with air, and apparently one cannot by any further treatment in this direction make up for the lack of free oxygen in the culture medium, since on this factor and on the subsequent access of oxygen from the outside mainly depends the final degree of reproduction up to the point at which inhibitory causes come in, such as those due to failing of essential nutrients, and formation of alcohol.

At ordinary temperatures yeast suspended in its nutrient liquid is a very complete and rapid absorber of dissolved oxygen, much more so in fact than might be imagined from the experiments of Pasteur just alluded to, which were made at a temperature of 6° C. I have studied the actual rate of absorption by varying proportions of yeast-cells by suspending these in *water* fully saturated with atmospheric oxygen, and noting at intervals the gradual disappearance of the oxygen.

The so-called Winkler process for determining the dissolved oxygen is

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[1] There is one fact which seems to be against the idea that this anaerobic reproduction was due to the ‘oxygen charge’ of the seed-yeast. If this were so one would expect that on varying the amount of seed-yeast the maximal number of cells produced anaerobically would be proportional to the seed-yeast employed, whereas it was found that the maximal cell-reproduction $N-n$ was constant and independent of the initial seeding. This is the result which might be expected if there was any residual oxygen in the culture-liquid, but the conditions of the experiments negative this explanation. Another possible explanation of this fact may be that during fermentation under strictly anaerobic conditions a small amount of peroxide of hydrogen may be produced, and that the ferrous salts in the nutrient liquid may be the ‘carrier’ of the oxygen of the peroxide to the yeast.

well adapted for this purpose, since the presence of the yeast-cells does not sensibly affect its accuracy. A description of the method has been given by Clowes and Biggs (Journ. Soc. Chem. Ind., April 15, 1904).

A stoppered bottle of from 300 to 400 c.c. capacity is filled with the water to be examined, due precautions being taken to avoid agitation during the process. About 1 c.c. of a strong solution of manganous chloride is then added, followed by 2 c.c. of a solution containing 33 per cent. of alkaline hydroxide and 10 per cent. of potassium iodide. The bottle, which must be quite full, is then closed with its stopper, taking care to avoid the inclusion of any air bubbles, and is inverted several times in order to mix the liquid thoroughly. The manganous oxide which is precipitated rapidly absorbs any free oxygen which is present, and in order to complete the reaction the bottle is put in the dark for fifteen minutes. Its contents are then acidified with from 2 to 3 c.c. of strong hydrochloric or sulphuric acid, which dissolves the precipitate, leaving the liquid slightly coloured with iodine. This liberated iodine is a measure of the original amount of free oxygen present in the water, and is titrated with a standard solution of thiosulphate, of which the oxygen value is known, using starch as the indicator.

If the experiment is repeated with another sample of the water which has been *fully aerated* at the same temperature, then the ratio of the amount of thiosulphate solution employed in the two experiments gives the degree of oxygen-saturation of the first sample when full saturation is represented as unity.

The curves in Fig. 7 show the rates of absorption of dissolved oxygen from fully aerated water at 15° C. by amounts of yeast corresponding to 1·0, 2·0, and 3·0 cells per unit volume respectively, the actual amounts of pressed yeast per 100 c.c. being 0·1, 0·2, and 0.3 grm.

It will be noted that above 1 cell per unit volume the absorption of oxygen is approximately proportional to the number of cells per unit volume, and that with the small amount of only 0·3 grm. of pressed yeast per 100 c.c. or 3·0 cells per unit volume, the absorption is complete in two and a half hours.

It might reasonably be expected that a reaction of this kind would show a tendency to slow down as the concentration of the dissolved oxygen gradually decreased. Absorption of dissolved oxygen by the yeast-cell progresses, however, as a *linear function of the time*, just as the invertive, fermentative, and reproductive actions do. There is no doubt a common cause controlling all these functions, which is to be found in the fact that they are all dependent on cell metabolism, and that the processes involved, although in some cases rapid as judged by ordinary standards of time, are very slow compared with the relatively rapid processes of diffusion by which the materials involved get access to the cell-protoplasm from the surrounding medium. In such cases diffusion can only become the limiting factor at very low outside concentrations, as shown by Slator for the fermentative faculty, and where diffusion ceases to limit the reaction there must always be a tendency to linear rather than logarithmic rate of change.

If the water from which suspended yeast has absorbed all the dissolved oxygen is again saturated with air by strong agitation, the cells recommence their absorption at almost the same rate, and it is only by repeating such an experiment several times, or by passing strong currents of air through the liquid for an hour or two, that any marked diminution in the absorbing power is noticeable.

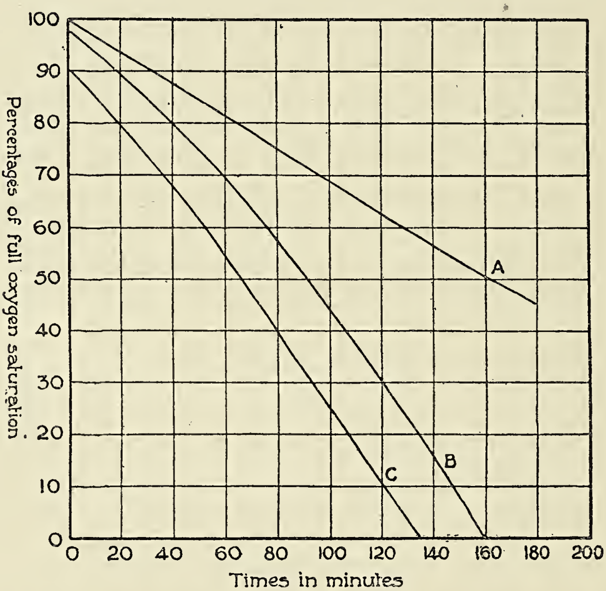

FIG. 7. Curve showing the rectilinear rate of absorption of dissolved oxygen by yeast at 15° C. Liquid used was water saturated with atmospheric air. The values on the line of ordinates give the percentages of full saturation remaining, and measure the absorption of oxygen. Seed-yeast used: A. 0·1 grm. pressed yeast per 100 c.c. = 1 cell per unit volume. B. 0·2 grm. pressed yeast per 100 c.c. = 2 cells per unit volume. C. 0·3 grm. pressed yeast per 100 c.c. = 3 cells per unit volume.

The main facts which have been established experimentally, with regard to the reproduction of yeast-cells in a nutrient liquid containing dissolved oxygen, and an excess of all the mineral and organic substances requisite for their full nutrition, may be summarized as follows:

1. When the available oxygen is limited to that initially contained in the liquid, the number of yeast-cells per unit volume tends to attain a maximum which is independent, or nearly so, of the number of cells of seed-yeast per unit volume, but is conditioned in the first instance by the initial amount of this dissolved oxygen.

2. Within certain limits of oxygen supply, the maximal reproduction is strictly proportional to the initial amount of this oxygen.

3. The rate of reproduction under these conditions is not logarithmic, but is a linear function of the time.

4. The dissolved oxygen does not remain as such in the liquid during the reproductive period, but is rapidly absorbed by the seed-yeast before cell-budding commences.

A due consideration of the important fact mentioned in (4), and of everything which it implies, enables us to give a satisfactory explanation of all the phenomena we are dealing with.

Although the main controlling influence on cell-reproduction is due to the free oxygen originally present in the nutrient liquid, this oxygen is absorbed very rapidly by the seed-yeast, the subsequent reproduction of which is brought about under conditions which, as regards the liquid itself, are anaerobic. We must therefore regard the potentiality of reproduction as being impressed on the cell at the very outset by the oxygen which it has absorbed, and that a quantitative relation exists between this absorbed oxygen and the number of subdivisions which the initial yeast-cells can finally make. The action is, in fact, one of *induction*, and all the known facts can be explained if we assume the equal partition of the available oxygen between the initial cells, and the consequent variability of the *oxygen charge* which these cells must receive when the ratio of the seed-yeast to the available oxygen varies.

In order to illustrate this point, let us consider three hypothetical cases, A, B, and C, in which we have a nutrient liquid such as a malt-wort, saturated with atmospheric air, and therefore containing about 0·5 c.c. of dissolved oxygen per 100 c.c. We will assume that A is seeded with 1 cell of yeast per unit volume, B with 2 cells, and C with 4 cells,[1] and that further access of oxygen is prevented during the reproductive period. Under such conditions we should expect to find, at the termination of the reproductive period, about 16 cells per unit volume in each case, the final cell-count being independent, or nearly so, of the rate of seeding. This result can be explained as follows: In all three cases the dissolved oxygen is absorbed within a short time by the seed-yeast, but it is manifest that, when this has taken place, the initial *oxygen charges* of the individual cells in A, B, and C respectively must vary inversely as the original cell-countings, and will therefore be represented by the ratios $1 : \frac{1}{2} : \frac{1}{4}$.

Since, within the limits of our experiment, the maximal cell reproduction is known to be proportional to the oxygen supply, the number of possible cell-divisions of any individual cell in B will only be one-half of that in A, but since the initial number of cells in B is double that of A, the final

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[1] These seedings approximately represent volumes of yeast-cells equivalent to 0·1, 0·2, and 0·4 c.c. per 100 c.c. of liquid.

limit of reproduction, as determined by cell-counting in a unit volume of liquid, should be the same in both cases. Again, the reduced number of subdivisions in C, due to the reduction of the oxygen charge on the individual cells to one quarter, is compensated by the fourfold number of seed-cells in this case.

This fundamental idea of the dependence of reproduction on the intensity of the initial *oxygen charge* also gives a complete explanation of the *rate* of reproduction being linear instead of logarithmic, for at each cell-subdivision the oxygen charge of the mother-cell must be halved, consequently the time required for the next subdivision of the mother- and daughter-cell will be double that required for the previous subdivision.

These considerations, taken in conjunction with the experimental data given in this paper, afford a quantitative proof of the correctness of the idea, first expressed by Pasteur, that the oxygen originally absorbed by the yeast from its surrounding medium is the *primum movens* which determines its subsequent power of reproduction.

The question now arises as to the limits of reproductive power which are imposed on the cell by this initial absorption of oxygen.

Although a yeast-cell can, under favourable conditions, continue to take in oxygen from the surrounding liquid if this is maintained saturated with air, the cell can only store up a limited amount of oxygen in a form available for subsequent reproduction. All attempts to increase this effective 'oxygen charge' by previously submitting the seed-yeast to strong aeration, or even by passing pure oxygen through the liquid, were unsuccessful. The yeast under these circumstances continues to take in oxygen, but its potentiality of reproduction is not thereby increased. Whether the whole of the surplus oxygen absorbed, over and above that required for the reproductive 'charge', is used up in ordinary processes of respiration appears to be very doubtful, and much more work requires to be done before this question can be definitely answered.

Meanwhile, the experiments which have been summarized in the curves of Fig. 4 give us some information about the actual values of an oxygen charge requisite to produce a given amount of cell-reproduction. In these experiments, in which the nutrient liquids contained known and graduated amounts of dissolved oxygen and were initially seeded at a uniform rate of one cell per unit volume, it is seen that a direct proportionality exists between oxygen supply and maximal cell-reproduction, up to a point within about 60 to 65 per cent. of complete saturation of the liquid with atmospheric oxygen. This point corresponds to an initial oxygen content of the liquid of about $0{\cdot}6 \times 0{\cdot}559 = 0{\cdot}335$ c.c. of oxygen per 100 c.c. of the liquid.

When the oxygen in the liquid is increased beyond this point up to full saturation with air, although further yeast reproduction takes place, the final cell-increase is no longer strictly proportional to the available oxygen initially present, and from the course of the curve we may conclude that, with a liquid seeded with yeast at the rate of one cell per unit volume, the stimulation to reproduction ceases when the initial oxygen supply is equivalent to about 0·5 c.c. per 100 c.c. of the liquid.

Since one cell of yeast per unit volume is equivalent to about 0·1 c.c. of yeast per 100 c.c. and the whole of the oxygen is rapidly taken up from the liquid, it is evident that under the above conditions the volume of oxygen taken up by each cell and constituting the 'oxygen charge' is about five times the volume of the cell itself.

PART II.

THE METABOLISM OF THE YEAST-CELL, WITH SPECIAL REFERENCE TO THE THERMAL PHENOMENA OF FERMENTATION.

The earlier ideas that the yeast-cell, by its mere presence, is able to exert an extraneous transforming influence on certain substances dissolved in the surrounding medium have now proved to be erroneous. All known facts point consistently to these changes taking place exclusively within the cell, and they therefore connote a constant centripetal flux of material through the cell-wall, and a counter flux of the metabolites of equal or nearly equal amount in the opposite direction.

We can obtain some idea of the extraordinary activity of this metabolic 'mill' by determining the ratio of the mass of the cell to that of the sugar fermented in a given time, or we can, if we prefer it, indicate the activity under given conditions by stating the time necessary for the cell to transform its own weight of sugar into alcohol and carbon dioxide.

Another way of illustrating the activity of the cell is to study the thermal phenomena of fermentation, and to refer the liberation of energy to the cell in such a manner as to show to what extent the temperature of the cell would be raised in a given time if the liberated heat were all concentrated on the cell, and had not to be shared by its environment.

The advantage of this second method is that it gives us a rough means of comparing the metabolic activity of a yeast-cell with that of a warm-blooded animal; but it takes for granted that we know the amount of heat energy evolved during the breaking down of a unit weight of sugar by the cell, a value which is still to some extent in dispute.

The earliest attempts to ascertain the actual amount of heat evolved during the splitting up of sugar into alcohol and carbon dioxide were made by Berthelot and by C. V. Rechenberg, whose methods, however, were entirely indirect, since they were based on a determination of the differences between the calculated heat of formation of dextrose and the calculated heat of formation of the equivalent amounts of alcohol and carbon dioxide.

Berthelot's final result, after making a small correction for the non-volatile products of fermentation, indicated that the gramme molecule of dextrose liberated on fermentation 32·07 kilogram-centigrade heat units, corresponding to 178·1 calories per gramme of sugar.

But the possible errors of such indirect methods are very great, and are all accumulated on the final result.

In 1895 Bouffard (Compt. rend., 1895, 121, 357) made the first attempt to determine the heat of fermentation by direct means, employing for this purpose a Berthelot's calorimeter containing a litre of grape-juice, to which had been added a further quantity of dextrose. When all due corrections were applied, Bouffard's experiments pointed to the heat of fermentation of dextrose being K 23·5, or 130 calories per gramme of dextrose.

A few years later, the problem was attacked by Adrian Brown (Journ. Fed. Inst. Brewing, vol. vii, 1901, p. 93).

The great difficulty which had been experienced by Bouffard was one which is incidental to all measurements of thermal effects produced by comparatively slow reactions, viz. that of determining the rate of cooling of the calorimeter and its contents. Adrian Brown reduced this possible error by employing as his calorimeter a fermenting vessel of about 1,300 gallons capacity, as used in a brewery under the 'high fermentation' system.

The liquid operated upon in this vessel was an ordinary brewer's wort, and observations were made at sufficiently short intervals almost to eliminate the cooling correction.

The amount of sugar (maltose) fermented between any two intervals corresponding to a definite rise of temperature was ascertained from the loss in specific gravity of the liquid after deprivation of its alcohol, and the application of the known 'divisor' for maltose. The specific heat of the wort being taken at 0·9678, the final conclusion was arrived at that the heat liberated by the fermentation of 1 grm. of maltose is 119·2 calories.

Within the last few years I have made various attempts to determine the heat of fermentation of maltose by a method substantially the same as that used by Adrian Brown. The principal points of difference were that I used larger vessels, varying in content from 1,800 to 14,000 gallons, and that the fermented sugar was estimated from the amount of alcohol formed, the exact ratio of alcohol to sugar at different stages of the fermentation of a malt-wort having been previously determined in the course of a separate investigation.[1]

Twelve such experiments were made, and a critical examination of the results points to a value of about 125 *calories* as representing the heat of fermentation of 1 grm. of maltose, this value including the heat due to hydrolysis of the maltose at the moment of its fermentation.

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[1] See Reports on Original Gravities in process of publication in the Journ. of Fed. Institutes of Brewing.

The immediate experimental data on which this result was founded consist of a determination of the actual amount of sugar fermented within short intervals of time corresponding to a rise of temperature of the liquid of from one to two degrees centigrade.

In order to refer the rise of temperature to a definite weight or number of yeast-cells in a given volume of the liquid, a series of cell-countings were made at the beginning and end of each time-interval, by means of the haemocytometer. If the observations were made at a time when cell-reproduction had ceased, the initial and final countings were, of course, practically identical, but in those cases in which the 'oxygen charge' had still not spent itself and the yeast was still reproducing, the arithmetical mean of the initial and final countings was taken as representing the average number of cells actively engaged in fermentation within the period.

The actual amount of maltose which had disappeared during each period of observation was determined from accurate estimations of the alcohol produced, and from these data were obtained the weights of maltose which had disappeared from 100 c.c. of the liquid in one hour, under the influence of a definite number of yeast-cells per unit volume of the liquid.

For convenience in further calculations and comparisons, this last value was further reduced so as to represent the weight of maltose per 100 c.c., which in one hour would have been fermented by a definite unit weight of yeast, or by a definite distribution of so many cells per unit volume, on the assumption (capable of proof) that under fixed conditions the amount of sugar fermented is proportional to the yeast present.

A convenient standard to take for the yeast-distribution is that of 10 cells per unit volume of $\frac{1}{4000}$ c.mm., corresponding to 4×10^9 cells per 100 c.c. This number of cells weighs, on an average, 1 grm.[1]

If, in any such given case, we take M to denote the weight of maltose fermented per 100 c.c. per gramme of yeast (10 cells per unit volume) per hour; c the calories liberated by the fermentation of 1 grm. of maltose; and s the specific heat of the yeast-cell, which may be taken as approximately 0·86; then, assuming all conditions constant, the potential increase of temperature of the cell, if all the liberated energy of metabolism were retained in it, is represented by $\frac{Mc}{s}$.

In the following Table is given a summary of the results of ten experiments, from which have been calculated in the last two columns the time required for a yeast-cell under stated conditions of temperature to ferment

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[1] This can be shown by washing a freshly cultivated yeast with water and then freeing it as far as possible from adhering moisture, either by repeated pressing between folds of filter paper or by distributing it over the surface of a porous tile. When one gramme of a top-fermentation yeast so prepared is thoroughly mixed with water and made up to 100 c.c. the cell-count per unit volume of $\frac{1}{4000}$ c.mm. varies very little from ten.

its own weight of maltose, and also the potential rise of temperature of the cell per hour, if all the heat due to metabolism had been retained.

The values are not strictly comparable, owing to the fermentations having been made at different times, and in some cases with different yeasts. It will be noticed that between about 14° and 16° C. the time required for a yeast-cell to ferment its own weight of sugar varies from about 8½ to 9½ hours, and that the heat generated during one hour is sufficient to raise the temperature of the cell by 15° or 16° C.

TABLE I.

a. Mean temperature of Expt.	*b.* Maltose fermented per 100 c.c. per hour, per one gramme of yeast.	*c.* Time required by yeast-cell to ferment its own weight of maltose.	*d.* Potential rise of temperature of yeast-cell in one hour. $\frac{Mc}{s}$.
	grm.	*Hours.*	
(1) 14·3° C.	0·104	9·6	15·1° C.
(2) 15·1°	0·101	9·9	14·6°
(3) 15·9°	0·105	9·5	15·2°
(4) 16·1°	0 115	8·7	16·7°
(5) 16·3°	0·123	8·1	17·8°
(6) 17·4°	0·119	8·4	17·2°
(7) 17·9°	0·133	7·5	19·3°
(8) 18·3°	0·134	7·4	19·4°
(9) 19·1°	0·130	7·6	18·8°
(10) 20·9°	0·178	5·6	25·8°

Although the entire range of temperature in these experiments is only about 6° C., the influence of increasing temperature on cell-metabolism is consistently shown throughout, and the results are such as to indicate a high 'temperature coefficient' for yeast.

During the course of his studies on alcoholic fermentation, A. Slator has introduced a new experimental method which in his hands has given, and is still giving, results of the greatest possible interest as regards the mechanics of fermentation (Journ. Chem. Soc., Trans., 1906, p. 128; ibid., 1908, p. 217). It consists essentially of measuring the fermentative change over a brief interval of time by change of manometric pressure due to the evolution of CO_2. By making the time intervals very short, various disturbing causes, such as changes of concentration, alteration in the activity or amount of the yeast, and the accumulation of the products, are reduced to a minimum.

Slator was thus able to prove satisfactorily that, other things being the same, the rate of fermentation of a sugar is proportional to the number of active cells in a given volume, i. e. to the 'density of the population'; and that the rate is almost independent of the concentration of the sugar except in very dilute solutions.

With this same apparatus, Slator also investigated the influence of temperature on the rate of fermentation, and for the first time obtained a satisfactory series of values for the temperature-coefficients over a range from 5° C. to 35° C.

The coefficients are large, but diminish with increasing temperature: for instance, a rise of temperature from 5° C. to 10° C. results in the fermentation at the higher point being 2·65 times as rapid as at the lower; whereas raising the temperature from 30° C. to 35° C. results in an increased rapidity of only 1·35 times. He also found that these coefficients are remarkably similar for brewery yeasts, distillery yeasts, and wine yeasts, so much so in fact that he was led to regard the temperature-coefficient as characterizing certain constant properties of the enzyme *zymase*, which is common to all alcoholic yeasts.

The following Table gives the temperature coefficients, as determined by Slator, for brewery yeasts (top fermentation); they are expressed as quotients for 5° C.:

TABLE II.

Temperature quotients for brewery yeast.

$V\,10°/V\,5°$	2·65
$V\,15°/V\,10°$	2·11
$V\,20°/V\,15°$	1·80
$V\,25°/V\,20°$	1·57
$V\,30°/V\,25°$	1·43
$V\,35°/V\,30°$	1·35
$V\,40°/V\,35°$	1·20

By making use of the above Table of temperature coefficients, taken from Slator's paper, and expressing as unity the amount of sugar fermented in a brief interval of time at 5° C. through the agency of a definite number of yeast-cells per unit volume, we can obtain a series of values which represent the progressive rates of fermentation expressed as a function of the temperature between 5° and 40° C. The values which I have recast in this way are given in Table III, and are also represented graphically in the curve of Fig. 8.

TABLE III.

Temperature.	*Rates of fermentation.*
At 5°	1·00
10°	2·65
15°	5·59
20°	10·05
25°	15·80
30°	22·59
35°	30·50
40°	36·60

Since this curve (Fig. 8) is based on true temperature coefficients, we can ascertain by means of its use the effect which any given rise of temperature would have on the rate of fermentation where the data have been previously obtained at any lower temperature. It can, for instance, be applied to any of the experiments recorded in Table I. Only one or two examples are necessary.

In the first experiment, recorded in Table I, in which the mean temperature of fermentation was 14·3° C., it was found that the maltose which was fermented per 100 c.c. per hour by 1 grm. of yeast (10 cells per unit volume) amounted to 0·104 grm. Let us see what the amount of maltose fermented would have been under precisely the same conditions if the temperature had been raised to 30° and 40° C. respectively, and how this would have affected (*a*) the time required for a yeast-cell to ferment its own weight of the sugar, and (*b*) the potential rise of temperature of the cell under adiabatic conditions.

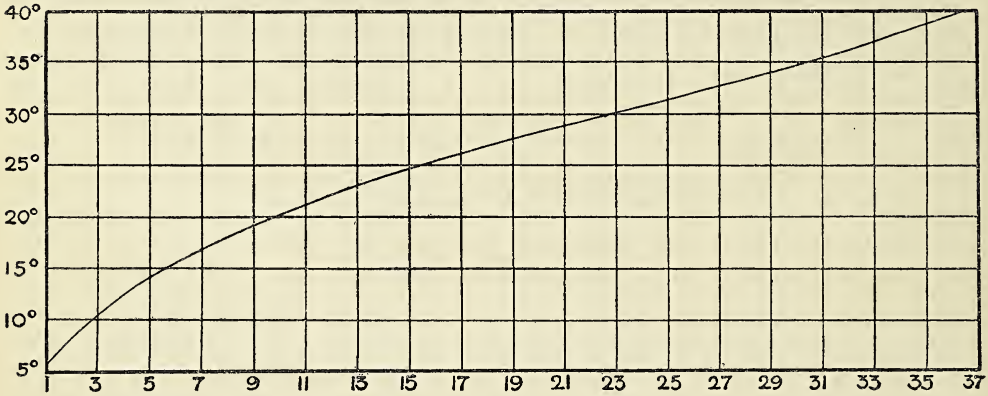

FIG. 8. Graphical representation of Table III, showing influence of temperature on fermentations. Amount of sugar fermented at 50° C. is taken as unity. Deduced from A. Slator's temperature coefficients for brewery yeast. See Table II.

On reference to the curve, we note in the first place that the ordinate of temperature corresponding to 14·3° C. cuts the curve at the co-ordinate 5·2, and that the ordinate corresponding to a temperature of 30° intersects the curve at 22·6. The amount of maltose fermented at 30° will therefore be $\frac{22\cdot6 \times 0\cdot104}{5\cdot2} = 0\cdot451$ grm. per 100 c.c. per 1 grm. of yeast per hour; ∴ the time taken for the yeast to ferment its own weight of maltose at 30° will be $\frac{1}{0\cdot451} = 2\cdot2$ hours, and the potential rise of temperature of the yeast-cell in *one hour* under these new conditions will be $\frac{0\cdot451 \times 125}{0\cdot86} = 75\cdot5^\circ$ C., instead of 15·1° C.

In the same manner it can be shown that at 40° C. the amount of sugar fermented per hour, per 100 c.c. per 1 grm. of yeast, would be still further raised to 0·731 grm., whilst the time occupied by a cell in fermenting its own weight of sugar under these conditions would be only 1·3 hours, and the potential adiabatic rise of temperature of the cell would be in one hour 106·2° C.[1]

Considerations of this kind bring home to us in a striking manner the intense nature of the metabolic processes which go on in a yeast-cell, and the large liberation of apparently wasted energy which accompanies them. It may be permissible for a moment to compare a yeast-cell in this respect with the human body. I am informed by Dr. Leonard Hill that the metabolism of a man quietly resting in bed can be taken as resulting in the production of 2,000 large calories per diem, or 83·3 large calories per hour. Assuming a body-weight of 65 kilograms, and a mean specific heat of 0.83, and further supposing the metabolism to continue at the same rate, then the heat evolved would be sufficient to raise the body-temperature of the man at the rate of about 1·5° C. per hour.

But, as we have just seen, the metabolism of a yeast-cell at about the temperature of the human body evolves heat sufficiently fast to raise its body-temperature 106° C. per hour, or about seventy times as fast as in the case of a man at rest.

We may now ask, what is the physiological significance of this enormous metabolism and liberation of energy which the yeast-cell exhibits in pursuance of its life-functions, and which seem so disproportionate to its requirements for reproduction and nutrition?

Are we to regard the phenomena as being to some extent accidental, and due to the fact that the living cell requires some small but essential part of the sugar molecule, the removal of which brings about a rearrangement of the residual portions of the molecule with consequent transformation of potential into kinetic energy? According to this view, the action of the yeast may be likened to the removal of the keystone of an arch and the partial demolition of an edifice.

This was substantially the explanation given by Pasteur many years ago. He regarded the reproductivity of the yeast-cell and its fermentative power as being correlative but inverse phenomena.

By a somewhat curious misinterpretation of experimental results, Pasteur believed he had evidence that when the yeast-cell was fully supplied with oxygen, and was therefore reproducing itself freely, its faculty for fermenting sugar was at a minimum, or even altogether in abeyance. On the other hand, its fermentative power was supposed to be at a maximum under anaerobic conditions, this being due to the yeast then taking the requisite oxygen from the sugar, thus destroying the sugar molecule as such.

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[1] It is not without interest to compare the energy liberated under these conditions in the yeast-cell with that evolved by the atomic disintegration of radium, which is said to produce sufficient heat to raise its own weight of water through 100° C. per hour.

That this was a wrong interpretation of the facts was conclusively shown by Adrian Brown (Journ. Chem. Soc., Trans., 1892, p. 61; ibid., 1894, 1911), who has brought forward unmistakable evidence, proving that yeast well supplied with free oxygen possesses as much or even more fermentative power than the same yeast under conditions of oxygen starvation; and more recently this has been confirmed by A. Slator. But the errors of great men live long after them, and it will no doubt require another generation of workers to eliminate the statement from the text-books.[1]

Whatever truth there may be in the view that the breaking down of the sugar molecule, and the resulting liberation of energy, is due to the cell protoplasm abstracting and appropriating some small constituent part of the sugar molecule, this hypothesis receives no support from the conjoint study of the reproductive and fermentative functions of the cell, which are certainly not inversely related to each other.

It will help us if we consider for a moment the behaviour of a yeast-cell bathed in a suitable nutrient liquid, at a time when the free oxygen originally dissolved in the liquid has been completely absorbed by the yeast, and when the 'oxygen charge' of the cells has completely spent itself in several successive generations of subdivision. As we have seen in Part I of this paper, no further reproduction is possible under these conditions, if access of oxygen from without is prevented, but the cells speedily acquire a static condition of equilibrium with regard to the medium, and this condition remains constant for some time. During this period it can be experimentally demonstrated that the average mass and composition of the cells remain constant; in other words, regarded from the point of view merely of cell-maintenance, the normal katabolic and anabolic processes, if they still exist at all, must just balance each other, and little or no further extraneous supply of energy is required to continue the life of a cell so placed. Notwithstanding this, we find under these apparently static conditions of maintenance an enormous activity in the metabolic 'mill', through which continues to pass an amount of substance which, under certain conditions of temperature, may amount to several times the mass of the cell in a few hours, and a corresponding liberation of energy sufficient to raise the cell-temperature to a very high point.

It may be argued that this apparent waste of energy is due to the necessity of the yeast-cell having to share its temperature with the surrounding medium, which is one of high specific heat, but under any circumstances it is difficult to see how any rise of temperature in the medium can be of any use to the cell under the above conditions, since such a rise can only stimulate the 'mill' to a further output of work which appears to be

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[1] Quite recently I have heard of a proposed improvement in wine-making which was entirely based on this fundamental error of assuming the inverse relation of the reproductive and fermentative faculties of yeast.

perfectly useless for the economics of the cell, and immensely disproportionate to any 'toll' which the mill requires to keep it in working order.

The explanation of this apparent paradox appears to lie in the fact that the conditions under which we generally cultivate yeast, that is to say, in comparatively large masses of liquid containing but a very limited supply of oxygen, are eminently artificial. They have been imposed by the exigencies of industries in which the fermentative faculty of the yeast-cells has been purposely enhanced, whilst the reproductive faculties have been restricted. These artificial conditions differ *in toto coelo* from the natural conditions under which the specific physiological characters of the Saccharomycetes have been evolved. If we wish to study the question in a philosophical manner, it is to the vineyard and orchard we must have recourse, rather than to the vats of the wine-maker, the brewer, and distiller.

The natural habitat of the various forms of yeasts is the outer skin of fruits, and especially those succulent fruits which contain abundance of sugar when ripe.[1] They may, for instance, be detected somewhat sparsely scattered over the skin of a ripe grape, awaiting their opportunity of gaining access to the stores of nutrient material from which they are separated by a thin semi-permeable membrane. This opportunity arises the moment the skin of the grape is ruptured, and such a rupture may be brought about by a variety of natural causes, such as the undue swelling of the ripe fruit after rain, the attack of birds, insects, or fungi, or by the accidental crushing of the berry by the foot of a passing animal.

The extraneous yeasts and their ascospores now find themselves in a medium rich in all the nutrient substances they require, and one to which atmospheric oxygen can gain ready and continuous access, owing to the small scale of the natural operations.

Hence, as one may readily see in the vineyard at vintage time, extremely rapid cell-multiplication takes place, which has not the same check imposed upon it as it has when the cultivations are made on a large scale in the wine-makers' vats. This rapid and constant building up of new cells, under the continued action of oxygen, requires a constant source of extraneous energy, which is furnished by the ancillary fermentative function of the cells. Moreover, since the cell-growth is going on in a medium of high specific heat, and under conditions in which there is a natural tendency for rapid equalization of temperature with the surroundings, it is manifestly to the advantage of the organism to have some other and more intense source of energy than that supplied by the respiratory processes. By a study of these natural cases, it becomes easier to understand the true

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[1] The origin of the wine-yeasts is obvious. That of the various races of brewers' yeast is not known with certainty, any more than the origin of some of our domestic animals and cereals.

relation of the fermentative to the reproductive processes, and why the yeasts have acquired their fermentative power.

It is but a special instance of a well-established generalization or ' law ' of nature, that the mechanism of every living organism is adjusted to the one great aim and object of reproduction.

That we can by means more or less artificial keep the reproductive power of a yeast in abeyance, whilst still availing ourselves of its fermentative power, has hitherto obscured the relation of the two functions, and hence has given rise to the somewhat exaggerated idea of the purposeless and prodigal waste of the yeast-cell regarded as a living unit.

Addendum.

It follows from the above considerations that, if we could devise means for measuring with exactness the amount of heat evolved during the disappearance of 1 grm. of sugar under the influence of yeast, whilst the cells are in process of active reproduction, this amount of heat ought to fall short of that produced by the fermentation of the same amount of sugar when there has been no yeast reproduction, the difference being due to the energy required for the observed yeast reproduction.

I have not succeeded hitherto in obtaining numerical results which can be relied upon as giving the ' heat of formation ' of yeast, but in those cases where yeast reproduction was still in progress within the observed time-intervals, I have generally found distinctly *less* evolution of heat per unit weight of sugar destroyed than where the cell-reproduction had ceased.

In one case, for instance, in which the fermentation of a malt-wort had been continued for 6½ hours, the maltose which had disappeared per hour was 0·117 grm. per 100 c.c., and the yeast *increase* per hour was 1·26 cells per unit volume, equivalent to 0·126 grm. per 100 c.c. of moist yeast, or 0·027 grm. of dry yeast. The ratio of the sugar used up, to the *dry* yeast formed, therefore had the high value of 1 : 0·23.

The heat developed per gramme of sugar fermented in this case amounted only to 114·4 calories, instead of 125, the normal value.

The Plasmodiophoraceae and their Relationship to the Mycetozoa and the Chytrideae.[1]

BY

E. J. SCHWARTZ, M.A., B.Sc.

With Plate XII.

SINCE the publication in the Annals of Botany, in 1911, of an investigation of the life-history and cytology of *Sorosphaera Graminis* (25), a great deal of work has been done on the Mycetozoa and Plasmodiophoraceae, and several papers have been published describing the cytology of various species and discussing the relationship between these two families. Pavillard, in the 'Progressus Rei Botanicae', 1910, gives an excellent account of the position at that date, and concludes that the Plasmodiophoraceae are a branch of the Mycetozoa that has become modified owing to having taken to a parasitic mode of life. Maire and Tison (13 and 14), in 1911, combat this view, and suggest that the Plasmodiophoraceae should form a separate family, which they consider probably owes its origin to the Chytrideae. Osborn (20), on the other hand, in his paper on *Spongospora*, lays stress on the similarities between the Plasmodiophoraceae and Mycetozoa, and describes a karyogamy similar to that observed by Jahn and Kränzlin in the Mycetozoa.

The present paper is the outcome of the study of many species of both the Plasmodiophoraceae and the Mycetozoa, and, while admitting that these families are related, I am in agreement with the opinion expressed by Maire and Tison that their differences are so great that the Plasmodiophoraceae should form a separate order intermediate between the Mycetozoa and the Chytrideae. I do not think that the differences between these families can be accounted for by the parasitic mode of life adopted by the Plasmodiophoraceae.

THE PLASMODIOPHORACEAE.

This family comprises a number of multinucleate amoeboid organisms, which live as parasites on various Phanerogamic plants; the best known, and perhaps the commonest, member of the family is *P. Brassicae*. The

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[1] This thesis, together with the work on the Plasmodiophoraceae previously published, viz. Root Diseases of the Juncaceae; Tumours on *Veronica Chamaedrys*; Life-history and Cytology of *Sorosphaera Graminis*, was approved for the Degree of Doctor of Science in the University of London.

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members of this family are characterized by a vegetative stage, in which the nuclei of the amoebae divide by a process that has been described by Maire and Tison as 'an intranuclear karyokinesis combined with an amitosis', and by a reproductive stage which closes with two karyokinetic nuclear divisions leading to spore formation.

The family includes the following seven genera:

1. *Plasmodiophora*, with free spores.
2. *Sorosphaera*, with spores collected into hollow spheres.
3. *Tetramyxa*, with spores in tetrads.
4. *Sorodiscus*, with spores collected into hollow, flattened spheres and ellipsoids.
5. *Molliardia*, with spores at present unknown.
6. *Spongospora*, with spores collected into spongy masses.
7. *Ligniera*, with spores collected into masses of varying shapes (e.g. spheres and ellipsoids).

Of these genera, the first five possess certain common characteristics in that they each contain only a single species, the presence of which in the host-plant always gives rise to hypertrophy of tissue with the formation of tumours or swellings either in the stem, leaf, inflorescence, or root. The genus *Spongospora*, represented by *S. subterranea*, causes no apparent hypertrophy to the tissues of the potato, on which it is parasitic.

The genus Plasmodiophora, Wor.

The cytology of *P. Brassicae* has been fully described by Nawaschin (16), Prowazek (23), and Maire and Tison (11). The statement made by Marchand, and quoted by me in my paper on *S. Graminis* (25), to the effect that the roots of Celery, Sorrel, and Melon had been attacked by this species, has been found by Griffon and Maublanc (2) to be an error. Nawaschin states that the groups of diseased cells are formed by the division of single or small groups of infected cells at the growing apices of the roots, and that the amoebae have no power to pass from cell to cell by penetration of the cell-wall; this appears to me to be the case in all the genera. The spores are said to germinate readily, but I do not find this to be the case. Although I have tried to germinate spores of various ages, including some which had been kept through the winter, in no case have I seen an actual germination. In cultures of the spores I have occasionally seen a number of small, pear-shaped flagellate bodies, but these may very probably have been due to other organisms, as the culture was not pure. Prowazek has described a karyogamy taking place on spore formation; this, however, has not been confirmed.

The genus Sorosphaera, Schröt.

This genus is marked by the spores being collected into hollow spherical masses, enclosed by a common membrane. It is represented by the single species *S. Veronicae*, which causes swellings on the stems, leaves, or inflorescences of various species of *Veronica*. I have found this parasite only in *V. Chamaedrys*, although it is stated by Lagerheim to be found also in *V. hederifolia* and in other species. I have seen *V. hederifolia* growing in the immediate vicinity of diseased *Chamaedrys* plants, the former remaining quite healthy. Diseased *Veronica* plants are not commonly met with, and can only be identified with the aid of the microscope. Tumours caused by eel-worms are indistinguishable to the naked eye from those caused by the *Sorosphaera*. These eel-worm tumours, which are much more common, I have seen on *V. Chamaedrys* and other species of *Veronica*.

The cytology and life-history of *S. Veronicae* has been described by Maire and Tison (11) and by Blomfield and myself (1). The first-named authors, in a recent paper (14), confirm the opinion expressed by Blomfield and myself that the infection takes place at the growing apex of the stem.

Winge (26), in his paper on 'Cytological Studies in the Plasmodiophoraceae', in remarking on the akaryote stage of the organism, states that the karyosomes of the nuclei of the amoebae disappear at the close of the vegetative period of its life-history, and that subsequently no fresh karyosomes are formed. He is of opinion that the akaryote stage is formed by the bulk of the chromatin passing out from the nuclei into the plasma of the amoebae. He does not, however, consider that the organism is at any period devoid of nuclei, since some of the chromatin always remains on the site of each nucleus. Subsequently fresh nuclei are formed on the sites of the former ones; these, however, contain no karyosome, and form the reproductive nuclei of the organism. This confirms the statement made in my paper on the cytology of *S. Graminis* (25), that the reproductive nuclei occupied the same sites as the early vegetative ones. Osborn (20), however, in his paper on *Spongospora*, states that there is a total disappearance of the vegetative nuclei, and that the reproductive nuclei are formed *de novo* on adjoining sites.

The genus Tetramyxa, Göbel.

This genus, which is represented by the single species *T. parasitica*, is characterized by the formation of spores in tetrads. Its life-history and cytology have been studied by Maire and Tison (14), who state that the nuclear phenomena are for the most part similar to those met with in the other genera, with the exception that no akaryote stage appears to follow the close of the schizont stage. They have, however, observed and figured some forms resembling the akaryote stage in the cells of young tumours, in which the disease was in an early stage and the infection recent. Should these forms prove to be the akaryote stage, this stage would seem to be displaced and would appear earlier than is the case in the other genera.

T. parasitica causes tumours on *Ruppia rostellata*; it has also been stated to have been found on *Zannichellia palustris* by Hisinger in 1887. Maire and Tison, however, have recently found it plentifully in *R. rostellata*, growing in close proximity to *Z. palustris*, which was uninfected. This throws doubt on the statement that the latter is a host-plant. I have found multinucleate amoebae in the root-hairs of plants of *Z. palustris* which were otherwise free from infection; these amoebae might be a species of *Ligniera*, or, on the other hand, they might be *T. parasitica*.

The genus Molliardia, M. and T.

In *M. Triglochinis*, M. and T., which is the sole representative of this genus, neither the akaryote stage nor spore clusters have been observed as yet. This species was formerly known as *Tetramyxa Triglochinis*, but has been removed from that genus, since the latter is characterized by the presence of tetrads of spores. It is the cause of swellings on the stems and inflorescences of *Triglochin maritimum* and *T. palustre*; these swellings are similar in structure and development to those of the *Veronica*. It has been described by Maire and Tison (14), who state that the mitoses in the schizont stage are similar to those of the other genera, with the exception that abnormal mitoses are not uncommon, and the presence of uninucleate amoebae in the plant-cells is frequent. It is not unlikely that a spore stage will be found, in which case *M. Triglochinis* will possibly be transferred to one of the other genera.

The genus Sorodiscus, Lager and Winge.

S. Callitrichis, Lager and Winge, is the cause of tumours on the stems of *Callitriche autumnalis*, and is the only species of this genus. It has been described by Winge (26), who states that it is very closely related to *Sorosphaera Veronicae*, from which it differs in having the spores arranged in flattened hollow spheres or ellipsoids. Winge has observed in *S. Callitrichis* the typical vegetative mitoses which, he states, occur simultaneously in all the nuclei of an amoeba; this statement applies also to the other genera. He states further that the axes of the dividing nuclei (i. e. their elongated karyosomes) are generally parallel; this is certainly not the case in the other genera I have examined. Winge also observed the two mitoses which occur just before spore formation, the number of chromosomes showing a reduction from sixteen to eight.

The genus Spongospora, Brunch.

S. subterranea has been described by Osborn (20), and agrees in most respects with other genera. As before stated, Osborn maintains that there is a complete disappearance of nuclei in the akaryote stage, and that fresh nuclei (reproductive) are formed on different sites. These reproductive nuclei fuse in pairs, and the resulting nuclei undergo two mitoses prior to the formation of spores. This karyogamy, observed by Osborn, has not been noted in any of the other genera. The vegetative nuclei divide in the manner peculiar to the Plasmodiophoraceae. Osborn, however, states that the chromatin is in the form of a ring around the elongated karyosome, whereas careful focusing shows that it is in reality an equatorial plate. Osborn was unable to get the spores to germinate, but Massee (15) states that he has seen a germination, the spore giving rise to a single myxamoeba.

The genus Ligniera, M. and T.

This genus, which is closely allied to *Sorosphaera*, was formed by Maire and Tison (13) to include those members of the Plasmodiophoraceae in which the spore-clusters are most usually of ellipsoidal and irregular shapes and are rarely spherical. A common characteristic of the members of this genus is that they do not give rise either to hypertrophy of tissue or to nuclear degeneration in the host-plant. To this genus the species described by myself and named *Sorosphaera Junci* and *S. Graminis* have been transferred.

Other species at present included in the genus are: *L. radicalis*, M. and T., found on roots of *Callitriche stagnalis*; *L. verrucosa*, M. and T., found on roots of *Veronica arvensis.*

To the above species three new ones, recently found by myself, have now to be added—viz. *L. Bellidis*, *L. Menthae*, and *L. Alismatis.* All the *Ligniera* agree closely in their life-histories and cytology, but, owing to the fact that they do not cause hypertrophy in their host-plants, and for other reasons referred to later, they are not so suitable for the observation of cytological details as are the members of the other genera. All the species of this genus are root parasites, and the infection in all cases takes place near the root apices. The infected cells, which are confined to the outer cortex of the root, are frequently found in longitudinal rows. In many cases the amoeboid stage of the organism is only to be found in the youngest roots. Root-hairs may also serve as channels for infection, but in this case the amoeba does not spread the infection beyond the cell which gives rise to the hair. The parasite may complete its life-history in the root-hair by the formation therein of a row of spores. Schizogony is rare, and the akaryote stage is seldom seem, it being probably of but short duration, as Osborn suggests.

In the roots of *Poa annua* the root-hairs are frequently swollen at their apices to form club-shaped chambers, in which the amoebae or spores of *L. Graminis* may be seen. The nuclei, however, of these amoebae are smaller than those in the cortical cells of the root. Figs. 9 and 10 show these hairs. It is to be noted that many of the *Ligniera* are parasitic on the roots of marsh- and water-plants, and further search will probably result in the addition of more new species. I have also observed that these parasites are frequently associated in the roots with typical mycorhiza. This I find to be the case in the roots of *Poa annua*, *Bellis perennis*, *Chrysanthemum Leucanthemum*, *Mentha Pulegium*, and, at times, *Juncus articulatus*. The mycorhizal cells have their nuclei enlarged and degenerate, and, as viewed under the microscope, do not give a healthy appearance.

The three new species of *Ligniera* are described below.

Ligniera Bellidis Schwartz, sp. nov. Amoebis raris in cellulis hospitis; sporis 5–6 $\mu \times$ 4–5 μ in acervulos diversiformes conjunctis. Hab. in radicibus Compositarum. Sevenoaks.

This species, which is found in the roots of daisies, is closely related to *L. Graminis* and *L. Junci*, but appears to be distinct, since grass and rush-roots in the immediate vicinity of diseased daisies were found free from infection. The habitat from which the diseased plants were obtained was a moist one on Fawke Common, Sevenoaks; the soil, however, was very poor and stony. The daisies were plentiful but the plants were very small; about 40 per cent. of them were infected. Microscopic examination of the diseased roots showed that the cortical cells were for the most part filled with spore-clusters of various shapes, of which the ellipsoid was the most common. Very rarely were any amoebae to be seen even in the youngest roots. The same parasite has been found by me on the roots of *Chrysanthemum Leucanthemum*, but the roots of other Composites growing in the diseased area were found to be unaffected. The disease could be imparted from the *Chrysanthemum* to *Bellis*, and vice versa. The structure of the diseased roots in both plants was similar, and the reserve plant-food in the root-cells was inulin.

Infection probably takes place at the growing root apices, but even in these regions of diseased roots only masses of spores are usually to be found. This is in striking contrast to the case of *M. Triglochinis*, in which amoebae only are to be seen. The vegetative amoeboid stage of *L. Bellidis* is shown in Fig. 7, and the akaryote stage in Fig. 8. This species, on account of the lack of amoeboid forms, is not suitable for the observation of nuclear divisions; the modes of division are probably similar to those met with in other genera.

Ligniera Menthae Schwartz, sp. nov. Amoebis in cellulis radicis hospitis; sporarum soris globosis aut acervulos diversiformes efformantibus; sporis 3–5 $\mu \times$ 4–5 μ. Hab. in radicibus plantae 'Mentha Pulegium' dictae.

This species I found at Chislehurst, in the roots of some plants of *Mentha Pulegium* which were growing in profusion on the border of a pond on the Common. Other marsh-plants growing with the *Mentha,* such as *Alisma ranunculoides, Bellis perennis,* and various species of *Juncus,* were examined, but were found to be free from the *Ligniera.*

Microscopical examination of the older roots showed the infection to be confined to the outer cortex, the cells of which were largely filled with spore-clusters. These clusters were mostly ellipsoidal in shape; spherical ones were also common, and sometimes a tetrad or a single or double row of spores were to be seen. The individual spore is similar to that of other species. In the young branch-roots amoebae were sometimes to be seen, although the *L. Menthae* displayed the same tendency to early spore formation as I observed in *L. Bellidis,* but not in so marked a degree. The vegetative nuclei are typical ones; they are, however, somewhat smaller than those of *L. Graminis.* I have not observed the typical 'cruciform' method of division of these nuclei, although the usual method of division is probably of that form. I have, however, observed in one of the amoebae some abnormal vegetative nuclear divisions in which the division was effected by an ordinary karyokinesis, as is shown in Fig. 6. This confirms the observation of Maire and Tison, who found a case of typical karyokinetic division in the schizont stage of *Molliardia.* The akaryote stage shown in Fig. 2 is comparatively common. I have not observed any karyogamy prior to spore formation, but the form is not well suited for cytological study owing to the difficulty of finding diseased material.

The amoebae are small and irregular in shape, and several of them may be seen in a plant-cell, so that schizogony is not uncommon. The infected cells occupied by the organism in its amoeboid form occur in small groups and are rarely isolated. The amoebae are shown in Fig. 1. I have only found this disease in the one locality mentioned above, but, on the other hand, I have not had the opportunity of examining plants from more than two other localities, so I am unable to speak as to the rarity or otherwise of its occurrence.

Ligniera Alismatis, Schwartz, sp. nov. Amoebis raris in cellulis hospitis; sporarum soris, iis *Spongosporae* similibus, aut sporis in ordinibus aut globose dispositis; sporis $3\,\mu \times 4\,\mu$. Hab. in radicibus plantae 'Alisma Plantago' dictae.

This species I found parasitic in the roots of *Alisma Plantago* growing in some trenches near Dunton Green, Kent. It resembles *L. Bellidis* in that the amoeboid form is very rarely seen. The spore-clusters are not exactly similar to those of other species of *Ligniera,* since in the latter the clusters usually exhibit an axial symmetry, while in *L. Alismatis* the boundaries of the spore-clusters are not, as a rule, so definite as in the other species, and to some extent they resemble those of *Spongospora.* The spores are very rarely collected into spheres or ellipsoids, which are the forms commonly met with in other species. The individual spores are approximately of the same size as those of *Spongospora.*

The life-history and cytology of the various members of the Plasmodiophoraceae is remarkably uniform, except for the case of *M. Triglochinis*, in which, apparently, there is no spore formation by the parasite in the host-plant. With regard to the karyogamy described by Osborn as taking place in *Spongospora*, this has not been observed in any of the other genera. I have re-examined my slides of *S. Veronicae* with a view of confirming Osborn's observation, but have found no such karyogamy as he describes, although this species is a favourable one for the observation of nuclear changes, the akaryote and early spore forms being well marked and plentiful. It would, I think, be easy to mistake overlapping nuclei or the close of a mitosis for a karyogamy. Winge also fails to see any signs of a karyogamy in *Sorodiscus Callitrichis*, which is also a favourable subject for observation. Of the karyogamy described by Osborn, Winge remarks: 'If a karyogamy at this stage is really found in *Spongospora*, we should rather conclude that this organism is not a Plasmodiophoracea.' Maire and Tison have also failed to find a karyogamy in either *S. Veronicae* or the other species studied and described by them; they suggest, as also does Winge, that a conjugation of amoebae follows the germination of the spores. This suggestion I also made in my paper on *S. Junci.* The refusal of the spores to germinate under artificial conditions is unfortunate; probably the germinated spore gives rise to a pear-shaped swarm-cell, and it is at this stage that a conjugation should be looked for.

MYCETOZOA.

In 1884 Strasburger observed a simultaneous mitosis of all the nuclei in the developing sporangium of *Trichia fallax*; this mitosis gives rise to the nuclei of the spores. This simultaneous nuclear division has since been observed in other species, and is stated by Pavillard (21) to be probably constant throughout the endosporous Mycetozoa.

In 1907 Jahn and Helene Kränzlin (8) identified this division as a heterotypic mitosis; according to this view, the reduction would be completed on the germination of the spore. In *Arcyria* and *Trichia* Kränzlin states that just prior to spore formation the nuclei in the sporangium associate in pairs and a karyogamy takes place, and that any nuclei which have not paired quickly degenerate. Kränzlin states further that a temporary enlargement of the nucleus follows, and that then synapsis takes place. In the exosporous genus *Ceratiomyxa* a karyogamy has been described by Olive (19) and Jahn (6), but their accounts do not agree.

Quite recently Jahn (7) has observed a conjugation of the myxamoebae formed after the germination of the spores, and has stated that his original contention of a karogamy prior to spore formation was due to an error. The nuclear fusions he had previously seen he now considers as a concomitant of nuclear degeneration, and not as sexual fusions. The reasons he assigns for this view are ample—viz. that the fusions are occasional, and do not give the appearance of a universal or simultaneous karyogamy; the fusions are also to be found in the stalk and in the neighbourhood of the membrane, and are accompanied by degeneration. Similar nuclear fusions have been observed by Winkler in the disorganizing tapetal cells of developing anthers. In Jahn's paper (7) on the sexuality of the Mycetozoa, published in 1911, he states that the swarm-cells are haploid, whereas the nuclei of the plasmodia are diploid, so that the sexual fusion is to be looked for at the commencement of plasmodium formation, and not at the stage preceding spore development. He figures mitoses in the haploid amoebae of *Physarum didermoides*, and diploid mitoses in young plasmodia, and he also figures a nuclear fusion of two haploid myxamoebae; he finds also typical karyokinetic divisions of the plasmodium nuclei.

The spores of the Mycetozoa, when germinated, as a rule give rise to ciliate swarm-cells, one of which emerges from each spore. In the exosporous *Ceratiomyxa*, however, each spore gives rise to eight swarm-cells, and in a few species of the endosporous Mycetozoa we get two or four swarm-cells from each spore. This I have observed to be the case in *Comatricha obtusata*, the spores of which each give rise to two swarm-cells. I have germinated the spores of *Fuligo septica* and *Lycogala miniatum*; the swarm-cells of the former are shown in Fig. 11. The *Fuligo* spores germinate in half an hour in rain-water if they are previously broken up by crushing them on a microscope slide, otherwise the germination is extremely uncertain, only one culture out of ten showing germination after three days; the spores, if kept for a week or more, lose their power of rapid germination. The swarm-cell is pear-shaped, and has a long flagellum at the pointed end, at which end also the nucleus is situated. The swarming lasts for about thirty hours and is followed by temporary encystment; after two or three days a few amoebae or small plasmodia may occasionally be seen. I have not succeeded in cultivating the organism beyond this stage.

Strasburger's statement that a simultaneous division of the nuclei of the developing sporangium takes place just prior to spore formation I have verified in the case of *Lycogala miniatum*, and this karyokinetic division is shown in Figs. 12 and 13.

The development of the sporangium of *Arcyria ferruginea* shows considerable difference to that of a typical endosporous Mycetozoon. In place of the universal karyokinetic division of the nuclei and consequent simultaneous formation and ripening of the spores we get a gradual or successive ripening from the outside inwards. Thus the innermost part of the young sporangium is plasmodial with nuclei having well-marked karyosomes; contiguous to this region is a narrow belt containing nuclei in which the karyosomes are replaced by granules of chromatin, and in the outer portion of this belt the plasma becomes discrete, and beyond this point is replaced by spores, as may be seen in Figs. 14 and 15. A slightly earlier stage of development to that just described is to be seen in Fig. 16, in which the karyokinetic nuclear divisions, which precede the spore formation, are to be observed in place of the spores. Among these dividing nuclei some are to be seen in the 'synapsis' stage; these are shown in Fig. 20.

In *Spumaria alba* and *Arcyria albida* I have observed the enlarged and degenerate nuclei described by Helene Kränzlin, but have not seen them approaching in pairs, nor have I seen any nuclear fusions.

In *Comatricha obtusata* I find that a karyokinetic nuclear division takes place within the spore, so that the ripe spore is usually binucleate. This division, which is not, however, quite universal, is shown in Fig. 18.

COMPARISON OF THE PLASMODIOPHORACEAE AND MYCETOZOA.

In the vegetative stage of the Plasmodiophoraceae we get the well-known intranuclear 'cruciform' type of nuclear division. This is altogether lacking in the Mycetozoa, where nothing resembling it has been observed. In the vegetative or plasmodial stage of the Mycetozoa Jahn has observed typical karyokinetic figures; Lister also has observed karyokinesis in the plasmodium of *Badhamia utricularis*, although he inclines to the opinion that direct nuclear division is the usual form. The akaryote stage which follows the vegetative stage in the Plasmodiophoraceae is also wanting in the Mycetozoa. These considerations seem to me of sufficient importance to justify the separation of the Plasmodiophoraceae from the Mycetozoa.

Osborn laid stress on the karyogamy observed by him in *Spongospora*, and, on the strength of its similarity to that described by Jahn and Kränzlin in the Mycetozoa, considered these processes as homologous and as evidence of close relationship. Since, however, Jahn has retracted his view, the existence of a karyogamy in the Mycetozoa becomes, to say the least, doubtful, and the argument used by Osborn has little weight. Jahn, however, as I stated above, also observed a karyogamy or conjugation of the nuclei of the young uninucleate amoebae developed from the swarm-cells. If, as is possible, a conjugation of the myxamoebae be found in the Plasmodiophoraceae, this would furnish evidence of relationship between this family and the Mycetozoa.

THE CHYTRIDEAE.

Němec (17) has described, in *Sorolpidium Betae*, a member of this family which is quite evidently closely allied to the Plasmodiophoraceae.

Among the points of resemblance between them may be mentioned the fact that *Sorolpidium* possesses two distinct forms of nuclei—viz. vegetative nuclei in which the chromatin is collected into a central karyosome, and reproductive nuclei in which the karyosomes are lacking. The resemblance is rendered the more evident by the mode of division of the vegetative nuclei which Němec states is by an amitotic division of the karyosome combined with an equatorial plate of idiochromatin. In the Plasmodiophoraceae the spores are, however, formed after two karyokinetic divisions, whereas in *Sorolpidium* the spore mother-cells form the resting stage, and later each cell nucleus undergoes two karyokineses, thus giving rise to four zoospores. The amoeboid vegetative stage in *Sorolpidium* is succeeded by the reproductive phase in which the organism divides into a number of uninucleate myxamoebae which form the spore mother-cells which are contained in a sporangium with an enclosing wall. Thus in the Plasmodiophoraceae we get clusters of spores, each spore of which is, in most genera, formed from a uninucleate amoeba; these clusters are usually enclosed by a common membrane, and constitute the resting stage of the organism. For the resting stage in *Sorolpidium* we get collections of spore mother-cells, each cell of which is formed from a uninucleate amoeba; these collections are enclosed in a membrane, and each spore mother-cell produces four zoospores.

Kusano (9) has also described a species of *Olpidium* parasitic on *Vicia unijuga.* He states that in the amoeboid vegetative stage the nuclei, which contain a prominent karyosome, divide amitotically, there being, however, no plate of idiochromatin as in the previous species. Later on the nuclei lose their karyosomes and become reproductive nuclei. These, like the corresponding ones in the Plasmodiophoraceae, undergo karyokinetic divisions, but give rise to the mother-cells of the zoospores. The zoospores conjugate in pairs, and the zygote gives rise to resting sporangia, which at first are binucleate, fusion of the nuclei taking place later. During the development of the resting sporangia the nuclei pass through a phase similar to the akaryote stage in the Plasmodiophoraceae.

I have observed in some few of the roots of *Mentha Pulegium* the presence of an *Olpidium* similar to those described above, but on account of inability to obtain sufficient material I have been unable to cover completely its life-history. This *Olpidium* is found in the cells in the form of amoebae of varying size, which apparently fuse to form a plasmodium. These amoebae differ from those of the *Ligniera* in being much larger, in having power of penetrating the cell-walls, and in their nuclei being much smaller; also they are not found in groups as are those of the *Ligniera*, and they are usually seen in the neighbourhood of the sporangia to which they give rise. The sporangium, which is surrounded by a membrane, contains the reproductive nuclei which probably give rise to the zoospores. This organism is shown in Figs. 21 and 22.

From a cytological point of view it is clear that these organisms are closely related to the Plasmodiophoraceae. The great similarity of the vegetative nuclear division, the somewhat similar akaryote stages, the karyokinetic divisions of the reproductive nuclei are all evidence of relationship. As differences we may note the formation of a membrane around the developing sporangium, and the fact that this body contains the spore mother-cells, and not the spores themselves. On germination the nuclei of these mother-cells undergo the karyokinetic divisions, whereas in the Plasmodiophoraceae these two divisions preceded the resting stage. Like the Mycetozoa, these species of *Olpidium* have both the vegetative and the reproductive forms of nuclei. The so-called spores of some of the Mycetozoa may, in reality, be 'sporangia', in that they give rise to more than one swarm-cell on germination, as for example *Ceratiomyxa* or *Comatricha.* The balance of the evidence of relationship seems to me to show that the Plasmodiophoraceae should form a separate class intermediate between the Chytrideae and Mycetozoa.

SUMMARY AND CONCLUSIONS.

1. The root parasites of *Bellis perennis*, *Mentha Pulegium*, and *Alisma Plantago* are members of the Plasmodiophoraceae belonging to the genus *Ligniera.*

2. The 'cruciform' type of vegetative nuclear division, and the presence of the akaryote stage is constant throughout the Plasmodiophoraceae, but both are lacking in the Mycetozoa, but are found in *Olpidium.*

3. The nuclei of the Plasmodiophoraceae, Mycetozoa, and Chytrideae are of two types: vegetative and reproductive.

4. There is an absence of karyogamy prior to spore formation in all three families.

5. In the Mycetozoa the vegetative and reproductive nuclear divisions are karyokinetic.

6. The Plasmodiophoraceae, though closely related to both the Mycetozoa and Chytrideae, are best considered as a separate class.

7. The spore mother-cells in the zoosporangium of *Olpidium* each give rise to four zoospores.

8. There is a conjugation in pairs of the zoospores of the Chytrideae.

9. There is a conjugation of the myxamoebae and a nuclear fusion in the Mycetozoa.

BIBLIOGRAPHY.

1. BLOMFIELD and SCHWARTZ: Some Observations on the Tumours of *Veronica Chamaedrys* caused by *Sorosphaera Veronicae.* Ann. of Bot., vol. xxiv, 1910.
2. GRIFFON and MAUBLANC: Notes de pathologie végétale et animale. Bull. d. l. Soc. Myc. de France, vol. xxvi, 1912, p. 469.

3. HARPER: Cell and Nuclear Division in *Fuligo varians.* Bot. Gazette, vol. xxx, 1900.
4. HORNE: Preliminary Note on *Spongospora Solani.* Ann. of Bot., vol. xxv, 1911.
5. JAHN: Myxomycetenstudien: VI. Kernverschmelzungen und Reduktionsteilungen. Ber. der deutsch. bot. Gesell., vol. xxv, 1907, p. 23.
6. ——: Myxomycetenstudien: VII. *Ceratiomyxa.* Ibid., vol. xxvi *a*, 1908, p. 342.
7. ——: Myxomycetenstudien: VIII. Der Sexualakt. Ibid., vol. xxix, 1911, p. 231.
8. KRÄNZLIN: Zur Entwicklungsgeschichte der Sporangien bei den Trichien und Arcyrien. Archiv f. Protistenkunde, vol. ix, 1907, p. 170.
9. KUSANO: On the Life-history and Cytology of a New *Olpidium.* Journ. Coll. of Agric. Tokyo, vol. iv, 1912, p. 3.
10. LISTER: On the Division of the Nuclei in the Mycetozoa. Journ. Linn. Soc. Bot., vol. xxix, 1893, p. 529.
11. MAIRE and TISON: La cytologie des Plasmodiophoracées et la classe des Phytomyxinae. Ann. Myc., vol. vii, 1909, p. 226.
12. ——————: Sur quelques Plasmodiophoracées. C.-R. Ac. Sc. Paris, vol. cl, 1910, p. 1768.
13. ——————: Sur quelques Plasmodiophoracées non hypertrophiantes. C.-R. Ac. Sc. Paris, vol. clii, 1911, p. 206.
14. ——————: Nouvelles recherches sur les Plasmodiophoracées. Ann. Myc., vol. ix, 1911, p. 226.
15. MASSEE: Corky Scab of Potatoes. Journ. Board of Agric., vol. xv, 1908, p. 592.
16. NAWASCHIN: Beobachtungen über den feineren Bau und die Umwandlungen von *Plasmodiophora Brassicae.* Flora, vol. lxxxvi, 1899.
17. NĚMEC: Ueber eine Chytridiacee der Zuckerrübe. Ber. d. Deutsch. Bot. Ges., vol. xxix, 2, 1911, p. 48.
18. OLIVE: Evidences of Sexual Reproduction in the Slime-Moulds. Science, N.S., vol. xxv, 1907, p. 266.
19. ——: Cytological Studies on *Ceratiomyxa.* Trans. Wisconsin Acad., vol. xv, Pt. II, 1907, p. 753.
20. OSBORN: *Spongospora subterranea.* Ann. of Bot., vol. xxv, 1911, p. 327.
21. PAVILLARD: État actuel de la protistologie végétale. Prog. Rei Bot., vol. iii, 1910, p. 474.
22. ————: A propos de la phylogénie des Plasmodiophoracées. Ann. Myc., vol. x, 1912, p. 218.
23. PROWAZEK: Ueber den Erreger der Kohlhernie, *Plasmodiophora Brassicae.* Arbeit. aus dem Kaiserl. Gesundheitsamte, vol. xxii, 1905, p. 396.
24. SCHWARTZ: Parasitic Root Diseases of the Juncaceae. Ann. of Bot., vol, xxiv, 1910.
25. ————: The Life-history and Cytology of *Sorosphaera Graminis.* Ann. of Bot., vol. xxv, 1911.
26. WINGE: Cytological Studies in the Plasmodiophoraceae. Archiv För Botanik, vol. xii, No. 9, 1912.

EXPLANATION OF PLATE XII.

Illustrating the paper by Mr. Schwartz on the Plasmodiophoraceae and their relationship to the Mycetozoa and the Chytrideae.

Fig. 1. Longitudinal section of portion of outer cortex of diseased root of *Mentha Pulegium.* × 800.

Fig. 2. The akaryote stage of *Ligniera Menthae.* × 800.

Fig. 3. Karyokinetic nuclear divisions of reproductive nuclei of *L. Menthae.* × 800.

Fig. 4. Karyokinesis leading to spore formation in *L. Menthae.* × 800.

Fig. 5. Spore clusters of *L. Menthae.* × 800.

Fig. 6. Karyokinesis in vegetative phase of *L. Menthae.* × 800.

Fig. 7. Amoeboid stage of *L. Bellidis.* × 800.

Fig. 8. Akaryote stage and spores of *L Bellidis.* × 800.

Fig. 9. Apex of root-hair of *Poa annua* infected by *L. Graminis.* × 800.

Fig. 10. Empty spores of *L. Graminis* in apex of root-hair of *Poa annua.* × 800.

Fig. 11. Swarm-cells, myxamoebae, and spore of *Fuligo septica.* × 800.

Fig. 12. Karyokinesis of nuclei in sporangium of *Lycogala miniatum.* × 800.

Fig. 13. Anaphase of mitoses in sporangium of *Lycogala miniatum.* × 800.

Fig. 14. Central plasmodial portion of sporangium of *Arcyria ferruginea.* × 800.

Fig. 15. Middle belt of sporangium of *A. ferruginea,* showing transition to reproductive nuclei and spores. × 800.

Fig. 16. Portion of sporangium of *A. ferruginea,* showing karyokinesis of reproductive nuclei prior to spore formation. × 800.

Fig. 17. Section of sporangium of *A. ferruginea,* showing central plasmodial portion and outer sporogenous portion. × 500.

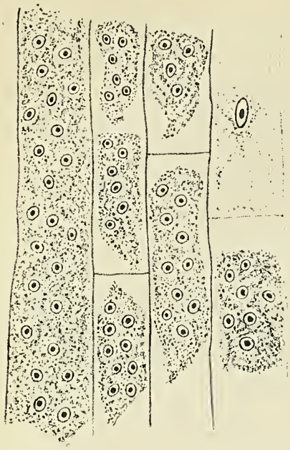
Fig. 18. Spores of *Comatricha obtusata,* showing nuclear division. × 800.

Fig. 19. Portion of sporangium of *Arcyria albida,* showing enlarged nuclei. × 800.

Fig. 20. Karyokinesis and synapsis in sporangium of *Arcyria ferruginea.* × 1,200.

Fig. 21. Developing sporangium of *Olpidium* in root of *M. Pulegium.* × 800.

Fig. 22. Ripe sporangium of *Olpidium.* × 800.



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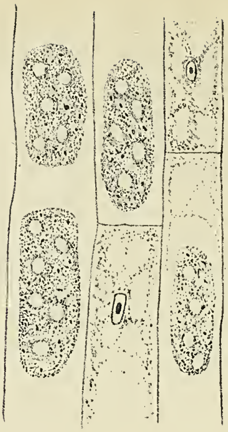

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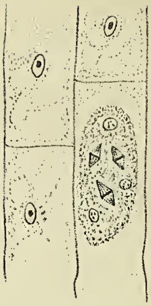

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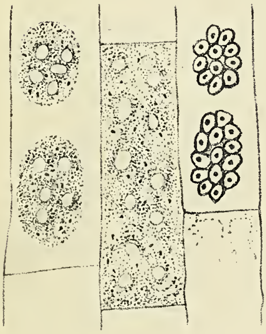

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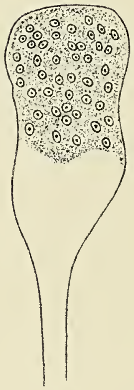

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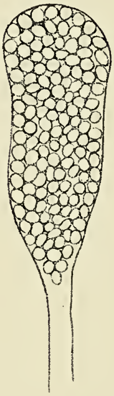

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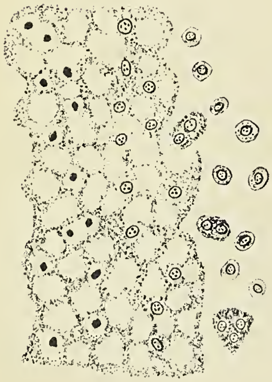

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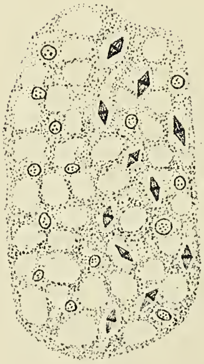

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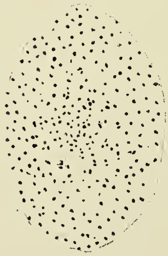

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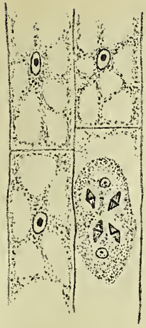

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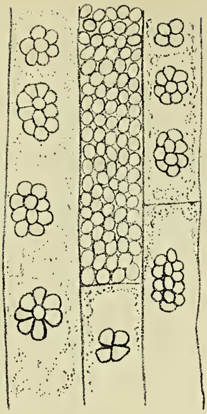

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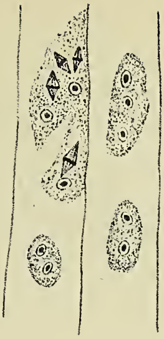

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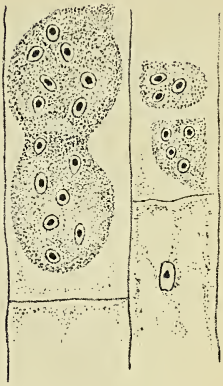

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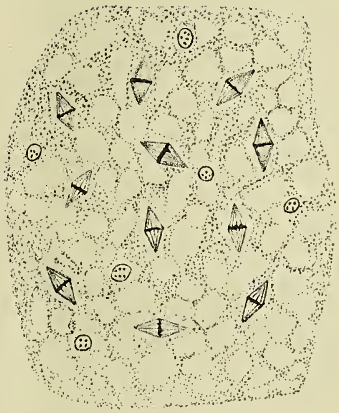

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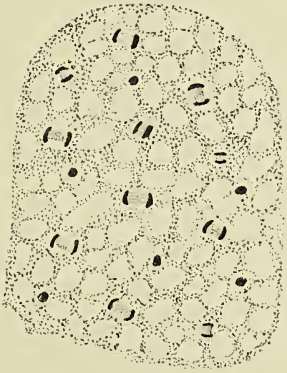

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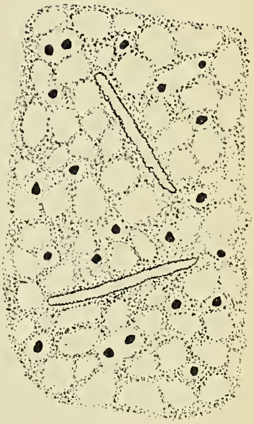

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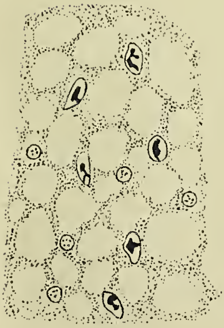

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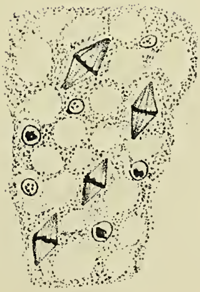

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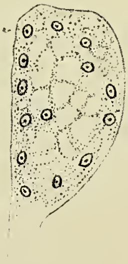

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The Structure and Life-history of Verrucaria margacea, Wahl., an Aquatic Lichen.

BY

ETHEL M. POULTON, M.Sc.,

University Scholar, University of Birmingham.

With Plates XIII and XIV.

VERRUCARIA MARGACEA is a crustaceous lichen found adhering to smooth stones in running water. The material examined was obtained chiefly from a clear, shallow stream in Sutton Park, Warwickshire, where the lichen was first observed in 1902 by Mr. W. H. Wilkinson. It was subsequently discovered independently by Professor G. S. West, at whose instigation I undertook the study of the organism. The lichen also occurs in great abundance in the streams of Cannock Chase, Staffordshire.

EXTERNAL CHARACTERS.

When young, *Verrucaria margacea* appears as very small, almost transparent membranous patches, circular in outline, and pale green in colour (Pl. XIII, Fig. 1). The crust at this stage is continuous and perfectly smooth. The small thalli are firmly adherent to the substratum, but may be peeled from the stone by means of a sharp knife. Increase in area eventually occurs and neighbouring thalli frequently coalesce to form a more or less extensive thallus of irregular contour (Fig. 2). A definite ridge usually marks the place where such union has occurred. The mature thallus is of a dark olive-green colour, and is very conspicuous when adherent to light-coloured stones. On darker stones the lichen is scarcely recognizable. Some of the older specimens examined were almost black, and the crust was often much wrinkled. The thallus is typically covered with perithecia which appear as small black spots. They have been observed on thalli as small as 2 mm. in diameter.

The perithecia are arranged in no definite order. Occasionally a slight zoning is observed, but this is merely a coincidence. The youngest perithecia are situated nearest the periphery of the thallus. In no case do the perithecia extend to the extreme edge of the thallus; there is invariably a zone devoid of perithecia, and pale green in colour. In many specimens

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this was further surrounded by a colourless layer consisting of interlacing fungal hyphae without algae. This is presumably the youngest portion of the thallus.

MINUTE STRUCTURE OF THALLUS.

The thallus exhibits considerable diversity of structure. A vertical section of the young thallus will reveal a large number of green algal cells scattered irregularly throughout a colourless network of fungal hyphae (Fig. 3). The alga is a form of *Protococcus viridis*, Ag. (=*Pleurococcus vulgaris* auct.).[1] Each cell is furnished with a lobed parietal chloroplast ; no pyrenoids have been detected. The algae occur either singly or in groups of two or three cells. If the thallus is very young, the algae are chiefly unicellular, and many of the cells are somewhat elongated, preparatory to division.

The fungal hyphae are absolutely uniform in structure, with rather short cells. In horizontal and vertical sections of the thallus, and in surface view, the hyphae have the appearance of a compact pseudo-parenchymatous tissue. There is thus no definite arrangement of the alga with reference to the hyphal network ; the thallus is strictly homoiomerous.

The older thallus exhibits an interesting structure. The algae are often disposed in short chains of cells (3–7 or more) at right angles to the surface of the thallus. In many cases the cells are connected to form a continuous chain, suggesting that they result from the division of a single algal cell (Fig. 4). A controlling factor in the distribution of the algal cells may be the excessive amount of moisture to which the algae are subjected. *Protococcus* cells frequently produce short filaments when immersed in water or culture solutions. (See Cultures, p. 245.)

A further type of thallus-structure has been observed—e. g. there is sometimes a rudimentary distinction into layers, short chains of algal cells occurring aggregated near the dorsal and ventral surfaces of the thallus. Separating these is a conspicuous layer of fungal hyphae, entirely devoid of algae. The hyphae are interwoven to form a pseudo-parenchymatous tissue absolutely identical and continuous with that in which the algae are embedded (Fig. 5). This type of structure has been observed on the same thallus as the homoiomerous type.

I have not succeeded in detecting the presence of haustoria connecting the hyphae with the algal cells. Owing to the close juxtaposition of the two constituents it is presumable that interchange of food materials takes place between alga and fungus, by osmosis through the cell walls.

An interesting feature of the thallus is the relatively great abundance of the alga. Green lichens are somewhat rare, and in the present instance,

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[1] The structure of the alga of the thallus, and its behaviour in culture solutions, are in precise agreement with Chodat's *Pleurococcus Nägelii*. (Polymorphsme des Algues, pp. 53 et seqq.)

although the fungus dominates from a reproductive point of view, the alga plays a prominent part in determining the general aspect of the thallus, responsible as it is for the green colour, even from the earliest stages.

REPRODUCTION.

1. *Vegetative.* I have been unable to gain any definite information as to the production of true soredia. On gently sweeping the surface of the thallus with a camel's-hair brush, a large number of fragments are obtained, having a structure identical with that of the ordinary lichen thallus (Fig. 6). These are frequently somewhat circular in outline and may be true soredia, but only in one or two instances have such fragments been observed in direct connexion with the upper surface of the thallus.

Any detached portion of the thallus appears to have the capacity of continuing its existence.

2. *The Perithecia.*[1] The perithecia are flask-shaped bodies wholly immersed in elevations of the thallus. When mature, each perithecium communicates with the exterior by means of an ostiole which is surrounded by the black outer investment of the perithecium. This black coat is very characteristic of the perithecium. It surrounds the perithecium on all sides except the base, and is readily visible through the transparent thallus which covers it. The development of the perithecium proceeds in the following manner:

From the base of the thallus numerous hyphae grow vertically and obliquely towards the substratum. By their growth they usually push upwards that portion of the thallus above their origin, so that it appears as a small protuberance. The hyphae branch profusely, septa soon appear, so that a pseudo-parenchymatous tissue, in the shape of a broad, smoothly conical mass, is produced beneath the thallus (Fig. 7). The cell walls of the outermost hyphae of the cone become impregnated with a dark brown material, the deposition commencing at that portion nearest the base of the thallus and gradually extending towards the base of the cone-shaped mass of hyphae. In old specimens this deposit is so abundant that the perithecial investment appears quite black. If perithecia are being formed in close proximity, the dark brown tissues of each may become continuous, so that a dark interrupted stratum is formed beneath the crust (Fig. 12).

During these changes in the outermost hyphae of the cone important changes are proceeding in the inner hyphae. These never become impregnated with the brown material. A cavity appears in the centre of the mass, which gradually increases in size. From the hyphae of the upper surface of this cavity short colourless threads (periphyses) are formed, and these hang

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[1] A good deal of work has been done with a view to determining whether sexual organs are formed. The evidence, however, appears to be too scanty to admit of a definite statement on this point.

down into the cavity. They are very abundant, and may be recognized at an early stage in the development of the perithecium. The periphyses are multicellular and contain granular protoplasm (Fig. 13). A channel is now formed from the apex of the cone through the thalline covering so that an ostiole is formed. Fuisting finds that the ostiole is formed in a lysigenetic manner in *Verrucaria*.[1]

The periphyses now line the upper part of the perithecium as a fringe and eventually extend through the ostiole (Pl. XIV, Fig. 14).

From the hyphae forming the floor and sides of the perithecium the asci are developed. Each ascus is a transparent, colourless sac containing very granular protoplasm (Fig. 14).

The contents become vacuolate and subsequently divide into eight portions, each of which becomes rounded off and invested with a cell wall.

The spores are colourless and spindle-shaped (Pl. XIII, Fig. 15). They contain granular protoplasm in which a large quantity of oil occurs. The application of osmic acid or alkanin solution will show that the oil is diffused in the general cytoplasm, and frequently forms one or more large refractive granules also (Fig. 16).

The spores are 12–16 μ long by 5–7 μ broad when in the ascus, and they are invariably unicellular. After their liberation a septum appears in the broadest portion, so that the spores become bicellular.

The spores usually germinate in the bicellular condition, although aseptate spores have occasionally been observed germinating. A colourless germ-tube is protruded from one or both extremities of the spore (Fig. 17, *s*). This usually becomes divided by transverse septa, and eventually branches. A number of germinating spores were kept under observation for two to three weeks. The spores were at first aseptate, but after a short time a septum appeared in most of the spores. A little later two more septa were formed, one in each half of the originally bilocular spore. At the same time the spores increased in size considerably. Their dimensions are 21–24 μ × 9–10 μ in this condition. In many cases germ tubes were put out from each locule of the spore (Fig. 17, *b*). All stages between aseptate and 3-septate conditions were observed. Quadrilocular spores often occur in nature some time after their liberation from the ascus.

Spores are frequently found germinating singly upon the surface of the thallus. A large number of spores, however, germinate while still in the perithecium and the resulting hyphae branch profusely. A closely interweaving mass of hyphal threads is thus formed (Fig. 18). This mass of hyphae and spores projects through the ostiole and is visible to the naked eye as a small white speck (the 'nucleus' of lichenologists). It is eventually expelled through the ostiole and floats freely in the water.

The tendency of the ascospores to germinate *en masse* while still in the

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[1] Bot. Ztg., 1868, pp. 369, 641.

perithecium may be regarded as a specialization in order to ensure the reproduction of the organism. A single spore floating in the water has a very remote chance of encountering a suitable algal cell under such conditions that germination, and enclosing of the alga by hyphae, could occur. Hence single spores appear to be of little use as agents of reproduction in the case of an aquatic lichen. On the other hand, a mass of interlacing fungal hyphae, exposing a large surface, would be more likely to entrap any free floating cells of *Pleurococcus*, and thus the requisite algal constituent of the lichen would be secured. I have frequently observed the algal cells in the meshes of such hyphae.

CULTURAL EXPERIMENTS.

Hanging drop and Petri-dish cultures of the lichen were made, in various culture media. The results are on the whole inconstant, but the following general statements may be made:

Dilute Knop Solution (0·1 per cent.–0·4 per cent.) favoured the growth of the algal constituent. In some cases the algal cells grew out into short filaments of 3–6 cells, and occasionally branching occurred (Figs. 10, 11). In other cultures the algae of the thallus became so numerous that the hyphal network was completely hidden. Algae which became detached from the thallus underwent vegetative division, resulting in colonies in the form of flat plates. The constituent cells were often polygonal in shape, due to compression (Fig. 9). In most cases, the fungal constituent eventually disappeared.

Agar and dilute Knop solution, *Agar and 2 per cent. Glucose solution.* Neither alga nor fungus appeared to be in a healthy condition whilst growing upon these media. In most cases the thallus turned pale and eventually died.

In no instances did the lichen thallus as a whole thrive. An attempt was made to synthesise the lichen by sowing fungal spores among algal cells obtained from a culture in dilute Knop solution. The apparatus used was a modification of that employed by G. Bonnier in his researches on the Synthesis of Lichens (Bonnier, p. 15). The algae continued to grow for several months, but the conditions of the experiment were evidently unfavourable to the continued growth of the fungus, as in every case the hyphae formed on germination of the spores eventually died.

SYSTEMATIC POSITION AND NOMENCLATURE.

There seems to have been a good deal of confusion regarding the synonomy of the lichen in question. After careful consideration of the original descriptions, and others to which I had access, I have concluded that the following are synonymous:

1. *Thelotrema margaceum*, Wahl., in Ach. Meth. Lich. p. 30, 1803.

2. *Pyrenula margacea*, Ach. L. Univ. p. 315, T. V. f. 3, 1810.
3. *Verrucaria margacea*, Wahl. Fl. Lapp. p. 465, 1812.
4. *Pyrenula margacea*, Ach. Syn. Meth. Lich. p. 127, 1814.
5. *Verrucaria margacea*, Wahl. Fl. Suec. p. 872, 1826.
6. *Verrucaria submersa*, Borr., E. Bot. Suppl. No. 2768, 1834.
7. *Verrucaria margacea*, Leighton, Ang. Lich. p. 62, Plate XXVI, 1851.
8. *Verrucaria margacea*, Leighton, Lich. Fl. p. 446, 1879.

Acharius has considered the first four as synonymous, Wahlenberg the first five. Leighton regards all the above as synonymous, Borrer the first six.

Acharius[1] includes *V. papillosa* among his synonyms, but the synonymy of *V. margacea* and *V. papillosa* is doubtful. The thallus of *V. margacea* does not closely agree with that described for *V. papillosa*, neither are the perithecia of *V. margacea* 'semi-immersed in thalline elevations'. Leighton[2] describes *V. papillosa* as a distinct species. His figure does not agree with *V. margacea.* On the whole it seems more natural to place *V. papillosa* as a variety of *V. margacea* as Leighton does later.[3]

Borrer[4] gives a description of *V. submersa*, also figures, which closely agree with *V. margacea.* He thinks, however, that *V. submersa* may be identical with *V. mucosa* as described by Wahlenberg[5] and Acharius.[6] It is, however, obvious from the descriptions of *V. mucosa* that it is quite a distinct species. Borrer also suggests a resemblance to *V. nitida*, which is equally distinct both in structure and habitat.

Mudd[7] recognizes two totally distinct organisms on the ground of difference in spores. He says (l. c., p. 286) 'I cannot see how Leighton reconciles the fig. of the spores of *V. submersa* in E. Bot. Suppl. 2768 with those of his *V. margacea.* In E. Bot. they are represented as elliptical, unilocular, sub-hyaline ; in his Brit. Ang. Lichens, as linear quadrilocular, and coloured'. Mudd then distinguishes *V. submersa*, Borr. (*V. chlorotica*, Ach. Syn. 94 in pt.) (l. c. p. 286), and *Thelidium cataractum*, Hepp. (l. c. p. 294), to which he refers *V. margacea* of Leighton.[8] The genus *Verrucaria* is regarded by Mudd as possessing colourless, unilocular spores, while those types having pale brownish, bi- or quadrilocular spores are included in the genus *Thelidium.* In other respects both descriptions agree fairly well with *V. margacea.*

This method of widely separating lichens which differ in the absence or existence of septate spores, but are otherwise closely similar, is unsound. The spores of *V. margacea* have been shown to be aseptate, uni- and tri-

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[1] L. Univ., p. 286.

[2] Ang. Lich., p. 52, Plate XXIV, Fig. 1.

[3] Lich. Fl., p. 447.

[4] E. Bot. Suppl. No. 2768.

[5] Fl. Lapp., p. 466.

[6] Syn. Meth. Lich., p. 93 ; L. Univ., p. 282.

[7] Mudd, Man. Brit. Lich., p. 294.

[8] Leight. Ang. Lich., p. 62, Plate XXVI, Fig. 3.

septate, at different periods of the life-history. The fact that the spores become triseptate at a comparatively late period probably accounts for some of the discrepancies in the above-mentioned descriptions. *V. margacea* is repeatedly said to have unilocular spores,[1] although in a few instances [2] triseptate spores are said to be characteristic of *V. margacea* or its synonyms.

It should be remembered in dealing with lichens that spore production is characteristic of the fungal constituent. The spores are incapable of reproducing the lichen thallus unless they are brought in contact with a suitable alga. Lichen spores are, therefore, more or less unreliable agents of reproduction. It seems not improbable that the late appearance of septa is a sign of degeneration.

This goes to show that spore-characters of lichens are not always reliable as a basis of classification unless the complete life-history of the organism is followed out.

In conclusion I have to express my gratitude to Professor West for his interest and assistance during the progress of the work, also to Dr. T. W. Woodhead for helpful suggestions.

SUMMARY.

1. *Verrucaria margacea* is a crustaceous lichen attached to smooth stones in streams. The young thallus is pale green, but when older it appears dark olive-green or almost black.

2. The algal constituent of the lichen is a form of *Protococcus viridis,* Ag. The fungus forms a compact, pseudo-parenchymatous network, absolutely uniform in structure throughout the whole thallus. Three types of thallus structure have been observed :

(*a*) In the young thallus the algae are disposed singly throughout the colourless fungal network.

(*b*) An older thallus shows the algae in the form of short chains at right angles to the surface of the thallus; the chains are uniformly distributed throughout the fungal network (homoiomerous).

(*c*) The chains of algae may be aggregated near the dorsal and ventral surfaces of the thallus.

(A tendency towards the heteromerous condition.)

3. The perithecia are immersed in elevations of the thallus, and are characterized by a firm, black outer investment.

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[1] Leight. Lich. Fl., p. 446. Mudd, Man. Brit. Lich., *V. submersa,* p. 286. Borr. : E. Bot. Suppl., No. 2768.

[2] Leight. Ang. Lich., p. 62. Mudd, Man. Brit. Lich., p. 294 (*Thelidium cataractum*). Lindsay : Brit. Lich., p. 306.

Periphyses line the ostiole and the upper portion of the perithecium, and from the base, asci containing ascospores are developed in the usual manner.

4. The spores are at first unilocular, later they become bilocular, and finally quadrilocular. They may germinate in any of these conditions.

The spores often germinate while still within the perithecium and the resulting hyphal network is eventually expelled through the ostiole and floats freely in the water. This no doubt serves to entrap any free-floating cells of *Pleuroccocus*, and it may be regarded as a specialization to ensure the union of the two constituents.

WORKS REFERRED TO.

Ach. L. Univ.: Acharius, Lichenographia Universalis, 1810.
Ach. Meth. Lich.: Acharius, Methodus Lichenum, 1803.
Ach. Syn. Meth. Lich.: Acharius, Synopsis Methodica Lichenum, 1814.
BONNIER, G.: Recherches sur la synthèse des Lichens. Annales des Sciences naturelles, Ser. vii, Bot. vol. ix. 1889.
CHODAT, R.: Polymorphisme des Algues. Genève, 1909.
CROMBIE: British Lichens, 1894.
E. Bot. Suppl.: Supplement to English Botany, 1834.
Leight. Ang. Lich.: Leighton, Angiocarpic Lichens. Proceedings of Ray Society, 1851.
Leight. Lich. Fl.: Leighton, Lichen Flora of Great Britain, 1879.
Lind. Brit. Lich.: Lindsay, Popular History of British Lichens, 1856.
Mudd, Man. Brit. Lich.: Mudd, Manual of British Lichens, 1861.
Wahl. Fl. Suec.: Wahlenberg, Flora Suecica, 1826.

EXPLANATION OF PLATES XIII AND XIV.

Illustrating the paper by Miss Poulton on the Structure and Life-history of *Verrucaria margacea.*

PLATE XIII.

Fig. 1. Lichen thalli growing upon stone. ½ nat. size.

Fig. 2. Thalli growing in close proximity and becoming coalescent. ½ nat. size.

Fig. 3. Vertical section of young thallus. *a*, algal cells scattered in hyphal network, *h*. × 750.

Fig. 4. Vertical section of older thallus, showing chains of algal cells, *c*. *h*, fungal hyphae. × 750.

Fig. 5. Thallus, vertical section, showing algae, *a*, aggregated near dorsal and ventral surfaces. *h*, fungal hyphae. × 750.

Fig. 6. ? Soredium. × 800.

Fig. 7. Vertical section of thallus, showing first stage in formation of perithecium. *a*, elevation of thallus caused by conical mass of hyphae, *b*. × 80.

Fig. 8. Ditto. *a*, base of thallus with algal cells; *b*, hyphae interweaving to form pseudo-parenchymatous mass of tissue, *t*. × 800.

Fig. 9. Algae from culture in 0·15 % Knop solution, showing colonial arrangement of algal cells. × 500.

Fig. 10. Algae from culture in 0·1 % Knop solution in form of short filaments. × 400.

Fig. 11. Ditto. × 800.

Fig. 12. Vertical section of thallus with perithecia. *a*, ordinary thallus; *b*, stratum of cells impregnated with brown deposit; *p*, perithecia. × 80.

Fig. 13. Inner lining of perithecium, *a*, with periphyses, *s*. × 1,000.

Fig. 15. Aseptate spores. × 500.

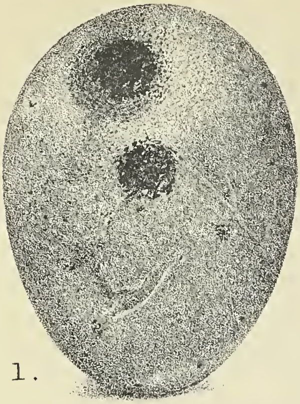
PLATE XIV.

Fig. 14. Young perithecium in vertical section. *t*, ordinary thallus; *o*, ostiole; *p*, periphyses; *a*, asci; *s*, spores. × 800.

Fig. 16. Simple spores after treatment with osmic acid. *o*. oil granules. × 500.

Fig. 17. Germinating spores. *s*, simple; *a*, 1-septate; *b*, 3-septate. × 500.

Fig. 18. Hyphal network expelled from ostiole of perithecium. × 400.



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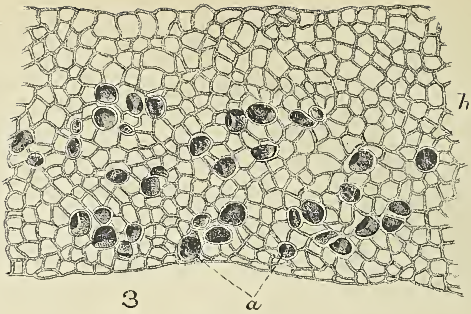

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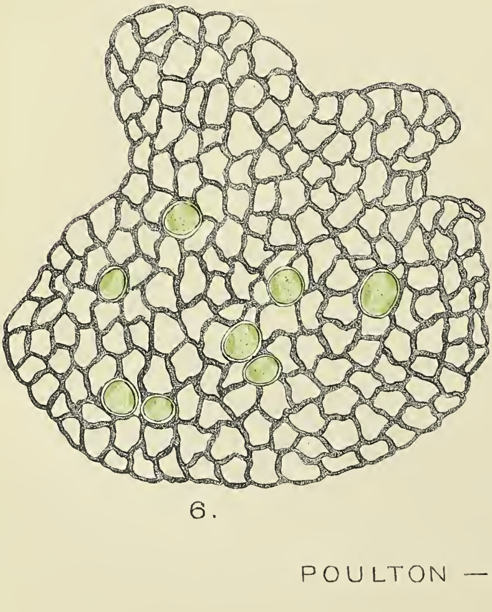

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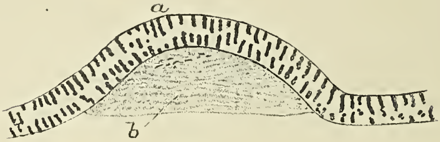

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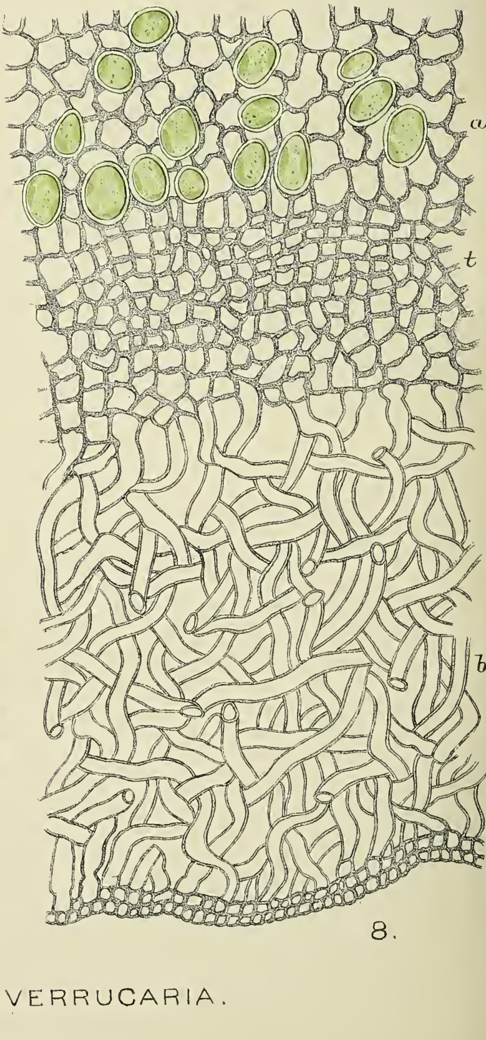

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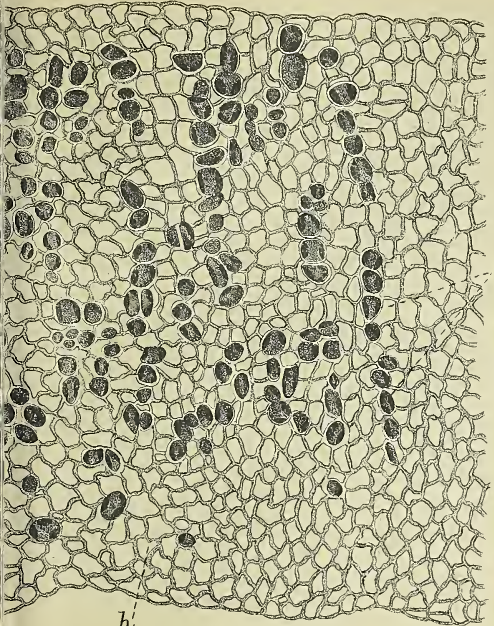
POULTON — VERRUCARIA.



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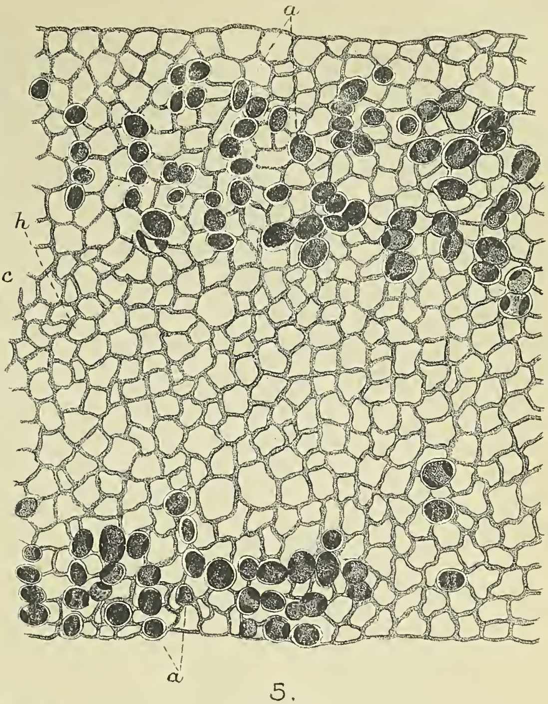

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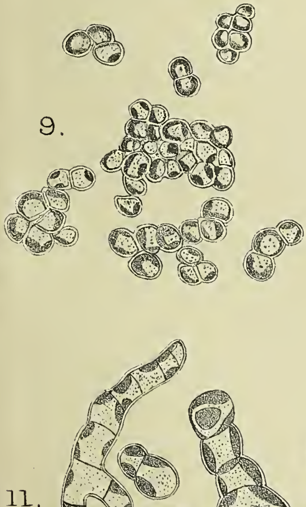

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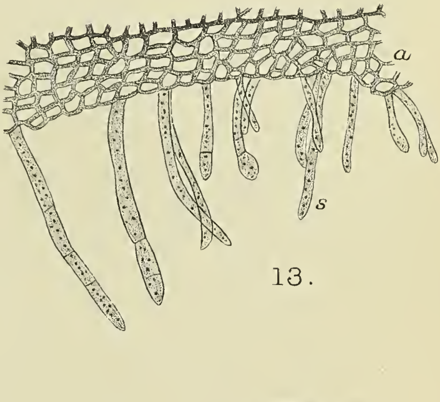

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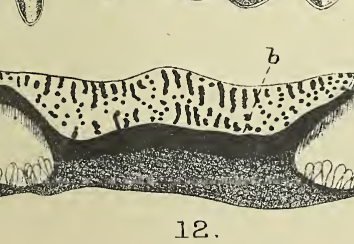

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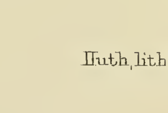

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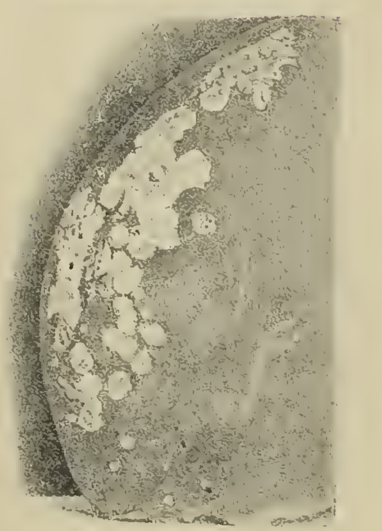

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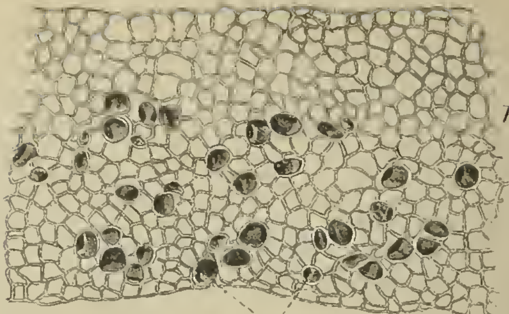

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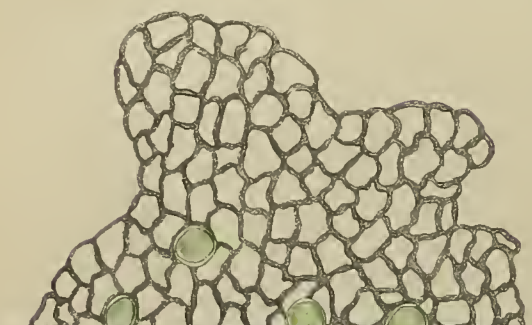

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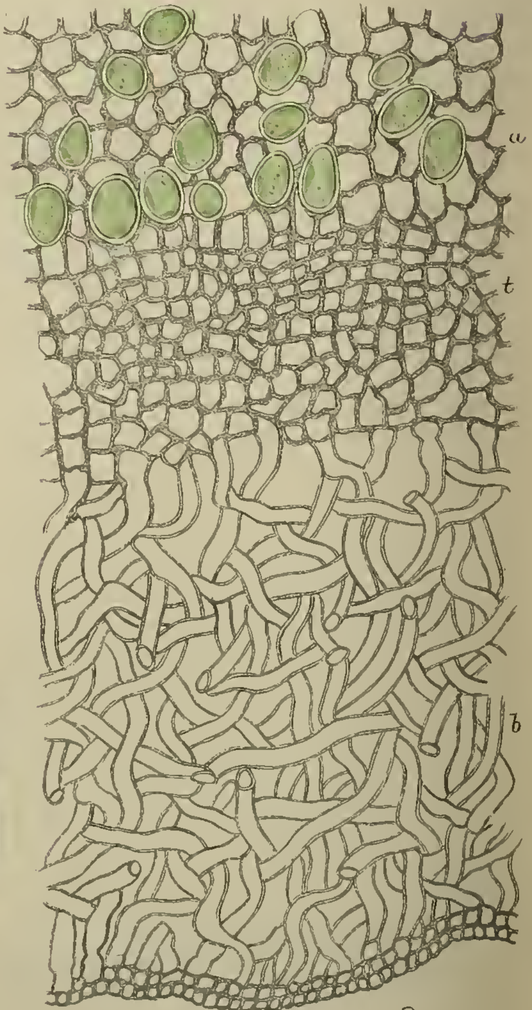

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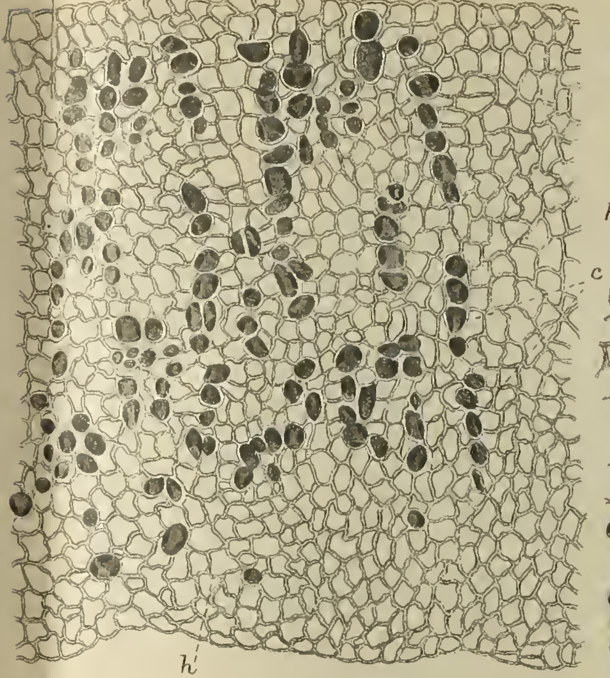

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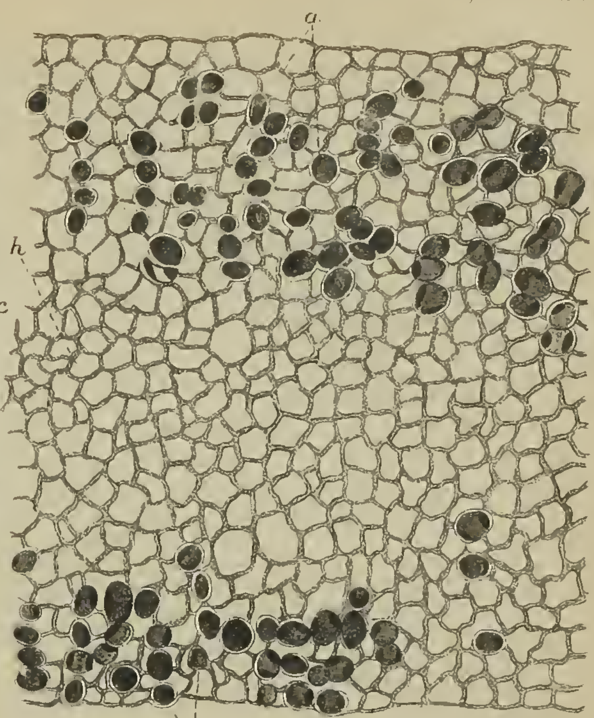

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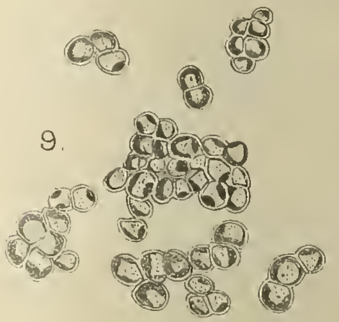

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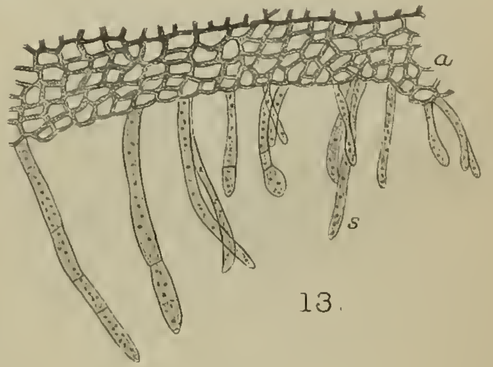

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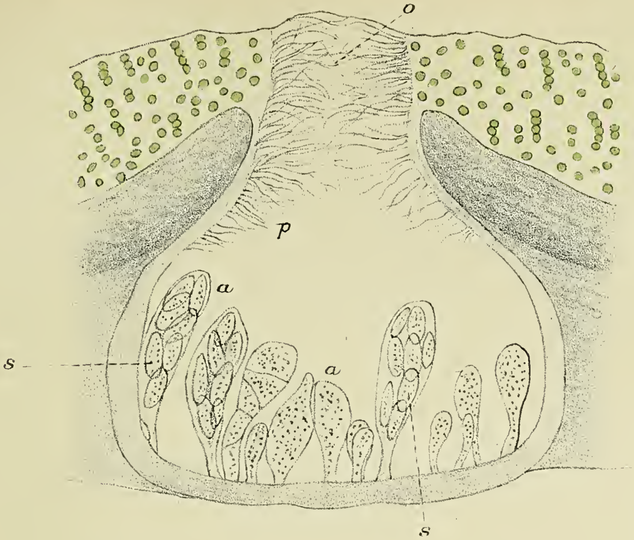
POULTON — VERRUCARIA.

Huth, lith et imp



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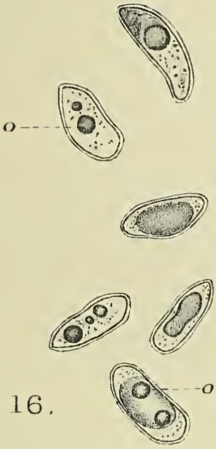

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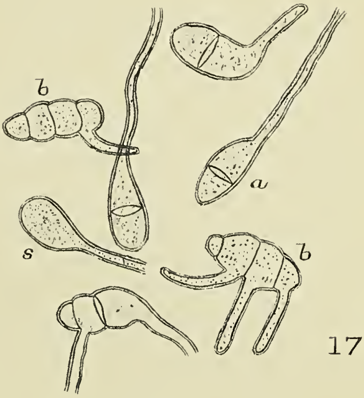

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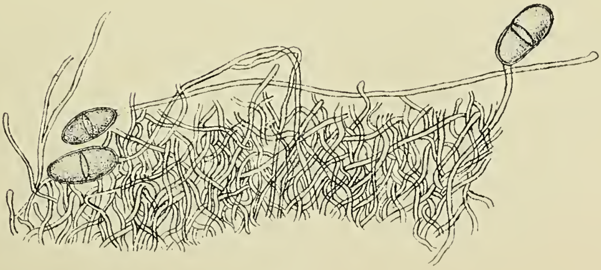

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Huth lith et imp.

POULTON — VERRUCARIA.

On Medullosa centrofilis, a New Species of Medullosa from the Lower Coal Measures.

BY

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With Plate XV and five Figures in the Text.

CONTENTS.

I. INTRODUCTION.

THE specimen of a Medullosean stem which forms the subject of the present investigation was obtained from the colliery at Shore Littleborough in Lancashire, and is of Lower Coal Measure age, as are also the only other British species of the genus which have, up to the present, been described—namely *M. anglica*[1] and *M. pusilla.*[2]

The coal-ball in which the stem occurred was divided by Mr. J. Lomax into three parts: from the portion named 3 a series of six transverse sections was obtained (sections A–F[3]), two slightly oblique transverse sections were available from part 2 (sections 8–9[4]), and a transverse and two longitudinal sections were cut from piece 1 (sections 24[5] and 25–6[6]). No information was obtained by which the distance between sections F and

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[1] Scott, D. H.: On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks. III. On *Medullosa anglica*, a new Representative of the Cycadofilices. Phil. Trans. Roy. Soc., Ser. B, vol. cxci, 1899. (Referred to subsequently as *M. anglica*, loc. cit.)

[2] Scott, D. H.: Studies in Fossil Botany. Part II, 2nd ed., 1909; On *Medullosa pusilla*, Proc. Roy. Soc., Ser. B, vol. lxxxvii, pp. 221–8.

[3] University College, London, Collection, Catalogue nos. O. 80 *a*, O. 80 *f*.

[4] " " " " " nos. O. 80 *g*, O. 80 *h*.

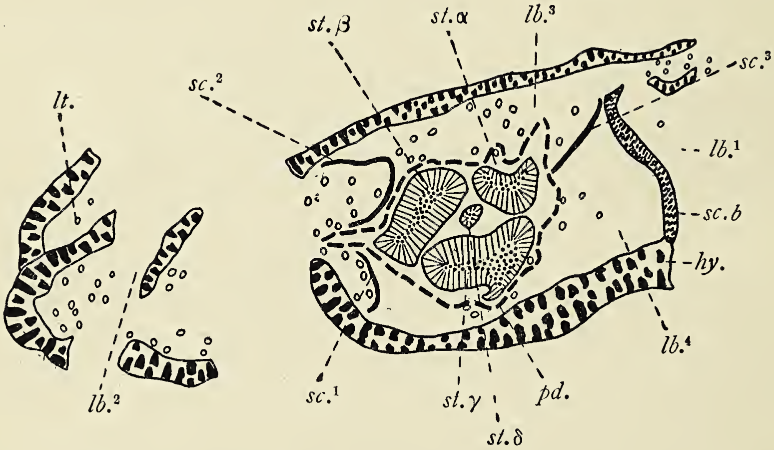
[5] " " " " " no. O. 80 *k*.

[6] " " " " " nos. O. 81 *a*, O. 81 *b*.

8, and between sections 9 and 24, could be estimated, so that the rapidity with which the changes occurred in the series of sections could not be determined.

II. GENERAL STRUCTURE.

The specimen consists of a portion of a stem surrounded by adherent leaf-bases. The diameters of the stem in the topmost section are roughly 5 cm. × 1·5 cm., including the leaf-bases; this estimate of the longer diameter is certainly too low, however, for part of one of the leaf-bases has either been torn off or else destroyed at some time during the process of petrifaction. The shape of the stem with its covering of leaf-bases was thus distinctly flattened (Pl. I, Fig. 1), though the form is, in all probability, exaggerated by lateral pressure.



TEXT-FIG. 1. Diagram of slide B. This diagram is intended to serve as a key to Plate XV, Fig. 1. × 2.

The ends of the long axis of the stem are occupied by prominent masses of tissue which represent leaf-bases, still in continuity with the stem. Practically the whole of one of these is present (*lb.*[2], Text-fig. 1), though its tissues are much displaced by the intrusion of Stigmarian rootlets; two bands of sclerenchymatous fibres, *sc.*[1] and *sc.*[2], mark the limit between the tissues of the adherent leaf-base and those of the stem proper.

Nearly opposite to this leaf-base, at the other end of the long diameter of the stem, is a second leaf-base, *lb.*[1], much less well preserved than *lb.*[2]; its outer parts have been almost entirely removed. A broad zone of anastomosing groups of sclerenchymatous fibres serves to delimit the tissues of this leaf-base; this zone, *sc.b.*, is very closely similar in its construction to the hypodermal layer of the leaf-base; it differs in the much more compact nature of the fibrous groups, the strands of parenchyma cells being larger in the hypoderma (*hy.*) than in the limiting band (*sc.b.*), and also in the fact that secretory canals are abundant in the hypodermal region, but are almost entirely absent in the limiting zone. In the sections 8 and 9, cut from portion 2 of the coal-ball, the limiting zone *sc.b.* is present only in the form of a band of sclerenchyma precisely similar to *sc.*^{1} or *sc.*^{2}. The passage from the simple sclerenchymatous band to the more complex limiting layer *sc.b.* could not unfortunately be traced, since it lay between sections F and 8. There can be no doubt, however, that in the lower parts of the adherent leaf-base its tissues are separated from those of the stem by a continuous sclerenchymatous layer; this layer became broken up into strands of fibres by the 'intrusion' of parenchyma tissue as the nodal region above was approached, and finally, by the increase in the parenchyma and the association of secretory canals, the limiting layer approximated to the hypoderma of the leaf-base, with which it became continuous as the petiole separated from the stem-tissue at the node.

The hypodermal layer is of the form described by Renault as the *Landriotii* type,[1] and the leaf-base as a whole shows a characteristic *Myeloxylon*[2] structure.

Lying between these two leaf-bases *lb.*^{1} and *lb.*^{2} are two much less prominent tissue masses, at either end of the short diameter of the stem; they are labelled *lb.*^{3} and *lb.*^{4} respectively. Their structure repeats on a smaller scale that of *lb.*^{1} and *lb.*^{2}: a band of sclerenchyma (*sc.*^{3}) occurs in the upper sections of the series, cutting off part of the leaf-base *lb.*^{3}; in the lower sections this band cannot be made out. It is possible, of course, that the regions marked *lb.*^{3} and *lb.*^{4} represent the true cortical tissues of the stem, but the presence of the band *sc.*^{3} in the upper part of the series, a band which appears to be characteristic of an adherent leaf-base at its lower end, would appear to render the view that they represent the extreme bases of leaves the more tenable one.

If this view be maintained, then the specimen represents a portion of a stem entirely surrounded by four spirally arranged adherent leaf-bases. No petiole becomes free from the stem during the course of the series, but a consideration of the nature of the 'limiting layer' (*sc.*^{1}, *sc.*^{2}, *sc.*^{3}, and *sc.b.*, Text-fig. 1) during the series shows that the leaves left the stem in the order *lb.*^{1}, *lb.*^{2}, *lb.*^{3}, and *lb.*^{4}. Of these, *lb.*^{1} represents the oldest leaf-base; it probably separated from the stem at a distance not very much above the level of the top section. The leaf-bases were decurrent down the stem for a considerable distance (for at least four internodes), so that in life the stem with its distant nodes presented a ribbed or winged appearance.

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[1] Scott, D. H.: Studies in Fossil Botany, 2nd ed., Pt. II, p. 445.

[2] Seward, A. C.: On the Genus *Myeloxylon* (Brong.) Ann. of Bot., vol. vii, p. 1, March, 1893.

In *Medullosa anglica*[1] the leaf-bases also appeared to clothe completely the stem surface; there also they were decurrent and confluent with the stem for a great vertical distance, at least 13 cm., so that in these respects the two plants show great similarity.

In *M. anglica*, Scott was able to determine the arrangement of the leaves, but in the present specimen this could not be definitely ascertained, but the phyllotaxis certainly does not appear to be consistent with that of *M. anglica*, where the divergence has been shown to be two-fifths.

The vascular tissues of the stem are entirely enclosed in a narrow zone of tissue *pd.*, which represents the beginning of periderm[2] development (Text-fig. 1). The vascular system consists of an outer ring of four steles, reduced by fusion to three in the upper part of the specimen, enclosing a central stele or star-ring (δ, Text-fig. 1). The greatest interest of the specimen lies in the presence of this star-ring, for hitherto such a structure has not been described in any of the Medulloseae of Lower Coal Measure age, although the Permian members of the genus *Medullosa* are all characterized by the occurrence of such strands.[3]

The structure of the steles is essentially similar in every case. The central portion of each is composed of a mass of primary tracheides intermixed with parenchyma; surrounding the primary xylem is a zone of secondary wood of variable thickness; but no phloem is preserved round any of the steles.

III. THE STELES.

The *size* of the individual steles varies considerably, as the maximum dimensions of transverse sections taken from the uppermost slide, A, will show:

Stele α	4·5 mm. × 3 mm.
„ β	7 mm. × 4 mm.
„ γ	9 mm. × 5 mm.
„ δ	2 mm. × 1·5 mm. (star-ring).

The *form* of the stele is somewhat irregular and varies in the course of the series; but the star-ring remains consistently oval throughout (cf. Pl. I, Fig. 1 and Fig. 2).

The whole of the interior of the steles is occupied by a mass of primary *wood* associated with thin-walled parenchymatous elements; the structure is strikingly similar to that of one of the steles of *Medullosa anglica*, except that there appears to be less variability between the pro-

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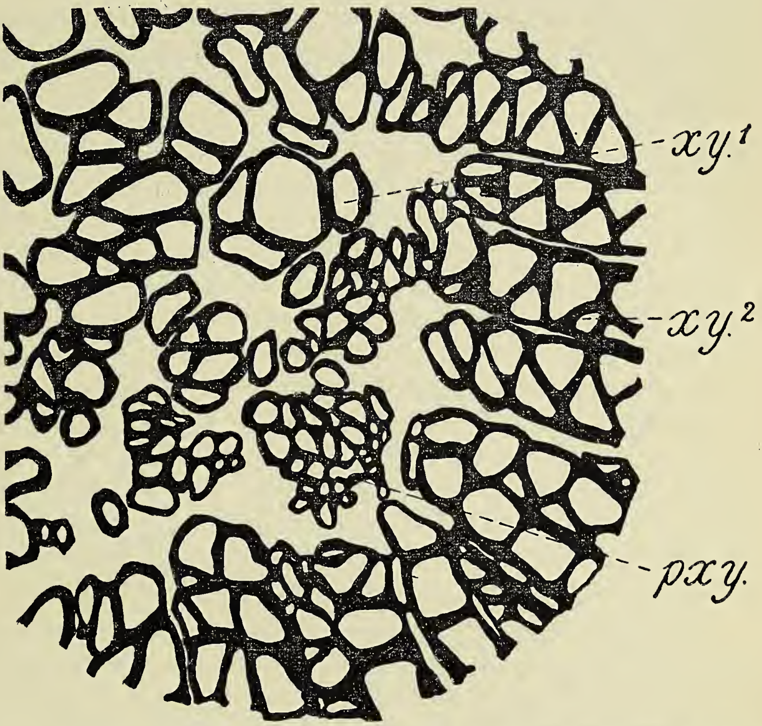
[1] *M. anglica*, loc. cit.

[2] Scott describes an internal periderm surrounding the steles in *Medullosa pusilla*, p. 222.

[3] Weber, O., und Sterzel, J. T.: Beiträge zur Kenntniss der Medulloseae. Ber. der Naturw. Ges. zu Chemnitz, vol. xiii, 1896.

portions, and in the arrangement, of the two constituents than is the case with *M. anglica.* The main bulk of the elements of the primary wood consists of multiseriately pitted tracheides which pass over almost insensibly into the similar, though somewhat smaller, elements of the secondary xylem.

Here and there towards the periphery of the primary wood occur large strands of tracheides, considerably smaller in size than the ordinary primary xylem elements, and showing spiral thickenings on their walls (*pxy.*, Text-fig. 2). These groups evidently indicate the position of the protoxylem elements, but from transverse sections it is impossible to say with certainty whether the development of the protoxylem was strictly exarch or slightly mesarch in nature, and the longitudinal sections did not enable the point to be decided. The structure of a stele is strikingly similar to that of a stele of *Medullosa pusilla,* in which also the position of the protoxylem group could not be determined with certainty. In the star-ring the strand of spiral elements is represented by three or four tracheides only (as seen in oblique section), and the development of the protoxylem appears to be almost certainly exarch. It is, therefore, probably exarch in the outer ring



TEXT-FIG. 2. Transverse section of part of stele, showing a strand of spirally thickened tracheides of the primary wood. × 70. $xy.^1$ = primary xylem; $xy.^2$ = secondary xylem; *pxy.* = strand of spirally marked tracheides.

of steles also. The well-marked peripheral strands of spirally marked tracheides are also characteristic of the steles of *M. anglica,* but the protoxylem in this case is undoubtedly mesarch in development.[1]

The conjunctive parenchyma of the primary wood is composed of thin-walled tissue, which on the whole is very badly preserved.

A zone of secondary wood occurs around each stele, its dimensions vary somewhat, and the thickness is usually greater on the side of the stele adjacent to the star-ring.[2] The number of secondary tracheides in a row is, on an average, eight to ten. The sculpturing of the secondary tracheides consists of multiseriate bordered pits which appear to be limited to the radial walls of the elements.

Medullary rays run from the primary wood of the stele to the limit of the secondary xylem, separating the radial series of tracheides into segments which are usually two to three cells broad (Text-fig. 2, *xy.*^{2}). The rays are usually very narrow and appear to be composed of narrow thin-walled parenchyma cells, considerably longer than they are wide; each ray is generally two or three cells in width. Here and there the rays appear to broaden out towards the periphery of the stele into wedge-shaped masses. In addition to the primary rays, secondary ones may arise in the secondary wood at varying distances from the central mass of primary tissue.

No trace of any phloem surrounding any of the steles can be distinguished, but lying immediately beyond the secondary wood is a narrow zone in which the tissue as a whole is not preserved, but in which secretory canals occur with great frequency (*m.c.*, Plate XV, Fig. 3). This zone probably represents the phloem and the pericycle of the stem; it is bounded on the outer side by the periderm (*p.d.*, Pl. XV, Fig. 3).

The Star-ring.—The tissues of the star-ring are, on the whole, very well preserved, and show quite clearly that the structure here is precisely similar to that of the peripheral steles, but on a smaller scale.

In the Permian Medullosas, in which 'star-rings' form so characteristic a feature, their preservation is, as a rule, far from good, and the structures are described as possessing a 'partial pith' surrounded by a zone of secondary wood and bast; the 'partial pith' consists of scattered primary tracheides and parenchyma,[3] though occasionally, as in *M. Solmsii*, Schenk *γ lignosa*, 'die markständigen Sternringe lassen kein deutliches Centralmark mit Primärtracheiden, auch keinen äusseren Bastbelag erkennen.'[4] It seems extremely probable that the star-rings of this last named species are of the

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[1] *M. anglica,* loc. cit., p. 89.

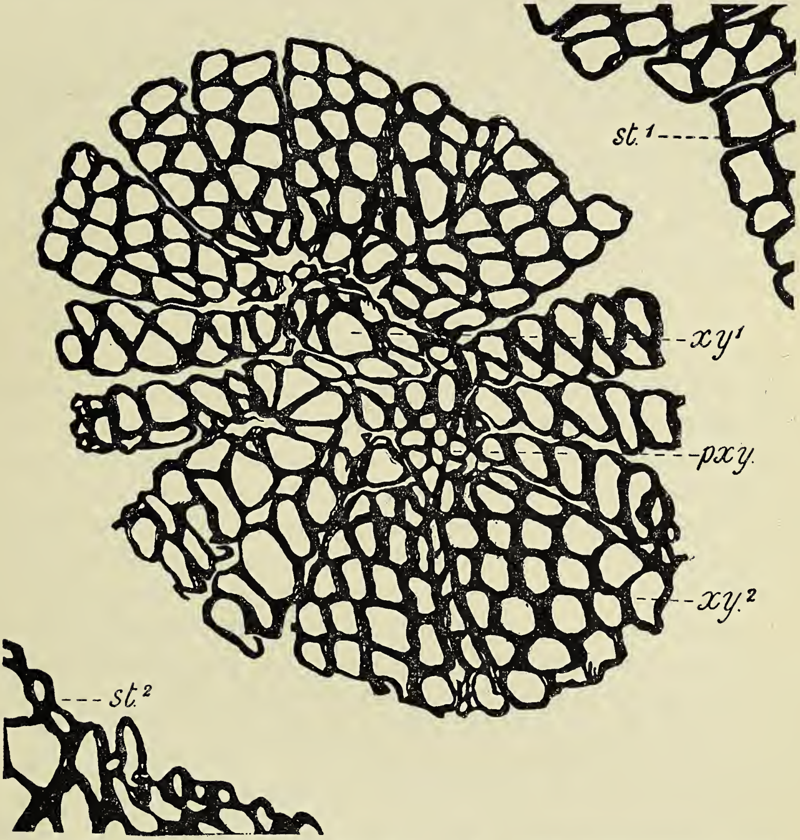
[2] Cf. *M. anglica,* loc. cit., p. 89, and *M. pusilla,* p. 223, both of which show a similar phenomenon.

[3] Weber, O., und Sterzel, J. T.: Beiträge zur Kenntniss der Medulloseae, loc. cit., cf. Text-fig. 23, 'Sternring' of *Medullosa Leuckarti*, Göpp. und Stenz., with Text-fig. 9, 'Sternring' of *M. stellata* v. *Cotta δ lignosa.*

[4] Loc. cit., p. 78 and Text-fig. 13.

type shown in the new specimen, in which the transition from primary to secondary wood is difficult to trace even when the preservation is good.

The 'partial pith' is evidently homologous with the primary wood of the stele, but in some of the Chemnitz fossils the proportion of parenchyma to wood elements was very different from the new specimen,[1] though in some cases poor preservation may account for the seeming difference.[2] So long ago as 1881 it was pointed out by Goeppert and Stenzel[3] that the star-rings differed from the peripheral steles only in size and shape, and this observation can clearly be extended to the new *Medullosa* also.



TEXT-FIG. 3. Transverse section of star-ring. × 53. *xy.*[1] = primary wood; *xy.*[2] = secondary wood; *pxy.* protoxylem; *st.*[1] and *st.*[2] = portions of two outer steles.

Course of the Steles.—The central stele or star-ring shows no change in the portion of stem which was available for investigation, so that neither its origin nor its ultimate fate could be determined.

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[1] Loc. cit., p. 54, Text-fig. 5.

[2] Loc. cit., p. 64, Text-fig. 9.

[3] Goeppert, H. R., und Stenzel, G.: Die Medulloseae. Eine neue Gruppe der fossilen Cycadeen. Kassel, 1881.

In the lower sections of the series other plant tissues have so intruded into the stem that only one of the outer ring of steles is represented in entirety; this is the stele termed α (Pl. I, Fig. 2), and it undergoes practically no change during the whole series; stele β also appears to have been materially unaltered. In sections 8 and 9, cut from the middle portion of the block, the steles γ^1 and γ^2 (Pl. II, Fig. 2) are represented by two fragments; in the next section F, γ^1 has partly fused with γ^2; this fusion takes place very rapidly, so that in section B a single stele γ has resulted (Pl. I, Fig. 1). Such fusion of steles with neighbouring steles appears to be of fairly common occurrence in the Permian Medulloseae, and Scott states that in *Medullosa anglica* 'there is evidence that the steles in their course through the stem underwent both fusion and division, though probably only at long intervals'.[1] According to Worsdell,[2] the very sinuous contour of many Medullosean stem steles was partly due to the perpetual anastomoses between the neighbouring steles.

IV. THE LEAF-TRACE BUNDLES.

The leaf-trace bundles were given off from the peripheral region of the outer ring of steles. Although the actual passage of a leaf-trace strand through the secondary tissues of the stele could not be traced in the sections, yet sufficient evidence was available to show that the leaf-trace xylem was chiefly furnished by the peripheral strands of spirally thickened tracheides so well marked in the primary xylem of the steles. The xylem of the leaf-trace passed out through the zone of secondary wood, and the latter rapidly closed up again after its exit. Although several leaf-trace strands were present, which had obviously only just been emitted from the stele, yet no sign of any secondary tissues could be found in connexion with them (Pl. XV, Fig. 4, $lt.^1$); hence it appears extremely probable that the outgoing leaf-traces were unaccompanied by secondary xylem.[3] This forms an important point of distinction from the leaf-trace in *Medullosa anglica*, in which fossil the outgoing trace is always surrounded by its own zone of secondary wood and bast.[4] No phloem is preserved in the stem, hence it is impossible to determine whether the outgoing strands are concentric in structure, but in any case they immediately begin to divide up into smaller collateral bundles, in which the protoxylem lies adjacent to the space representing the phloem (cf. $lt.^1$ and $lt.^2$ in Pl. I, Fig. 4).

In the zone of ill-preserved tissue lying between the ring of steles and the periderm, a fairly extensive group of short, reticulately marked tracheides,

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[1] *M. anglica*, loc. cit., p. 91.

[2] Worsdell, W. C.: The Structure and Origin of the Cycadaceae. Ann. of Bot., vol. xx, April, 1906, p. 129.

[3] In *M. pusilla* the leaf-trace immediately after its exit showed 'no obvious secondary wood'.

[4] Compare *M. anglica*, loc. cit., Plate II, Fig. 10.

very like transfusion tissue, is present in section A, lying close to an outgoing leaf-trace bundle. This group is of interest when it is recalled that Scott noted in two cases in *M. anglica*[1] that a leaf-trace after diverging from the stele was connected with it for a considerable distance by radial bridles of short tracheides, and he suggested that the peculiarity was correlated with the insertion of adventitious roots.

V. THE PERIDERM.

A continuous zone of well-preserved tissue encloses the vascular tissues of the stem (cf. *pd.*, Pl. I, Figs. 1 and 4). The zone, which is very irregular in outline, is usually about five or six cells in thickness, and the more or less regular seriation of the cells points to its secondary origin, while here and there traces of a cambium can be detected (*c.b.*, Pl. I, Fig. 4); secretory sacs are occasionally present (*m.c.*, Pl. I, Fig. 3). The tissue strongly recalls the secondary cortex of *Sutcliffia insignis*,[2] though the development is far less than in that fossil.

Since none of the external tissues show sign of death or of 'drying up', it is obvious that the zone, though morphologically periderm, cannot function as does the cork of the present day. The conclusion arrived at by Kisch,[3] in an investigation into the physiological anatomy of the periderm in the fossil Lycopodiales, applies equally well here: that, 'whatever the morphological nature of the secondary tissue, there is no evidence to show that any of it was other than secondary cortex.'

VI. THE LEAF-BASES.

The leaf-bases which are present are in all cases still in continuity with the stem. Their ground tissue is composed of thin-walled parenchymatous cells, throughout which are scattered numerous secretory canals (*m.c.*, Pl. I, Fig. 3). A well-marked hypodermal zone lies at the limit of the section, but the external layers are not in any case preserved; the hypoderma is of Renault's *Myeloxylon Landriotii* type (*hy.*, Pl. I, Figs. 1, 2, and 3).

The sclerotic strands of the hypoderma are usually two, occasionally three deep, and are somewhat irregular in form; they are similar to those of *Medullosa anglica*; the hypoderma thus differs from the simpler form in *M. pusilla*, where usually the strands are single, at the most two deep, and at the same time more regular in form. Corresponding with the

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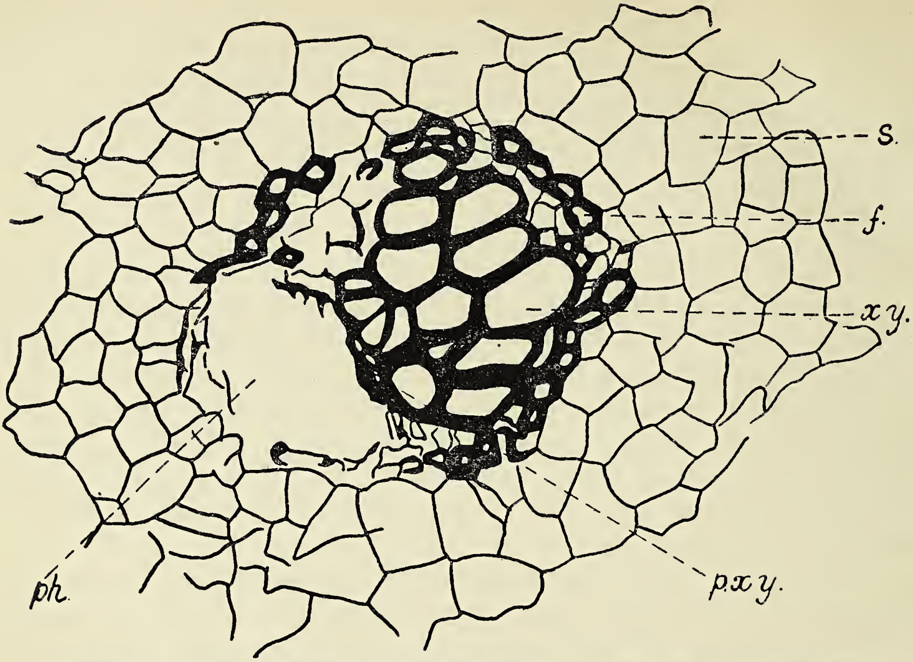
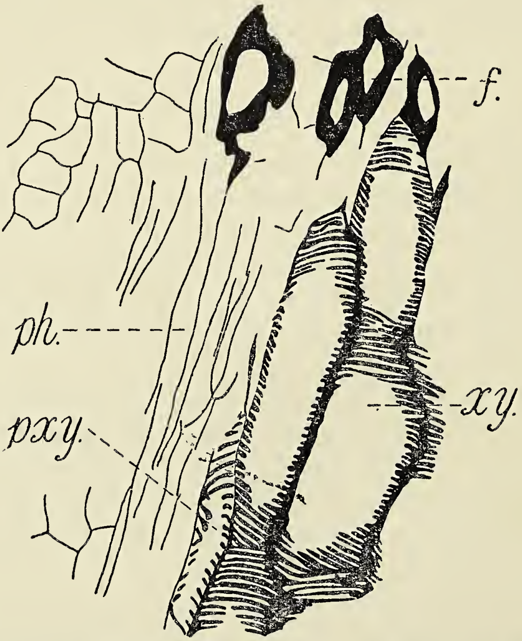
[1] *M. anglica*, loc. cit., p. 93.

[2] de Fraine, E.: On the Structure and Affinities of *Sutcliffia*, in the Light of a newly discovered Specimen. Ann. of Bot., vol. xxvi, No. civ, Oct. 1912, p. 1051 and Text-fig. 17.

[3] Kisch, M. H.: The Physiological Anatomy of the Periderm of Fossil Lycopodiales. Ann. of Bot., vol. xxvii, No. cvi, April 1913, p. 296.



TEXT-FIG 4. Transverse section of a leaf-trace bundle. × 105. *ph.* = phloem space; *pxy.* = protoxylem; *xy.* = xylem; *f.* = fibres; *s.* = sheath.



TEXT-FIG. 5. Oblique longitudinal section of part of a leaf-trace bundle. × 132·5. *pxy.* = protoxylem; *xy.* = xylem; *ph.* = badly preserved phloem elements; *f.* = fibres.

difference in size in the three species there appears to be a difference in the development of the hypodermal layers of the leaf-bases.

Very numerous leaf-trace strands are scattered throughout the ground tissue of the leaf-bases; they appear to be orientated in no definite manner, and branching and anastomosing of the bundles occasionally occurred (Pl. I, Fig. 3).

Each leaf-trace strand is collateral in type; the phloem is almost invariably represented by a space, on which the exarch protoxylem abutted (Text-figs. 4 and 5). The xylem consists of spirally thickened tracheides; it is surrounded by a single layer of thick-walled fibres, as in *M. pusilla*.[1]

Many of the leaf-trace strands are surrounded by a sheath of well-preserved cells, differing somewhat in appearance from the cells of the ground tissue; the cells of this sheath show in places a regular seriation of the elements, and in many cambial divisions are taking place. (Cf. *s.* in Text-fig. 4 with *s.* in Pl. I, Fig. 3.)

Speaking generally, the hypoderma, the vascular strands, and the gum canals are so closely similar in the new specimen to those in *Medullosa anglica*, that except for the difference in size it would be difficult to distinguish between the leaf-bases of the two fossils.

VII. AFFINITIES.

With regard to the affinities of the new specimen there is little to say, for the general organization of the stem, with its adherent leaf-bases entirely clothing the surface; the close similarity of the leaf-base, both in general structure and in histological details, with that of *Medullosa anglica* and *M. pusilla*; and finally, the agreement in practically every detail between the steles of the specimen and those of *M. anglica* and *M. pusilla*, leave no doubt as to its very close relationship with those fossils.

There are, however, certain features which serve to distinguish the specimen from these species. The *size* of the stem is distinctly smaller than that of any described specimen of *M. anglica*, and it appears to be intermediate between that species and *M. pusilla*: the dimensions given by Scott[2] for *M. anglica* are 10·5 × 3·7 cm., the present specimen as nearly as can be estimated measures 5 × 1·5 cm., while *M. pusilla* is 2·2 × 1·3 cm. Little importance would be attached to such a character considered alone, but, taken in conjunction with the following differences, it may possibly be of some diagnostic value.

The arrangement of the leaves could not be definitely determined, but it does not appear to be consistent with the two-fifths phyllotaxis of *Medullosa anglica*;[3] the stem in *M. pusilla* appears to have been covered

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[1] *M. pusilla*, loc. cit., p. 225.

[2] *M. pusilla*, loc. cit., pp. 221–2.

[3] It is possible that this may be a local peculiarity, and that the phyllotaxis may prove to be two-fifths when further specimens are available for examination; such a local variation in the leaf arrangement is not uncommon among living plants.

with three adherent leaf-bases, but four are present in the new specimen. A more important feature is the absence of any secondary tissues accompanying the leaf-trace on its passage from the stele into the cortex, a peculiarity which the specimen appears to share with *M. pusilla.*

The most distinctive character, however, is the organization of the stem steles into an outer ring of three or four steles, enclosing a central strand or star-ring: it is chiefly on account of this feature—a feature which may be considered as a foreshadowing of the structures so characteristic of the Permian members of the genus—that the specimen has been distinguished by a specific name;[1] at the same time there can be no doubt that the three species are very closely related.

In an account of the genus *Medullosa* by Weber and Sterzel,[2] the then known specimens of the genus were arranged in groups or form-cycles. Each form-cycle consisted of a typical species and included those specimens which were either varieties of the type or species closely related to it. Up to 1896 four of these form-cycles were recognized—namely those of *Medullosa stellata*, Cotta; *M. porosa*, Cotta; *M. Solmsii*, Schenk; and *M. Leuckarti*, Göpp. & Stenz.; the species of the genus provided by the English Coal Measures would appear to furnish a fifth, with *M. anglica*, Scott, as the type and the new specimen and *M. pusilla*[3] as closely allied species.

It has already been pointed out by Scott[4] that *M. anglica* approaches most closely to *M. Leuckarti* among the Permian Medulloseae, not only on account of the structure of the individual steles, but also because of the close agreement in leaf characters; moreover, *M. anglica*, *M. pusilla*, and *M. Leuckarti* appear to be the only hitherto described species in which secretory canals occur.[5] Finally, it is of interest to note in this connexion that the number of star-rings may be very few in *M. Leuckarti*; indeed, the specimen figured by Weber and Sterzel[6] with three peripheral sinuous steles enclosing five 'Sternringe' offers a striking comparison with the new specimen.

In conclusion, I am glad to take this opportunity to express my gratitude to Professor F. W. Oliver, in whose laboratory at University College, London, this investigation was carried out, not only for handing over the slides to me for description, but also for the advice he has given me during the course of the work.

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[1] If the protoxylem of the steles should prove to be exarch, this would provide a further feature distinguishing the stem from *M. anglica.*

[2] Loc. cit.

[3] Scott: *M. pusilla*, loc. cit.

[4] Scott: *M. anglica*, loc. cit., p. 114–5.

[5] Bancroft, N.: III. *Rhexoxylon africanum*, a new Medullosean Stem. Trans. Linn. Soc., Lond., Ser. 2, Bot., vol. viii, Pt. 2, 1913.

[6] Loc. cit., Tafel V, Fig. 2.

VIII. DIAGNOSIS.

Medullosa centrofilis, sp. nov.

Stem *small*, 5 × 1·5 cm., *including the leaf-bases,* completely clothed by spirally arranged, decurrent leaf-bases, *four in number.*

Vascular system of the stem composed of a ring of three or four steles, irregular in transverse section, *enclosing a central star-ring.* Interior of each stele occupied by primary wood.[1]

Secondary wood developed round each of the steles. Tracheides with bordered pits on the radial walls.

Leaf-traces leave the stele without any secondary wood; branching almost immediately into collateral strands.

Leaf-bases with the structure of *Myeloxylon Landriotii*, Ren.

Secretory canals abundant in the leaf-bases and around the steles of the stem.

Locality. Shore Littleborough Colliery, Lancashire.

Horizon. Lower Coal Measures.

The characters in which the species differs from both *M. anglica* and *M. pusilla* are italicized. It also differs from *M. anglica* in the absence of secondary tissues in the leaf-trace, and from *M. pusilla* in the structure of the hypoderma.

IX. SUMMARY.

1. *Structure* (Sections ii–vi, pp. 252–261). The specimen consisted of a stem entirely clothed by portions of four spirally arranged, adherent leaf-bases.

(a) *Stem.*

The vascular system consists of an outer series of three or four uniform steles, surrounding a single, central star-ring. The structure of the steles and of the star-ring is essentially similar to that of a single stele of *Medullosa anglica.* The steles are surrounded by a well-preserved zone of tissue, morphologically periderm, but functionally secondary cortex.

Leaf-trace strands are given off from the peripheral region of the outer stelar ring. They are not accompanied in their exit by secondary tissue, and immediately begin to divide up into collateral bundles.

(b) *Leaf-base.*

The ground tissue consists of thin-walled parenchyma, in which numerous mucilage sacs occur.

Many leaf-trace strands occur in the ground tissue; they are collateral, with exarch protoxylem abutting on a space representing the phloem. A single layer of fibres surrounds the xylem, and most of the strands are enclosed in a sheath, in the cells of which cambial divisions occur.

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[1] Protoxylem probably exarch.

The hypodermal zone is of Renault's *Myeloxylon Landriotii* type.

2. *Affinities* (Section vii, pp. 261–262). There is close agreement with *Medullosa anglica* and *M. pusilla*, not only in general organization but in the details of the leaf-base structure and of the vascular system of the stem. The small size, the differing phyllotaxy, the absence of secondary tissues from the leaf-trace when emerging from the stele, and, more particularly, the presence of the central star-ring, are the main reasons for assigning specific rank to the specimen.

The fossil is regarded as belonging to the same 'form-cycle' as *Medullosa anglica* and *M. pusilla*. The central star-ring foreshadows the type of structure characteristic of the Permian members of the genus, and more particularly such a type as *M. Leuckarti*.

LITERATURE.

BANCROFT, N.: III. *Rhexoxylon africanum*, a new Medullosean Stem. Trans. Linn. Soc. Lond., Ser. 2, Bot., vol. viii, Pt. II, 1913.

DE FRAINE, E.: On the Structure and Affinities of *Sutcliffia*, in the Light of a newly discovered Specimen. Ann. of Bot., vol. xxvi, No. civ, Oct. 1912.

GÖPPERT, H. R., and STENZEL, G.: Die Medulloseae. Eine neue Gruppe der fossilen Cycadeen. Kassel, 1881.

KISCH, M. H.: The Physiological Anatomy of the Periderm of Fossil Lycopodiales. Ann. Bot., vol. xxvii, No. cvi, April, 1913.

SCOTT, D. H.: Studies in Fossil Botany, 2nd ed., Pt. II, 1909.

——————: On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks. III. On *Medullosa anglica*, a new Representative of the Cycadofilices. Phil. Trans. Roy. Soc., Ser. B, vol. cxci, 1899.

——————: On *Medullosa pusilla*. Proc. Roy. Soc., B., vol. lxxxvii, 1914.

SEWARD, A. C.: On the genus *Myeloxylon* (Brong.). Ann. of Bot., vol. vii, p. 1, March, 1893.

WEBER, O., and STERZEL, J. T.: Beiträge zur Kenntniss der Medulloseae. Ber. der naturw. Ges. zu Chemnitz, vol. xvii, 1896.

WORSDELL, W. C.: The Structure and Origin of the Cycadaceae. Ann. of Bot., vol. xx, April, 1906.

EXPLANATION OF PLATE XV.

Illustrating Dr. E. de Fraine's paper on *Medullosa centrofilis*.

The photographs were taken by Mr. F. Pittock, of the Zoological Department, University College, London.

α, β, γ, γ^1 and γ^2 = steles: δ = star-ring; *lb.*^{1}, *lb.*^{2}, *lb.*^{3}, and *lb.*^{4} = leaf-bases; *sc.*^{1}, *sc.*^{2} = sclerenchyma fibres; *sc.b.* = fibrous zone; *hy.* = hypoderma; *pd.* = periderm; *lt.* = leaf-trace; *st.* = stele; *m.c.* = secretory canal; *s.* = sheath; *c.b.* = cambium.

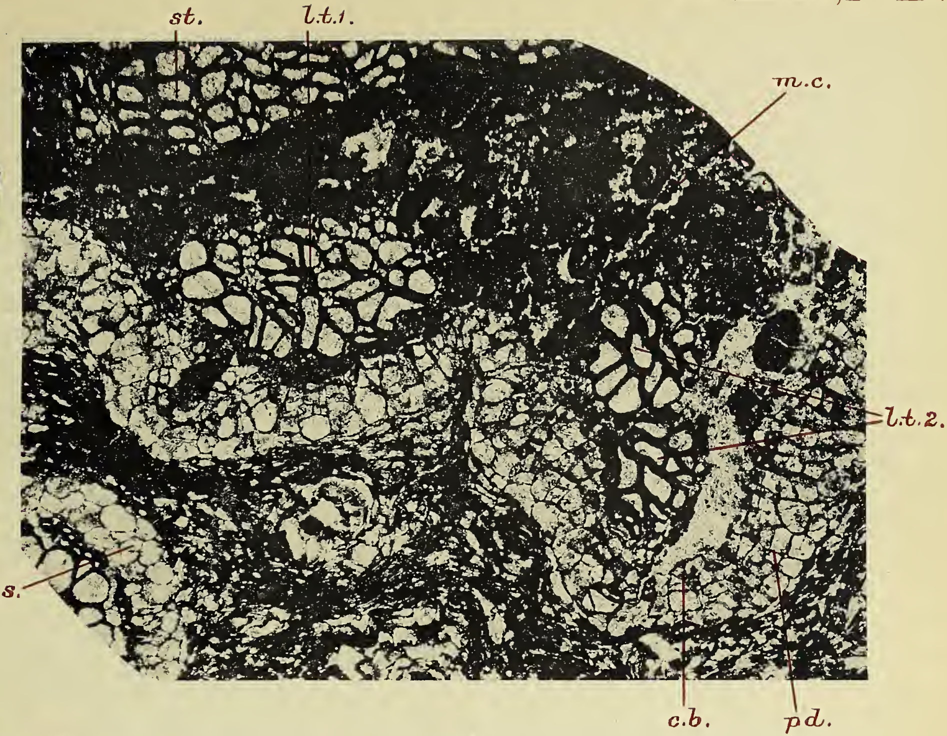
Fig. 1. Transverse section of stem (Section A), showing three of the leaf-bases: part of leaf-base 1 (on the right) omitted. × 2·5.

Fig. 2. Transverse section of the stem (Section 9). × 2·5.

Fig. 3. Part of Section B, showing part of leaf-base *lb.*^{2} cut off by the sclerenchyma zone *sc.*^{2}. × 17·5.

Fig. 4. Leaf-trace 1 which has just emerged from the stele, *st.*; a second leaf-trace, *lt.*^{2}, has divided. From section 8. × 17·5

DE FRAINE — MEDULLOSA CENTROFILIS.



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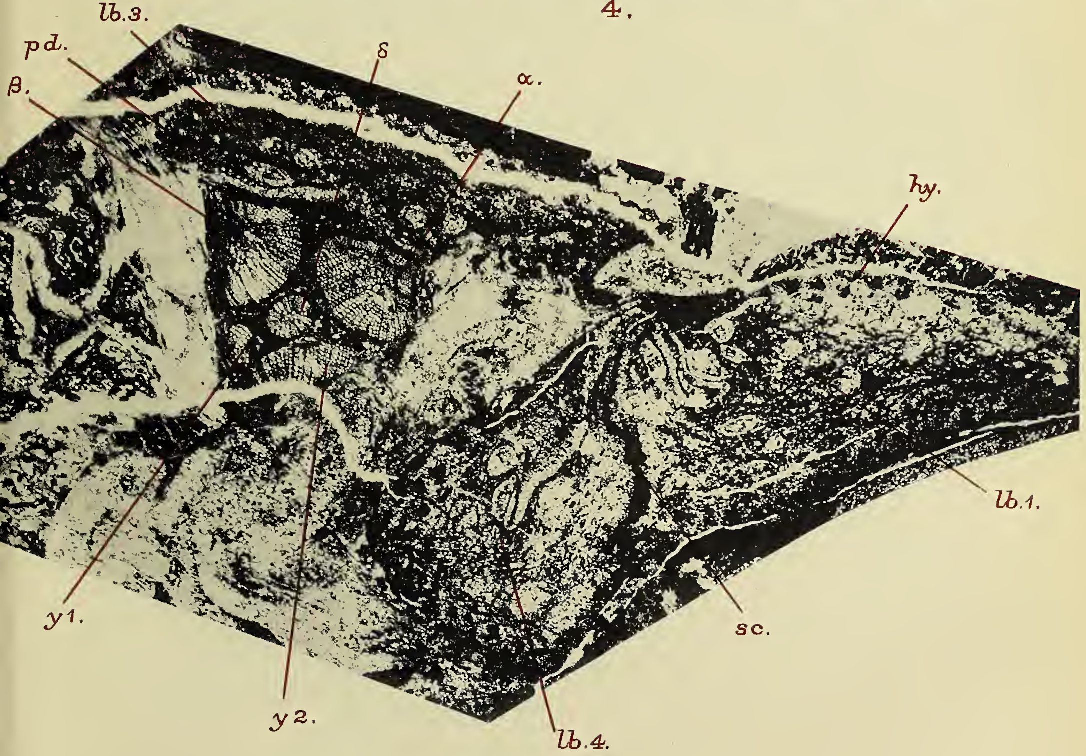

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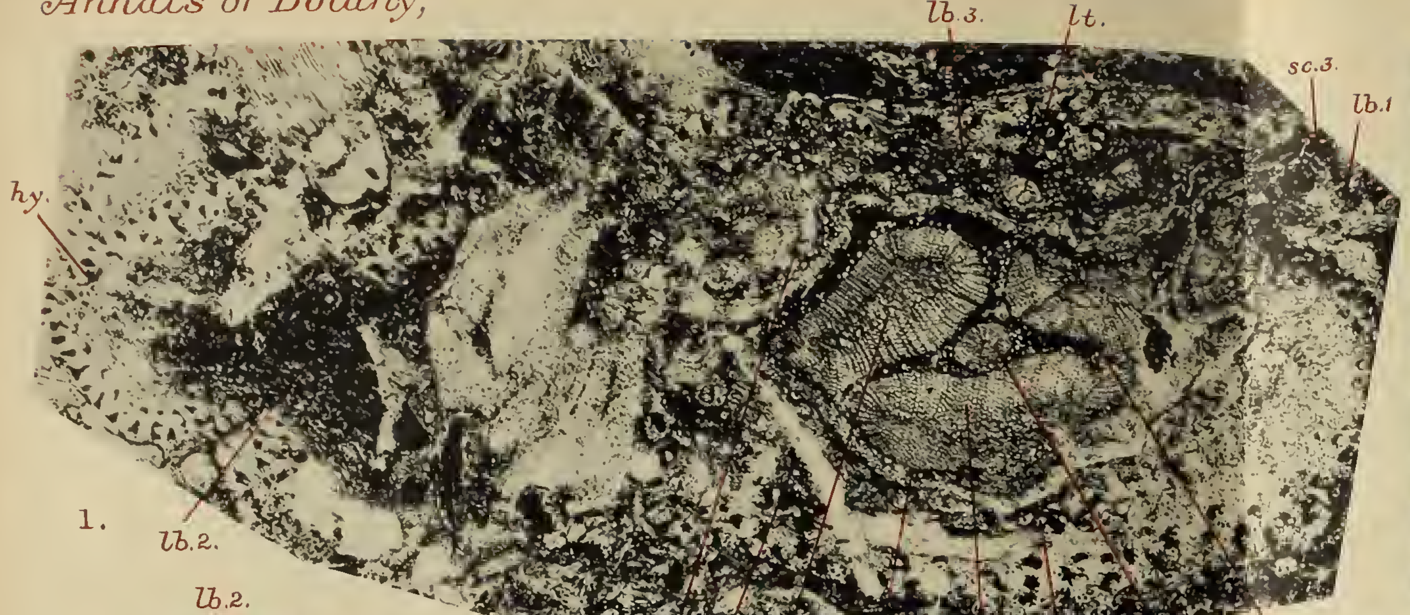

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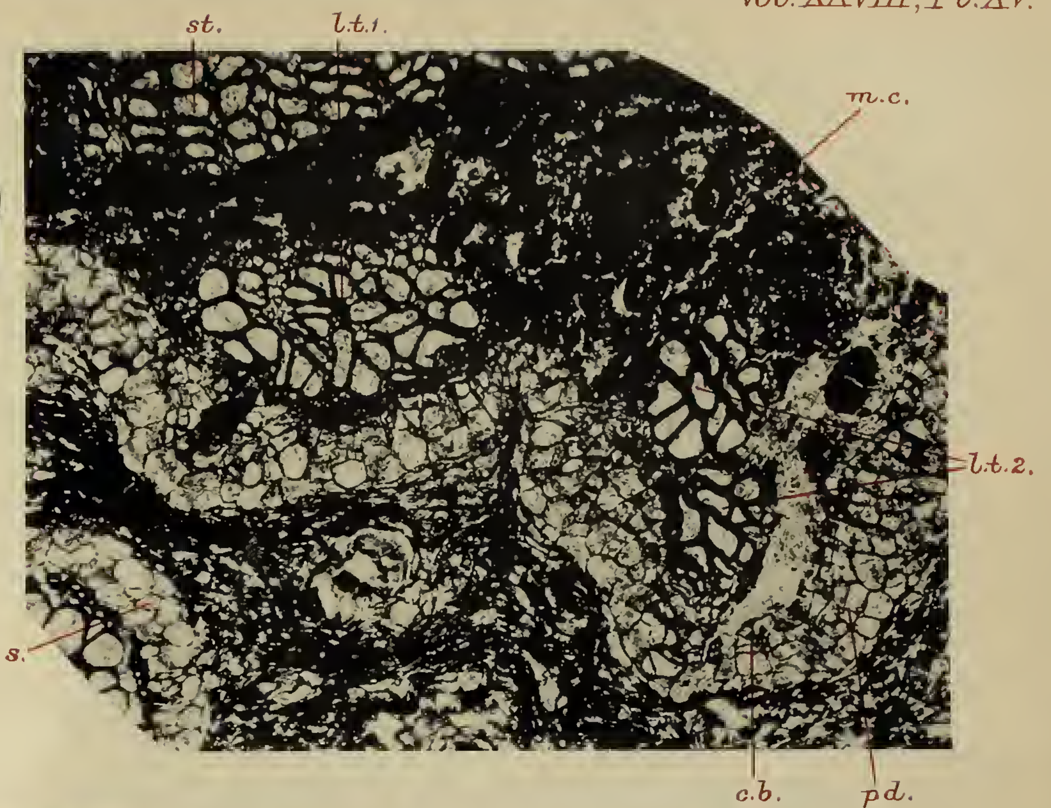

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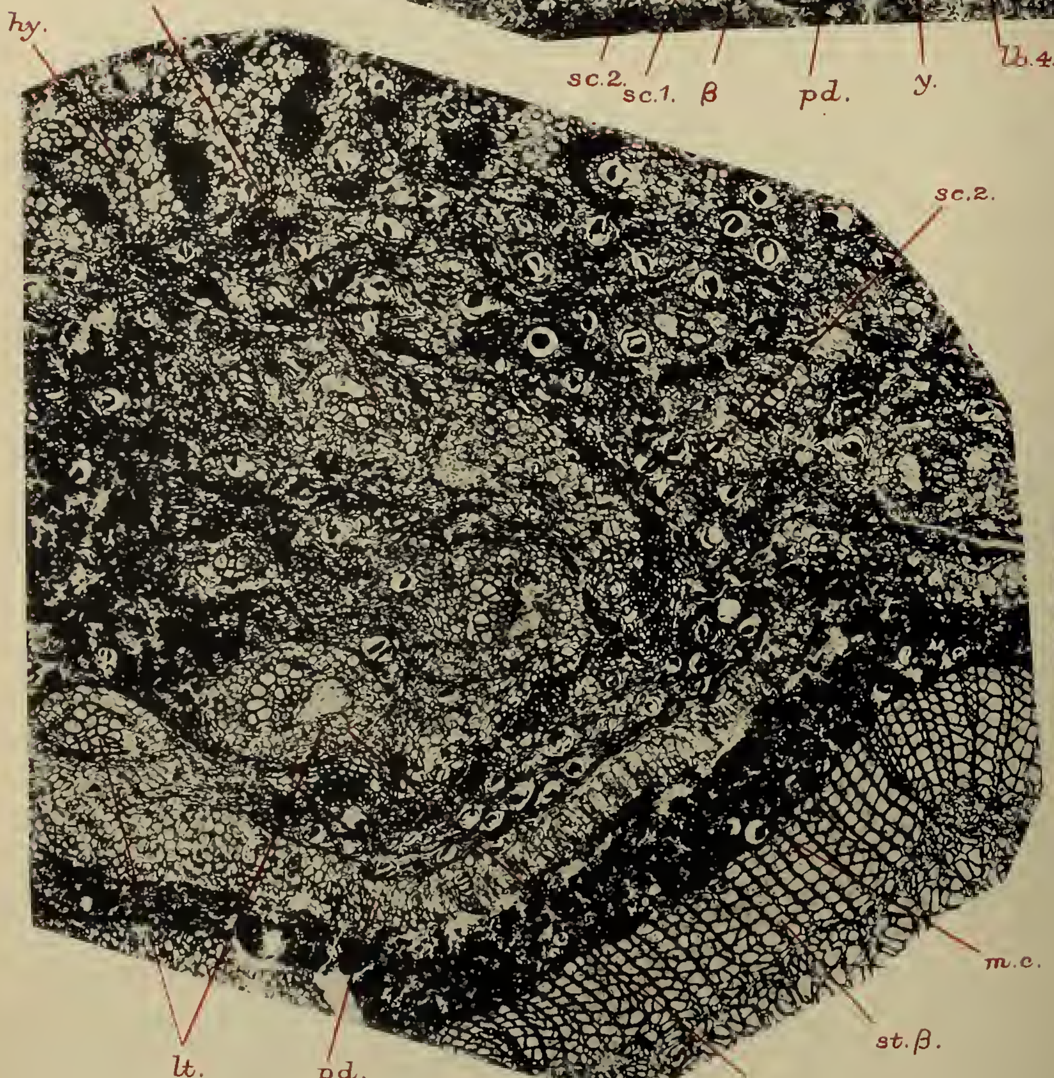

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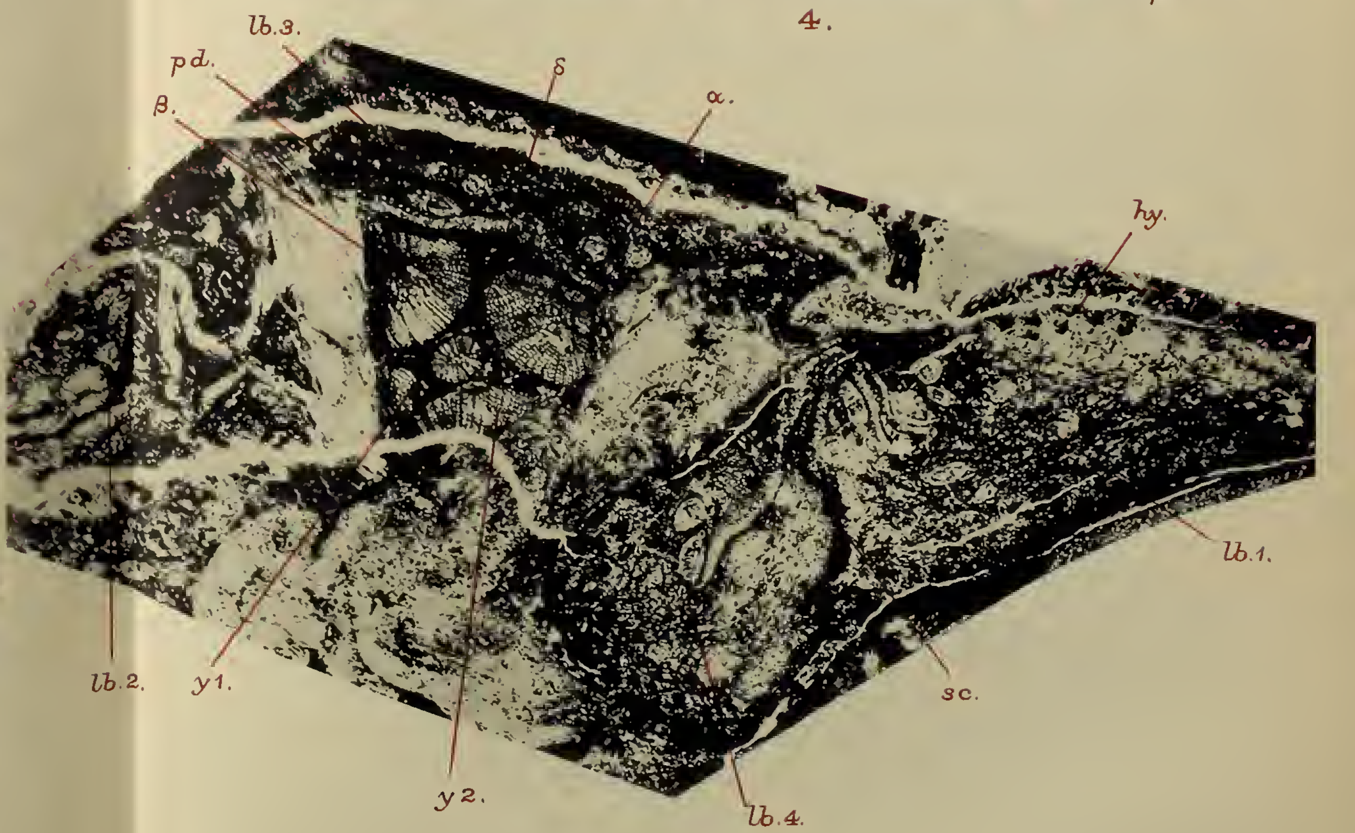

2.

DE FRAINE — MEDULLOSA CENTROFILIS.

Huth, coll.

The Genesis of the Male Nuclei in Lilium.

BY

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With Plates XVI and XVII.

A PAPER was published in 1913 by Professor V. H. Blackman and the author, giving an account of fertilization in *Lilium auratum* and *Lilium Martagon*.[1] As a good deal of material remained over from this investigation, it seemed worth while to add to it and to undertake a study of the development of the male nuclei.

It is a pleasure to take this opportunity of most sincerely thanking Professor Blackman for his generosity in handing over the material to the author.

The earliest stage examined was that in which the young pollen-grain of *Lilium auratum* contains a single nucleus, lying near the centre of the cell; its protoplasm is finely vacuolate throughout. Preparatory to the first post-meiotic division, the nucleus moves to one side of the pollen-grain and enters upon a spireme phase. The various stages of division follow one another rapidly (Pl. XVI, Figs. 1–3), and result in the formation of two similar nuclei which lie close to each other against the wall of the grain (Fig. 4). The innermost or tube nucleus now moves towards the centre of the cell, after which its membrane becomes more clearly defined and its outline is often seen to have become irregular, so much so that it is sometimes lobed, a condition, however, more commonly found after the nucleus has passed some little way down the pollen tube. Meanwhile the membrane of the generative nucleus has become clearly visible, and the cytoplasm immediately surrounding it is seen to be so dense as to form a differentiated area. The cytoplasm of the pollen-grain, at this time, is very vacuolate, the walls of the meshes appearing to be formed of densely granular protoplasm (Fig. 6). The preparations suggest that the dense appearance of the generative protoplasm is due to the granular matter which is concentrated in this part of the grain, whilst the remainder becomes more vacuolate. At first it is

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[1] Blackman and Welsford: Fertilization in *Lilium*. Ann. of Bot., vol. xxvii, 1913, p. 3.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

only these vacuoles which mark the limit of the generative cell (Fig. 6), but later on a membrane is differentiated (Fig. 7). The generative cell takes the shape of that part of the pollen-grain wall against which it lies, merely bulging out to cover the nucleus. This is more clearly seen in a rather later stage when the generative cell has moved away from the wall. Fig. 8 is a median longitudinal section of the ripe pollen-grain showing the peculiar shape of the generative cell, whilst Fig. 9 is a transverse section showing the nucleus lying in the centre of what appears to be a circular cell.

A deeply staining granule may always be found at either pole of the generative nucleus; they persist till a late stage, probably only disappearing when the cytoplasm of the sperm-cells disintegrates in the embryo sac. They may represent centrosomes, but no traces of spindle fibres were found near them. Granules are also sometimes found lying in a row, midway between the wall of the generative cell and its nucleus. They are extruded from the nucleus (Figs. 6, 7) and were seen to persist in the cytoplasm of the young sperm-cells (Pl. XVII, Figs. 24, 27), where they sometimes form a band-like structure. This is, however, better seen after they have passed from the pollen tube into the embryo sac, and is figured in the previous paper.[1] Their formation, later appearance, and the fact that they are often, but not always, present, suggest that they may be the vestiges of a blepharoplast.

In the ripe pollen-grain, before the anther has dehisced, the chromatin of the generative cell always stains more deeply than that of the tube nucleus; it is, indeed, already preparing to divide. Nuclear division, however, was never found to occur before germination in *Lilium auratum*, the nucleus being invariably in the spireme stage when passing into the tube. Material fixed two hours after pollination showed various stages; in some cases the pollen tube was only just growing out, whilst in others the young tube contained a generative nucleus in the anaphase condition. Fig. 10 shows a young pollen-grain which has germinated and in which the two nuclei have not passed into the tube. The cytoplasm has a very streaky appearance, indicating that the nuclei are carried out passively by the streaming protoplasm.

Unfortunately the pollen tubes of *Lilium auratum* at this stage are lying on the stigma and are so twisted that it was found almost impossible to study the stages of nuclear division with accuracy or to make satisfactory drawings of them. The generative cell of *Lilium Martagon* divides after the pollen tube has grown into the stylar canal, where it keeps a comparatively straight course; consequently a description of the division of the generative cell has been given of this plant instead of *Lilium auratum*. Sufficient preparations, however, were obtained of *Lilium auratum* to show that the stages of division are very similar in the two species.

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[1] Loc. cit., Blackman and Welsford, Plate XII, Fig. 6.

Soon after the generative cell of *Lilium Martagon* has passed into the tube its nucleus enlarges, often almost hiding the cytoplasm; in Pl. XVI, Fig. 11 it is seen to be much spread out and to be lying over one of the polar granules. The outline of the generative cell is still clearly visible at the stage shown in Fig. 12 before the split thread has divided to form the chromosomes. From this time the generative cell increases greatly in size and becomes less clearly marked, though it is differentiated from the more vacuolate protoplasm surrounding it. After the cytoplasm has spread out and before the chromosomes pass into the metaphase the chromatin material is often found to lie in a curved mass suggesting a wriggling movement (Figs. 13, 14); later, however, owing to the narrow space of the tube, it spreads out and the twelve longitudinally split chromosomes are formed (Figs. 15, 16, 17). No spindle fibres were seen at any stage of the division, the twenty-four long thin daughter-chromosomes being massed together as is shown in Pl. XVII, Fig. 19. The chromosomes now separate, and as the two groups move apart each is seen to be surrounded by a separate mass of cytoplasm (Fig. 20). The nuclei of the two male cells pass into the spireme condition and soon assume the curved or humped sausage shape with pointed ends so characteristic of the mature sperm. Nuclei at this stage were found at about 2 mm. from the apex of the stigma and presumably had at least a further 10 mm. to travel before reaching the micropyle; this they usually do whilst still surrounded by their cells (Figs. 26, 27), but sometimes the cells are left behind (Figs. 24, 25), or are attached to one end of the nucleus (Figs. 21, 24, 29). Fig. 27 shows two male cells of *Lilium auratum* about 3 mm. from the apex of the stigma.

Figs. 28, 30, 31 are drawn from pollen tubes dissected from the base of the style of *Lilium auratum* six days after pollination and about three days before fertilization will take place. The male cells are clearly seen, their nuclei being either curved or straight; the lobed tube nucleus lies a little way in front of the male cells. At this stage the tip of the pollen tube is generally somewhat enlarged and often thickened (Figs. 27, 28). The function of this enlargement was not ascertained; perhaps it may be of use in pushing apart the cells of the micropyle. In Fig. 32 two male cells are seen in the micropyle of *Lilium auratum*, but in Fig. 33 the nuclei have escaped from their cells and are lying free in the cytoplasm of the pollen tube. The pollen tube actually enters the embryo sac and preserves its shape, at any rate till after fertilization is accomplished, becoming filled with densely staining disintegrating matter after the escape of the two sperm nuclei. This was clearly shown in one preparation where a slight pressure on the cover-slip caused the tube to move slightly and brought its wall into prominence against the vacuolate cytoplasm of the embryo sac. The two male nuclei were invariably found to escape from their cells before leaving the pollen tube to pass across the embryo sac.

The male nuclei thus, either with or without their cells, are seen to travel from 10 to 16 mm. in five days in *Lilium Martagon*, or from 12 to 20 mm. in about seven days in *Lilium auratum*, before they effect fertilization. It is also a noticeable fact that, after the male cells are formed, the protoplasm of the tube no longer has an appearance of active streaming, but instead sometimes looks as if it had been disturbed by a moving body (Figs. 24, 25). The nuclei during their passage down the tube alter slightly; they never pass into the typical reticulate resting stage, but form a spireme with very few and very delicate cross-connexions (Figs. 24, 25); these connexions become more pronounced in the mature sperm nuclei, and especially so in those nuclei in the later stages of fusion, such as are shown in Figs. 1 and 6 in the previous paper. It seems, therefore, that the sperm nuclei in *Lilium auratum* and *Lilium Martagon* have undoubted motility for a considerable period, and it is suggested that this may be correlated with the fact that there is a minimum of cross-connexions between the chromatin threads during a great part of their existence. The cytoplasm of the male cells soon disintegrates after the loss of the nuclei, and doubtless gives rise to the structures termed 'X-Körper' by Nawaschin.[1]

The material was fixed in Flemming's fluid of various strengths and in Perenyi's fluid, and was stained with Benda's iron-haematoxylin or with Breinl's stain.

SUMMARY.

1. The vermiform nuclei of *Lilium auratum* and *Lilium Martagon* pass down the pollen tube in male cells and are usually only liberated from their cytoplasm after the pollen tube has entered the embryo sac.

2. The male nuclei are regarded as possessing motility from an early stage of their development.

3. The 'X-Körper' of Nawaschin are shown to be the disintegrating cytoplasm of the male cell

4. The history of the bands of granules sometimes found near the disintegrating cytoplasm suggests the possibility that they may be vestiges of blepharoplasts.

EXPLANATION OF PLATES XVI AND XVII.

Illustrating Miss Welsford's paper on the male nuclei of *Lilium*.

PLATE XVI.

Fig. 1. *Lilium auratum*. Single nucleus of pollen-grain preparing for division. × 1,200.

Fig. 2. *Lilium auratum*. Later stage of same. × 1,200.

Fig. 3. *Lilium auratum*. Ditto, showing the twenty-four daughter chromosomes separating from each other. × 1,200.

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[1] Nawaschin, S.: Näheres über die Bildung der Spermakerne bei *Lilium Martagon*. Annales du Jard. Botan. de Buitenzorg, 2^e sér., Suppl. iii, 1910.

Fig. 4. *Lilium auratum.* The tube and generative nuclei lying close together and showing no differentiation. × 800.

Fig. 5. *Lilium auratum.* The tube nucleus has moved towards the centre of the cell; its membrane is not yet clearly defined. The formation of the generative cell has begun. × 800.

Fig. 6. *Lilium auratum.* Young generative cell, showing a row of granules. The cell membrane is not yet complete and the cell is delimited by vacuoles. × 1,200.

Fig. 7. *Lilium auratum.* Young generative cell, showing the differentiation of cytoplasm and the extrusion of granules from the nucleus. × 1,200.

Fig. 8. *Lilium auratum.* Longitudinal section of a pollen-grain, showing the faintly stained tube nucleus and the more deeply stained generative nucleus lying in the generative cell. × 800.

Fig. 9. *Lilium auratum.* Transverse section of generative cell, showing one of the polar granules. Delicate cross-connexions can be seen between the threads of the spireme. × 1,200.

Fig. 10. *Lilium auratum.* A generative pollen-grain, showing the tube nucleus and generative cell being carried in a stream of protoplasm towards the tube. × 500.

Fig. 11. *Lilium Martagon.* Generative cell in pollen tube. The nucleus has already begun to enlarge preparatory to division. The two polar granules are shown. × 800.

Fig. 12. *Lilium Martagon.* Later stage, showing the longitudinally split thread. The generative cell is clearly shown. × 800.

Fig. 13. *Lilium Martagon.* Later stage; the spireme still shows the split in places. The cytoplasm of the generative cell stains very faintly. The curved shape of the spireme is indicated. × 1,200.

Fig. 14. *Lilium Martagon.* About the same stage as Fig. 13. × 1,200.

Fig. 15. *Lilium Martagon.* Shows the twelve split chromosomes lying in the generative cell. × 1,200.

Fig. 16. *Lilium Martagon.* Shows the twelve split chromosomes lying in the generative cell. × 1,200.

Fig. 17. *Lilium Martagon.* Ditto. × 800.

Fig. 18. *Lilium Martagon.* Chromosome 7 from Fig. 17. × 1,800.

PLATE XVII.

Fig. 19. *Lilium Martagon.* Metaphase in generative cell; one of the polar granules is shown. × 800.

Fig. 20. *Lilium Martagon.* Anaphase. The two groups of chromosomes are lying in the male cells. × 800.

Fig. 21. *Lilium Martagon.* A later stage. × 800.

Fig. 22. *Lilium Martagon.* The chromosomes have joined up. The generative cytoplasm is very thin and spread out. × 1,200.

Fig. 23. *Lilium Martagon.* A rather later stage; the spireme thread is thickening and the whole nucleus is beginning to show a curvature. × 1,200.

Fig. 24. *Lilium Martagon.* Two male nuclei at a slightly later stage than that shown in Fig. 23. One of the nuclei has moved away from its cytoplasm. Both masses of cytoplasm show a more or less band-like group of granules. These nuclei are about 8 mm. from the top of the ovary. × 800.

Fig. 25. *Lilium Martagon.* A rather older male nucleus, showing its curved, almost spiral shape. The protoplasm has a disturbed appearance. This nucleus is also about 8 mm. from the top of the ovary. × 1,800.

Fig. 26. *Lilium auratum.* Part of a pollen tube dissected from the base of the style. The two male cells are clearly seen, each with its curved nucleus and numerous granules. The tube nucleus is also shown. × 500.

Fig. 27. *Lilium auratum.* Two male cells in a bent-over pollen tube about two mm. from the apex of the stigma. × 1,200.

Fig. 28. *Lilium Martagon.* Two male cells in the tube; one of the nuclei has almost parted from its cytoplasm. × 800.

Fig. 29. *Lilium Martagon.* A male nucleus and its faintly stained cytoplasm lying in the pollen tube. × 800.

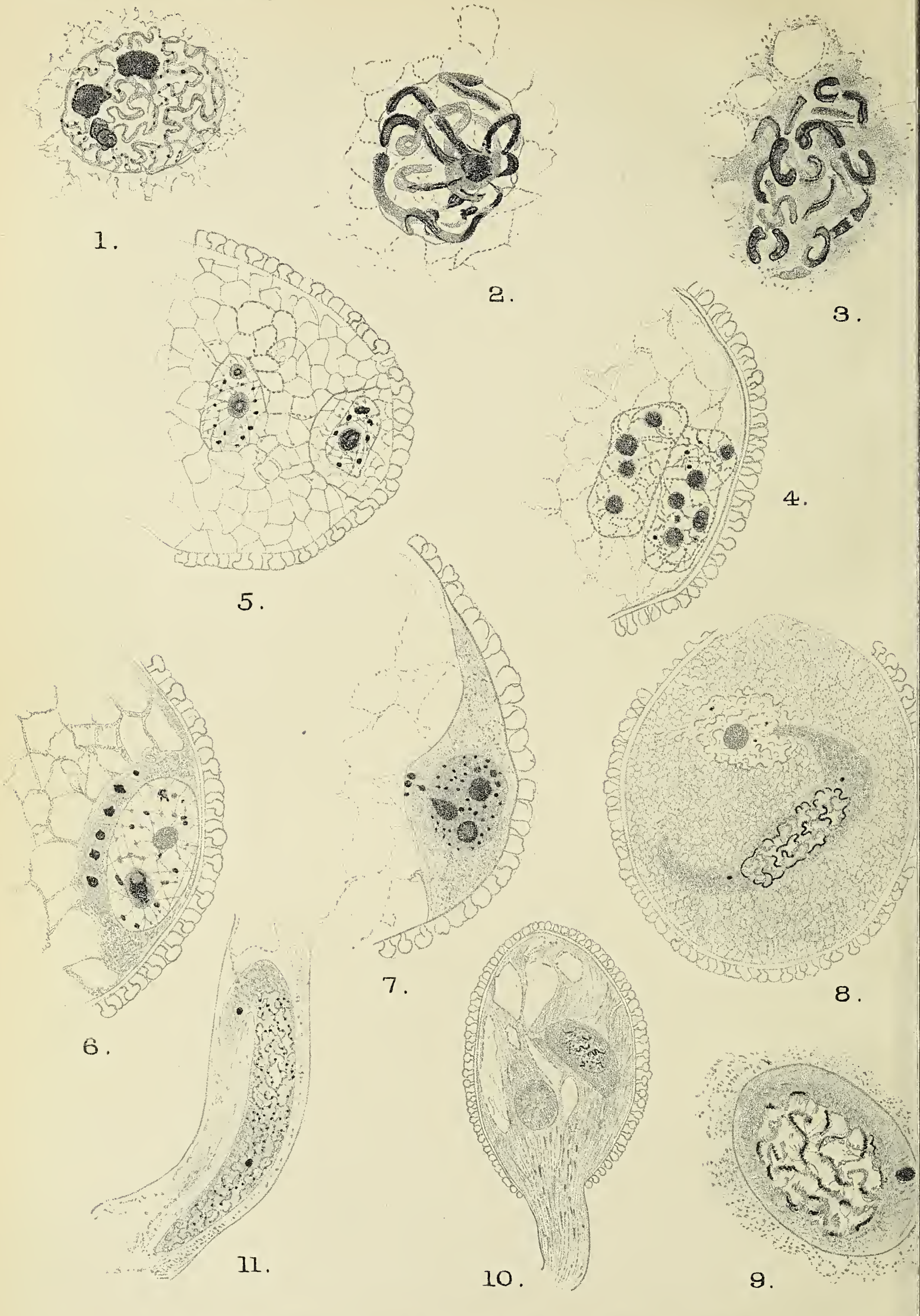
Fig. 30. *Lilium auratum.* A pollen tube from the base of the style, showing the thickened tip, the lobed tube nucleus, and one of the male cells. × 500.

Fig. 31. *Lilium auratum.* A pollen tube from the base of the style, showing the enlarged and thickened tip, the lobed tube nucleus, and the two male cells. × 500.

Fig. 32. *Lilium auratum.* Semi-diagrammatic drawing of the two male cells and the tube nucleus in the micropyle. × 300.

Fig. 33. *Lilium auratum.* Upper part of embryo sac, showing the pollen tube with its two male nuclei and the disintegrating cytoplasm of their cells. The egg cell and one of the synergids is also shown, the other has been destroyed by the entrance of the pollen tube. × 500.

Annals of Botany,



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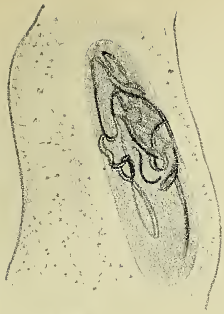

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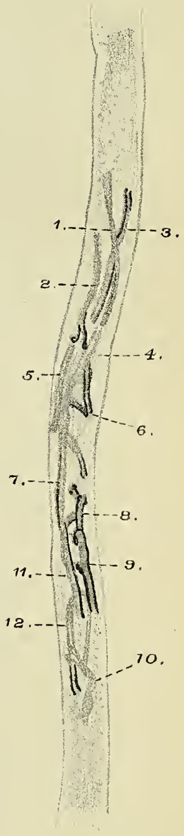

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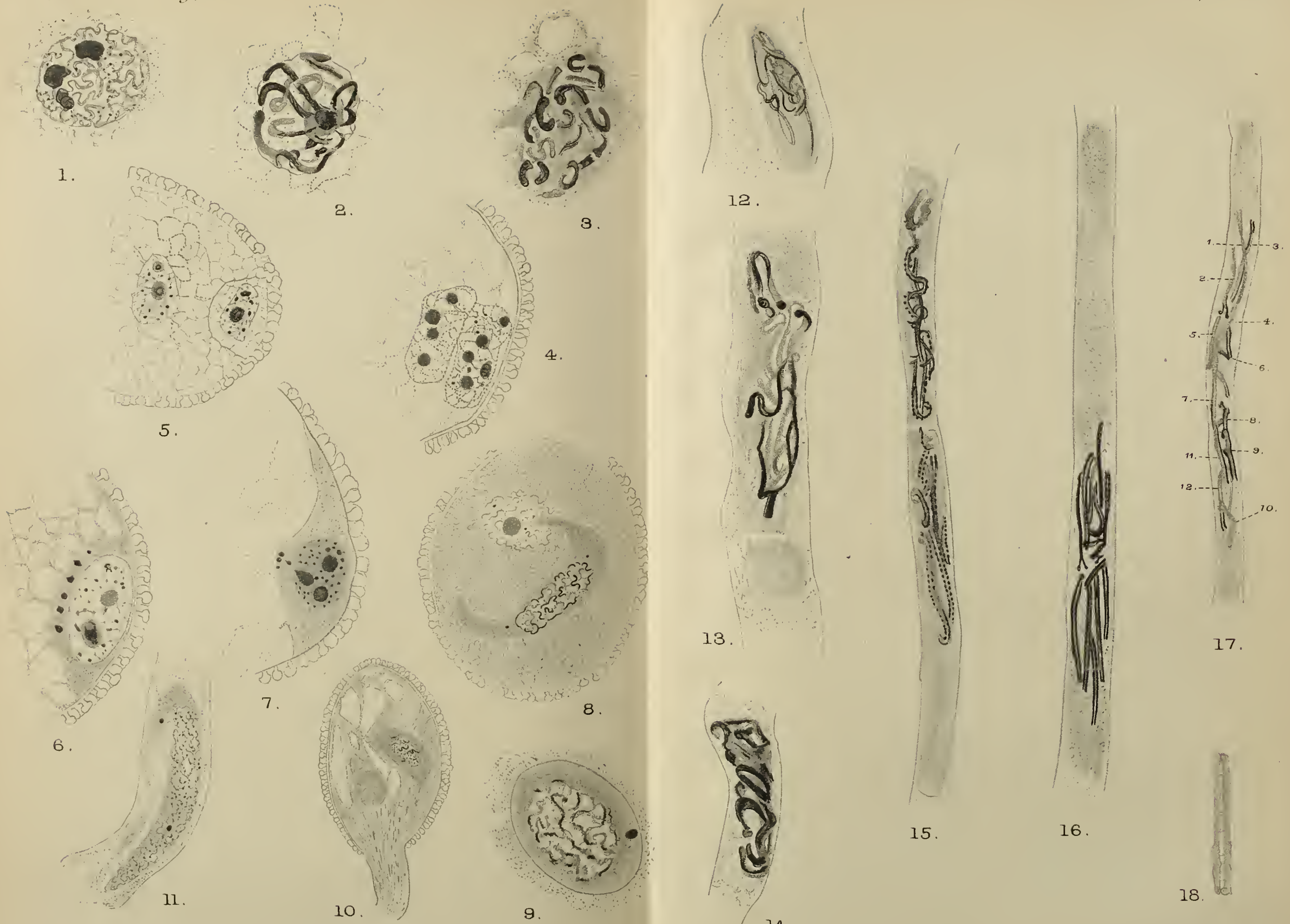

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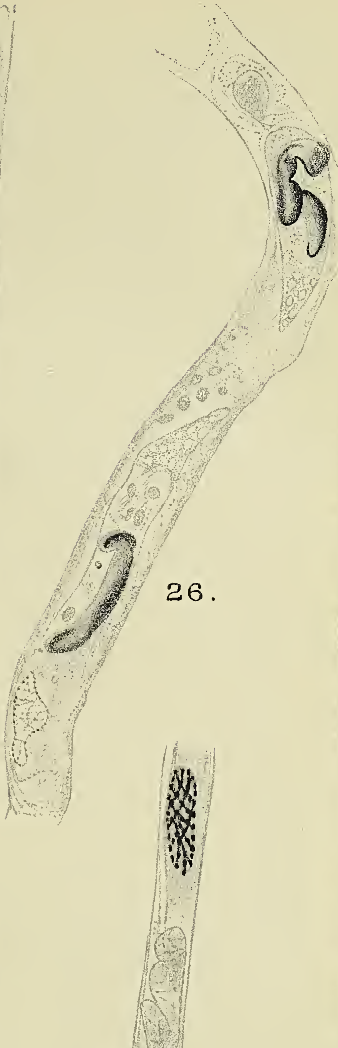

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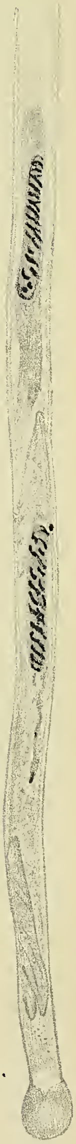

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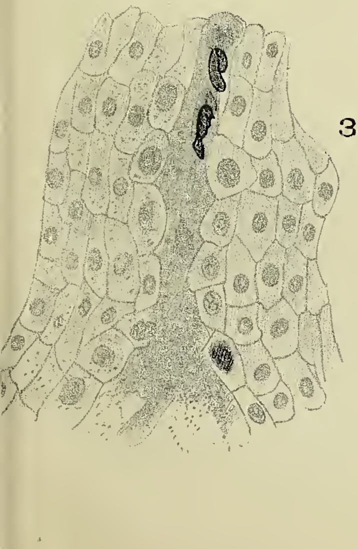

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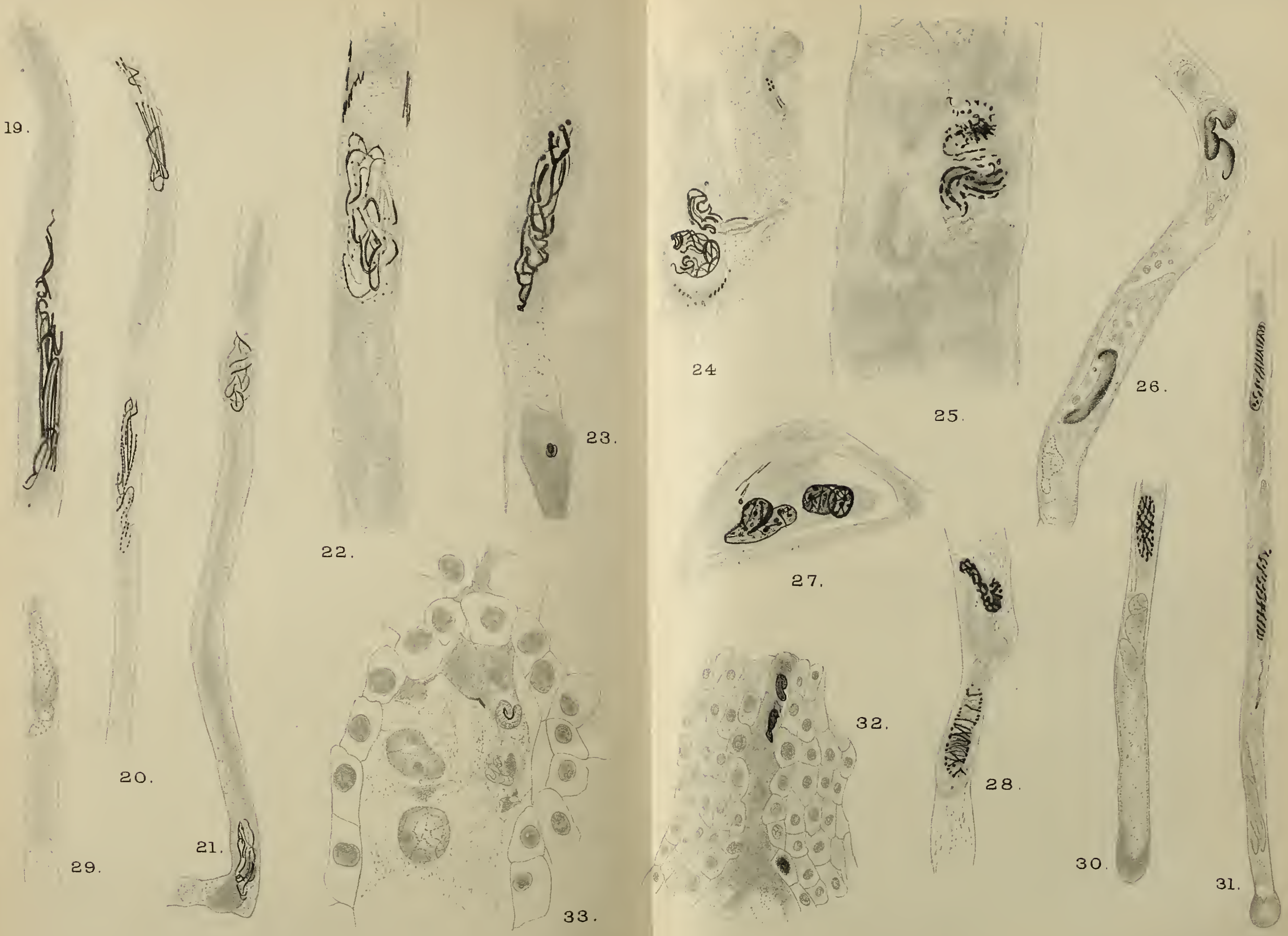

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WELSFORD—MALE CELLS IN LILIUM.

The Nature of the Double Spireme in Allium Cepa.

BY

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With Plates XVIII and XIX.

ALLIUM CEPA has repeatedly been the subject of cytological investigation, but there are several phases in its nuclear history which cannot be regarded as being clearly understood.

In the present investigation attention has been more particularly focused on the following points: the development and significance of the longitudinal fission in the spireme ribbon, the method of origin and grouping of the chromosomes in the prophases, and the origin and subsequent fate of the nucleoli.

In view of the recent investigations of Dr. Lawson (10, 11, 12) on the significance of nuclear osmosis as a factor in mitosis, the behaviour of the nuclear vacuole and its contents during the prophases has also been carefully studied.

METHODS.

Root apices have been almost exclusively used in the investigation. These were removed from plants which were grown in culture solutions or in soil. The bulbs used for the production of the roots were Sutton's 'Reading' and 'Ailsa Craig'.

The fixatives employed were Flemming's strong and weak solutions, either full strength or diluted with an equal volume of water, Hermann's fluid, and Farmer's acetic alcohol. Various strengths of chrom-acetic acid solutions were used, but were not found to be satisfactory.

In staining, Breinl's combination was extensively used and gave the best results, but Flemming's triple stain and Heidenhain's haematoxylin were also employed.

The sections were cut 2 μ–14 μ in thickness.

PROPHASES.

During the nuclear 'resting' stages the bulk of the chromatin is stored in the nucleoli; the remainder of the nucleus contains a fine faintly staining reticulum. Where these threads of the reticulum cross one another a slightly

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more granular structure may be seen, but this does not stain with chromatin dyes. This reticulum appears to differ very little from the general cytoplasm of the cell (Pl. XVIII, Fig. 1). It is impossible to say (at this stage) whether the threadwork is a continuous structure or not, but subsequent events suggest that it is of the nature of a thin fenestrated sphere.

One or more nucleoli may be present, and these are formed by subdivision in the hour-glass fashion described by Digby (1) for *Galtonia.*

The first indications of approaching mitosis are seen in the nuclear reticulum. The chromatin begins to aggregate in small band-like masses on this framework, as shown in Pl. XVIII, Fig. 2. It slowly spreads itself and builds up the spireme ribbon. It may have the granular structure of Pl. XVIII, Figs. 3 and 4, or it may be fairly homogeneous, as in Pl. XVIII, Figs. 5 and 6. Grégoire (7) maintains for *Allium Cepa*, and probably for all somatic nuclei, the existence of a discontinuous spireme. This point is extremely difficult of interpretation, for, when first formed, the spireme is a much-coiled structure, and the coils are often closely wrapped about the nucleolus (Pl. XVIII, Figs. 6 and 7), but there appears to be little doubt that at this stage it is a continuous ribbon. The ribbon is coiled quite irregularly (Pl. XVIII, Figs. 6, 7, 8) within the nuclear vacuole; no indication is seen of the arrangement into sixteen loops described by Schaffner (20).

The nucleoli are intimately associated with the formation of the spireme. Even in the 'resting' stages (Pl. XVIII, Fig. 1) they may show fine fibrils of stainable matter radiating out to the nuclear reticulum. During the organization of the spireme these fibrils stain more densely (Pl. XVIII, Figs. 5 and 6), and there seems little doubt that substances are passing out from the nucleoli from which the chromatic spireme is built. When the chromatin band is fully formed, the nucleoli are left as faintly staining bodies, often vacuolated, and still later they may be seen extruded into the cytoplasm (Pl. XVIII, Fig. 16). No fragmentation of the nucleoli was seen as described by Digby (1) for *Galtonia.*

Their ultimate fate was not followed, but it seems probable that they may become absorbed by the cytoplasm, for no trace of the core was ever seen in the cytoplasm of the daughter cell.

During the 'resting' stages the nucleoli often contain regularly shaped, highly refracting bodies, as shown in Pl. XVIII, Fig. 1 *a*. Leitgeb (13) and Digby (1) have described these bodies in the cells of *Galtonia candicans*, &c. The latter observer suggests that these structures probably originate from the nucleolus. In the present instance there can be no doubt that this is the case, since they are actually situated within the nucleolus. Unlike the structures in *Galtonia*, which stain with chromatin dyes, these in *A. Cepa* appear to be unstained. The structures are apparently absorbed during the development of the spireme, for when the latter is complete they have disappeared, and, as was shown above (Fig. 16), the nucleolar core is left as a faintly staining body within the cytoplasm (Pl. XVIII, Fig. 16).

These bodies may simply represent waste material produced by the nucleus during the 'resting' stages, which material might then diffuse into the cytoplasm during the early stages of mitosis, or represent some substance which is used in the process of organization of the spireme.

No useful purpose would be served here by detailing the various views which have been held as to the possible function of the nucleolus. It seems quite certain that it is a storehouse of reserve material which is used up in the formation of chromatin, but this may be only one of its functions.

From the early stages of its formation the spireme is seen to be a double structure. At first it consists of a double series of irregularly shaped granules lying side by side in the nuclear framework (Pl. XVIII, Figs. 3 and 4). Grégoire (7) has described this structure for several species of *Allium*, and has pointed out that the doubling is brought about by an alveolization of the chromatin band. Lundigård (14) also describes the split spireme for *Allium*. These observations also bear out Grégoire's (7) statement that the spireme is not built up of chromatin discs on a linin framework. No evidence is found to support the observations of Merriman (15) that the thread is a quadripartite structure.

The separation of the spireme into two parallel longitudinal bands is regarded as a definite fission, and, as will subsequently be shown, the split first makes its appearance in the anaphases of the preceding divisions, and not at the metaphase of the same division, as described by Schaffner (20), or the prophase, as stated by Grégoire (7), who says 'the longitudinal division is essentially a prophase phenomenon'.

The more or less isolated granules of the early spireme spread themselves over the nuclear groundwork and form a fairly homogeneous ribbon (Pl. XVIII, Figs. 6, 7, and 8). It is at this stage that the spireme becomes segmented. At first it appears to break up into a number of lengths. The number is, in all probability, eight, and each segment is a long, often twisted, double band. These segments are usually bent somewhere near their middle, and it is common to find the parts on either side of the bend with similar configurations (Pl. XVIII, Figs. 9, 10, 11, 12, and 13). Later these subdivisions break across transversely, and the sixteen chromosomes which characterize the somatic cells appear (Pl. XVIII, Figs. 14, 15, 16, and 17). In other words, there appears to be a preparation at this stage for the paired arrangement of the chromosomes, which will be described presently.

A short digression must be made here to describe other changes which have been taking place in the nucleus during the prophases.

The 'resting' nucleus occupies about half the cell volume (Pl. XVIII, Fig. 1); the organization of the spireme involves a very considerable increase in size of the nuclear vacuole, so much so that Lawson (10, 11, and 12) has termed these prophases the 'growth period'.

During the early stages of the prophase the nuclear vacuole has a very definite boundary—the so-called nuclear membrane. The growth of the vacuole is accompanied by the stretching or by the growth of this boundary to keep pace with the increased volume. Whilst the distension of the vacuole is progressing, the chromatic elements become closely pressed to its surface, so that there is little doubt that this growth is a result of increased turgidity due to the metabolic processes which must be taking place, and which result in the formation of the spireme segments.

The increased size of the nuclear vacuole is accompanied by a corresponding diminution in definiteness of its boundary. As the older stages of prophase are reached, the nuclear vacuole occupies almost the whole of the cell space, and some of the chromosomes have passed out into the general cytoplasm (Pl. XVIII, Figs. 9, 10, 11). Finally, the limits of the vacuole appear merely as a faint, irregular boundary of cytoplasm (Pl. XVIII, Figs. 9, 10, 14, 15, 16) without the slightest suggestion of a definite membrane as a distinct entity, which is suggested by Lawson (11), Němec (18), Merriman (15), and others.

Further, the form of the vacuole is not necessarily 'perfectly spherical', as described by Lawson (p. 154, 10) for *A. Cepa.* It may be quite irregular in outline, and is sometimes even bilobed (Pl. XVIII, Fig. 1 *b*), although this latter condition is not of frequent occurrence. Farmer (2) has shown that the form of the vacuole will not be determined by osmotic pressure alone, but that surface tension will play a part in the determination of its shape.

When the vacuole has reached its full size Lawson maintains that it collapses and that the nuclear membrane becomes wrapped around each chromosome. The collapse, according to this author, is followed by the drawing in of the cytoplasm, which is consequently compelled to assume a radiate structure, in which he sees the beginnings of the formation of the achromatic spindle.

No evidence whatever is found in the present investigation which supports these conclusions. The ragged edge of the cytoplasm can be recognized even when the chromosomes are passing on to the equatorial plate (Pl. XVIII, Fig. 16).

This is not the place to discuss the bearing of the above conclusion on the synaptic contraction during the heterotype mitoses, but it may be remarked that no useful purpose would be served by giving series of measurements showing the increase in size of the nuclear vacuole or the volume of the chromatin mass. This method of comparison would only be useful if the nuclei were of similar sizes and if they contained equal volumes of chromatin. In the somatic nuclei of *A. Cepa* it can hardly be assumed that the latter supposition is valid, whilst the former can easily be shown to be untrue.

To return to the prophases. When the sixteen chromosomes have been organized they pass on to the equatorial plate, and there they are seen to be arranged in pairs (Pl. XVIII, Figs. 16 and 17). The pairing is suggested not only by the approximation of the chromosomes, but also by the fact that members of a pair often show a similarity of form and size. It is not always possible to make out eight distinct pairs, but this is probably due to the fact that some of them lag behind the others in taking up their position, and that they are seen from different angles. Further, it seems probable that the members of a pair are both derived from the same subdivision of spireme (Pl. XVIII, Figs. 10, 16, 17). Müller (16) has recently shown that a similar pairing of chromosomes takes place in *Yucca*, but only among the larger chromosomes. Grégoire (8) denies the existence of paired chromosomes for *A. Cepa*. Gates (6) and Strasburger (22) have also described pairing of chromosomes in *Oenothera* and *Pisum* respectively, and Stomps (21) in the somatic nuclei of *Spinacia oleracea*.

METAPHASE.

Details of the method of attachment of the chromosomes to the spindle fibres have not been followed.

The chromosomes become V-shaped or form loops with one longer and one shorter arm.

Grégoire (8) states that they become attached by their bent parts, and that these are the first parts to separate. This is undoubtedly a common occurrence, but the ends sometimes separate before the middle parts (Pl. XVIII, Fig. 17).

Pl. XVIII, Figs. 17, 18, 19, 20, 21, and 22, show the separation of the daughter chromosomes. It will be noticed by a comparison of the figures that the split previously observed in the prophase is the one which now brings about the separation of the daughter chromosomes.

Sometimes the longitudinal split closes up during the later stages of prophase, and it should be noticed that when this is the case the re-formed split shows first of all as a series of vacuoles which gradually fuse and effect the separation of the daughter chromosomes (Pl. XVIII, Figs. 21, 22 *a*).

ANAPHASES.

As the daughter chromosomes pass to the poles they retain their V- or loop-shaped forms; they seldom appear to be straightened out in the passage to the poles of the spindle.

During the separation of the chromosomes the arrangement in pairs still remains evident (Pl. XVIII, Figs. 23 and 24). It should further be noticed that the members of a pair are very similar in form. The pairing cannot be seen amongst the whole sixteen chromosomes, but this is probably due to the fact that they are closely massed together, for it will be noticed that the pairing is extremely well seen in the chromosomes which stand out from the general tangle.

Attention should be directed to the chromosomes *x* of Pl. XVIII, Figs. 22, 23, and 24, which are quite different in form from the others, and may therefore be termed 'hooked' chromosomes.

It is whilst the daughter chromosomes are passing to the poles that the beginnings of the longitudinal fission are effected. It makes its appearance as a series of slits (Pl. XVIII, Figs. 22 *b*, 23, and 24). At this stage no lateral attachments between neighbouring chromosomes have been effected, so that in this case the split is not due to the lateral attachments pulling out the chromosomes, as suggested by Fraser and Snell (6) for *Vicia*.

TELOPHASE.

Lateral attachments between neighbouring chromosomes are now freely effected (Pl. XVIII, Figs. 25, 26, and 27). The free ends of the chromosomes are drawn inwards and fusions take place between these free ends, the result being that a somewhat flattened nucleus is formed in which the chromatin is present as a flattened sphere with rather large spaces in it (Pl. XVIII, Fig. 26). This is later followed by the expansion of the nucleus and the assumption of an oval or rounded form. With the distension of the nucleus the slits previously observed in the anaphase naturally become much larger, since now all the chromosomes are more or less connected one with another and form a hollow sphere. Polar views of the nucleus show that the sixteen chromosomes can still be recognized (Pl. XVIII, Fig. 28), although, as has been pointed out above, fusions take place between neighbouring chromosomes.

At this stage one or more chromatin knots have been formed on some part of the chromatin sphere (Pl. XVIII, Fig. 28). Each knot later develops the characteristic nucleolar vacuole, so that there is little doubt that they represent the beginnings of the formation of the nucleoli.

As the nucleus passes further into the resting stage the amount of chromatin diminishes in the nuclear network and increases in the nucleolus, the splits in the network open out still further, and finally the nucleus consists of the faintly staining fenestrated sphere almost entirely devoid of stainable matter.

During the telophases an enormous number of cross-connexions are made between neighbouring chromosomes, so that the boundaries of each become difficult to follow. A recognizable foundation for each chromosome cannot be seen during the resting stages, such as is suggested by Grégoire (7) for *Allium*, Müller (17) for *Najas marina*, and Overton (19) for *Calycanthus*, &c.

MITOSIS IN THE GAMETOPHYTE.

It has been shown above that *A. Cepa* has a longitudinally split spireme in its somatic nuclei.

The nuclei of the pollen-grains have been examined, and it may be stated at once that they also show a similar type of spireme to that present in the somatic cells.

Pl. XIX, Fig. 29, shows the 'roof' of a nucleus, and at this early stage it will be seen that the chromatin bands show well-marked splits. Pl. XIX, Fig. 30, shows a slightly older nucleus, and here again the longitudinal fission is apparent. The spireme which is ultimately formed is rather stout, and although it may not show the extensive splitting seen in Pl. XVIII, Figs. 10, 11, and 12, of somatic nuclei, there can be no question that a split exists (Pl. XIX, Figs. 31, 32, and 33). It is during the last stages of prophase only that the longitudinal fission is obscured, and this is precisely the stage at which it is least obvious in the somatic nuclei. Pl. XIX, Fig. 34, however, shows a well-marked fission in one of the very stout and deeply staining chromosomes.

The nucleus need not be followed through the subsequent phases of division since enough has been seen to clearly demonstrate that, apart from the chromosome number, there is no essential difference in the appearance of the spiremes in the somatic and gametophytic nuclei of *A. Cepa.*

It may, however, be pointed out that, as the generative nucleus passes into the resting stage, it becomes very much elongated and eventually suggests a vermiform type of nucleus; at the same time a well-marked vacuole is formed around it and the nucleus becomes suspended in this space by a spindle-shaped sheath of cytoplasm (Pl. XIX, Figs. 35 and 36). Both these nuclei also show distinct evidence of longitudinal fission in their chromatic elements.

DISCUSSION.

It is not proposed to discuss fully the results of this investigation at the present stage; other phases in the nuclear history are being examined, so that a full discussion must necessarily be left to a later date, when all the facts may be brought into line. Certain points, however, may be emphasized here.

It has been shown that both the somatic and the gametophytic nuclei have the double or longitudinally split type of spireme. This type of spireme in somatic cells is regarded by Overton (19), Sykes (23), and Takara (24) as representing a lateral approximation of the structures derived from the male and female gametes respectively, whilst Farmer and Digby (1 and 4) and Hof (9) regard it as representing a premature longitudinal fission which will effect the separation of the daughter chromosomes at the next division.

If any pairing of allelomorphs takes place at all it is obvious that the double type of spireme can hardly represent this phenomenon in *A. Cepa*, for the same type of spireme has been shown to exist in gametophytic cells where there can be no question of pairing of homologous chromosomes. It seems, rather, that this phenomenon is indicated by the pairs of chromosomes of similar form which may be seen in the prophases, metaphases, and anaphases of somatic cells. During these three phases of nuclear division there is ample opportunity for any interaction between the members of chromosome pairs.

In any case it now seems clear that in *Allium Cepa*, as in *Vicia Faba*, the double type of spireme does not represent the paternal and maternal threads lying side by side. It does appear certain, however, as pointed out by Farmer and Digby (1 and 2), Farmer and Shove (3), and Fraser and Snell (5), that it merely represents the premature fission of the chromosomes, a fission which is generally effected at the anaphase or telophase of the division preceding that at which it will become operative.

It has also been shown that in *A. Cepa* the longitudinal fission is recognizable at the anaphase of the preceding division and not the telophase as in *Vicia Faba*, and also that the fission is not effected by the pull of the lateral attachments between adjacent chromosomes, but that such lateral attachments merely accentuate a fission already formed within the chromosome. It would be extremely interesting to know exactly what brings about the fission at this early stage.

Further, the presence of a chromosome of a definite form—the hooked chromosome, *x* in Pl. XVIII, Figs. 22, 23, and 24—suggests that we may have here a feature of some special biological importance, but until it can be traced through all the nuclear phases of the plant it would be unwise to endow it with any particular function.

Finally, it may again be remarked that the facts which have emerged from this investigation lend no support to Lawson's recently propounded theories (10, 11, 12) to account for certain phenomena of mitosis. It has not been found possible to demonstrate in *A. Cepa* the presence of a nuclear membrane as a distinct entity, nor is it found that there is any sudden collapse of the nuclear vacuole which might call the spindle into being. It is quite true that Lawson's results were mainly derived from the study of meiotic cells, but he maintains that the processes are the same in both meiotic and somatic cells.

SUMMARY.

1. At the end of prophase chromosomes are arranged in eight pairs on the equatorial plate and the members of each pair roughly correspond with one another in form.

2. The paired arrangement is preserved during the anaphases. During

the anaphase the fission which will effect the separation of the daughter chromosomes at the subsequent division is marked out. During the stages 'hooked chromosomes' may be seen.

3. In the telophases lateral fusions take place between the chromosomes, and this has the effect of making the fission more evident.

4. A chromatin knot is formed by the fusion of parts of chromosomes and gives rise to the nucleolus. During the prophase the reverse series of changes take place; the nucleolus gives up its chromatin to the developing spireme.

5. In the 'resting' stages the nucleus consists of a faintly staining reticulum. The chromatin is stored in the nucleoli, and these are joined to the reticulum by the fine fibrillar connexions.

6. The spireme, which is longitudinally split, appears to be continuous at first, but later segments into eight subdivisions, and finally these segment and give rise to sixteen chromosomes.

7. No evidence is found in favour of the theory that the sudden collapse of the nuclear vacuole is responsible for the appearance of the achromatic spindle.

8. It was not found possible to demonstrate the presence of a distinct nuclear membrane.

9. The gametophytic nuclei, like the somatic, show the double type of spireme.

My heartiest thanks are tendered to Dr. H. C. I. Gwynne-Vaughan (Fraser) for providing facilities for the undertaking of this work in the laboratory of Birkbeck College and for valuable criticism during its progress.

BIBLIOGRAPHY.

1. Digby, L.: The Somatic, Premeiotic, and Meiotic Nuclear Divisions of *Galtonia candicans*. Ann. Bot., vol. xxiv, p. 727.
2. Farmer, J. B.: 'Nuclear Osmosis' and its Assumed Relation to Nuclear Division. New Phyt., vol. xi, p. 139.
3. Farmer, J. B., and Shove, D.: The Structure and Development of the Somatic and Heterotype Chromosomes of *Tradescantia virginica*. Quart. Journ. Mic. Sc., vol. xlviii, p. 559.
4. Farmer, J. B., and Digby, L.: Somatic and Heterotype Mitoses in *Galtonia candicans*. B. A. Report, Sheffield, 1910.
5. Fraser and Snell: The Vegetative Divisions of *Vicia Faba*. Ann. Bot., vol. xxv, p. 845.
6. Gates, R. R.: Somatic Mitoses in *Oenothera*. Ann. Bot., vol. xxvi, p. 993.
7. Grégoire, V.: La structure de l'élément chromosomique dans les cellules végétales. La Cellule, t. xxiii, 2e fascicule, p. 311.
8. ———: Les phénomènes de la métaphase et de l'anaphase dans la caryocinèse somatique. Ann. de la Soc. Sc. de Bruxelles, t. xxxvi.
9. Hof, A. C.: Histologische Studien an Vegetationspunkten. Bot. Centralbl., Bd. lxxvi, p. 221.

10. Lawson, A. A.: Nuclear Osmosis as a Factor in Mitosis. Trans. Roy. Soc. Edin., vol. xlviii, p. 137.
11. ————: The Phase of the Nucleus known as Synapsis. Trans. Roy. Soc. Edin., vol. xlvii, p. 591.
12. ————: A Study in Chromosome Reduction. Trans. Roy. Soc. Edin., vol. xlviii.
13. Leitgeb, H.: Krystalloide in Zellenkernen. Mitt. aus dem Bot. Inst. zu Graz, Jena, p. 115.
14. Lundigård, H.: Ueber Kerntheilung in den Wurzelspitzen von *Allium*. Svensk Botanisk Tidskrift, Bd. iv, p. 188.
15. Merriman, M. C.: Vegetative Cell-division in *Allium*. Bot. Gaz., vol. xxxvii, p. 178.
16. Muller, H. A. C.: Ueber karyokinetische Bilder in der Wurzelspitze von *Yucca*. Pringsh. Jahrb. f. wiss. Bot., Bd. xlvii, p. 99.
17. ————: Kernstudien an Pflanzen. Archiv f. Zellforschung, Bd. viii.
18. Nemec, B.: Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*. Pringsh. Jahrb. f. wiss. Bot., Bd. xxxiii.
19. Overton, J. B.: On the Organization of the Nuclei in the Pollen Mother-cells of Certain Plants. Ann. Bot,, vol. xxiii, p. 19.
20. Schaffner, J. H.: Karyokinesis in Root Tips of *Allium Cepa*. Bot. Gaz., vol. xxvi, p. 225.
21. Stomps, T. J.: Kerndeeling en synapsis bij *Spinacia oleracea*. Amsterdam, 1910.
22. Strasburger, E.: Ueber die Individualität der Chromosomen und die Pfropfhybriden-Frage. Pringsh. Jahrb. f. wiss. Bot., Bd. xliv, p. 482.
23. Sykes, M. G.: Note on the Nuclei of some Unisexual Plants. Ann. Bot., vol. xxiii, p. 341.
24. Takara, M.: Ueber Kernteilung bei *Morus*. Bot. Mag. Tokyo, vol. xxiv, p. 281.

EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Mr. T. Reed's paper on the Nature of the Double Spireme in *Allium Cepa*.

All figures were drawn with camera lucida under a 2 mm. apoch. hom. imm. Swift obj. and oc. 12 and 18. Magnification × 1450 and × 2100.

PLATE XVIII.

Fig. 1. 'Resting' stage, showing chromatin in nucleoli; lower nucleolus shows fibres joining it to faintly staining nuclear reticulum. × 1450.

Fig. 1*a*. Nucleoli with crystalline bodies. × 1450.

Fig. 1*b*. Nucleus with bilobed form. × 1450.

Fig. 2. Early prophase showing commencement of spireme formation. × 1450.

Figs. 3–4. Later stage of same. × 1450.

Fig. 5. Spireme with prominent nucleolar connexions. × 1450.

Fig. 6. Later stage of same; nucleolus with crystalline body. × 1450.

Fig. 7. Spireme, showing close relation with nucleolus. × 1450.

Fig. 8. Spireme with well-developed fission. × 1450.

Figs. 9–13. Segmentation of spireme to form subdivisions. × 1450.

Figs. 14, 15. The full number of somatic chromosomes formed. × 1450.

Figs. 16, 17. Pairing of chromosomes on equatorial plate as seen in polar view. 16 × 1450. 17 × 2100.

Figs. 18, 19, 20. Metaphases showing the opening of the fission which will effect the separation of the daughter chromosomes. × 1450.

Fig. 21. The chromosomes showing development of 'vacuoles' which will bring about their fission. × 2100.

Figs. 22 *a* and 22 *b*. Hooked chromosomes (*a*) from a metaphase, (*b*) from an anaphase. × 2100.

Fig. 23. Anaphase, pairing well seen in lower group. ‘Hooked’ chromosome (x) also shown. × 1450.

Fig. 24. Similar to Fig. 23 with ‘hooked’ chromosome (x), also showing commencement of longitudinal fission. The four large dots represent the arms of a pair of chromosomes cut transversely. × 1450.

Figs. 25, 26. Telophases, lateral fusions between chromosomes effected, and pulling out of the ‘slits’. × 1450.

Fig. 27. Later telophase. × 1450.

Fig. 28. Polar view of telophase, showing the sixteen chromosomes and nucleolar knot with characteristic nucleolar vacuole. × 1450.

PLATE XIX.

Fig. 29. Pollen-grain nucleus. ‘Roof’ of nucleus, showing the split spireme. × 1450.

Fig. 30. Older pollen-grain nucleus with well-marked split spireme. × 1450.

Fig. 31. Generative nucleus with longitudinally split spireme. × 1450.

Fig. 32. Prophase of pollen-grain nucleus with split spireme. × 1450.

Fig. 33. Later spireme in pollen-grain. × 1450.

Fig. 34. Portion of pollen-grain nucleus with one chromosome longitudinally split. × 1450.

Figs. 35–36. Generative nucleus of pollen-grain surrounded by vacuole and sheath of cytoplasm. × 1450.

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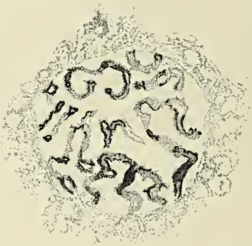

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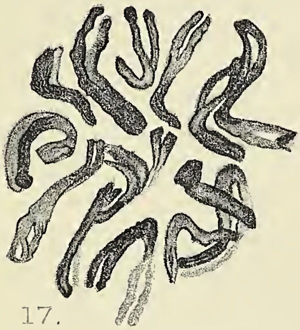

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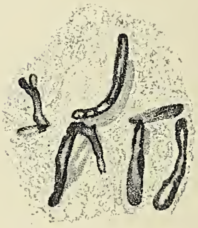

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T. Reed, del.

REED—CYTOLOGY OF ALLIUM.

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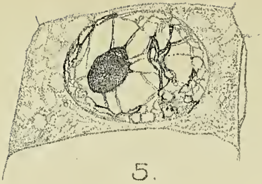

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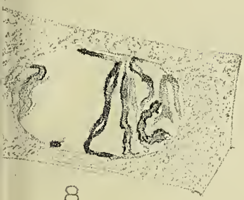

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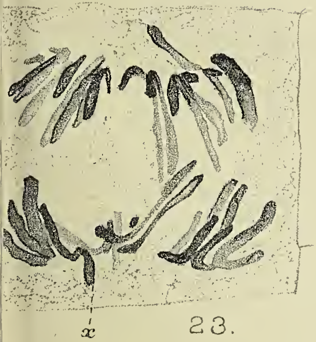

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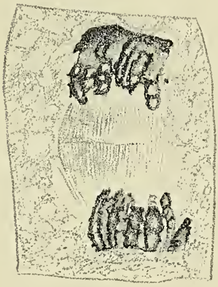

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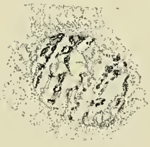

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REED—CYTOLOGY OF ALLIUM.

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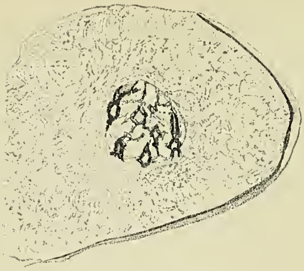

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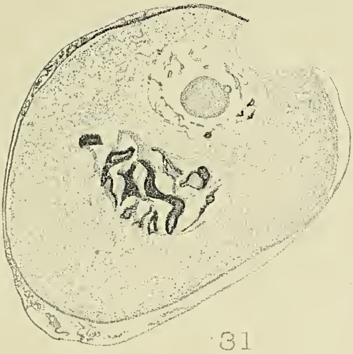

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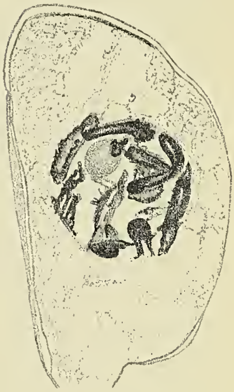

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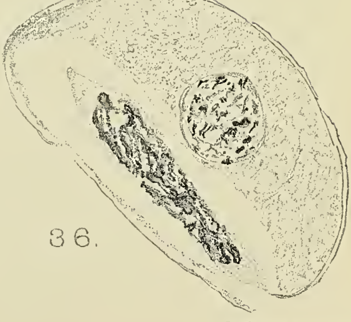

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REED—CYTOLOGY OF ALLIUM.

On the Action of Certain Compounds of Zinc, Arsenic, and Boron on the Growth of Plants.

BY

WINIFRED E. BRENCHLEY, D.Sc.,

Rothamsted Experimental Station, Lawes Agricultural Trust.

With seventeen Figures in the Text.

THE question of the toxic and stimulant action exercised by various inorganic substances on plant life is one round which much controversy has circled during recent years. Different investigators have frequently obtained discordant results with the same substance, some workers, perhaps, claiming for it a stimulative action, while others maintain its universal toxicity. The reasons for these discrepancies may be various, but the significance of one outstanding fact has not been realized. Experiments have been made with plants growing in different substrata, soil, sand, and water being the chief media. Results obtained with the one medium have often, without adequate proof, been assumed to hold good for another medium. Close examination of a great bulk of the literature of the subject has shown the fallacy of this assumption, and it is quite evident that a result obtained by one experimental method may apparently be contradicted by that obtained by another method.[1] In water cultures the experimental conditions are very largely under control, but the habitat of the plants is unnatural. In sand cultures the investigator is again able to regulate the supply of nutrients and poisons, but there comes into play the process of adsorption, whereby part of the dissolved substances are withdrawn from the solution and removed from the sphere of action by the grains of sand. In soil cultures the supply of nutriment to the plant is quite out of control, and also the worker has no adequate conception of the interaction occurring between the soil constituents and the inorganic substances supplied for test purposes.

As a general rule it is found that the quantity of an inorganic substance which exercises a toxic action when presented in water cultures is less than that required to exercise a like action in soil cultures. Again,

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[1] Vide Brenchley, W. E.: Inorganic Plant Poisons and Stimulants. Cambridge University Press, 1914.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

a strength of poison which is toxic in water cultures may exercise a stimulant action in soil, and further, a substance may stimulate in soil cultures which only exerts a toxic or indifferent action in water cultures, no matter how small the quantity in which it is presented.

Consequently, in considering this question of toxic and stimulant action, it is essential that the experimental methods are given due consideration. When that is done, it is possible to balance up the various results, and finally to obtain a more accurate idea of the true action of a substance than when one method only is considered.

The work in the Rothamsted botanical laboratory is confined to the water-culture method, and the action of zinc sulphate, arsenic compounds, and boric acid, chiefly on peas and barley, has been tested, to follow up the work published in 1910 on the action of copper sulphate and manganese sulphate on barley.[1]

In each scale of concentrations represented in the curves, a convenient intermediate strength is selected as a unit, and all other concentrations in the series are expressed in terms of that unit. Thus, with 1/1,000,000 as the unit, the scale of concentrations might run thus:—

10·	1/100,000
4·	1/250,000
2·	1/500,000
1·	1/1,000,000
0·5	1/2,000,000
0·1	1/10,000,000
0·05	1/20,000,000
0·	Control.

ZINC SULPHATE.

Zinc is one of the elements whose compounds are well known to be toxic to plant life. It is generally assumed that the flora of soils containing a considerable amount of zinc is specialized, partly in that only a limited number of species occur, and partly in that certain morphological differences are to be observed between plants of the same species on the calamine soil and on ordinary soil. The toxicity of the element has been fully proved by various investigators, and the point now at issue is that of a possible improvement of growth by means of zinc compounds applied in very small amounts. This point has been tested at Rothamsted, anhydrous zinc sulphate being used throughout the experiments.

Barley. High concentrations of zinc sulphate, 1/10,000 and upwards, kill barley plants. With 1/10,000 zinc sulphate, death does not ensue

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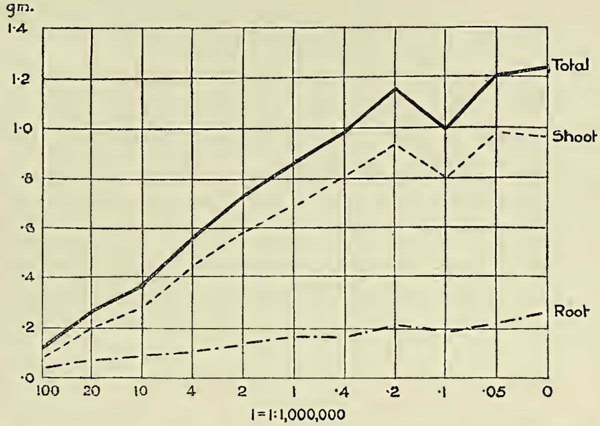
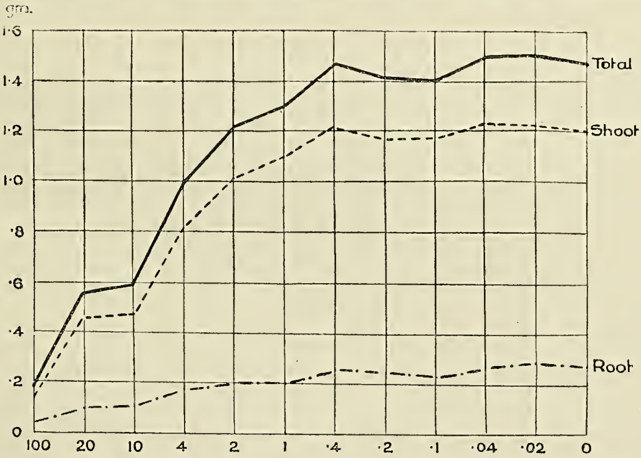
[1] Brenchley, W. E.: The Influence of Copper Sulphate and Manganese Sulphate upon the Growth of Barley.' Ann. Bot., vol. xxiv, pp. 571–83 (1910).

immediately, as for two or three weeks the shoot growth is apparently normal, though the roots are somewhat short and stout, with numerous stubby laterals. After about a month, however, the shoots appear small and weak, and by the time the experiments close, after a growing period of six to ten weeks, the shoots are quite dead. Higher concentrations, as 1/5,000, kill the plants at an earlier stage of development. As the strength of the zinc sulphate decreases, the growth of barley, both as regards the shoot and root development, steadily improves, the dry weights showing a regular upward curve. As a general rule 1/50,000 zinc sulphate allows



CURVE 1. Showing the mean values of the dry weights of fifteen series of barley plants ('Pedigree Plumage') grown in the presence of anhydrous zinc sulphate and nutrient salts. (March 7–April 16, 1910.)



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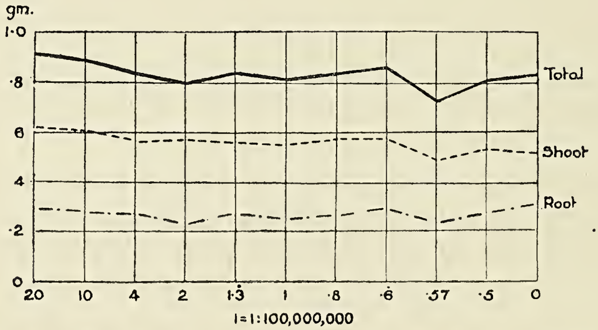
CURVE 2. Showing the mean values of the dry weights of ten series of barley plants ('English Archer') grown in the presence of anhydrous zinc sulphate and nutrient salts. (March 2–May 8, 1911.)

the plants to live for the full time of the experiment, though the development is weak. In only one experiment were such plants found to be dying.

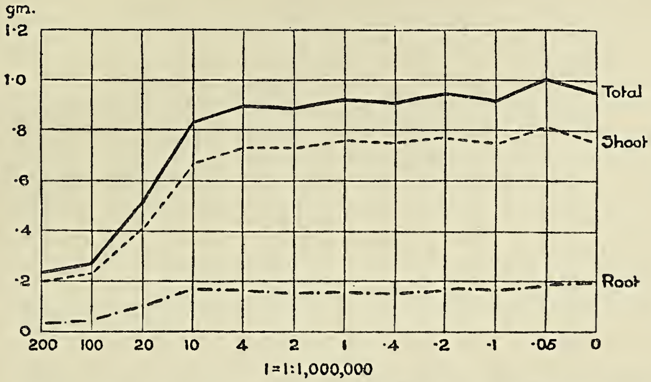
The dry weights of the plants continue to increase until the zinc sulphate reaches a dilution of 1/2,500,000 or 1/5,000,000. It is possible that the variety of barley may play a part in determining the actual critical point, as with 'Pedigree Plumage', grown in 1910, the concentration was 1/5,000,000, while with 'English Archer' in 1911, 1/2,500,000 was the strength concerned (Curves 1 and 2). On the other hand, the difference may be a seasonal variation, the experiments being made at about the same time of year. Below this strength the behaviour of the plants is somewhat erratic. In some cases a slight depression remains evident, in others no influence, either toxic or stimulant, can be observed, and again, slight increase in dry weight may be obtained with 1/10,000,000 or 1/20,000,000 zinc sulphate. Such diversity seems to indicate that barley is indifferent to the action of zinc sulphate when it is presented in quantities below the critical concentration of 1 in 2½ millions.



CURVE 3. Showing the mean values of the dry weights of ten series of barley plants ('Archer') grown in the presence of anhydrous zinc sulphate and nutrient salts. (April 14–May 25, 1913.)

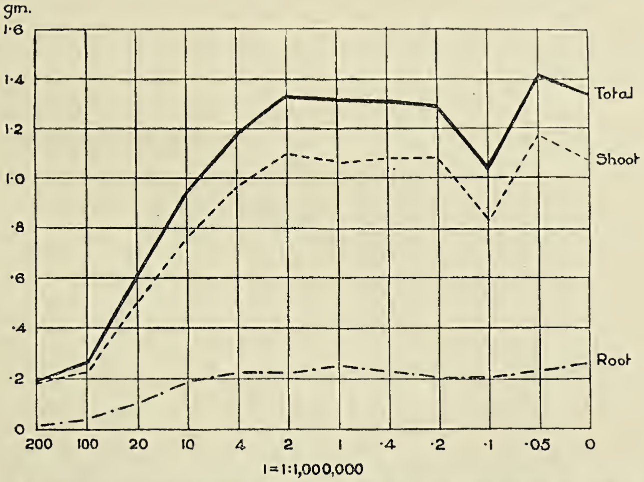
A further experiment was made to determine if stimulation set in at still lower concentrations. A series ranging from 1/5,000,000 to 1/200,000,000 was tested, and the dry weights of the plants yielded an approximately flat curve, the variations being such as would come within the range of experimental error (Curve 3). Hence it may be concluded that zinc sulphate does not act as a stimulant to barley when grown in water cultures, when present in any dilution down to 1/200,000,000.

Peas. The action of the greater strengths of zinc sulphate on peas is parallel to that on barley; 1/5,000 zinc sulphate kills the plants almost immediately, without permitting any growth, while 1/10,000 zinc sulphate permits a little shoot growth at first, but death ultimately results before the close of the experiment. Even these stunted little plants make great efforts to produce flowers and fruit before they die, little shoots an inch high bearing tiny dwarfed flowers or equally dwarfed pods. Rapid improvement in growth results with decrease in the quantity of the toxic agent. Again some slight



CURVE 4. Showing the mean values of the dry weights of nine series of pea plants ('Sutton's Harbinger') grown in the presence of anhydrous zinc sulphate and nutrient salts. (May 18–June 28, 1910.)

difference manifests itself as to the concentration of zinc sulphate which fails to exercise a toxic influence upon pea plants. In a series grown early in the summer (May–June), very little increase in the dry weights occurred



CURVE 5. Showing the mean values of the dry weights of five series of pea plants ('Sutton's Harbinger') grown in the presence of anhydrous zinc sulphate and nutrient salts. (Sept. 30–Dec. 20, 1912.)

with concentrations from 1/250,000 downwards (Curve 4). Later in the year (Sept.–Dec.) the toxic action was perfectly definite down to 1/500,000 zinc sulphate (Curve 5). Below these points little action is evident, but the dry weights of the plants grown with the small amounts of zinc sulphate tend to be very slightly below that of the control, though with 1/20,000,000 zinc sulphate the plants are rather above the normal in weight. No evidence of stimulation of peas was obtained with any strength of zinc sulphate down to a limit of 1/200,000,000.

ARSENIC.

Arsenic is one of the most interesting of the inorganic plant poisons. Inasmuch as very small quantities of arsenic are used in medical practice as stimulants, one is naturally led to wonder whether the action on plant protoplasm is parallel to that on animal protoplasm. Much work has been done with arsenic, but naturally enough the toxic action has claimed most attention, as it is by far the more obvious. Certain discrepancies occur in the results obtained, but further investigations make it probable that these are due to the fact that the common compounds of arsenic are derived from two distinct acids, arsenious ($H_2As_2O_4$) and arsenic ($H_2As_2O_6$), which differ in their action upon plant protoplasm. These two acids, with their compounds sodium arsenite and sodium arsenate, have been studied, chiefly in their action on peas and barley.

ARSENIOUS ACID.

Some of the earlier experiments at Rothamsted were made with arsenious and arsenic acid instead of with their compounds.[1] Arsenious anhydride is not very soluble in water, so considerable care and much time were needed in making up the stock solutions of the poison.

Barley. Barley is very susceptible to the action of arsenious acid, 1/50,000 As_2O_3 killing the plants without allowing any preliminary growth. Little growth occurs in any concentration down to 1/100,000 or 1/250,000 As_2O_3. Below 1/100,000 As_2O_3 'Pedigree Plumage' barley (grown in June and July, 1910) improved considerably in growth, while with 'English Archer' (grown from March to May, 1911) the increase in dry weight did not set in until concentrations below 1/250,000 As_2O_3 were reached. The toxic action rapidly increases with the concentration, but there is evidence to show that a slight depression is still caused by as little as 1/10,000,000 As_2O_3, though the growth of the plants with this strength approaches the normal very closely (Curve 6). According to the dry weights no stimulation is induced even with 1/50,000,000 As_2O_3, but if stimulation is judged by the appearance of the green plants, then concentrations of arsenious acid from 1/1,000,000 to 1/5,000,000 cause improvement in growth. The curious point is that certain of the plants with poison appear to be much stronger than the controls, whereas the dry weights of the same plants fall considerably below those of the controls.

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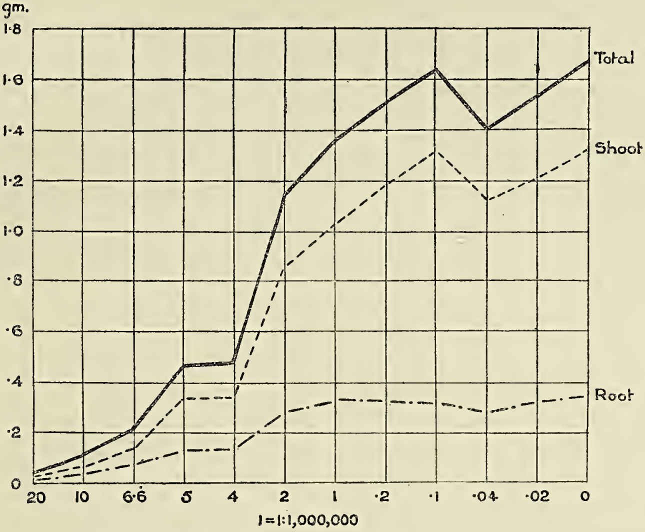
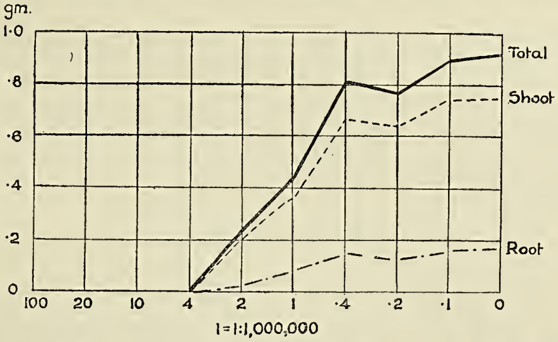
[1] In actual practice one always deals with arsenious and arsenic anhydride (As_2O_3, As_2O_5) which on solution in water give the corresponding acids.

Peas. Peas are far more sensitive than barley to the higher strengths of arsenious acid. With 1/50,000 As_2O_3 no growth at all is possible, even at the very beginning, but the little plants are killed straightway. With 1/100,000 As_2O_3 the shoot grows a very little at first, but the roots are killed, and within a short time the plant dies. This seems to be the critical point, as below this concentration rapid improvement occurs down to a strength of 1/2,500,000 As_2O_3. From this point onwards the improvement is less well marked, and it is just possible that a slight toxic action is still manifest with 1/10,000,000 As_2O_3, though this may mark the limit of toxicity and the setting in of indifferent action (Curve 7). The concentrations have not been pushed further, so that the question of stimulation by smaller doses of arsenious acid is not settled.



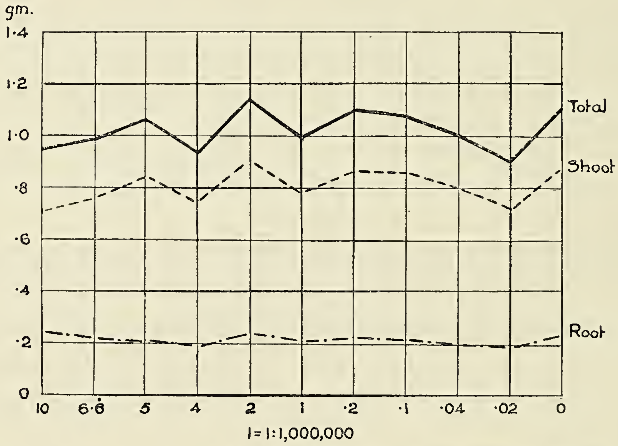
CURVE 6. Showing the mean values of the dry weights of ten series of barley plants ('English Archer') grown in the presence of arsenious acid and nutrient salts. (March 16–May 9, 1911.)



CURVE 7. Showing the mean values of the dry weights of ten series of pea plants ('Sutton's Harbinger') grown in the presence of arsenious acid and nutrient salts. (June 8–July 21, 1910.)

ARSENIC ACID.

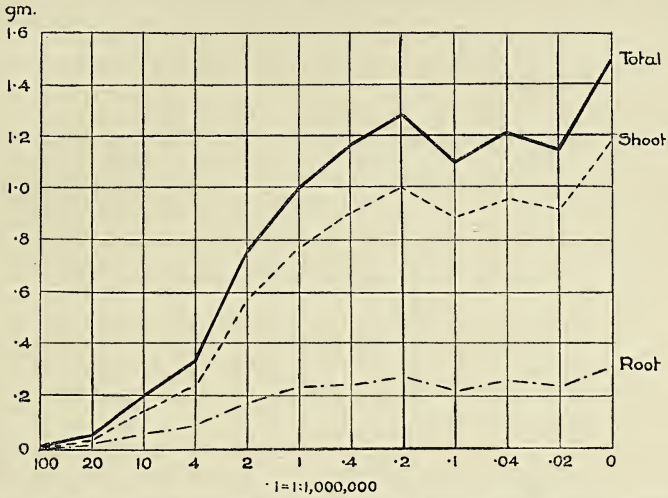
Arsenic acid is far less toxic than arsenious acid in its action on barley. Concentrations ranging from 1/100,000 to 1/50,000,000 arsenic acid gave a series of plants in which no definite toxicity was manifested, though the shoot growth with the highest strength (1/100,000 As_2O_5) indicated a slight depression, which, however, was not evidenced by the roots (Curve 8). The whole series of plants were of a particularly dark green colour, which contrasted very strongly with the yellowish green tinge shown by a series of plants of the same age ranged alongside, which were growing in the presence of zinc sulphate. The general appearance of the plants with arsenic acid was very favourable. Higher strengths of the poison have not yet been tested, so the degree of toxicity of arsenic acid for barley is uncertain.



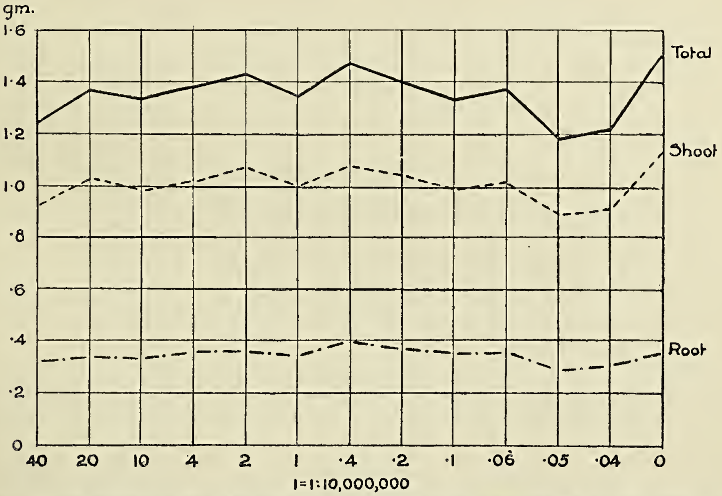
CURVE 8. Showing the mean values of the dry weights of ten series of barley plants ('English Archer') grown in the presence of arsenic acid and nutrient salts. (Feb. 28–April 24, 1911.)

SODIUM ARSENITE.

Barley. The highest strength of sodium arsenite which admits of any appreciable growth in barley is 1/100,000, but the plants are very weak indeed, and are liable to succumb at the least provocation. With decreasing concentration rapid improvement in growth is noticed, which is specially well marked in plants grown in the summer (May–July). In spite of this, however, a marked depression continues evident even with as little poison as 1/50,000,000 (Curve 9). Although the dry weights indicate this continued toxic action, the appearance of the plants points to a possible stimulus with concentrations of 1/5,000,000 and less. This depression was not completely eliminated even when the strength of the poison was reduced to 1/250,000,000 (Curve 10). The sodium arsenite appears to retain its toxicity for barley to a remarkable degree, so that very small quantities do not act as indifferent agents, but as consistent poisons.

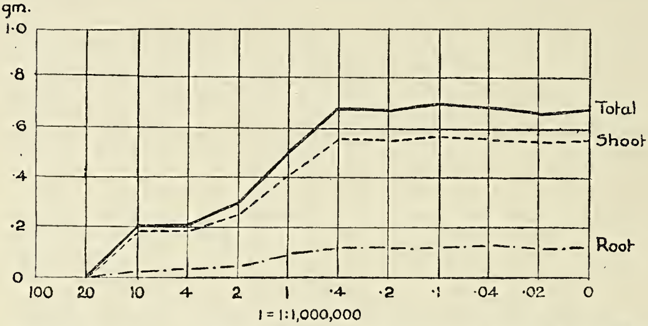


CURVE 9. Showing the mean values of the dry weights of nine series of barley plants ('English Archer') grown in the presence of sodium arsenite and nutrient salts. (March 11–May 15, 1911.)

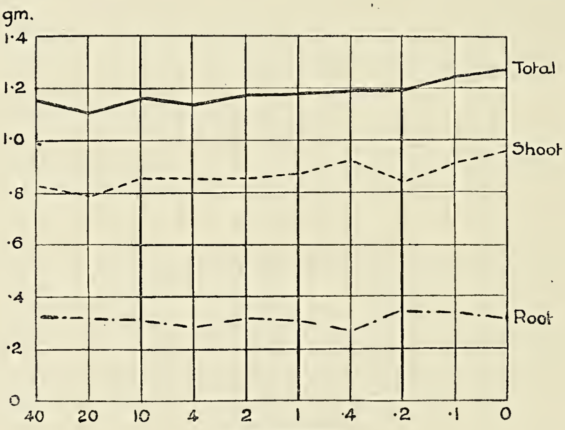


CURVE 10. Showing the mean values of the dry weights of ten series of barley plants ('Archer') grown in the presence of sodium arsenite and nutrient salts. (Feb. 10–April 18, 1913.)

Peas. The results obtained with peas grown in two different seasons are remarkably divergent in some respects. As with barley, 1/100,000 is the greatest strength of sodium arsenite in which the plants can grow at all, and as the solution is made more dilute the amount of growth increases steadily. With peas ('Sutton's Harbinger') grown in the summer months of 1911, the limit of toxicity was found to be 1/2,500,000 sodium arsenite, beyond which growth was unaffected, the dry weights of both root and shoots showing practically flat curves (Curve 11). On the other hand, with plants grown in the early part of 1913 (January to March), strong growth was made even with 1/250,000 sodium arsenite, but although with decreasing amounts of poison a gradual improvement in growth occurred, the plants never quite attained the normal development in any concentration down to 1/100,000,000 sodium arsenite. This constant depression only affected the shoot growth, as the roots were practically normal in development with strengths of sodium arsenite below 1/1,000,000.



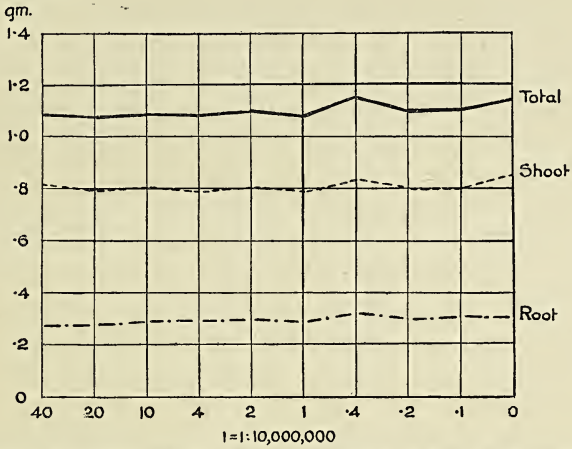
CURVE 11. Showing the mean values of the dry weights of ten series of pea plants ('Sutton's Harbinger') grown in the presence of sodium arsenite and nutrient salts. (Aug. 10–Sept. 18, 1911.)



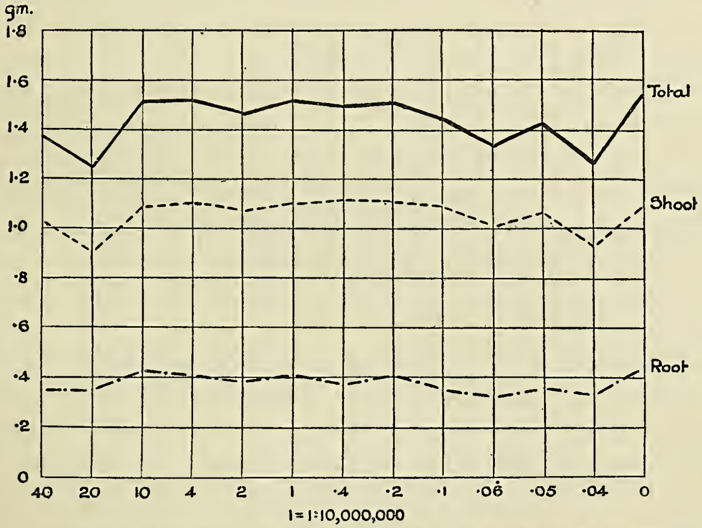
CURVE 12. Showing the mean values of the dry weights of five series of pea plants ('Sutton's Harbinger') grown in the presence of sodium arsenite and nutrient salts. (Jan. 16–March 25, 1913.

SODIUM ARSENATE.

Comparatively few experiments have been carried out with this arsenic compound, but the indications are that sodium arsenate does not act as either a toxic or stimulative agent within wide limits of concentration. With both peas and barley it is possible that some slight depression occurs with 1/250,000 sodium arsenate, but below this strength, down to 1/100,000,000, the dry weights of the plants either approach the normal or else give a somewhat irregular curve which is probably of little significance (Curves 13 and 14).



Curve 13. Showing the mean values of the dry weights of five series of pea plants ('Sutton's Harbinger') grown in the presence of sodium arsenate and nutrient salts. (Jan. 16–March 25, 1913.)



Curve 14. Showing the mean values of the dry weights of ten series of barley plants ('Archer') grown in the presence of sodium arsenate and nutrient salts. (Feb. 11–April 18, 1913.)

Boric Acid.

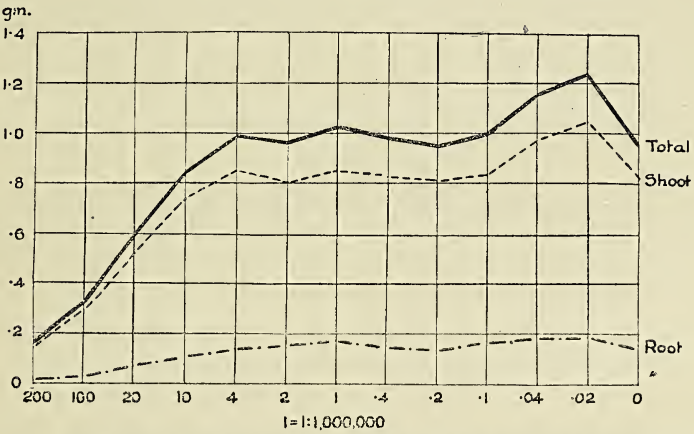
The chief experiments to test the action of boric acid on plants were carried out with peas and barley, but other tests were also made with lupins. The latter plants grew more successfully than is usual for them in water cultures, and although the results must not be considered as conclusive, still they give certain indications as to the reaction of the genus to the poison, so a short account is here included.

Barley. The initial experiments were carried out in July and August, 1910, at a time of year peculiarly unfavourable to the growth of barley under the conditions appertaining to water cultures. Seedlings were treated with various amounts of boric acid grading from 1/5,000 to 1/20,000,000 in nutrient solution. In a very short time the effect of the boric acid was evident in the general dark green coloration of the plants supplied with it, except in those cases in which the concentration was so high as to cause decided poisoning. At a very early date the lower leaves of the latter plants presented a curious mottled appearance, turning yellow with big brown spots. Some trace of this discoloration was evident even with plants receiving only 1/500,000 boric acid, but here only the tips of the leaves were affected. Towards the end of the experiment the shoots were badly attacked by rust and finally died off, so only the dry weights of the roots were determined in order to get some gauge of the effect of the poison. These dry weights indicated that 1/100,000 B_2O_3 exercised a very depressing action on the plants, but below this concentration no conclusions could be safely drawn.

The above experiment was repeated twice in 1911, once in March and April, and again in May and June, but the concentrations were pushed still further, from 1/5,000 to 1/50,000,000 boric acid. In the first set all plants with more than 1/2,500,000 boric acid showed some trace of discoloration in the leaves within a week from the start, those with the higher concentrations already having the upper parts of the leaves spotted and very brown at the tips. This browning was seen first at the tips of the lower leaves, and then spread downwards as brown spots which coalesced, until finally the whole leaf was involved. The other leaves from below upwards were affected at a later stage with the higher strengths of boric acid, the mode of progression of the poisoning action being always the same. The plants were cut for drying after six weeks' growth, at which time all those with weak solutions of boric acid were particularly dark green in colour, while all those with 1/2,500,000 boric acid and less showed a possible stimulus to the eye when compared with the control sets. On examining the dry weights of the plants it is evident that this stimulus was more apparent than real, the only plants which rose above the probable level of experimental error being those grown with 1/25,000,000 and 1/50,000,000 boric acid (Curve 15).

In the later experiments a similar course of events was observed, except that a possible stimulation was obtained with concentrations below 1/10,000,000 boric acid, the difference being probably the result of the later season.

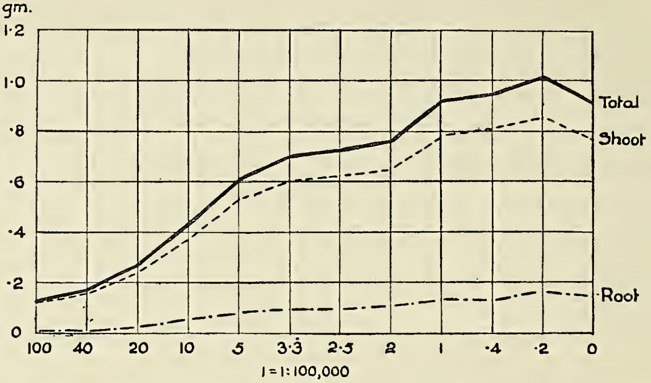
From a comparison of curves and figures it is evident that boric acid has a decidedly toxic action on barley, if it is supplied in too great amount. Below a certain concentration, about 1/250,000 boric acid, the action is indifferent, but there are indications that very low strengths, below 1/10,000,000, may exercise a stimulant action, but the concentrations have not yet been carried down low enough to ascertain this definitely. The results were somewhat unexpected, as, when judged by the eye, some of the indifferent concentrations of boric acid seemed to cause strong stimulation, the colour of the plants also being particularly healthy.



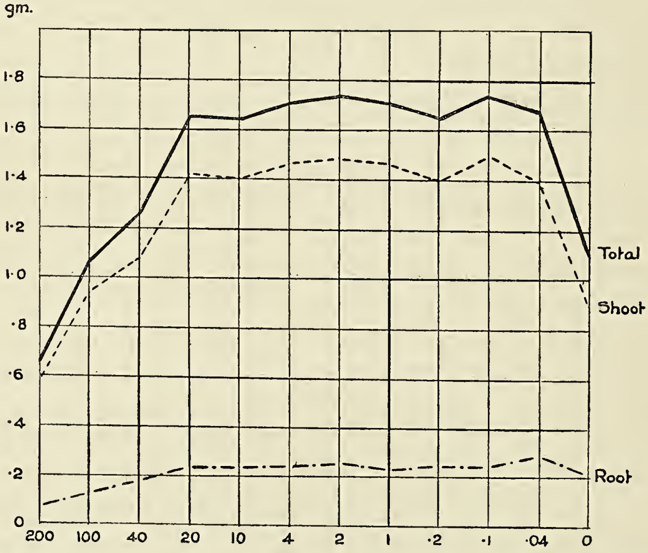
CURVE 15. Showing the mean values of the dry weights of ten series of barley plants ('English Archer') grown in the presence of boric acid and nutrient salts. (May 1–June 20, 1911.)

Peas. In the initial experiments in July and August, 1910, the range of concentrations was from 1/5,000 to 1/20,000,000 boric acid. Even with the strongest of these concentrations the plants were not killed, but they were very poor and weak. All the lower leaves died and the edges of nearly all the remaining leaves were brown and shrivelled, while the roots were curiously long and thin. As the concentrations decreased the plants improved until with 1/100,000 boric acid they seemed normal to the eye, the dry weights indicating some stimulation with this strength. All the stimulated plants showed the characteristic dark green colour which seems to be associated with the presence of boron in the nutritive solution. An interesting morphological feature was the strong development of small side shoots from the base of the plants in the presence of medium amounts of boric acid, 1/100,000 downwards. This gave rise to a certain bushiness of growth, which was less evident as the concentration of the stimulant decreased.

With plants grown later in the same year, in September and October, it was found that 1/1,000 boric acid was necessary to kill the plant at once. Even with as high a concentration as 1/2,500 the peas made some little attempt to push out shoots before succumbing, but the root growth was checked with a less amount of poison than the shoots could stand. Once again 1/100,000 boric acid seemed to indicate the point at which the toxic action ceased and the stimulative action set in (Curve 16).



Curve 16. Showing the mean values of the dry weights of ten series of pea plants ('Sutton's Harbinger') grown in the presence of boric acid and nutrient salts. (Aug. 29-Oct. 24, 1910.)



Curve 17. Showing the mean values of the dry weights of ten series of pea plants ('Sutton's Harbinger') grown in the presence of boric acid and nutrient salts. (May 12-June 30, 1911.)

The next year similar results were obtained, except in that the toxic effect of the acid was rather less evident; 1/50,000 boric acid produced stimulation, which was maintained at the same level down to a concentration of 1/25,000,000 B_2O_3. The slight difference in the two years' results may be due to a combination of causes, such as the earlier date of the experiment (May and June) and differences in the seed and the season (Curve 17).

Further tests in 1912 and 1913 bore out the general accuracy of these observations. 1/100,000 boric acid seems to be a definitely critical strength for peas, generally indicating the point of cessation of the toxic influence and the setting in of the stimulative action, although rarely it introduces an indifferent range of concentrations below the stimulating strength.

The general outcome of the tests seems to be that boric acid needs to be supplied in relatively great quantities to be fatal to pea plants, and that the toxic action gives place to a stimulative influence high up in the scale of concentration. As far as experiments have already gone, it seems that the stimulation is not a progressive one, as the effect of 1/100,000 boric acid is as good as that of 1/20,000,000 boric acid, a flat curve connecting the two.

Lupins. The tests with white lupins gave no conclusive results, as for some reason it proved very difficult to get satisfactory plants in water cultures. When they are grown under such conditions the roots always tend to get more or less diseased and covered with a thick hanging slime, probably fungal in nature. In the presence of boric acid the roots remain in a much healthier condition, the slime being conspicuous by its absence, which suggests that the acid has in this case a strong antiseptic action which protects the roots. With high concentrations the lower leaves of the plants are badly affected, just as with peas and barley, turning brown and withering at an early date.

Various experiments have been made with yellow lupins, but these again are very difficult to grow in water cultures, as they are apt to drop their leaves for no apparent reason. Generally speaking, the evidence goes to prove that boric acid is toxic down to a concentration of about 1/50,000, and that below that strength stimulative action sets in. It is difficult to get a true control with which to make comparisons, as the plants without boric acid are encumbered with the slime on their roots, which naturally interferes with normal growth, while the plants in the presence of boric acid have the advantage due to the antiseptic action of the boron. The effect of the boron poisoning is again evident in the dying off of the lower leaves, which become drooping and flaccid, and finally drop off. The lupins grown with boron are very active in the putting forth of lateral roots, so much so that the cortex of the roots is split along the line of emergence of the laterals, which are very numerous and crowded.

DISCUSSION OF RESULTS.

It is quite usual to find that in different series of plants grown under similar conditions, the degree of toxicity of a particular strength of poison varies within certain limits. This may probably be accounted for by difference in the variety of plant grown, and also by difference in season. There is no ground for assuming that each variety of a given species will react in precisely the same way to an external factor, and consequently such variation may reasonably be expected. Seasonal variation is of two kinds:

(1) Variation at different periods of the same year.

(2) Variation in different years.

As a general rule plants are able to withstand a higher concentration of poison in the summer months than in the early spring and late autumn. It is quite conceivable that the limit of toxicity is lower at the latter times than in the late spring and summer. As the concentration of the poison approaches the indifferent point, the balance between the toxic action of the external agent and the normal functioning of the plant itself becomes more finely adjusted. If the plant is growing vigorously, as it is in summer—the optimum growing period—then its vitality is sufficient to enable it to successfully withstand rather more of the poison than it can endure in the early spring and autumn, when growth is more sluggish and life activity is at a lower ebb.

As a similar variation is observed between plants grown at the same period in different years, it may be deduced that physiological factors, such as temperature and light, play their part in influencing the *degree* of toxicity of a given poison.

TABLE SHOWING THE HIGHEST INDIFFERENT STRENGTHS OF POISON IN DIFFERENT SERIES OF PLANTS.

Period.		*Zinc Sulphate.*		*Boric Acid.*	
		Barley.	*Peas.*	*Barley.*	*Peas.*
1910	March–May	1/20,000,000			
”	May–June	1/10,000,000	1/5,000,000		
”	June–Aug.				1/50,000
”	Aug.–Oct.				1/100,000
1911	March–April			1/5,000,000	
”	March–May	1/25,000,000			
”	May–June			1/250,000	1/25,000
1912	Sept.–Dec.		1/20,000,000		1/100,000
1913	March–June				1/100,000
”	April–May	1/5,000,000			
”	April–June		1/10,000,000		
		Arsenious Acid.		*Arsenic Acid.*	
1910	June–July	1/10,000,000 Still toxic	1/10,000,000 Still toxic		
1911	Feb.–April			1/500,000	
”	March–May	1/50,000,000 Still toxic			
		Sodium Arsenite.		*Sodium Arsenate.*	
1911	March–May	1/50,000,000 Still toxic			
”	May–July	”			
”	June–Aug.		1/50,000,000 Still toxic		
”	Aug.–Sept.		1/2,500,000		
1913	Jan.–March		1/100,000,000 Still toxic		1/25,000,000
”	” ”		”		1/1,000,000
”	Feb.–April	1/250,000,000 Still toxic			

A. *Zinc and Arsenic.* The facts brought out by the foregoing experiments show that there is no sign that zinc sulphate and various compounds of arsenic stimulate peas and barley under any circumstances, and that the toxic action is very marked, even with quite small quantities of the substances.

This failure to obtain stimulation of peas and barley with very small quantities of *zinc sulphate* might be criticized from the standpoint of defective nutrition. The plants are grown in the same solution right through the experiment, and it might be argued that stimulation does occur in the plants with the least amounts of the sulphate, but that as they exhaust their food solution before the end of the experiment growth ceases, so that they are ultimately overtaken by the plants with more sulphate, which continue their growth longer, as their supply of nutrients is not exhausted so early. Analyses of the plants and the food solutions show that this supposition does not hold good. At the close of an experiment with barley in which the plants had made good normal growth, the used nutrient solutions were carefully sampled after the removal of the plants, duplicate samples being taken after all the solutions of a given initial concentration had been mixed together. The nitrate nitrogen in these samples was then determined. Also, the nitrogen present in the dried plants was estimated, and the results tabulated and compared.

POTASSIUM NITRATE PRESENT IN ORIGINAL SOLUTION = 1 GRM. PER LITRE = 0·6 GRM. PER BOTTLE (1 PLANT).

	KNO_3 *left in sol. per litre. grm.*	KNO_3 *lost from sol. per litre. grm.*	KNO_3 *lost from sol. per bottle. grm.*	*av. wt.* KNO_3 *taken from sol. per plant. grm.*	KNO_3 *not accounted for per bottle. grm.*
2	0·885	0·114	0·068	0·042	0·026
3	0·785	0·214	0·128	0·108	0·009
4	0·699	0·300	0·180	0·142	0·039
5	0·576	0·423	0·254	0·213	0·040
6	0·477	0·522	0·313	—	—
7	0·398	0·601	0·361	0·323	0·037
8	0·327	0·662	0·397	0·371	0·025
9	0·280	0·719	0·431	0·413	0·018
10	0·338	0·662	0·397	0·373	0·024
11	0·232	0·767	0·460	0·420	0·039
1	0·237	0·762	0·457	0·428	0·029

2 1/10,000 $ZnSO_4$
3 1/50,000 „
4 1/100,000 „
5 1/250,000 „
6 1/500,000 „
7 1/1,000,000 $ZnSO_4$
8 1/2,500,000 „
9 1/5,000,000 „
10 1/10,000,000 „
11 1/20,000,000 „
1 Control.

The above table shows that at the close of the experiment over 20 per cent. of the initial nitrate was left in the solutions by the plants that had made the most growth. A like result was obtained from similar analyses of solutions in which barley plants had been grown in the presence of varying amounts of boric acid, as in every case at least 50 per cent. of the original nitrate remained in the solution. The mineral constituents were supplied in such excess in the food solution that it was impossible that their supply should have been exhausted, the nitrogen being the only food constituent liable to complete removal. Consequently it may safely be assumed that, other things being equal, in solutions of inorganic compounds in progressive concentration, the depression and absence of stimulation of plant growth are the direct result of the deleterious action of the compounds upon the plant, and that they are not bye-results caused indirectly by interference with food supply outside the plant. An interesting point is the consistent amount of nitrate that is not accounted for by the analyses of the solutions and the plant. In the course of the experiment there is unavoidable loss due to mechanical manipulation, and possibly to bacterial action, as a little solution is always adherent to the roots when they are removed for washing, and bacteria may lock up in themselves a certain amount of organic nitrogen that is not accounted for by the methods used in detecting nitrate nitrogen in the solutions. Bacterial counts were also made to see whether denitrification played much part in determining the results, but no regularity was found in the numbers of bacteria present in the various solutions.

The general outcome of the experiments with *arsenic* on peas and barley is that on the whole arsenious acid and sodium arsenite are more poisonous in their action than are arsenic acid and sodium arsenate. This distinction is more sharply marked with the acids themselves than with their salts, especially with regard to peas. Calculation of the actual quantities of arsenic in the various concentrations used show that the difference of toxicity is real, and that the same quantity of arsenic presented in the different forms of arsenic and arsenious acid varies in its action, being more toxic in the latter case. The reason for this is not obvious. A very tentative hypothesis may perhaps be put forward—that the acid radical formed from the lower oxide is absorbed by the plant in the usual way, and when within the plant tissues undergoes a process of oxidation, the necessary oxygen being withdrawn from the protoplasm, to the detriment of the latter substance. If this be true, it would indicate a double toxic action, partly due to the specific toxicity of the arsenic, which would manifest itself with greater strengths of arsenic acid, and partly due to a physiological action induced by the breaking down of part of the protoplasm to provide oxygen for the purpose of oxidation.

B. *Boron.* Boric acid differs very considerably from zinc sulphate and arsenic compounds in its action upon plants. Although it is toxic in high concentrations, very considerable quantities are necessary to kill peas and barley, as the presence of even 1/5,000 boric acid will admit of a certain amount of growth. With decreasing concentrations barley shows a cessation of the toxic influence and the setting in of an indifferent action, but it is possible that very small amounts may cause improvement in growth. Peas, on the contrary, are very definitely stimulated, the improvement usually setting in immediately the toxic action ceases to be evident. The results suggest that boric acid does exert a very real stimulative influence. With barley, however, the toxic action is rather strong, and makes itself felt far down the range of concentrations, so that with intermediate strengths of boric acid the stimulative influence is cancelled by the toxic action, giving rise to an apparent indifference. With peas, on the other hand, the toxic action is far less marked, and apparently ceases with a higher strength of boric acid, so that the stimulative influence is able to become evident at a higher concentration, and without the intervention of a range of indifferent strengths. With lupins no definite conclusions can be drawn, partly on account of cultural difficulties, and partly because of the counter action introduced by the antiseptic effect of the higher strengths of boric acid on the roots, but the probability exists that definite stimulation is caused by the weaker strengths of boric acid.

Thus, with boron the stimulation may be due to a direct action on the plant or to an antiseptic action which keeps the roots healthy and free from slime. This result of this antiseptic action is visible with lupins, but not with peas, so that it is not definitely determined how far the increase observed in growth is a direct effect or an antiseptic effect.

SUMMARY.

1. Zinc sulphate in high concentration is very toxic to barley and peas, and no evidence of stimulation has yet been obtained with any strength of the poison down to a lower limit of 1/200,000,000.

2. Arsenious acid is more toxic in its action on peas and barley than is arsenic acid, peas being particularly susceptible to the former poison. This distinction holds good for sodium arsenite and sodium arsenate, though in a less degree. Again no stimulation is evident with the smallest quantities so far tested.

3. Boric acid is less poisonous than zinc sulphate or arsenic compounds, especially with peas. Barley shows stimulation to the eye with some of the weaker strengths of poison, but this is not borne out by the dry weights. Peas, on the other hand, are definitely stimulated with relatively high concentrations of boric acid. The action of the greater strengths of the poison is well marked in the leaves, which tend to become brown, and to die in a characteristic manner.

Observations on the Seedling Anatomy of Certain Sympetalae.

II. Compositae.

BY

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Department of Agriculture, University of Leeds.

With eleven Diagrams and two Figures in the Text.

Introduction.

SINCE the present investigation was first undertaken two or three years ago, the problem of seedling anatomy has undergone considerable change. The stimulus which investigators received in the first years of the present century has resulted in the detailed examination of hundreds of seedlings from all the great groups of plants. The results obtained are for the most part qualitative, and have been used chiefly as evidence for or against certain phylogenetic theories. Recently, however, Compton (1) has initiated another line of research in connexion with this subject. This author was able to show that in the Leguminosae the type of vascular anatomy in the seedling is correlated with the *size* of the latter, a result which, if it can be applied generally, goes a long way towards destroying any phylogenetic importance which seedling anatomy may have possessed. But in other groups examined by the present writer, while the type of seedling structure is generally correlated with the size of the seedling, there are not infrequently rather disconcerting exceptions, some of which will be noted later.

In the first part of the present investigation (7) it was indicated that the possession of the Anemarrhena type of seedling structure does not necessarily involve affinity with the Liliaceae. The discovery of this type in the highly evolved Bignoniaceae appears considerably to weaken the phylogenetic theories of Miss Sargant (8, 9, 10, 11). The physiological aspect of the subject has recently been approached by Hill and de Fraine (6), who have attempted to show that the problem of seedling anatomy is largely, if not wholly, physiological, i. e. that the environment (using the term in its widest sense) is chiefly responsible for the amount of vascular tissue present in the seedling, and therefore, to a certain extent, for the number and kind of strands present and the type of seedling structure.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

When the present research was first begun, it was hoped to include a description of the anatomy of seedlings selected, as far as possible, from all the Natural Orders in Engler's Campanulatae. But while a considerable amount of work has been accomplished in this direction, little of interest can be reported outside the Compositae. The present paper, therefore, is restricted to the latter group.

HISTORICAL.

Considering the large size of the Natural Order Compositae, it is a surprising fact that little detailed research has been done on the seedling anatomy of these plants. This is perhaps accounted for by the view that little of phylogenetic importance could be expected in a group which is generally believed to include the most highly evolved plants known. It is hoped, however, that the results here recorded throw some light on the connexion which undoubtedly exists between the different 'types' of seedling structure, if not on the phylogenetic question as a whole.

What are, perhaps, the earliest observations on this subject worth noting were published in 1881. In that year Gérard (5) gave a description of the seedling anatomy of two species belonging to the Compositae; this description has been confirmed in the present research. Three years later Vuillemin (12) published a long paper on the anatomy of the Compositae. His researches, however, were actuated by a desire to evolve a system of classification, based on the anatomy, which would be of use in the identification of the vegetative parts of medicinal plants. It is not surprising, therefore, to find that, although many seedlings were carefully studied from different points of view, there is only a short general account of the 'transition' phenomena, which, as the author remarks, agrees with the more detailed description given by Gérard and cited above.

For the rest, the very general references by Dangeard (3) and by Thomas (12) to the seedling structure in this group, and the more complete descriptions by Chauveaud (2), should be noted.

METHODS.

The seedlings for this research were grown at Birkbeck College, London, from seeds presented by Mr. W. Hales, A.L.S., Physic Garden, Chelsea, to whom I am deeply indebted for generous assistance. The seeds of upwards of seventy species were selected, of as many shapes and sizes as possible, and of these about fifty germinated. The seedlings were cut and the sections stained according to the methods described in the first part of this report (7).

I would like to take this opportunity of tendering to Miss F. A. Randell, B.Sc., my sincere thanks for the very valuable assistance she has rendered in preparing, cutting, and staining many of the seedlings.

TYPES OF VENATION IN THE COTYLEDONS.

The venation of the cotyledons in the Compositae is perhaps one of the most remarkable features in the seedlings examined. In Diagram I, in which the vascular bundles are represented by dotted lines and crosses, the five types of venation observed are given. It must be remarked that in all cases the smallest bundles have been omitted from the diagram.

Type V, which was seen in only one example—*Cichorium Intybus*—is one of the simplest. Although several bundles are present in the upper portion of each seed-leaf, only a single one enters the hypocotyl, and, as will be seen later, this is associated with the simplest type of transition.

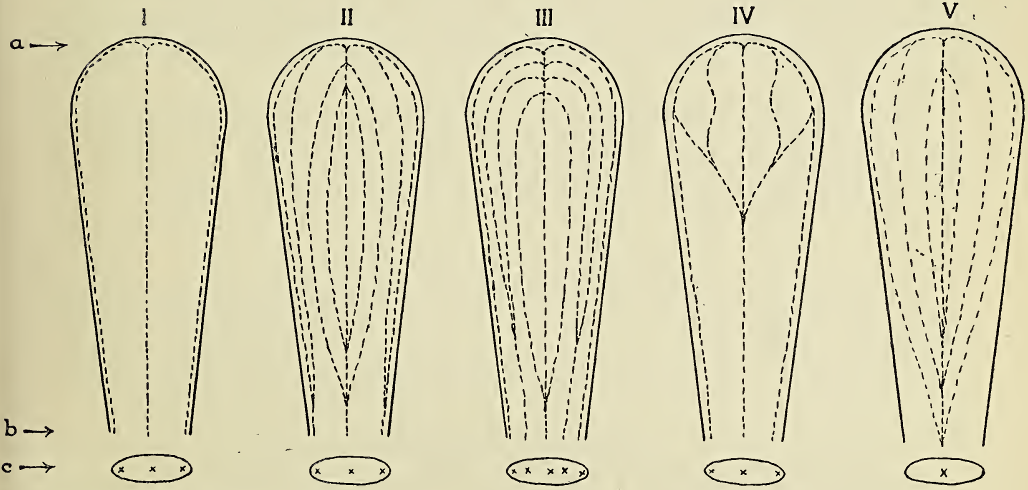

DIAGRAM I. Types of venation in the cotyledons. *a* = apex, *b* = base, and *c* = transverse section of base of cotyledon. Vascular bundles represented thus - - - - and *x*.

Type I, also, is very simple, but here three bundles enter the hypocotyl. The remaining ones are really variants on I and V. In II and III the chief strands are more numerous, but they arise in the same manner as in I and V. At the base of the cotyledon in II (as in I also) there are three vascular strands, but in III five well-marked bundles enter the hypocotyl. Type IV, which is seen in *Arctium majus*, differs from I chiefly because of the peculiar branching of the chief strands near the apex of the cotyledon. This will be described later under the species.

Two rather remarkable facts in connexion with the venation must be noted. The first is that, so far as has been observed, the chief strands never end blindly in the tissue of the mesophyll. The branches which arise near the base of the seed-leaf, whether from the midrib or from the laterals, generally pursue an arcuate or sinuous course and reunite with the bigger bundles at a higher level. The second fact, which is really only an accentuation of the first, is that the extreme lateral bundles on either side invariably unite with the midrib at the apex of the cotyledon.

To describe this in the reverse way. The first sections at the apex of the seed-leaf show no vascular bundles. Then, in the general case, a broad strand of vascular tissue arises. At first it is indefinite in outline, but lower down it separates into three distinct bundles, one being the midrib, the other two following the outline of the cotyledon and giving rise to the laterals. In the majority of cases, as we pass down the seed-leaf all the bundles branch, but the branches produced always fuse with one or other of the chief bundles before the base is reached, only the midrib and the two laterals persisting. The two exceptions to this are seen in III and V: in the former case four laterals (in addition to the midrib) persist, while in V all the laterals produced unite with the midrib before the latter enters the hypocotyl.

The importance of this peculiar venation in connexion with the transition cannot easily be over-estimated. This subject will be referred to again after the seedling anatomy has been described in more detail.

DESCRIPTION OF SPECIES.

The vascular anatomy of most of the species examined will now be described.

A. TUBULIFLORAE.

Tribe III, Astereae.

Solidago ulmifolia, Muhl. Seedlings small (3–7 cm. long), each with two equal cotyledons, which are united along one side for a long distance before fusion occurs on the other margin.

Seedlings A and B. Near the base of each seed-leaf there are three vascular strands, of which the two laterals are very small (Diagram 2, I). On the other side, where the cotyledons first unite, the lateral from one cotyledon fuses with its fellow from the other (Diagram 2, II), and almost immediately the fused bundle, moving round in a clockwise manner (as seen from above), unites with one of the midrib bundles. On the other side, fusion of the laterals is followed by the passage of the double strand into the centre of the hypocotyl, where it remains quite distinct for some distance (Diagram 2, III and IV), finally moving slightly clockwise to join one of the xylem-strands in the cotyledonary plane. In the meantime, the midrib of each cotyledon has passed nearly to the centre of the hypocotyl; the xylem in each case proceeds to undergo rearrangement, and the phloem group bifurcates. A transverse section at this level shows three xylem-masses, of which the intercotyledonary strand near the centre gradually merges into one of the xylem-masses as described above. In the xylem-strands situated in the cotyledonary plane, the protoxylem becomes exarch, the phloem-groups unite laterally in pairs, and a diarch root-structure results (Diagram 2, V).

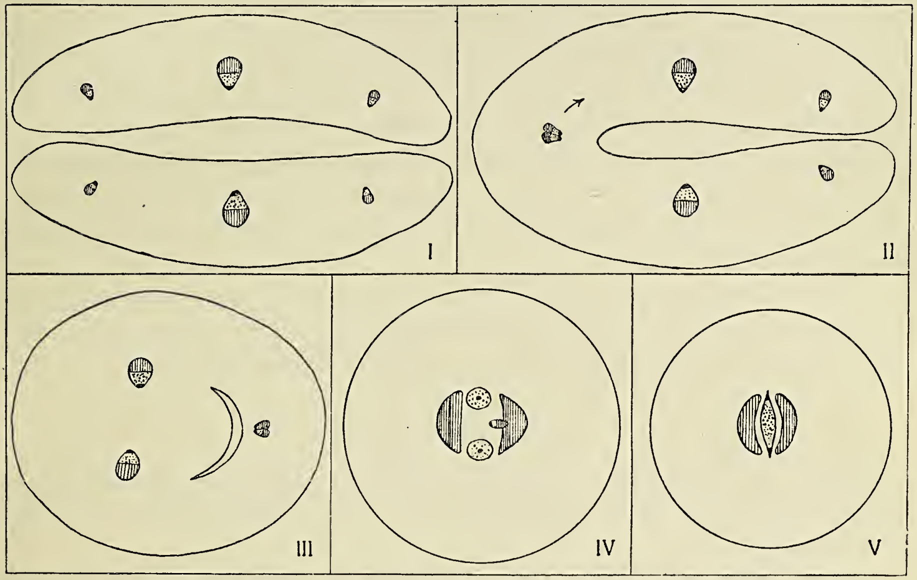

DIAGRAM 2. *Solidago ulmifolia.* 'Transition' from base of cotyledons to root. In this and the following diagrams, protoxylem is shown in black, metaxylem dotted, and phloem hatched.

Seedling C is slightly different from the above. Before the first fusion of the cotyledons along one side, each of the laterals on that side fuse with the corresponding midrib, making the whole structure asymmetrical. At a lower level, new bundles appear on the same side of the two midribs, fuse together as soon as the cotyledons on that side unite, and immediately disappear without ever having been connected with any of the other vascular strands. The behaviour of the remaining vascular bundles is exactly as in *Seedlings A and B*.

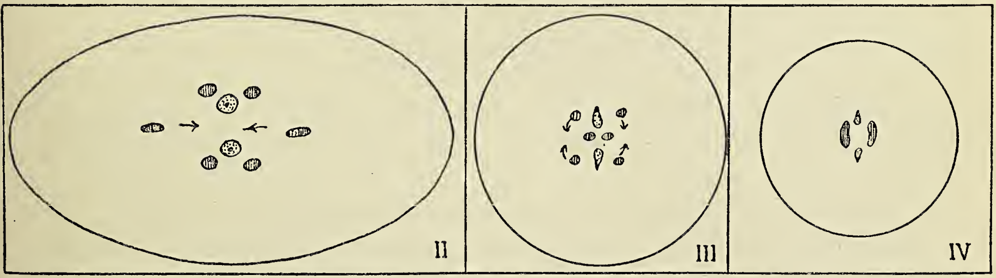

DIAGRAM 3. *Charieis heterophylla.* (For I see Diagram 2, I.)

Charieis heterophylla, Cass. Seedlings smaller than in *Solidago ulmifolia*, with anatomy correspondingly simpler, though of the same general type. A fairly conspicuous cotyledonary tube is present, the fusion of the cotyledons taking place as in *Solidago*. Though the vascular strands are very minute, the details of transition are quite clear. The following differences from *Solidago ulmifolia* may be noted: After fusing in pairs in the cotyledonary tube, the laterals pass inwards in the upper part of the hypocotyl, and on reaching the centre (Diagram 3, II and III) they gradually disappear or join on to the midrib bundles. In the latter, the xylem gradually becomes rearranged, so that quite high up in the hypocotyl the two protoxylems are external. The phloem part of each bundle bifurcates, and the halves fuse in pairs on either side of the diarch xylem-plate (Diagram 3, IV).

Aster Amellus, Linn. Seedlings small (about 2·5 cm. long), with long, fleshy seed-leaves and a short cotyledonary tube. The transition, which is not very clearly shown, is like that in *Charieis heterophylla*.

Tribe IV, Inuleae.

Inula Helenium, Linn. Seedlings small and possessing rather long, slender tap-roots. Transition similar to that described for *Charieis heterophylla*.

Buphthalmum speciosum, Schreb. Seedlings small. Hypocotyl slender, but seed-leaves comparatively large and foliaceous, each possessing a distinct petiole.

The transition is exactly as in *Charieis heterophylla*, with the exception that in the hypocotyl the intercotyledonary strands apparently disappear before they reach the centre. The whole of the rearrangement in the xylem takes place in the upper part of the hypocotyl.

Tribe V, Heliantheae.

Silphium perfoliatum, Linn. Seedlings large (7–12 cm. long) and fleshy. Seed-leaves very large, and hypocotyl stout and comparatively short.

As usual in this Order, the single vascular bundle at the apex of the cotyledon first divides into three, after which, in correspondence with the large size of the cotyledons, each main strand gives off a large number of smaller bundles. At a lower level, these begin to re-fuse with the larger strands, and at the base of each cotyledon only five vascular strands remain —a large median one and two smaller laterals on either side (Diagram 4, I). In the pronounced cotyledonary tube, the extreme lateral (and smallest) bundles fuse with the corresponding bundles from the other cotyledon, and the composite structure produced, after decreasing in size, moves round and joins on to one of the remaining lateral strands (Diagram 4, II). At a still lower level in the cotyledonary tube, the remaining lateral bundles fuse in pairs, so that four equal vascular strands enter the hypocotyl (Diagram 4, III). Almost immediately, the xylem in each case becomes rearranged and the protoxylem assumes an exarch position, while the phloem groups, of which eight have been formed by division of the original four, fuse in pairs, and a tetrarch arrangement is produced (Diagram 4, IV and V).

The root is long and fairly stout, and for the most part a large pith is present. This, however, becomes smaller as the apex is approached, but at no place does it disappear altogether.

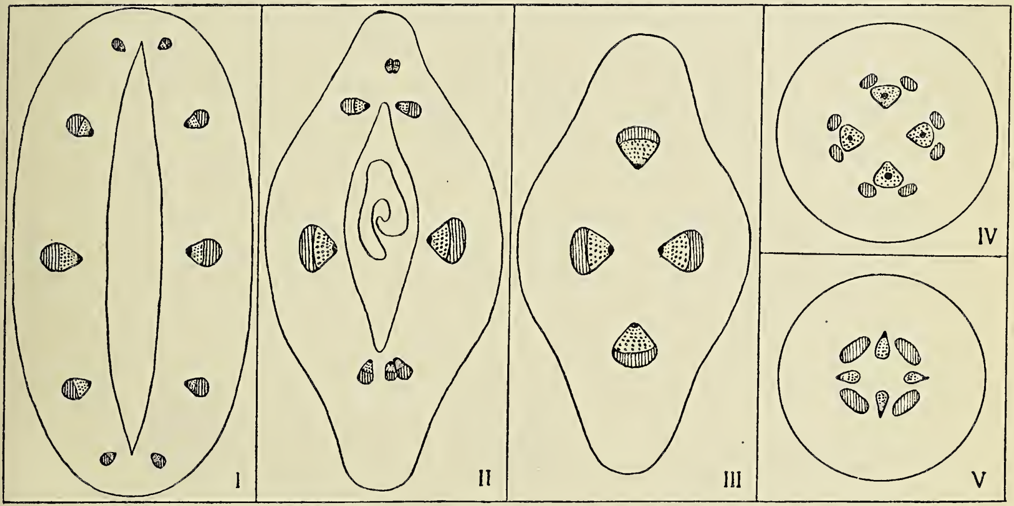

DIAGRAM 4. *Silphium perfoliatum.*

It may be observed here that, though the seedling is large, the transition takes place at a high level in the comparatively short hypocotyl.

Xanthium Strumarium, Vahl. Seedlings generally a little larger (never less than 10 cm. long) than in *Silphium perfoliatum*, but possessing the same general characters.

In the upper part of the seed-leaf the behaviour of the vascular bundles is the same as in *Silphium perfoliatum*. After division and subsequent re-fusion of the strands, there are present at the base of each cotyledon a large number of vascular bundles—viz. a large midrib, two smaller laterals near the margin, and, in the intermediate positions, numerous still smaller bundles. In the large cotyledonary tube the midrib bifurcates, its protoxylem becomes exarch, and the whole mass of vascular tissue assumes a form which it retains until the root is reached.

The remaining strands pass unchanged into the hypocotyl. In the upper part of the latter, the extreme lateral bundle on either side fuses with its fellow from the other cotyledon, and in pursuing its way down the hypocotyl the resulting strand bifurcates, the protoxylem becoming external during the process.

Thus a tetrarch root is formed in which, as one might expect in so large a seedling, there is at first a large pith. The smaller lateral bundles of the cotyledon continue unchanged for a long distance in the hypocotyl. Finally, however, owing to the decrease in size of the latter as it passes into the root, the whole of the vascular tissue becomes arranged nearer the centre, and the bundles, fusing laterally, form a continuous ring of xylem and a ring of phloem which is only broken where it passes the protoxylem groups.

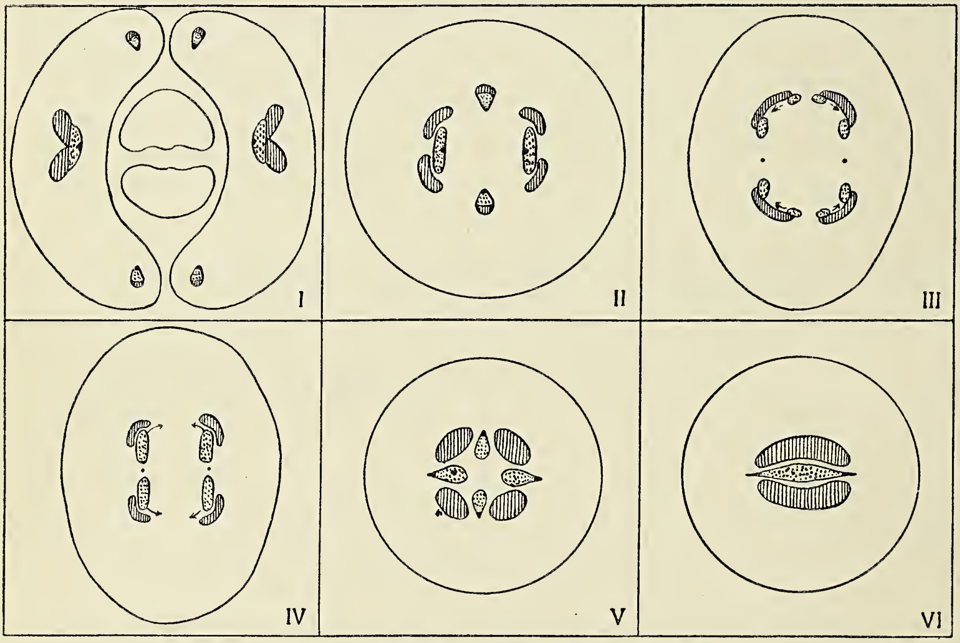

DIAGRAM 5. *Heliopsis laevis.* Seedling A.

The rearrangements are thus initiated in the seed-leaves, and the region of transition is very elongated.

Heliopsis laevis, Pers. The seedlings were of the small, slender type, but the seed-leaves were long and rather fleshy, and also were unequal in size.

Seedling A. In the cotyledons there is the usual branching and re-fusion of the vascular bundles, as a result of which six strands (three from each seed-leaf) pass into the short cotyledonary tube (Diagram 5, I). The two in the cotyledonary plane are very large. Each of these bifurcates, the xylem assuming the form of a flat ribbon with the small protoxylem elements in a median position. In this condition, the midrib strand traverses the greater portion of the hypocotyl. The lateral strands pass inwards, and each pair, after fusing completely, separate again, and, moving laterally, join on to the strands in the median plane (Diagram 5, II, III, and IV). There are thus formed two broad ribbons, each consisting of almost continuous masses of xylem and phloem. Near the base of the hypocotyl the median protoxylem strands become really exarch, and about the same time the original lateral strands separate once more, and, fusing in pairs with the protoxylem exarch, form the intercotyledonary strand of the tetrarch root (Diagram 5, V).

At this stage the tetrarch arrangement is radially symmetrical, but changes now occur which result in a reduction of the symmetry. The intercotyledonary xylem strands become gradually smaller and the four phloem masses fuse in pairs, forming two large phloem groups situate in the cotyledonary plane. The stele is now bisymmetrical, a symmetry which it retains to the root-tip. Gradually the two intercotyledonary xylem masses disappear, or, as in one case, they pass inwards and form the core of the diarch root (Diagram 5, VI).

Seedling B. The above is the usual course of events in this species, but in one case the tetrarch stage, so clearly shown in all the other examples, is entirely omitted. From the 'ribbon' stage, in which the vascular tissue is enormously elongated laterally, one passes straight to the diarch root by a simple closing in of the vascular tissue followed by fusion in pairs of the phloem groups. It is noteworthy that in this case no two seedlings were exactly the same in all details.

Zinnia pauciflora, Linn. Seedlings 4–6 cm. long, each with an elongated hypocotyl and a definite cotyledonary tube.

The transition, though not very clear, is, so far as can be determined, almost exactly the same as in *Bidens pilosa*.[1] At the base of each seed-leaf, however, there are five vascular bundles, of which the very small ones near the margin fuse with their fellows from the other cotyledon as soon as the cotyledonary tube is reached. At a lower level, the fused bundles unite with the other laterals on the same side, so that four strands enter the hypocotyl. Thenceforward the transition is like that in *Bidens pilosa*. Rearrangements in the midrib are always initiated in the lamina, and the region of transition is very elongated.

Rudbeckia amplexicaulis, Vahl. Seedlings about 3 cm. long, and possessing petiolate seed-leaves which fuse to form a cotyledonary tube.

The transition, which takes place at a high level, is very similar to that in *Charieis heterophylla*, the chief difference being that here the fused lateral strands generally disappear before they reach the centre of the hypocotyl.

Leptosyne Douglasii, D. C. Seedlings 2–4 cm. long, very slender, and

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[1] See p. 313.

possessing linear seed-leaves which unite at the base to form a very short cotyledonary tube.

In each seed-leaf there are three principal strands present, the two laterals fusing with their fellows in the upper part of the hypocotyl (Diagram 6, I, II). The fused bundles immediately pass inwards, but remain at some distance from the centre throughout the hypocotyl. The larger midrib strands undergo slight rearrangement, the protoxylem becoming exarch, but the phloem remaining more or less in position. Near the base of the hypocotyl, the four midrib strands approach nearer the centre, and the xylem elements (the number of which fluctuates) now become loosely arranged and tend to intermix. At this level (Diagram 6, III), though the structure simulates a tetrarch arrangement, it does not form an ordinary tetrarch root. The xylem is four-angled, but the angles consist, on the whole, of larger elements than the rest, although all the lignified tracheides and vessels are spirally thickened. In addition the phloem groups are situated *outside* and *not between* the angles. This condition obtains not only in the greater part of the hypocotyl, but also nearly to the root-tip. Finally, a gradual rearrangement occurs: some of the lignified elements disappear and the rest become more closely arranged to form a regular triarch root. Similarly with the phloem, three groups being formed which take up positions between the xylem angles (Diagram 6, IV, V).

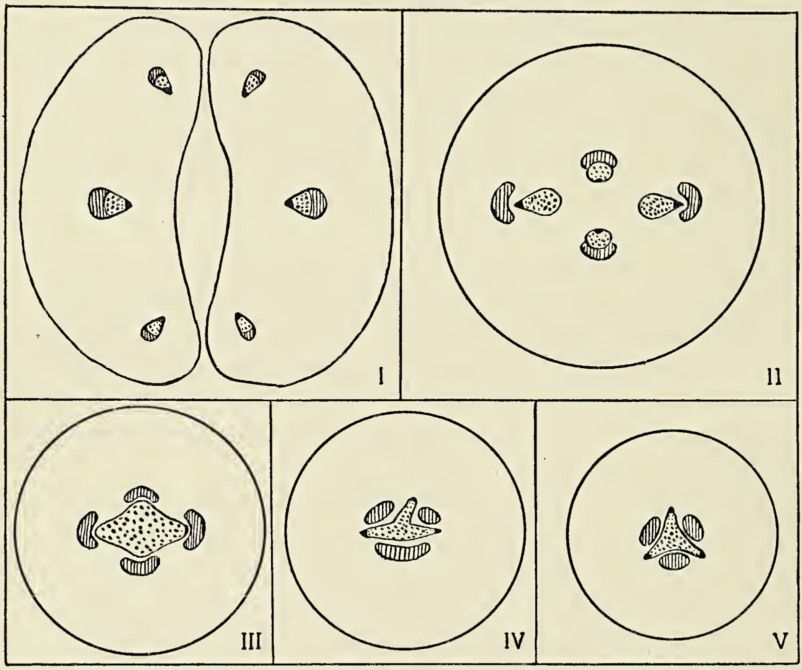


DIAGRAM 6. *Leptosyne Douglasii.*

In one or two cases there were indications near the root-apex of further reduction to a diarch arrangement.

Bidens pilosa, Linn. Seedlings 5–7 cm. long, slender, and each possessing two equal, linear seed-leaves which unite at the base to form a cotyledonary tube.

Near the apex of the seed-leaf there are three vascular bundles—the usual median strand and two smaller laterals—formed by division of a single large strand. At a lower level, small median bundles occur which subsequently unite with the median strand. In the upper part of the hypocotyl the median bundle divides, and the branches pass to the corners of the almost square stele, leaving behind a small strand of protoxylem in the cotyledonary plane (Diagram 7, I, II). The metaxylem branches thus formed consist in each case of 1–3 lignified elements, the protoxylem of only a single vessel. The lateral strands from the two seed-leaves fuse in pairs in the cotyledonary tube. At a lower level, the metaxylem in each case disappears, leaving behind a single, more or less lignified, protoxylem

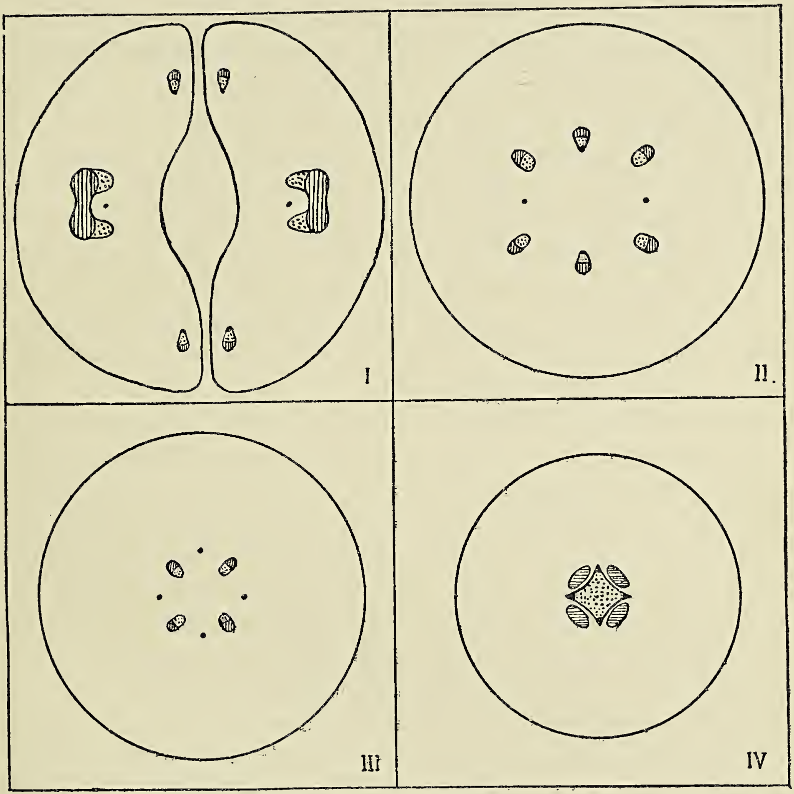


Diagram 7. *Bidens pilosa.*

element (Diagram 7, III). The stele is therefore perfectly symmetrical at this stage and in all the succeeding sections to the root-tip. Lower down, the metaxylem of the corner bundles becomes reduced to a single strand which passes inwards towards the centre, where new lignified elements arise in such large numbers that the pith disappears. The groups of phloem and protoxylem having closed in also, a typical tetrarch stele results (Diagram 7, IV).

In these seedlings there is a good deal of variation in the level at which transition first begins. In the midrib, for example, in some cases, the phloem bifurcates and the xylem divides into three before entering the hypocotyl.

Seedling with three cotyledons. In this case one of the cotyledons was much larger than the other two. In the upper part of the hypocotyl the strands from each cotyledon behave as in the ordinary seedlings; consequently, for some distance there are six protoxylem groups, and six other masses each consisting of xylem and phloem. Finally, the protoxylem and the metaxylem bundles from the smallest cotyledon die out, and rearrangements occur which result in the production of a tetrarch root.

Tridax procumbens, Linn. Seedlings small (generally less than 4 cm. in length), each possessing two unequal cotyledons which unite at the base to form a very short cotyledonary tube.

In the sessile cotyledons the vascular bundles are of the type, and follow the course, characteristic of the Compositae. A noteworthy feature is the early preparation for transition in the median bundle. As soon as the numerous vascular strands of the upper part of the cotyledon have given place, by fusion, to the usual midrib and two lateral bundles, the former bifurcates (Diagram 8, I). The two xylem portions thus produced remain in continuity by means of the protoxylem (which moves slightly to take up an exarch position), but the phloem halves diverge and become widely separated. Lower down, in the short cotyledonary tube, the lateral strands fuse in pairs, and the resulting bundles pass inwards nearly to the centre, where for a time they remain symmetrically disposed in the intercotyledonary plane. In the upper part of the hypocotyl, these bundles move round in an anti-clockwise direction (as seen from above), and finally come into contact with the two branches of the bifurcated midrib bundles situated at diametrically opposite corners of the square stele. This is the condition characteristic of the hypocotyl (Diagram 8, II).

Near the base of the hypocotyl the symmetry is restored by the disappearance of part of the original intercotyledonary strands, and the complete fusion of the remainder with the corner bundles (Diagram 8, III). In the latter, at this level, no protoxylem can be distinguished. In the root most of the pith becomes replaced by vessels, and in the almost solid xylem core there are occasionally four narrow angles simulating a tetrarch structure. Finally, the xylem narrows considerably in the intercotyledonary plane, the four phloem groups fuse in pairs, and near the root-tip there is a typical diarch stele (Diagram 8, IV).

In one seedling in which the cotyledons were comparatively thick, the bundles in them showed less division and re-fusion. A further point of difference concerns the intercotyledonary bundles; these moved to the same side in the hypocotyl, instead of assuming the diagonal position.

Galinsoga parviflora, Cav. Seedling about 2·5 cm. long, with a very slender hypocotyl. The transition region is elongated, and the rearrangements are like those in *Heliopsis laevis*, *Seedling B*.

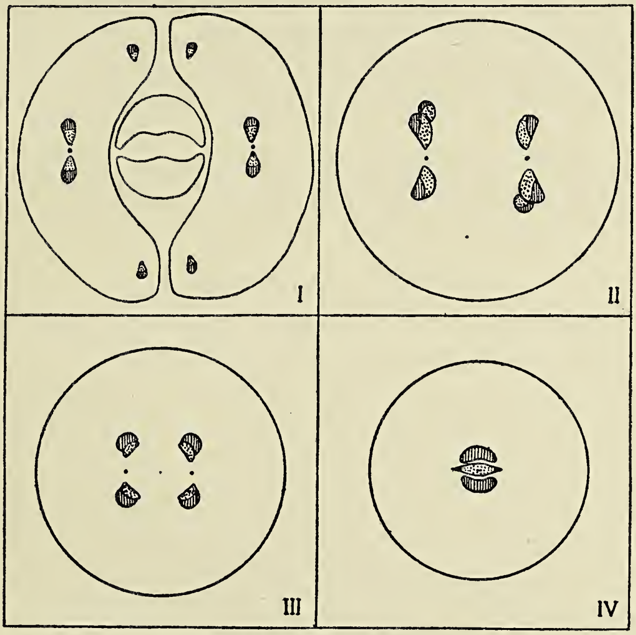


DIAGRAM 8. *Tridax procumbens*.

Madia sativa, Molina. Seedlings slender, over 12 cm. long, and possessing comparatively large, foliaceous cotyledons.

The details of transition are exactly like those in *Heliopsis laevis*, *Seedling B*, and it is worth noting that though these seedlings were twice the size of *Seedling A* in the species just referred to, there was only the slightest trace of a tetrarch structure.

Tribe VI. Helenieae.

Baeria coronaria, A. Gray. Seedlings about 3 cm. long and extremely slender.

Little vascular tissue is present. The two laterals in each seed-leaf fuse with the midrib before they reach the short cotyledonary tube, and the details of transition conform to Van Tieghem's Type 3.

Gaillardia aristata, Pursh. Seedlings 4–6 cm. long, with prominent cotyledons and very short hypocotyls. Transition exactly as in *Charieis heterophylla.*

Tagetes erecta, Linn. Seedlings 5–6 cm. long with narrow, rather fleshy cotyledons.

The transition, first described by Gérard (5, p. 369), is similar to that described for *Heliopsis laevis*, *Seedling A*, the following being the chief points of difference: the strands produced by fusion of the laterals from the cotyledons gradually die out while still some distance from the centre of the hypocotyl. Near the base of the latter they reappear internally to the lateral phloem groups, only to disappear finally in the root, which then possesses a diarch arrangement. Thus, a semi-tetrarch structure, with four protoxylem groups and two or four phloem masses, is present for a long distance.

T. patula, Linn. Seedlings externally like those of *T. erecta.*

Transition similar in all details to that in *Heliopsis laevis, Seedling B.*

Tribe VII. Anthemideae.

Anacyclus Pyrethrum, DC. Seedlings about 6 cm. long. Transition as in *Tridax procumbens*, a diarch root being produced.

Achillea pyrenaica, Sibth. Seedlings about 3 cm. long, each with two equal cotyledons, which, uniting at the base, produce a cotyledonary tube. The transition is almost exactly as in *Charieis heterophylla.*

Matricaria eximia ? Seedlings about 2 cm. long. Vascular strands very minute, with transition as in *Charieis heterophylla.*

Tanacetum vulgare, Linn. Seedlings generally 4–5 cm. long, with comparatively large seed-leaves, and a very long cotyledonary tube.

Transition is similar to that in *Charieis heterophylla*, but the fused laterals disappear in the cotyledonary tube. In one seedling which was larger than the rest, however, the lateral (cotyledonary) strands persisted for a considerable distance in the hypocotyl.

Chrysanthemum carinatum, Schousb.
C. Sibthorpii ? } Seedlings slender, 4–6 cm. long; cotyledons equal in size and comparatively fleshy. Transition almost exactly as in *Charieis heterophylla*, except that the intercotyledonary bundles may join those in the cotyledonary plane by moving in a clockwise direction, or may disappear before they reach the centre of the hypocotyl.

C. Parthenium, Bernh.
C. viscosum, Desf. } In these seedlings, which are slightly larger than in the last species, the general course of events is the same, but the intercotyledonary strands generally travel to the centre of the hypocotyl, where the xylem portion forms the core of the diarch plate. In all the

species of *Chrysanthemum* the arrangement of the median strand begins in the long cotyledonary tube, and is soon completed.

Artemisia annua, Linn.
A. Absinthium, Linn. } Seedlings very short (2–3 cm. long) and slender; the vascular strands throughout are correspondingly small, and the transition is exactly similar to that in *Chrysanthemum Parthenium*, the root being diarch in structure.

Tribe VIII. Senecioneae.

Senecio clivorum, Maxim. In all the seedlings, which varied in length from 7–10 cm., the hypocotyl was comparatively short (less than 1 cm.), and the seed-leaves, which were spatulate in shape and were furnished with long petioles, were distinctly unequal in size.

The venation of the cotyledons is not peculiar. The numerous bundles present in the leaf-blade fuse in various ways to give rise to a large midrib and two small laterals, all of which run down the petiole and enter the hypocotyl almost unchanged. In the upper part of the latter each midrib bundle undergoes modification: the phloem bifurcates, and the two halves travel laterally, while in the wood portion the protoxylem begins to take up an exarch position. The intercotyledonary strands produced by the fusion of the laterals in pairs pass to the centre of the hypocotyl, and after a longer or shorter distance has been traversed, they gradually die out. The phloem groups having previously assumed a lateral position and fused in pairs, the midrib xylem masses meet at the centre, and a typical diarch root is produced. Altogether the transition is like that in *Charieis heterophylla*.

In the region of the root-tip, after the pith has completely disappeared, there is often a return to an almost tetrarch condition: this is brought about by the enlargement of the xylem in the intercotyledonary plane, and the subsequent more or less complete division of the phloem masses.

Tribe IX. Calenduleae.

Silybium Marianum, Gaertn. Seedlings 8–10 cm. long, and rather massive.

The course of the vascular strands in the cotyledon is the same as in *Silphium perfoliatum* (p. 308). The bundles formed by the fusion of the four laterals on either side of the midrib are smaller than the latter, and their protoxylem groups never become exarch. With the decrease in size of the hypocotyl in passing downwards, the intercotyledonary strands travel centrewards, then dwindling in size, they move laterally in a clockwise manner (as seen from above), and fuse with the flattened midrib. There is no real tetrarch arrangement, but at the base of the hypocotyl the midrib xylem groups, each of which by this time has a definite protoxylem, meet at the centre, the phloem groups unite laterally, and the diarch root is complete.

Dimorphotheca pluvialis, Moench. This species is interesting in that the small crop of seedlings grown included many tricotyledonary examples, while the rest showed all stages between these and seedlings with the normal number of cotyledons. Reference to Text-Fig. 1 (p. 319) will show that the larger number is due to the splitting of one of the two original cotyledons.

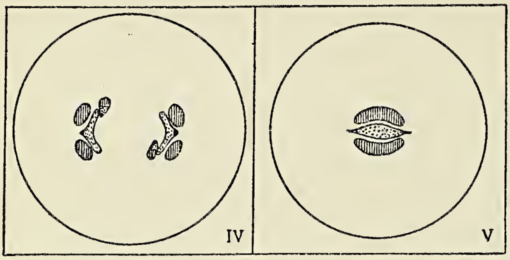


DIAGRAM 9. *Silybium Marianum.* (For I, II, and III see Diagram 4.)

The seedlings are generally about 4·5 cm. long, and are very slender. There is no cotyledonary tube, and the transition is very similar to that described for *Tridax procumbens*, but of a much reduced type, the vascular bundles being very small.

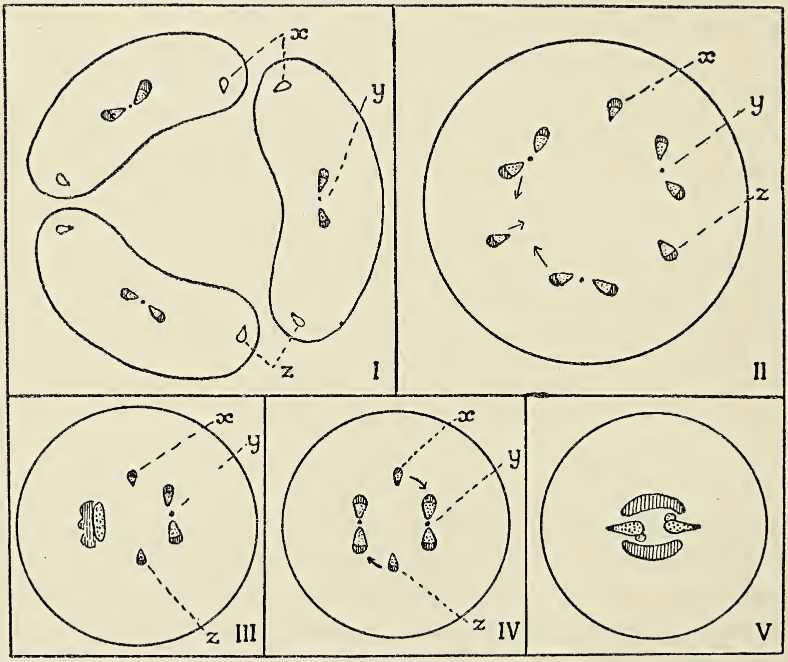
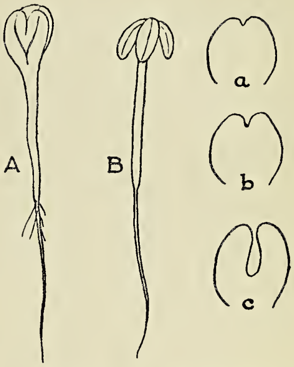


DIAGRAM 10. *Dimorphotheca pluvialis.* 'Transition' in seedling with 3 cotyledons. (For description see text.)

Seedlings with three cotyledons. A comparison of Diagram 8 with Diagram 10 will reveal the peculiar features of the transition in the tricotyledonary seedlings. Working from root to cotyledons, the diarch stele gives place in the hypocotyl to four collateral strands—the two large ones in the plane of the diarch plate (corresponding to the plane of the cotyledons in an ordinary seedling), and two small ones in a plane at right angles to the diarch (intercotyledonary plane in an ordinary seedling), (Diagram 10, IV and V). If two of the cotyledons really correspond to one in the dicotyledonous seedling, it follows that half the vascular tissue should supply one of the three cotyledons, while the other half should do duty for the remaining two cotyledons; which is what actually happens. One of the large bundles (y) and half of each small one (x and z) pass to a single cotyledon, while the remaining halves of the small strands form one of the lateral bundles in each of the other cotyledons. The other large strand divides into three—two large bundles, each forming a midrib, and a small bundle which bifurcates so as to supply the two lateral strands required (compare with Diagram 10, I, II, and III).



TEXT-FIG. I. *Dimorphotheca pluvialis.* A and B represent seedlings showing two stages in the production of the tricotyledonous condition. *a*, *b*, *c* (from actual specimens) show stages in division of one cotyledon into two.

Thus the anatomy, also, supports the view that two of the seed-leaves are homologous with one in the dicotyledonous seedling.

Tribe X. Arctotideae.

Arctotis calendacea, Linn. (*Cryptostema calendulaceum*, R. Br.). Although the seedlings are much larger than those of *Charieis heterophylla*, the transition is similar, the lateral strands of the cotyledon, after fusing in the hypocotyl, passing to the centre to form the core of the diarch root. The transition, which is completed at a high level, begins in the cotyledonary tube.

Tribe XI. Cynareae.

Arctium majus, Bernh. In this species the seedlings, which are generally about 6–8 cm. long, are each provided with two rather fleshy, equal cotyledons, which possess a peculiar venation. Near the base of each seed-leaf there are the usual three bundles—a large midrib and two small laterals. The latter follow the margins of the cotyledon to the apex, where they are joined by the midrib and its chief branches, which arise in the following manner. In the upper half of the seed-leaf the midrib gives off its branches, which run outwards at an acute angle and join the laterals somewhat nearer the apex; but before doing so each gives rise to a branch which runs approximately parallel with the midrib, and like the latter joins the lateral strands at the apex. Smaller branches are also given off by all these bundles. The character seen in most of the Compositae examined, i. e. fusion of the chief bundles at the apex of the cotyledon, is easily demonstrated in this species.

The transition is much like that seen in *Charieis heterophylla.* The xylem of the lateral strands remains distinct in the centre of the hypocotyl for a long distance before finally merging into the xylem of the midrib bundles to form the diarch plate.

Saussurea albescens, Hook. Seedlings 6–9 cm. long, and rather massive. The internal morphology is very similar to that in *Silybium Marianum*, the chief difference being that here the transition begins at a high level. In the midrib bundles the phloem bifurcates, and the protoxylem becomes exarch while still in the cotyledons.

Onopordon tauricum, Willd. Seedlings very large, with ovate and equal cotyledons which join at the base to form a large cotyledonary tube. The transition is almost exactly as in *Tridax procumbens*, a diarch root being produced.

Crupina Crupinastrum, Vis. In this species the seedling is similar in size and external morphology to that of *Silybium Marianum*, and differs from the latter only in a few particulars with regard to transition. In both there are five strands at the base of the cotyledon. The extreme laterals from one cotyledon fuse with the corresponding bundles from the other as soon as the cotyledonary tube is reached. The fused structure, instead of moving round in a clockwise manner as in *Silybium Marianum*, passes inwards, and is joined by the remaining lateral strands on the same side, the three fusing together. All this occurs in the cotyledonary tube, so that only two bundles in addition to the two midribs enter the hypocotyl. These four strands, of which the intercotyledonary ones are the smaller, are symmetrically arranged. In the midrib the bundles become tangentially flattened, and gradually the protoxylem takes up an exarch position. In the intercotyledonary bundles the phloem, also, spreads out and is joined by the bifurcations of the midrib phloem. At a lower level, the xylem of these bundles gradually decreases in size and passes inwards. Near the centre it is joined by the xylem from the cotyledonary plane, with which it fuses, producing a diarch root.

It should be noted that here, though the arrangement in the hypocotyl suggests a tetrarch root-structure, the latter is never attained. In the intercotyledonary strands the xylem never becomes exarch, and the phloem, though assuming a flat ribbon-like form, never bifurcates.

Amberboa (*Volutarella*) *muricata*, DC. Seedlings 4–6 cm. long, and with comparatively fleshy seed-leaves. The transition is similar to that in *Crepis rubra*.

Serratula tinctoria, Linn. Seedlings 9–12 cm. in length, with long, almost linear cotyledons, and a very extended cotyledonary tube. The main features of the cotyledons and of the rearrangement of the vascular bundles are very similar to those in *Charieis heterophylla*, but the region of transition is much longer. In some cases the intercotyledonary strands are retained in the centre almost to the root-tip.

Centaurea macrocephala, Puschk. Seedlings both externally and internally similar to those of *Silphium perfoliatum*. A tetrarch root with a large pith is first produced, which lower down decreases in area in transverse section, so that the pith becomes smaller and the vascular tissue takes up a position nearer the centre. The xylem in the intercotyledonary plane now becomes reduced. One by one the lignified elements disappear, the phloem groups fuse in pairs laterally, and a diarch root is produced.

C. babylonica, Linn. Seedlings not so large as in the last species, and vascular strands correspondingly smaller. The chief features of the transition are similar to those in *Crupina Crupinastrum*. The intercotyledonary bundles decrease immediately after their formation by fusion of the four lateral strands, and when only slightly rearranged they pass inwards and fuse with the cotyledonary bundles. At quite a high level, a diarch root-structure obtains.

Carthamus tinctorius, Linn. In all essentials the seedling of this species (which was also examined by Gérard (5, p. 367), is like a much reduced one of *Centaurea macrocephala*, exhibiting exactly the same features as *Centaurea babylonica*. The seedling is not nearly so massive as that of the former species, and the vascular bundles are very much smaller. In the very long cotyledonary tube the extreme lateral strands fuse in pairs, and almost immediately die out or fuse with the other lateral bundles. The latter next fuse in the same order, and of the four strands which enter the hypocotyl, the two very small ones in the intercotyledonary plane immediately pass to the centre to form the core of the diarch root. Thus there is only the slightest trace of a tetrarch structure. At a fairly high level the midrib strands undergo rearrangements which result in the production of a diarch stele.

Tribe XIII. Cichorieae.

Cichorium Intybus, Linn. (garden variety). Seedlings short (not more than 5 cm. long) and rather stout. The cotyledons are broad and ovate in shape, and fuse at the base to form a fairly long cotyledonary tube. In each seed-leaf there is a definite midrib with smaller veins which run more or less parallel with the margin. These join on to the midrib very gradually, the last fusion occurring in the cotyledonary tube. Only two bundles enter the hypocotyl, and these become rearranged according to Van Tieghem's Type 3.

Picris echioides, Linn. Seedlings 5–6 cm. long, with slender hypocotyl and large foliaceous cotyledons. Transition as in *Charieis heterophylla*, with only slight variations on the latter.

Tragopogon dubius, Scop. Seedlings 10–12 cm. in length, with short hypocotyl and long, linear cotyledons.

Though the seedlings differ so much externally from those of *Centaurea macrocephala*, the transition is almost exactly similar. In the upper part of each cotyledon, there are three main vascular bundles with numerous smaller ones. At the base these give place to five strands which in most seedlings follow the course described for *Centaurea macrocephala*. An almost perfect tetrarch root is formed, which becomes diarch at a lower level.

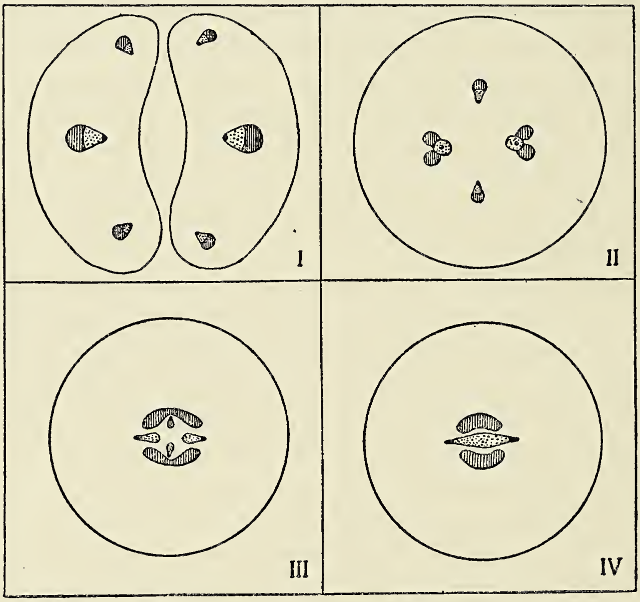


Diagram 11. *Hieracium alpinum*.

In one seedling the outermost bundles in the cotyledons disappeared soon after fusion. In the same specimen there were two extra small bundles, one on either side of the midrib, which persisted for some distance in the hypocotyl. It was not clear whether these small strands finally died out or fused with some of the other bundles.

Lactuca sagittata, Waldst. Seedlings small (less than 5 cm. long); cotyledons equal in size and rather fleshy. Transition almost exactly as in *Charieis heterophylla*.

Crepis rubra, Linn. Seedlings about 4 cm. long, each with a distinct cotyledonary tube. Transition as in *Charieis heterophylla*, with the exception that the intercotyledonary strands, instead of passing directly to the centre of the hypocotyl, travel obliquely and fuse with the midrib bundles. By further rearrangement a solid diarch xylem-plate is formed.

Hieracium alpinum, Linn. Seedlings 7–9 cm. long, with comparatively large, more or less lanceolate seed-leaves which join the hypocotyl without forming a cotyledonary tube.

The numerous vascular strands which occupy the upper part of the cotyledon give place at the base to three, the two lateral bundles being very small (Diagram 11, I). The rearrangement in the midrib strand begins at the base of the cotyledon, but the protoxylem does not become really exarch until the root is reached. In the upper part of the hypocotyl the corresponding laterals fuse in pairs, the resulting bundles passing inwards towards the centre (Diagram 11, II and III). During its passage, the protoxylem becomes exarch, and the metaxylem gradually disappears. At a lower level there is a bisymmetrical tetrarch arrangement, the protoxylem groups being devoid of metaxylem, and the phloem being disposed in two flattened masses in the intercotyledonary plane (Diagram 11, III). Finally, the protoxylem elements enlarge, merge into the metaxylem at the centre, and give rise to a diarch root (Diagram 11, IV).

In other seedlings examined the same sequence was found, but owing to the extreme smallness of the lateral bundles, the features were not exhibited with the same diagrammatic clearness in all cases.

THEORETICAL CONSIDERATIONS.

In a former paper on the seedling structure of the Tubiflorae (7) certain theoretical conclusions were put forward, to one of which allusion has already been made (vol. xxvi, p. 742). It was also shown that in the matter of classification no assistance is to be derived from seedling anatomy. In the Tubiflorae, genera, which on other grounds are held to be nearly related, differ markedly in the vascular anatomy of their seedlings, e.g. *Incarvillea* and *Eccremocarpus* (N. O. Bignoniaceae).

The present case is rather different. In the fairly large number of seedlings examined, it cannot with reason be held that more than one type of transition has been discovered: for, although there are two extremes—diarch and tetrarch—all stages from the one to the other have been described in the preceding pages. The diarch type seen in *Cichorium Intybus* and *Baeria coronaria* passes by way of *Solidago ulmifolia, Charieis heterophylla*, and others (in which the laterals persist for a longer or a shorter distance without giving more than the slightest indication of tetrarchy) to *Hieracium alpinum*, *Heliopsis laevis*, and others (in which the laterals persist to form the weak intercotyledonary poles of a tetrarch root); and thence to such plants as *Silphium perfoliatum* (in which a fully symmetrical, tetrarch root is invariably present). That these are essentially stages of the same type is also indicated by the variations not uncommonly found in the seedlings of certain species. To take a single instance, in one seedling of *Heliopsis laevis*, the tetrarch stage present in the others was entirely omitted. Many smaller variations were noted in other cases, all of which unite in giving the observer the impression that even the extreme 'types' are only variations on one method of transition. Throughout the group this sense of similarity is apparent, and, as in other cases where evolution has been at work, it is impossible to draw hard and fast lines between the two extremes. The case of *Heliopsis laevis* again demonstrates the uselessness of seedling structure as an indicator of affinity.

It is very difficult to determine the cause or causes of such variations; and it is equally difficult to determine which type—diarch or tetrarch—of seedling structure is primitive and which derived. Many authors have discussed the general question of the evolution of seedling structure. In her 'Theory of the Double Leaf-trace' Miss Thomas (12) examines the evidence and concludes that the tetrarch type is primitive, and has given rise to the diarch by reduction. To the present writer this conclusion seems to require extreme conservatism on the part of the seedling: for, unless we assume that the first seedling had a certain type of structure, and that in the ages that have passed since it existed, either the structure has remained unaltered or has changed once and once only, the term 'primitive' becomes meaningless and the discussion futile.

But is such an assumption justified? Are we at liberty to assume that while the mature parts of plants have varied enormously, the young parts have suffered little or no structural alteration? Comparatively little work has been published on the physiology of seedlings, but sufficient has been done to indicate that seedlings can easily be altered artificially. Evans (4), working on *Luzula*, showed that by sowing the seeds at different depths in the soil a hypocotyl of corresponding length could be produced; and when other methods are used and other species experimented with, it will probably be shown that within certain limits the size of the seedling organs can be regulated. Apart, however, from mere speculation, Evans's work indicates that seedlings can be made to vary by artificially altering the environment. There is no doubt that great changes have occurred on the earth's surface during even recent geological times, and if this be so, it is highly probable that seedlings also have altered in accordance with the changed conditions. The different sizes and shapes which seeds have assumed, and the various functions adopted by seedling organs (in connexion with storage, transference of food material, &c.), indicate that the seedling is the reverse of conservative, and that it is really very susceptible to changes of all kinds. There seems, therefore, a high degree of probability that not only have seedlings not been more or less at a standstill during recent geological times, but that they have been continually evolving.

Several writers have emphasized the fact that in general seedlings have either a diarch or a tetrarch root. The connexion between these two types has been shown above. Only in exceptional cases is some other arrangement found to hold, e. g. in the Liliaceae (10). If, in the general case, evolution of the seedling anatomy has not been an extremely slow process, it is quite possible that diarchy and tetrarchy have been gained and lost many times during the evolution of the Angiosperms. This may help to explain the presence of the two types of seedling structure in the Compositae, which by universal consent is the most highly organized Natural Order in the Plant Kingdom. In this group, if there are cases, as Miss Thomas assumes, where the tetrarch structure is becoming reduced to diarch, there are as certainly seedlings in which the diarch structure is giving place to tetrarch.

In the general description of the venation of the seed-leaves (p. 305) it was shown that there are always present more or less numerous laterals in the cotyledon. On a first consideration it appears that the conditions which regulate these laterals determine the type of root to be produced; that is, that the *size* of the laterals and the *level* at which they unite with the bundles in the cotyledonary plane might well determine the type of root structure. But the problem is not so easily solved. Compared with the respective midrib bundles, the lateral strands in the species which possess fully symmetrical tetrarch roots are often no larger than the laterals which form the core of the diarch root in the diarch species; while in such examples as *Bidens pilosa* and *Zinnia pauciflora*, both of which are strongly tetrarch, they are much smaller and are more or less insignificant.

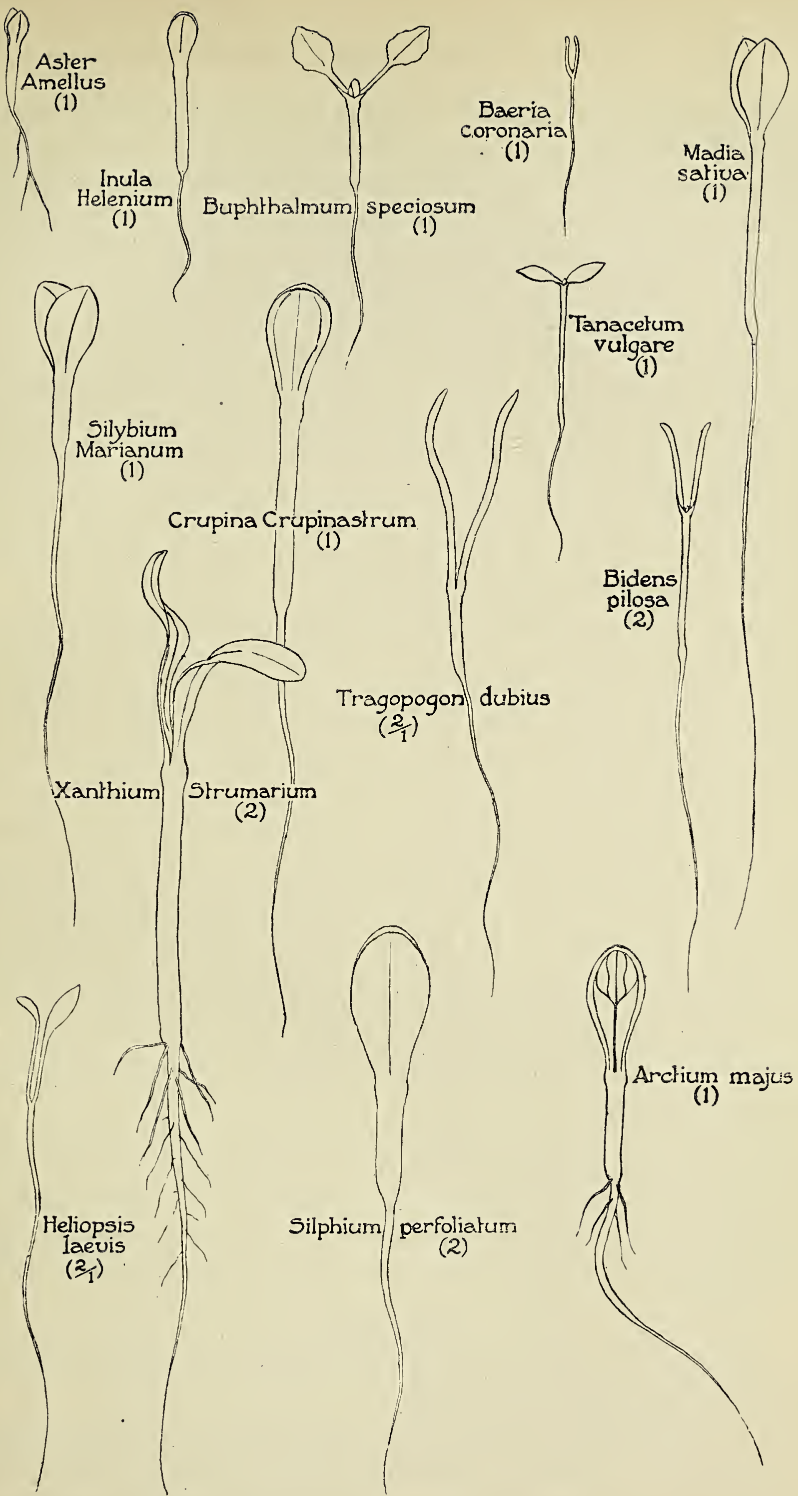
The level at which the laterals unite with the midrib strand varies greatly. In the extreme cases it is true that in the diarch seedlings this union occurs in the cotyledon or in the upper part of the hypocotyl; while in the extreme tetrarch seedlings the union occurs near the base of the hypocotyl. But between these two extremes there is every gradation, as will be seen from the descriptions in the preceding pages. To the present writer it seems obvious that there must have been some other factor at work in addition to the size of the laterals and the level at which these unite with the midrib bundles.

According to Hill and de Fraine (6), 'size of seedlings almost, if not quite, determines whether or not the laterals shall penetrate the hypocotyl or fuse with the main cotyledonary strand before its entrance,' i. e. size of seedling determines whether a diarch or a tetrarch arrangement shall be produced. A similar conclusion was reached by Compton in studying the Leguminosae (1). In the Compositae, as well as in the Tubiflorae, it is generally true that large seedlings are tetrarch and small seedlings diarch, but there are many conspicuous exceptions. An inspection of the outline-drawings given in Text-fig. 2 will save discussion. The drawings are all to scale. The roots of those marked (1) are diarch throughout, those distinguished (2) are tetrarch throughout, while those marked ($\frac{2}{1}$) are first tetrarch, but finally become diarch. The difference in size between *Arctium majus* or *Crupina Crupinastrum* and *Baeria coronaria* need not be emphasized, but it must be noted that all these possess the same type of seedling structure. Again, compare *Arctium majus* or *Crupina Crupinastrum* with *Silphium perfoliatum*; the first two are diarch, the last tetrarch throughout, though one can hardly say that the difference in size warrants such a difference in vascular anatomy. An examination of the seedling of *Bidens pilosa* serves not only to emphasize this point, but also to throw light on another, i. e. the relation between the amount of vascular tissue present and the type of seedling structure.

If, as has been cautiously advanced by Hill and de Fraine (6), there is a real relation between the size of the organ and the amount of vascular tissue present, it follows that organs of the same size should (if the vascular tissue be arranged in the same way) possess the same amount of vascular tissue, not in a transverse section, but in the whole organ; and further, that in an organ like the hypocotyl, which presumably, in the ordinary case, everywhere performs the same function, the vascular tissue present in any transverse section should equal in amount the vascular tissue in any other transverse section of the same area.

To take the first case. In the seedling of *Fagus sylvatica*, Hill and de Fraine appear to pit the area of vascular bundles in a transverse section of the seed-leaf against the surface area of the cotyledon. Now, as the vascular bundles decrease in size towards the apex of the seed-leaf, the area of the bundles depends very much on the position of the transverse section, and it is improbable that one would be able to select the corresponding section in a large number of seedlings. Furthermore, the relation between the amount of vascular tissue in a transverse section and the surface area of the leaf is not at all obvious. It appears, on the other hand, that the total amount of vascular tissue present in the organ should be taken into account, and should be correlated either with the cubic contents of the leaf or with the surface area. Here again, it is almost impossible to make precise measurements. A cursory examination, however, of almost any species will reveal the fact that while the type of venation remains the same, the number and extent of the smaller bundles vary greatly, even when the leaves are of the same size.

With regard to the second point raised above, in most hypocotyls, measurements show that in successive transverse sections of the hypocotyl the amount of vascular tissue is approximately the same, or it decreases slightly towards the base of the hypocotyl. This is what one would expect on any theory postulating a definite connexion between size of organ and the amount of vascular tissue present. In the hypocotyl of *Bidens pilosa*,



TEXT-FIG. 2. Some of the seedlings used in the present paper (drawn to scale). (1) indicates that the root is diarch throughout; (2) indicates that the root is tetrarch throughout; ($\frac{2}{1}$) indicates that the root is first tetrarch, but becomes diarch at a lower level.

however, there is much variation; near the top of the hypocotyl the vascular bundles enlarge, while a little lower down they decrease to a very small number of elements in each case before finally increasing at a still lower level and attaining a comparatively large size. This is only one out of several instances where fluctuations occur in the size of the bundle in the hypocotyl. Everywhere in the latter organ, apparently, the vascular bundles do the same work, yet fluctuate in size.

Finally, from a careful study of a large number of seedlings, and after making measurements into which it seems impossible to read anything like constancy or uniformity, the present writer is forced to the conclusion that other factors are at work in addition to those which are purely physiological. It is possible that two sets of factors are constantly influencing the seedling: (1) those factors which are purely physiological, and which are concerned with changes in the environment; and (2) factors which are connected with the nature of the organism.

It seems impossible to avoid the impression that changes occur in the seedling which cannot be correlated with changes in the environment. These changes in the seedling, due to the nature of the organism, occur continuously, and are probably to a certain extent guided by the first set, or physiological factors. But even when the latter are quiescent—as they must be when there are no changes in the environment—the innate factors are probably always active, and are constantly producing morphological changes in the various seedling-organs. The two sets of factors will sometimes be antagonistic, in which case the physiological factors, which are based on the immediate needs of the plant, will be dominant and will determine the changes which occur. When the physiological factors are quiescent, and new structures are produced by the innate factors, so long as these new structures do not unfit the seedling, they will persist; but when a new structure begins to clash with the environment the physiological factors will be brought into play.

In general, seedlings are limited to two extremes of vascular structure, the diarch and the tetrarch. The course of evolution is probably not to be represented by a straight, nor by a zigzag line, but by a spiral with a large number of turns, the spiral representing the fluctuations between the diarch and tetrarch structure, the axis the general line of advance of the seedling.

SUMMARY.

1. The present paper deals with the seedling structure of about fifty species belonging to the Compositae.

2. All the seedlings belong either to the diarch or to the tetrarch type.

3. Variations in vascular structure occur not only in nearly related species, but in different examples of the same species, indicating that seedling anatomy is of no value in questions of affinity.

4. It is held (*a*) that the evolution of the vascular structures of seedlings is probably not an extremely slow process; but that (*b*) tetrarchy and diarchy have probably been interchanged several times during the evolution of Angiosperms.

5. It seems probable that physiological factors are not sufficient to account for all the structures found in seedlings.

6. There are many exceptions to the rule that size of seedling is correlated with the type of structure.

BIBLIOGRAPHY.

1. COMPTON, R. H.: An Investigation of the Seedling Structure in the Leguminosae. Journ. Linn. Soc. (Botany), vol. xli, 1912.
2. CHAUVEAUD, G.: L'appareil conducteur des plantes vasculaires et les phases principales de son évolution. Ann. Sci. nat. (Bot.), 9^{e} sér., t. xiii, 1911, p. 113.
3. DANGEARD, P. A.: Recherches sur la mode d'union de la tige et de la racine chez les Dicotylédones. Le Botaniste, 1889, p. 75.
4. EVANS, W. E.: Effect of Environment on the Hypocotyl in the Genus *Luzula*. Notes, Roy. Bot. Garden, Edinburgh, No. xviii, 1907, p. 105.
5. GÉRARD, R.: Recherches sur le passage à la tige. Ann. Sci. nat. (Bot.), 6^{e} sér., t. xi, 1881, p. 367.
6. HILL, T. G., and DE FRAINE, E.: A Consideration of the Facts relating to the Structure of Seedlings. Annals of Botany, vol. xxvii, 1913, p. 257.
7. LEE, E.: Observations on the Seedling-anatomy of Certain Sympetalae. I. Tubiflorae. Annals of Botany, vol. xxvi, 1912, p. 727.
8. SARGANT, E.: The Origin of the Seed-leaf in Monocotyledons. New Phytologist, vol. i, 1903, p. 197.
9. ——————: A Theory of the Origin of Monocotyledons. Annals of Botany, vol. xvii, 1903, p. 1.
10. ——————: Evolution of Monocotyledons. Bot. Gaz., vol. xxxvii, 1904, p. 325.
11. ——————: Reconstruction of a Race of Primitive Angiosperms. Annals of Botany, vol. xxii, 1908, p. 121.
12. THOMAS, E. N.: A Theory of the Double Leaf-trace founded on Seedling Structure. New Phyt., vol. vi, 1907, p. 77.
13. VUILLEMIN, P.: Tiges des Composées. Paris, 1884.

(A more complete list will be found in the previous paper (7).)

Some Experiments on the Effect of External Stimuli on the Sporidia of Puccinia malvacearum (Mont.).

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With seven Figures in the Text.

In certain preliminary experiments in which the sporidia of *Puccinia malvacearum* were germinated near a fragment of hollyhock leaf, it was observed that all the germ tubes grew in one direction, and pointed towards the leaf fragment. This at first suggested positive chemotropism. More critical work, however, revealed the fact that it was an effect produced by the unilateral illumination of the sporidia. This led to the investigation of the tropic influences affecting the germinating sporidia of *Puccinia malvacearum.*

De Bary[1] first raised the question as to why the germ tubes of parasitic fungi turn towards, and penetrate the tissues of their host-plants. Even before the publication of the work of Pfeffer on external stimuli de Bary suggested that 'physical irritations' or chemical stimuli might play a part in these phenomena. Later, as a result of numerous experiments on the chemotropism of Fungi, Miyoshi[2] concluded that certain chemical substances attract the germ tubes of Fungi, whilst others repel them. Nordhausen[3] accepted Miyoshi's results and investigated the biology of the penetration of plant tissues by *Botrytis* and other facultative parasites.

More recently Massee[4] has concluded that the germ tubes of Fungi are attracted to their hosts by chemotropic substances secreted by the cell sap. In 1906 Fulton[5] repeated many of Miyoshi's experiments, and concluded that in the light of known facts, no simple explanation such as the theory

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[1] Vergl. Morph. und Biol. d. Pilze, 1884.

[2] Bot. Zeitung, Bd. lii, p. 1, 1894.

[3] Beiträge zur Biol. parasitärer Pilze. Jahrb. f. wiss. Botanik, 1898.

[4] On the Origin of Parasitism in Fungi. Phil. Trans. Roy. Soc. B., 1904.

[5] Chemotropism of Fungi. Bot. Gazette, 1906.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

of chemotropism will explain the entrance of the germ tubes of parasitic Fungi into the tissues of the host-plant. He also found that various Fungi show positive hydrotropism, but that an over-abundance of moisture may cause a negative reaction in certain Fungi.

From a large number of experiments on the effect of tannin upon the germination and growth of many Fungi, Cook and Taubenhaus[1] in 1911 found that this substance has a tendency to retard the growth of Fungi. They found also that the parasitic forms are more sensitive to the action of tannin than the saprophytic forms they used. It should be noted, however, that the parasites employed were not of the obligate class, as they could be cultivated upon nutrient media apart from the living host-plant. These investigators suggest also that tannin may, to some extent, serve as a protective agent against the attacks of parasitic Fungi.

Up to the present the Fungi used in this kind of work have, for the most part, been saprophytes or facultative parasites. We have, therefore, very little information respecting the influence of external stimuli on the germ tubes of obligate parasites. The causes which determine the penetration of the epidermis of the host-plant by the germ tube of the sporidium of *Puccinia malvacearum* are obviously somewhat different from those operating in the case of infection by uredospores or aecidiospores. In infection by the latter, entrance is effected through the stomata, and a very considerable amount of growth can occur at the expense of the reserve material in the relatively large spore before the fungus enters into its normal relationship with the host-plant. On the other hand, the sporidium is a small thin-walled spore with very little reserve material, and consequently, in order to succeed, its germ tube must promptly enter the tissues of the host. What determines this entry is unknown. In order to obtain some insight into the stimuli affecting the germinating sporidia, experiments were designed to test the effects of fragments of various leaves, of light, of gravity, and of water upon them.

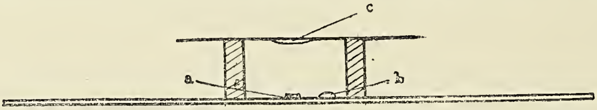

FIG. 1. A sectional elevation of a Van Tieghem cell as used in these experiments. *a*, fragment of a sorus with ripe teleutospores; *b*, a small drop of water; *c*, a drop of gelatine.

Most of the experiments were carried out in Van Tieghem's cells, and large pure sowings of sporidia were obtained from germinating teleutospores by the device shown in Fig. 1. A small fragment of hollyhock leaf bearing a portion of a teleutospore sorus (*a*) was fastened to the floor of the cell by a small quantity of vaseline. Near this, but not in contact with it,

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[1] The Relation of Parasitic Fungi to the Contents of the Cells of the Host-plants (Toxicity of Tannin). Bull. 91, Delaware Coll. Agri. Exp. Station, Feb. 1911.

was placed a drop of water (*b*) to ensure a saturated atmosphere. A large rectangular cover-slip was used, and the whole slide with the cell was inverted so that the sporidia, when produced, would fall on the cover-glass (*c*). A good sowing of sporidia was usually obtained in twelve hours. The sporidia were found to germinate equally well when sown in a thin film of water, or on the surface of 2 per cent. gelatine. For most of the experiments the sporidia were allowed to fall on a drop of 2 per cent. gelatine spread on the cover-glass of the cell. In this way the possibility of the direction of the germ tubes being altered by the necessary movement of the cell was avoided.

The first series of experiments was arranged to test whether fragments of various leaves, placed near the germinating sporidia, exercised any influence on their germination or on the subsequent direction taken by the germ tubes. The leaf fragment in each case was cut with a clean scalpel, washed with distilled water, roughly dried on filter paper, and placed on the surface of a drop of gelatine on the cover of a cell. The sporidia were sown around the leaf fragment by the device already described, and all the cultures were kept in the dark. These were examined after twenty-four hours and the results were as follow. For fragments of the leaves of *Althaea rosea*, *Ranunculus Ficaria*, *Stellaria media*, *Oxalis* (sp.), *Fuchsia* (sp.), *Solanum tuberosum*, *Sinapis nigra*, *Crataegus Oxyacantha*, *Tropaeolum* (sp.), *Geranium Robertianum*, *Mercurialis perennis*, *Tradescantia* (sp.), *Quercus Robur*, and *Citrus Aurantium*, vigorous normal germination was obtained. For fragments of the leaves of *Primula vulgaris* and garden geranium (*Pelargonium*) germination was, to a large extent, inhibited. Although in these cases no normal germ tubes were produced, some cases of abnormal germination were observed in all the tests. Fragments of the leaf of *Eucalyptus globulus* showed similar inhibitory effects, although some normal germ tubes also appeared in this case.


FIG. 2. Sporidia in a culture near to a fragment of a garden geranium leaf showing abnormal germination. × 100.

From these results it is clear that the germination of the sporidia in close proximity to most of the leaf fragments was quite normal and vigorous. No difference could be detected between the behaviour of the sporidia germinated near a fragment of the leaf of the normal host-plant, *Althaea rosea*, and that of sporidia near leaves of many of the other species used in these experiments. Whilst no positive stimulatory results were obtained, it has been mentioned that certain negative effects were seen with leaf fragments of *Pelargonium*, *Primula vulgaris*, and *Eucalyptus globulus*. None of the sporidia germinated normally in the vicinity of fragments of the leaves of *Pelargonium* and *Primula vulgaris*. The sporidia in these cultures, however, made many abortive attempts at germination. Instead of putting out a normal germ tube the sporidium became distended, giving rise to one or more bud-like structures (Fig. 2). The contents of these were granular and contrasted strongly with the hyaline appearance of the normal germ tubes. These abortive germ tubes did not grow any further.

Similar abnormal germinations were observed where fragments of the leaves of *Eucalyptus globulus* were used, but here the inhibitory effect on germination was not so strongly marked; for some of the sporidia in each culture germinated normally. In one of these experiments with *Eucalyptus* a series of counts was made of the actual numbers of sporidia which, respectively, germinated normally, abnormally, or not at all. These counts were taken in successive areas, moving from the leaf fragments outwards to the margin of the drop. Table I shows the results of these counts.

TABLE I. (Exp. 35, Series II.)

		Total No. of spores in area.	*No. of spores showing normal germination.*	*No. of spores showing abortive germination.*	*No. of spores not germinated.*	*% of normal germination.*	*% of abnormal germination.*
Area next leaf fragment	1	66	13	44	9	19	66
	2	80	17	35	28	21	43
	3	57	16	9	32	28	15
	4	55	11	5	39	20	9
	5	46	13	1	32	26	2
↓	6	68	62	—	6	91	—
Area most remote from leaf fragment	7	55	55	—	—	100	—

The two columns on the extreme right give percentages obtained from the figures in the corresponding counts. The percentage of sporidia which germinated at all, near the fragment, was small, but increased on moving away, until on the margin of the drop 100 per cent. of the sporidia germinated normally. More striking, however, is the fact that the percentage of abnormal germinations was greatest near to the leaf fragment, and gradually diminished to nil on the margin of the drop.

It seemed possible that in the experiments with the fragments of garden geranium leaf, the substance which affected the germination of the sporidia might be localized in the glandular hairs of the leaf. An experiment was accordingly set up, in which the sporidia were allowed to germinate near to a fragment from which the epidermis had been carefully removed. In this case excellent germination took place. It has been mentioned above that where a fragment of the leaf of *Geranium Robertianum* was used, quite normal and vigorous germination resulted. The material of this species used had very few glandular hairs.

A number of experiments was carried out in which the sporidia were sown upon the epidermis of short pieces of the petiole of leaves of *Althaea*, *Pelargonium*, and potato respectively. After three days the epidermis on which the sporidia rested was stripped off, fixed in alcohol, and either stained in erythrosin and mounted in glycerine or embedded and cut in serial sections. No normal germination was noted in the case of *Pelargonium*, the sporidia having behaved as in the cover-glass cultures. On the leaves of *Althaea* and potato, however, the germ tubes were normal and their tips showed a slight swelling which was pressed against the epidermis (Fig. 3).

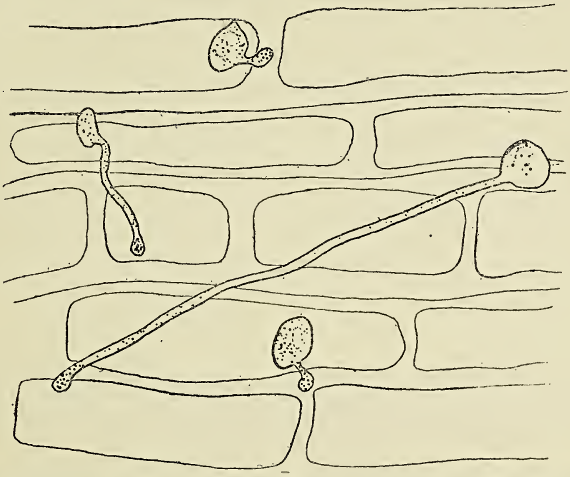

FIG. 3. Sporidia germinating on the epidermis of a fragment of the petiole of a potato leaf. Normal germination with a slight swelling at the tip of each germ tube pressed against the epidermis, but no penetration of the cuticle. × 300.

No case of penetration of the epidermis by the germ tube was seen in the case of the potato, though on *Althaea* the tip of the germ tube had penetrated the epidermis and formed an infection vesicle within the epidermal cell. This normal infection has already been described and figured by Kellerman,[1] Rathay,[2] Eriksson,[3] and myself.[4]

My attention was called to the negative heliotropism of the germ tubes by a preliminary experiment in which they appeared to be directed towards a fragment of leaf on the drop of gelatine. More critical examination of

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[1] Sitzber. Phys. Medic. Soc., Erlangen, 1874.

[2] Ueber das Eindringen der Sporidien-Keimschläuche der *P. malvacearum* in die Epidermiszellen der *Althaea rosea*. Verh. k. k. Zool. Bot. Ges., Wien, 1881.

[3] Der Malvenrost. Kungl. Svenska Vetensk. Hand., Bd. xlvii, 1911.

[4] On Some Relations between *P. malvacearum* and the Tissues of its Host-plant. Memoirs of the Manchester Literary and Philosophical Society, 1913.

this experiment suggested that the direction was influenced by the shadow cast from the leaf fragment and not by the substance of the leaf. The effect of light on the direction taken by the germ tubes was therefore investigated.

Several pairs of cultures were set up in which sowings of sporidia were made on the surface of a drop of gelatine as already described. One from each pair was placed on the laboratory bench in such a position that it was illuminated from one side by the light from the window. The control cultures were placed in the dark. After 16 hours all the sporidia in every cell had germinated equally well. Whilst the germ tubes of those in the dark were indifferent as to direction, the germ tubes of those which were illuminated from one side had all grown away from the light. Fig. 4, A and B, represents one pair of these tests. In this figure and in Figs. 5 and 6 the position of the spores and the direction of the germ tubes are accurately indicated, the figures having been drawn with the aid of the camera lucida.

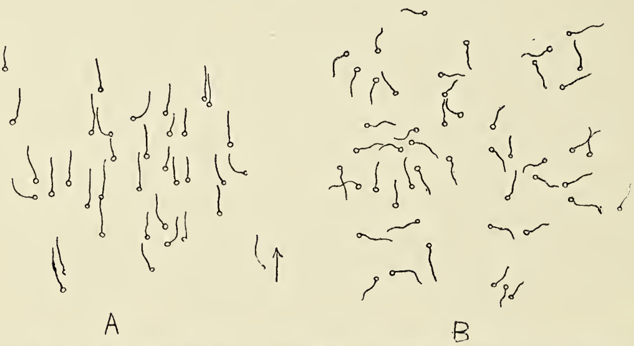

Fig. 4. A. Sowing of sporidia showing germ tubes growing away from the light of the window. The arrow indicates the direction of the light. B. Control to the experiment figured in A carried out in the dark: the germ tubes are growing in all directions. × 45.

In order further to test this effect of light on the direction of the germ tubes another double series of cultures was set up. One from each pair of these was placed on the bench as before in front of the window. The controls, instead of being placed in darkness, were rotated horizontally on a klinostat which was placed in front of the same window. As before, all the germ tubes in the cultures at rest were directed away from the light, while in the control cultures they had grown in all directions. The resulting appearance was so similar to that shown in Fig. 4 that illustration is unnecessary.

A still further confirmation of this effect of light was obtained by the following experiment. One of the cells was arranged as before, except that a minute partition of tin-foil, about 1 mm. high and 1 cm. long, was fixed on the surface of the gelatine. The culture was placed before the window with the partition parallel to the window. Fig. 5 shows the result; the germ tubes on the light side (A) of the partition (*p*) are all growing towards the latter, i. e. away from the light. On the other side (B), however, the spores lying in the zone of shadow have their germ tubes directed towards the partition, i. e. further into the shade. A little further away they are growing in all directions, whilst entirely beyond the influence of the shadow they are pointing away from the light of the window. A control culture with a similar partition in the dark showed, as before, the germ tubes growing indifferently in all directions.

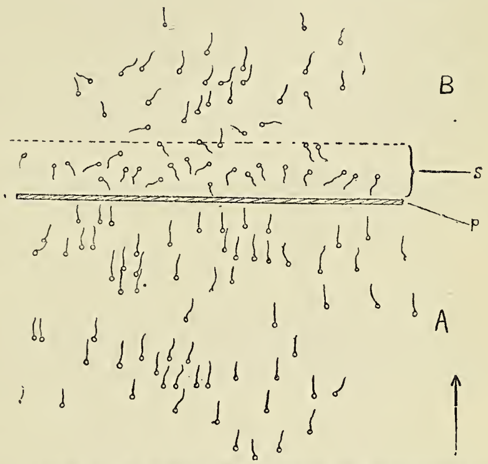

FIG. 5. A sowing of sporidia on both sides of a partition (*p*) of tin-foil; the arrow indicates the direction of the light, and *s* is the shadow between the dotted line and the partition. × 45.

Miyoshi[1] found in his work on the chemotropism of Fungi that light exercised no influence upon the germinating spores of the Fungi he used in his experiments. It should be noted, however, that he only experimented with one obligate parasite, viz. the uredospores of *Uredo linearis*.

For purposes of comparison similar series of experiments to those described were set up in which aecidiospores of *Puccinia poarum*, the conidia of *Botrytis* (sp.), *Alternaria*, *Penicillium glaucum*, and *Peronospora parasitica* were employed. The results of these experiments testing the effect of light, as well as those already described for the sporidia of *P. malvacearum*, are summarized in Table II.

TABLE II.

Spore.	*No. of tests separately made.*	*Medium of hanging drop.*	*Effect of unilateral light.*
Sporidia of *P. malvacearum*	25	2 % gelatine	All germ tubes directed away from light.
Aecidiospores of *P. poarum*	6	2 % gelatine	Indifferent to light.
Botrytis (sp.) . . .	12	2 % gelatine	All germ tubes directed away from light.
Alternaria (sp.) . .	4	2 % gelatine	Indifferent to light.
Penicillium glaucum .	6	2 % gelatine	,, ,,
Peronospora parasiticum	6	Water	,, ,,

Fulton[2] in investigations on the chemotropism of Fungi obtained results which he regarded as negativing Miyoshi's conclusions. He did not specially test the effect of light, but states that it probably does not enter as a tropic factor. This appears to be so for some cases. My experiments for *Botrytis*,

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[1] Loc. cit.

[2] Loc. cit. p. 105.

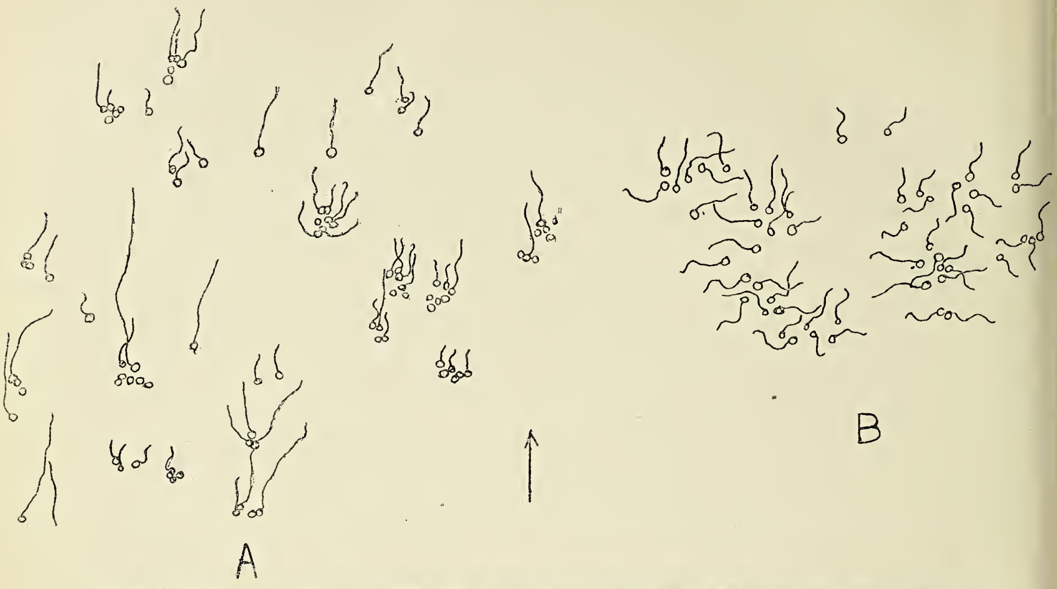

FIG. 6. A. Sowing of the conidia of *Botrytis* showing germ tubes growing away from the light of the window. The arrow shows the direction of the light. × 45. B. Control experiment carried out in the dark; the germ tubes growing in all directions. × 45

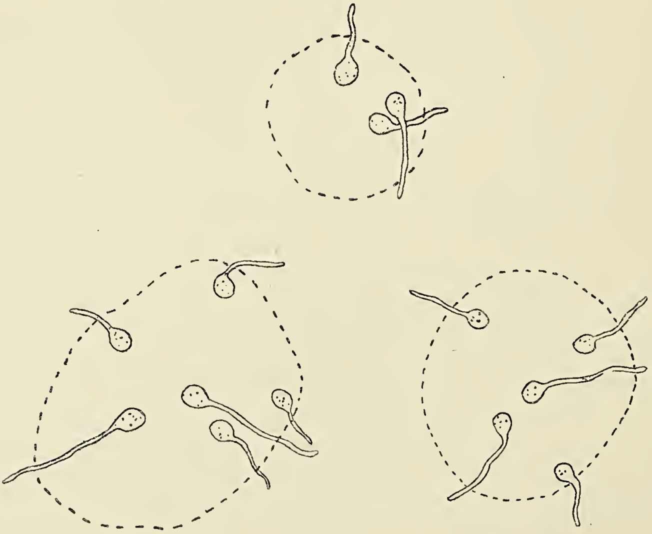

FIG. 7. Sporidia germinating in droplets of condensation water. All the germ tubes are directed out of the droplets which are indicated by the dotted lines. × 100.

which was one of the species Fulton used, show conclusively that the direction of the growth of the germ tubes was influenced by light. Fig. 6 A represents one of these experiments with *Botrytis* and Fig. 6 B is the corresponding control which was kept in the dark.

The effect of water on the germination of the sporidia is of some interest. In certain preliminary experiments where the sporidia germinated in the droplets of water which condensed on the cover of the cell, it was observed that the germ tubes were invariably directed towards the margin of the droplet and often grew out of it (Fig. 7). It was not decided how far this was an expression of negative hydrotropism or merely of the need of the growing germ tubes for oxygen. It may be mentioned that where the sporidia were germinated in an atmosphere saturated with water vapour they frequently produced germ tubes which formed a slight swelling at the tip and grew into the gelatine.

The growth and direction of the germ tubes did not appear to be influenced in any way by gravity.

SUMMARY AND CONCLUSION.

The results of the experiments made on the effect of various stimuli on the germinating sporidia of *Puccinia malvacearum* may be briefly summarized thus:

1. The sporidia of *P. malvacearum* have been shown to be negatively heliotropic, and this is also the case for the conidia of a species of *Botrytis*; whilst with conidia of *Penicillium*, *Alternaria*, *Peronospora*, and aecidiospores of *Puccinia poarum* no irritability to light was apparent.

2. Other influences shown to affect the germ tubes are moisture and contact. As regards moisture, the germ tubes tend to grow out of a drop of water into the moist atmosphere around. On the other hand, germ tubes, on the surface of gelatine in a moist atmosphere, tend to penetrate the gelatine. These influences are difficult to analyse satisfactorily. With respect to contact, the tip of the germ tube swells and becomes closely applied to the epidermal surface of both the host and non-susceptible plants. This may, perhaps, be a result of the contact.

3. On the normal host-plant a very slender growth from the swollen end of the germ tube penetrates the cuticle and outer wall of the epidermis, and this brings about true infection. This has not been seen in any case on plants other than the normal hosts although numerous attempts at infection were made.

4. No evidence of chemotropic influences radiating from a fragment of leaf laid on a drop of gelatine could be obtained. There were no indications of positive chemotropism of the germ tubes towards the normal host or of negative chemotropism of the germ tubes towards fragments of non-susceptible leaves.

5. In the case of certain leaves (*Pelargonium*, *Eucalyptus*, *Primula*) with glandular secretions, a definite toxic effect was evidently exerted on sporidia and germ tubes in the immediate neighbourhood of the fragment of the leaf. This is a special phenomenon presumably related to the presence of glandular secretions and cannot be extended generally to leaves that are not susceptible to infection.

So far as the results bear on the question of why the normal host should be infected whilst other plants are not, they simply confirm this fact without explaining it. They do not support any of the explanations so far offered of this natural immunity or susceptibility, nor do they indicate the direction in which an explanation is to be sought.

The experiments, however, result in indicating that the germ tube is irritable to light, to a certain degree of moisture, and to contact. These irritabilities are possibly advantageous in bringing the tip of the germ tube of a sporidium into the most favourable position for infection, but the problem as to what permits or determines actual infection still remains.

In conclusion, I wish to acknowledge my indebtedness to Professor W. H. Lang for suggesting this investigation and for his continual advice and assistance in the course of the work. I am also grateful to Mr. D. Thoday for his helpful interest in the experiments.

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A New Araucarioxylon from New Zealand.[1]

BY

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With Plate XX and three Figures in the Text.

TWO years ago Mr. J. Allan Thomson, Palaeontologist to the Geological Survey of New Zealand, sent me a specimen for description of which he wrote: 'I have a fine specimen of wood with bark preserved, from the base of the Cretaceous as developed at Amuri Bluff.' Later on he kindly gave me further information about its geological horizon: 'At Amuri Bluff the wood sands lie beneath a calcareous conglomerate which contains ammonites, belemnites, Trigonia, Inoceramus, and Aporrhais. This in turn is overlain by mudstones with saurians (Cimiliosaurus). There has been so much discussion as to whether certain Tertiary beds higher up in the section are conformable or not to the Cretaceous that the fact that there are undoubted Cretaceous beds is not always made clear.'

Though the necessary work on the mollusca in the beds above the wood-containing beds is not yet completed, it is clear that our fossil is certainly Cretaceous, and probably of mid-Cretaceous age.

The wood contains several interesting details in its structure, and the surprising paucity of fossil remains of Araucarians in the very region where they are now native renders its description desirable, particularly at present, when the Araucarineae are being so much discussed.

DESCRIPTION OF THE SPECIMEN.

General. The fossil consists of part of a small trunk or branch, and is at present roughly circular in outline and about 8 cm. in diameter. It may have been part of a much larger stem, and certainly must have been at least a little larger when alive because it is now entirely decorticated. When first observed the trunk appears to consist of a smaller woody core, surrounded

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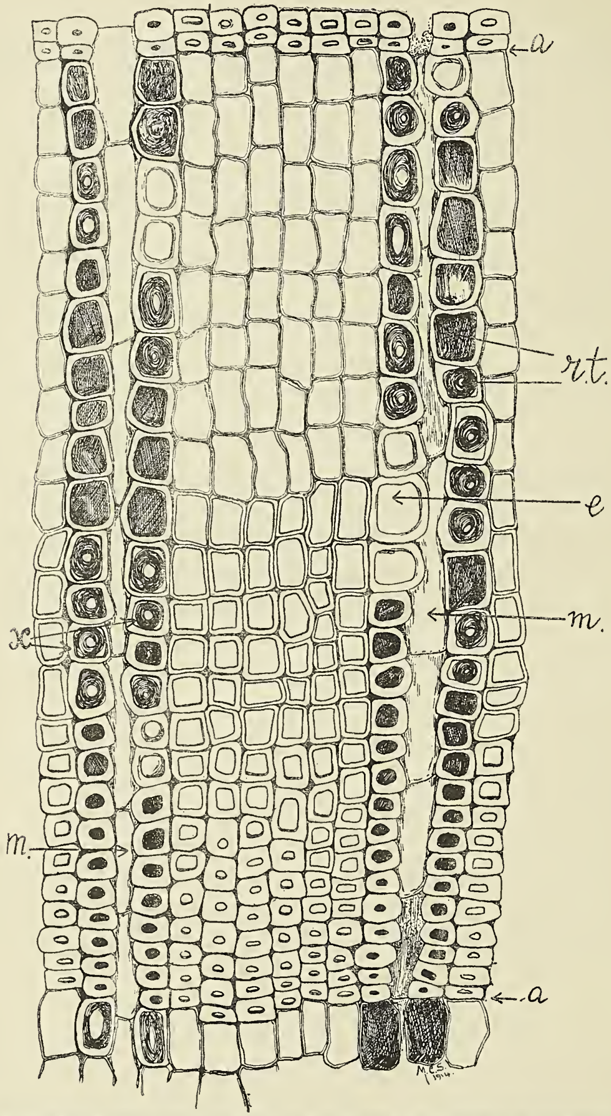
[1] Published by permission of the Director of the Geological Survey of New Zealand.

by a thick bark, as is seen in Pl. XX, Photo 1. This deceptive appearance depends on *a peculiarity of petrifaction*: the central region, of darker and denser structure, is about 3–4 cm. in diameter, and rather irregular in outline; its boundary bears no relation to the limits of the annual rings, but cuts sharply across them, as can clearly be seen in Photo 1, *s*; the outer zones of the fossil, as at *c*, are also composed of petrified rings of secondary



TEXT-FIG. 1. Transverse section of part of the wood, showing the sharp alternation between spring and autumn wood, *a*, *a*, limits of annual rings. *m*, medullary rays, bordered by special thick-walled tracheides *r.t.* *e* shows one of these with thick wall, but empty of resin; *x* shows some with thick walls and resin deposit round them.

wood: the distinction between the two regions of the same woody trunk depends on the fact that the core is silicified and the outer zones preserved in carbonates. The contrast between the two regions is very well marked when weak hydrochloric acid is poured on the cut surface: the inner zones are not affected at all by the acid, but stand out like a placid island in a surrounding sea of excited effervescence. It is noticeable that the inner zones of the wood of the carbonate region (that is, those which lie just outside the part preserved in silica) are very poorly preserved, or are entirely eliminated. It looks as though the change in the petrifying medium had upset the preservative properties of the mineral. The outer zones of the wood, though in the carbonate matrix, are fairly well preserved. The mineralization of this specimen suggests a number of interesting problems concerning the mode of penetration of two different petrifying fluids, the possible priority of one and its replacement by the other, the time required for the preservation of the wood, &c.; but this one specimen does no more than suggest, it does not solve the problems it raises.

Topography of the Stem: *Pith.* Owing to imperfect petrifaction, and also to a break in the centre of the stem, little of the pith remains. It is clear, however, that it was small (i. e. not more than 1 mm. in diameter), and that the cells composing it were rather large and irregular in outline.

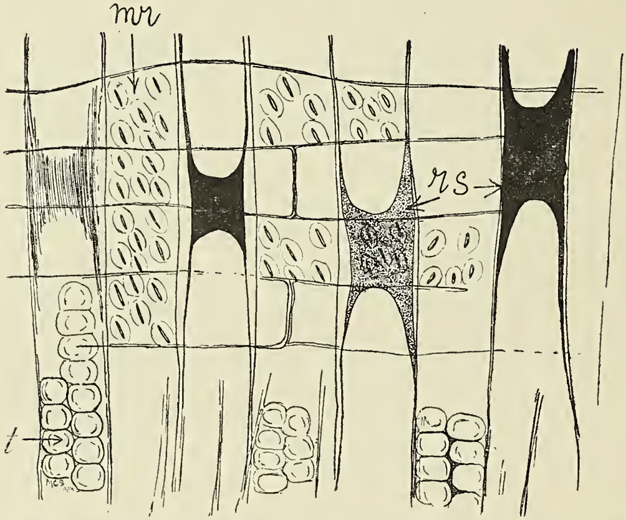
Growth rings are exceedingly well marked. The autumn wood cells are so thickened as almost to obliterate the cell lumen. The size of the rings varies from about 1 mm., consisting of about 36 cells, to small rings of about 10 cells in radial sequence. On an average the spring wood is about equal to the autumn wood in extent (see Photo 3), but owing to its markedly thinner walls it tends to be crushed in many parts of the specimen.

Wood elements. The radial diameter of the tracheides varies from 25 to 36 μ on an average, the last cells of the autumn wood being much less than this (see Text-fig. 1, *a*). Thickened 'resin' containing tracheides are noticeable on either side of the ray, and will be described below. The general build of the tracheides both of spring and autumn wood is indicated in Text-fig. 1 and Photos 3 and 4. But few pittings are to be seen in transverse section; in longitudinal section the preservation is not very sharp, but a number of elements show the typical Araucarian pitting, as indicated in Text-fig. 2, *t*. In the majority of the elements which show pitting at all, the pits are biseriate, alternating, and mutually hexagonally compressed. Special tracheides contain tannin (see below). Wood parenchyma was not observed.

Medullary rays. Simple, uniseriate rays are numerous throughout the wood. In radial extension they tally with 3–4 tracheides. The rays are principally 3 and 4 cells high, as judged from 133 counts in various regions of the sections.

6 rays were 1 cell high,
20 „ „ 2 cells „
42 „ „ 3 „ „
43 „ „ 4 „ „
15 „ „ 5 „ „
6 „ „ 6 „ „
1 „ „ 7 „ „ and none were higher than seven.

The cells of the ray are all alike, and undifferentiated into various types of elements (see Text-fig. 2 and Photos 5, 6 and 8). In radial sections the ray pitting can clearly be seen, at *mr*, Text-fig. 2, and in Photo 6 in several cells along the ray *mr*, also in Photo 8 on a larger scale of magnification. These pits are in groups of five or six, and are bordered. The slit is very noticeable in some cells, and is placed obliquely, which is the case in all living Araucarians, according to Thomson ('13).



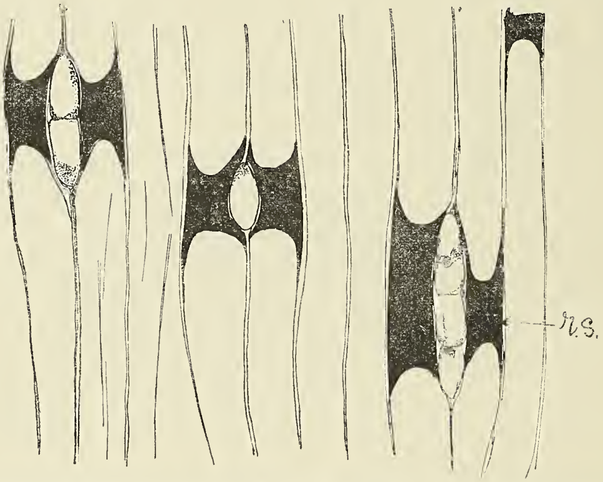
TEXT-FIG. 2. Radial section of wood showing 'resin spools', *rs*; the pitting on the tracheides *t*, and the pitting on the ray-cells *mr*.

Resin-containing tracheides. In both radial and tangential sections the most noticeable feature is the extensive 'resin' deposit in the elements neighbouring the medullary rays (see Photos 5, 6, 7 and 8, and Text-figs. 2 and 3). In transverse sections the radial series of tracheides running on either side of the medullary ray are generally differentiated from the others more or less noticeably, both by the thickening of their walls and by the filling up of their lumina by resin-like, thick contents. These can be seen at *rt* in Photo 2, and more clearly in Photos 3 and 4. Owing to the opaque nature of the fossil, even Photo 4, clearly as these cells show in it, does not convey quite the same noticeable contrast between these cells and the other tracheides as is evident to the eye of the observer. Text-fig. 1 perhaps gives a fairer idea of the appearance of the woody tissues. These cells show up particularly well where the summer wood is just a little crushed, as in Photo 3, where, outlining the slightly wavy rays, the blackened and relatively uncrushed resin-containing tracheides on either side of them show up distinctly. This is enlarged in Photo 4. The deposit of 'resin' collects in the form of large discs or 'spools' opposite the middle of the medullary rays, the lateral extensions of these discs running up and down the containing cell-wall for some distance (see Text-figs. 2 and 3 and Photos 5, 6, 7, and 8). As a consequence of the great quantity of this deposit there is a black, wing-like mass on either side of the medullary rays, as seen in a low-power view of the tangential section (see Photo 7), which gives the wood an unusual appearance. The rays are also rendered very conspicuous in radial section (see Photo 8).

The extent of this phenomenon seems to be greater in this fossil than in any other described form.

Some mention of such an appearance on a lesser scale has been made by Penhallow ('07), Lignier ('07) and Jeffrey ('12), but without great emphasis or much illustration. I had nearly finished my work on this new fossil, and concluded that it was very exceptional in the extent of its 'resin-spool' tracheides, when Professor Thomson's ('13) paper reached me. He gives, for the first time, adequate treatment and illustration to this peculiar feature; and as his work has largely forestalled what I had to say on the subject, I cannot do better than refer the reader to his paper, pp. 23–28, as I agree with his conclusions. The present fossil, however, seems to show this character even more markedly than the plants he mentions, so that its description appears justified and may help to draw attention to an interesting feature in the evolution of resin-containing tissue which has been considerably overlooked.

In 1907 Penhallow (pp. 53–58, Text-figs. 4–7) described the wood of living Araucarians as showing 'more or less numerous elements containing resin' which may be scattered, but are more commonly in rows next the medullary rays. He said: 'The walls of the tracheides may be much thicker just opposite the ray where the resin plate comes,' and he thought that in *Dammara* and *Araucaria* these resinous tracheides may be of specific value. Lignier ('07, Pl. XVII, Fig. 2), from his new species *Cormaraucarioxylon crasseradiatum*, gives an outline drawing of a portion of a medullary ray with specially thickened tracheides on either side. Regarding Lignier's and Penhallow's interpretation of these tracheides as having a thickened wall, Jeffrey ('12, p. 538) says: 'Lignier has described the thickening up of the tracheides adjacent to the rays in certain Araucarian woods from the French Jurassic. It seems entirely probable that he has mistaken resin-filled tracheides for thick-walled ones.' Jeffrey continues, saying that his own Fig. *f* ' makes it clear that the apparently thick-walled tracheides are in reality only tracheides more or less occupied by a plugging exudation from the rays'. While I think that the excessive thickness indicated by Professor Lignier is probably due to resin deposited round the wall (cf. Text-fig. 1, *x*), it is certain that the view that these tracheides do have thickened walls is correct. Professor Jeffrey's figures are on a very small scale of magnification, and are taken from a specimen in which the phenomenon is not nearly so fully developed as in other cases, and they do not suffice to disprove the view that these tracheides have thickened walls *as well* as resinous contents. Reference to my Pl. XX, Photo 4, as well as Text-fig. 1, will make it abundantly clear that in this fossil the walls are thicker than in the adjacent tracheides, (1) because there the thick walls are, and (2) because where the rest of the wood cells with their thinner walls are somewhat crushed and distorted, as in Photo 3, the rows on either side of the ray are rounded and uncrushed, and stand out as of sturdier build even in the cases where they are empty of their resin contents. In the living *Agathis australis*, where a similar, though less marked, phenomenon occurs, the thickening of the wall in the region of the ray is readily observable and is well figured by Thomson ('13, Pl. V, Fig. 44 *b*).



TEXT-FIG. 3. Tangential section showing the 'resin spools' *r.s.* on either side of the rays.

Penhallow made a comparison between these 'resin-spools' and the trabeculae of Sanio. This could only have been suggested by the ordinary cases where the amount of the deposit is very small. Had such an example as the present fossil been the first to be observed, no such comparison could have been made.

COMPARISON WITH PREVIOUSLY DESCRIBED SPECIES FROM NEW ZEALAND.

The poverty of records of fossil Araucarians from this region has already been remarked. Ettingshausen ('87, '91) has described a few impressions of foliage and imperfectly preserved woods from beds then called Tertiary, but some of which are now known to be Cretaceous. From foliage impressions he founded a species *Araucaria Haastii* in 1887 (p. 154, Pl. II, Figs. 1, 2) which has an axis richly set with leaves which are truly Araucarian in appearance, and agree closely with the living *A. chilensis*, Mirb. His *diagnosis* of the species takes into account only the external morphology, nevertheless following this diagnosis he appends the remark, 'A petrified wood has been found in the Tertiary strata at Malvern Hills which agrees best with that of *Araucaria*, with which species I classify it'. He then figures and describes a few details of the wood, which is poorly preserved, including it in the name *A. Haastii* just given to *foliage* impressions, without even referring to the fact that there is not the slightest evidence that the wood and the leaves belonged to the same plant. Modern palaeo-botanical diagnosis will not, of course, allow this name to stand; but for the present purposes of this paper I shall continue to call the wood *A. Haastii*.

Ettingshausen describes another Araucarian foliage branch with smaller leaves, under the name *Araucaria Danai*. The original specimens of the Paratypes, kindly sent me by the Geological Survey of New Zealand for examination, are very poorly preserved and I should hesitate very much to base a species on such foliage.

Under the one name *Dammara Oweni* Ettingshausen describes the impression of a cone, a cone scale, a leaf, and a piece of wood (!), all detached fragments, about which he had no evidence that they really belonged to each other. Consequently, they cannot properly be included under the one name. This material, however, does not seem to me to be well enough preserved to justify the founding of three fresh names for it, though an ardent species-monger will be bound to do so some day. The wood which is included in this comprehensive name *Dammara Oweni* appears truly to be a different species from the so-called *A. Haastii*, having very much larger pits, with closely adjacent hexagonal areas instead of the small, rounded, and separated borders of *A. Haastii*.

These records, it is evident, are of little phylogenetic or morphological value.

The New Zealand Geological Survey kindly sent me the type specimen of the wood described by Ettingshausen as *A. Haastii*, with permission to cut it for comparison with the present fossil. It is part of a trunk, larger than the new one now described, and is very poorly preserved, so that accurate comparison is not possible.

In the following points the new specimen differs from *A. Haastii*. *A. Haastii* has rather larger tracheides (about 45 μ as against 25 to 36 μ), with less rounding at the corners; the bordered pits are rounded and separated by considerable areas of tracheide wall; the rays are higher than in the new fossil, averaging 2 to 5 cells and running up to as many as 16; and the 'resin-spools' are not evident.[1] Consequently, the new specimen cannot be the same plant as the so-called *Araucaria Haastii*, and as I am not aware of any other specimen with which it might be included, I will therefore proceed to name and diagnose it as a new species.

Araucarioxylon novae zeelandii, sp. nov.

Diagnosis. Characters of a typical *Araucarioxylon*. Growth rings exceptionally well marked, tracheides uniform, 25 to 36 μ in diam., radial rows uninterrupted. Tracheides adjacent to medullary rays with noticeably thickened walls, filled with 'resin' which makes very large and conspicuous 'spools' on either side of the ray cells in tangential and radial section. Rays uniseriate, undifferentiated, 1 to 7 cells high, principally 3 and 4 cells high.

Type. Block and sections, Geological Survey, New Zealand.

Age. Cretaceous [Mid-Cretaceous?].

Locality. Amuri Bluff, New Zealand.

The existence of such well-defined growth rings in an Araucarian of Cretaceous age in this region is of special interest because it affords evidence which is strongly presumptive of well-marked seasons. Mr. Allan Thomson writes that this is the first piece of evidence obtained in New Zealand on this point either from plant or animal fossils. It is well known that among living Araucarians annual rings are by no means always clearly developed. A *résumé* of the various views on the annual rings in this family is given by Professor R. B. Thomson ('13, p. 32), and he says that in a Cretaceous species he has found poorly marked growth rings of irregular occurrence. The present specimen, however, has such extremely well marked rings that there is every reason to see in them true annual

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[1] Though I greatly object to naming such poorly preserved material, as this specimen is an original type of Ettingshausen's, and as I have examined new sections of it, and as its name cannot stand, I must propose a new name for it. The pitting is very poorly preserved and I cannot see the ray pitting at all, but the round widely separated pits leave me in doubt if it is an Araucarian. ? *Araucarioxylon Ettingshauseni*, sp. nov.

Diagn. Tracheides average 45 μ in diam.; bordered pits rounded and separated; rays chiefly 2 to 5 cells high, up to 16. 'Resin-spools' not evident.

Type. Ettingshausen's wood included in his foliage species *Araucaria Haastii*; very poorly preserved.

Age. Doubtfully Cretaceous.

rings. Their slight irregularities are only those to be found any day in a fir or pine trunk which has grown normally in a climate with well-marked seasons like our own.

The value of growth rings in the Gymnosperms, where the foliage is evergreen, is very much greater than in the Angiosperms, where they may be due to simple leaf-fall, which, as we know from plants now growing in the tropics, may take place irrespective of the seasons. Gothan ('08) in his paper dealing with the climates of the Jurassic and Cretaceous epoch lays much stress on this.

Such an appearance as seen in Photos 1 and 3 in an Araucarian, therefore, makes the presumption in favour of the existence of well-marked seasons where it was growing an exceedingly strong one.

SUMMARY.

The specimen is petrified in an interesting manner, with a central core of silicified tissues surrounded by wood preserved in mineral carbonates.

It is described as a new species because it differs greatly from the more imperfect specimens of fossil Araucarians hitherto recorded from that region.

It is marked out from all other fossils of the kind by the extreme development of its rows of thickened tracheides on either side of the rays, which are filled with large 'resin-spools'.

It has also much more regular and strongly marked annual rings than are customary in Araucarians.

It is held to afford good evidence that the region, now New Zealand, had well-marked seasons in Mid-Cretaceous times.

PAPERS REFERRED TO.

ETTINGSHAUSEN, Baron C. von ('87): Beiträge zur Kenntniss der fossilen Flora Neuseelands. Denkschriften K. Akad. Wiss. Wien, vol. liii, pp. 143–192, Pls. I–IX.

——————— ('91): Contributions to the Knowledge of the Fossil Flora of New Zealand. Trans. New Zealand Institute, vol. xxiii (for 1890), pp. 237–310, Pls. XXIV–XXXIII. [Descriptions of species translated from the Denkschrift. K. Akad. Wiss. Wien, vol. liii, 1887.]

GOTHAN, W. ('08): Die Frage der Klimadifferenzierung im Jura und in der Kreideformation im Lichte palaeobotanischer Tatsachen. Jahrb. K. Preuss. Geol. Anst., vol. xxix, pp. 220–42, Pls. XVI–XIX.

JEFFREY, E. C. ('12): The History, Comparative Anatomy and Evolution of the Araucarian type. Proc. Amer. Acad. Arts and Sci., vol. xlviii, No. 13, pp. 531–40. Pls. I–IV.

LIGNIER, O. ('07): Végétaux fossiles de Normandie, IV. Bois divers. Univers. Caen., Mém. Soc. Linn. Normand., vol. xxii, pp. 239–332, Pls. XVII–XXIII.

PENHALLOW, D. P. ('07): A Manual of the North American Gymnosperms, exclusive of the Cycadales, but together with certain Exotic Species. Boston, 1907. Pp. 374, Pls. LV.

SEWARD, A. C., and FORD, S. O. ('06): The Araucarieae, Recent and Extinct. Phil. Trans. Roy. Soc., Lond., B., vol. cxcviii, pp. 305–411, Pls. XXIII–XXIV.

THOMSON, R. B. ('13): On the Comparative Anatomy and Affinities of the Araucarineae. Phil. Trans. Roy. Soc., vol. cciv, pp. 1–50, Pls. I–VII.

EXPLANATION OF PLATE XX.

Illustrating Dr. Marie Stopes's paper on a new Araucarioxylon from New Zealand.

All figures illustrating *Araucarioxylon novae zeelandii*, sp. nov. Photos 1, 3, and 4 taken by Mr. Herring, and 2, 5, 6, 7, and 8 by Mr. Pittock.

1. Part of the area of the petrifaction, showing the whole central core of silicified wood (this shows up darker than the rest, its broken outline marked at *s*) surrounded by less well preserved zones in carbonate, the outer regions at *c* showing again the annual rings and the wood tissue fairly well preserved. (× slightly over 2 diam.)

2. Small part of transverse section. *rt*, resin-containing tracheides bordering annual rings.

3. Transverse section showing clearly the alternation of spring and autumn wood; the spring wood slightly crushed, through which the resin-containing tracheides bordering the medullary rays stand out very clearly.

4. Part of Photo 3 highly magnified. *aa*, limit of annual ring. The contrast between the thin-walled summer wood and the thicker-walled, resin-containing tracheides running on either side of three rays shows up very clearly at *x*.

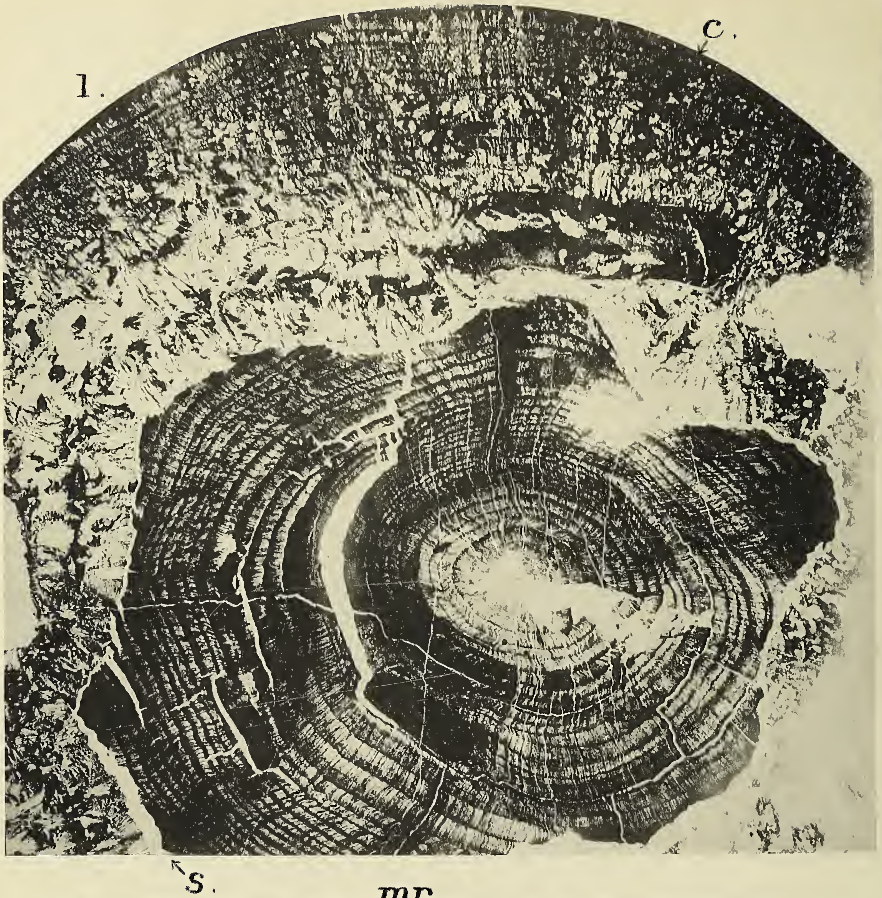
5. Radial longitudinal section of the wood showing the masses of the 'resin-spools'.

6. Portion of a similar section, showing also the pitting of the ray cells along ray marked *mr*.

7. Tangential section of wood, showing the rays with the wing-like masses of resin adjacent to each.

8. High power view of radial section, showing two medullary rays largely obscured by the quantities of 'resin-spools'. Below *mr* the pitting of the ray cells can be seen.

1.



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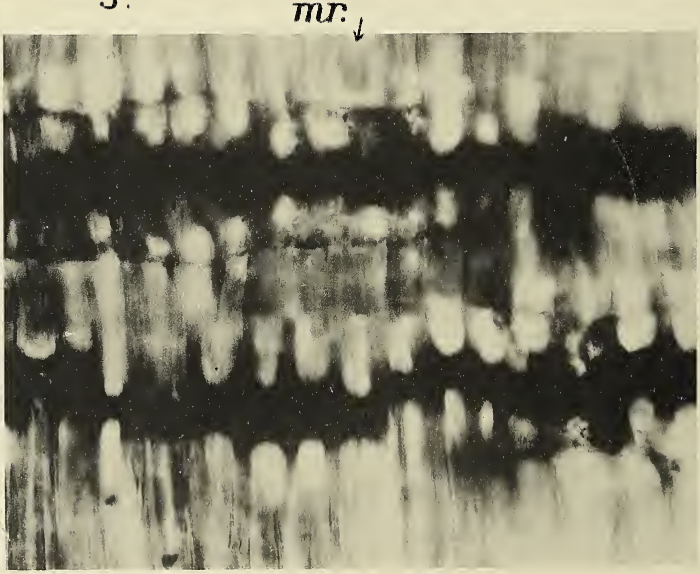

STOPES — ARAUCARIOXYLON.

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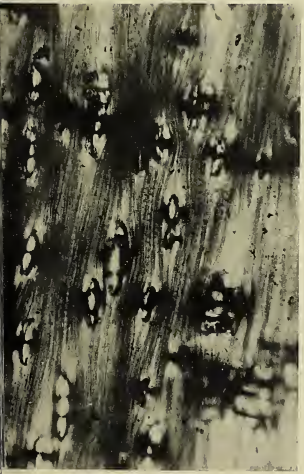

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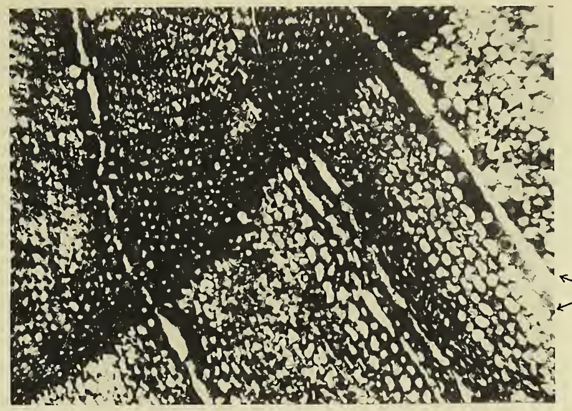

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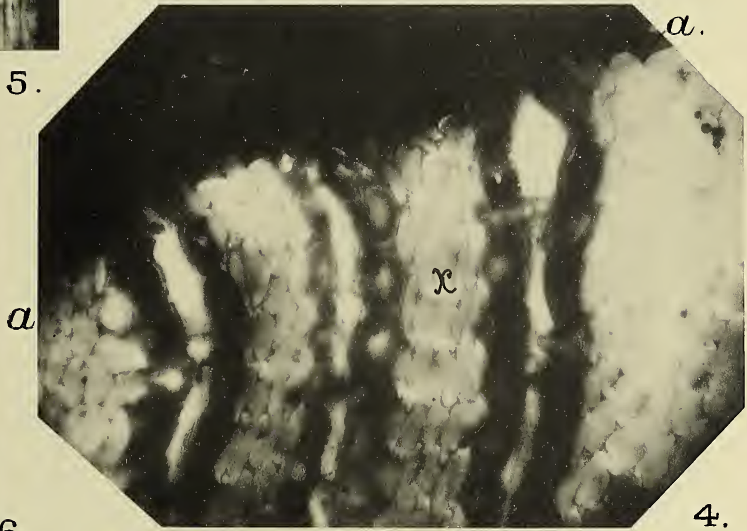

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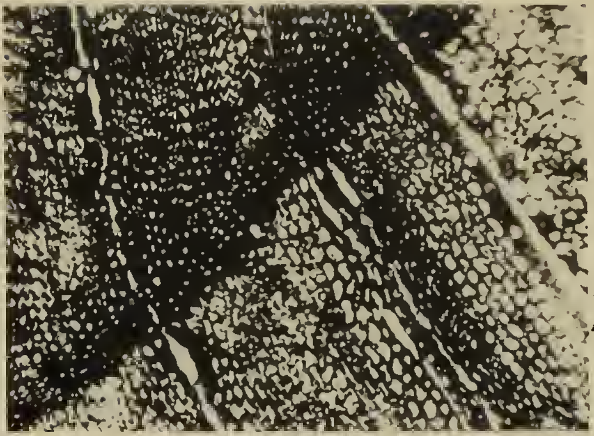
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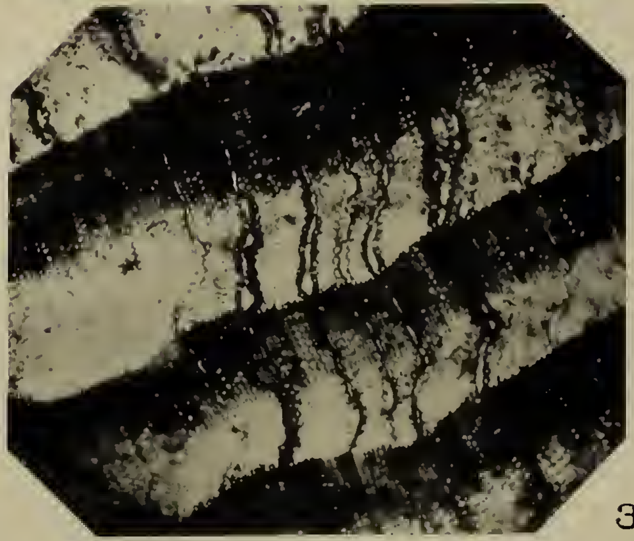
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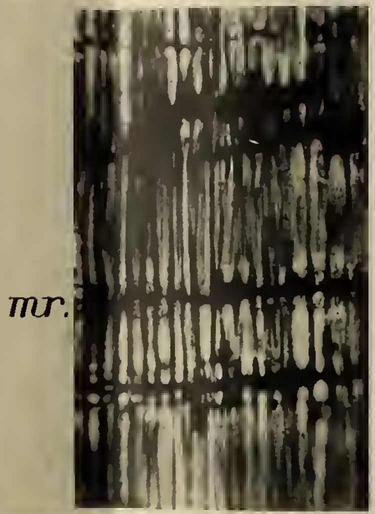
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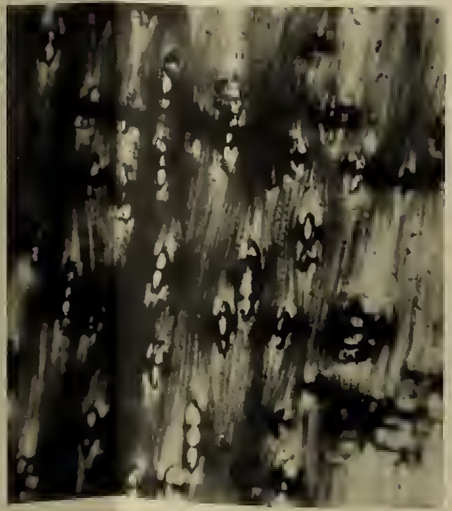
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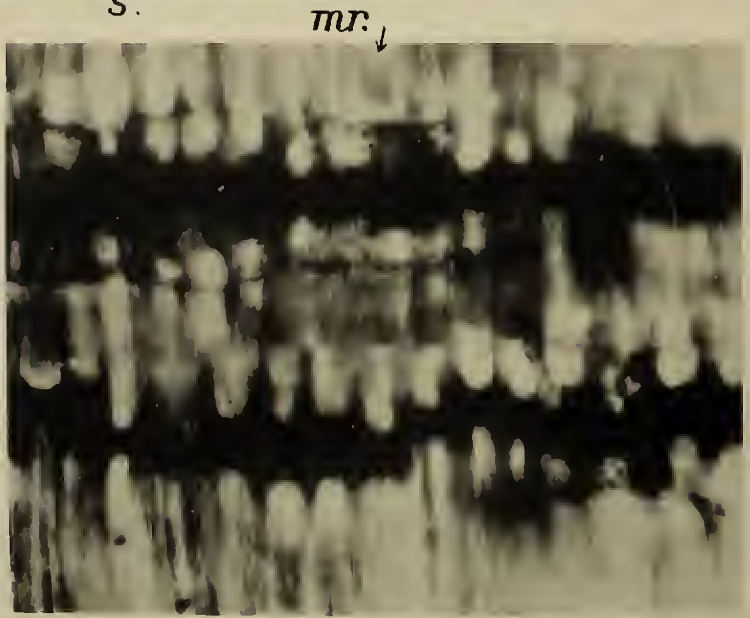
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On a ‘Mixed Pith’ in an Anomalous Stem of Osmunda regalis.

BY

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

Professor of Botany in Queen's University, Belfast.

With Plate XXI.

IN a paper published by Dr. R. Kidston and myself in the year 1910 an account is given of a fossil plant, *Osmundites Kolbei*, Seward, which deserves particular attention because it is the first member of the Osmundaceae that has been described with a ‘mixed pith’.[1] That is to say, the medulla of this plant is not homogeneous, but contains a number of well-defined tracheal elements scattered throughout its tissue. It is, in fact, exactly the same as the mixed piths that are present in the steles of several of the Zygopterideae (*Ankyropteris Grayii*, *A. corrugata*, &c.). The discovery of the existence of a mixed pith in the Order Osmundaceae attracts special interest to an anomalous stem of *Osmunda regalis* that was met with in the course of the advanced class-work at Birkbeck College, London, and was kindly placed at my disposal by the Head of the Botanical Department. The plant in question was not grown in cultivation, but so far as could be ascertained was collected at random in a wild condition from a moist situation in Cornwall.

A number of sections, perhaps a dozen or more, were cut from the lower end of the stem by the students of the class, in all of which the anomaly probably occurred. A few of these were recovered and came into my possession together with the remainder of the stem. Further sections were then taken from the exposed end of the stem, but, unfortunately, after three or four had been obtained the anomaly abruptly came to an end.

In the region of the anomaly the stem was 8 mm. in diameter and the stele, which contained a ring of nine separate xylem strands, about 2·5 mm. In the stele the xylem, phloem, and porose layers are perfectly normal, but the parenchyma of the pith contains a large number of scattered tracheal elements (Figs. 1 and 2). In one section there are well over sixty of these

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[1] Kidston and Gwynne-Vaughan: On the Fossil Osmundaceae, Pt. IV. Trans. Roy. Soc. Edin., vol. xlvii, 1910, p. 455.

[Annals of Botany, Vol. XXVIII. No. CX. April, 1914.]

medullary tracheae distributed quite irregularly over the whole area of the pith. They occur as isolated elements or in small groups up to as many as nine together. In a few cases they are seen in contact with the tracheae of the normal xylem ring, but they do not extend into the medullary rays. As seen in transverse section they are very irregular in form and vary much in size, closely resembling in these respects the parenchymatous cells of the rest of the pith. A few, however, are somewhat larger and more or less vesicular or sack-like in appearance. Since the surfaces of the end walls of most of them are visible in a transverse section they must be quite short in the vertical direction. Indeed, some of them seem to be broader transversally than they are long (Fig. 1). The elements in question are entirely tracheal in character. They are quite dead and empty, with thick and well-lignified walls. Their pitting is irregularly scalariform, passing over in many cases to a reticulate or even a porose marking (Fig. 3). A very similar reticulate-porose marking is also characteristic of the medullary tracheae of *Osmundites Kolbei* (Fig. 4).

The whole length of the stem, which was about 55 mm. long, was carefully cut into successive transverse sections, but no more medullary tracheae were encountered until a point was reached about 8 mm. from the apex, where they appeared once more and persisted for about a dozen sections. Above this point the stem was cut longitudinally, but did not disclose any medullary tracheae. In this upper anomalous region the medullary tracheae are essentially the same in character as those in the lower region, but they are fewer in number, not exceeding nine in any one transverse section (Fig. 5).

The presence of this unusual type of pith in our specimen is undoubtedly due to injury. Indeed, the pith is obviously in an unhealthy condition throughout the whole length of the stem. It is traversed in all directions by cracks which in alcohol material are filled with a yellow mucilaginous substance. They are formed partly by the tearing apart of the pith cells and partly by the breaking down of the same, and are usually bordered by the remains of the disintegrated cells (Figs. 1 and 5). It is seen that the medullary tracheae are mostly found in close relation to these cracks, from which in Fig. 5 the mucilage has been removed by the action of eau de Javelle.

It is not clear whether the plant was suffering from some constitutional disease or whether it had received a definite external wound. The fact that there is no trace of such a wound and that the anomaly reappears in a region just below the apex seems to point to the former suggestion. At the same time a traumatism may appear at a considerable distance away from the wound that causes it, and in this case the wound may have been inflicted on a part of the stem below that collected. The lower anomalous region may have been formed shortly after the reception of the injury, and since the plant would still be in a debilitated condition, a weak place in the stem would probably result, where it would be very likely to snap off when the specimen was collected.

In *Osmundites Kolbei* a mixed pith was found throughout the whole length of the specimen examined, and there is no reason to believe that it is not a perfectly constant and diagnostic character of the plant. Apart from the larger size and vertical elongation of the medullary tracheae (Figs. 4 and 6), it is essentially the same type of pith as that in the specimen of *Osmunda regalis* described above. It may be held, therefore, that if the normal differentiation of the pith of the present-day *Osmunda regalis* is interfered with by traumatic conditions, some of its elements are liable to revert to their earlier phylogenetic habit and are still able to give rise to tracheae instead of parenchyma. We have thus a return to an ancestral type of pith-structure that was normal in the Cretaceous fossil *Osmundites Kolbei*. If this is so, additional support is given by our specimen to the theory that the pith of the Osmundaceae is truly stelar in origin; that it arose by the conversion of the central tracheae of an originally solid mass of xylem into thin-walled parenchyma; and that this transformation did not take place simultaneously in all of them, but some retained their tracheal characters long after the rest had become converted into parenchyma.

The fact that valuable phylogenetic information may be obtained from traumatic variations in structure has already been pointed out in the Gymnosperms, both fossil and recent, by Professor E. C. Jeffrey.[1] He has shown that tracheidal cells appear traumatically in the medullary rays of the secondary wood of *Cunninghamia sinensis*, in the normal secondary wood of which they are entirely absent. It is interesting to note that these traumatic ray-tracheides are to be found in tissues that are formed some time after the reception of the wound and in a region some distance away from it. Again, in *Sequoia sempervirens* the normal secondary wood is entirely devoid of resin-passages, yet such appear traumatically, as in the case above, subsequent to and at some distance from the wound. Jeffrey considers that these facts may be taken as evidence of the descent of the plants concerned from abietinous ancestors in which ray-tracheides and resin-passages respectively occurred in the normal secondary wood.

It would no doubt be dangerous to place any great phylogenetic weight upon the peculiarities of traumatic tissues considered apart and by themselves. At the same time I agree with Professor Jeffrey in so far that when traumatic characters 'are supported by a considerable body of collateral evidence, especially if such evidence is derived from extinct species', they

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[1] Jeffrey: (i) Traumatic Ray-tracheides in *Cunninghamia sinensis*. Annals of Botany, vol. xxii, 1908, p. 593. (ii) The Wound Reactions of Brachyphyllum. Ann. of Bot., vol. xx, 1906, p. 383. (iii) Comparative Anatomy and Phylogeny of the Coniferales. Pt. I, The Genus *Sequoia*. Mem. Boston Soc. Nat. Hist., vol. v, 1903, p. 441. Pt. II, The Abietineae. Ibid., vol. vi, 1905, p. 1.

may be safely taken as strong confirmatory evidence in the consideration of phylogenetic hypotheses.

My thanks are due to Dr. R. Kidston for kindly taking the photographs used in illustration.

SUMMARY.

1. An anomalous stem of *Osmunda regalis* is described, the pith of which at certain points contains scattered medullary tracheae.

2. It is held that this gives support to the theory that the pith of the Osmundaceae is phylogenetically stelar and not cortical, and that it arose by the progressive conversion of the central tracheae of a solid xylem strand into parenchyma.

EXPLANATION OF PLATE XXI.

Fig. 1 is from a drawing. The others are from untouched photographs.

Fig. 1. *Osmunda regalis.* Transverse section from the lower end of the specimen, showing the mixed pith and the greater part of the xylem ring. × 52.

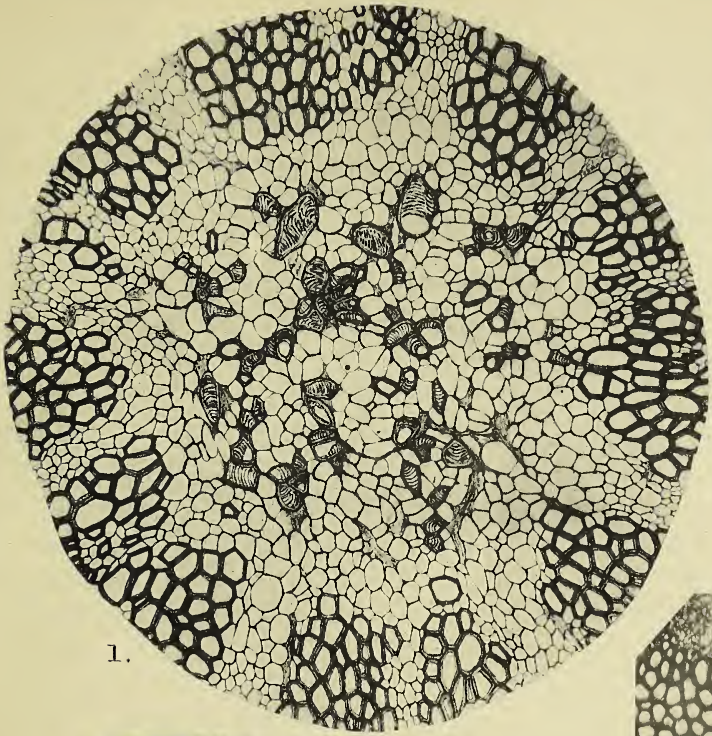
Fig. 2. *Osmunda regalis.* Photograph of another section from the same region. × 50.

Fig. 3. *Osmunda regalis.* Surface view of the wall of one of the medullary tracheae to show porose pitting. × 200.

Fig. 4. *Osmundites Kolbei.* Surface view of the wall of one of the medullary tracheae. × 200.

Fig. 5. *Osmunda regalis.* Transverse section of the upper anomalous region, showing the traumatic cracks and a few medullary tracheae. × 50.

Fig. 6. *Osmundites Kolbei.* Longitudinal section through the stele, showing the elongation of the tracheae in the 'mixed pith'. × 10.



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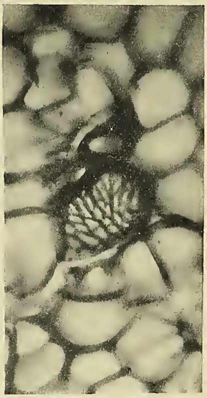

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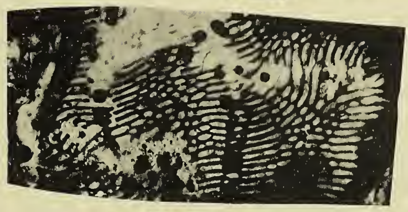

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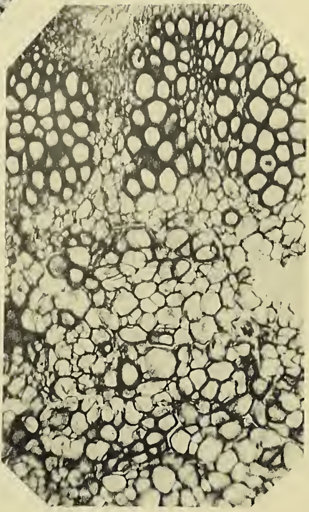

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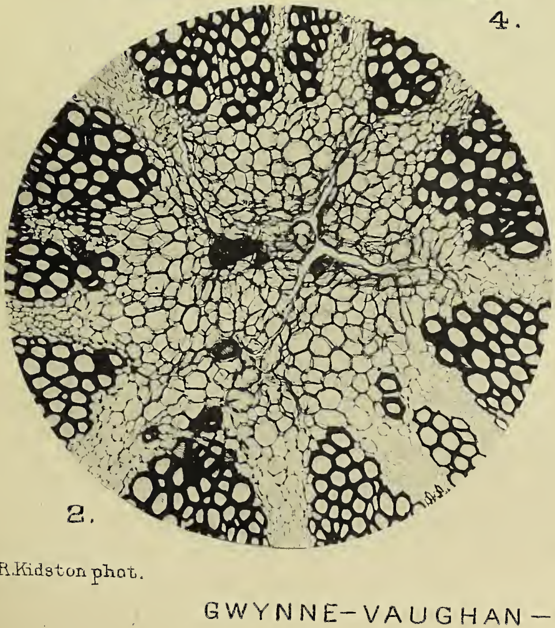

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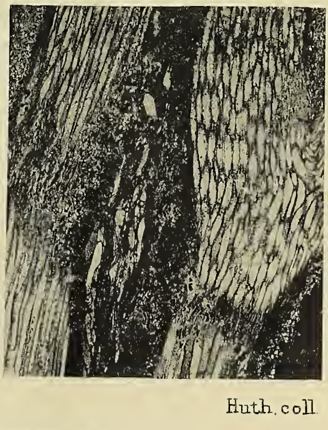

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R. Kidston phot.

Huth, coll.

GWYNNE-VAUGHAN—OSMUNDA.

Note on Anomalous Bulbils in a Lily.

BY

M. L. GREEN, B.A.

With two Figures in the Text.

BULBILS 'taken from the stem of *Lilium Fortunei giganteum*[1] 4 feet from ground' were sent to Kew by Messrs. Little and Ballantyne for examination. They afforded a very interesting case for investigation, as they had formed flowers *in situ*, and these flowers showed various degrees of abnormality. Three bulbils were examined with the following results. The first contained five fleshy bulbil scales, five perianth leaves on which were some reddish excrescences, and five stamens (Fig. 1, A–E). Various degrees of transition were exhibited in this flower between bulbil scales and perianth leaves, and also between bulbil scales and stamens. Fig. 1, D, shows that the perianth leaves have thickened bases which are obviously of the nature of bulbil scales. The stamens in the same flower were not uniform in size and shape. Some of them were normal. One stamen (Fig. 2, G) showed a little abnormality in having a frontal petaloid appendage springing from the connective. This appendage was adnate to the filament for a short distance. Another stamen (Fig. 2, H) was still more abnormal in having a thickened base of the nature of a bulbil scale, a filament of the texture and form of a perianth leaf, and an anther at the apex. The petaloid filament had two lobes and a central ridge of tissue on which was a little crest. This crest is homologous with the petaloid appendage seen in Fig. 2, G. The differentiation of parts in the flower just described was on the whole distinct. In the other two bulbils, however, this was far from being the case: they consisted, like the previous one, of five fleshy scales, but most of them represented transition stages between bulbil scales and perianth leaves. Then came five members, some of which resembled perianth leaves and others stamens, the transitional stages being more advanced than in the first bulbil examined. On some of these last-mentioned perianth leaves were excrescences similar to those mentioned above. The excrescences seem to be homologous with the papillae at the

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[1] *Lilium Fortunei*, Lindl. = *Lilium tigrinum*, var. *Fortunei*. It is doubtful what is meant by '*Lilium Fortunei giganteum*'.

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base of the perianth leaves in *Lilium tigrinum* and other species, although, of course, they are in a very undeveloped state. The arrangement of parts in these flowers is spiral (Fig. 2, J) instead of cyclic as in the normal flower. In all cases examined the pistil was very rudimentary.

FIG. 1. A, Bulbil, natural size. B, another view of the same, × 2. C, the same as B, but showing particularly the five bulbil scales. D, Two bulbil scales removed, showing the perianth leaves with their swollen bases. E, Perianth leaves drawn back exhibiting the stamens.

The question arises as to the morphological value of the bulbils. Are they to be regarded as vegetative organs or as representing flowers? The latter hypothesis is supported by the following considerations:

1. The bulbils were situated on the stem, namely, 4 feet from the ground, evidently in or near the inflorescence.

2. The stem of *Lilium* does not, under normal conditions, branch vegetatively, so the bulbils could scarcely represent condensed vegetative shoots.

3. A somewhat similar anomaly has been recorded for *Gagea arvensis* by Wirtgen in Flora, 1846, pp. 353–68, where the bulbils undoubtedly represent abortive flowers. A few details of this anomaly may be interesting here.

The bulbils usually appeared at the base of the main axis of the inflorescence in the leaf axils and represented the lower flowers. Some of the bulbils produced stalks, and these in turn bore flowers. Several single small bulbils on the ramifications ot short-flower stalks were observed which bore abortive flowers, and this the writer considers to be a clear indication that most of these bulbils are to be regarded as undeveloped or abortive flowers. Further, there also existed on the same inflorescence a transition from the rudimentary flower to the bulbil. The bulbil flowers were by no means uniform, varying in a similar manner to those described above.

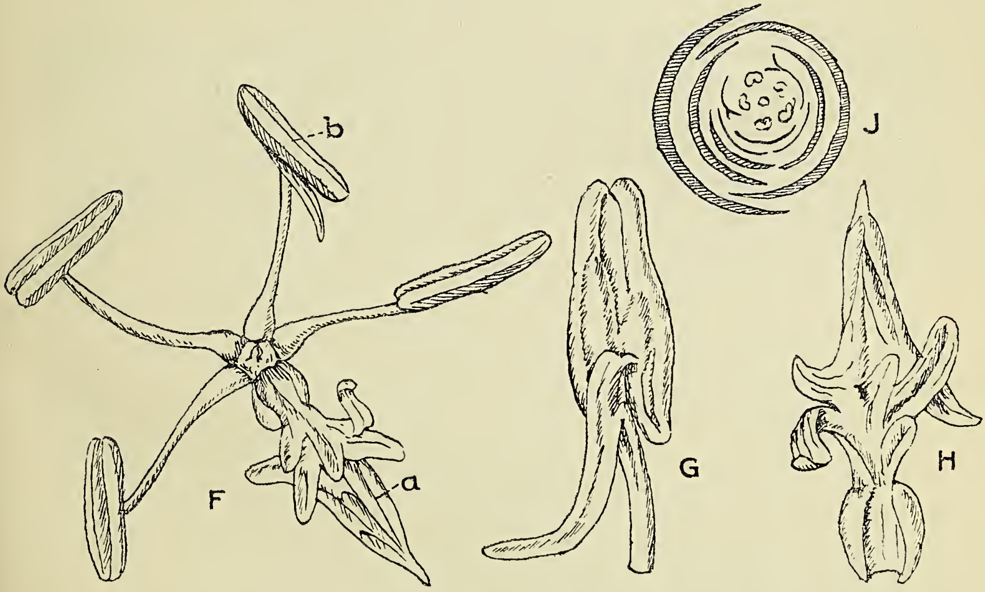

Fig. 2. F, Stamens enlarged. G, Stamen *b* enlarged, showing anterior petaloid appendage. H, Stamen *a* showing the transition between bulbil scale and stamen (front view). J, Floral diagram showing spiral arrangement of flower.

4. The only strictly comparable case to the one under consideration is described by H. Hesselman in Act. Hort. Bergiani, Band III, No. 1 A, pp. 1–19, under the title, 'Ueber die Ausbildung von Bulbillenblättern als florale Blätter bei *Lilium bulbiferum*, L.'

In this instance the bulbils were sessile in the leaf axils, those in the upper axils occurring along with the flowers. The outermost 1–3 leaves of all the bulbils were normal bulbil scales. The lower bulbils had inside these some pale, slightly sepal-like leaves, but no stamens, whereas the upper ones had, in addition to sepal-like leaves, thin and broadened perianth leaves with the fiery red colour of *Lilium bulbiferum*, and from one to four stamens. Some of the stamens were like a bulb-scale at the base, with a filament and anther above, whilst others were normal.

My thanks are due to Mr. W. C. Worsdell for valuable help and suggestions during the preparation of this note.

SUMMARY.

An account is given of anomalous bulbils taken from the stem of *Lilium Fortunei giganteum.* These bulbils exhibit various degrees of transition between the bulbil scale and the perianth leaf, and also between the bulbil scale and the stamen. Reasons are given for considering the bulbils as homologous with flowers, and two other cases are cited comparable to the one dealt with.

On the Classification of Seed-Leaves.

BY

T. G. HILL

AND

E. DE FRAINE.

IN a highly interesting study of Syncotyly and Schizocotyly, Mr. Compton,[1] whilst in agreement with our general conclusions,[2] adversely criticizes us with regard to what we have termed 'subsidiary cotyledons'.

The classification of cotyledons was a by-way in our main work on seedling-structure, and one to which we do not attach a vast amount of importance; but since Mr. Compton, who has much first-hand knowledge of the subject-matter, finds difficulties in understanding our position, it is not unlikely that others also will find difficulties; hence it appears desirable to write a few words in reply.

It was found, in our work referred to above, that although the seed-leaves, more especially of the polycotylous Gymnosperms, might be exactly similar externally, the behaviour of their vascular bundles in the transition phenomena might be very different.

1. In some cases the vascular strand of a cotyledon gave rise to one pole of the root-structure.

2. In other instances, the vascular bundles of two adjacent cotyledons gave origin to one pole of the root-structure.

3. And in some examples, the trace of a seed-leaf played no part in the transition phenomena, but joined on to any adjacent vascular tissue in no definite fashion.

From the constant occurrence of these phenomena, the seed-leaves were respectively divided into three classes: (i) whole cotyledons, (ii) half-cotyledons, and (iii) subsidiary cotyledons.

We expressly stated that this classification was based *on the behaviour of the seed-leaf traces in the transition region*.[3] Whether we were justified

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[1] Compton, R. H.: An Anatomical Study of Syncotyly and Schizocotyly. Ann. Bot., 1913, vol. xxvii, p. 793.

[2] Hill, T. G., and de Fraine, E.: On the Seedling Structure of Gymnosperms. Ann. Bot., 1908, vol. xxii, p. 689; 1909, vol. xxiii, p. 189.

[3] Hill and de Fraine: Seedling Structure of Gymnosperms. II. Ann. Bot., 1909, vol. xxiii, p. 221.

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'in arguing from anatomy to morphology in this matter' is doubtless a matter of individual opinion; it is certainly a classification which is remarkable in the fact that it does not break down when applied, and further, it may be remarked that anatomy is frequently a very valuable aid in determining morphological status.

Mr. Compton cites *Cupressus torulosa* Series C, *Abies sibirica* Series B, and *Abies amabilis* as examples illustrating that the above classification, even when restricted solely to the vascular strands, cannot be applied strictly. We have referred to our written descriptions of the transition phenomena in these plants, and quite fail to see any difficulty whatever in the interpretation with regard to the first and last named plant. The instance of *Abies sibirica* presented some difficulties which are fully considered and met in the paper.[1] Of the large number of polycotylous Gymnosperm seedlings examined, this particular example, *Abies sibirica*, is one of a very few, if not the only one, in which the interpretation of the value of the cotyledons did not assert itself obviously. There is much to be said for a classification which is so generally applicable; a conception is not, we think, to be condemned because one particular example does not at first sight appear to fit.

In further support of his contention, Mr. Compton cites his observations on *Phacelia tanacetifolia*.[2] No illustrations are given of the vascular rearrangements in this plant, but from his account we should say that our classification finds considerable support.

It does not appear necessary to examine in detail all the examples of this plant examined by Mr. Compton; two, however, may be dealt with at some little length.

Hemitricotyl A: In this seedling there was one entire and one bifurcated cotyledon. The vascular bundle of the former gave origin to one pole of the diarch root, whilst the two bundles, which did not fuse together before entering the hypocotyl, from the deeply forked cotyledon, together formed the other pole of the diarch root-structure. The interpretation is clear—there were two whole cotyledons, one of which was so deeply split as almost to form two half-cotyledons.

In the case of Tricotyl B there were three cotyledons, the bundles of which divided into two and gave rise to a triarch root-structure. At a lower level the triarch arrangement became reduced to diarch. Mr. Compton, who traces the vascular changes from the root to the seed-leaves, calls this the 'diarch-triarch' structure. This method of increase in the number of protoxylems is not uncommon among tricotyls. It is *not* 'the method followed by the vascular bundles of so-called subsidiary cotyledons in Coniferae', as Mr. Compton states. According to our reading of the facts, there were no subsidiary cotyledons in this example of *Phacelia*: there

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[1] Hill and de Fraine, loc. cit., 1909, p. 191.

[2] Loc. cit., p. 811.

were three whole cotyledons, the vascular bundles of which gave rise to a triarch root-structure which subsequently became reduced to diarch, a phenomenon not at all uncommon in the Coniferae.

With regard to the other seedlings of *Phacelia*, the following analysis is perfectly obvious from Mr. Compton's description.

Tricotyl C and Hemitetracotyl D. Three whole cotyledons. Root-structure triarch throughout.

Tetracotyl E. Four whole cotyledons. Root-structure tetrarch above, but successively reduced at lower levels to triarch and diarch.

It is clear that Mr. Compton quite misunderstands our conception, and this, in part, is possibly due to the fact that he worked from below upwards, whilst we, in common with most other investigators of seedling anatomy in this country, worked from the cotyledons downwards. This appears from the following quotation:[1]

'The most striking feature in the schizocotyls of *Phacelia* is the fact that the vascular strands [presumably of the root, below the collar] do not split to correspond with the split of the cotyledons. In all cases (except two, where the division occurred in the lamina) the number of xylem bundles is increased by the addition of new ones, not by the division of old. On tracing the strands from above downwards, we may say that in these cases the traces of certain cotyledons or half-cotyledons do not contribute to the structure of the root, but die out in the hypocotyl. This is the characteristic[2] of "subsidiary cotyledons"—a category of plant-members which apparently cannot be maintained.'

In all our work, the *first* organization of a root-structure, whether it occurred high up or low down in the hypocotyl or below the collar, was taken as a factor in our classification.

This appeared the obvious course to take, for the fact that the number of poles might be altered at a lower level results in chaos, as Mr. Compton has found. If the structure of the root at a lower level is to be taken, what particular level is to be selected, and what is to guide us in our selection? It is not at all infrequent, especially when the number of cotyledons varies, that the number of poles in a root, with the exception of diarch roots, becomes reduced and sometimes increased as we pass towards the apex. For instance, a pentarch root might show a reduction to tetrarch and finally to triarch just above the apex; it is possible that if the seedling had been allowed to grow for a week or so longer and then examined, a further reduction to diarchy might be shown.

The difficulty could be overcome by referring the structures in question to a diarch organization, on the assumption that this is primitive; and this no doubt would be excellent philosophy, but it does not appeal to us as science.

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[1] Loc. cit., p. 812.

[2] As we have already remarked, it is not.

Mr. Compton correlates the final root-structure with the cotyledonary bundles, and takes no count of the root-structure which first results from the arrangement of the seed-leaf traces; we do exactly the reverse, and it appears that this is the cause of the misconception.

The polycotylous condition is an heritable quality, and since it appears to be generally, but not universally, agreed that the primitive condition was dicotyledonous, the presence of many seed-leaves, many of which may be whole cotyledons, in a seedling has to be accounted for.

This is, of course, a difficult matter, since we cannot 'have our cake and eat it'; in other words, we cannot dissect a seedling and also examine its progeny.

It appears not improbable that in the course of descent the half or the subsidiary cotyledons of an ancestor should become of increasing importance in succeeding generations until they became whole or half-cotyledons respectively.

This was our meaning in saying that a half-cotyledon could arise by the 'promotion' of a subsidiary cotyledon. The same goal can be arrived at in different ways, so that, for us, it is not unthinkable that a 'half-cotyledon' is sometimes the half of a whole cotyledon, whilst in other instances it represents a promoted subsidiary cotyledon.[1]

In view of Mr. Compton's account[2] of the 'Theories of the Anatomical Transition from Root to Stem', which is an admirable exposition of the different views of the various schools of anatomists especially in relation to seedling structure, it would appear to be unnecessary to consider in detail M. Dauphiné's criticism[3] of our work on the Centrospermae.[4] It may, however, be remarked that this particular investigation was a continuation of our work on seedling anatomy, carried out from the phylogenetic point of view and commenced some thirteen years ago; in which investigation we, in common with most other British workers in the same field, considered the vascular tissues in the gross. In writing the paper on the Centrosperms we fully recognized the high value of M. Chauveaud's work on the same group, but we did not think it desirable to make reference to it since such reference, to be of any value, would have involved a long consideration of the views of the different schools of thought and would have tended to obscure our main line of work.

UNIVERSITY COLLEGE,
LONDON.

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[1] See Hill and de Fraine: Ann. Bot., 1909, vol. xxiii, p. 222.

[2] New Phyt., 1912, vol. xii, p. 13.

[3] A. Dauphiné: Sur le développement de l'appareil conducteur chez quelques Centrospermées. Bull. Soc. Bot. France, 1913, vol. xiii, p. 312.

[4] Hill and de Fraine: On the Seedling Structure of Certain Centrospermae. Ann. Bot., 1912, vol. xxvi, p. 175.

THE ANNALS
OF THE
BOLUS HERBARIUM
(South African College)

EDITED BY H. H. W. PEARSON, SC.D., F.L.S.

HARRY BOLUS PROFESSOR OF BOTANY IN THE SOUTH AFRICAN COLLEGE, CAPETOWN
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BY

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Studies in the Phylogeny of the Filicales.

IV. Blechnum and Allied Genera.

BY

F. O. BOWER, F.R.S.

With Plates XXII–XXXII and twenty-six Figures in the Text.

THE Ferns now included in the comprehensive genus *Blechnum* number about 140 species, and are very widely spread geographically. They vary considerably in habit, some being small erect Tree-Ferns, others having an elongated creeping rhizome, while some assume even a climbing habit. In their leaves also they show great diversity, for in some there is a strong dimorphism of the sterile and fertile fronds, the latter having narrow segments and standing usually erect, the former being broader in expanse and more or less inclined. In others the sterile and fertile leaves resemble one another both in form and position; others, again, among the climbing species bear three distinct types of leaf, of which two are sterile. The leaves are usually simply pinnate, but some species show more complex branching. The 'sori', which as we shall presently see are really the result of the extension and fusion of true sori, are elongated, running parallel to the midrib of the segment, and are protected by an indusial flap, which has its margin directed towards the midrib. But there is some diversity in the position which the sorus holds relatively to the apparent margin of the leaf; sometimes it appears to be near to the margin itself, in other species it appears to be distinctly intramarginal, but it is always in close proximity to the midrib even where the fertile pinna is a broad one.

The genus thus variable has undergone vicissitudes of classification. Originally Linnaeus founded the genus *Blechnum* in 1753. The Index to the Linnaean Herbarium, printed as a Supplement to the Proceedings of the Linnean Society (1911–12, p. 45), shows five species included in the genus. Of these three are still retained (*B. australe*, *occidentale*, and *orientale*), but the other two are now separated in the genus *Woodwardia* (*W. radicans* and *virginica*). Thus not only was the genus undivided, but Linnaeus included in it also Ferns now segregated in *Woodwardia*. In the Synopsis Filicum of Swartz (1806) *Woodwardia* was separated, though the

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

genus *Blechnum* remained otherwise still undivided. But in 1809 Willdenow again divided the genus, which had meanwhile grown in number of species. Those with dimorphic leaves and with the sorus apparently marginal fell into the genus *Lomaria*, which was placed near to *Struthiopteris*; those with their leaves conforming, and with their sori distinctly intramarginal, remained under the name of *Blechnum*, a genus placed in relation with *Pteris* and *Vittaria* on the one hand, and with *Woodwardia* on the other. Kaulfuss (1827) also kept the two genera distinct, but placed them side by side in his Blechnoideae.

Presl in his Tentamen (1836) not only maintained the distinctness of the genera, but placed them widely apart in his system. He ranked *Blechnum* in his Tribe IV, Aspleniaceae, which included *Cystopteris*, *Onoclea*, *Doodia*, and *Woodwardia*, while he placed *Lomaria* in his Tribe VI, Adiantaceae, together with other genera now constituting the Pterideae. The distinction of the two genera thus recognized by Willdenow and accentuated by Presl was adopted by Sir William Hooker, and by Baker and various other writers. Sir William placed both genera under the heading 'Lomarieae' in the Species Filicum (vol. iii, p. 1), together with *Sadleria*, *Woodwardia*, and *Doodia*. But in the Synopsis Filicum, though the rest remain in his Tribe VII, Blechneae, he places *Lomaria* in the preceding Tribe VI of the Pterideae. It may further be noted that *Onoclea* (including *Struthiopteris*) is far removed in Hooker's system, being placed in his Tribe II, Dicksonieae.

But already in 1856 Mettenius had expressed a contrary opinion (Fil. Hort. Lips., p. 60). He points out that the different forms of the fertile and sterile leaves are not a good criterion, since various steps between complete similarity and complete difference may be found. Neither is the position of the receptacle relatively to the midrib and margin a good criterion, for it may vary even in the different parts of the same leaf (*Bl. Gilliesii*, Taf. IV, Figs. 15, 16). These and other considerations led him to accept the position already stated by Schlechtendal (Adumbr. 34), that the genera *Lomaria* and *Blechnum* must be again thrown together. This conclusion has been adopted by Luerssen (Rab. Krypt.-Fl., iii, p. 111), by Prantl (Das System der Farne, 1892, p. 16), by Diels (Engler u. Prantl, i. 4, p. 245), and by Christ (Farnkräuter, p. 176, &c.). It is thus seen that the fusion of the genera is generally accepted, though it is still the custom to subdivide the genus into sub-genera, such as (1) *Eu-Blechnum*, (2) *Salpichlaena*, and (3) *Lomaria*. One of the objects of this investigation will be to test by developmental inquiry the validity of these conclusions.

We have seen that Swartz placed his undivided genus *Blechnum* in near relation to *Onoclea* (*Struthiopteris*). Many of the later writers, after the division of the genus into *Blechnum* and *Lomaria*, placed one or the other, or both, apart from this affinity; and the comparison, though early made, was in danger of passing entirely out of view. For instance, Diels places the Woodsieae-Onocleinae quite apart from the Asplenieae-Blechninae, and does not indicate their relationship in the text (Engler u. Prantl, i. 4, pp. 164 and 245). Christ also separates *Onoclea* and *Struthiopteris* (Farnkräuter, p. 284) widely from *Blechnum* (p. 176), as though there were no near kinship between them. It will be another object of this inquiry to see whether there is not ground for bringing again into notice a relationship which has thus been allowed to drop.

On the other hand, there are the closely related genera of *Woodwardia*, *Doodia*, and *Sadleria*. The synonymy of these genera indicates their near alliance with one another, while in all the leading systems they have been placed in juxtaposition with *Blechnum*. The justification for this must be examined, with a view to their possible phyletic seriation.

Again, *Stenochlaena*, which has been referred to *Lomaria* by Willdenow, to *Onoclea* by Swartz, and to *Acrostichum* by Baker, will require careful examination, to test the position now assigned to it with the Ferns above named. *Brainea* also, though a more isolated genus, will have to be compared, as it has been referred to this affinity by certain writers.

It will be necessary to submit these Ferns to anatomical and developmental study. The vascular system of many of them is very imperfectly known, and in some has never been adequately examined. But the most important point of all for this inquiry will be the characters of their sori. The differences in the relation of the sori to the apparent leaf-margin in the various species now included in *Blechnum* have never been submitted to adequate developmental examination. Nor has any developmental comparison been made of them with the divergent genera *Woodwardia*, *Doodia*, and *Sadleria*, nor yet with *Stenochlaena*. The only comparative observations bearing on the development, and they are very imperfect and restricted in their scope, are those of Burck (Indusium der Varens, Haarlem, 1874). Mettenius, however, made valuable comparative observations on the more mature state of the sorus in numerous species (Fil. Hort. Lips., p. 60). Nevertheless, the field may be held to be here still open for more detailed comparative study of the sori than has yet been made. It would be desirable, no doubt, to extend the observations to other criteria than those named. This must, however, stand over for the present; and something, at all events, will be gained which may lead to more confirmed opinions than are at present possible, if the relationships above indicated are tested more fully through the characters of anatomy and the development of the sorus.

But there is yet another relationship that will have to be considered, since it has been very definitely indicated by systematists. It is the relation of the Ferns named to the Asplenieae. This, however, is an ulterior question. Before it can be adequately discussed it will be necessary to have some clearer knowledge of the probable phyletic relationships of the

Blechnineae among themselves, for they are probably more nearly related to relatively primitive types than are the Asplenieae.

There remains lastly to be mentioned in this connexion the genus *Plagiogyria*, which formed the subject of the first of the memoirs of this series (Ann. of Bot., xxiv, p. 423, April, 1910). Though separated by Mettenius as a distinct genus, the species included under that name were merged by Sir William Hooker in *Lomaria*, to which genus they had originally been assigned by Kunze. For reasons detailed in my paper quoted above, they are shown to constitute a substantive genus, which is relatively primitive in its characters, and may be held to be a 'synthetic' type. It shows relations downwards by various characters with the Simplices, but the question here will be rather its relations upwards. The similarity to certain Pterideae is striking, though the nearness of actual relation may be doubtful. But obviously, as the systematic history shows, the relation to (*Lomaria*) *Blechnum* seems to be a closer one, and it is apparent chiefly in the dimorphism of the leaves, and the position and mode of protection of the sori.

Plagiogyria.

Recognizing that there is probably some true relation between *Plagiogyria* and *Blechnum*, it is necessary to be clear on the origin and nature of the marginal flap which covers the young sori, thus providing a habit-character upon which the comparison of these Ferns was originally based. Its general appearance was represented in my paper on *Plagiogyria* (Ann. of Bot., vol. xxiv, p. 423, Figs. 1, 2, 14, 15), but certain developmental details were there omitted. Transverse sections of the very young fertile pinnae of *Pl. semicordata* show that the lateral flap of the pinna is referable in origin wholly to a regular marginal segmentation (Pl. XXII, Fig. 1, *a*). There is a single oval vascular strand initiated in the midrib of the pinna. As the pinna develops, single superficial cells grow out into the characteristic glandular hairs, whose terminal cells resemble closely those studied in detail by Gardiner and Ito in *Blechnum* (Ann. of Bot., vol. i, p. 27). As development proceeds, the margin of the pinna enters upon less regular segmentations, with a large increase in the number of anticlinal divisions (Pl. XXII, Fig. 1, *b*). This is in preparation for its rapid extension, and the thinning out of its margin as a membranous protection, while the curvature is such that it folds over and protects the lower surface of the pinna. Here certain large and plasmatically rich superficial cells show great activity of growth, and provide after segmentation the mother-cells of the sporangia.

Sections of a pinna as it approaches maturity show the final result in *Plagiogyria* (Pl. XXII, Fig. 1, *c*). The midrib is traversed by a single collateral vascular strand of oval form, sharply delimited by a brown-coloured endodermis. The adjoining tissue of the midrib is mechanically strengthened.

The wing of the pinna, on leaving the midrib, is composed of five cell-layers. In passing towards the margin it may be seen to dilate, sometimes by increase in number of layers, at other points by simple enlargement of its cells. These dilatations are not constant in position, and an examination of numerous transverse sections shows that they may vary in position and extent in the individual pinna. In the case shown in Pl. XXII, Fig. 1, *c*, there are two dilatations, the one nearer the midrib than the insertion of the sporangia being due to the increase in number of layers, the other nearer to the margin than the sorus being due to dilatation of the individual cells. It is important to note these dilatations and the way in which they are produced, and their irregularity, as they are similar in nature to features which appear in *Blechnum*. In both cases they may produce swellings on the convex surface of the pinna, at or near to the point of its greatest curvature. The margin has developed in accordance with the segmentation already seen in (Pl. XXII, Fig. 1, *b*). It consists usually of three layers of cells, as against five or six of the rest of the wing.

There can be no doubt from its mode of origin, as well as from its structure, that here the indusium-like flap is nothing more than the result of outgrowth and thinning of the margin of the pinna, which has thus become specialized for protective purposes. It may be a question whether or not this similarity to *Blechnum* is an index of phyletic relation, or only a parallel development, and the same question may arise also as regards the Pterideae. As bearing on this it may be noted that the vascular condition of the axis and of the leaf-trace in *Plagiogyria* is of a more primitive type than that of *Blechnum*, while the origin of the vascular supply to the roots lies laterally from the point of origin of the leaf-trace, not directly opposite it, as is so very constant a feature in the Blechnoid series. These divergent features indicate some degree of aloofness of *Plagiogyria* from *Blechnum*, and the more primitive position of the former, which the sporangial characters bear out; as also does the absence of flattened scales from the surfaces of axis and leaf. Nevertheless there is an underlying similarity that justifies the comparison with *Blechnum* recognized by the early Pteridologists, and so constantly maintained by them.

Matteuccia intermedia, C. Chr.

It has been pointed out above how, from the time of Willdenow and other early writers, a relation had been recognized between *Onoclea* and *Struthiopteris* on the one hand and *Blechnum* on the other. There is a general similarity of habit, both types including upright and creeping forms with relatively simple pinnation. Sorally, the obvious objection would seem to be in the presence of a 'true' indusium in the former, and its absence in the latter. But apart from this, both show dimorphism of their leaves, while the sori are essentially superficial, borne on the branching veins and covered by the recurved leaf-margin. If, then, a non-indusiate representative of the Onocleinae were found, that discovery would materially strengthen an otherwise valid comparison instituted by the instinct of the older systematists.

Such a type has been found in *Matteuccia intermedia* n. sp., C. Chr. I recently inquired of the Director of the Calcutta Garden whether he could supply to me specimens of *Matteuccia* (*Struthiopteris*) *orientalis*. He kindly obtained for me specimens sent under that name from Darjeeling. I also obtained a supply of plants, which appeared to be correctly named, by purchase from Messrs. May, of Edmonton. But when the plants from these two sources came to be grown side by side in the Glasgow Garden, it was obvious that they were not identical in habit. Moreover, as already recorded (Ann. of Bot., xxvi, p. 301), the Darjeeling specimens were found to have no indusium. This fact made the determination so doubtful that specimens were sent to Dr. C. Christensen. He reports to me by letter that 'the form is extraordinarily interesting to me. I have some months ago described a species of *Matteuccia* (*M. intermedia*) as new. I have received it from Professor Sargent, and it was collected in the Province of Shen-Si, North China, by William Purdon. The plant from Darjeeling is no doubt the same species. . . . I have also observed the total absence of an indusium in my *M. intermedia*, but in my specimens of *M. orientalis* I have not seen an indusium, probably because of the specimens being mature. . . . The full description of *M. intermedia* was sent to Prof. Sargent, and it should appear in the Botanical Gazette during the year.'[1]

Thus a peculiar importance attaches to the origin and details of the sorus in the Fern from Darjeeling, now named *Matteuccia intermedia*. The venation of the fertile pinna is as is shown in my former memoir (Ann. of Bot., xxvi, Pl. XXXVI, Fig. 36), and the position of the sori is such that they form a regular line on either side of the midrib, but there is no vascular commissure joining laterally the branch veins upon which each distinct and circumscribed sorus is seated. Sections transversely through the young pinnæ show the relation of the sorus to the midrib and the margin as in Pl. XXII, Fig. 2, *d–g*. There is a single vein in the midrib. The origin of the sorus is distinctly intramarginal (*d*), with a raised receptacle upon which the sporangia are borne in basipetal succession (*e, f, g*). There is at no time any sign of an indusium, but the sorus is nevertheless adequately protected by the curved leaf-margin, in which in the young state the successive cleavages can be readily seen (*d, e, f*), as in *Plagiogyria*: this is an indication of its being, as in that case, the true margin. Longitudinal sections show that laterally the sori are quite distinct from one another (*h*). The whole condition of the naked sorus, with its basipetal succession of sporangia, suggests a comparison with *Alsophila*. But an examination of

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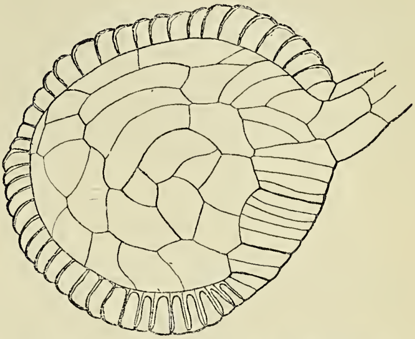
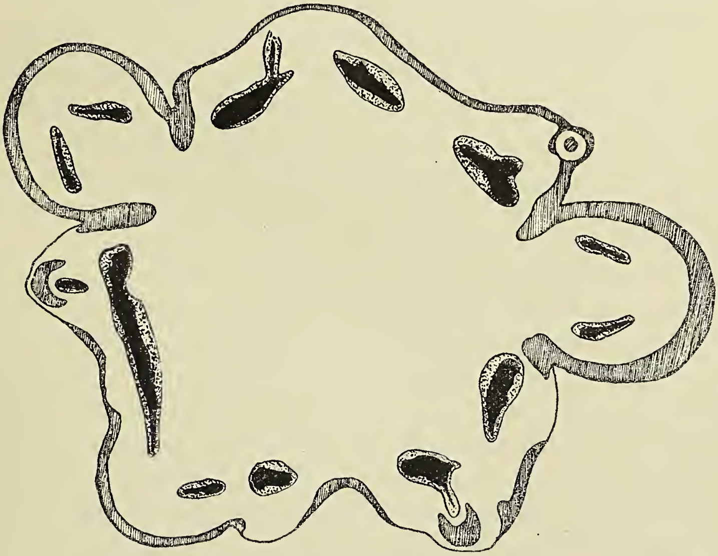
[1] Bot. Gazette, vol. lvi, 1913, p. 337.

the sporangia themselves shows a similarity rather with *Lophosoria*, for the sporangium is large, with, it may be, over fifty cells in the annulus (Text-fig. 1), which shows signs of slight obliquity, but the succession is partially or completely interrupted at the insertion of the stalk. Glandular hairs of the Blechnoid type are present, though sparingly, on the young pinna.



TEXT-FIG. 1. Sporangium of *Matteuccia intermedia*, C. Chr. × 125.

Matteuccia intermedia is thus seen to be a non-indusiate type, with dimorphic leaves and with sori protected by the reflexed leaf-margin, while the succession of the sporangia is basipetal. The sori form rows parallel to the midrib, and on either side of it, as in *Blechnum*, but they are not connected by commissures as is the rule in that genus. The axis of *Matteuccia* shows an open dictyostele, with a very bulky pith (Text-fig. 2). The leaf-trace originates as a pair of strands separate from the first, and remaining for some distance upwards without further subdivision. There is thus a basis, soral and anatomical, for comparison with the simpler types of the Blechnoid Ferns which goes considerably beyond the mere characters of external habit. The relations downwards are clearly with the Cyatheoid Ferns, and especially with the more primitive types, such as *Alsophila* and



TEXT-FIG. 2. Transverse section of the stock of *Matteuccia intermedia*, C. Chr. × 3.

Lophosoria. But, on the other hand, it is now more evident than before that the relationship upwards between *Matteuccia* (*Struthiopteris*) and the genus *Blechnum*, so clearly recognized by the early systematists, has the support of more detailed comparison than they had instituted. The new facts appear to suggest that the Blechnoids probably sprang from some early Cyatheoid source, and the nearest indication of the connexion is seen in the Fern so happily named by Christensen, *Matteuccia intermedia.* With this general indication, which coincides with the opinions of early sytematists, the detailed study of the Blechnoid Ferns may be entered upon.

Blechnum.

For purposes of the present description, and in accord with general opinion since the time of Mettenius, the genus *Blechnum* is accepted in its wider sense, as comprising the sub-genera (1) *Lomaria*, (2) *Salpichlaena*, (3) *Eu-Blechnum.* As there is reason to believe that *Lomaria* most nearly represents the phyletically prior type—a question which will be discussed when the facts are before us—this sub-genus will be taken first. *Lomaria* differs from *Eu-Blechnum* in the relation of the sorus to the ostensible margin of the leaf. Presl, in his Tentamen (1836, p. 141), speaks of the 'indusium marginarium' of *Lomaria.* But in the case of *Blechnum* (p. 101) he speaks of 'indusium lineare, scariosum, margine libero, costam respiciente'. And later (p. 102), he writes of *Blechnum* more explicitly thus: 'Attamen margo frondis semper evidentissime liber est, et indusium proprium adest, cum in Lomariis, quibuscum hae species confundi possunt, indusium spurium e margine frondis replicato et alterato exoritur.' Clearly he held the indusium in the two genera as essentially different things, and since his time the question has never been cleared up by comparative and developmental observations. Evidence will here be adduced from comparison of the development in numerous species, which leads to the conclusion that the protective organ is phyletically the same throughout the genus *Blechnum* in its extended sense, and it will be styled the '*phyletic margin*'. This true margin of the pinna, or 'indusium', comes to be apparently intramarginal in *Eu-Blechnum* owing to the formation of a new structure, which will here be styled '*the flange*'. Its origin will be traced by comparison of the species to be described; but its formation does not alter the nature of the protective 'phyletic margin', notwithstanding that the position of the latter may no longer be ostensibly marginal in cases where the flange has attained large proportions. The 'phyletic margin', which then appears as an indusial covering to the sorus, and has usually been so described, is still held to maintain its identity. But it will be shown that it may undergo considerable further modifications by segregation into short lengths; this will be seen in genera held to be derivative from the simpler Blechnoid type, such as *Woodwardia* and *Doodia.*

It was necessary to make these points of terminology clear at the outset for purposes of lucid description. But the comparative discussion of the facts now to be detailed will be deferred to a later page. The description of the numerous species on which observations have been made will be so arranged that those species in which the structure of the fertile pinnae is the simplest, and which accordingly conform most nearly to the type seen in *Plagiogyria* or in *Matteuccia intermedia*, will be taken first, and from these the description will proceed to those which show a higher complexity.

i. The Sorus of Blechnum and its Relation to the 'Flange'.

(A) *Blechnum discolor* (Forst), Keys.

This is a large upright species which may become subdendroid in habit. Runners may arise from the base of the plant, which, like the main axis, show a dictyostelic structure essentially similar to that seen in *B. tabulare* (see below). The leaf-trace comes off from the sides of the foliar gap in the form of two equal strands, which are distinct in origin from the first, as in *Matteuccia*. Higher up in the petiole of the fertile leaf, branching of the strands may occur, and the system settles down as a group of three, the lateral strands showing the usual adaxial hooks. The fertile pinna is similar in position and character to that of *Plagiogyria*, and like it is supplied by a single strand in the midrib (Pl. XXII, Fig. 3, *f*, *g*, *h*). From this lateral strands arise supplying the lateral expansions, and these are themselves curved strongly downwards as in *Plagiogyria* and *Matteuccia*, so as to protect the sori borne upon the lower surface. The lateral expansions show considerable irregularity of outline as seen in the transverse section; in this they resemble what has been seen in *Plagiogyria*, though here the irregularity is more marked. It is seen in Fig. 3, *g*, *h*. Passing outwards laterally from the midrib, the thickness of the flap is seven or eight layers of cells. It then widens out to a considerably increased thickness at the point of greatest curvature, narrowing again where the sorus is inserted, and finally thinning out to a single layer where the margin is membranous or 'indusioid'. The effect of this on the external appearance of the whole pinna is to give it roughly a four-angled form, with one of the flat sides facing upwards, and marked by longitudinal ridges, which, as they project alternately more and less strongly, form rows of rounded bosses. This is a form which is pretty general for the fertile pinnae of the section *Lomaria*, to which the simpler species belong.

The sori appear as linear masses of sporangia, seated laterally in the concavity of the lower surface, and are usually continuous. The receptacle is supplied by veins which run out obliquely from the midrib of the pinna. As they reach the receptacle, each widens out laterally in a fan-like manner, so as to meet the next, and thus a continuous vascular 'commissure' is formed beneath the continuous receptacle (Fig. 3, *g*; compare also Text-fig. 14, p. 400). But it is important to note that this is not constant. Sections may be found in series cut from a fertile pinna in which both sorus and commissure are interrupted. Such a section is shown in Fig. 3, *h*, and such cases are important for comparison with *Plagiogyria* and *Matteuccia*, in neither of which are the sori or the commissures continuous.

In *B. discolor* the origin of the wing of the pinna is, as usual, from segmentation of the marginal series, but it is rather more robust than in *Plagiogyria*. It is seen in an early stage in Fig. 3, *a*, where clearly the whole is referable in origin to a normal segmentation. Already extra periclinal divisions may be seen at × , which foreshadow the thickening later formed at the angle of curvature of the transverse section (compare Fig. 3, *g*, *h*), and constitute those ridges which are a marked external feature in the mature state. Such ridges initiated early, with extra periclinal divisions as a frequent feature, are common in the genus; they are, however, variable in their position, size, and mature structure; they may even vary at different levels on the same fertile pinna, as may be seen on examination of the pinna from without, as well as from serial sections through it (compare Pl. XXIII, Fig. 5 of *B. lanceolatum*). Up to the condition shown in Fig. 3, *a*, there is no definite sign of initiation of the 'indusial' margin. The interest will naturally centre round the origin of those protective flaps which are so prominent in older stages (Fig. 3, *f*, *g*, *h*). Their position is apparently marginal, as in *Plagiogyria*, but their actual relation to the marginal initial is rather indeterminate. As the marginal segmentation proceeds, the marginal cells become smaller (Fig. 3, *b*, *c*, *d*, *e*), and may finally divide up as in Fig. 3, *c*. As a rule in this species, it appears that the marginal cell does not itself directly furnish the indusial growth, but at an early stage divisions begin in an adjoining segment (Fig. 3, *b*, *c*, *d*, *e*), accompanied by more rapid growth, so that the flap (*i*) begins even at this early stage to project. It should be remarked that the sections drawn have been selected from a very large number examined, and are believed to represent typical examples. But there is a good deal of variety of detail in nearness of origin to, or even coincidence of the indusium with the marginal cell itself. Such conditions as those shown suggest the very first stages of a 'phyletic slide' of the indusium, or phyletic margin, from a strictly marginal position as it is in *Plagiogyria*, to a superficial position. That such a 'slide' has taken place is, in point of fact, the working hypothesis which will accompany the description in this and other species.

The cells which will give rise to the sorus itself have meanwhile become recognizable, by their deeper prismatic form (Fig. 3, *c*); they begin to project (Fig. 3, *d*, *e*), and commonly form a group showing the features of a slightly basipetal sorus, those at the centre being more advanced than those on either side. The position of the sorus is very near to the indusium as a rule, but it is variable. The basipetal sequence is not long maintained, nor is it very marked even in favourable cases (Fig. 3, *e*, *f*). Later on there may be a transition to a mixed condition of the sorus, by interpolation of younger sporangia between those already formed (Fig. 3, *g*); this is the usual state in strongly developed pinnae.

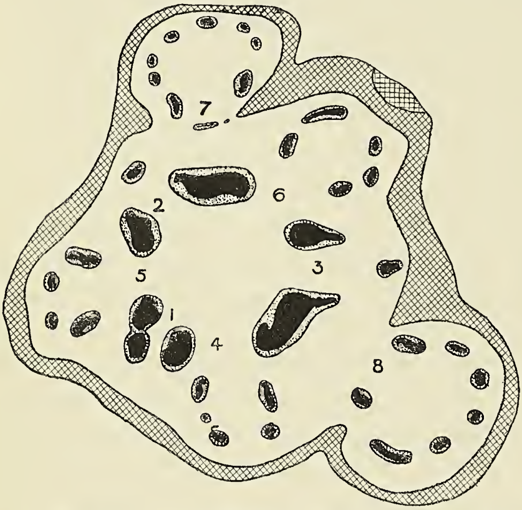
Commonly, a vascular commissure appears, as already noted, which runs parallel with the midrib, and connects the successive veins one with another, so that in each transverse section a vascular tract will be present below the sorus (Fig. 3, *f*, *g*). But exceptions occur where the commissural development is incomplete, a condition shown in Fig. 3, *h*, where no vascular tissue nor any sorus is seen. This interruption of the sorus and commissure, going along with the prevalent basipetal condition of the sorus, is believed to indicate a relation of *B. discolor* to primitive types, such as *Matteuccia*, in which these two characters are constant. The similarity of the leaf-trace to that of *Matteuccia*, already noted, points in the same direction. Nevertheless, the usual condition in well-developed fertile pinnae of *B. discolor* appears to be the more advanced one, with continuous vascular commissures, and with sporangia of ages more or less mixed, borne in a continuous band above it, and not located in circumscribed sori, as in *Matteuccia*.

The dermal appendages in *B. discolor* are chiefly brown scales on the axis and leaf-bases; scales of smaller size extend up the leaf, and cover the young pinnae. But here there are present also numerous glandular mucilaginous hairs of the usual *Blechnum* type.

(B) *Blechnum tabulare* (Thunb.), Kühn.

This subdendroid species, frequently known under its synonym of *Lomaria Boryana* (Swartz), Willd., was obtained in Jamaica, and large plants from that source living in the Glasgow Garden have provided developmental material. It spreads from the West Indies to the Falkland Islands, and is found in South Africa. In characters it is near to *B. capense* (L.), Schlecht (= *Lomaria procera*), but it differs markedly in texture, and in the black, narrow scales on its stem and leaf-bases. It is a dwarf Tree-Fern. Sir W. Hooker (Sp. Fil., vol. iii, p. 27) gives the height of the stem as 1–2 feet, but Gardner (MS.) quotes it as 4 feet high near to the summit of the Organ Mountains.

The vascular system of a large axis has been examined, and it was found to show characters which are usual for the Blechnoid types (Text-fig. 3). It is dictyostelic, with a massive pith. In the drawing the foliar gaps of the leaves are numbered in succession according to the level at which the gaps were traversed in the section, 1 being cut nearest to the base of the gap which it traverses. Following this series a clear idea of the vascular system will be obtained. Number 1, being cut just where the gap first opens, shows it giving off at its base the stele of a root. This is a very usual feature in *Blechnum*. In 2 the root-stele is seen passing outwards, while from the sides of the gap the initiation of the foliar trace may be noted. In 3 the two strands which constitute it are more clearly defined, while in 4 they have separated from the axial meristeles, and have already subdivided. The traces 4–8 indicate the behaviour usual for the leaf-trace in the larger types of the genus. The two larger strands of the series into which the trace divides are on the adaxial side, and show the usual hooks of xylem ; the smaller strands vary in number and in size. As the trace emerges into the petiole, it usually consists of the two adaxial strands with five or six smaller strands disposed in a curve between them (compare 7 and 8). It may be added that the vascular supply from the rachis to the pinna consists in this species of two equal strands. Plainly the vascular system is essentially of the same type as that seen in *Matteuccia*, but here the vascular tracts are more subdivided, in accordance with an hypothetical advance, and perhaps also the large size. Still more does *B. tabulare* show an advance in vascular structure as compared with *Plagiogyria*.



TEXT-FIG. 3. Transverse section of the stock of *Blechnum tabulare* (Thunb.), Kühn. The numbers indicate the succession of the leaf-gaps from below upwards. × 4.

The species is strongly dimorphic, the fertile leaves standing more erect than the sterile. The development of the fertile pinna shows the usual marginal segmentation of the wings. But the relation of the protective 'indusial' flaps to the marginal cells does not appear to be constant. Sometimes the 'indusial' structure seems plainly to be derived from the marginal cell itself (Pl. XXIII, Fig. 4, *a*, *b*) ; in other cases it seems to arise as clearly from an abaxial segment (Fig. 4, *c*, *d*, *e*, *f*), so that the true margin as defined by segmentation lies towards the upper, or adaxial face of the developing pinna. It is possible that this apparent variability may have its explanation in differences of position of the actual sections relatively to the apex and base of the pinna, a point which will come out more clearly in the description of the facts to be given for *B. lanceolatum*. Probably the sections *a*, *b*, where the flap is truly marginal, came from the middle region of the pinna, and those shown in *c*, *d*, *e*, *f*, from points nearer the apex or base, where the flap may be in varying degree progressively intramarginal. Such data are important as giving the opportunity of seeing the very first steps in origin of that assimilating flange which becomes so prominent a feature in the *Eu-Blechnum* section of the genus. Moreover, they provide an interesting comparison with the very similar condition in *B. discolor.*

Another important question in this species is the order of appearance of the sporangia. There is a considerable constancy in the basipetal sequence, though the number of sporangia in each section may be small (Fig. 4, *g*). For instance, three whole slides of sections were looked over without a single departure from it being seen. But exceptions are occasionally found, and one is shown in Fig. 4, *h*.

(C) *Blechnum lanceolatum* (R. Br.), Sturm.

This is again a species with a relatively simple structure of its fertile pinna, which shows no marked flange, or only one which is variable, owing to enlargements of the tissues of the wings rather than to any definite tissue formation. It is ranked with the '*spicant*' group by Hooker, but it has a more simple form than most of the species of that affinity. The outline and venation of the fertile pinna are shown in Fig. 5. The plant has a short ascending stock and a terminal tuft of strongly dimorphic leaves. A supply of material was sent to me by Mr. C. E. Foweraker, Waimate, S. Cant., New Zealand, to whom my best thanks are due.

Transverse sections of a fairly advanced pinna, taken at such a point as *x–y* in Fig. 5, show an outline as in Fig. 6, *a*. The contour of the section is almost circular, owing partly to the curvature of the regions right and left of the midrib, partly to the great distension of the tissues below the receptacles, which appear as assimilating tissues with stomata. The margins appear directly continuous into the 'indusial' flaps, which are curved over to protect the sori. There are sometimes two, sometimes only one vascular strand in the midrib, while beneath each sorus is a longitudinal commissure connected with the midrib by oblique veins. A vascular supply also branches off towards the upper surface of the pinna, each twig of which terminates in a gland, as is seen in some other species. The relation of this to the receptacle is shown in Fig. 6, *b*. The sorus is continuous, as in other species. The sporangia are spread over a rather wide flattened surface, and arise almost simultaneously; but, as development shows, there are indications of a basipetal succession, while later there may be seen some slight mixture of various ages together.

A transverse section of a young fertile pinna shows a segmentation as in Fig. 6, *d*. It is here obvious that the lateral flap results from the usual marginal segmentation, and the marginal cell itself is seen rather strongly deflected towards the lower surface. The deep cells which intervene between it and the midrib give rise to the sporangia, while the indusial flap which covers them is usually and normally formed from it; at least in the middle region of the pinna it is so, though at the basal and apical ends this may be departed from. A slightly later stage is seen in Fig. 6, *e*, in which it is plain, by comparison with Fig. 6, *d*, that the indusial flap has sprung from the marginal cell. Fig. 6, *f*, which shows a still more advanced stage, with the segmentation of the sporangia beginning, indicates again that the indusial flap is developmentally the extension of the margin of the pinna. It may be noted that there is some evidence of a gradate sequence of origin of the sporangia, as shown by this drawing.

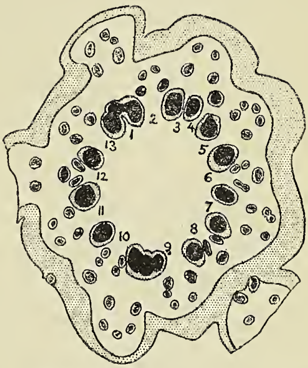
It will be seen from Fig. 5 that the indusial flap, which may be held to be the 'phyletic margin', stops short of the apex and the base of the pinna, and that it is there replaced by another distinct margin. Such a condition is explained by Fig. 6, *g*, which was cut from near the base of the pinna. It shows the 'indusial' flap projecting apparently from the under surface, at some distance from the actual margin. A similar condition may be seen in sections from near to the apex. Fig. 6, *c*, shows a section of a younger pinna near to its apex. On the right-hand wing two wedge-shaped cells are seen; one of these is presumably the source of the 'indusial' flap, the other is presumably the margin of the 'flange'. A somewhat similar condition is seen on the opposite wing, though not so clearly. Such conditions bring into prominence the question of the true and phyletic relation of the 'indusial' flap to the 'flange' or apparent margin of the pinna. This question can only be decided after a wide basis for comparison has been established, by examination of various species, both in external form and by means of sections.

Three species from the sub-genus *Lomaria* have now been described as regards the structure and development of the fertile pinna. They have been selected as outstanding examples from the sub-genus in respect of simplicity; they show in the form of the maturing pinna, and in the early stages of its development, the nearest similarity seen in the genus to what is found in the similarly dimorphic genera of *Matteuccia* and *Plagiogyria*. These are all alike in the absence from the fertile pinna of any distinct flange, though in varying degree there are indications of its origin in the three species. But substantially the 'indusial flap' is the recurved leaf-margin, as it is in *Matteuccia* or *Plagiogyria*. We shall next proceed to examine such further species from the sub-genus *Lomaria* as show a distinct 'flange', apart from the indusial flap, but in which that flange is not a dominant feature. It will be found, however, that though of small size in these species, still it is actually present. The species are—*B. attenuatum* (Sw.), Mett.; *B. L'Herminieri* (Bory), Mett.; *B. spicant* (L.), Wither, and *B. capense* (L.), Schlecht. They will be taken in the order in which they stand in the Synopsis Filicum.

(D) *Blechnum attenuatum* (Sw.), Mett.

This is a species which is sometimes creeping in habit, but more usually erect, and may often assume a climbing habit, attaching itself by means of roots to the stems of larger plants. Material was obtained in Jamaica, and also from the Botanic Gardens of Edinburgh and Glasgow. The axis contains a dictyostele, with very numerous, narrow leaf-gaps. In Text-fig. 4 there are thirteen of them, and they have been numbered. according to their position in the circular dictyostele. As their number is so large their succession from below and their relation to the leaf-traces and root-traces may be readily deduced.

The large meristele between gaps 9 and 10 is preparing to give off a root-trace by separation of a central tract of the meristele. The process is shown more advanced in the meristele on the opposite side of the axis, which is beginning to form by involution the gap numbered 1. The central tract of the meristele is here nearly separated, while in the case of gap 4 the separation of the root-trace is seen completed, and thus the foliar gap is fully formed. From the meristeles on either side of a gap thus opened a strap-shaped leaf-trace strand is then given off, as seen in gaps 8 and 12. These very soon divide each into two (see gaps 6 and 10), and later into larger numbers (gaps 5, 7, 9). It is thus evident that the vascular system is on the same plan as that of *B. tabulare*, but complicated by a greater crowding of the leaves, and a much larger proportion of vascular tissue, as seen in the transverse section. This, together with the relatively large bulk of the strands, is in accord with the climbing habit, and affords an interesting basis for comparison with the climbing *Stenochlaena*. It corresponds to *B. tabulare* in having a single root arising from the base of each gap, and in the fact that the leaf-trace consists originally of only two strands.



TEXT-FIG. 4. *Blechnum attenuatum* (Sw.), Mett. Transverse section of the stock; the leaf-gaps are numbered for convenience of reference, but not in order of their natural sequence. × 4.

The mature fertile pinna of *B. attenuatum* shows an outline as seen in Pl. XXIV, Fig. 7, *e*. There is no marked flange, but the whole section is roughly four-angled, owing to the presence of two rounded ridges, right and left of the midrib. The latter is traversed by two vascular strands, with a slight surface-involution between them. Right and left of this are glands (hydathodes) of flask-like form, opening upon the adaxial surface. To each of them a vascular strand passes; it is derived from the commissure which runs below each of the receptacles and extends outwards into the indusial flap. The latter is clearly of the nature of a portion of the leaf surface, for the tissues of the thicker portion of the median region of the pinna extend out into it, without any sudden change, graduating imperceptibly into the flap, till at last a single layer of cells is reached. There is, however, a preponderance of lacunar mesophyll in the median region. The sori are restricted to the thinner region, where the mesophyll is either reduced or absent. But they extend for a considerable distance along the flaps, while a vascular supply stretches beneath them outwards from the commissures. Thus the character of the flaps suggests that they are lateral regions of the pinna expanse.

The development of the fertile pinna has been traced in Pl. XXIV, Fig. 7, *a–e*, and it has been found that the marginal cell, with its usual alternating cleavages (*m*), finally takes its place at the extremity of one of the rounded ridges. The indusial flap is here of later origin, as the figures themselves will show. In the young state (Fig. 7, *a*) the foliar wing is clearly referable in origin to cleavages from a marginal cell (*m*). As the development proceeds, an upgrowth appears at some distance from the margin and upon the lower surface. This is the indusial flap (*i*), which is thus initiated as a superficial outgrowth (Fig. 7, *b*, *c*). It gradually increases, with inclined cleavages (*c*, *d*), thinning out to only two layers (*d*) and finally to a single one. It thus appears that the indusial flap is no longer the direct product of a marginal segmentation, but is slightly deferred in its time of appearance and is produced as an upgrowth from the lower surface.

The sporangia arise almost simultaneously, and, though they do not exactly synchronize in their segmentation, there is not any marked 'mixed' condition of the sorus. Fig. 7, *d*, shows the early cleavages which define the procambial strands, one of these leading to the gland (*h*), the other passing to the receptacle of the sorus.

(E) *Blechnum L'Herminieri* (Bory), Mett.

Material of this species was obtained from the Blue Mountains, Jamaica, and from the Glasgow Botanic Garden. The plant is erect and subdendroid, but does not assume a climbing habit. The axis and leaf-base are covered with dark scales, and the leaves are dimorphic, the pinnae of the fertile leaves being very narrow. Hooker remarks (Syn. Fil., p. 176) that it 'is probably the same species as the Australian and Polynesian *lanceolata*'. But this identity was not actually adopted by Hooker himself or by later writers.

The fertile pinnae show in transverse section a similar outline to those of *B. attenuatum*, but on a less bulky scale of construction. There is no flange that can be observed externally. The very youngest stages were not observed in this species. In the earliest seen there were already two distinct projections on the developing wing (Pl. XXIV, Fig. 8, *a*). They are plainly the indusial flap (*i*) and the margin (*m*), as in *B. attenuatum*, and their ultimate origin is probably the same. The indusium soon projects as a protective covering over the region where the sporangia arise. They are formed from the deep prismatic cells seen in Fig. 8, *a*, *b*, and usually show some indication of a gradate sequence (Fig. 8, *c*), but this is not constant, and in Fig. 8, *d*, which shows a rather more advanced condition, there are signs of irregularity. The wedge-shaped cell marked *m* is a fairly constant and easily recognized feature, and marks the structural margin. This is specially noted for comparison with other cases, such as *B. filiforme* and *Stenochlaena*. There are evidences of branchings of the vascular supply below the sorus to supply glands, as in *B. attenuatum* (Fig. 7, *c*), but the whole structure, though very like that species, is on a less elaborate scale.

(F) *Blechnum spicant* (L.), Wither.

This familiar species corresponds in essentials to those above described, but, as will be seen, it shows a slight degree of advance in the complexity of its fertile pinnae.[1] The mature stem is upright and contains much sclerenchyma, which forms an external cortical band, and a medullary mass with projecting angles. Its vascular system, consisting of the usual dictyostele, lies between these, surrounded by softer parenchyma (Text-fig. 5). The meristeles vary in number in the transverse section according to the leaf arrangement; they are usually about four in number and have a form similar to those in *B. attenuatum*. Here, as elsewhere in the genus and with striking regularity, each meristele first gives off a root-trace from its central region, which passes bodily out. A leaf-gap is thus formed, the sides of which, at a slightly higher point, give off the leaf-trace itself as two strap-shaped strands, which enter the leaf-base without further division. But here one of them may give off a single smaller strand, which usually splits into two (see Luerssen, Rab. Kr.-Fl., vol. iii, p. 114). These lie between the larger strands, forming with them a simple horseshoe. This is a rather simpler structure than in the larger *B. tabulare*, or *attenuatum*, while all may be held as derivatives of the type seen in its simple form in *Matteuccia*, or still more simply in *Plagiogyria*.

TEXT-FIG. 5. Transverse section of the stock of *Blechnum spicant* (L.), Wither. × 8.

The general characters of the fertile pinna are adequately shown in the

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[1] For habit of pinnae of this species see Rab. Krypt.-Flora, vol. iii, p. 109, and Figs. 84, 85; for an account of the vascular system, l. c., p. 110. For the vascular condition of the seedling see Chandler, Ann. of Bot., vol. xix, p. 373.

figures in Rabenhorst, but the venation of the tip in relation to the sorus, in cases where the latter stops short of it, is better seen in Pl. XXV, Fig. 9. It shows the sorus distinctly intramarginal, with a flange on either side, into which short vascular strands pass. This is an advance on any of the species hitherto described ; in them the flange was a relatively insignificant structure without any vascular supply of its own. The sori are shown in Fig. 9 as shaded tracts stopping short of the apex; a vascular commissure runs beneath each, connecting the veins, but this also stops short where the sorus ends. The arrangement of the veins and the relation of the commissure to them suggests very strongly that it is actually an extension of the anadromic branch of the furcate veins. This was the view held by Mettenius (Fil. Hort. Lips., p. 60, Taf. V, Fig. 5).

The development of the fertile pinna begins in the usual way, by alternate cleavages of a marginal cell (Pl. XXV, Fig. 10, *a*). But here the marginal cell takes its place quite definitely at the apex of the flange, which, as we have seen, is here of larger size proportionately than in previous examples. It may be seen to hold that position in Fig. 10, *b*, *c*, *d*, and the whole flange is referable in origin to it. But very soon a stronger growth appears on the abaxial face, at a point distinctly back from the margin (Fig. 10, *b*). It consists of a broad weal, several cells in section. The slope of this, which faces obliquely to the midrib, soon shows deeper cells (Fig. 10, *c*), which, undergoing segmentation, produce sporangia (Fig. 10, *d*). Though almost simultaneous in their time of origin, there are slight signs of a gradate sequence of the sporangia. It is closely adjoining the young sporangia on the side away from the midrib that the 'indusium' arises. We note that it is here relatively late in its origin, and is remote from the margin. Its structure when mature is shown in Rabenhorst's Fig. 85. Its late appearance as compared with the species hitherto described, and its intramarginal position, may be connected with its smaller size when mature, as an example of a principle of wide application. In respect of the time and place of appearance of the 'indusium', *B. spicant* may be held to be a more advanced type than those previously described, and to show a more clear case than any of them of the 'phyletic slide' of the indusium to the lower surface of the pinna.

(G) *Blechnum capense* (L.), Schlecht.

This very widely spread species, better known under its synonym of *Lomaria procera*, is of coarse habit, with stout ascending or erect stem, covered thickly with scales which extend also up the leaves, and form a very perfect protection to them while young. But in addition there are the glandular hairs of the *B. brasiliense*-type, which are so prevalent through the genus. Normally the leaves are strongly dimorphic and the fertile pinnae narrow, without any very prominent flange. But intermediate states are common. Sometimes the upper part of a leaf is fertile, and the lower sterile with broad pinnae, or the pinnae of one side of the leaf may be fertile and those of the other sterile, or the pinnae themselves may be sterile at the base and fertile at the tip. There is perhaps no species that is so variable in this respect as *B. capense*. But, in addition, types are sometimes found in which the pinna appears as in *B. brasiliense*, with the linear sori close right and left of the midrib, and the flange, which is usually small in this species, widened out into a broad expansion with an extensive venation of its own. The extraordinary instability which is thus seen in *B. capense* may be compared with that to be described below in *B. punctulatum*, but it will be seen that the two species have brought about the change in rather different ways, though the result of obliteration of the dimorphism is the same in both.

The vascular system of the axis is in essentials on the plan above described; the petiole also, except that it shows a rather higher complexity owing to subdivision of the leaf-trace strands. In a large petiole at the level of the lower pinnae, the horseshoe-like series of strands may number ten or eleven, but the two on the adaxial side are always the largest and show the usual xylem-hooks.

In the development of the fertile pinna the usual marginal segmentation is found, and the whole of the lateral wing is referable to it (Pl. XXV, Fig. 11, *a*). The initial cell itself remains at the margin of the flange, but the latter does not normally develop to any great size. Some distance inwards from the margin a massive upgrowth appears upon the lower surface (Fig. 11, *a*, *b*). This produces the receptacle and indusium, which together are rather more robust than in *B. spicant*, and the indusium soon projects as a body of considerable dimensions, overarching the receptacle (Fig. 11, *c*). In the mature state there is a strong vascular commissure, which runs under the receptacle, and, as in *B. spicant*, the venation extends outwards into the flange, which is here quite of appreciable size.

(H) *B. gibbum* (Lab.), Mett.

This species need not be examined in detail. It is a subdendroid type, in habit very like *B. discolor*, and the leaves are commonly dimorphic. But the point of interest for us is that the fertile pinnae are again variable. Sometimes they are narrow, with a hardly perceptible flange, but in other cases there may be considerable expansion of it, so as to supply a large assimilating surface. It is thus susceptible of an instability similar to that of *B. capense*. A state of this species which very nearly resembles *B. brasiliense* has even been described as var. *blechnoides*.

The species above described are all included by systematists in the sub-genus *Lomaria*. They give a fair representation of that type and show that it includes chiefly upright, or even subdendroid, forms; they illustrate various conditions of the fertile pinna from those in which a 'flange' is virtually absent both in the mature and in early developmental stages (e.g. *B. lanceolatum*), a state which compares with *Matteuccia* or *Plagiogyria*; to those in which it is present, and even developed to a considerable size, as in *B. spicant* or *B. gibbum*. There is an underlying uniformity in the form and anatomical structure of the whole plant, notwithstanding fluctuations of detail, and a similarity in the scales and glandular hairs, which indicate that the series is a natural one of real affinity. This, indeed, has never been a matter of serious question.

Examples will next be taken of *Eu-Blechnum*, which is characterized by the more or less complete absence of dimorphism. Here it may be difficult, or even impossible, to tell the sterile from the fertile leaves until they are examined from below. The sori then appear as linear structures parallel to the midrib, and lying as a rule close to it on either side. This state has been seen to occur abnormally in *B. gibbum* and *B. capense*, and it thus appears that the two sub-genera are closely allied, while we shall anticipate that the development of the fertile pinnae will differ rather in detail than in principle. The facts show that the anticipation is justified.

(I) *Blechnum* (*Eu-Blechnum*) *brasiliense*, Desv.

This is a large, erect, subdendroid species, with the bud covered by dark scales, which remain permanently on the axis and leaf-bases. In addition to these, the mucilaginous glandular hairs described in detail by Gardiner and Ito (Ann. of Bot., vol. i, p. 27) are present, a type that is prevalent in the genus. The leaves are all alike in outline and their pinnae relatively broad. It will be unnecessary here to describe more than the origin of the sorus and of the largely developed flange. The wing of the fertile pinna arises in the same way as that of the sterile, that is by the usual marginal segmentation (Pl. XXV, Fig. 12, *a*), and the sorus does not make its appearance until the wing has attained considerable size. It originates as a rather massive upgrowth at some distance from the marginal segmentation, which has here no direct part in its formation (Fig. 12, *b*, *c*). The upgrowth soon shows a greater activity on the side remote from the midrib, so that it becomes tilted over, facing centrally (Fig. 12, *d*). Very soon the 'indusium' appears, overarching the receptacle which bears the sporangia. It grows rapidly (Fig. 12, *e*), taking a strongly curved form, and its apex coming in close relation to the projecting midrib; the result is a very perfect protection of the receptacle. Upon this the sporangia arise, with the usual segmentation. Their order of succession in the sorus at first shows clear signs of a gradate sequence (Fig. 12, *d*, *e*), but this is not maintained, and in later stages the sorus becomes pronouncedly of the mixed type. The progression from the more primitive to the derivative state is here, as in some other cases in Ferns, reflected in the individual development. Beneath the sorus is the usual vascular commissure, which is indicated in Fig. 12, *e*, though still young. It is interesting also to note in this drawing that a stoma is seated on the swelling of the 'indusium'.

Similar observations were also made on *B. occidentale*, a species with erect axis and long lanceolate scales. It is unnecessary to give any detailed description, but it was obvious that the origin of the flange is directly from the marginal segmentation, and that the appearance of the sorus and 'indusium' is very late, and in position superficial, as in *B. brasiliense*. This is in fact the type for the sub-genus *Eu-Blechnum*.

There are, however, certain types related to *Eu-Blechnum* in the soral characters, which differ in the general conformation of their leaves and in general habit. Some are doubly pinnate, a state seen in *B. Fraseri*, and in *Sadleria*: or the habit may be climbing, as in *B.* (*Salpichlaena*) *volubile*. These must also be examined for purposes of comparison.

(J) *Blechnum Fraseri* (A. Cunn.), Luerss.

For material of this species I am indebted to Dr. Cockayne, F.R.S., and to Mr. H. Carse, of Kaiaka, Auckland, New Zealand. Its stem is sub-erect, with the leaves bipinnatifid. Hooker remarks that it is 'very dissimilar in habit to all other species, combining a frond like one of our common *Lastreae* with the fructification of a *Lomaria*' (Syn. Fil., p. 182). But various intermediate stages between simple pinnation and double are illustrated in this species, while the same is also seen at times in *B. diversifolia*, Mett. Moreover, at the apex of the leaf it runs into single pinnation, as in other species. These intermediate states serve as a connecting condition between this and the usual Blechnoid type.

The fertile segments show a marked midrib, with an area of dark green tissue on either side, limited by a slightly projecting paler line, which is continuous round the sinus of the next segments above and below. From this the paler indusial flaps slope downwards like a curtain on either side. A transverse section of an almost mature pinna shows a single vascular strand in the midrib, and lateral commissures (Plate XXVI, Fig. 13, *f*). There is a marked flange on either side, made up of lacunar mesophyll, covered by epidermis with stomata, but it is not nearly so extensive as in the *Eu-Blechnum* types. The indusial flaps curve sharply inwards, so as closely to meet the enlarged midrib. It is found that the sporangia may extend on to it, beyond the insertion of the flange.

The development begins by segmentation after the usual plan, with segments alternately from the sides of the marginal cell (Plate XXVI, Fig. 13, *a*). Very soon a growth begins upon the lower surface at a point distinctly intramarginal from the genetic margin, and its cells segment obliquely, so that it is at first relatively massive (Fig. 13, *b*, *c*, *d*). But as its growth proceeds it becomes thinner, till at its extreme margin it becomes only a single layer of cells thick (Fig. 13, *e*, *f*). Meanwhile it curves towards the median line, so that, as the segment has here enlarged to form the midrib, the new growth inclining towards it covers in the concave lower surface. The part of this surface directly overarched becomes the receptacle, which is here flattened. Vascular tissue, constituting a strand on either side of the midrib, has meanwhile been formed internally at a point slightly nearer to the midrib than the receptacle itself (Fig. 13, *e*, *f*). This is the commissure usual in the genus, and from it a vascular extension may be continued towards the margin of the flange. The latter has meanwhile enlarged, its tissues become lacunar, and it may bear stomata (Fig. 13, *f*). The sporangia are produced upon the flat receptacle in close order, and almost simultaneously, but traces of a basipetal sequence are evident, as indicated by the various stages of segmentation which may be seen side by side. For instance, in Fig. 13, *e*, the middle sporangium of the five shown is slightly in advance of the others. This basipetal sequence, slight though it is, appears to be maintained with a high degree of constancy, though occasional departures from it have been observed, but there is no confirmed condition typical of the Mixtae. The area over which the sporangia are spread is worthy of note. They may extend beyond the flange on to the 'indusial' margin. This may be held as an indication of its foliar nature, and that it is not a mere indusium such as is seen in certain other types of Ferns.

The petioles of *B. Fraseri* are thinner than in other species, and show the usual two-hooked adaxial strands, and between them one or two smaller strands constituting a relatively simple horseshoe.

(K) *B.* (*Salpichlaena*) *volubile*, Klf.

This tropical American species is, like *B. Fraseri*, bipinnate, but differs from it greatly in size, and in having adopted a climbing habit somewhat like *Lygodium*. A good representation of it is given in Hooker's Garden Ferns, Plate XV. The leaves are not dimorphic as a rule. The pinnules are very large, with 'the fructification of a *Blechnum*', i. e. sori parallel to and closely adjoining the midrib, and with a very wide expanse of assimilating 'flange'. No material has been available for developmental study, but the mature structure leaves no room for doubt that the development would prove to be of the ordinary Blechnoid type.

Hooker, in his Garden Ferns, points out the 'very close resemblance to our *Lomaria volubilis*'. The difference lies in the latter being dimorphic, with narrow fertile pinnules. But the detailed drawing in the Species Filicum, vol. iii, Pl. CL, Fig. 3, clearly shows the presence of a rudimentary flange. We have seen in certain species of *Blechnum* how variable the development of the flange may be, especially in *B. gibbum*, so that there can be no definite line drawn between the Blechnoid and the Lomarioid states. This fully justifies the merging together of the two Ferns named as forms or varieties of the species *B. volubile*, Klf., which has been done by

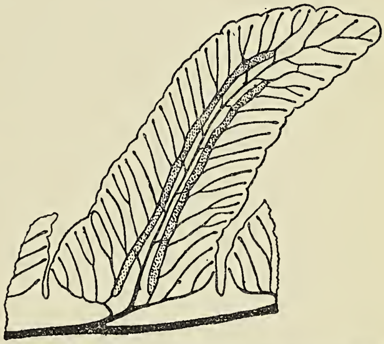
Diels (Nat. Pflanzen-Fam. i. 4, p. 247), Christ (Farnkräuter, p. 183), and by Christensen (Index Filic., p. 161).

The two distinct conditions coexisting in what is otherwise a uniform species illustrates again the instability of development of the 'flange'. But the question which of these states is the more primitive must be left open. It cannot be rightly assumed *a priori* that in the *Lomaria* type the lamina is reduced ('stark in der Spreite reducirt', Diels, l. c.). A decision on this point can only be based on grounds of broad comparative and physiological consideration. The question will be taken up later.

Sadleria cyatheoides, Klf.

This Fern is maintained as the type of a substantive genus, though in many respects it is very closely allied to *Blechnum*. It is subdendroid in its habit, and a distinguishing mark is the double pinnation of its leaves. But as *B. Fraseri* and *volubile* both show a similar condition, this cannot be held as a very valid generic distinction. The specific name points a comparison with the Cyatheaceous habit; a similarity there exists which may be accepted as having more than a mere external significance.

The pinnules of the fertile leaf have a distinct Blechnoid character. One of them is represented whole in Text-fig. 6, as viewed from below. The venation springs wholly from a single strand given off from the midrib of the pinna, and its branching is like that of *Blechnum*. On either side of the midrib runs the sorus, with a vascular commissure, and 'indusia' arching towards the midrib. Transverse sections show that the relations are the same as those in *Blechnum*, while there are indications of a basipetal sequence of origin of the sporangia, but this does not appear to be strictly maintained. I owe the supply of material of this Fern to the kindness of Professor Campbell of Stanford University.



TEXT-FIG. 6. *Sadleria cyatheoides*, Klf. A pinnule, showing the venation and its relation to the fusion-sori. × 3.

Comparative Conclusions on the typical Fusion-Sorus and 'Flange' in Blechnum.

The reader who has carefully followed the details described in the foregoing pages will have already perceived the morphological progression which they indicate. The description started from a type of Fern with dimorphic leaves and superficial sori, which were protected by a simple curling downwards of the leaf-margin, and its development as a thin, even a single-layered, protective flap. But there is no 'true' indusium. This state is seen in *Matteuccia*, and also in *Plagiogyria*, both of which genera have been closely related by the older systematists with *Blechnum* itself. In the former genus there is a convex receptacle in each isolated sorus, and a clearly marked gradation in the appearance of the sporangia; but in *Plagiogyria* the receptacle is flattened, and there are indications of a 'mixed' state. An irregularity in thickness of the lateral wings of the pinna has been noted already in *Plagiogyria*: it becomes more marked in various species of *Blechnum*, and it contributes to the peculiarity of the outline as seen in section.

In the first-named species of *Blechnum*, such as *B. discolor*, *tabulare*, and *lanceolatum*, the pinnae as seen in section conform very nearly to the type of *Matteuccia* or *Plagiogyria*. But indications are already seen that the protective flap does not always coincide with the margin, as defined by segmentation; the divergence appears most marked at the apex and base of the pinna. Proceeding through the series of species described, this divergence increases, both in time and place of origin of the 'indusial flap', till the *Eu-Blechnum* condition is arrived at, as seen in *B. brasiliense*. The series suggests that *the true margin by descent is the indusial flap*, and that it has undergone a 'phyletic slide' from its original marginal position to a position on the lower surface; consequently it is the 'flange' that now originates by direct continuance of the marginal segmentation. If this be the true history, then the 'flange' is really a new structure, of secondary origin by descent, but taking a prior place in the ontogeny in accordance with its importance as an assimilating organ. For by its formation the assimilating tissue of the fertile pinna is largely increased, spongy parenchyma and stomata being produced upon it. The consequence of its appearance is that the dimorphism is obliterated, and all the leaves, sterile or fertile, take a similar form. The biological advantage of better nutrition of the sporophylls thus gained is too obvious to need insistence. This is a brief and bare statement of the comparative conclusion. It might of course be possible to invert the thesis, and to suggest that the series illustrates an abortion of a broad lamina, and that the Lomarioid state is really the derivative. But this view would present various comparative difficulties, especially in relation to the sorus and indusial flap.

On the view stated above, the Blechnoid fusion-sorus arose from a gradate type with isolated sori like those seen in *Matteuccia*, or possibly in *Plagiogyria*. These were seated on distinct veins. The formation of commissures connecting the veins, and the consequent running together of the separate sori into the Blechnoid fusion-sorus, is a step easily understood when starting from a type like *Matteuccia*. Also the progression from the gradate to the mixed condition, illustrated in the ontogeny of the species described, would readily follow with the other changes. It does not appear, if the thesis be inverted, from what known type of Ferns *Eu-Blechnum* could have arisen, while some of the steps towards *Lomaria* would be inconsistent with a general experience in the phylogeny of the Filicales. Finally, a biological probability of the change would need to be established of greater strength than that advanced above, which explains the progression from § *Lomaria* to *Eu-Blechnum*.

ii. Progression towards an Acrostichoid Type.

Having arrived at a probable view regarding the origin of the Blechnoid state, we may now proceed to consider certain further developments from it, which are believed to have been phyletically derivative. The first of these is the ' Acrostichoid ' type, the origin of which may be illustrated by a comparison of such species as *B. Patersoni*, *B. penna-marina*, *B. filiforme*, and *B.* (*Stenochlaena*) *sorbifolia*.

(L) *Blechnum Patersoni* (R. Br.), Mett.

As originally described, this species was characterized by having a simple leaf. It is a low-growing, very tough, and leathery plant, rather widely spread from Ceylon to Australia and New Zealand. Cultivated specimens frequently show irregular lobing of the strongly dimorphic leaves, while through the var. *pinnata*, and through *B. elongatum*, Blume, various steps may be found to the pinnate type so usual in the genus. It would then seem probable that the habit of the species has originated by simplification of the leaf-outline from the common pinnate type of *Blechnum*.

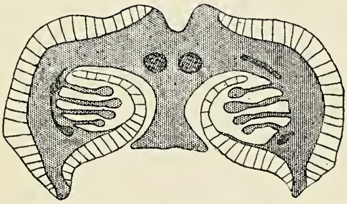

a. *b.*

TEXT-FIG. 7. *Blechnum Patersoni* (R. Br.), Mett. *a*, transverse section of the stock; *b*, tangential section of the stock, showing the relation of roots to leaf-traces. × 6.

The anatomy of *Blechnum Patersoni* conforms on a relatively simple scale to the characteristics of the genus. Transverse sections of the rather short stock show the usual dictyostele (Text-fig. 7, *a*). At the base of each leaf-gap, just before it opens, a root-trace passes off. Closely above it, from right and left of the gap, the two equal foliar traces spring. These pass obliquely outwards without further subdivision into the petiole. Tangential sections show how regular is the disposition of these (Text-fig. 7, *b*). The root-trace may at times be slightly diverted to right or left, but it commonly lies immediately below the leaf-trace. The whole scheme is one of the most regular observed in *Blechnum*, but the leaf-trace is simpler than is frequently the case in the larger species. It corresponds rather to that of *Matteuccia*. It is possible that its simplicity may be correlated with xerophytic reduction, which has undoubtedly played its part in the moulding of the species.

The sporophylls are upright, very narrow, and commonly unbranched. Text-fig. 8 shows the outline of the transverse section, with its two vascular strands in the midrib. The margins are strongly rolled downwards, and the indusial flap fits closely into the curves of the enlarged midrib, giving very perfect protection to the sori. Superficially there are deep watery cells, shown diagrammatically in the drawing, which contracting at maturity unroll the lateral flaps, and allow freedom to the spores.

From early stages it is seen that there is as usual a row of marginal initials, with alternate segmentation (Pl. XXVI, Fig. 14, *a*). A considerable wing is thus formed on either side. Upon the abaxial surface of this, and at a distinctly intramarginal point, an upgrowth arises (Fig. 14, *b*, *c*). From this the indusial flap springs, while the receptacle lies between it and the midrib (Fig. 14, *d*). But the form of the receptacle is here not convex when mature, but may be distinctly concave (Fig. 14, *d*, *e*); moreover, it may clearly be seen that the sporangia of different ages are intermixed. These features are here more marked than in any of the preceding species; they suggest that *B. Patersoni* is derivative and advanced as compared with them. Further, it is seen that the area of the sorus is not sharply restricted, but spread over a considerable space between the midrib and the indusial flap, a point which will be important for comparison with the species to be subsequently described.



TEXT-FIG. 8. Transverse section of a pinna of *Blechnum Patersoni* (R. Br.), Mett. Semidiagrammatic. × 24.

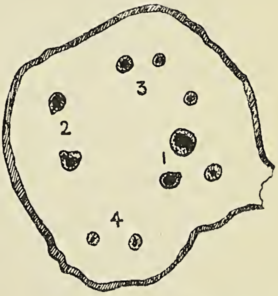
Mettenius figures for *B. Patersoni* a specially large type of sporangium with long and thick stalk, and a head with a continuous oblique annulus; and he states that such sporangia are found seated on the most projecting point of the receptacle (Fil. Hort. Lips., p. 61, Pl. IV, Fig. 7). In face of his circumstantial statement and of the drawing which he gives, I do not express any doubt of these interesting and comparatively important facts;

but I have not been able myself to find such sporangia in my material. The details of the sporangia are variable, but the annulus is certainly interrupted at the insertion of the stalk in the great majority of cases. It is oblique and takes a sinuous course, as in *Matteuccia*, though the number of cells is less than in that genus. The characters which are thus prevalent in this species appear to be general for *Blechnum*.

The general conclusion from study of *B. Patersoni* is that it is a type xerophytically reduced in form and structure; that its sporophyll (though usually unbranched) is of a relatively advanced type, with its strongly intramarginal origin of the indusial flap, the extended and flattened receptacle, and the mixed sorus. But on the other hand, the slightly oblique but typically interrupted annulus of the sporangia appears to be a character reminiscent of earlier forms, and comparison relates them with that of *Matteuccia*.

Blechnum penna-marina (Poir), Kuhn (= *Lomaria alpina* (Spreng.).

Specimens of this species were received from Dr. Cockayne, F.R.S., and also from Mr. C. E. Foweraker, District High School, Waimate, New Zealand; some material was also obtained from Kew, from specimens from the Falkland Islands. It is a creeping species with elongated rhizome and strongly dimorphic leaves. Of these the sterile are coriaceous, with short obtuse pinnae. The fertile leaves are taller, with pinnae of the type of *B. spicant* as seen externally, but shorter and broader towards the base, and when mature the sporangia appear spread over the whole under surface, which is itself unusually broad. There is, in fact, a definite approach to an 'Acrostichoid' condition.



TEXT-FIG. 9. *Blechnum penna-marina* (Poir), Kuhn. Transverse section of the stock; the leaf-gaps numbered in succession from below upwards. × 15.

The leaf-trace comes off as two small strands from the dictyostele of the axis, the latter corresponding in its relation to the leaf- and root-traces to the usual Blechnoid construction, but drawn out in accordance with the elongation of the rhizome. This is apparent from Text-fig. 9. Here there are five meristeles of the axis. The positions of the leaf-traces are numbered successively from below upwards: 1 shows the root-trace just separating, and the meristeles beginning the formation of the leaf-trace strands; 2 shows these more advanced; in 3 the leaf-trace strands have separated, and are passing out; in 4 they are further out, and the leaf-base is beginning to take its outline. Clearly the type is the same as in *B. Patersoni*, but greatly extended.

The structure of the young pinna follows the type of *B. attenuatum*, *L'Herminieri*, and *Patersoni* in having the indusial flap intramarginal, and the margin itself showing as a distinct projection (*m*), though it is only of small size. This is seen in Pl. XXVII, Fig. 15, *a*, which was cut near to the apex of a pinna: it shows also that the sporangia do not arise simultaneously, but that various ages are intermixed. The pinnae widen out considerably downwards, having a more or less cuneate form: and a section lower down in a pinna of more advanced age is seen in Fig. 15, *b*. Here it appears that a very considerable space intervenes between the midrib and the margin; and the sporangia, which vary greatly in age, are distributed over the whole concave surface. The section has been cut so as to follow the course of a vein, and some sporangia may be seen to lie beyond its distal end. Below the third from the margin the commissure has been cut through. If sections be cut transversely to the veins, and between the commissure and the midrib, the appearance will be as in Fig. 15, *c*. Here the young vascular strands are seen all separate, while the whole surface, not only above them but also between them, is occupied by sporangia. Clearly they may be here produced over the whole surface, and are not restricted to the position above the veins or the commissure. Comparison with Fig. 15, *b*, shows that a non-soral condition has been reached, with the sporangia spread over a broad band of leaf-surface on either side of the midrib. It is, in fact, an approach to an 'Acrostichoid' state, and this will be interesting for further comparison.

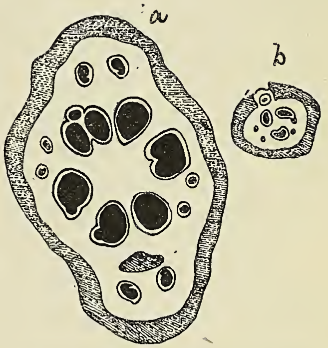
Blechnum filiforme (A. Cunn.), Ettingsh.

This remarkable plant has been definitely placed in the *Lomaria* section of the genus *Blechnum*, though it has passed under various synonyms. It was styled *Osmunda reptans* by Banks and Solander, and *Stenochlaena heteromorpha* by J. Smith, a name which probably points its most natural affinity. It is figured in Hooker's Species Filicum, vol. iii, Tab. CXLIX. It is a scandent species, rooting below in the soil, and climbing up supports to a considerable height. The sterile leaves in the basal region are 'Pimpinelloid' in outline, and sharply toothed; but those of the climbing region resemble those of other Lomarias. The fertile leaves are externally of the usual Blechnoid type, but with very long and narrow pinnae, which shrivel when mature, so that their upper surface becomes concave, and the lower soral surface convex. This, as it is covered with sporangia over a broad area, gives an 'Acrostichoid' appearance to the fully ripe pinna.

The Fern is a native of New Zealand and Fiji. Supplies of material were received from Dr. Cockayne, F.R.S., and also through his instigation from Mr. Esmond Atkinson, of the Department of Agriculture, Wellington, New Zealand, to both of whom my best thanks are due.

There is a marked difference in size of the creeping and the climbing regions of the axis. This is shown in Text-fig. 10, *a*, *b*. But the structure is upon the usual Blechnoid plan, with minor variants. The creeping region shows relatively few meristeles, in accordance with the scattered distribution of the leaves; in this it resembles *B. penna-marina*: the origin of the root-traces and leaf-traces is the same, the latter consisting also of only two strands. But in the climbing region the leaves are more crowded, and the axis larger, while the ring of meristeles shows more numerous gaps, and the meristeles themselves are very large, with bulky xylem. Still the *Blechnum*-type is maintained; it is only the size and the complexity of the system, not its plan, that is changed. It is important that this likeness to other Blechnums should be demonstrated, as in certain external features this species is so divergent from the genus at large.

The development of the fertile pinna resembles that of *B. Patersoni*. It starts with the usual marginal segmentation (Pl. XXVII, Fig. 16, *a*). The indusial flap is initiated in an intramarginal position (Fig. 16, *b*, *c*). But as the marginal activity is not continued, the flange remains small (Fig. 16, *d*, *e*), and in some cases may not project appreciably from the curved surface of the pinna (Fig. 16, *f*). The marginal cell itself takes sometimes a glandular appearance, which together with its position and shape serves to mark its identity, which might otherwise be missed. Meanwhile the cells on the upper surface of the pinna enlarge, and become watery. The receptacle, which is now covered in by the indusial flap, produces sporangia, which show various ages intermixed and are spread over a fairly wide area, as in *B. penna-marina*. Later, the thin watery cells in the neighbourhood of the flange begin to collapse; their thin outer walls are thrown into folds, with the effect that the margin is inverted, as may be observed in the mature pinna (Fig. 16, *g*).



TEXT-FIG. 10. *Blechnum filiforme* (A. Cunn.), Ettingsh. *a*, transverse section from a climbing stem; *b*, from a creeping rhizome. × 6.

It appears from these data that the pinna is of the type of *B. Patersoni*; that it shows slightly 'Acrostichoid' characters; that the flange is liable to be arrested, and appears indeed to be merely vestigial, as is seen in Fig. 16, *f*, *g*. As an 'Acrostichoid' type it is not so advanced as *B. penna-marina* in point of spread of the sorus. But it will be seen that in the general characters of the plant it shows a nearer approach to the *Stenochlaena* type than any other species of the genus that has been investigated.

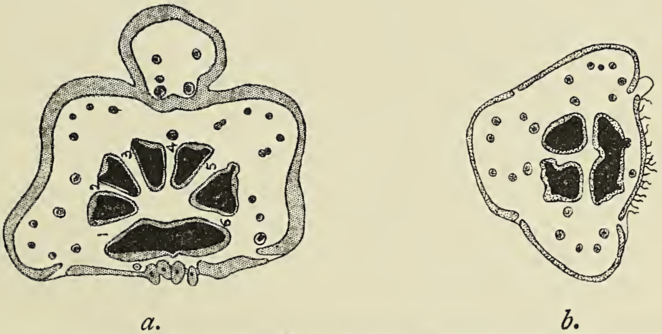
Stenochlaena sorbifolia (L.), J. Sm.

This Fern, which is very widely spread throughout the tropics, is maintained as a representative of the substantive genus *Stenochlaena*, J. Sm., by Dr. C. Christensen. It has undergone unusual vicissitudes of nomenclature, having been referred to *Acrostichum*, L., *Onoclea Lomariopsis* (Fee), Sw., *Chrysodium*, Luerss., and *Polybotrya*, Keys. It will be seen from its detailed characters that it is properly placed as a Blechnoid derivative, a position which is suggested in the arrangement of Diels (E. & P., i. 4, p. 251) and of C. Christensen (Index Filicum, p. xxxvi). It may, indeed, be a question whether there is any valid generic distinction. Christ, on the other hand, places it under the old generic name of *Lomariopsis*, Fee, in a position relatively remote from *Blechnum* (Farnkräuter, p. 39). The genus *Stenochlaena* as defined by Christensen includes eleven species, of which *S. sorbifolia* and *palustris* are the best known. They are widely climbing Ferns, and are characterized by an extraordinary variability of leaf-form. In *S. sorbifolia* a 'Pimpinelloid' type is found in the lower parts of the plant, which may match very perfectly those of *B. filiforme*. But the sterile leaves scattered upon the climbing axes are of a Blechnoid type. The fertile leaves are, however, uniform, and resemble those of species of *Blechnum*, except that the soral region is wide, and everted at maturity, as in *B. filiforme*, so as to expose the very numerous sporangia spread over its surface. It is in fact a pronounced 'Acrostichoid' type. This species was collected twice in Jamaica, and the details given below are derived from that material.

Some idea of the great plasticity of the sterile leaves of this plant will be gained from the drawings of Christ in his Farnkräuter, p. 40. Further reference may be made to Karsten's description under the old name of *Teratophyllum aculeatum*, var. *inermis*, Mett., of a Moluccan Fern referred by Christensen (l. c., p. 632) to *Stenochlaena aculeata* (Bl.), Kze. (Buit. Ann., vol. xii, p. 143, Pl. XIV). Here there are two types of sterile leaves in the climbing region, one of which corresponds to the ordinary sterile leaves of *S. sorbifolia*, the other is closely appressed to the surface of the support, and Karsten ascribes to these leaves a water-collecting function.

The climbing stem of *S. sorbifolia*, which has relatively long internodes, is rather variable in size (Text-fig. 11, *a*, *b*). Where it is appressed to the supporting trunk its side is flattened, and the roots are chiefly located there. In addition, rhizoid-like hairs may serve as an additional attachment. The transverse section appears roughly polygonal, owing to the fact that the insertions of the leaves extend as ridges downwards. The stem and leaf-bases are covered by a broad band of sclerenchyma, which is, however, interrupted here and there by bands of lacunar tissue, as in *Pteridium* and other Ferns (Text-fig. 11). The general construction is obviously a modification of the *Blechnum*-type. There is a girdle of meristeles, here of very large size in accordance with the climbing habit. The largest is seen to be opposite the support. The leaf-gaps have been numbered in Text-fig. 11, *a*, for purposes of reference. Opposite gaps 2 and 4, a root-trace has arisen in the usual Blechnoid way. The leaf-traces consist of a variable number of strands, usually about four to six, forming a horseshoe of a relatively advanced type, as compared with such species as *B. filiforme* and *alpinum*.

The development of the fertile pinna has been only imperfectly traced owing to the youngest stages not having been obtained. But sufficient has been made out to give a basis for comparison with those species of *Blechnum* which approach *Stenochlaena* in other respects. The fertile pinna has either one or two vascular strands in the midrib, or sometimes even a third may be present between the two larger ones. The form of the transverse section is apparently as in the simpler species of *Blechnum*, such as *B. lanceolatum*; there is no obvious flange, but a curved wing on either side of the midrib, which thins off at the margin, but not as a rule to a single layer of cells (Plate XXVIII, Fig. 17, *a*). The concave surface of this is covered over a very considerable area by hairs with glandular heads of the *Blechnum* type, and sporangia; the latter are not grouped in any definite sori, nor are there any projecting receptacles (Fig. 17, *c*). The origin of the sporangia sometimes appears to be almost simultaneous, many appearing in a given section to be of like age (Fig. 17, *b*). But other sections show clear evidence of a 'mixed' character, though the succession never seems to be long maintained. If a longitudinal section be cut so as to traverse one of the wings vertically to its surfaces, the phalanx of sporangia appears to be continuous (Fig. 17, *d*). The veins severed transversely are widely apart, and quite distinct from one another; there is in fact no vascular commissure present, linking the veins together as is usual in *Blechnum*. It is seen that sporangia arise from the whole leaf-surface intervening between them. The condition is distinctly 'Acrostichoid', and there is no isolation of distinct sori.

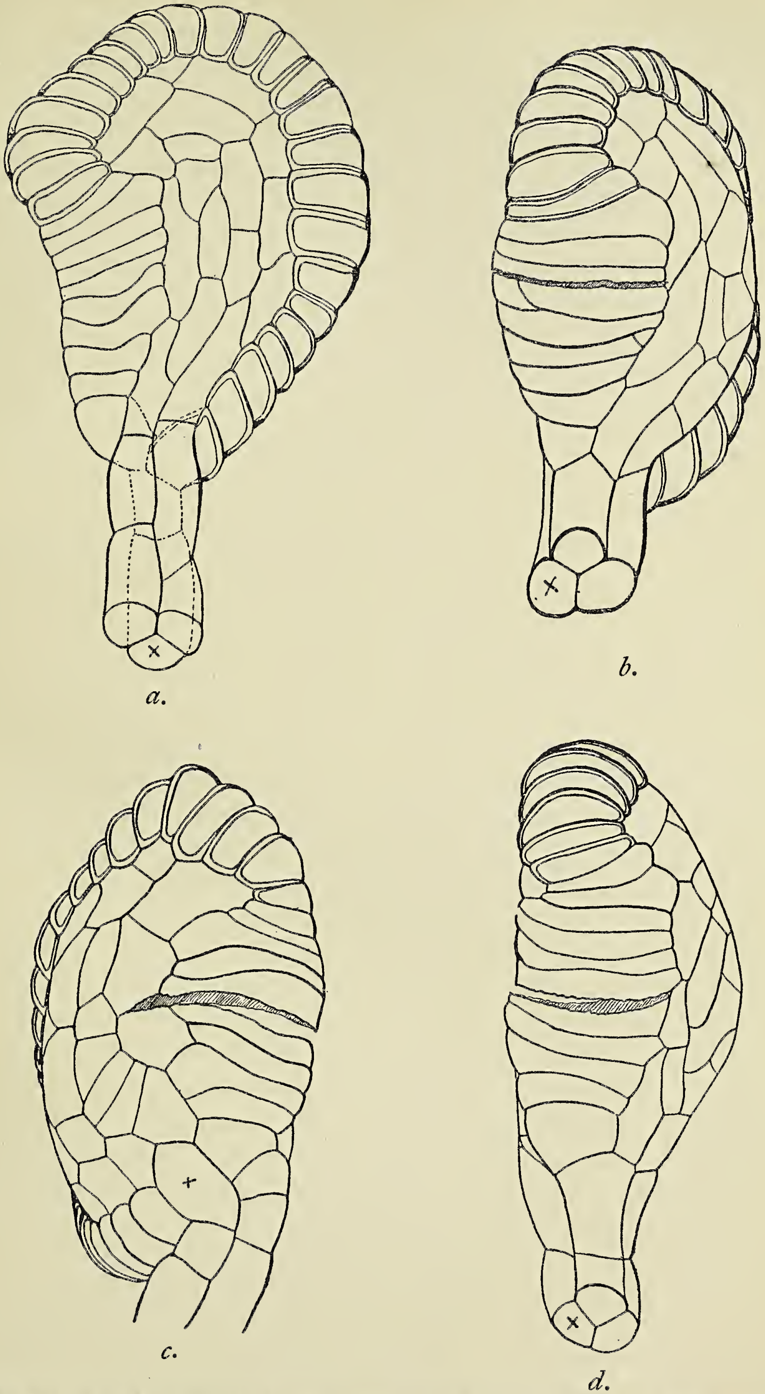


TEXT-FIG. 11. *Stenochlaena sorbifolia* (L.), J. Sm. Transverse sections of the stock; *a* and *b* show different sizes and complexities of construction. × 4.

The question remains how this condition arose. Already, what has been seen in *B. alpinum* and in *B. filiforme* suggests very strongly the answer. The latter species, resembling *Stenochlaena* so closely as it does in habit and in leaf differentiation, provides the initial point of habit similarity. From this a general probability of similar characters of the fertile pinnae may be anticipated. The more perfect 'Acrostichoid' condition has been seen in *B. alpinum*, and a commissure was there present, at least in most cases. Moreover, a flange is clearly seen there. But in *B. filiforme*, which is nearer to *Stenochlaena* in habit, the marginal flange was variable, and apt to be reduced to vestigial proportions. This suggested an examination of *Stenochlaena* in this respect. It was found that frequently there is virtually no sign of a flange (Fig. 17, *c*), or only a very slight waviness of the outline of the section (Fig. 17, *a*, *b*). In other cases the irregularity of outline was more marked (Fig. 17, *e*), while occasionally a slight flange, even with characteristic segmentations, may be seen (Fig. 17, *f*). Such conditions, inconstant though they are, when taken together with the facts of anatomy and of external form, support the hypothesis that *Stenochlaena* is really a Blechnoid type, of origin from some forms like *alpinum* and *filiforme*; that it has assumed a climbing habit as prefigured by *B. filiforme*, with peculiar differentiation of the sterile leaves. The structure of its fertile pinnae appears simple; possibly it was primitively so, but more probably it came about by obliteration of the flange; there was also a spread of its soral area, so as not only to merge the sori into a continuous band, but also to spread it into a wide 'Acrostichoid' surface. But the commissure was either non-existent from the first, or it became obliterated.

We thus arrive at an 'Acrostichoid' type as derived from a Blechnoid, and ultimately from a probable Matteuccoid source. It may be a question whether similar lines of progression may not account for other 'Acrostichoid' types, which have gone under the names of *Lomariopsis* and *Polybotrya*. But such questions must be left open for further study.

As the sporangia of *Stenochlaena* appear to be fair examples of those of the Blechnoid affinity, they have been examined carefully, and illustrated by drawings (Text-fig. 12, *a–d*). Their structure seems to be rather variable, and in size and number of the cells of the annulus they fall short of *Matteuccia* (compare Text-fig. 2). But at least in the great majority of cases the annulus appears to be interrupted at the stalk. A close examination shows an intermediate state between the oblique continuous annulus and the vertical and interrupted; this has been observed elsewhere in forms which on other grounds have been held as transitional (cf. *Dennstaedtia apiifolia*, Phil. Trans., vol. 192, p. 73, and Fig. 131). In the present case the point of interest lies in the detail of the insertion of the stalk in its relation to the annulus. Text-fig. 12, *a*, shows a sporangium seen from the side. Immediately below the capsule the stalk consists of three rows of cells, though lower down it may be more complex. Of these, two rows are continuous up to that face of the sporangium which corresponds to the 'central' face of the Gleicheniaceous or Cyatheaceous type (see Land Flora, p. 556). The third row, marked ×, runs up to the face corresponding to that described as 'peripheral' in the sporangia of those Ferns. Text-fig. 12, *d*, shows



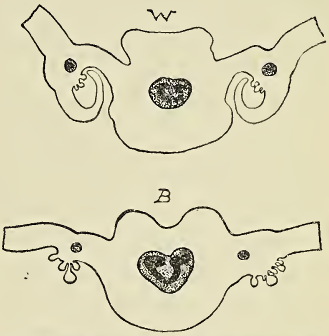
TEXT-FIG. 12, *a–d*. Sporangia of *Stenochlaena sorbifolia* (L.), J. Sm., seen from different points of view. × 125.

different sporangia in various aspects, so as to give an idea of the relations of stalk and annulus. The latter is very slightly oblique. Not unfrequently it may be seen that cells of the series of the annulus are in contact, as is shown by the dotted lines in Text-fig. 12, *a*. On the other hand, Text-fig. 12, *d*, shows the position of the corresponding row of cells as seen from the outside. Here it appears as though the cell marked × was itself one of the continuous series of the annulus. Such conditions indicate that the sporangium of *Stenochlaena sorbifolia*, which resembles that of many Blechnums, is but little removed from the type with a continuous oblique ring. It is in fact just such a sporangium as might have been anticipated in Ferns derived from a gradate type, with oblique annulus. The view has already been suggested that they sprang, through some Matteuccioid line, from a Cyatheoid, and ultimately from a Gleichenioid source, and the condition of the sporangia accords with that suggestion.

Brainea insignis (Hk.), J. Sm.

This is a monotypic genus represented by a small Tree-Fern, which has clearly a Blechnoid habit. It is generally placed in close relation to *Blechnum*, a position which is probably correct. It may, however, be a question how the almost 'Acrostichoid' state of the fertile pinnae originated; but the analogy with *Stenochlaena* seems to lie ready to hand. Anatomically there is similarity to *Blechnum*, *Doodia*, and *Woodwardia*. The leaf-trace as seen in the base of the petiole of a large leaf is a complex one, consisting of two larger strands on the adaxial side, and a varying number of smaller strands (2 to 10 or 12) constituting the remainder of the horseshoe. These connect together downwards, till where the leaf-trace comes off from the foliar gap it may be represented by only two strands, though at times it may be more from the very first. This state resembles that seen in the larger Blechnums, or in *Woodwardia*. The transverse section of the axis shows very numerous masses of gritty sclerenchyma; but putting those on one side, the disposition of the vascular system of a large stem is dictyostelic, with details similar in essentials to that seen in other large and advanced Blechnoid Ferns. *Brainea* bears superficially scales of the *Blechnum* type, and also the mucilaginous glandular hairs characteristic of these Ferns. Comparison on these points thus confirms the general affinity assigned to *Brainea*, but with a special relation to the more advanced types of the Blechnoid Ferns.

Brainea has, like *Blechnum*, conforming leaves, though the fertile pinnae may be slightly narrower than the sterile, and show crinkled margins. In outline and venation they also resemble *Blechnum*, but differ in the fact that there is no indusial flap, while the sporangia spread over a wide area of the lower surface, but stop short of the margin. The fertile pinna shows a single vascular strand in the midrib, which runs out with the usual pinnate venation to the tip. The best understanding of the rather complex 'Acrostichoid' soral condition is obtained by examining first the apical region of a pinna (Pl. XXIX, Fig. 18). The distal veins may be sterile, but soon small groups of sporangia appear, forming isolated circular sori at points at, or outwards from, their bifurcation. Further down the venation is connected by vascular commissures forming arches, which finally become continuous, and serve as a basis for a connected soral line on either side of the midrib. Further back from the apex, the soral bands may extend outwards along the veins, so as to constitute broad, apparently 'Acrostichoid' tracts. If a transverse section be cut through such a pinna in the young state it will appear as in Text-fig. 13, B; and a comparison with a similar section of *Woodwardia* (Text-fig. 13, W) shows the remarkable similarity which may exist. The outline and vascular arrangements are the same; the difference lies in the absence of the indusium in *Brainea*.



TEXT-FIG. 13. W, transverse section of the midrib and sori of *Woodwardia radicans* (L.), Sm., showing the indusial flaps. B, a similar section of the pinna of *Brainea insignis* (Hk.), J. Sm., showing the same relations, but with the absence of an indusium. × 35.

The details of development of the fertile pinna have their bearing on the relation of this Fern to the Blechnoids. The wing of the pinna shows as usual the marginal cell with alternate segmentation (Pl. XXIX, Fig. 19, *a*). Very soon, cells project upon the lower surface, forming a rounded upgrowth, upon which young sporangia may soon be recognized (Fig. 19, *b*). This corresponds in position and in office to the sorus of *Blechnum* or *Woodwardia*, but differs in the entire absence of the indusial flap. It had been anticipated that possibly vestigial indications of an indusium might be seen in *Brainea*, which would confirm the comparison; but none have been found. At first the succession of the sporangia is basipetal, those in the centre of the upgrowth being most advanced (Fig. 19, *a*); but this is not usually maintained, and younger sporangia are found between those more advanced, showing thus a progression towards a 'mixed' condition of the sorus (Fig. 19, *b*, *c*). Moreover, the locality of the sorus is not restricted to the commissure close to the midrib, but is liable to spread towards the margin, following especially the outward course of the veins (Fig. 19, *c*). Using these facts according to the usual comparative method, the following conclusion may be drawn: That *Brainea* is a type sprung from a gradate ancestry with restricted sorus, and that it has progressed to a mixed condition, and has lost the strict limits of its soral area, the sporangia having spread outwards, and assumed an 'Acrostichoid' character. The type from which it arose—as indicated by the soral condition, the anatomy, and the dermal appendages—is clearly Blechnoid, and ultimately Matteuccioid. But in assuming the soral character known as 'Acrostichoid' it has probably proceeded along a line independent from that of *Stenochlaena,* while the appearance shown in Fig. 18 suggests that it may have passed through a stage with interrupted sori, of the type seen in *Woodwardia* and *Doodia.* It will, indeed, be seen below how the indusium in *W. areolata* may be strongly reduced (Pl. XXIX, Fig. 25); it only requires the complete absence of the indusial flap in such a pinna to give a source from which *Brainea* might readily have arisen.

From the observations thus detailed, it is seen that *Blechnum* has given rise to a number of derivative forms showing the soral characters of the old collective genus *Acrostichum.* Further, there is reason to think that these have not all followed one phyletic line of origin; more probably the change of character has appeared more than once within the Blechninae.

iii. The Disintegration of the Fusion-Sorus of the Blechnoid Type.

From the comparative study of *Matteuccia* and *Plagiogyria,* and then of a number of species of *Blechnum*, it has been seen that probably from an original type with a simple flattened pinna, or pinnule, bearing isolated sori upon the veins, and protecting them by in-rolling of the reduced leaf-margins, which formed flaps thinning off in an 'indusioid' manner, a more complex structure than the original one has arisen. Vascular commissures appeared, linking the veins laterally together; the soral development accompanied these extensions, so that the originally isolated sori became threaded together to form continuous lines on either side of the midrib. They may be styled the 'fusion-sori' of the *Blechnum* type. Further, the comparison of numerous species of *Blechnum* has made it appear probable that a concomitant, or early consequence of the fusion, was a 'phyletic slide' of the protective flap from the margin to the lower surface of the pinna. It is still regarded as the phyletic margin, though its insertion comes to be more or less distinctly superficial. But meanwhile the activity of growth and segmentation was continued at the margin itself, resulting in the formation of the 'flange', which assuming ultimately large dimensions, became the chief assimilating surface of the *Eu-Blechnum* type of fertile pinna.

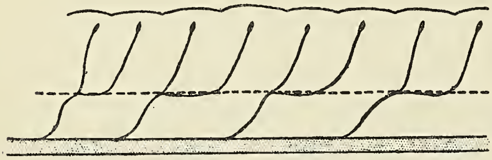
It is clear that the soral lines thus resulting from fusion are not themselves sori in the sense of the term as it is used in the Cyatheoid or Onocleoid Ferns. In point of origin they may be compared with what is seen in the *Saccoloma-Lindsaya* series (see 'Studies in Phylogeny, III,' Ann. of Bot., 1913, p. 459), and a similar condition appears also to have arisen in the Pterideae.

But a further derivative state is arrived at by the subsequent breaking up of these 'fusion-sori' into short isolated portions, a phenomenon which has frequently been the subject of observation and remark in *Blechnum* itself, and has been more or less clearly recognized as leading to such conditions as are seen in *Woodwardia*, and in *Doodia*. It is not right to assume that such disintegration will necessarily follow, in converse, the lines of the original construction of the fusion-sorus. Before any opinion can be formulated on such a question, it will be necessary to examine the facts, and so to ascertain how far there may be correspondence between the soral fragments resulting from the disintegration of the Blechnoid fusion-sorus and the original sori from which, according to the views stated above, the fusion-sorus was constructed.

An essential question at the outset will be the relation of the commissure to the original venation. This has already been considered by Mettenius (Fil. Hort. Lips., p. 60). He there describes the origin of the commissure as a phenomenon of branching of veins, which link up together as a consequence of part of their course running parallel to the midrib. So long as the course of the strands and not their constitution is taken into account, this explanation may serve, as it apparently would do quite well in the case of a normal pinna-apex of *Blechnum fraxineum*, Willd. = *longifolium*, H. B. K. (Plate XXIX, Fig. 20, *d*). But further examination shows that there are two types of xylem involved—that with long narrow conducting tracheides, which are characteristic of the veins; and that with shorter, broader, almost brick-shaped tracheides, which are of the nature of storage tracheides, and are characteristic of the receptacle, and especially of the commissure. The distinction of these two is shown somewhat conventionally in *B. Fraseri* (Fig. 21). Here it was possible to see plainly that the continuous commissure differs in structure from the veins, which run past the commissures in a plane nearer to the upper surface of the leaf, but intimately related to them. Having recognized the existence of these two types of xylem, we may now consider the question of the disintegration of the 'fusion-sorus', and their relation to the process.

A very simple example of disintegration has been seen and delineated by Mettenius (Fil. Hort. Lips., Pl. IV, Fig. 21) in *Blechnum capense*, in the case of a leaf which was only partially fertile. At the transitional region each vein was found to bear its own sorus, and often its own indusial flap. Fortunately, I collected in Jamaica material of this same species in like condition, and Fig. 22 has been drawn to show the relations of the sori at the transitional region. Below, the normal Blechnoid state is seen, with continuous sorus and commissure on either side of the midrib, and with the curled-over margin of the sterile flanges. These gradually uncurl higher up, till the pinna becomes flattened like a sterile pinna. Following the fusion-sori from below upwards, the indusial flap is seen to be interrupted, at first irregularly; but finally it appears as small isolated flaps upon the several veins. The vascular commissure, at first continuous, becomes also interrupted, and often appears as mere streamers of storage xylem to right and left of the vein; and finally, as minute processes of such tracheides rising from the vein into the minute receptacle. The appearance is here as though the fusion-sorus were resolved into a series of isolated sori, somewhat like those of *Matteuccia*, from which it may be supposed to have sprung. But here each is still covered by a persistent portion of the indusial flap, a structure not represented in *Matteuccia*.

It may thus be that the fusion-sorus may be disintegrated into portions corresponding in position at least to its original constituents. But even here the correspondence is not very exact; and in other cases there may be greater irregularity. This is seen in the transitional forms so well known in *B. spicant*. Examples have been noted by Luerssen (Rab. Krypt.-Fl., p. 112, Fig. lxxxvi). The apical part of such a type is shown in Pl. XXIX, Fig. 23. The distal part is sterile, with pinnate venation, while the lower veins bifurcate. By various transitional steps it is seen how, in the lower fertile tract, these furcate veins are linked together by commissures, which in the normal pinna constitute with them continuous vascular connexions running parallel to the midrib. The constitution of these is thus in part from deflexion of the anadromic branch of the furcate vein, partly from the commissure of storage tracheides. The relation of these two constituents is suggested by the diagram, Text-fig. 14. The two constituents may run parallel, or independently. This is already indicated by the diagram, but it is made clearer by a drawing from the leaf of *Blechnum fraxineum* (Fig. 20, *f*), in which it is seen that beneath the greater part of the receptacle both the conducting xylem of the veins and the storage xylem of the commissure are present. But the veins on the one hand may take a separate course into the assimilating flange, and on the other hand the storage xylem of the commissure may form a bridge of its own, even with an independent process in a plane below that in which the conducting veins run. This accords entirely with the condition seen in *B. Fraseri* (Pl. XXIX, Fig. 21). It is important to realize the existence of these two allied but distinct systems of vascular tissue. They appear to find an analogy in the state of the fertile regions of *Platycerium*, as already noted by Mettenius (Fil. Hort. Lips., Pl. IV, Figs. 1–3).



TEXT-FIG. 14. Scheme of venation of *Blechnum*, showing the midrib, and forked veins arising from it. The dotted line indicates the commissure.

We may return now to the case of *Blechnum boreale*, and the disintegration of the fusion-sorus which it shows (Pl. XXIX, Fig. 23). At first sight it would appear that the result of the disintegration is the more or less regular resolution into the original sori. But though there is a relation of the separate sori to the several veins, each is covered in by its portion of the indusial flap, that is (on the present view) by a portion of the original (phyletic) leaf-margin; while frequently a vascular process, projecting on the anadromic side, represents a part of the commissure, which is a derivative structure. It is thus apparent that the resolution is not simply and directly into the original sori, but into fragments of the fusion-sorus, involving bodies which did not form part of the primitive sori.

Woodwardia and *Doodia*.

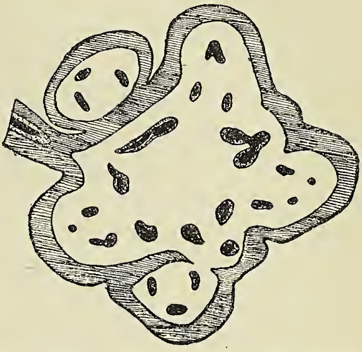
Such disintegration of the fusion-sori as that described is not uncommon in the genus, especially in leaves which are half sterile and half fertile. Particularly good examples have been seen in *B. cartilagineum*, as grown at Kew (Pl. XXIX, Fig. 24). It has also been observed occasionally in *Sadleria*, which is to all intents and purposes a bipinnate *Blechnum*. But it has become stereotyped in the genera *Woodwardia* and *Doodia*. There are so many published figures showing the relation of the sori of these Ferns to the venation that it seems unnecessary to produce new ones. It will suffice to quote Mettenius, Fil. Hort. Lips., Pl. VI; Christ's Farnkräuter, Figs. 569–72; and Diels, in Engler u. Prantl, i. 4, Fig. 134. Special allusion may be made to the case of *Woodwardia areolata*, in which there is strongly marked dimorphism, though most of the genus are conforming, as in *Eu-Blechnum*. The broad sterile leaves are plagiotropic, but the narrow fertile leaves stand erect, and the disintegrated fusion-sori form lines on either side of the midrib. Intermediate conditions have been seen between the sterile and fertile pinnae, and from one of these Pl. XXIX, Fig. 25, has been drawn. It shows the same relations as seen in *W. radicans* and other species, but here the indusial flaps are of small size, arching inwards from the loops of the vascular commissure. Further reticulations of the veins are to be noted, which give the species its name and indicate by their presence a character more advanced than others of the genus.

The hypothetical position as regards *Woodwardia* and *Doodia* is this. That these genera were derived from a Blechnoid source, by partition of the fusion-sorus into isolated portions. Each of these corresponds to a loop of the commissure. In that case the indusial flap will correspond to a part of the phyletic leaf-margin, and the broad assimilating 'flange' with its venation will be the correlative of the similar 'flange' seen in the more advanced types of *Blechnum*. Further, in the case of *W. radicans*, and other relatively primitive Woodwardias with free venation, there is no dimorphism of leaves, their condition being as in *Eu-Blechnum*, from which the genus was probably derived. But in the reticulate, and thus probably derivative *W. areolata*, a dimorphism has again been assumed; the erect fertile leaves have their reduced assimilating flange reflexed for purposes of protection of the sori. There seems good reason to believe that this dimorphism is secondary ; it compares with that in *B. Patersoni*, and a somewhat similar condition is also seen in *Doodia caudata*.

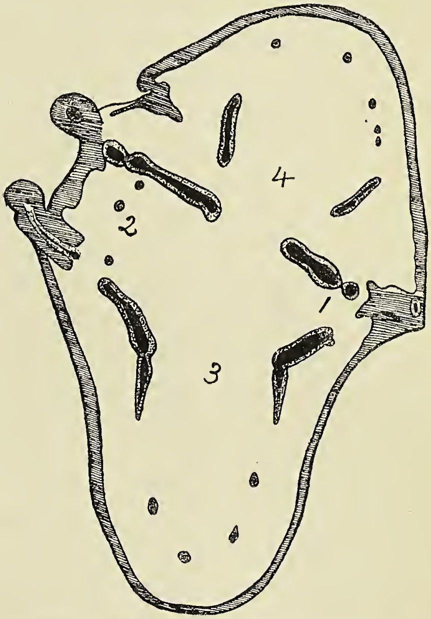
The development of the fertile pinna has been followed out in the last-named species. It is specially interesting because the terminal lobe of the sporophyll frequently shows the fusion-sori on either side of the midrib uninterrupted for considerable distances. This may be held as a retention in the apical region of the leaf of that state which was characteristic of its ancestry, according to the hypothesis above stated. The lateral wing of the pinna arises, as usual, with segments from alternate sides of the marginal cell. The wing, as in *B. brasiliense*, attains considerable size before any sign of the sorus appears (Pl. XXX, Fig. 26, *a*). A rather broad upgrowth then makes its appearance far within the margin ; it gives rise at the marginal limit to the indusial flap (x , Fig. 26, *b*), while several deep cells lying more towards the midrib are the parent-cells of sporangia. These parts come rapidly forward (Fig. 26, *c, d*), and take the characters respectively of an inward-turned indusium, and of sporangia upon a slightly convex receptacle. In order of their appearance they show a slight basipetal sequence. Below the receptacle runs the vascular commissure, here cut transversely. The development of the fertile pinna of *Woodwardia radicans* shows similar steps. It will not be necessary to do more than illustrate this by a single section (Pl. XXX, Fig. 27), which shows the very large indusial flap strongly curved towards the midrib, covering the receptacle with its slightly basipetal sequence of sporangia. Below this runs the commissure. Clearly the two genera closely coincide ; but whereas in *Woodwardia* the assimilating flange is broad and flat, in *Doodia* it is reduced, and its margin reflexed (as in *W. areolata*), probably in accordance with a xerophytic habit.

It is well known that in *Doodia* the parts of the fusion-sorus thus isolated do not maintain the regular linear sequence which is so characteristic of *Woodwardia*. Steps leading to the irregularity which they show may be seen illustrated in any large series of specimens. Commonly, the sori on either side of the midrib form two series, one nearer to, the other further from it. In that case their position is alternate, and they may be held to be the result of displacement of an originally linear series. But this explanation will only account for the simplest cases, and it seems probable that an initiation of new sori must have taken place at points previously untenanted. Of this there is abundant evidence in other affinities among Ferns.

The rough anatomy of *Doodia* and *Woodwardia* has been examined, and presents features of interest. As an example of the former genus, *D. maxima*, J. Sm. (= *D. blechnoides*, Cunning), was taken, as being a larger species, in place of the small and possibly reduced *D. caudata*. A transverse section is shown in Text-fig. 15. It is clear that the dictyostele is like that of *Blechnum*, as also the departure of its root-traces and leaf-traces. The latter come off at first as two strands, one or both of which very soon abstrict to form one or more smaller strands. The vascular supply thus passes into the leaf, sometimes as three strands, sometimes as four—a condition which may be matched in species of *Blechnum* of medium size. But in *Woodwardia radicans* the case is more complicated. A section of a stock of relatively small size is shown in Text-fig. 16. Numerous root-traces are given off, and frequently without that definite relation to the foliar gap and leaf-trace which is so characteristic of *Blechnum.* The point of special interest is, however, the mode of origin of the leaf-trace. The foliar gap opens widely, and four of them are identified by numbers in the section: 1 shows a gap cut near to its base; a root-trace is being given off from one of the meristeles, but the leaf-trace has not yet appeared; 2 is traversed at a higher point, and already three small strands have arisen which will form the central region of the leaf-supply, but not the larger lateral strands; a root-trace is passing off from one of the meristeles; 3 shows the leaf-gap at its widest, and the larger lateral strands are detaching themselves from the meristeles which bound it, while four smaller strands, which had clearly originated before and separately from them, are ranged so as to form the horseshoe-like series; 4 shows the leaf-trace completed, and the gap narrowing. It is thus seen that the origin of the leaf-trace in *W. radicans* differs from the plan which is usual in *Blechnum.* There, the trace consists of two strands, or more, but the smaller median strands are derived by abstriction from the larger. Here, the median strands originate independently from the foliar gap. This indicates for *Woodwardia*



TEXT-FIG. 15. Transverse section of the stock of *Doodia maxima*, J. Sm. It shows the inconstancy of the strands of the leaf-trace. × 3.



TEXT-FIG. 16. Transverse section of the stock of *Woodwardia radicans* (L.), Sm. The leaf-gaps are numbered in their succession from below: at gaps 2 and 3 the median strands of the trace are detached before the lateral strands have separated from the meristeles of the axis. × 3.

an anatomical advance on *Blechnum*, a point to be placed in relation to the more advanced condition of its fertile pinna.

The facts thus appear to indicate that *Woodwardia* and *Doodia* are Blechnoid derivatives, in which the fusion-sorus has been disintegrated, while in *Doodia* a displacement of the separate portions has also occurred. But the displacement thus seen in *Doodia* is a relatively minor matter compared with those displacements which are next to be considered. These are associated with bold curvatures of the Blechnoid fusion-sorus, accompanied by its disintegration, as before, into detached portions. Their study will begin from typical species of *Blechnum*, and the modifications there seen will form the basis for comparisons, leading on to those states which are characteristic of *Scolopendrium* and *Asplenium*.

The comparison may start from *Blechnum fraxineum*, Willd. (= *B. longifolium*, H. B. K.), a commonly cultivated species, with rather simple leathery leaves bearing relatively few pinnae. It belongs to *Eu-Blechnum*, and the character of the normal pinnae is shown in Pl. XXIX, Fig. 20, *d*, as regards venation and sori. In this there is nothing calling for special remark. But the interest centres round those points where the pinnae are attached, and especially those pinnae which appear successively smaller as the large terminal segment is approached. This region of a normal leaf is represented in Fig. 20, *e*, which shows the basal part of a large terminal lobe, together with the bases of the two next lower pinnae. Here are plainly conditions of the sori and of the venation which represent additional pinnae not fully formed. Possibly it is a species whose relatively simple leaf-structure is the consequence of reduction from a more fully pinnate ancestry, and the outward archings or diverticula of the sori may be recognized as partial pinnae (Fig. 20, *e*, i–vi). It is important to note that each of these archings arises opposite to the departure of a lateral vein from the midrib, while the main strand of it forms a midrib in cases where the arch is large; clearly this is of the nature of a stunted pinna.

It has already been noted that the venation of the pinna and the vascular supply to the receptacles, though intimately connected, constitute two systems. Their behaviour in these stunted pinnae illustrates this. At the apex of the arch (iii) in Fig. 20, *e*, where the interruption of the sorus and indusium is not fully carried out, as it is at (iv), a disposition of the vascular tissue is seen which gives some insight into the behaviour of the storage-xylem. Fig. 20, *f*, shows it on a larger scale. The indusial flap is not actually interrupted, but the series of sporangia ceases at the apex of the curve. The veins which run nearer the upper surface of the leaf are shaded relatively lightly, and constitute a connected system. But what this transparent preparation brings out prominently is, that the xylem of the receptacle consists of short storage tracheides, distinct in form from the long conducting tracheides of the venation ; and that they project towards the lower surface as a continuous band. They are conventionally shaded more darkly in the drawing. At the extreme distal apex of the arch shown in Fig. 20, *f*, the band of storage tracheides separates from the vein, and forms an independent commissure, passing in a lower plane across the course of one of the veins. It has even formed an independent process at the apex of the curve. This shows clearly how distinct the receptacular xylem may be from the conducting veins.

Comparison suggests that relatively simple-leaved types such as *B. fraxineum*, having few pinnae, originated from a more complex one such as *B. brasiliense*, with many pinnae ; and the latter, very possibly, from some doubly pinnate source, a state which actually exists in *B. Fraseri* and *volubile*, and in *Sadleria*. But this phyletic aspect does not exhaust the interest of the case. The facts may be regarded from the point of view of the individual leaf-development. Starting in the ontogeny from the relatively simple juvenile forms, we may proceed to those leaves which show the full normal development of the species. The state shown in *B. fraxineum* might then be held to be an imperfect realization of the more complex ancestral form in a type which had passed into a less complex state. The archings outwards of this fusion-sorus seen in Fig. 20, *e*, would thus be the expression of a tendency towards separate pinna-formation as in the ancestry, but imperfectly carried out in the individual, which had thus advanced phyletically in the direction of a simpler leaf.

It is the relation of these imperfect pinna formations to the veins departing from the midrib which stamps their pinna character. We pass now to certain developments which stand in strong antithesis to them and are not of the nature of pinnae. They arise between the veins. As their point of origin is different, so their morphological interpretation must be different. They are believed to have led to important modifications of the Blechnoid type. The key to these changes is to be found in varieties of a well-known species, viz. *B. punctulatum*, Sw. The normal plant shows the ordinary characters of § *Lomaria*. It is strongly dimorphic, with narrow fertile pinnae, but these show occasional signs of interruption of the fusion-sori, especially where the condition as regards their breadth is intermediate between the sterile and fertile. The species is native in South Africa and Java.

Blechnum punctulatum, Sw., var. *Krebsii*, Kunze.

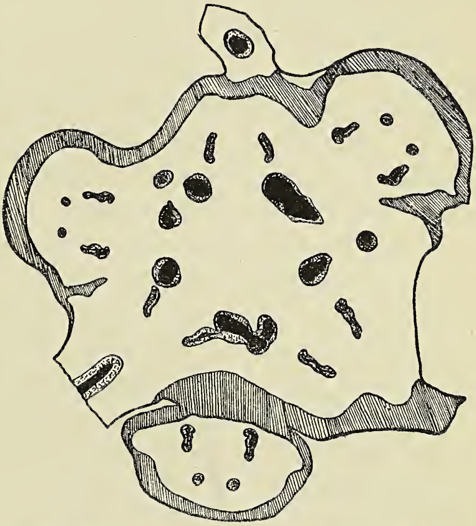
The normal plants of the species present no features of importance beyond those mentioned. It has been referred by various authors to § *Blechnum* or to § *Lomaria*, and is in fact intermediate in character, though the fertile leaves are markedly narrower than the sterile. But the interest is in the abnormal or varietal forms, which early drew the attention of pteridologists and have been repeatedly figured and discussed. The point of interest in them lies in the fact that they illustrate various intermediate conditions between *Blechnum* and *Scolopendrium*, and that the broader the fertile leaves are, the nearer they approach to the latter state. Pl. XXX, Figs. 27 *bis* and *tris* show respectively a leaf and a portion of a leaf of the var. *Krebsii*, in which the width of the fertile pinna is a striking feature.

The varieties of *Blechnum punctulatum* which are associated with the extreme form named var. *Krebsii* were discovered near Grahamstown by Krebs. The first detailed description was given by Kunze (Die Farnkräuter, 1847, p. 176, Taf. LXXIV) under the name of *Scolopendrium Krebsii*, Kunze, including with it various forms, some with broader leaves and sori of the *Scolopendrium* type, others narrower and Blechnoid. The former he regarded as the normal type, the latter as derived from it by contraction. He notes the habit as of *Lomaria punctulata*, but compares it, on the other hand, with *Scolopendrium pinnatum*, J. Sm., on the ground of the pinnate form of the leaf. Mettenius (Fil. Hort. Lips., p. 67) evidently shared this view, ranking the plant with *Scolopendrium*, and regarding the narrower forms as derivative. But Sir William Hooker (Species Filicum, iii, p. 30, and Bot. Mag., No. 4768) includes it under *Lomaria punctulata*, Kze., as an abnormal form. He remarks, 'I have specimens from Mr. Atherstone in my herbarium clearly showing the passage from *L. punctulata*, Sw., to *Scolopendrium Krebsii*, Kze.' Such forms have been described as *B. punctulatum*, var. *Atherstoni*, Pappe; examples hardly diverging from the *Blechnum* condition of the sorus were sent to me from Durban by Mr. J. Medley Wood, F.L.S. Hooker derived all these varieties from a '*punctulata*' source thus: 'I possess fertile fronds, however, where the pinnae are unusually broad at the base, the rest of the pinna being contracted; then the sori within the broader portion are irregularly waved and partially broken up into short pieces, which have a tendency to an oblique direction (not parallel with the costa). Where the whole of the fertile pinna takes a manifestly broader form throughout, I find the sorus broken up into *Woodwardia*- or *Doodia*-like involucres, more or less oblique with regard to the costa, still preserving an imperfect contiguity. But when the fertile pinnae are at the broadest the sori are completed separated into very oblique, almost transverse, double sori, distant from the costa, sometimes half-way between it and the margin; and if one of the more transparent specimens of these be held up between the eye and the light, and examined with a pocket lens, it will be seen that the costal areoles formed by the transverse receptacular veins of *Lomaria* are singularly elongated, together with the receptacular vein itself, carrying its portion of the sorus with it, taking nearly the shape of an inverted letter V. The broken portion of the sorus or involucre is, if I may so say, carried along with it, and thus is formed an abnormal double involucre, the two opening face to face and resembling those of *Scolo-*

pendrium.' It will hardly be necessary here to do more than to illustrate by drawings the position so correctly stated by Sir W. Hooker in 1860.

The varieties of *B. punctulatum* have been briefly mentioned by later writers, but no further detailed examination of them is yet to hand. They occur in the shady gorges in the neighbourhood of Durban, whence a supply of living plants and of dry specimens was kindly sent to me by the veteran, Mr. J. Medley Wood, A.L.S., of the Durban Botanic Garden. Writing some years ago in the Natal Colonist, 1879, he noted the rarity of the intermediate, and the prevalence of the extreme forms; and he suggested that the former may be dying out, in which case their connexion with the species would tend to become more obscure than it now is.

That, notwithstanding the peculiarities of the sporophyll in the var. *Krebsii* of *B. punctulatum*, it is still substantially a *Blechnum* is indicated by its vascular structure. The leaf-trace comes off as two strands, which soon divide into four. Their separation from the dictyostele of the axis is, as in *Blechnum* generally, preceded by the giving off of a root-trace from the large meristele of the axis, immediately above which the foliar gap is formed (Text-fig. 17). The correspondence of vascular arrangement with that in *B. attenuatum* is uncommonly close. It is, then, with some degree of confidence that we are dealing with a development from a *Blechnum* type that we proceed to examine the soral peculiarities of the *Krebsii* varieties.



Text-fig. 17. Transverse section of the stock of *Blechnum punctulatum*, var. *Krebsii*, showing that the structure is of the *Blechnum* type. × 3.

The slightest observed deviations from the ordinary Blechnoid fusion-sorus, as it is seen in the typical *B. punctulatum*, consists in an outward arching of it: such archings *lie between the veins* which connect the commissure with the midrib of the pinna. Sometimes the arching is very slight (Pl. XXXI, Fig. 28, *a*), but where it becomes pronounced it is commonly associated with a partition of the fusion-sorus into short lengths, with very irregular limits (Fig. 28, *b*). The pinnae which show these characters are wider than the normal fertile pinnae, so that the peculiarity goes along with expansion of surface. Except for the greater irregularity of the interruptions and the outward arching of the detached portions, there is a similarity to the state already noted in *B. boreale* (Fig. 23). The usual point for the interruptions of the fusion-sorus to appear is towards the anadromic end of the arch. Several intermediate conditions are shown in Fig. 28, *e*; in one case the vascular commissure is complete but the indusial flap is interrupted; in another, the indusial flap is still continuous but the commissure interrupted, with an isolated tracheide lying in the gap. In a third, both flap and commissure are interrupted. In such cases, while the vein-endings run outwards towards the margin, a process of storage xylem of the commissure underlies the detached ends of the receptacle. More advanced states of interruption of the fusion-sorus are seen in Fig. 28, *c*, *d*. These show how the detached portions may be extended outwards towards the margin of the widening pinna, finally taking an arrangement in pairs, with the indusial flaps facing one another.

Two other complications are apt to be introduced which are important for comparison with derivative forms, and they are shown in their simplest terms in Fig. 28, *c, d*. The first of these is the formation of secondary branches of the veins, which lie in the space between the forks of the primary veins. Frequently these run out to the margin of the pinna without any sorus attached to them. But this is not always so. At the points marked × in Fig. 28, *c*, *d*, there are one or two small supernumerary sori attached to such branches; in the more elaborate types, and especially towards the base of the pinnae, these appear more regularly. They must be held to be new formations, and not directly derivative from the disintegration of the fusion-sori, like the rest. It will be seen that the former of these innovations becomes a regular feature in *Scolopendrium*. The latter is important for comparison with certain types of *Asplenium*.

It is necessary first to put the phenomena thus seen in *B. punctulatum*, var. *Krebsii*, into relation with questions of pinnation, as illustrated in *B. longifolium*. It will be noted on comparison of the drawings, and especially their basal parts, that the margins of the pinnae of *B. Krebsii* are sinuous, also that indentations of the margin lie peripherally to the pairs of sori; the convexities are obliquely opposite to the spaces between the forks of the primary veins, and accordingly they subtend morphologically the primary veins themselves. So far, then, as there is any indication of a further pinnation, each of the pinnae (as marked by the convexity of the margin) is served by one of the primary veins. The correctness of this conclusion is indicated by a comparison with the distal end of the pinnae of *B. Fraseri* (Pl. XXIX, Fig. 21). There, a pinnule subtends and is traversed by each primary vein, though the fusion-sorus does not there show the archings and interruptions seen in *B. Krebsii*.

In order to maintain the comparison on the one hand with *Blechnum* and on the other with *Scolopendrium*, the development of the sorus of the var. *Krebsii* has also been traced. The initial steps accord with those of species of *Blechnum* of a rather advanced type. If sections be cut of a pinna not strongly divergent from the normal *Blechnum* arrangement of sorus, the structural correspondence is very close with such a species as *B. spicant.* Fig. 29, *g*, shows this; there is a considerable flange, with the sorus distinctly intramarginal in origin. The indusial flap is rather late in its development, while the sporangia may show in their early stages distinct traces of a basipetal sequence. This is not, however, maintained.

But if sections be examined which traverse a diverticulum of the fusion-sorus, so that the two sides of the curve be cut through, the appearance presented is as in Fig. 29, *f*, *h*. The former represents a very early state, in which a deep depression of the surface indicates where the soral development is to take place, while right and left procambial strands mark the position of the vascular supply. The points of greatest convexity which lie above these show by wedge-shaped superficial cells the places of origin of the opposed indusial flaps. Fig. 29, *h*, shows a more advanced state, though the deep depression remains as in the younger state. The slight convexities right and left have grown out into indusial flaps, consisting at their bases of two layers of cells, but thinning to a single layer at their margins. Beneath them are the vascular strands corresponding to the commissures of *Blechnum*; seated on them are the sori, the sporangia showing again some indication of a basipetal sequence. The whole structure is plainly a duplication of a single fusion-sorus of *Blechnum*, with the two receptacles face to face, as they would be in consequence of the sinuous curving of the ordinary fusion-sorus.

The conclusion which follows is then this. That in the var. *Krebsii* we see a Blechnoid Fern which has widened its fertile pinna beyond the normal; that this widening is accompanied by a sinuous curving, and often also by disruption of the fusion-sorus and by extra branchings of the veins; beneath all this there may be recognized, by the characters of the venation and the margin sinuosities, an imperfectly developed secondary pinnation, such as is actually seen to exist in *B. Fraseri* and in *Sadleria.*

Scolopendrium.

But *B. punctulatum*, var. *Krebsii*, was originally described by Kunze as *Scolopendrium Krebsii.* This raises the question of the relation of *Scolopendrium* to *Blechnum.* It is best to approach it through a comparatively simple and regular species, such as *S. vulgare*, L. The relation of the sori to the venation in this Fern and to the sinuosities of the margin is shown in Pl. XXXI, Fig. 30. It is seen that the primary veins arising from the midrib bifurcate twice, or sometimes more. On the outermost branches of each group, and facing outwards from the centre of the branch system of each primary vein, are the well-known elongated sori. As the sori connected with the successive primary veins face one another, the consequence is the arrangement typical of *Scolopendrium* (see E. u. P., i. 4, Fig. 123, B).

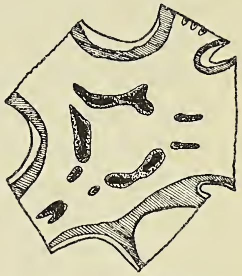
Moreover, the indentations of the sinuous margin correspond to these pairs of sori, and the convexities to the region lying between the forks of the primary veins. In fact, the relations in the Hart's Tongue are exactly those seen in the var. *Krebsii.* In both there is evidence of a suppressed pinnation.

A strong support of the correctness of this view is found in many monstrous forms of *Scolopendrium vulgare*, and especially in those designated var. *laciniata.* These are characterized by the occurrence of many projections from the margin, which give a laciniate outline. The position of these laciniae is very constantly *intersoral.* They thus appear in just the same position as those slighter convexities of the normal leaf of *Scolopendrium* or of var. *Krebsii.* If these are true indications of a suppressed pinnation, then the laciniate variety of *S. vulgare* is a retrogression towards the pinnate ancestry.

But the most interesting conditions for comparison with *B. punctulatum*, var. *Krebsii*, are found in *Camptosorus* (*Scolopendrium*) *rhizophyllus* (L.), Link, and *C. sibiricus*, Rupr., in the various regions of the very acuminate leaves (see E. u. P., i. 4, Fig. 123, c). In the narrow excurrent apex the leaf frequently shows parallel sori of the Blechnoid type, but more or less interrupted, though often continuous for considerable distances. In a middle region soral arches are found, curved outwards as in *B. Krebsii* (Pl. XXXI, Fig. 28, *a*). In the basal region the lamina widens out into considerable lobes, on which the disposition of the sori is very irregular (compare Mettenius, Fil. Hort. Lips., Pl. V, Fig. 6). Sometimes they are paired, as in *Scolopendrium*; sometimes they are isolated, as in *Eu-Asplenium*; sometimes back to back, as in *Diplazium.* Such conditions may be very nearly matched sometimes in the basal regions of the pinnae of *B. punctulatum*, var. *Krebsii.*

The development of the typical double sorus of *Scolopendrium* follows the same lines as that of var. *Krebsii*, but with differences of minor detail and of proportion. It has already been studied by Burck (Indusium der Varens, Haarlem, 1874). In the very young state a depression of the lower surface is first seen (Pl. XXXII, Fig. 31, *a*), at the margins of which the indusial flaps soon appear as upgrowths with the usual segmentation. Below them, right and left, may be seen the procambial strands. A later stage (Fig. 31, *b*) shows that the region between these strands is clear of vascular tissue, and the surface rises here into a ridge between the two receptacles a development not seen in var. *Krebsii.* The two indusial flaps have meanwhile enlarged, being mostly only a single layer in thickness, but thicker nearer their bases. They overlap more fully than in var. *Krebsii*, and very perfectly protect the receptacles. These are formed immediately over the vascular strands, but are further apart than in var. *Krebsii*, owing to stronger development of the leaf-area between them. Though indications of a basipetal succession may be seen in the early stages, the sorus becomes later distinctly 'mixed' in character. A comparison of these earlier stages of the double sorus of *Scolopendrium* with those of var. *Krebsii* leaves no doubt that the two are very closely allied.

Transverse sections of the stem of *S. vulgare* show that the vascular arrangement is essentially that of the Blechnoid type, with a dictyostele (Text-fig. 18). The root-steles come off in the same way from the middle of the meristeles of the axis just below the leaf-gaps, but each may divide in its course through the cortex, so as to supply several roots instead of only one. Above the point of departure of the root-stele, the foliar gap opens and gives off from each margin a strap-shaped vascular strand; the pair of these constitutes the leaf-trace. These arrangements coincide with those of the simple Blechnoid Ferns. Proceeding up the leaf-stalk, the pair of leaf-trace strands may fuse together to form the complex X-shaped structure known as characteristic of this genus and of *Asplenium* (Russow, Vergl. Unters., p. 100, Taf. X, Fig. 16; Luerssen, Rab. Krypt.-Flora, iii, p. 120). Lastly, the young parts are densely covered by ramenta, which bear at their apex a large mucilage gland, similar in character to the well-known mucilage glands of *Blechnum*. All of these characters together strongly support the near relationship of *Scolopendrium* to *Blechnum*, and especially to some of the less specialized types of that affinity; while the peculiar soral features are explained through var. *Krebsii*, as derived from the simpler Blechnoid type by its becoming sinuous and ultimately disintegrated in relation to increased assimilating area.



TEXT-FIG. 18. Transverse section of the stock of *Scolopendrium vulgare*, Sm. × 3.

Asplenium.

The relations between *Scolopendrium* and *Asplenium* have always been recognized as very close, and the varieties of *B. punctulatum*, and of other Blechnoid derivatives, offer strong evidence that this large genus has sprung from a Blechnoid source. The genus is, however, so large and so variable in form that it is not possible at present to treat it fully. I shall hope to return to this on some future occasion. It includes some hundreds of species and has been subdivided variously by systematists, some including all those species in a single genus, others dividing them among as many as ten distinct genera. But whatever the systematic arrangement may be, there is general agreement that the whole body of species form a very natural group. Some are simple in the outline of the leaf, others run into high degrees of branching, and this character has been used by most writers as one of the bases of their systematic arrangement. There is some reason to think that here, as in *Blechnum* and *Scolopendrium*, the simplest types have been condensed from an ancestry where the leaves were more freely branched.

It will be best to start from those simpler and more regular types where the sori are disposed obliquely on either side of the midrib and overlap one another, their indusial flaps all facing acroscopically. This arrangement is characteristic of § *Thamnopteris*, Presl, and of §§ *Eu-Asplenium*. There is, however, a difference within these subgenera in the inclination and the overlapping of the sori, according to the breadth of the leaf. Where, as in *A.* (*Thamnopteris*) *nidus*, L., the leaf is very broad and the sori long, they often stand out almost at right angles to the midrib, and are crowded so that the overlapping is very close. But in those species in which the pinnae are narrow and elongated, and especially at the acuminate apices of such leaves, the sori may be only very slightly inclined to the midrib, or even almost parallel with it, and may overlap one another very little or not at all. A middle position is seen in such a species as *A. obtusatum*, Forst. (Pl. XXXII, Fig. 32).

Examples of the arrangement of the sori in narrow-leaved species are seen in Hooker's Species Filicum, iii, Pl. CXC–CXCV. In *A. horridum*, Klf., and especially at the excurrent apex of the pinna, the position of the elongated sori is parallel to the midrib, and with the indusial flap directed towards it, just as in *Woodwardia*. This was particularly well seen in New Zealand specimens collected by T. F. Cheeseman, from which the drawing has been made (Pl. XXXII, Fig. 33). Another good example is seen in *A. serra*, Langsd. & Fisch., collected by Purdie from Santa Martha (Fig. 34). If a comparison be made of such cases with *Woodwardia*, and especially with such a case as that shown by Mettenius for *W. virginica* (Fil. Hort. Lips., Taf. VI, Fig. 2), the similarity is unmistakable, while the oblique position and the overlapping of the sori are more pronounced in the latter than they are in the species of *Asplenium* quoted.

But there remain to be considered those more complicated dispositions of the sori found in the section § *Diplazium*, Swartz. Here two sori are frequently placed back to back upon a single vein. A rather complicated and irregular example of this is shown in Pl. XXXII, Fig. 35, in the case of *A.* (*Dipl.*) *celtidifolium*, Kze., from material collected in Jamaica. The underlying principle of arrangement of the sori is foreshadowed by *B. punctulatum*, var. *Krebsii* (Fig. 28, *c*, *d*). Here accessory soral tracts are seen in the spaces between the branches of a main vein (marked × in figures), which spaces are usually without sori in *Scolopendrium* and have, for grounds explained above, been held to correspond to a suppressed pinnation. The presence of these sori may here be held to be a consequence of a less perfect suppression of an extra pinnation than that seen in *Scolopendrium* itself. These extra sori were noted and lettered (*b*) in Mettenius's Fil. Hort. Lips., Pl. V, Fig. 7.

If, now, these extra sori were extended downwards beyond the point of branching of the vein that supports them—a condition which may be actually seen in var. *Krebsii* and in *Camptosorus* (compare Mettenius, l. c., Taf. V, Fig. 6, *bb*)—the result would be that of *Diplazium* with the sori back to back on an unbranched vein.

The most complex Asplenioid forms are found in those gigantic Ferns associated under the name of *Callipteris*, Bory (= *Anisogonium*, Presl). They are characterized by having the veins variously anastomosing. A particularly interesting example is seen in *Diplazium* (*Callipteris*) *ceratolepis*, Christ, from Costa Rica, described by Dr. Christ in Bull. Soc. Bot. Belg. 35, p. 203. It appears to connect the *Callipteris* type with the Blechnoid model, and to show in a less condensed form the same method of soral arrangement as is seen in *Scolopendrium*, while the relation of the pinnules to the soral arches corresponds to that of the suppressed pinnae of the latter. Its leaf is very large and pinnate; the pinnae are pinnatifid. The nervation of a part of one of them is shown in Fig. 703 of Christ's Farnkräuter, p. 222. It differs from most types of *Asplenium* in having arched veins running nearly parallel to the midrib between the main veins, corresponding in position to the commissural arches of *B. punctulatum*, var. *Krebsii*, or of *Brainea*. Like the latter Fern, the indusium appears to be absent, while the sori spread far outwards along the veins. This Fern would repay a complete study. But this, as also the more exhaustive analysis of the whole genus *Asplenium*, must be left over for the present.

The development of the sorus of *Asplenium* is quite in accord with the conclusions which follow from the comparisons thus made. Its origin is after the model of the most advanced Blechnoid types. The marginal segmentation of the pinna appears as usual, and produces a lateral wing. After this has attained considerable size the indusium and receptacle make their appearance at a point distinctly intramarginal. This is shown in Pl. XXXII, Fig. 36, *a*, *b*, *c*, for the bulky species *A. obtusatum*, Forst. The indusium develops quickly, and in this species it consists at first of a single layer of cells ; later it may become more massive at the base. The concave surface covered by it is the receptacle, from the deep cells of which the sporangia arise. These show a distinctly ' mixed ' character in the order of their appearance. The relations of the sorus respectively to the margin and to the midrib are thus seen to be substantially the same as those of advanced types of *Blechnum*, or of the less-divergent varieties of *B. punctulatum*, such as var. *Atherstoni*.

Sections were also cut from the leaves of *A. nidus*, L., in various stages, and they showed substantially the same arrangements as *A. obtusatum*, except that the sori were more crowded upon a still more fleshy leaf. As an example of a Diplazioid type, *A.* (*Dipl.*) *celtidifolium*, Kze., was cut. The leaf is here more delicate in texture, but, putting this aside, the structure of the single sori was again on the same plan. Where the sori were of the *Diplazium* type, this results merely from the duplication of the soral characters back to back upon the same vein, or, where the branching of the vein has begun, upon the vein in various degrees of division. The real nature of these duplicated sori has been explained above (p. 412), and their structure accords with the views already expressed.

Finally, though the anatomical facts are not very distinctive, they may be stated here. The axis of various species examined contains a dictyostele with leaf-gaps, from the sides of which the two broad straps of the leaf-trace spring. The root-traces arise from the outer limit of the meristeles at points below the openings of the leaf-gaps. Thus the system is of the same type as that of *Blechnum* (Text-fig. 19, *a*). Passing into the petiole, the two strap-shaped vascular strands may remain separate, as they do in the large *A. marginatum* (Text-fig. 19, *a*), or, especially in smaller species, they may coalesce upwards to form those peculiar X-shaped vascular tracts so fully described by Luerssen (Rab. Krypt.-Flora, iii, pp. 149, &c.). This is seen in *A. alatum* (Text-fig. 19, *b*). In this they show a close similarity to what is seen in *Scolopendrium*. These features strengthen the relation of *Scolopendrium* to *Asplenium*, which has never been questioned. And further, the varieties of *Blechnum punctulatum* link both of these without any doubt to the genus *Blechnum* as ultimate derivatives from that type of Ferns.

TEXT-FIG. 19. *a*, transverse section of the stock of *Asplenium obtusatum*, Forst. The leaf-trace resembles that of *Matteuccia*. × 3. *b*, a similar section of *Asplenium alatum*, H. B., which shows the fusion of the strands in the petiole. × 12.

COMPARATIVE TREATMENT AND PHYLETIC CONCLUSIONS.

In the above pages data are given relating to a very considerable series of Ferns which, both on the ground of old and persistent opinion of systematists, and on a basis of more recent and exact comparative study, are believed to be naturally allied. The attempt must now be made to draw together these results with a view to phyletic conclusions. In a previous memoir of this series, numerous criteria have been cited which may be available in Ferns for such an end (Ann. of Bot., vol. xxvi, p. 292, &c.). But it is not possible in every case to use all of the criteria there mentioned. Sometimes the facts themselves may be wanting, and that is so in regard to the whole gametophyte generation in many of the Ferns here examined. Again, when Ferns have progressed from a more primitive to a relatively advanced state, certain of the available criteria lose their validity, owing to the fact that all those types compared may have attained to a full state of development as regards that particular feature. For instance, advanced Ferns generally possess flattened scales; therefore, in relatively advanced families the criterion of form of the dermal appendages loses much of its value, or all of it. In the present case such comparisons will take only a minor place. Again, in relatively advanced Ferns the usual vascular system of the axis is dictyostelic, with a divided leaf-trace. This is the case for virtually all of the Ferns here treated, and accordingly the vascular comparisons also lose much of their value. Further, in the more advanced Leptosporangiate Ferns the sporangium itself becomes very generally standardized; that is, relatively uniform in size, structure, and spore number. This is so in most of the Ferns here considered, so that this criterion also becomes only of minor importance. The elimination of such useful criteria as these, or the diminution of their value, throws the weight of argument upon those that remain. In the present case, venation, the form and construction of the sorus, and of certain parts accessory to it, will have to bear the brunt of the comparative arguments, while external form, dermal appendages, and anatomy will also have to take their proper, though a minor, share in the comparisons.

EXTERNAL FORM.

Throughout the Ferns of Blechnoid affinity there is some degree of inconstancy in the position of the axis, and in its proportion to the other parts of the shoot. In the case of the Onocleineae the genus *Onoclea* has an elongated creeping rhizome, while *Matteuccia* (*Struthiopteris*) has a compact and upright stock. But even in the latter case the upright stock is commonly preceded by an elongated, runner-like tract, in those cases where branching occurs in relation to the leaf-bases. This is well illustrated in *M. germanica*. A somewhat similar arrangement is seen also in *Plagiogyria*, a genus of less certain affinity with the Blechnoid Ferns (Ann. of Bot., vol. xxiv, p. 423, &c.). In the genus *Blechnum* both creeping and compact-upright stocks are represented, while many species have an intermediate or ascending position; there is a preponderance of erect or ascending species over those of creeping habit, while not a few are definitely subdendroid. From the creeping habit an easy transition has been to the climbing condition, which appears in *B. attenuatum*, *filiforme*, and *volubile*, but finds its fullest development in § *Stenochlaena*. Of the Blechnoid derivatives, *Woodwardia*, *Doodia*, and *Brainea* show a preponderance of the compact, upright, or ascending habit, and it is shared in some degree by *Asplenium* and *Scolopendrium*.

There is nothing very distinctive in the facts relating to this feature. But it is material to note that the subdendroid habit, which is common, accords well enough with what is seen in the Cyatheoid Ferns, with which, through *Matteuccia*, a phyletic relation would exist if the comparisons given in the above pages are valid. On the other hand, the creeping habit has its prototype among the Cyatheoid Ferns in *Lophosoria*, and ultimately in the Gleicheniaceae, where it is the prevalent condition. In a previous memoir (Ann. of Bot., vol. xxvi, p. 293) it was concluded that the creeping was the relatively primitive type in the Cyatheoid series, and the upright the derivative state. A similar view may now be extended to the Blechnoid Ferns.

The leaves in the majority of the series show no peculiar basal developments such as are seen in *Plagiogyria*. But in *Matteuccia intermedia* the petiole does widen out towards the base, showing thus some degree of similarity to that isolated genus. There is a preponderance of the simple pinnation, though frequent marginal serrations indicate a potential further branching, which is actually realized in the doubly pinnate *Sadleria*, in *B. Fraseri*, and in the climbing *B. volubile*. Such facts accord readily enough with a derivation of the relatively simple leaf-forms by condensation from a more fully branched Cyatheoid type, as do also the facts of the venation. This is of an open forked type, connecting downwards with a marked midrib. In some cases fusions of the veins occur in the expanded sterile lamina, as in *Woodwardia areolata*; such cases may, with *Onoclea sensibilis*, be held to be, as regards their venation, relatively late and derivative states. In the sorophylls of *Blechnum* and its derivatives the formation of soral commissures becomes a marked and constant feature; this will be specially taken up later in relation to the sorus. Putting it for the moment on one side, the preponderating type of venation in the Blechnoid Ferns is of an open forked type—as in the Cyatheoid and Gleichenioid Ferns; and vein-fusions are only occasionally met with in isolated species. Such facts indicate for the family a relatively primitive position.

But the most characteristic feature of the leaves for purposes of comparison is the heterophylly (dimorphism or trimorphism). It may be a question whether this is a trustworthy basis for any wide comparison, however valuable it may be within narrow circles of affinity. It was chiefly on this character that the allocation of *Plagiogyria* in relation to *Blechnum* was based by the earlier writers. But a better knowledge of the details of anatomy and of the soral characters tends to loosen the comparison rather than to draw it closer. Whether or not there be a real relationship to *Plagiogyria*, the heterophylly of *Matteuccia* and of *Onoclea*, combined as it is with other features of correspondence, is a valid point establishing their relation with *Blechnum*, and suggesting what is the starting-point for our comparison of Blechnoid Ferns; viz. that in them heterophylly was a relatively primitive state. The lines of advance which emerge by comparison of the facts stated above illustrate various ways in which these Ferns have broken loose from the physiological disability of heterophylly. The essential point is the securing of an equal assimilative area in their sporophylls to that of their sterile leaves (compare p. 382, above). The several ways in which this has been accomplished will be best treated in relation to the sorus, as exemplified in *Woodwardia* and *Doodia*, in the *Krebsii* var. of *B. punctulatum*, and in the genera *Scolopendrium* and *Asplenium*. We note, meanwhile, the broad fact that the majority of those types which we regard as Blechnoid derivatives appear more nearly homophyllous than are the relatively primitive *Matteuccia* and the primitive *Lomaria* section of *Blechnum*. Speaking generally for this phylum, the progression has been from heterophylly to homophylly; and not only comparison of the detailed facts, but also the line of physiological probability above indicated, shows that the progression has been as thus stated.

One of the most remarkable modifications of form seen in this phylum is that related to the climbing habit. The transition from a ground-growing to a climbing state is easily grasped. Comparison of specimens of *B. attenuatum* in its native habitat provides the intermediate steps; elongated internodes, a supply of grasping roots, and slight structural alteration of the axis combine to fit a creeping type to a climbing habit. In *B. attenuatum* the usual type of heterophylly is seen. But in other climbing types more pronounced modifications appear. For instance, in *B.* (*Salpichlaena*) *volubile* we see a climbing, doubly pinnate type, in which the leaves themselves are prehensile and may show a pronounced heterophylly (*Lomaria* type), or the sporophylls may closely resemble the sterile leaves (*Blechnum* type). In others, however, a still higher degree of heterophylly is seen, and these provide the most pronounced examples of heterophylly in the Filicales: for in *B. filiforme* and *Stenochlaena sorbifolia* a trimorphism of the leaves is seen combined with a high degree of variability of the sterile leaves. The lower parts of the plant are rooted in the soil and bear sterile leaves of the 'Pimpinelloid' type. In the climbing region the sterile leaves are of the 'Blechnoid' type, while the sporophylls are narrow, but with a tendency to an 'Acrostichoid' condition. From these remarks it appears that the series of the Blechnoid Ferns has been unusually plastic in point of external form, a quality which has doubtless contributed to its successful seizure of stations by a very large number of species.

DERMAL APPENDAGES.

A very wide area of fact has shown the correctness of the conclusion that of the dermal appendages of Ferns the prior condition was the hair, composed of a single row of cells, branched or unbranched; and that the flattened scale is a derivative state, and is shown by Ferns phyletically more advanced. This conclusion was clearly stated by Prantl in his memoir on the Schizaeaceae (p. 38), and that family illustrates the point as well as any that could be quoted. In certain series of Ferns such hairs bear terminal cells of a glandular character, often with mucilaginous contents; in other cases the contents may be of the nature of resins or essential oils. The presence of such terminal glands often provides valuable features for comparison.

Long ago, Gardiner and Ito made a careful investigation of such glandular hairs in *Osmunda* and *Blechnum* (Ann. of Bot., vol. i, p. 27). The glandular cells might in *Blechnum* be terminal either on a simple hair or on a flattened scale, and the secretion might be mucilage or resin. Throughout the genus *Blechnum* such hairs with large terminal glands are found. They occur also in *Matteuccia intermedia*. Similar glands, terminal either on hairs or flattened scales, are seen also in *Brainea* and *Stenochlaena*; in *Doodia* and *Woodwardia*; in *Blechnum punctulatum*, var. *Krebsii*, and *Scolopendrium*; and in *Asplenium*. They are, in fact, general for the Blechnoid Ferns. The simple hairs of *Plagiogyria* also bear each a terminal gland of like character (Ann. of Bot., 1910, p. 427). The form of the dermal appendage which bears the gland may vary from a simple hair to a broad and flattened scale; and both types may be present on the same plant. When this is so, the scales are specially prevalent on the axis and base of the leaf, while the simple hairs preponderate distally. In *Plagiogyria*, hairs only are present, and there are no scales. This is probably a primitive state, and it appears to isolate *Plagiogyria* from the rest of the Ferns here treated. In *Matteuccia intermedia*, as in most Ferns of Cyatheoid affinity, broad scales cover the stock and leaf-stalk, but they diminish in size and number upwards, till on the pinnae they are replaced by simple hairs. With varying size and proportion, the same may be said of the genus *Blechnum*, but some of its species show a peculiarly effective protection of the young leaves by plentiful broad filmy scales. A climax of such scaly development, extending here to the distal end of the leaf, is seen in *Woodwardia radicans*, where simple hairs are almost entirely replaced by them; and a similar state is seen in *Asplenium obtusatum*.

These facts are not very distinctive, but they have their value. Taken quite generally, they show that there is an underlying common type of dermal appendage for the Ferns compared. Further, they indicate a progression from a condition where the primitive hair is prevalent, to one where the derivative scale is prevalent; and the latter state is found in those genera of our series which on other grounds of comparison are held as the more advanced, such as *Woodwardia* and *Asplenium*.

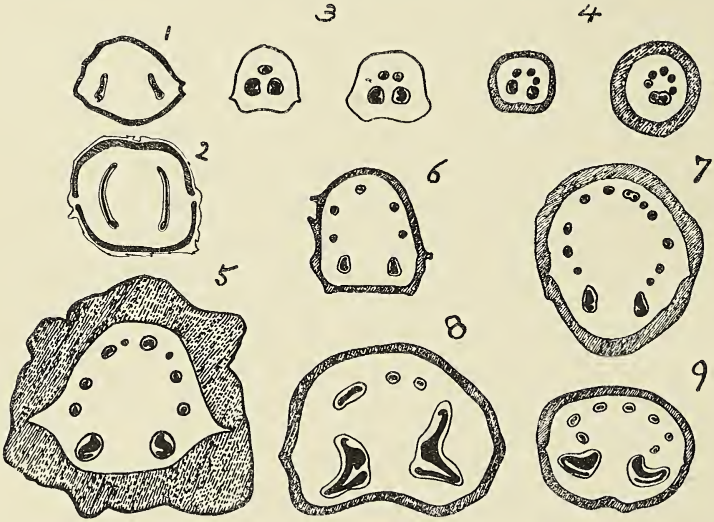
VASCULAR ANATOMY.

All the Blechnoid Ferns and their derivatives that have been studied here show a dictyostelic structure of the axis, and a more or less divided leaf-trace. Thus they may all be held to have attained a relatively advanced state, and their comparison must proceed upon minor points. It is true that *Plagiogyria* has a dictyostele which still remains very close to solenostely, and that its runners may be solenostelic, while its leaf-trace is undivided (cf. Ann. of Bot., vol. xxiv, p. 428). But it is uncertain whether in *Plagiogyria* we really see a prototype of the Blechnoid Ferns. At all events it is by far the most isolated, as it is also the most primitive of the Ferns here considered. But of the true Blechnoid Ferns, de Bary (Comp. Anat., pp. 312–13, quoting from Stenzel, Nova Acta Acad. Leopold., Bd. 28) points out that in *Blechnum spicant* the vascular supply to a lateral axis arises as a single thread-like strand, while in *Struthiopteris* (*Matteuccia*) *germanica* it is tubular (solenostelic) in its lower region, and a similar tube-like system has also been seen in *Diplazium giganteum*.

More recently, Chandler (Ann. of Bot., vol. xix, pp. 365–410, Pls. XVIII–XX) has investigated the vascular development of young plants of *Blechnum gibbum* and *spicant*, *Doodia aspera*, and *Asplenium nidus* and *bulbiferum*. These all show essential similarity. For young plants of *Doodia* the matter has been summed up by him (ibid., p. 403) in the statement that 'the type of vascular elaboration met with in *Doodia aspera* is probably best, regarded as a short cut to the adult dictyostelic arrangement', and that it is attained without the previous siphonostelic (or solenostelic) state—or with such a state very transitory indeed. It thus appears that a solenostelic structure is not altogether unknown in these Ferns, though they have all passed on to more complex arrangements in their mature state.

All of these Ferns are dictyostelic, but the details may vary. A good central type is that seen in *Blechnum Patersoni*, where the transverse section shows eight leaf-gaps (p. 387). Just below the level of opening of each gap a root-trace is given off, by detachment of the middle portion of a meristele; by this means the gap is opened, and at a higher level two equal leaf-trace strands are detached from the sides of the gap, and pass outwards as the leaf-trace. The systems of *B. tabulare*, *attenuatum*, *spicant*, and *alpinum* differ from this only in the number of the leaf-gaps traversed in a single section, and such minor details. The same may be said also for *Matteuccia intermedia*. In the climbing species (*B. filiforme* and *Stenochlaena sorbifolia*) a similar arrangement is found, but with the proportions of the strands, and especially of their xylem, much larger, and with the gaps narrower; while in accordance with the climbing habit the roots tend to be concentrated on the side next the support, where an unusually large meristele may sometimes be found. The *Blechnum* type also extends to *Woodwardia* and *Doodia*, and it is found to underlie the structure seen also in *Scolopendrium* and *Asplenium*. But there is little in the character of the dictyostele of any of these which can be laid hold of for comparative purposes.

The leaf-trace provides more interesting material. The relatively primitive type of this series is the leaf-trace of two equal strands, right and left. It is seen in *Matteuccia*, and in *B. Patersoni*, *tabulare*, *spicant*, *attenuatum*, *alpinum*, *filiforme*, and *punctulatum*; also in *Doodia*, *Scolopendrium*, and *Asplenium*. It may readily be regarded as derived from a single strand of a trace of a type such as is seen in *Plagiogyria* (Ann. of Bot., xxiv, p. 430, Text-fig.). In some cases, as in *Matteuccia*, *B. Patersoni*, *spicant*, *alpinum*, and *filiforme*, and many species of *Asplenium*, such as *A. marginatum*, the two leaf-trace strands maintain their identity undivided for a considerable distance. But in others they subdivide soon to form a 'horseshoe' of which the lateral strands are larger and hooked. This is seen in *B. tabulare*, *attenuatum*, *punctulatum*, *brasiliense*, in *Brainea* and



TEXT-FIG. 20. Transverse sections of the petioles of various Ferns of the Blechnoid series. (1) *Matteuccia intermedia*; (2) *Asplenium celtidifolium*; (3) *Blechnum Fraseri*; (4) *Blechnum attenuatum*; (5) *Blechnum tabulare*; (6) *Blechnum brasiliense*; (7) *Brainea insignis*; (8) *Woodwardia radicans*; (9) *Sadleria cyatheoides*.

Sadleria, and in *Doodia blechnoides*. It may be held as an advance upon the simpler type, and the result is seen in varying degrees of complexity of the petiolar system (Text-fig. 20).

But in *Stenochlaena sorbifolia* and in *Woodwardia radicans* the trace is more complex at its origin. The lateral strands are present as before, but lower down than their insertion smaller leaf-trace strands arise separately from the foliar gap, and pass into the median region of the horseshoe series. Technically, the leaf-trace consists here not of two strands, but of five or six, a condition of advance as compared with the usual *Blechnum* type (Text-figs. 11, 16).

Lastly, median vascular fusion makes its appearance in the petiole, in certain forms. This may be seen in a rather unusual position occasionally in *B. attenuatum* (Text-fig. 20, 4), but it is a marked feature in those genera which we regard as derivative, viz. *Scolopendrium* and *Asplenium*. It may be regarded as a secondary feature (see Rab. Krypt.-Flora, iii, pp. 120, 151).

The result of these anatomical comparisons is a general support of the natural affinity of the Ferns in question. But they do not give much detailed guidance in their phyletic seriation. The fact is clear, however, that the more complex leaf-traces are found in those types which on their soral characters are held to be the more advanced, such as *Stenochlaena* and *Woodwardia*. Also the vascular fusions in the leaf-base, which are so frequent in *Scolopendrium* and *Asplenium*, mark these genera out by a feature which is certainly derivative from the common type. Thus, so far as it goes, the anatomical evidence is in accord with the soral conclusions, and supports them.

SORAL CHARACTERS.

In proportion as other criteria fail us in greater or less degree, stress must be laid upon the soral characters for the phyletic treatment of the Blechnoid Ferns; and fortunately their sori provide a considerable range of divergent detail. On a basis of modern comparison, as well as on that of traditional opinion of the older systematists, the soral relation has been recognized between *Matteuccia* and *Blechnum* (compare Ann. of Bot., xxvi, Pl. XXXVI, Figs. 26–37). While both are dimorphic, with marginal protection of the sori, one important point of difference between them consists in the presence of a 'true' indusium in *Matteuccia* as hitherto described, and its absence in *Blechnum*. But it is now seen that this is not a constant distinction. The 'true' indusium is absent from the new species *M. intermedia*, C. Chr.; it is not even present in that species in the youngest state of the sorus. Accordingly, the starting-point for the nearer comparison of the Blechnoid Ferns will be a type corresponding to *Alsophila*, in having a basipetal sorus seated upon a vein, without any indusium. The chief soral difference between *Alsophila* and *M. intermedia* lies in the minor facts that in the latter the sori form a regular line on each side of the midrib of the narrow pinna, and that they are protected by the strongly deflexed margins which curl over them; in fact, that it is dimorphic. But in neither are there any vascular commissures connecting the sori laterally. The pinna of *M. intermedia* is, in fact, of the Cyatheoid type, but modified in slight degree for protection of the sori which it bears. The Blechnoid pinna, however, shows with high constancy an advance on this, in the presence of a vascular commissure which links together the line of sori into what has here been called the 'fusion-sorus'.

(*a*) *The Fusion-sorus.*

It has been amply shown, in many species of the genus *Blechnum*, especially in the least-modified of them belonging to § *Lomaria*, and in the early stages of their development, that the sori are, like those of *Matteuccia*, basipetal. This sequence may be departed from as the individual grows older, and markedly so in the more advanced *Eu-Blechnum* types, while the basipetal sequence may also disappear in those Ferns which have become 'Acrostichoid': also in such ultimate derivatives as *Woodwardia* and *Doodia*, or *Scolopendrium* and *Asplenium*, the 'mixed' type of sorus is prevalent. These facts very greatly support the position adopted here; for the progression from a simple or a basipetal sorus to the mixed type has been traced now in so many sequences, that it may be held as a general conclusion that the latter is relatively late, and derivative from the former. Applying this in the present case, *Matteuccia* and the Lomarioid Blechnums will be held as relatively primitive, and *Eu-Blechnum*, *Woodwardia*, *Doodia*, *Scolopendrium*, and *Asplenium* as relatively derivative types. The transition from the one to the other is, however, by only slight degrees, and no sharp line can be actually drawn; this in itself suggests that there has been a progression.

Occasionally the vascular commissure may be incomplete in the normal pinnae of simple Blechnums, as in *B. discolor* (Fig. 3, *h*). But very instructive conditions are found in pinnae of various species which are intermediate between the sterile and the fertile states (compare Mettenius, Fil. Hort. Lips., Taf. IV, Fig. 21, also Pl. XXIX, Fig. 22). In these the commissures are variously interrupted at the transitional point, and the details strongly suggest that the origin of the commissure has been by a lateral widening of the vascular supply of the receptacle right and left. In fact, that its origin has been just the same as is seen in *Saccoloma* or *Lindsaya* (compare Ann. of Bot., vol. xxvii, Pl. XXXIV, Figs. 20, 21), or in the series of the Pterideae as explained by Prantl (Engler's Bot. Jahrb., iii, p. 403,), where *Pellaea* has separate sori, but in *Pteris* they are connected laterally, and have a vascular commissure. It thus appears that in three phyla, for which there is no reason to assume any common evolutionary advance in this respect, a lateral fusion of sori is seen, resulting in each case in long continuous receptacles, with a connected vascular supply beneath them. The identity of the original sorus of the Cyatheoid type, seen in *Matteuccia*, is thus lost in *Blechnum*, just as that of the Dicksonioid sorus is lost in *Saccoloma* or *Lindsaya*, or the *Pellaea*-type of sorus is lost in *Pteris*. In each of these cases it must be remembered that in strict terminology 'sorus' should be applied to the original type. The derivative state might properly be styled a 'fusion-sorus'.

The protection of the fusion-sorus of *Blechnum* is by the recurved margin of the pinna. That this is the real nature of the so-called 'false indusium' is shown by comparison of *Matteuccia* or *Plagiogyria*, not only in the mature state, but also as regards early development. Further, the structure shows in its different forms various stages of the reduction in complexity, from that of an ordinary leaf-margin to a single layer of cells in extreme cases. It cannot be doubted, from comparison of the genera named with the simplest of the Blechnums, that the morphology of the organ in question is the same throughout. It is usually styled an 'indusium', but in the use of the word a reservation must be made. It must not be held to connote a morphological entity for Ferns at large, but be used in a general sense to cover any membranous structure which protects young sporangia of Ferns. For already several types of indusium, phyletically quite distinct from one another and different in their origin, are known. For instance, there is the umbrella-like covering of the sorus in *Matonia*, which arises from the distal end of the receptacle; the scale-like indusium of *Hemitelia* and *Nephrodium*; the basal cups (often two-lipped) of the Dicksonioid Ferns and Hymenophyllaceae, which are surface growths from the leaf below the marginal receptacle; the marginal flaps of the Pterids, and of the Blechnoids. It is impossible, from what we know of the ontogeny of these different organs, and the difference of their positions, to hold that they are morphologically identical. They appear to be independent responses along distinct phyletic lines to the same requirement for protection of the young sporangia.

Another question of morphological interest is, what is the real nature of that 'flange' which makes its appearance in the Blechnoid series? Its origin has been traced in a number of species, from those which are Lomarioid, in which it is small or even absent, to those which are Eu-Blechnoid, in which it is of considerable size. They illustrate its inception as a consequence of a 'phyletic slide' of the indusial margin from the genetic margin to the lower surface of the pinna. The indusial flap has been held to be the 'phyletic margin', because its identity is maintained throughout the series, though its position alters. What then is the flange? It must be of the nature of a new formation, emerging by gradual steps which the series of species above described sufficiently demonstrates. This suggestion is no new one. The first statement of such a view that I am aware of was pointed out to me by Professor Lang, in the Posthumous Papers of Griffith, Calcutta, 1847. On p. 444 of the Journals, Griffith wrote : 'It would be a curious circumstance if all indusiate Ferns were to be found reducible to a *marginal production* of the reproductive apparatus. I will bear this in mind, as certain forms of *Pteris* or its affinities lead me to suspect that in these tribes the indusium may be a long way from the margin, and yet be *quoad* origin marginal. . . . The transition to this might reasonably be suspected.' Again, in Griffith's Posthumous Papers (Notulae, vol. ii, pt. i, p. 600), under the heading *Blechnum,* he writes : 'The general involucre is referable to the inflexed margin of the frond, as in *Pteris*, in some species of which the frond is actually produced beyond the inflection. We may hence look for any amount of dislocation from the costa, forming a very natural passage into *Pteris*.' 'In no case does an involucre not having this origin open internally.' 'It follows hence as a matter of course that the intramarginal vein is far within the margin. Thus a section will be formed, characterized by the prolongation of the margin of the frond beyond the vein to which they belong, *Blechnum*, *Doodia*, *Woodwardia*.' Considering the time when these passages were written they are most striking, for not only do they adumbrate a view now supported by a wide area of developmental observation, but they anticipate in a most remarkable fashion the evolutionary aspect of such matters.

A second suggestion of the same nature, but based upon developmental evidence, was made by Burck, in his work Indusium der Varens, Haarlem, 1874. He recognized the indusium as truly the foliar margin, which had curved downwards, while the 'flange' resulted from intercalary cell-division from the convex surface.

But the most important observations bearing on this question of a 'phyletic slide', and the formation of a new margin to the leaf, are those made by Prantl in his memoir, Die Schizaeaceen, 1881. They provide an analogy only, as the Schizaeaceae are not nearly related to the Blechnoid Ferns. Prantl there demonstrated (pp. 39–46) that in all of the Schizaeaceae the sporangia spring from marginal cells of the fertile segment, but that in many of them, and especially in *Aneimia* and *Mohria,* the originally marginal sporangia are subsequently pushed to the lower surface by unequal growth of the tissues below them. This is illustrated very clearly in his Pl. VIII, Figs. 129–140, for *Mohria.* Below the sporangia which originate from single marginal cells, a new formation, showing a marginal segmentation like that of the leaf itself, appears on the adaxial side, which grows into a flap styled by Prantl an 'indusium'. It appears to take up the function and to continue the marginal growth of the leaf in the same way as the 'flange' does in *Blechnum.* Moreover, the vascular strands do not stop short at the insertion of the sporangia in *Mohria*, but extend slightly beyond that point into this new marginal growth, just as they extend into the 'flange' in *Blechnum.* Prantl remarks (p. 44) that this is an indication how a dorsal position of the sorus may be held to arise from a terminal (marginal) position. The argument here corresponds closely to that in the case of *Blechnum.* It is true the bodies involved are different. But in both cases a thing which is originally marginal is shunted to the lower surface by strong unequal growth below it. In *Mohria* what has happened is more clear in the individual development than it is in *Blechnum*; moreover, the final result is less marked than it is even in the section § *Lomaria.* But the condition of *Eu-Blechnum* is much more advanced still, for there not only is the 'flange' very large when mature, but the original marginal growth of the leaf is continued directly into it, and the indusium and sorus appear as later structures, even originating from it. Here the transition from a marginal to a superficial position is complete, and finds no direct reflection in the ontogeny of its phyletic history.

Biologically, such transitions appear to be most natural and intelligible. They secure for the young sporangia a better position in regard to protection while young, and to dispersal of their spores when mature. And this being so, it is no matter for surprise that such changes should appear in more than one phylum of Ferns. But whereas in the Schizaeaceae it is seen in a typical representative of the Marginales; in *Blechnum*, a representative of the Superficiales, the sorus was already upon the lower surface, and it is the indusial margin only which is shunted.

(*b*) *Modifications of the 'Fusion-sorus'.*

The disability of the strongly dimorphic type of *Blechnum* as regards nutrition may be obviated, as in the type of *Eu-Blechnum*, by the formation of the flange, and consequent enlargement of the assimilative area. But other methods are seen in other types, which are accompanied by modifications of the fusion-sorus itself. These take the form of its disintegration and breaking up into separate portions. The methods of this, exemplified as an abnormality in *Blechnum* itself, and seen permanently in *Woodwardia*, *Doodia*, *Scolopendrium*, and *Asplenium*, have been described in detail above, and may all be regarded as connected with enlargements of the assimilating area, with which the soral developments do not keep pace. The consequent breaking up of the fusion-sorus into parts may happen in different ways, which result in the characteristics of the well-known genera above named. Their various features are believed to be based on the following factors, which the study of the Ferns of Blechnoid origin have on analysis supplied:

(1) Disintegration of the fusion-sorus, without any outward arching of the parts. This is seen abnormally in *Blechnum* itself, and becomes a regular character of the genera *Woodwardia* and *Doodia*; also of *Asplenium*, usually with an oblique position and overlapping of the sori.

(2) An outward arching, with further disintegration at the apex of the arch, so that each is represented by two parts. This is seen in *Blechnum punctulatum*, var. *Krebsii*, and becomes a permanent feature in *Scolopendrium vulgare.*

(3) Additional branching of the veins in the region between the limbs of their bifurcation, which, as it corresponds to a convex sinuosity of margin subtending the primary vein, is held to represent a suppressed pinnation. This is seen in *B. punctulatum*, var. *Krebsii*, and becomes a regular feature in *Scolopendrium vulgare.*

(4) Formation of additional sori upon these veins; their position is such as to face the central line of the suppressed pinna, thus corresponding to that of the Blechnoid sorus throughout. But the consequence is that they are back to back with the relatively prior sori. This is seen in *B. punctulatum*, var. *Krebsii*, and in *Camptosorus.*

(5) Continuation of these sori downwards towards the midrib, so as to extend below the point of branching of the vein. Thus they are placed back to back with the prior sori, and on the same vein. This is the condition characteristic of *Diplazinm*, and may be seen occasionally in *Camptosorus.*

These factors, in various combinations, may be held to provide an explanation of the several types of *Scolopendrium* and *Asplenium* along phyletic lines, as derivatives from a Blechnoid source. The leaves in which they may be traced have probably had an origin from a more highly branched ancestry, and traces of suppressed pinnation have been noted above, especially in *Scolopendrium* and in the var. *Krebsii.* A further biological consideration has probably been a progressive increase of the assimilating area. The Lomarioid type, from which all of these Ferns appear to have been derived, had excessively narrow fertile pinnae with recurved flaps; in fact, assimilation was sacrificed to protection of the sori. The material for nourishing the developing sori had then to be brought to them *via* the stock, since the narrow sporophylls could not suffice for forming it on the spot. Obviously, an increase of the assimilating area of the sporophylls themselves would then be an advantage. And this was obtained, either by the formation of the flange, as in *Eu-Blechnum*, or by expansions which involved the soral lines themselves, and led to their sinuosities and their ultimate disruption, as in the var. *Krebsii*, in *Scolopendrium*, and *Asplenium*; or to their interruption without marked curvature, as in *Woodwardia* and *Doodia.*

In contemplating thus the breaking up of the fusion-sorus from the bio-evolutionary point of view, it is necessary to consider it also morphologically, and to see how the soral unit fares in the changes in question. There is good reason to believe that the fusion-sorus of *Blechnum*, with its continuous line of receptacle and its underlying vascular commissure, originated from separate sori of such a type as those of *Matteuccia intermedia*, arranged in linear sequence. That they were gradate sori, like those of *Matteuccia*, is indicated by the prevalence of a basipetal sequence of their sporangia in the simpler forms of *Blechnum*, and even in many that are more advanced. Accordingly, the commissure is a secondary formation, as it is also in *Pteris* and in *Saccoloma*; if this be so, the term 'fusion-sorus' is correctly applied. When this fusion-sorus is interrupted, as it is in *Woodwardia*, or *Scolopendrium*, or *Asplenium*, the interruptions do not always coincide with the number or position, or the limits of the original sori. Consequently, each isolated portion of the fusion-sorus cannot be held to be the exact equivalent of the sorus *Matteuccia*. It would doubtless be inconvenient to mark this discrepancy by any change of terminology. The 'sorus' of Ferns must still be so designated in a general sense, as it always has been. But in using the term in the case of these genera, the discrepancy which it covers and obscures should be clearly borne in mind.

Another and a distinct line of modification of the Blechnoid sorus is that which leads to 'Acrostichoid' developments. By this is meant the extension of the soral area, so as to spread over an enlarged surface, which is not necessarily restricted to the position above the veins. The fusion-sorus is itself an example of such lateral spread, i. e. transversely to the veins. But in other types the spread may be along the course of the veins, and in the area between them. Examples in a minor degree have been given in *B. Patersoni*, *penna-marina*, and *filiforme* (pp. 387–90). It only requires an extension of such developments as are there seen to produce the condition of *Stenochlaena*, or of *Brainea*. These show the full features of the old genus *Acrostichum*. In such cases the receptacle becomes flattened, and the basipetal sequence of the sporangia is lost, while the numerous sporangia appear to be more independent of protection while young. In *Stenochlaena* there is reason to believe that the 'flange' has been reduced, while the indusial leaf-margin is flattened, and even everted as maturity comes on. In *Brainea*, on the other hand, it is the 'indusium' that is aborted, and the spread of sporangia is outwards upon the 'flange', following especially the course of its veins, which correspond in position and character to those of *Eu-Blechnum*. It thus seems probable that these two Acrostichoid derivatives of *Blechnum* have arisen independently, as parallel developments. They illustrate what is becoming abundantly plain, that *Acrostichum* is not a genus in the phyletic sense, any more than *Polypodium* is a genus. Both are states or conditions of soral construction, which may have been arrived at along several distinct phyletic lines. They both show a common tendency of the more advanced Leptosporangiate Ferns to become less dependent upon protection of their young sori than the relatively primitive types from which they sprang.

It has already been shown that, among the Blechnoid Ferns, transitions are common from the more primitive gradate sorus to the 'mixed' type, and that the transition may often be traced in the individual as it passes from the young to the mature state. Such facts accord with the experience in other phyla, such as the Davallioid and the Cyatheoid Ferns (compare Land Flora, pp. 595–600; Ann. of Bot., vol. xxvi, p. 316; Davie, Ann. of Bot., xxvi, p. 263).

The transition from a gradate to a mixed sorus is usually accompanied by a transition from the oblique to the vertical annulus, and from the complete to the interrupted ring. This has been seen with special clearness in *Odontosoria* and *Lindsaya* (Ann. of Bot., xxvii, p. 460, Pl. XXXIV, Fig. 22). Some transitional states might therefore be anticipated in the Blechnoid series. It will be remembered that in *Plagiogyria* a complete oblique annulus is present, with lateral dehiscence (Ann. of Bot., vol. xxiv, Pl. XXXIII, Fig. 18, *a–d*). The same is the case in the Cyatheoid Ferns, with which *Matteuccia* is specially related. In some of these, and especially in *Cyathea* itself, there has been a considerable reduction in size of the sporangium. But this is not the case with those of *Lophosoria* (Ann. of Bot., xxvi, Pl. XXXV, Figs. 18–20), where the sporangium is relatively large, with continuous oblique annulus of numerous cells. A comparison of these with the sporangia of *Matteuccia* shows a considerable similarity in size, while the annulus is still more complex in the number of its cells. But it is only slightly oblique, and is interrupted at the insertion of the stalk (Text-fig. 2). The sporangia of *Blechnum* are again smaller. They appear to be of very uniform type throughout the genus, and are adequately represented by those of *Stenochlaena* (Text-fig. 12, *a–d*). The stalk is composed of only three rows of cells; the annulus also consists of fewer cells than in *Lophosoria* or *Matteuccia*, and it is only slightly oblique, showing complete or sometimes partial interruption at the insertion of the stalk. These characters are in fact just such as might be expected in Ferns derived from an ancestry with oblique annulus, in which the oblique position is no longer a matter of biological importance. They accord with the general hypothesis advanced in this memoir, though they are not so distinctive as the facts from the *Dicksonia*, *Lindsaya*, and *Davallia* are seen to be.

From the above paragraphs it will be apparent that comparisons in respect of several distinct criteria lead to similar conclusions, with greater or less clearness. They have been based on external form, on dermal appendages, on the vascular anatomy, on the characters of the sorus, and on the sporangium. The general result has been to indicate a progression from a Cyatheoid source, such as is represented by *Matteuccia intermedia*, through the large and varied genus *Blechnum*, to certain derivative genera, culminating in *Scolopendrium* and *Asplenium*. Not only do the several lines of comparison mutually support one another, but also a line of biological probability has been pointed out, which gives a reasonableness to the comparative conclusions.

SUMMARY.

1. For reasons which have been stated in the course of this memoir, the Ferns above treated as Blechnoid and Blechnoid derivatives are believed to represent a true phyletic sequence.

2. Its origin has been traced in relation to the Cyatheoid Ferns.

3. An actual point of probable contact has been found in *Matteuccia intermedia*, C. Chr.

4. From such a source appear to have sprung several divergent lines. The main line leads through § *Lomaria* to *Eu-Blechnum*.

5. This involves the origin of the 'flange', and diversion of the 'phyletic margin' to indusial functions, as the structure styled by the older writers the 'false indusium'.

6. Minor lines led to Acrostichoid derivatives, respectively in *Stenochlaena* and *Brainea*.

7. Interruption of the fusion-sorus, foreshadowed as an anomaly in *Blechnum*, led to the states shown in *Woodwardia* and *Doodia*.

8. An outward arching of the fusion-sorus of *Blechnum*, ultimately combined with interruption, foreshadowed in the varieties of *B. punctulatum*, gives the key to the origin of *Scolopendrium*.

9. An outward swinging of the interrupted fusion-sori, variously combined with archings and new formations of partial sori, and various branchings of the leaf, give the several types of *Asplenium*.

10. The relation of *Plagiogyria* to the whole series is problematical. It seems probable that it is an isolated, as it is certainly a relatively primitive, genus.

11. All the Ferns here considered belong to the *Superficiales*. But in the methods of their advance they show interesting parallels with representatives of the *Marginales*.

GLASGOW, *Dec.* 1913.

EXPLANATION OF FIGURES IN PLATES XXII–XXXII.

Illustrating Professor Bower's paper on *Blechnum* and Allied Genera.

PLATE XXII.

Fig. 1. Transverse sections of young pinnae of *Plagiogyria semicordata*, in successive stages of development. *a* shows that the whole wing of the pinna is referable in origin to marginal segmentation. *b* shows a more advanced state, in which the extreme margin is growing out to form the thin 'indusial' flap, which arches over the receptacle below it. Mucilage-hairs are also seen. *c* shows a section of a mature pinna, with fully formed flap covering the mature sporangia. × 125.

Fig. 2. Sections of young pinnae of *Matteuccia intermedia*, C. Chr. *d–g* are cut transversely, *h* is a longitudinal section. The sori are all separate and intramarginal, while the segmental margin of the pinna grows directly into the protective 'indusial flap'. × 125.

Fig. 3. Sections of the young pinnae of *Blechnum discolor*, Keys. *a*, a very young wing of a pinna, showing regular marginal segmentation. *b*, *c*, *d*, successively older pinnae, showing the relation of origin of the indusium, or phyletic margin, to the segmental margin (*m*) of the wing. *e*, slightly older, with basipetal sorus forming. *f*, older still, with vascular commissure and vein shaded. *g*, an older wing, with sorus showing slightly mixed condition. *h*, section of a fertile pinna, showing that both sorus and commissure are interrupted, for they are absent from the section. In *b–e*, the indusium is indicated by *i*. *f*, *g*, *h* are orientated naturally, but *a–e* are inverted. *a–f* × 125; *g*, *h* × 50.

PLATE XXIII.

Fig. 4. Sections illustrating the development of the indusial flap and sorus in *Blechnum tabulare*, Kuhn. *a*, *b* show a segmentation which indicates that the origin of the flap is truly marginal. *c–f* indicate that the flap originates from a point which is intramarginal. *g* shows a later stage, with a sorus of basipetal sequence. *h* is an occasional example of the mixed character which may supervene later. All the drawings × 125.

Fig. 5. A single fertile pinna of *Blechnum lanceolatum*, Sturm. *x–y* shows the plane in which the section Fig. 6, *a*, might have been cut. × 6.

Fig. 6. *Blechnum lanceolatum*, Sturm. *a* shows a fertile pinna in transverse section; no obvious flange is present; the section came from the middle region of the pinna. *b* is part of a similar section, showing a vascular strand passing to the upper surface. *c–g*, successive stages of development of the wings of the pinna. For details see text. *a*, *b* × 50; *c–g* × 125.

PLATE XXIV.

Fig. 7. *Blechnum attenuatum* (Sw.), Mett. *a–d*, successive stages in development of the wing of the pinna. *i* is the indusial flap; *m* is the flange; *h* is the young hydathode. Section *e* shows a mature pinna in transverse section. *a–d* × 125; *e* × 8.

Fig. 8. *Blechnum L'Herminieri* (Bory), Mett. Similar sections showing the relation of origin of the relatively small flange and the indusium. *a–c* × 125; *d* × 50.

PLATE XXV.

Fig. 9. *Blechnum spicant* (L.), Wither. Tip of a fertile pinna, showing the venation in its relation to the distal end of the fusion-sori. The commissure appears to be a mere joining up of the anadromic branches of the dichotomous veins. × 6.

Fig. 10. *Blechnum spicant* (L.), Wither. Sections showing successive stages of development of the flange and indusium. × 125.

Fig. 11. Similar sections from the pinna of *Blechnum capense* (L.), Schlecht. In *a* the segmentation is particularly clear, and the intramarginal origin of the indusial flap. × 125.

Fig. 12. *Blechnum brasiliense*, Desv. Similar sections, showing an extreme intramarginal origin of the indusial flap and sorus. × 125.

PLATE XXVI.

Fig. 13. Similar sections from the pinna of *Blechnum Fraseri* (A. Cunn.), Luerss. *a–e* × 125; *f* × 50.

Fig. 14. *Blechnum Patersoni* (R. Br.), Mett. Similar sections showing the segmentation, the intramarginal origin of the indusial flap and sorus, with its mixed condition. *a–e* × 125.

PLATE XXVII.

Fig. 15. *Blechnum penna-marina* (Poir), Kuhn. Sections of the pinna, of which *a* and *b* are transverse, and *c* longitudinal. The relations of origin of the relatively small flange and of the indusial flap and sorus are as before, but the receptacle is flat, and widely spread in an Acrostichoid manner. *a–c* × 125; *b* × 50.

Fig. 16. *Blechnum filiforme* (A. Cunn.), Ettings. Similar sections, showing the segmentation and origin of the indusial flap and sorus. In *e* the flange is present, but small. In *f*, *g* it appears to be merely vestigial. *a–f* × 125; *g* × 50.

PLATE XXVIII.

Fig. 17. *Stenochlaena sorbifolia* (L.), J. Sm. Similar sections. *a*, *b*, *c*, transverse sections, and *d*, a longitudinal section; these show the flattened concave receptacle with 'Acrostichoid' spread over a large area; the identity of the sori is completely lost. *e*, *f* show indications of a flange, but it is not seen in *g*. All × 125.

PLATE XXIX.

Fig. 18. *Brainea insignis* (L.), J. Sm. Distal end of a pinna, showing the venation, and the relation of the non-indusiate sori to it. × 10.

Fig. 19. *Brainea insignis* (L.), J. Sm. Sections of the pinna (*a–c*), showing that the initial stages closely resemble those of *Blechnum*, but with no sign of an indusial flap. × 125.

Fig. 20. *Blechnum fraxineum*, Willd. *d*, apical region of a pinna, showing the venation, and its relation to the fusion-sori. *e*, a lower region, showing partial pinnae, I–VI. *f* shows details of the pinna, III in the preceding figure, under higher power. *d*, *e* × 2; *f* × 15.

Fig. 21. Portion of a pinna of *Blechnum Fraseri*, Luerss., showing the relation of the venation to the commissure. × 15.

Fig. 22. Part of a pinna of *Blechnum capense* (L.), Schlecht, showing the transition from the sterile to the fertile state, with disintegration of the fusion-sorus. × 2.

Fig. 23. Apical region of a pinna of *Blechnum spicant* (L.), Wither, showing venation, and partial disintegration of the fusion-sorus. × 3.

Fig. 24. Portion of a pinna of *Blechnum cartilagineum*, Sw., showing disintegration of the fusion-sori. × 3.

Fig. 25. *Woodwardia areolata* (L.), Moore. Part of a pinna transitional between sterile and fertile, showing vein-fusions, and the indusial flaps, here much reduced. × 2.

PLATE XXX.

Fig. 26. *Doodia caudata* (Cav.), R. Br. *a–d*, successive sections illustrating the development of the indusial flap and sorus. × 125.

Fig. 27. *Woodwardia radicans* (L.), Sm. A similar section, showing an advanced state similar to that in *Doodia* or *Blechnum brasiliense*. × 125.

Fig. 27 *bis*. Whole leaf of *Blechnum punctulatum*, var. *Krebsii*, considerably reduced.

Fig. 27 *tris*. Three pinnae of the same leaf, slightly enlarged, to show the soral arrangements.

PLATE XXXI.

Fig. 28. *Blechnum punctulatum*, Sw., var. *Krebsii*, Kunze. Portions of pinnae, showing different states of disintegration of the fusion-sorus. *a* shows a condition very near to the normal for *Blechnum* at the apex, but strongly arched outwards below. *b* shows a rather more advanced state of disintegration. *c*, *d*, still more advanced arching and disintegration, so as to resemble *Scolopendrium*. At × in these drawings extra sori are present. *a–d* × 2. *e* shows more in detail the outward arching and disruption of the fusion-sorus. × 10.

Fig. 29. *Blechnum punctulatum*, Sw., var. *Krebsii*, Kunze. Sections of young fertile pinnae, for comparison of *g* with the normal type of *Blechnum* as seen in Figs. 10–12, and of *f*, *h* with what is seen in *Scolopendrium* in Fig. 31. × 125.

Fig. 30. *Scolopendrium vulgare*, Sm. Portion of the lateral flap of a leaf, with midrib, showing the venation and sori. Slightly enlarged.

PLATE XXXII.

Fig. 31. *Scolopendrium vulgare*, Sm. *a*, *b*, successive stages in development of the sorus, for comparison with Fig. 29, *f–h*. × 125.

Fig. 32. *Asplenium obtusatum*, Forst. Part of a pinna showing venation and sori. × 3.

Fig. 33. *Asplenium horridum*, Kaulf. The same. × 3.

Fig. 34. *Asplenium serra*, Langsd. et Fisch. The same. × 3.

Fig. 35. *Asplenium* (*Diplazium*) *celtidifolium*, Kze. The same. × 3.

Fig. 36. *Asplenium obtusatum*, Forst. Sections of fertile pinnae, showing successive stages of development of the sori. *a* and *b* × 125; *c* × 50.

FIG. 1

FIG. 2

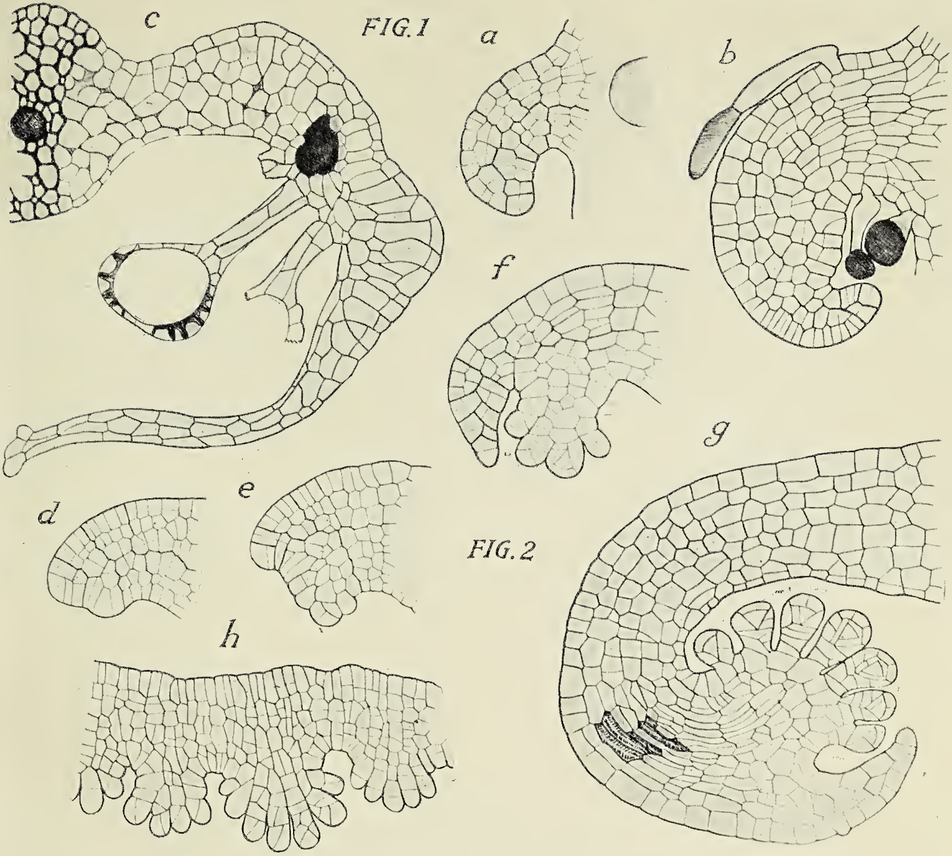
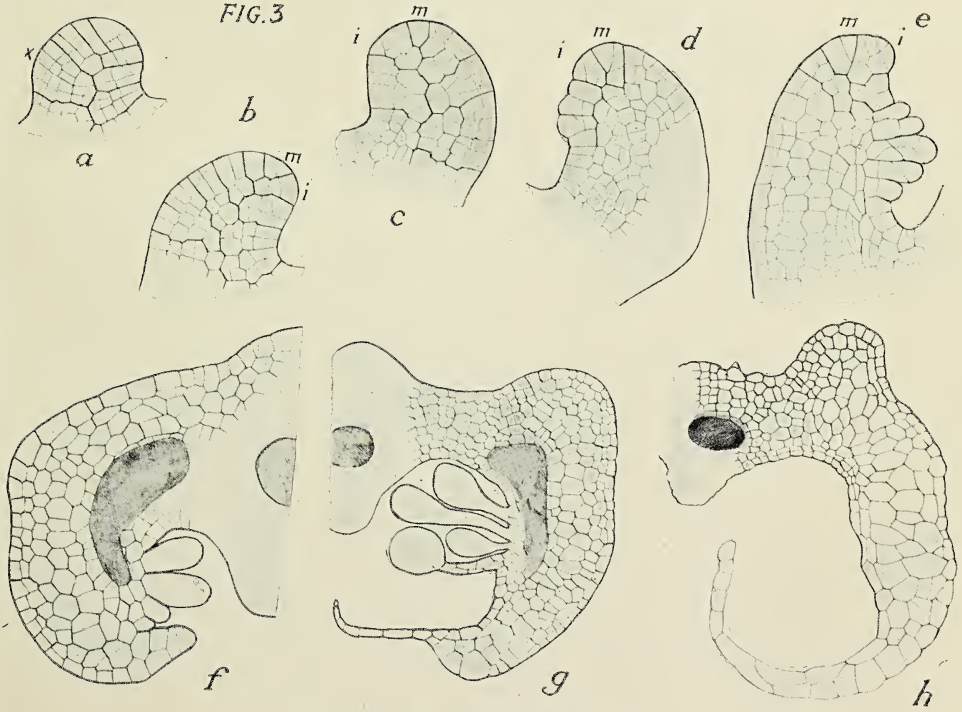


FIG. 3



BOWER—BLECHNUM, ETC.

FIG. 4

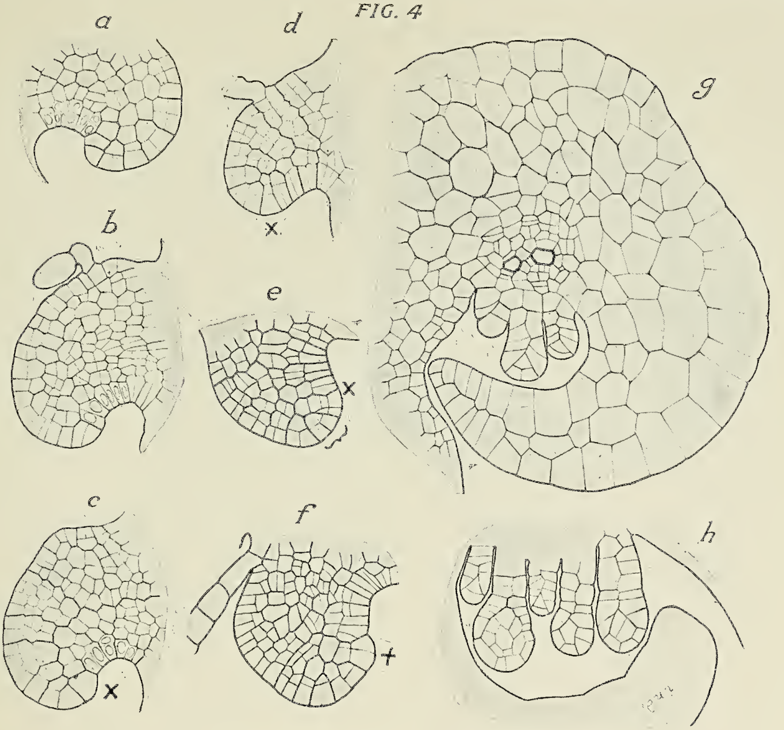


FIG. 5

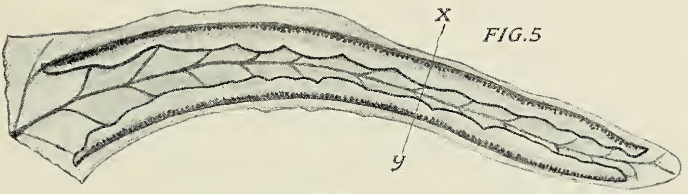
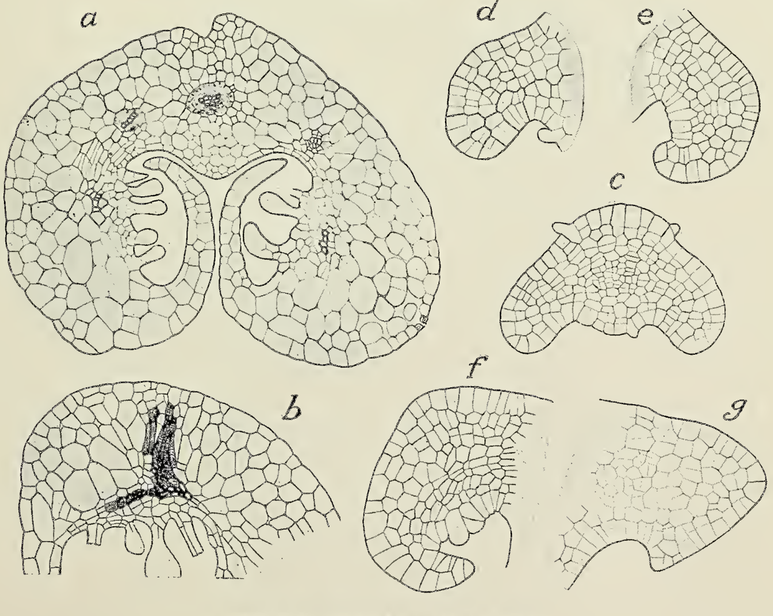


FIG. 6



BOWER—BLECHNUM, ETC.

FIG. 7

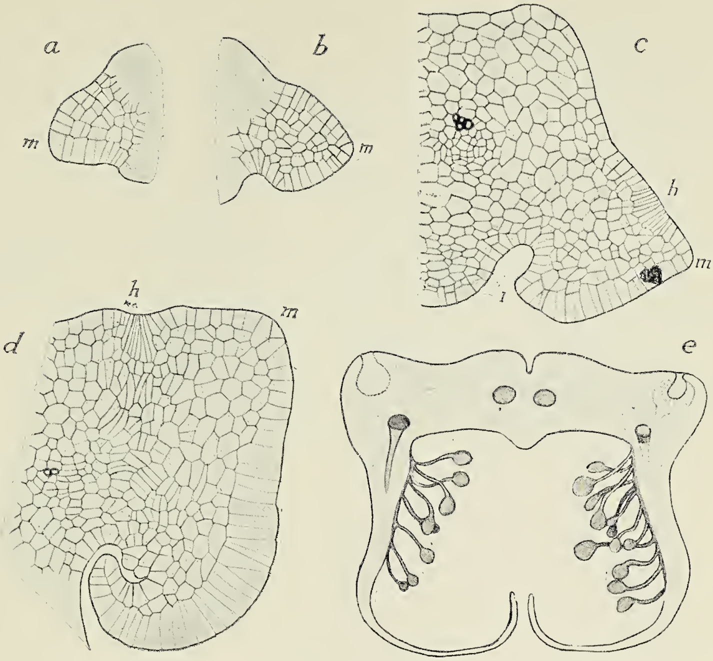
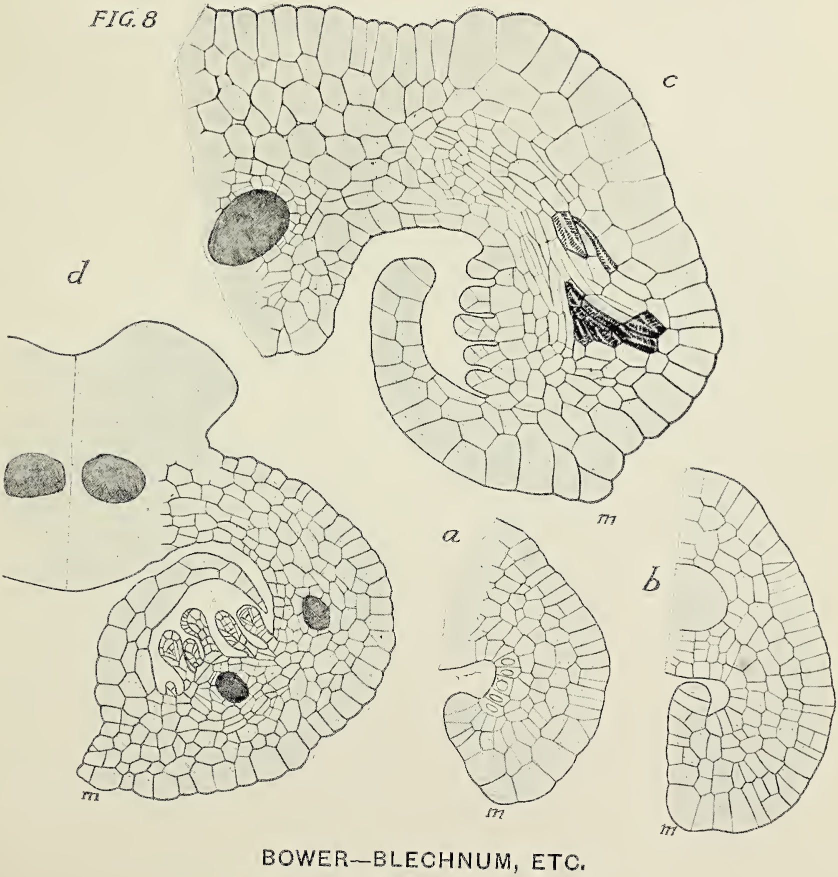


FIG. 8



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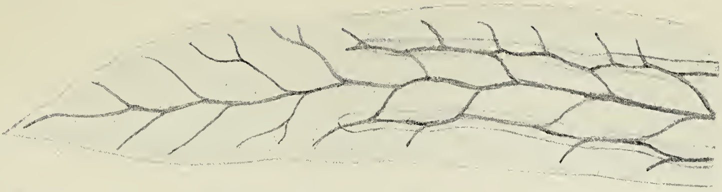


FIG. 9

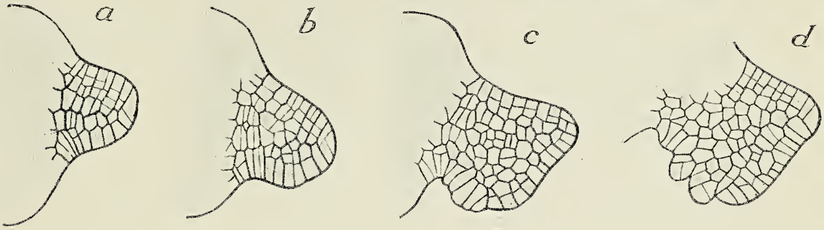


FIG. 10

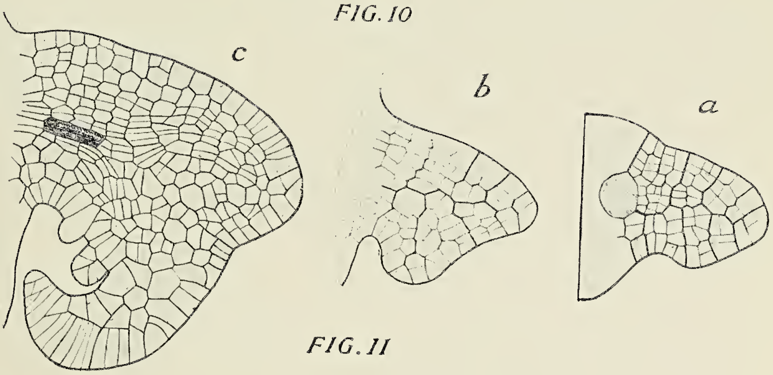


FIG. 11

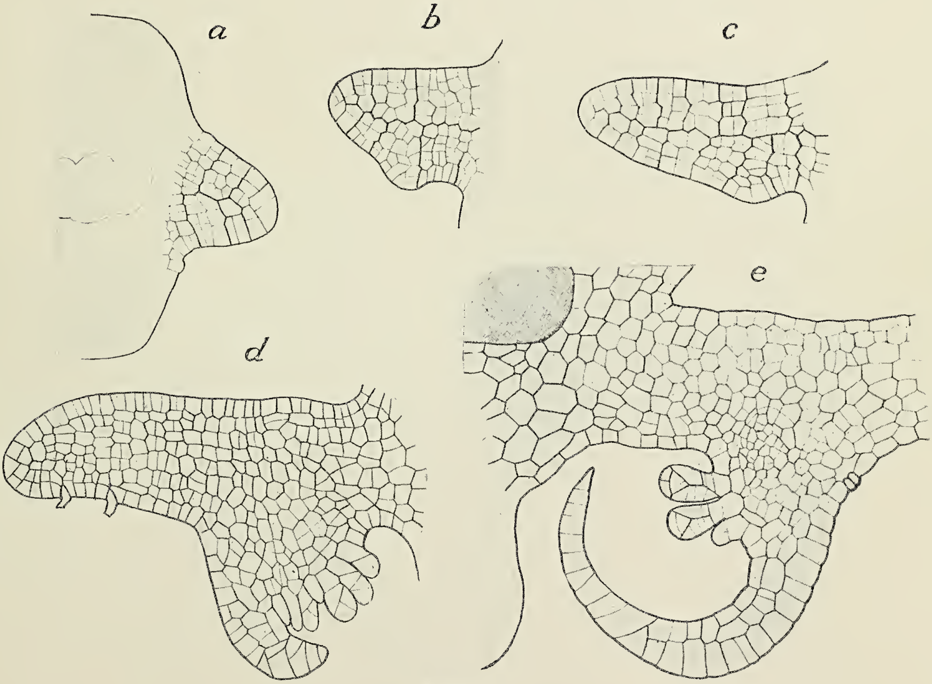


FIG. 12

BOWER—BLECHNUM, ETC.

FIG. 13

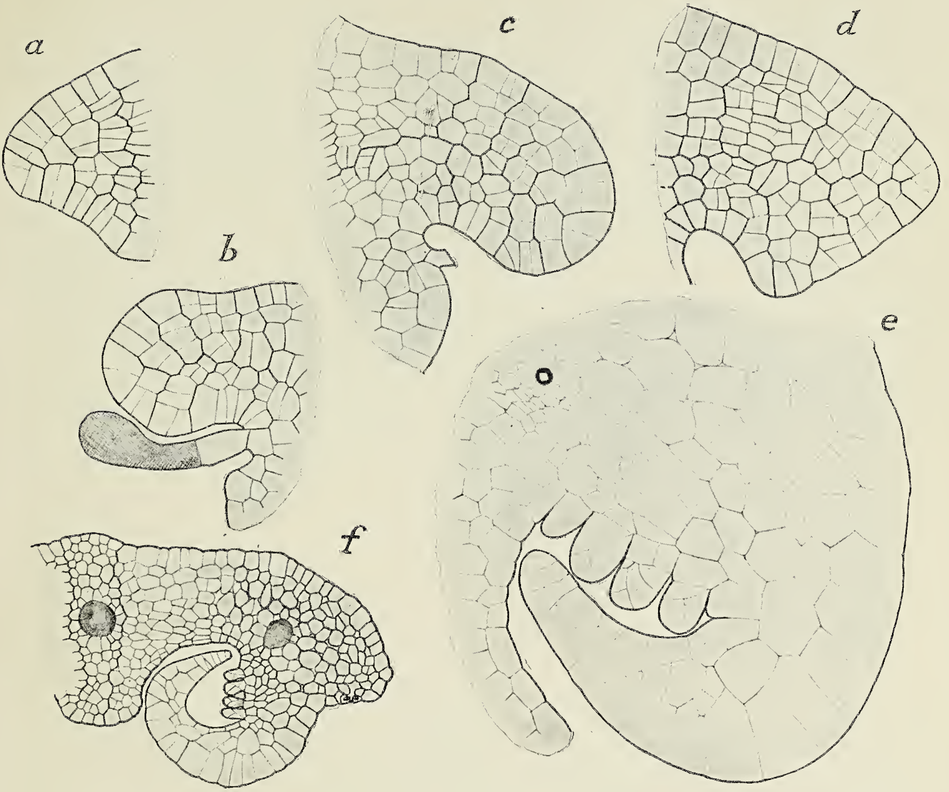
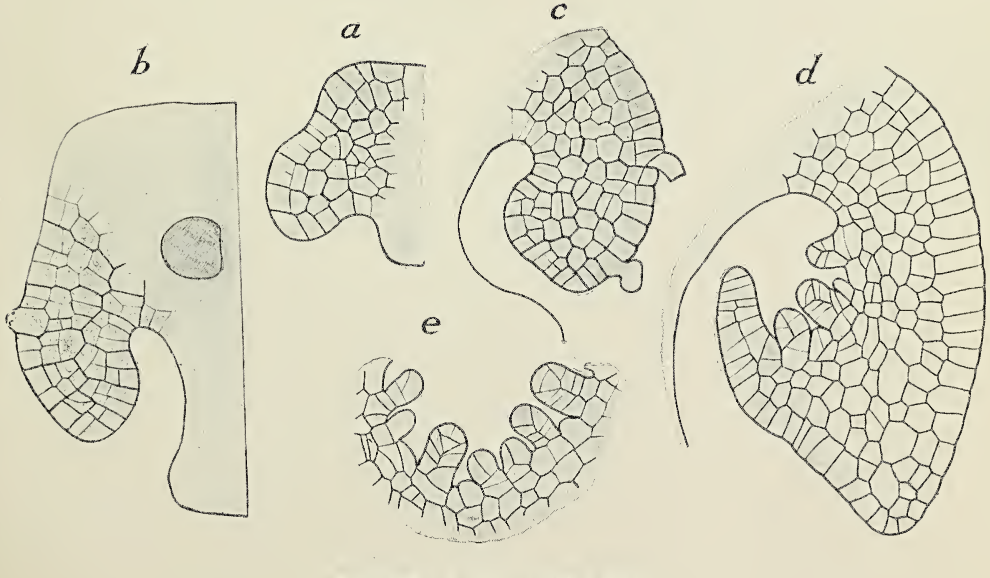


FIG. 14



BOWER—BLECHNUM, ETC.

FIG. 15

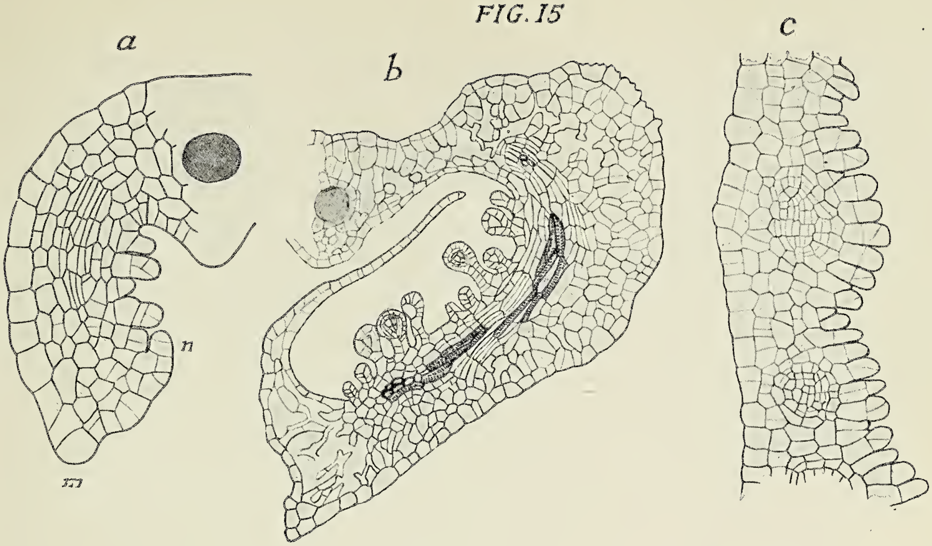
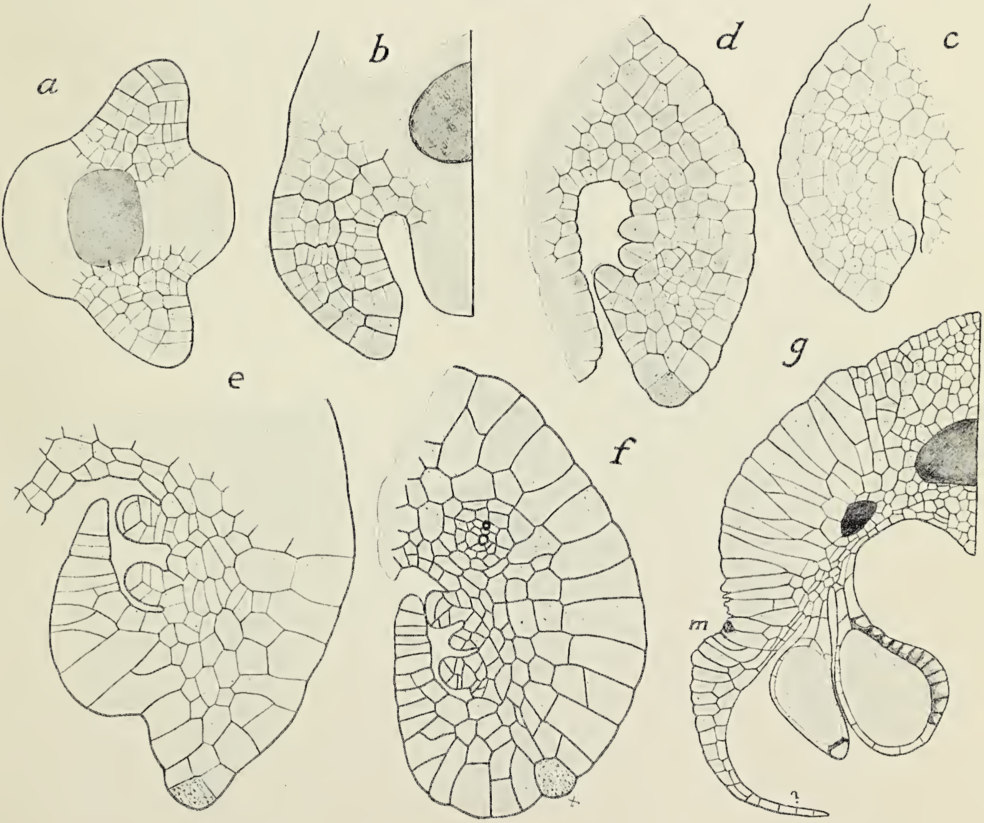
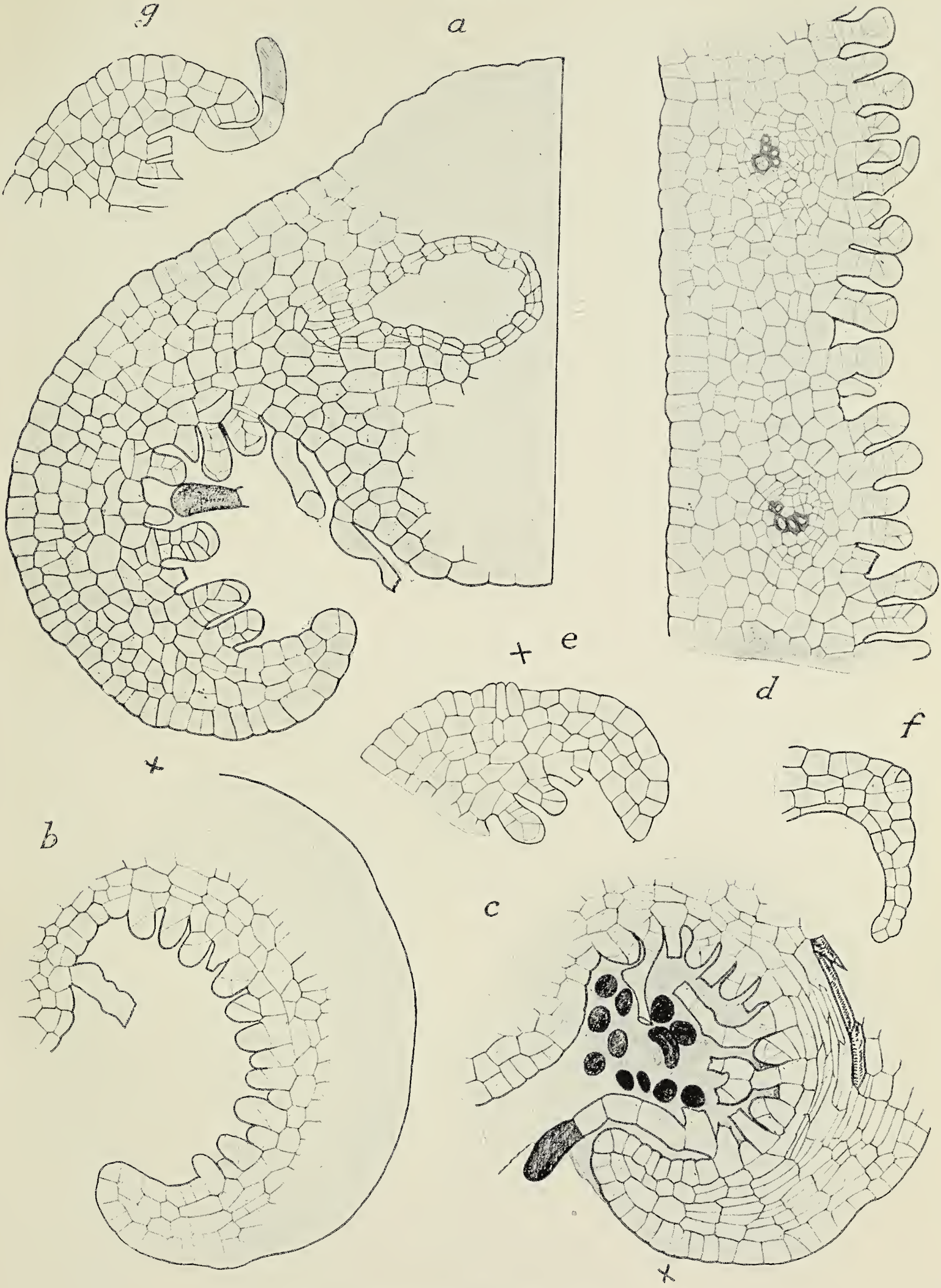


FIG. 16



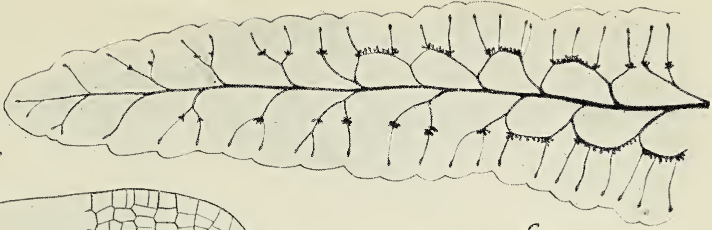
BOWER—BLECHNUM, ETC.

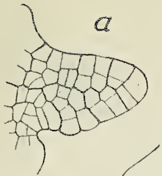
FIG. 17

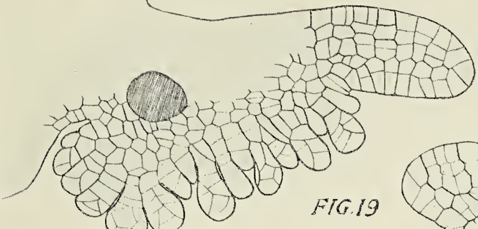


BOWER—BLECHNUM, ETC.

FIG. 18







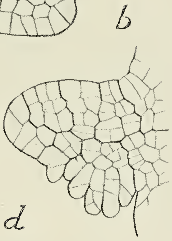


FIG. 19

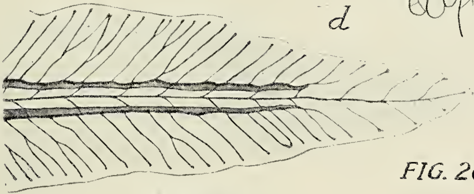
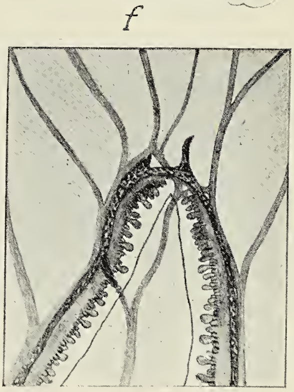


FIG. 20



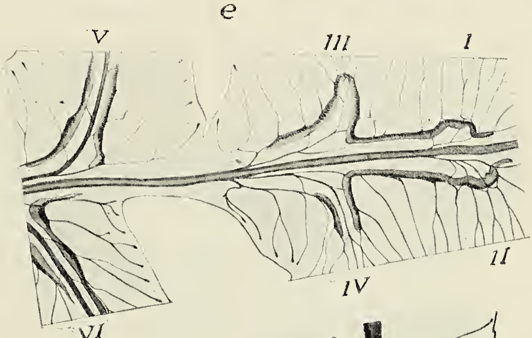
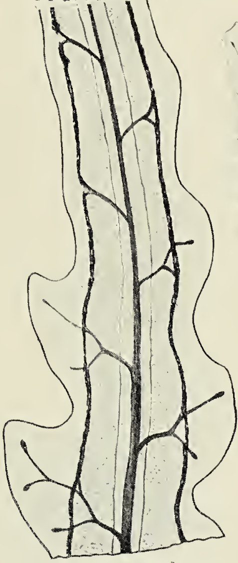


FIG. 21



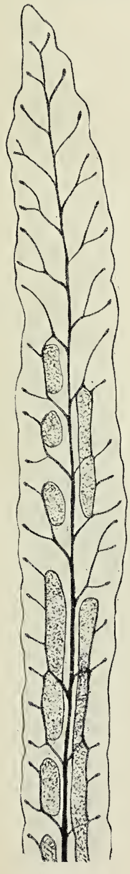


FIG. 23

FIG. 25

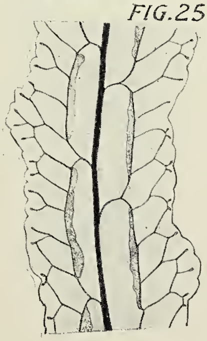
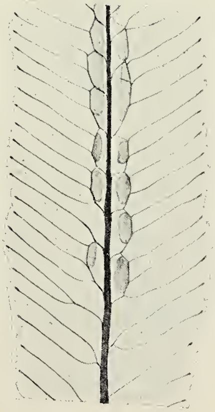


FIG. 24



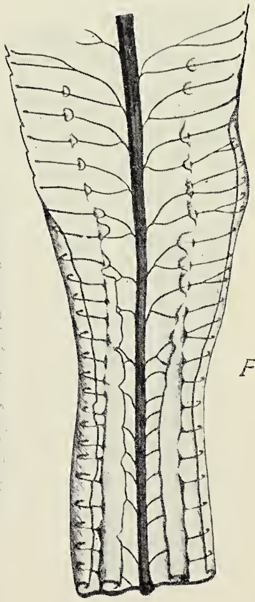


FIG. 22

BOWER – BLECHNUM, ETC.

FIG. 26

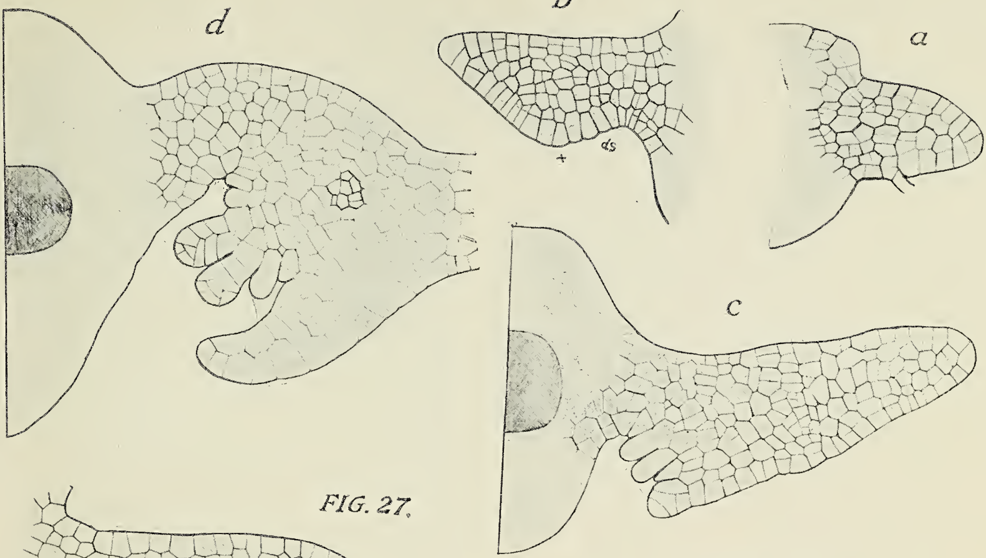


FIG. 27.

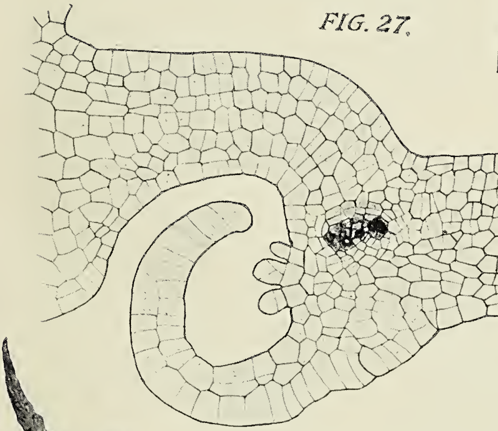
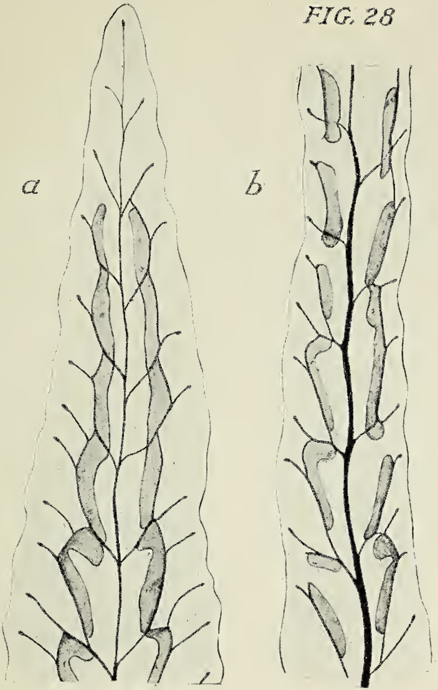


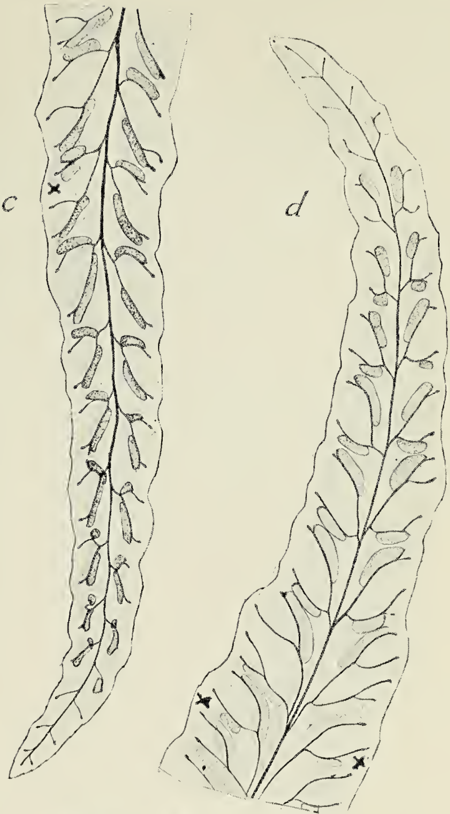
FIG. 27. tris.

FIG. 27. bis.

BOWER—BLECHNUM, ETC.

FIG. 28





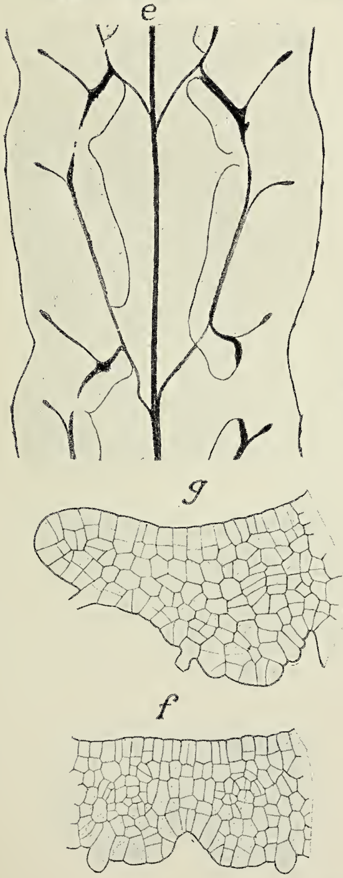


FIG. 29

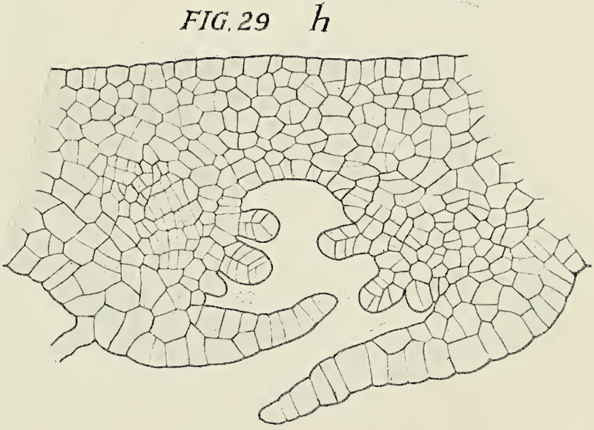
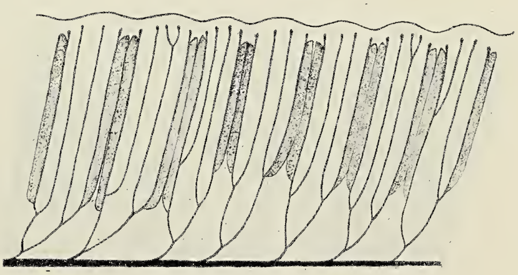


FIG. 30



BOWER—BLECHNUM, ETC.

FIG. 31

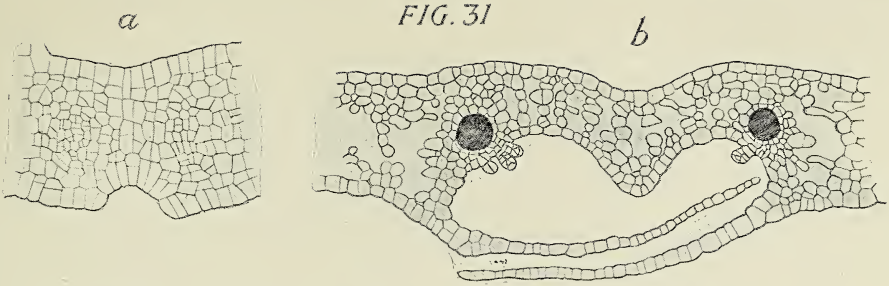


FIG. 32

FIG. 34

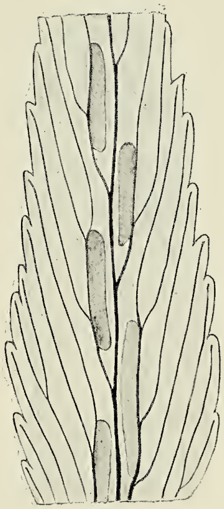


FIG. 35

FIG. 33

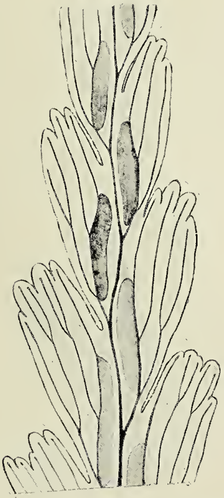
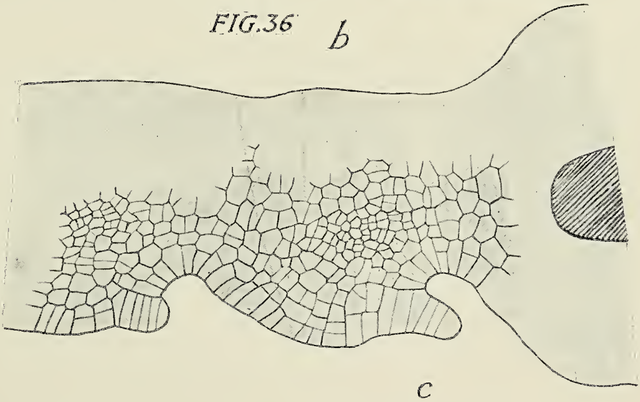
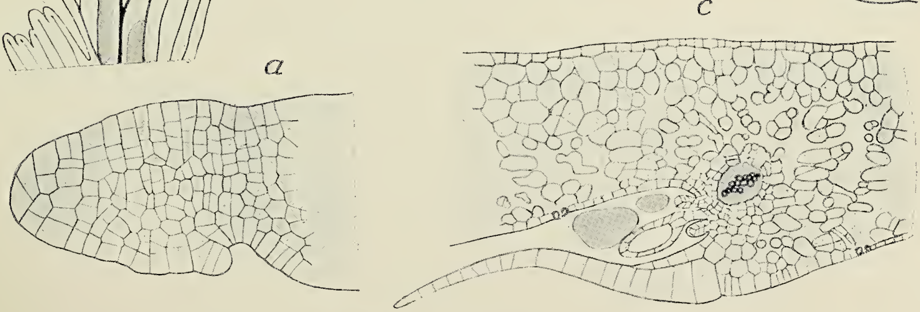


FIG. 36





BOWER—BLECHNUM, ETC.

Observations on the Cytology of the Chroococcaceae.

BY

ELIZABETH ACTON, M.Sc.

With Plates XXXIII and XXXIV.

I. Introduction.

THE controversial question of the structure of the cell in the Myxophyceae (or Cyanophyceae) has formed the subject of many papers. None of these, excepting Chodat's ('96) on *Chroococcus turgidus*, has been restricted to unicellular forms, a few have mentioned them incidentally along with filamentous forms, while the remainder neglect them entirely. Yet it is in the study of the unicellular species, as being the most primitive, that one might hope to find the key to the confusion which prevails at the present time on this question.

A detailed investigation of *Chroococcus macrococcus*, taken up partly in order to determine the systematic position of this Alga by finding out whether it possessed a fully organized nucleus or not, yielded such unexpected results with regard to the structure of the cell that it was thought advisable to examine other species of *Chroococcus*. Later, the investigation was extended to other members of the Chroococcaceae. Other species examined in addition to *Chroococcus macrococcus* were—

Chroococcus turgidus
,, *limneticus*
,, *minor*
,, *schizodermaticus*
Gloeocapsa sp.
Aphanothece prasina
Merismopedia elegans
,, *glauca*
Gomphosphaeria lacustris
Coelosphaerium Kützingianum
Dactylococcopsis sp.

II. Recent Literature.

The numerous papers which have appeared dealing with the cytology of the Cyanophyceae have been reviewed by so many authors, that it seems unnecessary to give an account of any but the more recent.

The papers of Kohl ('03), Phillips ('04), Olive ('05), and Guilliermond ('05), all contain excellent reviews. A criticism of these four papers forms the subject of a special paper by Zacharias ('07).

Interest chiefly centres round the question of the presence or absence of a nucleus, and its behaviour during division if present. Kohl, Olive, and Phillips agree on the presence of a nucleus which divides mitotically; though they differ on the question of the arrangement of the chromatin and on the details of division. It seems probable that both Olive and Phillips have mistaken central granules for chromatin.

The rod-like chromosomes of Kohl can hardly be explained as central granules, though stained preparations, in which under a $\frac{1}{12}''$ objective only central granules can be seen, do sometimes under a $\frac{1}{6}''$ objective show structures simulating karyokinetic figures with rod-like chromosomes; with the greater magnification the rod-like bodies are resolved into granules. Zacharias, who through the kindness of Dr. Kohl has been permitted to examine some of his preparations, believes that they are projections, &c., from the central body.

Gardner ('06), who has investigated *Oscillatoria limosa* and *O. splendida*, two species upon which Kohl worked, states with regard to mitosis in these: 'The process in the nuclei of these two plants is purely and simply amitotic.' He also makes the following statement in the course of a comparative review of the papers by Kohl, Olive, and Phillips: 'After very careful and prolonged investigation I am unable to subscribe to any of the conclusions just quoted concerning the mitosis of the nucleus in any of the Cyanophyceae upon which they have worked.'

Kohl believes that there are numerous small granular chromatophores, while Olive and Phillips agree that there is a single large chromatophore. All find granules of two kinds—central granules and cyanophycin granules.

Fischer ('05), on the other hand, denies the existence of a nucleus, though he admits the presence of a single chromatophore. He maintains that the chromatin-like, pseudo-mitotic masses in the centre of the cell are due to the presence of 'anabaenin', which is found sometimes in chromosome-like masses and sometimes in granules (central granules of other authors). The anabaenin is a transformation product of glycogen, and there is a steady stream of glycogen from the chromatophore to the central body. He believes that this laying down of anabaenin in the centre of the cell is a means of removing the excess of carbohydrate material formed by the chromatophore. He states that anabaenin is a carbohydrate, thus differing from the majority of authors, who believe that the central granules, with which he identifies anabaenin, are of a proteid nature. He has observed cyanophycin granules in the central body as well as in the chromatophore.

Gardner ('06) finds that there is a small refractive structure in the centre of the cell with a definite outline for each species, and regards this as a true nucleus. It does not, however, divide mitotically. He describes nuclei of three types. 'The diffuse type is characterized by having the chromatin distributed throughout the nucleus in the form of thin, plate-like or small angular pieces, or more or less branched and knotty thread-like masses, according to the species and to the shape and size of the cell in which it is located; or even a combination of these may be found in the same species. In whatever shape the chromatin is found, the essential characteristic is its quite equal distribution throughout the nucleus. . . . Within this type may be found quite a continuous series, showing how a very primitive nucleus becomes modified by gradual steps in the direction of the nuclear structure found in higher plants. . . . The differentiation seems to be proceeding towards the spireme formation found in the dividing of the nucleus of the higher plants.'

The second type he finds only in the genus *Dermocarpa*. 'The type is characterized by having the chromatin united into a very definite fine network, on which, particularly at the junction of the threads, are small granules or knots, presumably of chromatin, since they stain like the remainder of the thread. This whole network occupies a very large part of the cell, leaving only a very narrow zone outside, next to the cell-wall.

'Only a slight modification of the first type is necessary to produce the third or primitive mitosis type, which has been found in a single species only (*Synechocystis*). In this type the chromatin unites into a single contorted thread, quite irregular and indefinite in shape. This thread breaks into a definite number of pieces preparatory to cell division.

'In all the species studied, with the possible exception of *Synechocystis*, the nucleus divides amitotically.'

He describes granules of two kinds, which for convenience he terms α and β granules. These presumably correspond to central granules and cyanophycin granules respectively. There is no definitely organized chromatophore.

It will be seen that Gardner strikes out quite a new line with regard to the form and arrangement of the chromatin. At the same time, judging from his figures, the amount of chromatin present in proportion to the size of the cell is very great, and it is difficult to believe that so much chromatin is really present in the cells of such a primitive group as the Cyanophyceae.

Guilliermond ('06) states that the central body consists of a hyaloplasm with a deeply staining reticulum. The arrangement of this reticulum would seem to correspond to that of the chromatin in the more advanced of Gardner's diffuse type; but Guilliermond describes this reticulum as consisting of an achromatic ground substance containing granules of chromatin. He thinks that it ought to be considered as a true chromatic network, and compares it with the 'appareil chromidial' described by zoologists in certain *Protozoa*. Division is amitotic. There is no spireme nor chromosomes, neither do the chromatin granules divide. The only suggestion of mitosis is that in some more highly developed species the threads of the network tend to become drawn out in parallel lines, though the anastomoses do not disappear and the reticulum is not broken.

The cortical layer does not constitute a true chromatophore, but contains the pigment in a state of solution. In the central body he observes (*a*) 'corpuscules métachromatiques' (Kohl's central granules); (*b*) 'corps nucléoliformes' of A. Meyer; and in the cortical cytoplasm cyanophycin granules.

Swellengrebel ('10), who has investigated only one species, *Calothrix fusca*, describes a central body consisting of an alveolar achromatic ground substance in which are embedded granules and filaments of chromatin. There is no very marked distinction between the groundwork of the central body and the surrounding cytoplasm, and sometimes the chromatin is diffused throughout the whole cell, so that the distinction between central body and cytoplasm seems to be lost. Division is amitotic.

Brown ('11) in a species of *Lyngbya* finds a nucleus which, in the stages between division, resembles the resting nucleus of higher plants, except for the absence of a limiting membrane. It contains a mesh of fine fibres, along which small granules are scattered; the fibres are embedded in a clear substance resembling nuclear sap.

Dobell ('12) in a paper on Spirochætes briefly mentions three species of Blue-green Algae, and states that there is a definite central body in these species.

It will be seen from the foregoing account that, with the exception of Fischer, all investigators since 1902 conclude that there is a definite nucleus. Since, however, none of them agree on the details of its structure even when examining the same species, the value of these conclusions seems somewhat questionable. The evidence in support of the theory of a nucleus which divides mitotically is certainly inconclusive. With regard to the structure of a nucleus which divides amitotically there seems to be more agreement. Judging from figures, the achromatic ground substance of Guilliermond and the chromatin of Gardner occupy similar positions in the cell. Both stain more deeply than the surrounding cytoplasm, yet Guilliermond believes the substance to be achromatic, while Gardner thinks it is chromatin. As stated before, it is difficult to believe that there is as much chromatin present as Gardner describes.

The results described above are based almost entirely on work done on filamentous forms. On comparing these with my own results, it appears that the structure of the cell as described by Guilliermond most nearly approaches the condition in the Chroococcaceae.

III. TECHNIQUE.

The method of fixation employed naturally depended on the material to be examined. Both wet and dry methods were used.

In the case of gelatinous forms like *Aphanothece* and *Gloeocapsa* wet smears were made on the slide and fixed and stained without drying. If fixed in bulk, the material was afterwards embedded and cut with a microtome, as the fixing reagents caused coagulation of the gelatinous material, making it difficult to spread out on the slide.

Forms like *Chroococcus* were usually fixed by allowing a drop of the material to almost dry on the slide, and then fixing and staining in the ordinary way. These preparations were usually fixed in absolute alcohol. There was very rarely evidence of shrinkage or of plasmolysis in specimens fixed in this way, except in the earlier preparations of *Chr. macrococcus*, probably because the films were never allowed to become absolutely dry, and also possibly because the tough envelope afforded some measure of protection to the cell. Wet methods were always used as a control. *Chroococcus turgidus* and *Chr. macrococcus* were found in such enormous quantities among the flocculent material from certain sphagnum bogs that it was possible to fix in bulk, and also to embed and cut microtome sections of the material.

The fixing reagents used were absolute alcohol, Flemming's weak chrom-osmium-acetic, alcoholic-picric, alcoholic sublimate, and 2½ per cent. formalin.

The thick outer covering of the cell proved a very great obstacle to satisfactory staining. Many stains, e. g. safranin and gentian violet, stained the membrane intensely, preventing examination of the cell contents, while others only penetrated with difficulty.

The stains that gave the best results were Loeffler's methylene blue, Delafield's haematoxylin, and iodine-green-fuchsin as used by Kohl ('03). This combination stain was very difficult to manipulate, as the exact proportion of the two solutions required seemed to vary with the condition of the material and the species under investigation. Repeated trials had to be made in each case, but when the right proportions were found the results were excellent. It was never found to be profitable to take back through the stains, if overstained with one or the other reagent. With Loeffler's methylene blue and Delafield's haematoxylin, preparations were overstained and the excess stain washed out.

Brilliant blue and Bismarck brown were used for the identification of cyanophycin granules and central granules respectively. Unfortunately, brilliant blue stains everything in the cell, and it was found best not to overstain to bring out the cyanophycin granules.

IV. THE PROTOPLAST OF CHROOCOCCUS TURGIDUS (Kütz.), Näg.

Although *Chroococcus macrococcus* is perhaps the most interesting member of the Chroococcaceae, it cannot be considered as typical of the group. Also it is impossible to discuss its systematic position without comparing its cytology with that of other members, so that it will be described separately at the end of this paper.

Chroococcus turgidus was examined in great detail, and as the structure of the protoplast in this plant appears to agree almost entirely with that of the remaining species except *Merismopedia elegans*, I propose to first describe fully *Chroococcus turgidus*, and then to deal with any facts of interest in connexion with other species.

Among the literature on the Cyanophyceae there only appear to be three papers mentioning *Chroococcus turgidus* as one of the species examined.

Palla ('93) makes special mention of it only with regard to the cyanophycin granules, the presence of which he was unable to demonstrate.

Nadson ('95) worked at several unicellular species, among which was *Chr. turgidus*. His conclusions were briefly as follows: The protoplast shows an alveolar structure in the sense of Bütschli, and the outer portion functions both as cytoplasm and chromatophore. The pigmentless inner portion is only distinguishable from the outer portion by the fact that it contains a more strongly staining substance, and that in this region the so-called chromatin granules (metachromatin granules) are exclusively or especially concentrated. There are three kind of granules in the cell-chromatin granules (metachromatin granules), reserve granules, and plasmatic microsomes. The latter are only found in *Merismopedia* and *Aphanocapsa*. They are small granules of plasmatic substance occurring at the junctions of the meshes of the alveolar protoplasm. The chromatin granules are especially, but not exclusively, concentrated in the central body, and are variously distributed in different species. The reserve granules are situated in the peripheral portion of the protoplasm. Division is usually direct.

Lastly, Chodat ('96) has a special paper on *Chroococcus turgidus*. He fails to find a definite nucleus. The central portion is quite often coloured in addition to the peripheral portion, and there can be no reason therefore to distinguish a special chromatophore. Mucilages, soluble amides, and cyanophycin can appear in the protoplast. The distribution of these substances varies exceedingly according to physical conditions, but they may accumulate in the central region as a network, and confer on it the peculiarities which give it an appearance analogous to a nucleus.

The present investigation extended over a period of three years, and comprised material collected from three different sources at different periods of the year. The variation in the distribution and staining capacity of the granules in the different collections, due probably to physical conditions, as suggested by Chodat, was well shown.

As a result of these investigations, it has been found impossible to divide the work into descriptions of ' cytoplasm ', ' nucleus ', and ' granules ', in the usual way. The best plan seems to be to state first the actual results obtained by staining reactions, and to discuss these results afterwards.

In the living cell one can only see that the protoplast is densely packed with rounded granules of a fairly uniform size. Some of these seem to be more refractive than others. Satisfactory results could not be obtained with living staining methods, on account of the tough thick membrane. In only one or two cases did there seem to be any indication of deeper staining in the central region.

In considering the results obtained from fixed material, observations which were made early in the work, before the structure of the protoplast was fully understood, will be mentioned briefly, since they appear to coincide with results mentioned by other authors. But it will be shown later that these results, in my own case at any rate, were only due to imperfect observations.

Staining with brilliant blue brought out deeply staining granules in the peripheral region of the cell, and with Bismarck brown granules in the central region. The number of granules reacting with Bismarck brown varied considerably. They were not confined entirely to the central region, but were chiefly concentrated there. These granules apparently correspond to the central granules of Kohl ('03), metachromatin granules of Guilliermond ('05), and volutin of Wager and Peniston ('10). These will be called metachromatin granules in the remainder of the paper. The granules reacting with brilliant blue correspond to the cyanophycin granules of Kohl and others.

Preparations stained with Delafield's haematoxylin showed the metachromatin granules stained a dark red colour, sometimes appearing almost black. In most cases these granules were almost uniform in size, but some material showed, in addition to these metachromatin granules, larger irregular granules which were apparently hollow. In the peripheral region could be seen granules very faintly stained of the same colour as the rest of the groundwork.

In addition to these granules, in several dividing cells, V-shaped threads, which might be compared to the spindle threads of a mitotic figure, were seen joining a few of the metachromatin granules.

Similar results were given with Loeffler's methylene blue. Granules in the central region stained dark blue, and in some cases there were apparently large irregular granules with a reddish tinge. The larger the granules, the redder they appear. Delicate threads could be seen here and there joining the granules. The groundwork and granules in the peripheral region were stained a very greenish blue.

With the iodine-green-fuchsin combination the cells appeared to stain uniformly red, with reddish granules distributed throughout the cell. These were not quite so conspicuous in the central region, but could be seen in sections. In certain parts of the cell, however, not isolated threads, but a definite reticulum of fine threads could be seen joining the granules, giving the appearance of a nuclear network. Finally, it was seen that the reticulum extended throughout the whole cell (Pl. XXXIII, Fig. 6). This necessitated the revision of the whole work and preparation of new slides. The following conclusions were only arrived at as a result of careful examination of hundreds of specimens.

The protoplast undoubtedly consists of a ground substance traversed by a very regular reticulum of delicate threads. This reticulum extends throughout the whole cell right up to the cell-wall (Fig. 6). At the junctions of the meshes of the reticulum, granules occur of fairly uniform size. These appear to correspond to Nadson's 'plasmatic microsomes', although he only described them for *Merismopedia* and *Aphanocapsa*. They are a constant feature of every species examined by the author, and the name 'plasmatic microsomes' will be used for the sake of convenience in the remainder of the paper in describing these granular thickenings of the reticulum.

The reticulum, with its granular thickenings at the nodal points, is seen most clearly in slides stained with iodine-green-fuchsin. With this stain, the ground substance, reticulum, and plasmatic microsomes stain red. In the central region, however, the microsomes are not so clearly marked, sometimes appearing to take on a bluish tinge. In good preparations the reticulum can be seen with other stains, but more often portions of it only can be seen in different parts of the cell. This reticulum has a very small mesh and can only be seen with a high magnification (Fig. 2).

The granules in the central region, which stained deeply with Delafield's haematoxylin, were found to be situated at the junctions of the meshes, the fact that the reticulum was imperfectly observed giving rise to the idea that these granules are sometimes joined by V-shaped threads. It is interesting to note in this connexion that Gardner ('06), in speaking of the α (central) granules, says: 'There often appears to be a connexion between two granules, but I presume that this is simply the deeply stained protoplasm, the colour of which is not washed out.' Since they occupy the same position, i. e. at the junctions of the network, the metachromatin granules must be identical with the plasmatic microsomes in the centre of the cell, or, in other words, the plasmatic microsomes in the central region have the characteristic reactions of metachromatin. They stain deeply with haematoxylin, Loeffler's methylene blue, &c., and take on a reddish blue tint with iodine-green-fuchsin.

The latter stain does not appear to differentiate very clearly the various granules in the cell. Even the large metachromatin granules can often only be recognized because they are refractive.

Kohl ('03) states that the metachromatin granules are not stained with this combination, and that chromatin should stain green blue to blue violet. I find that metachromatin is often uncoloured, but at times gives the colour reactions which Kohl ascribes to chromatin. In Fig. 6 the dark granules stained a distinct green blue, yet they correspond in size and number to the metachromatin granules in other cells of this collection. Many of them contained very little metachromatin. At the same time the author does not deny the possibility of these granules being true chromatin, especially considering the position of the chromatin in those forms which have a distinct central body.

The large irregular, apparently hollow, metachromatin granules were found to be simply large accumulations of metachromatin, or, perhaps more correctly, clusters of metachromatin granules filling up a whole mesh of the network (Fig. 2). The granules are very refractive and thus give a dark edge on focusing, causing the appearance of a darkly stained rim (Fig. 2). Possibly these correspond to the hollow granules of Kohl and others.

It is possible that these clusters of granules, taken in conjunction with the imperfectly observed network, may have given rise to the statement of Phillips ('04) that 'The chromatin is aggregated in hollow vesicles in the resting cell. These vesicles give out chromatin to the net spireme very much like the nucleolus of the higher plants, and they may represent it.' He also describes on division a network of threads connecting chromatin granules. It will be shown that the metachromatin does actually diffuse into the ground substance.

It has been stated before that the number of metachromatin granules in the central region varies with the condition of the material. When the number is large, the groundwork seems to stain equally in all parts, but if the number of deeply staining granules is comparatively few, the ground substance differs in parts in its capacity for staining. This is especially well shown in slides stained with methylene blue. Certain parts of the ground substance stain more deeply than the rest, but they do not stain nearly as deeply as the metachromatin granules. Plasmatic microsomes, faintly stained like the remainder of the ground substance, can be seen in the central region.

These deeply staining areas are generally circular or elliptical in shape, and sometimes appear to be surrounded by a colourless rim. There may be either numerous small areas (Fig. 4), a few larger areas, or in some cases the whole central region seems to stain.

The very regular shape of these areas seems to suggest that they are of the nature of vacuoles. If so, they are certainly not due to degeneration, for they are almost always found in dividing cells. Since the cells in which they occur always contain few metachromatin granules, while the majority of the cells in the collection are rich in metachromatin, it is probable that they are due to diffusion of the metachromatin when accumulation of reserve has reached its limit.

With iodine-green-fuchsin these areas remain colourless or slightly blue, which is another argument in favour of diffusion of metachromatin (Fig. 5).

The cyanophycin granules were also found to be situated at the nodal points of the reticulum in the peripheral region of the cell. They are not situated in the absolute periphery of the cell. There is always a region immediately within the cell-wall in which the majority of the plasmatic microsomes stain only with cytoplasmic stain (Fig. 3).

Division is brought about by the constriction of the cell into two approximately equal halves, caused by the ingrowth of the cell-wall. There is perhaps a tendency for the reticulum in the central region to be drawn out into more or less parallel lines, though the cross-connexions are not lost (Fig. 7). Again, the threads of the reticulum appear to stain more deeply in some cases in the central region, but this may only be accidental.

V. OTHER SPECIES OF THE CHROOCOCCACEAE.

All the remaining species examined are characterized by a reticulum with plasmatic microsomes. The size of the mesh of the reticulum is approximately the same in the different species, so that in the smaller ones there are necessarily fewer granules (Figs. 12, 13, and 14). The other species of *Chroococcus* offer no features of particular interest.

The species of *Gomphosphaeria*, *Coelosphaerium*, and *Dactylococcopsis* examined were too small for a clear interpretation of results, but do not appear to differ from the *Chroococcus* type.

Aphanothece prasina is interesting because the envelope in this species is easily penetrated by stains, and so intravital methods of staining were possible. The action of methylene blue on the living cell is as follows: the metachromatin granules in the central region take up the stain almost immediately, and in a short time have stained so intensely that the central region is marked off as a dark mass; one or two granules in the periphery also stain. In the peripheral region of the cell the reticulum can clearly be seen stained a little darker than the ground substance, and also the unstained cyanophycin granules (Fig. 14).

Gloeocapsa sp. is worthy of special mention, because Olive ('04) has definitely described mitosis in *Gloeocapsa*. He says: 'The segmented spireme in *Gloeocapsa* appears to consist of a simple more or less spiral thread, having about eight chromatin granules held by the linin and situated in the middle of the cell, with its long axis corresponding to the long axis of the cell'; and again, 'Finally, the most necessary requirement of mitosis is fulfilled in that a longitudinal fission of the chromosomes occurs. This is plainly evident in the case of *Gloeocapsa*, in which the simple spireme threads divide lengthwise, beginning at the two ends and splitting thence progressively to the middle of the thread.'

Now in *Gloeocapsa* sp. the reticulum connecting the plasmatic microsomes in the centre of the cell appears to stain more deeply than the remainder of the reticulum (Fig. 13). This certainly simulates a segmented spireme stage, and is of quite frequent occurrence in some preparations. It probably accounts for Olive's 'mitosis'. It may be a stage in the specialization of the central portion of the network, but is possibly an artifact due to slight shrinkage of the central region.

The genus *Merismopedia* differs rather strikingly from the species described up to the present, since one species, at any rate, has an 'incipient nucleus'[1] in the dividing stages. Two species only were available—*M. elegans* and *M. glauca*. The former occurred very sparingly in some plankton material from Sutton Park, which also contained *Chr. limneticus* in fairly large quantities. *M. elegans* was unfortunately overlooked when the slides were prepared, and this species was in consequence overstained in most preparations. One slide, however, stained with Delafield's haematoxylin showed a fairly large colony with cells in all stages of division. Some of these are shown in Figs. 8 and 9. The stain had been completely washed out, with the exception of a deeply staining portion which is evidently the 'nucleus'. The position of this in the dividing cells is shown in Fig. 8. The cells also contained a few colourless refractive granules, which were probably metachromatin granules.

The 'nucleus', before division takes place, is situated in the centre of the cell. Constriction of the cell takes place in two planes simultaneously, but the 'nucleus' appears to constrict, if not to actually divide, before it is reached by the constriction of the cell-wall (Fig. 9, *b*).

The structure of the 'nucleus' is somewhat similar to that of *Chr. macrococcus*, which will be described later. It is a restriction of deeply staining plasmatic microsomes to a small definite area in the network. There is some indication that this substance is distributed along the network

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[1] The term 'incipient nucleus' has been suggested by Professor G. S. West, in his forthcoming publication in the Cambridge University Press, 1914, to describe the nuclear structure found in the Myxophyceae, and the word 'nucleus' will be used in this sense in the remainder of the paper. The reasons for using the term are given in detail by Professor West, and it is therefore unnecessary to discuss them here.

after division, and collects again in the central region when the next division is about to occur.

In this way, diminution in size by repeated division is prevented, and the central position of the 'nucleus' accounted for, since it is obvious that the 'nucleus' as a whole cannot travel to the centre. This suggestion needs to be more definitely established, but is supported by the following facts: In a group of four cells which were separating after division and increasing in size, the 'nucleus' was not so deeply stained, while the network was fairly distinct, especially round the region of the 'nucleus' (Fig. 9, *a*), but in cells showing a deeply stained 'nucleus' the network and plasmatic microsomes could hardly be distinguished (Fig. 9, *b*). Again, in one cell the network and microsomes were quite distinct, but no 'nucleus' could be distinguished (Fig. 9, *c*).

Merismopedia glauca, on the other hand, appears to have no definite nuclear body. This species occurred in abundance in some collections from North Wales, and though most of the colonies showed cells in a state of active division, no trace of a nuclear restriction of the network could be seen.

The cells only appear to divide in one plane at a time by a gradual constriction of the cell-wall, as in *Chroococcus turgidus* (Fig. 10). Most of the cells contained a few very large metachromatin granules, which almost filled the cell and at first gave rise to the idea that the cells were reproducing by gonidia.

No indication of a single chromatophore or of numerous small chromatophores was seen in any of the types examined.

VI. CHROOCOCCUS MACROCOCCUS, Rabenhorst.

Chroococcus macrococcus is a member of the Chroococcaceae of frequent occurrence in sphagnum bogs. The cells are solitary or associated in groups of two or four. They are spherical or somewhat elliptical in shape, and have a thick lamellose sheath. The diameter of the cells, including the sheath, varied from 30 μ to 64 μ, and of the protoplast from 24 μ to 30 μ in the specimens examined, but they were mainly from cultures and rather less than the usual size. The colour of the protoplast is not constant, but varies from a golden brown to a dark brown. At times an orange tinge is given to the cells by the presence of several blood-red pigment spots.

(a) *Cell-wall.*

The cell-wall is lamellose and often very thick, the outer layers frequently splitting away very irregularly (Pl. XXXIV, Figs. 20 and 21). It appears to consist of alternate layers of two substances.

In cuprammonia it swells rapidly but is not dissolved in thirty minutes, while in concentrated sulphuric acid only the darker layers remain. The whole wall is soluble in caustic potash solution. On testing with picro-sulphuric acid the thin darker layers take on a yellow tinge, showing the presence of chitin. No colour reactions for cellulose could be obtained.

Generalizations as to the nature of the cell-wall in the Cyanophyceae seem to have been derived from studies of filamentous form only. Hegler ('01) states that the sheath is chitinous, the gelatinous coat pectose in nature. Kohl ('03) observes that in the Cyanophyceae the membrane and sheath consist largely of chitin with some cellulose, the gelatinous sheath of pectose. The amount of cellulose present is often not sufficient to give the usual colour reactions. Speaking of *Tolypothrix*, he says, p. 92, '. . . in der Scheide das Chitin, in der Membran der Cellulose prävaliert'. Massart ('02) says the walls are not of cellulose, and a gelatinous sheath is often present. These observations cannot include forms like *Chroococcus macrococcus*, in which the envelope consists of alternating layers of pectose and chitin.

The irregular splitting away of the outer coats is probably due to the fact that the chitinous layers are incapable of rapid expansion, while the pectose layers swell with ease. The ecdysis of the older layers is always more noticeable in cells which are actively dividing after a period of rest.

Culture experiments were made to ascertain the effect of various media on the development of the cell-wall. It was found that solid media produced a one-sided development of the cell-wall. Cultures on damp earth showed, in three weeks, a considerable increase in size of the cell-wall on the side nearest the earth (Fig. 22). This was shown also in agar cultures, but not to the same extent, development being much slower.

The most striking results were obtained by removing material which had been for eighteen months on an agar culture to a 2 per cent. KNO_3 solution. The cell-wall elongated rapidly on one side, and, as the cells divided, corrugated stalks were formed (Fig. 23).

(b) *Protoplast.*

In the living condition the protoplast is too densely coloured to show much structure. Often it is not evenly coloured in all parts, and so an appearance simulating a star-shaped chromatophore arises. Cells which are golden brown in colour are frequently crowded with large refractive globules. These appear to be yellow, but whether they contain pigment or not has not been determined. They are not soluble in alcohol and do not blacken with osmic acid. One or more blood-red pigment spots can also be seen.

The pigment is not easily dissolved out of the cells, so all the earlier preparations were cleared in a 2 per cent. solution of KOH before staining. There was too much shrinkage in these preparations for detailed investigation, but they were useful in showing that a portion of the protoplast is definitely marked off from the remainder by its staining properties, having an affinity for chromatin stains. These two parts of the protoplast will be distinguished as 'nucleus' and cytoplasm.

Sections cut from carefully prepared material showed that the 'nucleus' is reticulate in character. With Delafield's haematoxylin the whole 'nucleus' appears to stain deeply, but at the nodal points of the reticulum granules are present which stain more intensely than the body of the 'nucleus' (Figs. 16 and 17). With iodine-green-fuchsin the body of the 'nucleus' does not appear to stain at all, or else takes on a slight bluish tinge, but the granules stain an intense blue and so the 'nucleus' is sharply defined. The reticulum stains red, as does the cytoplasm.

In cutting the material, sections thinner than 10 μ were found to be unsatisfactory. Unfortunately, this means that the section does not necessarily pass through the 'nucleus', and, in fact, only two sections through the 'nucleus' were found. These are shown in Figs. 16 and 17, and prove beyond doubt that the portion of the 'nucleus' which reacts with chromatin stains is confined to the surface. Traces of a network could be seen inside the 'nucleus', but this may have been the inside view of the surface reticulum.

The author is inclined to think that the interior of the 'nucleus' consists of a sap vacuole, but a thin slice through the 'nucleus' which would show this clearly has not been obtained. This suggestion is supported by the fact that the 'nucleus' frequently appears flattened and in a state of collapse.

At first sight the cytoplasm appears to be coarsely reticulate, except in the neighbourhood of the 'nucleus', but closer investigation shows that a fine reticulum is present which passes insensibly into the reticulum of the nucleus. This certainly corresponds to the reticulum present throughout the ground substance of the other members of the Chroococcaceae, but its definite character is disappearing.

The coarsely reticulate appearance is due to the presence of cell-sap vacuoles, which break up the ground substance. The amount of vacuolization varies, being much more marked in the older cells; the region immediately surrounding the 'nucleus' is the last to be broken up. The effect of this vacuolization is to destroy the regularity of the finer reticulum, which becomes very much distorted, and in parts can hardly be distinguished (Fig. 15). The thickenings at the nodal points, which are characteristic of the network in other types, have almost disappeared, but traces of them still remain, especially near the 'nucleus' (Fig. 15). Metachromatin granules are scattered through the cytoplasm.

In *Chroococcus macrococcus* there is evidently a distinct morphological differentiation in the protoplast. The fine reticulum, characteristic of the whole protoplast in simpler types, has been restricted (in its definite character) to one portion of the cell, and associated with it is a very definite accumulation of chromatin at the nodal points. In the remainder of the protoplast this fine reticulum is disappearing, and a coarser one, characteristic of higher plants, is arising.

This distinction appears to me to be sufficient to warrant the separation of the protoplast into 'cytoplasm' and 'nucleus'. Division, although it takes place by constriction of the cell, is initiated by the 'nucleus'. This divides and separates, and then the constriction in the cell-wall appears (Fig. 19). So that there is a physiological differentiation as well as a morphological one.

The 'nucleus' is certainly primitive. It divides by constriction, and there is no evidence whatever of mitosis (Fig. 18). It seems to permanently remain in a condition resembling the resting stage of the nucleus in higher types.

Repeated division of the cell seems to diminish the comparative size of the 'nucleus', for in the older cells the 'nucleus' is always small. Possibly reproduction takes place in some other way, as it seems essential that rejuvenescence of the 'nucleus' must take place sooner or later. No trace of fragmentation of the nucleus, as described by Gardner ('06) in *Dermocarpa*, has been seen in *Chroococcus macrococcus*, although in examining living material I have occasionally observed groups of eight daughter-cells free within the old cell-wall.

(c) *Systematic Position.*

Though the protoplast of the Alga described above is more highly developed than that of other Chroococcaceae, the connexion between them is clearly shown, and there is no reason therefore to separate it from the group on this account. It is undoubtedly a member of the Cyanophyceae, and agrees in all essentials with *Chroococcus macrococcus*, Rabenh.

Hassall (45) describes an Alga under the name of *Haematococcus insignis*, which he suggests should be placed in a separate genus, '*Urococcus*', characterized by transversely corrugated prolongations. The cells are described as being spherical and blood-red, with numerous vesicles and ringed prolongations. It is not unlikely that this Alga is identical with *Chroococcus macrococcus*. It has been shown that the *Chr. macrococcus* is capable of developing a ringed prolongation on being removed from a solid medium to a liquid one. The two algae are identical in size, but though *Chr. macrococcus* often contains several large blood-red pigment spots, it has never been described as completely blood-red in colour. This may possibly result from a prolonged resting condition.

Kützing ('49) refers to *Haematococcus insignis* as *Urococcus insignis*, and states that the stalk is short or absent, and the 'nucleus' blood-red. This is, of course, still more in agreement with *Chr. macrococcus*.

It is also possible that *Chr. macrococcus* is identical with a *Chroothece* described by Hansgirg ('84). Hansgirg's description of *Chroothece Richterianum* is in exact agreement with the characters of *Chr. macrococcus*, except as regards colour. He finds in the cell an orange-coloured, *rarely blue-green* 'chromatophore' with ray-like processes spreading into the periphery of the cytoplasm. The apparent star-shaped orange 'chromatophore' can often be seen in *Chr. macrococcus*, but it has never been described as blue-green. He finds in the older cells of *Chroothece* a one-sided development of the cell-wall on damp earth, but not in the younger cells, nor in the water forms. As the colour of *Chr. macrococcus* is very variable, it seems not unlikely that the two species are the same.

VII. Summary.

It is not surprising that in many of the Chroococcaceae there should be no nucleus, not even a primitive one. In the course of evolution there must have been a stage in which there was no nucleus, or in which the cell was all nucleus, depending upon the point of view, and we should expect to find this condition in some of the more primitive unicells. The Cyanophyceae are undoubtedly a very primitive and ancient group, and it is not unlikely that some of the early stages in the evolution of the nucleus may be shown in these plants. The type represented by *Chroococcus turgidus* may be a very early stage, in which the difference in function between the inner and outer region of the cell has not yet produced any morphological differentiation.

There is in this species a protoplast consisting of a matrix or ground substance traversed by a reticulum of delicate threads, and at the junctions of these threads small granular thickenings occur. There is no difference in the size of the mesh of the reticulum in any part of the cell. Thus there is no differentiation in form. But there is a difference in composition. In the portion immediately beneath the cell-wall the plasmatic microsomes do not stain deeply with any stains except cytoplasmic ones, and seem to be of the same nature as the reticulum. Further inwards, the plasmatic microsomes appear to contain accumulations of material, and stain deeply with brilliant blue, and in the central region the microsomes contain accumulations of metachromatin, which, although it is not true chromatin, is undoubtedly allied to it. The close connexion between the metachromatin granules and chromatin has probably been the cause of much confusion in the earlier papers on the Cyanophyceae. They react with most chromatin stains, though not all,[1] and Macallum ('99) has shown that they contain

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[1] Iodine-green-fuchsin, which invariably stains the nuclei intensely blue and the cytoplasm red in other forms which may be found on the same slide, such as Desmids and Protozoa, never seems to give more than a purplish tint to the central granules.

masked iron and organic phosphorus, and are therefore nuclein compounds, but states that they are not true chromatin.

Fischer denies the nuclein nature of these granules, and says they are simply accumulations of a carbohydrate reserve to which he gives the name 'anabaenin'. It is quite possible that in some forms, and at certain times in other species, the granules in the central region are entirely carbohydrate in composition. The plasmatic microsomes served probably first of all as centres for the accumulation of the excess of food-material elaborated by the pigment in the peripheral region. This would naturally be in the first place of a carbohydrate nature. But as evolution proceeded we might expect that the central region would become a centre for further metabolic activity, and so gradually nuclein compounds would be found as reserve, and finally true chromatin would be evolved. It would be somewhere about this stage that such a form as *Chroococcus turgidus* arose. A few of the plasmatic microsomes in the central region may consist of true chromatin, but this has not been definitely proved.

The difference in staining capacity shown by various parts of the ground substance at certain times has still to be considered. These deeply staining areas correspond in arrangement sometimes to the chromatin shown by Gardner ('06) in his Figs. 4, 5, 9, but the arrangement is not constant; it varies from numerous small areas to one or two large ones, as though the smaller areas gradually merged into each other. They do not stain nearly as intensely as chromatin should, and they are not a constant feature, so that there is no reason to regard them as nuclear structures.

It would appear that when the accumulation of reserve material reaches its maximum the central granules begin to diffuse into the ground substance. A similar diffusion of the central granules into the ground substance has been mentioned by Gardner,[1] and diffusion of both volutin and chromatin into the cytoplasm has been described by Wager and Peniston[2] in the Yeast-plant.

The majority of the cells showing this diffusion are in a state of active division, and so the dissolved material would gradually be utilized in growth. Then, possibly, a resting period occurs in which nuclein material is again stored up in the central granules. When this accumulation reaches its maximum, active division again sets in, and so eventually the formation

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[1] These granules (α granules) disappear before the spore reaches maturity. 'They do not disappear at once, but become gradually smaller and finally disappear entirely.' The α granules probably give up their material either to form chromatin or to form the β granules, and the former is more likely, since they are so closely united to the chromatin.

[2] 'It is possible that in the yeast-cell there is a constant interchange of chromatin between nucleus and cytoplasm.' Speaking of volutin granules, he states: 'there can be no doubt that they become dissolved in the cytoplasm, which becomes intensely stained with methylene blue just at this stage.'

of chromatin becomes connected with the part division and is confined, in highly developed cells, to that part of the protoplast which is connected with division, i. e. the nucleus.

If this suggestion as to the lines on which nuclear structures have evolved be true, then a stage should exist in which part of the reticulum is definitely marked off and true chromatin should be present, also this portion of the reticulum should have a definite relation to division of the cell.

This stage is actually found in *Chroococcus macrococcus* (Fig. 15). In this species a small portion of the reticulum always differs very markedly from the rest of the protoplast. The plasmatic microsomes contain true chromatin, and this portion of the reticulum has the power of dividing before the cell-wall constricts.

The peripheral portion has also begun to change in structure. The fine reticulum can still be seen in places, especially in the neighbourhood of the nucleus, but the plasmatic microsomes have almost disappeared, and the reticulum is very much broken up and distorted by cell-sap vacuoles.

The nucleus in *Chr. macrococcus* seems to remain permanently in a condition which resembles the resting nucleus of higher plants, except that a nucleolus and nuclear membrane are not present.

The exceedingly small and delicate reticulum found in these unicellular forms has not been described for filamentous species. Probably it does not occur, though this needs verification.

Vacuolization may have entirely crushed out this fine reticulum in the cytoplasm, giving rise to a coarser one, and at the same time the reticulum of the nucleus may have become irregular and thickened, giving rise to the achromatic reticulum of Guilliermond ('05) with chromatin granules embedded in it, or to something approaching the highest modification of Gardner's diffuse type of nucleus, in which 'differentiation seems to be proceeding towards the spireme formation found in the dividing nucleus of the higher plants'. It is not unlikely that the 'spireme' condition of the nucleus is a permanent one in some low types. This is, however, merely a suggestion, and the filamentous forms have yet to be investigated from this point of view.

VIII. CONCLUSIONS.

1. In the Chroococcaceae a highly specialized nucleus of the type found in higher plants does not occur.

2. There is a gradual transition in the structure of the cell from an almost undifferentiated condition in the lower types to a somewhat specialized one, of which *Chroococcus macrococcus* represents the highest type examined, and *Merismopedia elegans* an intermediate stage.

3. The protoplast consists of a ground substance traversed by a reticulum of delicate threads, with thickenings at the nodal points. These are

'plasmatic microsomes' and serve as centres for accumulation of reserve materials elaborated by the pigmented parts of the protoplast. The nature of the accumulation varies in the different regions of the cell.

4. In the majority of species examined by the author there is no definite demarcation of central and peripheral regions, but roughly speaking the microsomes in the central region accumulate metachromatin and correspond to the 'Centralkörner' of Kohl, and in the peripheral region accumulate cyanophycin and correspond to cyanophycin granules.

5. *Chroococcus turgidus* may be taken as an example of this type; though it is possible that it may represent a slightly higher one, since a few of the plasmatic microsomes in the central region occasionally give a true chromatin reaction. There is also in this species a region, just within the cell-wall, in which the plasmatic microsomes are undifferentiated, reacting only with cytoplasmic stains.

6. In *Chroococcus turgidus* the number of metachromatin granules varies greatly in different specimens. If the accumulation of metachromatin is excessive, it appears to diffuse into the ground substance, and a period of active division sets in. At this time the majority of the microsomes in the central region react only with cytoplasmic stains, but one or two very large metachromatin granules can usually be seen.

7. Division takes place in this type by the constriction of the cell into two approximately equal halves, caused by the ingrowing cell-wall. Occasionally the reticulum in the central region seems to stain a little more intensely in dividing cells, and there is a tendency for the threads to become drawn out in parallel lines, though the cross-connexions are not lost.

8. Metachromatin may represent a stage in the formation of chromatin.

9. In *Gloeocapsa* sp., many of the cells show a deeper staining of the network in the central region simulating the 'spireme' stage described by Olive ('05). This may be a more advanced stage in specialization of the central region, but it is probably an artifact.

10. In *Merismopedia elegans*, which represents a higher type, there is a definite 'central body' or 'nucleus' at the time of division. This is not of the same type as the nucleus of higher plants, but is simply an accumulation of chromatin, or some substance allied to it, at the nodal points of a small definite area in the centre of the cell. There is some evidence to show that this 'nucleus' gradually distributes itself along the reticulum after division, to appear again in the centre of the cell prior to the next division. Division of the 'nucleus' takes place before it is reached by the ingrowing cell-wall.

11. *Chroococcus macrococcus* represents the highest type found. Here there is a definite 'nucleus' and cytoplasm. Only the peripheral portion of the 'nucleus' stains deeply with chromatin stains, and contains a fine reticulum with chromatin at the nodal points. The interior of the 'nucleus' is

probably a sap vacuole. The cytoplasm is simply the ground substance of the former types broken up by cell-sap vacuoles, which give it a coarsely reticulate appearance. The fine reticulum is present but is very much distorted, except in the neighbourhood of the 'nucleus'. The plasmatic microsomes are very small and indistinct.

12. It is suggested that evolution of nucleus and cytoplasm has taken place along the following lines: The excess of food-material elaborated by the pigment was first stored in the plasmatic microsomes as a carbohydrate—cyanophycin. As more and more material was elaborated, the reserve in the central region became more complex, and the proteid metachromatin granules were formed. In time, the accumulation of nucleo-protein became restricted to a very limited area in the cell, so as to ensure its equal distribution on division, and this restriction only occurred on division, as in *Merismopedia elegans.* In this way, part of the cell became physiologically and morphologically separated on account of its function in connexion with division. This area may be called the 'nucleus'. At a later stage the 'nucleus' became stable and was always present, as in *Chroococcus macrococcus.* The ground substance also altered in character, forming a definite cytoplasm as described above.

In conclusion, I wish to express my thanks to Prof. G. S. West for suggesting the investigation, for continual help and encouragement in the work, and for providing the greater part of the material examined.

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LITERATURE CITED.

BROWN, W. H. ('11): Cell-division in *Lyngbya.* Bot. Gaz., vol. li, No. 5, 1911.

CHODAT, R. ('96): *Chroococcus turgidus.* La Nuova Notarisia, 1896, p. 98.

DOBELL, C. ('12): Researches on the Spirochaetes and related Organisms. Archiv für Protistenkunde, Bd. xxvi, no. 2, 1912, p. 117.

FISCHER, A. ('05): Die Zelle der Cyanophyceen. Botan. Zeitung, Bd. lxiii, 1905, p. 51.

GARDNER, N. L. ('06): Cytological Studies in Cyanophyceae. Univ. of California Publications, Botany, vol. ii, no. 12, 1906.

GUILLIERMOND, A. ('05): Contributions à l'étude cytologique des Cyanophycées. Acad. des Sci., Comptes rendus, t. cxli, 1905, p. 427.

HANSGIRG, A. ('88): Ueber *Bacillus muralis,* Tomaschek, nebst Beiträgen zur Kenntniss der Gallertbildungen einiger Spaltalgen. Bot. Centralbl., Bd. xxxv, p. 54.

HASSALL, A. H. ('45): British Freshwater Algae. I, 1845, p. 324.

HEGLER, R. ('01): Untersuchungen über die Organisation der Phycochromaceenzelle. Pringsh. Jahrb. f. wiss. Bot., Bd. xxxvi, 1901.

KÜTZING, F. T. ('49): Species Algarum. 1849, p. 207.

Kohl, F. G. ('03): Ueber die Organisation und Physiologie der Cyanophyceenzelle und die mitotische Teilung ihres Kernes. Jena, 1903.

Macallum ('98–'99): On the Cytology of the Non-nucleated Organisms. Trans. Canadian Institute, vol. vi, 1898–9.

Massart, J. ('02): In Recueil de l'Inst. Bot. Univ. de Bruxelles, t. v, 1902, p. 251.

Nadson, G. ('95): Ueber den Bau des Cyanophyceen-Protoplastes. Scripta bot. horto Univ. Imp. Petropolit., vol. iv, 1895.

Olive, E. W. ('05): Mitotic Division of the Nuclei of the Cyanophyceae. Beih. Bot. Centralbl., vol. xviii, 1905.

Palla, E. ('93): Beitrag zur Kenntnis des Baues des Cyanophyceen-Protoplastes. Jahrb. f. wiss. Bot., Bd. xxv, 1893.

Phillips, O. P. ('04): A Comparative Study of the Cytology and Movements of the Cyanophyceae. Contributions from the Botanical Laboratory of the University of Pennsylvania, Phil., vol. ii, no. 3, 1904.

Rabenhorst, L. ('65): Flora Europaea Algarum, Sectio ii, p. 33.

Swellengrebel, N. H. ('10): On the Cytology of *Calothrix fusca*. Q.J.M.S., new series, vol. liv, 1910, p. 623.

Wager, H., and Peniston, A. ('10): Cytological Observations on the Yeast Plant. Ann. Bot., vol. xxiv, No. 93, 1910.

Zacharias, E. ('07): Ueber die neuere Cyanophyceen-Litteratur. Botan. Zeitung, Bd. lxv, 1907.

EXPLANATION OF PLATES XXXIII AND XXXIV.

Illustrating Miss Acton's paper on *Chroococcaceae*.

All figures × 2,500 approximately, unless otherwise stated.

PLATE XXXIII.

Fig. 1. *Chroococcus turgidus*: A, actual appearance of cell; B, semi-diagrammatic optical section showing protoplasmic reticulum. *p.m.* = plasmatic microsomes, *m.* = metachromatic granules. Stained with Loeffler's methylene blue after fixing with absolute alcohol.

Fig. 2. Portion of reticulum, showing accumulations of metachromatin at nodal points. Stained with Delafeld's haematoxylin after fixing with Flemming's solution.

Fig. 3. *Chroococcus* [? *Chr. minutus* (Kütz.), Näg.] stained with brilliant blue after fixing with absolute alcohol. *c*, cyanophycin granules.

Fig. 4. *Chroococcus turgidus*. Cells which contain few metachromatin granules, showing differential staining in the ground substance; A, actual appearance; B, semi-diagrammatic optical section. Stained with Loeffler's methylene blue after fixing with absolute alcohol.

Fig. 5. As Fig. 4. Stained with iodine-green-fuchsin after fixing with absolute alcohol. The unstained portions of the ground substance correspond to the deeply stained portions in Fig. 4.

Fig. 6. Section showing that reticulum extends throughout the cell. The darker granules are metachromatic granules, stained with iodine-green-fuchsin after fixing with formalin.

Fig. 7. Cell showing slight elongation of network in central region which sometimes occurs in dividing cells. Stained with iodine-green-fuchsin after fixing with absolute alcohol.

Fig. 8. *Merismopedia elegans*, × 1,500; a colony showing the position of the 'nucleus' in dividing cells; *n* = nucleus. Stained with Delafield's haematoxylin after formalin.

Fig. 9. A, Cells which are enlarging and separating after division; the 'nucleus' is beginning to disappear. B, Cells about to divide; in one cell the 'nucleus' has actually divided, though the cell is only just beginning to constrict. The reticulum is very indistinct and plasmatic microsomes are small. C, Cell in which no 'nucleus' could be seen. The network and plasmatic microsomes are very distinct.

Fig. 10. *Merismopedia glauca*, × 1,500; dividing cells—no 'nucleus' can be distinguished.

Fig. 11. Dividing cells, showing large metachromatin granules and distinct network.

Fig. 12. *Gomphosphaeria lacustris*, stained with iodine-green-fuchsin after formalin.

Fig. 13. *Gloeocapsa* sp., stained with iodine-green-fuchsin after Flemming's solution. The apparent thickening of the network in the central region is probably only an artifact due to slight contraction.

Fig. 14. *Aphanothece prasina*: A, stained living with methylene blue, showing metachromatin granules and network in central region, cyanophycin granules in peripheral region; B and C, stained with iodine-green-fuchsin after fixing with chrom-acetic.

PLATE XXXIV.

Fig. 15. × 2,500. *Chroococcus macrococcus*. Section through protoplast, not cutting the nucleus. *v*, vacuole; *m*, metachromatin granules; *n*, 'nucleus'. Stained iodine-green-fuchsin after fixation in weak chrom-acetic.

Figs. 16 and 17. × 2,500. Sections through 'nuclei' stained with Delafield's haematoxylin. The interior of the nucleus is seen to be unstained and free from granules.

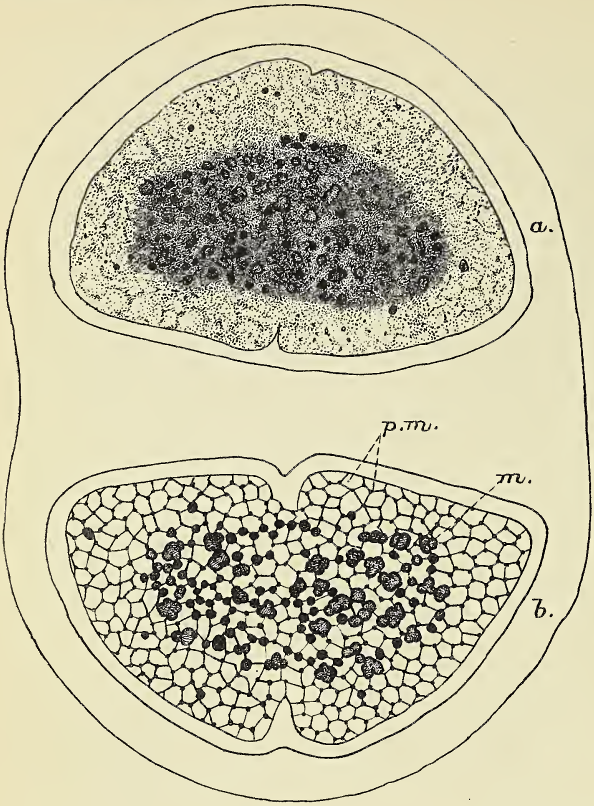
Fig. 18. × 2,500. Nucleus stained with Delafield's haematoxylin, showing division by constriction. The nuclear reticulum is still quite distinct.

Fig. 19. × 1,450. Cells showing nuclei dividing previous to constriction of cell-wall. Stained with Delafield's haematoxylin after absolute alcohol.

Figs. 20 and 21. × 540. Illustrating the splitting away of the outer envelope of the cell in the normal condition.

Fig. 22. × 540. From a three-weeks-old culture on damp earth. The cell-wall shows a one-sided development.

Fig. 23. × 540. From an eighteen-months-old culture on agar after three weeks in a $2\frac{1}{2}$ per cent. KNO_3 solution. A distinctly corrugated stalk is formed.



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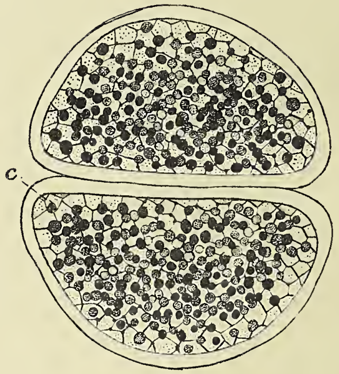

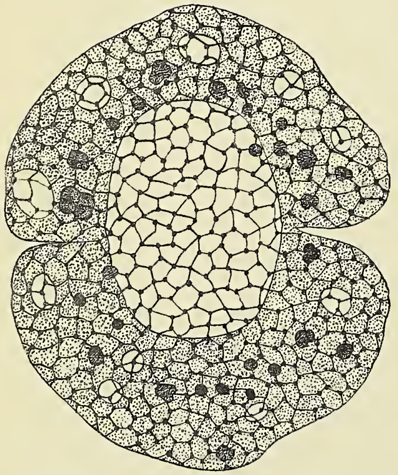

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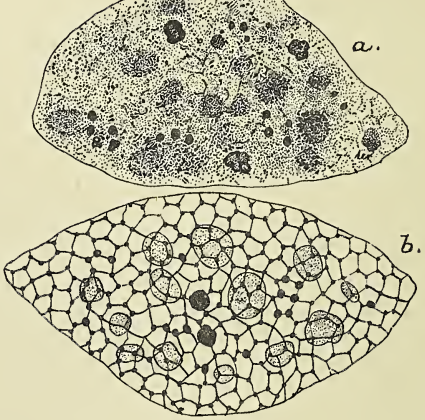

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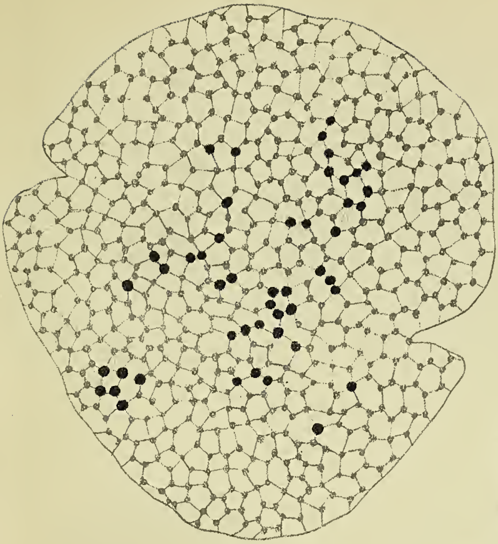
CYTOLOGY OF THE CHROOCOCCACEÆ.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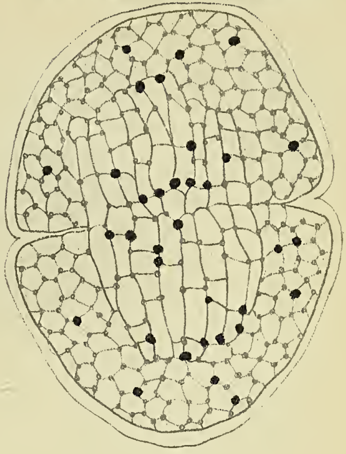
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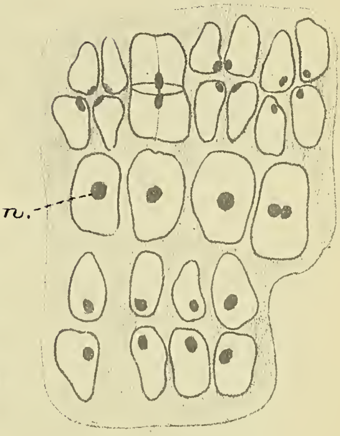

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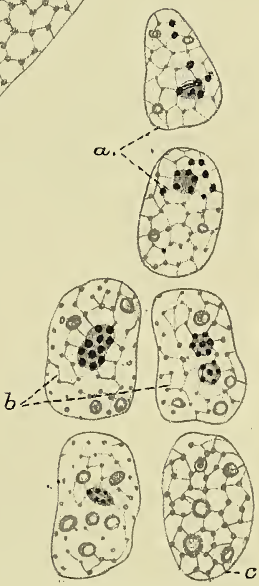

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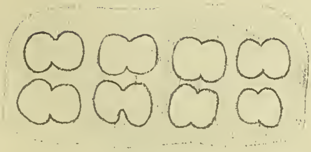
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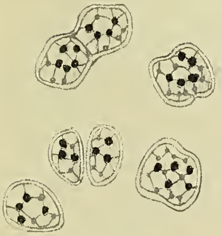
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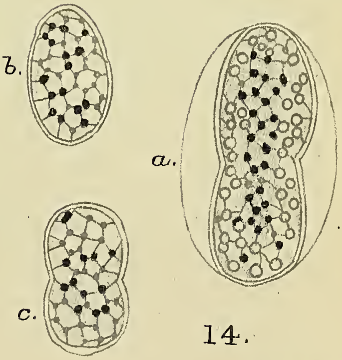

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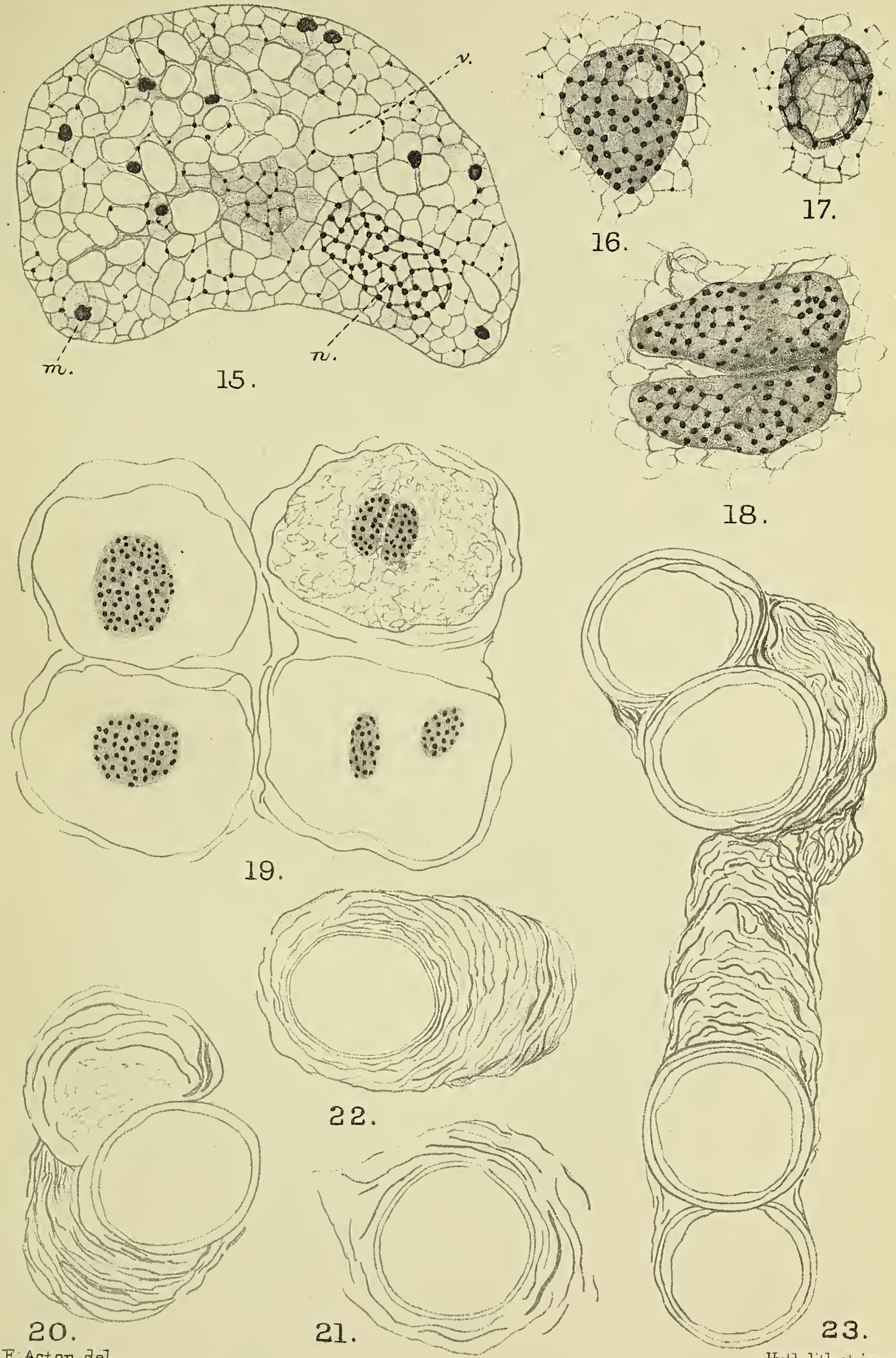

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CYTOLOGY OF THE CHROOCOCCACEÆ.

Cytological Studies of the Zygospores of Sporodinia grandis

BY

MARY LUCILLE KEENE.

With Plates XXXV and XXXVI.

THE study of the nuclear processes which take place when multinucleate gametes fuse, constitutes a problem of very great interest. In general, in sexual reproduction of both plants and animals, the sex cells which fuse are uninucleate, but there are numerous exceptions to this to be found in the Plant Kingdom, especially among the Fungi. In the Ascomycetes as *Pyronema,* in the Oomycetes as *Albugo,* in the Zygomycetes as *Sporodinia*, we find that the sexual fusion regularly takes place between gametes, each of which contains several nuclei. While most of these forms have been investigated more or less thoroughly, there is no final agreement with reference to the essential features of the nuclear phenomena.

Harper (21) has carefully investigated *Pyronema*, and has described the fusion of multinucleate gametes, the nuclei of which fuse in pairs. Claussen (3), in recent studies on the same form, also described a fusion of multinucleate gametes, but was not able to determine the fusion of the nuclei in pairs. He considers the nuclear fusions which occur later in the young ascus as the sexual act.

In the Oomycetes, there has been considerable evidence brought forward as to the nature of the fusions of the sex cells. Stevens (28) describes a fusion of multinucleate gametes in *Albugo Bliti*, and, later (30), in *Albugo Ipomoeae-panduranae.* He also describes the development of the oosphere of *Albugo Tragopogonis,* in which the supernumerary nuclei are said to disorganize, a uninucleate condition resulting. A single male nucleus then fuses with the egg nucleus. The fusion nucleus undergoes many divisions, so that the winter oospore is multinucleate.

Davis in 1900 (14), and Stevens in 1901 (29), both find that the mature oosphere of *Albugo candida* is uninucleate. One male nucleus is said to fuse with the nucleus of the oosphere, and a uninucleate oospore is produced.

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Both admit, however, that multiple fusions of nuclei may occur even in *Albugo candida.*

Davis (16) has studied *Vaucheria geminata*, a coenocytic Alga, and has found that the oosphere is multinucleate when first formed, but, by a process of rapid and complete degeneration of all the nuclei except one, the egg at the time of fertilization contains only one functional nucleus.

Although there has been a great deal of investigation in the Zygomycetes, a group of Fungi which furnishes very favourable material for the study of the fusion of multinucleate gametes, the results are contradictory. All investigators have encountered structures that they have described under different names and to which they have attributed various functions. All have admitted that the interpretation of these structures was difficult. Therefore it has seemed advisable to make an attempt to substantiate the results of one or the other group of workers, and to explain, if possible, the nature and function of the various structures found in developing zygospores.

As early as 1820, Ehrenberg (18) described the process of conjugation in a form that he termed *Syzygites*, now known as *Sporodinia,* one of the Mucorineae. In 1864 de Bary described, in much greater detail, the same form. According to de Bary, 'the true fruiting branches are branched dichotomously repeatedly. On every two of the hyphae are formed pear-shaped enlargements. The suspensors grow toward each other, meet, the ends are cut off by partitions, and in this manner the gametes are formed. The membranes on the contiguous surfaces are dissolved away, the contents fuse, and so results the zygospore. The same is encircled by three membranes, the outermost of which is dark brown, warty, and cutinized, formed by the membranes of the copulating gametes. The two inner ones belong to the zygospore itself' (17).

Through the work of Brefeld (2) in 1872, and also that of Van Tieghem and Le Monnier (31), the Mucorineae are well known from a morphological point of view. These authors, however, have gone no further into the internal structure of these forms than to say that the protoplasm contains many nuclei.

In 1894–5 Dangeard and Léger (11), using the more modern methods of histology, undertook to work out the internal processes of the developing zygospores of *Sporodinia grandis.* They describe the young zygospore as a cask-like structure, with an outer cutinized envelope, which has characteristic papilla-like projections. Internally, the protoplasm is dense and homogeneous, containing many nuclei. The separating walls are dissolved, leaving the gametes in free communication. The lateral walls show a central brown spot and a number of small openings which favour interchange with the suspensors which contain nuclei and protoplasm. The protoplasm is reticulate with meshes towards the surface. Nuclei of two kinds appear, some of which are two or three times the size of the ordinary nuclei and have a dense, spherical, red-staining nucleolus. The smaller nuclei are finally reduced to nucleoli that stain red.

At an older stage, when oil begins to accumulate in the centre of the zygospore, the nuclei are still evident in the protoplasm. No difference in the size of the nuclei is noted at this time. In the mature zygospore, the oil globule is very large and the protoplasm has become reduced to a parietal layer, dense at certain points and vacuolate over the rest. Mucorine crystals are present at this stage.

Maurice Léger (22) made a more extensive study of this form. He described many of the structures that more recent study has brought out. He describes the pyriform swellings that fuse as similar, and emphasizes their absolute isogamous nature. His account varies little from that given by himself and Dangeard, up to the point where the zygospore is formed. He lays some stress on the formation of a peculiar structure in the walls adjacent to the suspensors, and interprets it as a canal. He also describes the disintegration of some nuclei. The protoplasm becomes vacuolate, and appears as a sponge filled with oil. The nuclei disappear, and at the same time certain deep-staining bodies, difficult of interpretation, become evident. They arise as small spherical bodies, towards the extremities of the zygospore. Léger is uncertain regarding their origin, but concludes that they arise either from the large nuclei or from special portions of the protoplasm. He was unable to detect any details of structure. These spheres are isolated at first, but ultimately arrange themselves in a spherical zone around an oil mass. He describes these masses as varying in size in the different zygospores, but as being uniform within the same one. These small spheres Léger calls 'sphères embryogènes', and the larger ones resulting from their fusion he calls the 'sphères embryonnaires'. He justifies the terms on the basis of his interpretation of their ultimate function, namely, that they act directly in the reproduction of the plant when the zygospores germinate.

In germination, the spheres enlarge and fuse, although he can discern no definite organization in them. Characteristic nuclei appear again; they divide and pass out into the hyphae. The oil is absorbed little by little.

In summarizing, Léger emphasizes the importance of the 'embryo spheres', saying that these bodies constitute a sort of embryo which develops into a new plant at the time of germination.

In 1901 Gruber (20) undertook the further investigation of this form and failed to find the structures described by Léger. He was unable to observe either fusion or disintegration of the nuclei. He suggests that we have to do with processes similar to those displayed in the Oomycetes, but gives no evidence in support of this suggestion.

Dangeard (10), in a short paper in 1906, describes nuclear fusions in the zygospores of *Mucor fragilis* and *Sporodinia grandis*, and points out that copulation occurs progressively. The nuclear fusions present the ordinary conditions of fusion of the karyoplasm and, ultimately, of the nucleoli. This results in the presence in the zygospore of a large number of nuclei doubled by copulation. Dangeard here points out the fact that the two fusing structures are not to be regarded as gametes but as gametangia, being 'sexual energids' further undifferentiated morphologically.

In another paper in 1906, Dangeard (9) takes up the structure and development of the Mucorineae. His chief studies were made on the form *Mucor fragilis* because it contains a small number of nuclei. It was only after describing the nuclear fusions in *Mucor fragilis* that Dangeard was able to return to *Sporodinia* and describe the fusions there. The account varies but little from that given in previous accounts up to the place where nuclear fusions occur. Dangeard describes the nuclear fusions as occurring after the endospore has been established, and at the time when the protoplasmic portions become vacuolated.

In the mature zygospore, the vacuolate structure gives way to an alveolar structure, in which is contained the oil and other reserve substances. The zygospores contain chromatic bodies which are made up of a dense homogeneous substance which stains red with the triple stain. These chromatic bodies or corpuscles are usually very large, and two or three appear in a section. Their position is not constant, and they vary in size within the same zygospore. Their mass is without structure, and they are contained on the inside of a vacuole. Dangeard likens them to the coenocentra described by Stevens in *Albugo*. He describes similar structures as occurring in the suspensors, and also in the mycelium. In the older zygospores, Dangeard suggests that the large corpuscles numbering 10–20 are merely mucorine crystals which have undergone reconstruction. They have increased in volume and lost their crystalline nature. Dangeard then goes into an account of the formation of the membranes of the zygospore, which does not concern the immediate problem in hand.

Lendner (23), in 1908, recognizing the incompleteness of the reports on the fecundation of the Mucors, undertook, in connexion with some other work on their morphology, to work out the cytology of *Sporodinia*. His observations on the earlier stages agree with the majority of the previous workers. He emphasizes, for the first time, the activity of one of the gametes, and describes its penetration into the other. 'In some rare cases, it has been possible to observe a rather large mass which I have considered as one of the fusing nuclei, although the density of the protoplasm at this time is very great, rendering observation of the nuclei very difficult. . . . The middle membrane having disappeared, the protoplasm remains dense along the median line, and the small nuclei are still very numerous. It is not rare to encounter at this time two nuclei symmetrically placed. The part played by the nuclei which accumulate at the periphery is to preside at the formation of the membrane. I have not seen any disintegration as indicated by Dangeard. Later the two large nuclei come together, touch and unite to form a single mass occupying the exact centre. Finally, in the more advanced stages, one finds only a single body from which the protoplasm radiates.'

Lendner carries his investigations only to partly mature zygospores. Furthermore, he uses but a single stain, anilin-safranin, which is not sufficient to differentiate the various structures found within the zygospore.

In 1911 Moreau (26) investigated several forms: *Sporodinia grandis*, a *Mucor*, and *Zygorynchus*. In *Sporodinia* and *Mucor* he merely corroborates the work of Dangeard. In *Zygorynchus* he went further into the details of development and found varying structures. All the nuclei in this form are said to disintegrate, except four, which fuse in pairs, leaving two nuclei in the mature zygospore.

In January, 1912, Miss McCormick (25) published a preliminary note on some investigations on the closely related form, *Rhizopus nigricans*. She described many nuclei in the younger stages. All of the nuclei disintegrate except two. These, in connexion with coenocentra, increase greatly in size without the occurrence of any nuclear fusions. According to Miss McCormick, these coenocentra were examined by Professor Stevens, who considered them as similar to the structures described by him in *Albugo*. Miss McCormick indicates in her work that the coenocentra have their origin at the point of contact of the suspensors before the gametangia are cut off. The coenocentra persist until late, and there are many nuclei of the same size as those of the mycelium in the mature zygospore. Neither fusion nor division of the nuclei was observed by her. She describes the presence of a single large oil globule in the mature stages, while, in the younger stages, the oil is diffused throughout in small globules.

In a general brief review of the literature that has been available on this problem, it is evident that the details of nuclear processes in the fusion of the gametes are in doubt. On the one hand, Léger, Dangeard, and Moreau hold that numerous nuclei are present at all stages, and that nuclear disintegration occurs in the zygospore. Dangeard and Moreau describe nuclear fusions which occur between many pairs of nuclei. On the other hand, Lendner in *Sporodinia* and Miss McCormick in *Rhizopus* find numerous nuclei in the younger stages only. At maturity all the nuclei, except two, disintegrate. Dangeard and Miss McCormick describe structures comparable to the coenocentra of Stevens.

METHODS.

Sporodinia grandis was used for the most part in this investigation. The fungus was first found growing on mushrooms in October, 1911, in the vicinity of Columbia, Missouri. Isolations were made and the cultures were grown on bread. In the course of 72 to 96 hours, the development of zygospores occurred in most of the cultures. These were fixed in all stages, several different fixing agents being used, as Flemming's, chrom-acetic, Gilson's, Carnoy's, and picro-acetic. The best fixation of the earlier stages was secured with the weak solution of Flemming. For the older stages a strong solution of Flemming, diluted with twice its volume of water, gave better results, especially where the outer coat had become cutinized. The material was washed in water, dehydrated by the usual percentages of alcohol, and infiltration with paraffin was secured by the use of xylol. The older stages, however, are very brittle, due to the thick walls of the zygospore, and the ordinary methods were not satisfactory for the study of serial sections. It was found, after considerable experimenting, that if the zygospores were allowed to stand in a weak solution of sodium hydroxide for 24 to 48 hours, the outer brown coat became softened and somewhat transparent. It was then a relatively simple matter to dissect the zygospores from their thick coats and the suspensors. In other cases, the material was carried through the killing and fixing process without dissection. In all cases after treatment with sodium hydroxide, the material was washed in running water for 12 hours in order to counteract any tendency to plasmolysis. It was then fixed at once in various reagents, here again the best results being obtained with the diluted solution of Flemming's strong. It was necessary to expose the older material to this solution for 48 hours, as the coats are very slow of penetration. After washing in water, the usual grades of alcohol were used, followed by the grades of alcohol and cedar oil, with an exposure of 2–7 days in pure cedar oil. The chloroform and xylol methods were also used, but were not as satisfactory. The material was then embedded in paraffin and sectioned at 5 μ for the younger stages, and 7–30 μ in the case of the older.

In staining, Flemming's triple stain of safranin, gentian violet, and orange G was used almost entirely, although Haidenhain's iron-haematoxylin with Bismarck brown and safranin as counter-stains, and Delafield's haematoxylin and eosin, were used in a large number of preparations. These all proved highly satisfactory in their differential powers, and brought out the same structures in all cases.

LIFE-HISTORY AND DEVELOPMENT.

As has already been noted, *Sporodinia grandis* readily produces zygospores on bread cultures. The ends of certain aerial hyphae enlarge and fuse. Externally, there is little or no difference between these two hyphae or sexual branches. In some cases, one branch appears slightly smaller than the other, but this seems to be the result of physiological rather than morphological conditions. The protoplasm flows out into the ends, gradually growing denser until the ends of the hyphae are densely filled, while the regions further back are vacuolated. These observations can easily be made in fresh material.

When the internal structure of the branches is studied, however, there is found to be a slight variation in the activity of the sexual branches. Léger describes two types of branches: in one, the protoplasm fills both of the sexual branches; in the other, the protoplasm is retracted from the wall. According to my studies, this appears to be due to a difference in age. In the earlier stages the protoplasm fills the branches, but later a retraction of the protoplasmic mass occurs (Pl. XXXV, Fig. 3). This retraction is usually restricted to one branch, but occasionally both show it to some extent. Even in the latter case, however, the retraction is more marked in one branch than in the other. The writer, contrary to the results obtained by Miss McCormick, has been unable to distinguish any marked differential staining between the two protoplasts of the two sexual branches. A granular substance usually fills the intervening space where the protoplasm has drawn back from the wall. This was particularly evident in preparations stained with iron-haematoxylin and Bismarck brown, for in such preparations the granular substance always stains brown. This is probably the substance, described by Vuillemin (33, 34), which forms the secondary membrane before resorption occurs.

Many nuclei are found distributed in the protoplasm of the young sexual branches. Nuclear divisions were not observed, but there seems little doubt that they occur about this time, because there is a marked increase in the number of nuclei, which cannot well be explained on the basis of subsequent migrations. The nuclei are extremely small (Pl. XXXV, Fig. 1 *a*). The main part of the nucleus is granular in appearance, and stains violet or slightly red in Flemming's triple stain. The central part of the nucleus stains a brilliant red, and appears to be a constant organized structure. It is very similar in appearance to the nucleoli of the nuclei of the higher forms, but the fact that this structure is constant through all the stages of development would suggest that it is chromatin in nature rather than that it is a nucleolus. The true nature of this material will remain a question, however, until nuclear divisions have been observed. Lendner describes the nuclei as being formed of two small masses closely associated, which divide simultaneously and give the double division described by Maire in the Basidiomycetes. The writer, however, has made a careful study of the nuclei in the germinating spores where division must occur, but has been unable to find any variation from that described above.

Vuillemin (33) and Dangeard (9) have offered evidence as to the nature of the fusion of the contiguous walls of the gametangia. It will suffice here to say that the two walls coalesce, forming a single separating partition between the two branches. Previous to the cutting off of the gametes from the suspensors, large vacuoles are formed a short distance back in the swellings. The formation of these vacuoles usually takes place before the resorption of the separating wall. These vacuoles enlarge, becoming wedge-shaped. On their inner margins, a narrow isthmus of protoplasm is left connecting the protoplasm of the suspensors with that of the gametangia. Towards the margin of the gametangium, however, the point of the wedge-shaped vacuole cuts through the protoplasm, forming a cleavage furrow that extends to the wall of the gametangium (Pl. XXXV, Fig. 4). In some cases, the cleavage furrow appears to be cut in without the aid of the vacuoles. The line of cleavage remains distinct, and very soon a new wall is built in from the outer wall of the gametangium (Pl. XXXV, Fig. 5). Dangeard has suggested that this wall is in the form of a diaphragm which gradually closes in, but he offers no evidence to support his view. The relation of this diaphragm is very evident in the present preparations. As the wall is built in, the circular opening is gradually closed, like a diaphragm coming clear together, and cutting through the narrow isthmus of protoplasm. When the wall closes, the cellulose-like material continues to pile up for a short time, forming a papilla-like projection which the remaining strands of protoplasm also tend to draw out. This is the ‘canal’ described by Léger. Vuillemin has failed to recognize this structure in his study of the membrane of the zygospore. In Pl. XXXV, Fig. 5, the inner edge of the wall is shown as it cuts into the protoplasm at this point. The vacuoles and nuclei are somewhat elongated in the plane parallel to the long axis of the isthmus. The final cleavage is caused, either by the closing of the diaphragm, or by a furrow which precedes the new membrane so closely as to be indistinguishable. Thus it can be seen that this little structure that has received the passing attention of many, and had various functions allotted to it, is an unimportant result of the delimiting of the gametangium. In no cases have the orifices described by Dangeard and Léger been observed.

As soon as the wall which separates the gametes from the suspensors is complete, and in numerous instances at the formation of the large vacuoles, the wall separating the gametangia is dissolved from the centre outwards. As has been said, the contents of one gametangium usually completely fill its cavity, while in the other there may be, and usually is, a marked retraction of the protoplasmic mass. When the separating wall is resorbed, there is a marked activity on the part of that portion of the mass that has extended clear to the wall, while the protoplasm of the other gametangium shows a further retraction before the influx of the active protoplasm. The masses retain their individuality for a short time after the resorption of the wall, and the region of contact reacts somewhat differently to the stains (Pl. XXXV, Fig. 6). Ultimately, however, the line of fusion is obliterated, and it is impossible to distinguish any difference between the two masses of protoplasm. Gradually, as the mixing of the protoplasm progresses, it passes from a dense homogeneous nature to a vacuolar condition. It is at this stage that occur what appear to be nuclear fusions. They are first evident along the line of contact of the two protoplasmic masses, and later throughout the whole structure as it becomes vacuolated. In fusion, the nuclei present the usual appearance of fusing nuclei. They come to lie in pairs, the nuclear membrane disappears between them, and the deeper staining central masses previously described come to lie within a single membrane. Ultimately, these two masses fuse, and one larger nucleus results containing a single deeply staining central portion (Pl. XXXV, Fig. 7 *a*).

The question at once arises as to whether this condition just described might not be a process of division of the nuclei instead of fusion. The process might be viewed from either standpoint, and it is probable that the divisions described by many of the previous workers on this problem have been so interpreted. As has been said, the nuclei are so small that even under the highest magnification available, approximately 2,000 times, it is impossible to be absolutely certain on this point. There are, however, several resultant conditions that have seemed to justify the present interpretation. In the mycelium, in the germ-tube of the germinating spore, in the sporangia, and in the gametangia before resorption of the separating wall, the nuclei are small and not well defined (Pl. XXXV, Figs. 1, 2, 7 *a*). In later stages, however, the nuclei are larger, and the nuclear membrane is more distinct.

The fusion of the nuclei results in the presence of nuclei of two sizes in the young zygospore. The protoplasm assumes a thready nature, and throughout the whole zygospore there is evidence of a nuclear disorganization (Pl. XXXV, Fig. 8). This appears to be restricted to the smaller nuclei. A characteristic zonation occurs about this time. The majority of the nuclei that are undergoing disintegration appear to be concentrated in a zone around the periphery, while those towards the centre retain their normal form. When the nuclei begin to disorganize, the outer membrane remains distinct for some time, but the inner portion loses its granular appearance. The central red-staining mass increases in size as if being dissolved, and stains less densely. The whole nucleus appears eventually as a large red-staining mass (Pl. XXXVI, Fig. 11 *c*). The nuclei of the suspensors present a similar appearance as they degenerate, following the delimitation of the gametangium.

About this time, there appear numerous spherical bodies of cytoplasmic origin. That these are not products of the disorganizing nuclei seems evident from their early structure and staining reactions. At first they stain lightly violet. They are generally globular in shape, although they vary somewhat, and are coarsely reticulate. They stand out as distinct individual structures. Many originate near the periphery of the zygospore, but others are found through the central portion during the period of zonation. These new cytoplasmic bodies are confusing at first, and only a careful study of their function and fate has placed them as definite cell structures. The structures described by Leger under the name of 'sphères embryogènes' are most certainly identical with these bodies. Dangeard described them as 'chromatic corpuscles', but did not study them closely, and arrived at no conclusions as to their function, apart from comparing them to the coenocentra of Stevens.

That these bodies are not nuclei is evident upon a close study of their structure, and is more forcibly emphasized by their subsequent behaviour. They gradually increase in size and number (Pl. XXXVI, Figs. 10, 10*b*), as many as forty having been counted in one section of a zygospore cut at 10 μ. Fusions occur between them without reference to size or number. These fusions seem to be due to the rapid increase in size of the bodies, which causes a coalescence.

As these structures enlarge, they show a more definite organization. Earlier they appear much as if they were vacuoles filled with a bluish staining substance, and only the highest powers of magnification and careful staining bring out their reticulate nature. Later, however, they gradually lose their coarse reticulation, becoming sponge-like with a denser centre (Pl. XXXVI, Fig. 10 *b*). In no cases, under various killing reagents, stains, and in careful serial sections, could anything that resembled chromatin or a nucleolus be detected within these bodies. In no instances furthermore, were the small previously described nuclei found within these structures, but, in all cases, the nuclei can be seen in meshes of the protoplasm between these structures and the vacuoles which are also numerous at this time (Pl. XXXVI, Figs. 9, 10).

These structures continue to enlarge and fuse until, in the zygospores taken from cultures at the end of six weeks, there appears from one to three of these bodies (Pl. XXXVI, Fig. 11). Through tests with osmic acid and chloroform with fresh material, it is found that, from their first appearance, these plastid-like bodies are associated with the formation of oil. In fresh material, they appear as large globules, due to the fact that they are completely saturated with oil, to the point even that if slightly pressed the oil exudes in a large globule or many small ones. This oil is readily soluble in chloroform. The oil is not very refractive and is amber coloured. It assumes the shape of the plastid-like body, and is not always in the form of a definite rounded globule.

These plastid-like bodies may be related to the elaioplasts described by Zimmermann (38) in the stems of *Psilotum*, and in the perianth leaves of *Maxillaria picta*, and in several other forms. Wakker (37), in 1888, first described and named the elaioplasts as found in the epidermal cells of *Vanilla planifolia*. He described the elaioplast as somewhat larger than the nucleus, possessing a well-defined outline and a peculiar yellowish colour. Raciborski (27), in 1893, described elaioplasts in the tissues of *Ornithogalum*, *Albuca*, *Funkia*, and *Gagea*. Zimmermann and Raciborski describe the structure of the elaioplast as being finely granular, due to the presence of a number of small refractive bodies. Areas of lesser refraction may occur within it.

Raciborski describes the elaioplasts of *Gagea* as somewhat different from this. They possess a plasmatic envelope with a lesser development of the stroma. Walker and Zimmermann have shown that the elaioplast consists of a plasmatic matrix saturated with oil droplets.

Garjeanne (19), in a paper on the oil bodies of the Jungermanniales, concludes that the oil bodies here originate in the vacuoles, the oil droplets lying in a semi-fluid substance. The oil bodies are said to multiply by division in the earlier stages, but when once formed remain unchanged.

Beer (1), in 1909, describes elaioplasts in the floral regions of *Gaillardia*, a Composite. He describes the elaioplasts as appearing first as refractive granules in the cells, and as being loosely aggregated at first, but becoming compact later. He concludes that the mature elaioplasts are the result of the aggregation of numerous plastids, and may be formed in several ways in the same cell. He suggests that the close massing of the plastids into compact elaioplasts is probably connected with their degeneration, and that it is unlikely that the elaioplasts have any particular function for the life of the cell. The author has made brief preliminary studies of the oil bodies in the endosperm and cotyledons of the seed of *Ricinus communis*. There appears to be a close resemblance between these and the plastids that are found in *Sporodinia*. These oil bodies and plastids have been described by many others in the tissues of many monocotyledonous and dicotyledonous plants.

It would appear from these comparisons that these large plasmatic bodies occurring in the zygospore of *Sporodinia* are closely related to, if not identical with, the elaioplasts described by these various investigators, and that these oil plastids must be recognized as occurring in the Fungi, as well as in the higher plants.

These oil plastids are undoubtedly the 'sphères embryonnaires' of Leger, and they may also be the nuclei of Lendner.

Léger describes two of the embryo spheres as being present in all cases, and maintains that they fuse upon germination of the zygospore.

In the material at hand, however, these plastids have been found at various stages in material from six to twelve weeks old. In most of the zygospores a single plastid appears, and in year-old zygospores only one large plastid is usually found, although several small ones may be present. In some instances at this age, the amber-coloured oil substance has disappeared and only the plastid remains. This would seem to be a further indication that the oil is a reserve food-substance. In many of the zygospores at the end of a year, there appears to have been no change, and they appear exactly as they did at the end of eight weeks.

According to Miss McCormick, the large structures which she finds associated with the nucleus have been identified by Stevens as similar to the coenocentrum described by him in *Albugo.* Miss McCormick describes this in *Rhizopus* as arising at the point of contact of the suspensors before the gametangia are cut off. This may be similar to the zonation described in *Sporodinia.*

Various writers have described the presence of coenocentra in the Fungi, and also the occurrence of oil.

Wager (35), in 1896, on *Cystopus candidus*, describes the presence of a coenocentrum and says, in connexion with the formation of oil, ‘Small oil droplets in considerable numbers are to be found. These oil drops gradually fuse together, and at certain stages one large oil drop with a number of small ones is to be seen. . . . All the oil drops gradually fuse together into a somewhat irregular lumpy mass, and finally into the large central oil sphere of the ripe oospore.’

Wager (36), in 1900, in studies on *Peronospora*, describes the presence of a dense granular mass in the centre of the oosphere, which becomes converted into a homogeneous ill-defined body. This central body appears to play some part in bringing the sexual nuclei together.

Davis (14) describes a coenocentrum in *Albugo candida.* It is described as being composed of deeply staining, slightly granular protoplasm. ‘It is sometimes surrounded by a zone of lightly staining protoplasm through which delicate radiations may be traced. Later the structure contracts somewhat, rounds itself off, and becomes a very dense body with a firm outline.’ In a later paper, Davis (15) describes coenocentra associated with the nuclei of the eggs in *Saprolegnia.*

Stevens (29), in 1901, summarizes briefly his own observations on the structure of the coenocentrum. He studied *A. Portulacaceae*, *A. Tragopogonis*, and in an earlier paper (1899) *A. Bliti.* He says, ‘In *A. Portulacaceae* the coenocentrum is the least developed, consisting simply of a zone of darkly staining cytoplasm which contains at its centre the alveolar region. In *A. Bliti* the structure is more prominent owing to the presence of a distinct central globule. In *A. Tragopogonis* the central globule seems to be formed by the trophoplasm of the central region, or rather by the coalescence of the contents of its vacuoles. This globule at a later period becomes granular, the granules staining like nucleoli. *A. candida* possesses the most highly developed coenocentrum, which, from its earliest formation, is thickly beset with coarse granules that in size and staining reaction agree with the nucleoli of this species.' He describes a form of oil which is found in globules. 'This results finally in a condition where the entire central region is occupied by a curious irregular structure which stains much as the oils have earlier, but which is certainly not of fluid consistency.'

It would seem highly probable from these comparisons that there is a close similarity between these structures of *Albugo* and the large oil plastids found in the mature zygospores of *Sporodinia grandis*.

The fate of the nuclei through these varying changes is important and significant. Considerable difficulty, however, was encountered in matters of technique. Owing to the thick brittle walls of the zygospores, it was necessary to cut the material at 15–30 μ, so that the nuclei did not stand out as clearly as in the preparations of younger material. It was found that the nuclei persist in much the same form as earlier (Pl. XXXVI, Fig. 11 *a*). In the oldest stages obtained, the nuclei show a somewhat greater affinity for the safranin, and in many cases the whole karyoplasm stains red. They retain, however, their granular appearance. They are scattered through the cytoplasm, lying around and between the plastids as in the earlier stages. At the time of the appearance of the single large plastid, the nuclei are found in close proximity to the surface of the plastid. The protoplasm is found as a comparatively thin parietal layer in such stages, surrounding the plastid.

In the earliest stages in the fusion of the sexual branches, and also in portions of the mycelium, certain red-staining crystals are evident. These are undoubtedly the mucorine crystals described by many working with the Mucors. These crystals may be either reserve or waste substances. In the mature zygospore, there appears an irregularly knotted or densely granular substance of intensely red-staining material, aggregated for the most part on one side of the zygospore. It reacts to the stain much as the mucorine crystals just described, but also strongly suggests the disorganized nuclei. Until germination of the zygospores has been carefully worked out, however, the exact nature and function of this material will remain a question. These structures of the zygospore have been observed in material varying from eight weeks to three months after the zygospores had been formed in the cultures. Therefore it would seem that no further changes take place until germination.

From Léger's observations, it would seem that the zygospore, in germination, uses up the oil reserve, that the oil plastid is dissolved, and that the nuclei undergo division, forming the nuclei of the next vegetative generation. This is purely hypothetical, however, and remains as a problem for further study.

Investigations with *Rhizopus nigricans* and *Zygorynchus moelleri* are well under way. While these are by no means complete, yet I am convinced that the essential features are identical with those of *Sporodinia. Zygorynchus* has fewer nuclei, but the zygospores are so small that new difficulties are to be encountered. The nuclei in *Rhizopus* are somewhat larger than in *Sporodinia,* and the oil plastids appear at about the same stage of development. A single large plastid is characteristic of the mature zygospores of both *Rhizopus* and *Zygorynchus.*

SUMMARY.

1. In the early stages of copulation of the sexual branches, there is no difference morphologically between the two branches. Later, there is a characteristic retraction of the protoplasm of one of the gametangia. This may or may not be of sexual significance.

2. The nuclei of the gametangia are small and show the same structure, size, and staining reaction as those of the mycelium.

3. Vacuoles and cleavage furrows are formed. A new membrane is formed by ingrowth from the lateral walls. A central thickened portion is formed.

4. The wall between the gametangia is dissolved, and the gametes fuse, the protoplasm of one flowing into that of the other.

5. Nuclear fusions occur progressively as the protoplasmic masses mix.

6. Two sizes of nuclei result:
    (*a*) Large nuclei, the results of fusions.
    (*b*) Small nuclei which fail to fuse.

7. The formation of the second wall of the zygospore then takes place, and the unfused nuclei, as well as those of the suspensors, begin to show degeneration.

8. Mucorine crystals are present through all the earlier stages.

9. There next appear globular cytoplasmic structures, with which, from their first appearance, oil is associated. They fuse irregularly, and produce ultimately one or two large bodies which are saturated with oil, as determined by the osmic acid test and the solubility in chloroform.

10. These large cytoplasmic bodies are undoubtedly related to the elaioplasts described in many plants, and have for their function the elaboration of an oil for reserve food.

11. In the mature zygospore there are numerous characteristic nuclei formed by the fusion of the two nuclei. The protoplasm is reduced to a thin parietal layer surrounding the oil plastid. This state of affairs persists until germination.

This work has been done under the direction of Dr. George M. Reed, and I wish, in conclusion, to express to him my very great indebtedness for his many suggestions and kindly criticisms.

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BIBLIOGRAPHY.

1. BEER, R.: Elaioplasts. Ann. Bot., vol. xxiii, 1909, pp. 63–72.
2. BREFELD, O.: Botanische Untersuchungen über Schimmelpilze. Leipzig, 1872.
3. CLAUSSEN: Zur Entwicklungsgeschichte der Ascomyceten, *Pyronema confluens.* Zeitschrift für Botanik, vol. iv, 1912, pp. 1-65.
4. DANGEARD, P. A.: Sur les oospores formées par le concours d'éléments sexuels plurinucléés. Compt. rend., vol. cxi, 1890, p. 382.
5. ——————: Recherches sur la reproduction sexuelle des champignons. Le Botaniste, sér. 3, 1894, p. 282.
6. ——————: Considérations sur les phénomènes de reproduction chez les Phycomycètes. Le Botaniste, vol. iv, 1895, p. 249.
7. ——————: La reproduction sexuelle des champignons. Le Botaniste, vol. vii, 1900, pp. 89–130.
8. ——————: La sexualité chez les champignons. Revue scientifique, vol. iv, 1905, pp. 225-9, 262–70.
9. ——————: Les Mucorinées. Le Botaniste, vol. ix, 1906, pp. 227–53.
10. ——————: La fécondation nucléaire chez les Mucorinées. Compt. rend., vol. cxlii, 1906, pp. 645–6.
11. DANGEARD and LÉGER: La reproduction sexuelle des Mucorinées. Compt. rend., 5 mars 1894, pp. 547–9.
12. ——————: La reproduction sexuelle des Mucorinées. Le Botaniste, vol. iv, 1894, pp. 4–11.
13. ——————: Recherches sur la structure des Mucorinées. Compt. rend., vol. cxviii, 1894, p. 430.
14. DAVIS, B. M.: The Fertilization of *Albugo candida.* Bot. Gaz., vol. xxix, 1900, p. 299.
15. ——————: Oogenesis in *Saprolegnia.* Bot. Gaz., vol. xxxv, 1903, pp. 233–50.
16. ——————: Oogenesis in *Vaucheria.* Bot. Gaz., vol. xxxviii, 1904, p. 81.
17. DE BARY, A.: Beiträge zur Morphologie und Physiologie der Pilze. 1864, p. 74.
18. EHRENBERG, C. F.: Verhandlungen der Gesellsch. nat. Freunde, Berlin, Bd. i, 1820.
19. GARJEANNE, A.: The Oil Bodies of the Jungermanniales. Flora, vol. xcii, 1903, p. 457.
20. GRUBER: Über das Verhalten der Zellkerne in den Zygosporen von *Sporodinia grandis.* Ber. d. d. bot. Gesellsch., vol. xix, 1901, p. 51.
21. HARPER, R. A.: Sexual Reproduction in *Pyronema confluens.* Ann. Bot., vol. xiv, 1900, p. 321.
22. LÉGER, M.: La structure et développement de la zygospore du *Sporodinia grandis.* Revue gén. Bot., vol. vii, 1895, p. 481.
23. LENDNER, A.: Recherches histologiques sur les zygospores du *Sporodinia grandis.* Herb. Boissier, sér. 2, t. viii, 1908, p. 77.
24. LUNDSTRÖM, A. N.: Oelplastiden. Bot. Centralbl., vol. xxxv, 1888 (abstract), p. 177.
25. MCCORMICK, F.: Development of the Zygospores of *Rhizopus nigricans.* Bot. Gaz., vol. liii, 1, 1912, p. 61.
26. MOREAU, F.: Fusions de noyaux et dégénérescence nucléaire dans la zygospore. Bull. Soc. Mycol. Fr., vol. xxvii, 1911, p. 334.
27. RACIBORSKI, M.: Elaioplasten bei Liliaceen. Bot. Centralbl., vol. lvii, 1894 (abstract), p. 305.
28. STEVENS, F. L.: The Compound Oosphere of *Albugo Bliti.* Bot. Gaz., vol. xxviii, 1899, p. 149.

29. STEVENS, F. L.: Gametogenesis and Fertilization in *Albugo*. Bot. Gaz., vol. xxxii, 1901, p. 77.
30. ——————: Oogenesis and Fertilization in *Albugo Ipomoeae-panduranae*. Bot. Gaz., vol. xxxviii, 1904, p. 300.
31. VAN TIEGHEM et LE MONNIER: Recherches sur les Mucorinées. Annales des Sc. Nat., Bot., sér. 5, t. xvii, 1873, p. 261.
32. ——————: Nouvelles recherches sur les Mucorinées. Ann. des Sc. Nat., Bot., sér. 6, t. i, 1875, pp. 5–175.
33. VUILLEMIN, P.: Importance taxonomique de l'appareil zygosporé des Mucorinées. Bull. Soc. Mycol. Fr., t. xix, 1903, p. 106.
34. ——————: Évolution des quatre assises protectrices de la zygospore chez quelques espèces de Mucoracées. Ann. Mycol., vol. ii, 1904, p. 487.
35. WAGER, H.: On the Structure and Reproduction of *Cystopus candidus*. Ann. Bot., vol. x, 1896, p. 295.
36. ——————: Fertilization of *Peronospora parasitica*. Ann. Bot., vol. xiv, 1900, p. 263.
37. WAKKER, J. H.: De Elaioplast. Bot. Centralbl., vol. xxxiii, 1888 (abstract), p. 139.
38. ZIMMERMANN, A.: Elaioplasten. Beitr. zur Morph. u. Physiol. d. Pflanzenzelle, vol. iii, p. 185.

EXPLANATION OF FIGURES IN PLATES XXXV AND XXXVI.

Illustrating Miss Keene's paper on *Sporodinia grandis*.

(All figures were drawn with the aid of the camera lucida and with the Zeiss apochromatic objectives and compensating oculars. Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11 were drawn with obj. 16 mm., oc. 12. Figs. 1 *a*, 7 *a*, 7 *b*, 8 *a*, 9 *a*, 10 *a*, 10 *b*, 11 *a*, 11 *b* were drawn with obj. 1·5 mm., oc. 4. Fig. 1 was drawn with obj. 16 mm., oc. 8.)

PLATE XXXV.

Fig. 1. Germinating spore with two germ-tubes, showing numerous nuclei.

Fig. 1 *a*. Nuclei of germ-tubes.

Fig. 2. A small portion of the mycelium, showing size and structure of nuclei.

Fig. 3. Young branches at copulation.

Fig. 4. Gametangia with separating wall dissolved, showing fusion of cytoplasmic masses. Also shows formation of wedge-shaped vacuoles.

Fig. 5. Portion of suspensor and gametangium, showing formation of new delimiting wall and its relation to isthmus of protoplasm.

Fig. 6. Gametangia following the resorption of the separating wall, showing the penetration of one gamete into the other.

Fig. 7. The young zygospore at the time of nuclear fusions.

Fig. 7 *a*. Nuclei of the zygospore, showing fusions.

Fig. 7 *b*. Mucorine crystals.

Fig. 8. Zygospore at the time of zonation and first appearance of the oil plastids.

Fig. 8 *a*. Nuclei.

PLATE XXXVI.

Fig. 9. Partly mature zygospore, showing increase in size and number of the oil plastids.

Fig. 10. Oil plastids and area of densely staining material, probably disorganizing nuclei.

Fig. 10 *a*. Nuclei from zygospore.

Fig. 10 *b*. Oil plastid shown under same magnification as nuclei.

Fig. 11. Mature zygospore, showing single large plastid and several small ones.

Fig. 11 *a*. Nuclei of mature zygospore.

Fig. 11 *b*. A portion of the plastid shown in Fig. 11, under higher magnification in order to bring out its reticulate nature.

Fig. 11 *c*. Disorganizing nuclei.

Annals of Botany,


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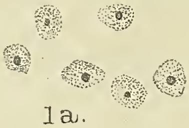
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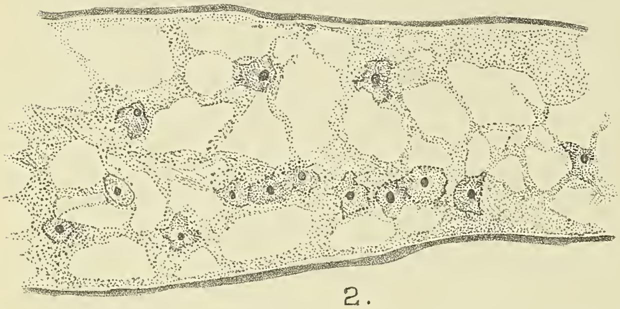
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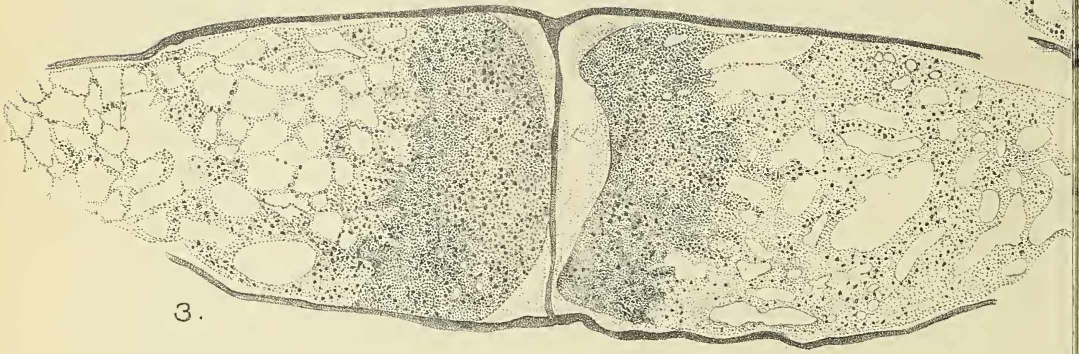
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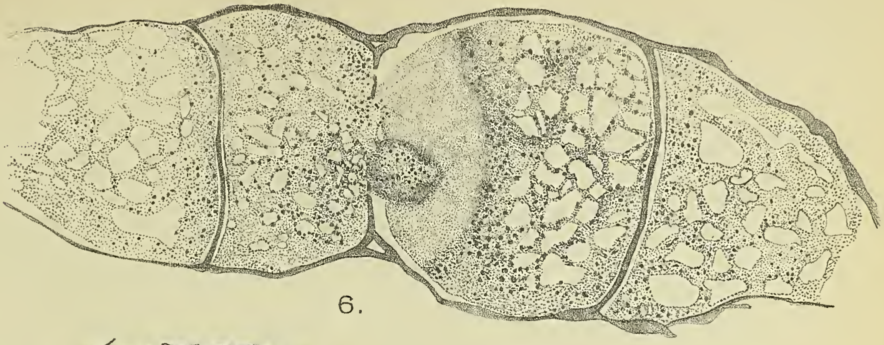
KEENE — SPORODINIA



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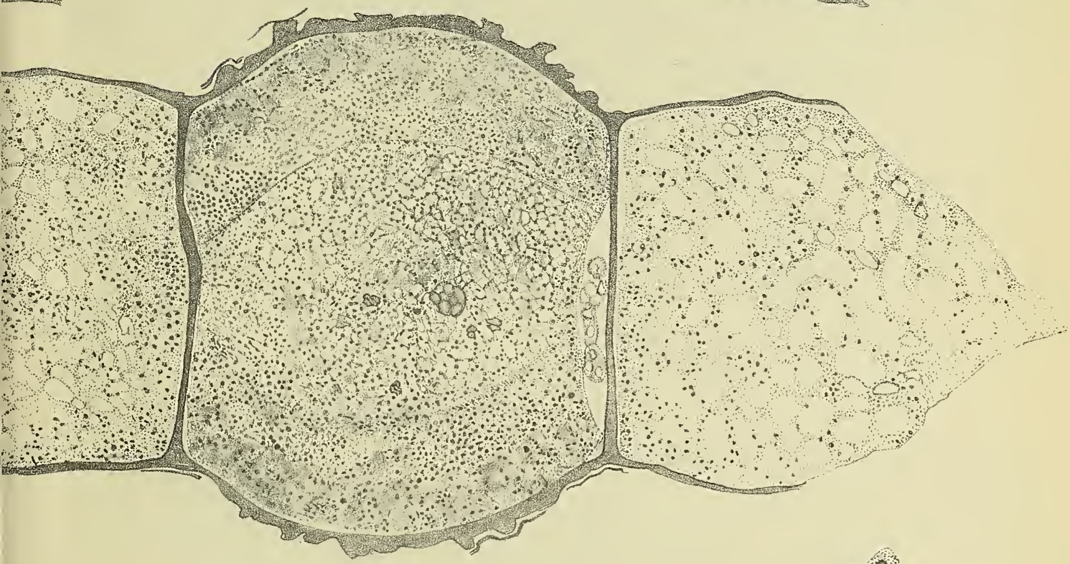

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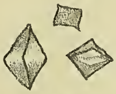

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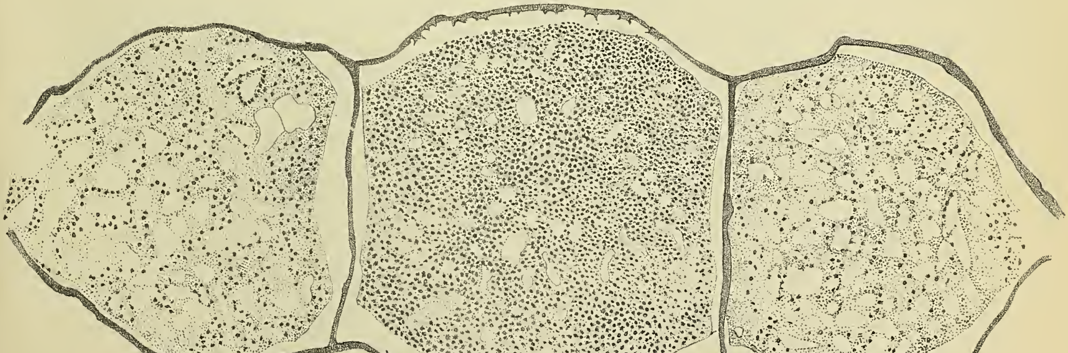

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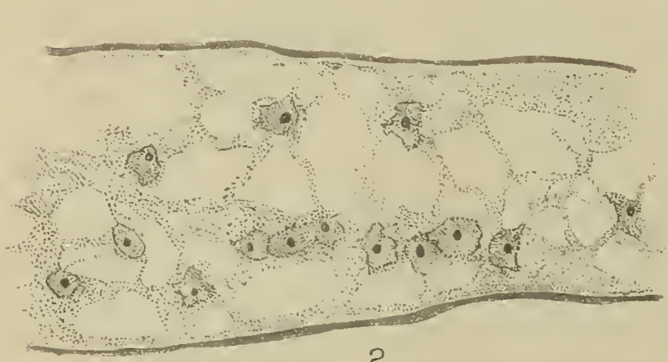

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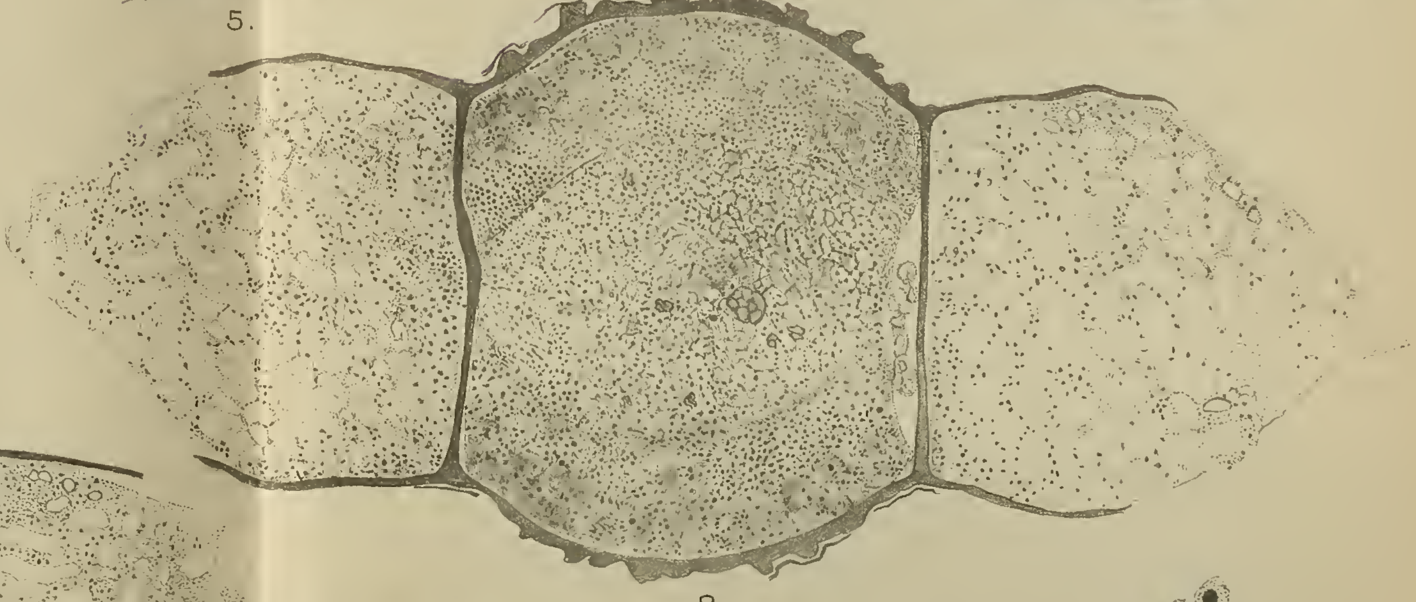

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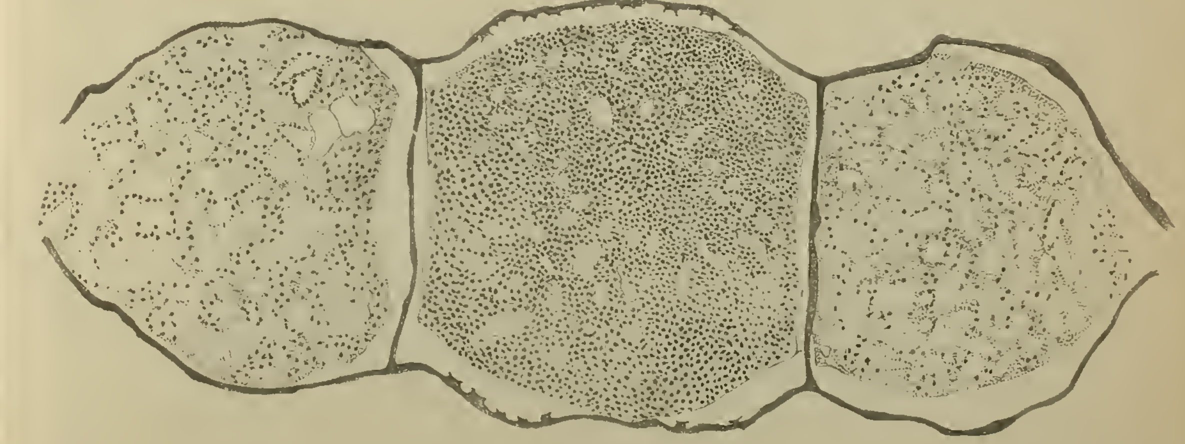

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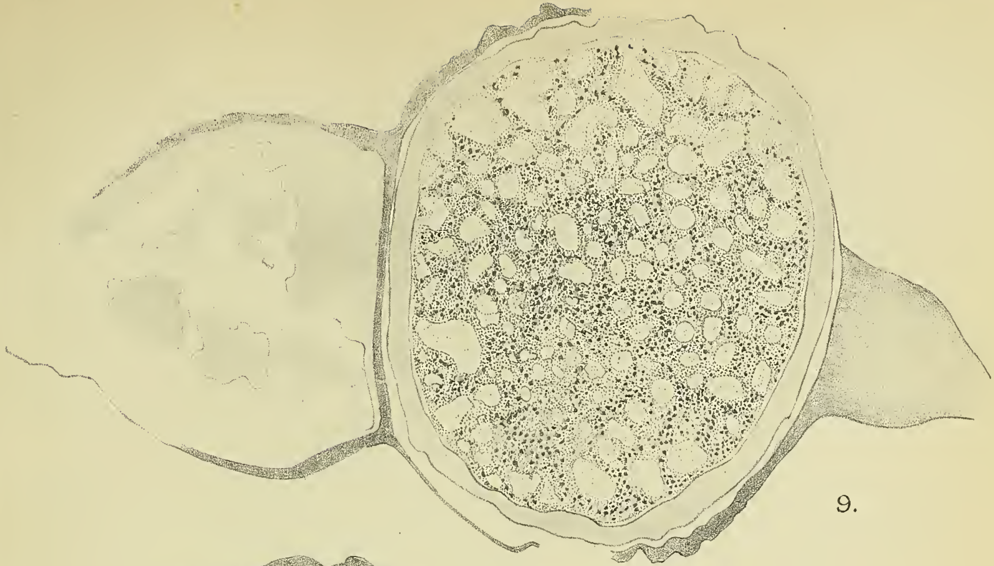
M.L.K. del.

KEENE — SPORODINIA

Huth lith et imp



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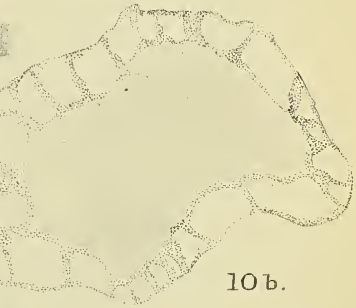

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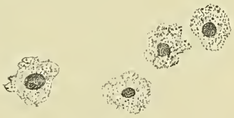

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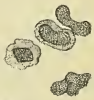

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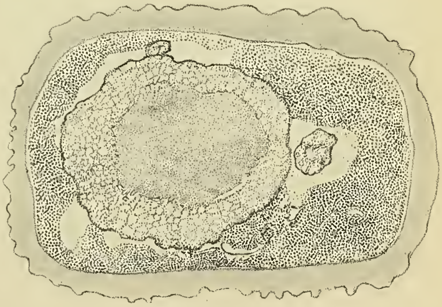

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M.L.K. del.

Huth lith et imp.

KEENE — SPORODINIA

Some Jurassic Osmundaceae from New Zealand.[1]

BY

EDMUND W. SINNOTT.

With Plate XXXVII.

THE vexed question of the morphology of the pith in vascular plants is still a point of dispute among botanists, but at present there seems to be a pretty general agreement of opinion, that in the great majority of the Pteridophyta the pith should be regarded as derived from the cortex, and as morphologically identical with it.[2] Many anatomists, however, contend that in at least two families, the Osmundaceae and the Ophioglossaceae, medullation has been achieved by a different process, the pith arising in an intrastelar fashion, and having no morphological connexion with the cortex. These two groups of Ferns present more evidence in favour of such a view than do any other vascular plants, and the discussion of the problem of stelar morphology has to a large extent become centred about their structure and phylogenetic history. All new facts concerning the anatomy of living or fossil members of these two families are therefore of particular interest.

Dr. A. J. Eames and the writer recently spent some time in New Zealand as Frederick Sheldon Travelling Fellows in Botany from Harvard University, and were fortunate in securing several petrified stems of Osmundites from the Jurassic rocks of the North and South Islands. A description of their structure and a discussion of its bearing on the morphology of the Osmundaceae is the purpose of the present paper.

Material was obtained from two localities: one, the Jurassic 'Fossil Forest' near Waikawa, Southland, at the extreme southern tip of the South Island; and the other (for a specimen from which the author is much indebted to Professor P. Marshall), the Jurassic rocks of Kawhia, North Island. The Kawhia material is in an excellent state of preservation (Pl. XXXVII, Fig. 1), whereas that from Waikawa is somewhat compressed, and most of its structure, except for the xylem and sclerenchyma, has been destroyed (Pl. XXXVII, Figs. 2 and 3).

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[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 62.

[2] A few investigators, however, deny the morphological value of the endodermis.

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

The structure of the stem and leaf-bases of both sets of material is very similar. The stele is of moderate dimensions (5 mm. to 8 mm. in diameter) and surrounds a true parenchymatous pith. This pith is excellently preserved, and is uniform in character in the Kawhia specimen, but almost structureless in those from Waikawa. In the latter material, however, there are in the pith numerous strands of xylem, elliptical in shape, with an exarch protoxylem group at each end (Figs. 2, 3, 5, and 6). There may be as many as four of these in a single section. They are precisely similar to the typical diarch root bundles which are distributed throughout the cortex. About each strand is a definite, sharply limited, and well-preserved parenchymatous cortex (Figs. 5 and 6). There is little doubt that we are here dealing with roots which in some way have managed to enter the pith of the stem.

That such an interpretation is the correct one is further emphasized by the occurrence of a similar condition in living species. Fig. 8 shows a section of the stem of *Osmunda cinnamomea* with a root obviously present in the pith. Fig. 7 shows the same stem a little further along, where a dichotomy occurs, and makes plain the fact that the root has invaded the pith from the cortex through the branch gap. In a similar way, probably, the roots in our fossil were able to make their way into the pith.

There are no positive indications of internal phloem or endodermis. The external phloem is normal in structure and distribution.

The leaf-gaps in the xylem are extremely narrow, and in the Waikawa specimen, where the xylem has been more or less compressed and distorted, they often appear to be absent. In the Kawhia material, however, which has suffered little disorganization, it is evident that leaf-gaps, although very narrow, are invariably present (Fig. 1). As is the case in some other fossil Osmundaceae, and in certain living species as well, the gap is not always complete immediately at the departure of the leaf-trace, but becomes so before the trace has passed very far into the cortex.

The leaf-trace is the typical elliptical monarch strand of the family. The protoxylem is often mesarch in position for a short distance, but soon becomes endarch.

The base of the petiole is without a broad stipular wing (Fig. 4). Its vascular bundle is surrounded by a circular ring of sclerenchyma, and there is a large island of the same tissue on each side of the ring, as well as several other smaller spots. The bundle is the typical single arch, and has a large patch of sclerenchyma inside each lateral bay.

The specimens from the two localities are very similar, and they display a close agreement in structure with *Osmundites Dunlopi*, a species described by Kidston and Gwynne-Vaughan (1) from Gore, New Zealand, a locality about forty miles from Waikawa. *O. Dunlopi* possesses the same compact stele as our fossils, with leaf-gaps very narrow, and sometimes apparently absent. Its state of preservation is very much poorer than that of our Kawhia specimen, however, which shows the universal presence of very narrow gaps, and it is logical to infer that, were the structure of *O. Dunlopi* less crushed and disorganized, gaps would be evident there also. The agreement in petiolar structure, which is an important diagnostic feature in the Osmundaceae, is even more precise, for both *O. Dunlopi* and the fossils under consideration show two large patches of sclerenchyma at the base of the stipular wings, and two others inside the lateral bays of the leaf-trace, thus differing from all other members of the family. The occurrence of roots in the pith of the Waikawa specimen is not considered a diagnostic character.

The resemblance of the fossils here described to *O. Dunlopi* is therefore close enough to warrant our regarding them as members of that species, which seems to have been widely distributed in New Zealand in Jurassic time.

DISCUSSION.

It remains to discuss what bearing the structure of these fossils has on the morphology of the Osmundaceae. The view that the living members of the family primitively possessed an amphiphloic siphonostele with wide leaf-gaps, similar to that of most modern Ferns, and that the present structure has been derived by reduction from such a condition, with the loss, in most cases, of internal phloem and endodermis, has been put forward by Faull (2), and by Jeffrey (3). This hypothesis has met with vigorous opposition from the majority of anatomists, most of whom contend that in the Osmundaceae, and also in the Ophioglossaceae, the pith has had an entirely different origin from that in all other Pteridophyta, and that it has been derived by the direct conversion into parenchyma of the vascular elements in the centre of the primitive protostele, without having any original morphological connexion with the cortex.

Which of these views is the correct one cannot be settled *a priori* by an appeal to any general law of evolution. It may well be that the pith of vascular plants has been derived in two entirely different ways, for it often happens that two very similar structures are morphologically different, and have had an entirely independent evolutionary origin. But the fact that in the vast majority of all medullated vascular cryptogams, and apparently in the higher plants as well, the pith is at least primitively in direct connexion with the cortex, and morphologically equivalent to it, and the presence in fossil and living Osmundaceae themselves of many structures which are hard to account for, unless we assume that reduction from the ordinary condition has taken place in this series, furnish a very strong presupposition in favour of the theory of a uniform method of medullation among all vascular plants. The burden of proof is clearly placed upon those who maintain that there have been exceptions to the normal course of evolution, and very strong evidence must be brought forward before their claim can be considered as established.

That such evidence is actually at hand, is the contention of many eminent anatomists. Facts recently called to notice, particularly by the researches of Kidston and Gwynne-Vaughan (1) on the fossil Osmundaceae, have led to the establishment of a plausible hypothesis for the origin of the family, which assumes that they have been derived from the Zygopterideae. These were the dominant Ferns of the Carboniferous, and might therefore be supposed to be the ancestors of the Mesozoic Osmundaceae. Many of the Zygopterideae show a well-marked internal region of shortened vascular elements, and a peripheral zone of longer ones, and some species show a considerable accumulation of parenchyma in the centre of the stele. Considering the anatomy of the stem alone, the transition seems easy, on the commonly accepted theory, between such forms and the condition of a 'medullated monostele' like that of *Osmunda regalis.*

A serious objection to this derivation of the Osmundaceae, and one which is recognized by the authors just mentioned, is the striking dissimilarity of the two families in the vascular supply of the leaf. The typical complex zygopteridean leaf-strand is as widely different from the simple arch of the Osmundaceae as could well be imagined, and as it has been demonstrated that the anatomy of the leaf is of great importance in determining relationships, since it is even more slow to change than that of the stem, anything like a close connexion between the Osmundaceae and the typical Zygopterideae, at least, is not to be thought of.

This difficulty is met, however, by assuming that it was the very primitive Zygopterideae which gave rise to the Osmundaceae. The leaf-trace of such a genus as *Clepsydropsis* is much less complex than that of the other members of the family, and is probably more primitive, but it is still typically diarch with two widely separated protoxylem groups. The writer (4) has recently called attention to the fact that the structure of the leaf-trace, just as it leaves the stele, is remarkably conservative throughout the Filicales, and has shown that the Osmundaceae and Ophioglossaceae, alone among Ferns, are characterized by a single, mesarch protoxylem at the base of the leaf-trace. This monarch condition is a distinctive feature of the ancient and modern members of both families. Evidence was at the same time presented for the derivation of the leaf-trace of the other Fern groups by an amplification of the diarch condition of something like the *Clepsydropsis* type. If the separation of Ferns on the structure of their leaf-traces into a primitively monarch and a primitively diarch group is a natural one, it is evident that the Osmundaceae fall into one division, and the Zygopterideae into the other. Of course one may derive almost any type of leaf-trace from the simple condition of *Clepsydropsis*, and it is very likely that the Zygopterideae and Osmundaceae, and all other modern families of Ferns as well, may have descended from an ancestor of this general type. The upshot of the whole matter seems to be that, so far as their anatomy is concerned, the evidence for a close relation of the two families in question is not very convincing.

Much stronger evidence in favour of the intrastelar origin of the pith is furnished by the structure of the Jurassic genera *Zalesskya* and *Thamnopteris* (1), which from the character of their leaf-trace and petiolar bundle seem clearly to belong to the Osmundaceae, though both are protostelic, with no parenchymatous pith. In the centre of the stele, however, is a sharply differentiated region of short, wide vascular elements, which is held to be the forerunner of a true pith.

The idea that these fossil Ferns are the direct ancestors of the typical medullated Osmundaceae is very plausible, but, unfortunately for the theory, *Zalesskya* and *Thamnopteris* are definitely protostelic, and have no traces even of wood parenchyma. Moreover, no indisputably intermediate forms between them and a medullated condition have yet been found.

The nearest approach to such a transitional type is *Osmundites Kolbei*, described from the Jurassic of South Africa by Kidston and Gwynne-Vaughan (1). The stem of this species is in a poor state of preservation, with the central cylinder much flattened, but the authors find in the pith one or two definite strands of vascular tissue, which they interpret as remnants of the originally solid vascular core which have failed to be converted into parenchyma, and which form part of a true 'mixed pith'. It is in this connexion that our Waikawa fossil is of interest, for it is in a much better state of preservation than *O. Kolbei*, but like that species shows definite strands of vascular tissue in the pith. As we have pointed out, however, these are very obviously roots, for the vascular bundle is diarch, like all Osmundaceous roots, and is surrounded by a definite cortex of its own which is sharply marked off from the disintegrating tissue of the pith. It is thus very doubtful if the vascular elements in the pith of *O. Kolbei* are to be regarded as remnants of an originally solid protostele. The evidence from more perfectly preserved material, and from living species as well, indicates that they are much more probably roots.

It must therefore be admitted that no indubitably transitional forms between the protostelic Osmundaceae, and those with a true pith, have yet been discovered. Nor is the evidence at hand sufficient to prove that the latter must have come from the former through such a type as *Thamnopteris*. It is very possible that protostelic and siphonostelic Osmundaceae existed side by side since the origin of the family, for other groups of Ferns exhibit similarly fundamental differences in the topography of the stele between closely related forms. The protostelic *Lygodium* and the siphonostelic *Aneimia* are both members of the Schizaeaceae, and in the single genus

Gleichenia there are some species with, and others without, a pith. *Gleichenia pectinata*, for example, has a pith continuous with the cortex through the wide leaf-gaps and morphologically identical with it, according to the ordinary interpretation. If the gaps should become much narrower, affecting only the xylem of the stele, and if the internal phloem and endodermis should disappear, the pith in this genus would undoubtedly be explained by many as merely a large aggregation of the parenchymatous tissue, which is abundantly present throughout the xylem of the protostelic species. Such an explanation would of course not be tenable if the members of the reduction series from *G. pectinata* to the hypothetical form in question were known.

The theory proposed by Jeffrey supposes that the ancient Osmundaceae were in somewhat the same state as the genus *Gleichenia* is in to-day, and possessed some forms with a solid stele, which gave rise to the *Thamnopteris* type, and others with a true pith and wide leaf-gaps, from which the Jurassic and modern siphonostelic forms have been developed by reduction. *Thamnopteris* and other protostelic Osmundaceae are considered on this view as divergent lines which led nowhere, and finally disappeared.

Strong evidence in favour of this theory is presented by such fossils as *Osmundites Skidegatensis*, which is characterized by internal phloem and the continuity of pith and cortex through the wide leaf-gaps. The more recently described *O. Carneri* (5) from Paraguay shows a similarly large stele and wide gaps. Such forms provide just the intermediate condition which Jeffrey's theory postulates between a normal siphonostele and the reduced state of the modern Osmundaceae. The importance of these fossils has been minimized because of their supposedly recent geological horizon, but in the case of *O. Skidegatensis* there is now doubt as to whether it should not be referred to the Jurassic instead of to the Cretaceous; and *O. Carneri* is very possibly Jurassic.

A shortening of the internode, and a narrowing of the leaf-gaps in such forms, would produce a stele much like that of *O. Dunlopi*, *O. Kolbei*, and others, where gaps are very narrow and often apparently absent. We have already considered the probability that the invariable presence of leaf-gaps could be determined if preservation were sufficiently perfect. On the intrastelar theory of the pith these narrow but persistent gaps fail of an explanation, for no sign of a deep and narrow indentation opposite the leaf-trace is evident in the cylinder of *Zalesskya* or *Thamnopteris*. On the reduction theory, however, the gaps are of significance as a notable retention of the character of phyllosiphony, which is so distinctive of all siphonostelic members of the Pteropsida.

The controversy over the morphology of the stele in the Osmundaceae will doubtless long continue, and can be settled conclusively only by a discovery of the fossil ancestors of the family, and by an actual reconstruction of the steps in its phylogeny. On the existing evidence it is possible to frame theories only, and the question at hand is as to which of the two so far in the field is most worthy of adoption by plant anatomists.

The test of a good hypothesis is the number of facts it will explain. Measured by this standard the reduction theory has many advantages over the intrastelar. It explains the remarkable structure of *O. Skidegatensis* and related forms. It accounts for the presence of narrow but persistent leaf-gaps in the xylem of other species of *Osmundites*, and of the modern Osmundaceae. It accounts for the universal presence of a true parenchymatous pith in all species of *Osmundites* and in modern forms, and the absence of a 'mixed pith' or of any intermediate condition between a true pith and a solid protostele. It explains the occasional presence in certain living species of internal phloem, internal endodermis, and medullary sclerenchyma.

These facts are either unaccounted for by the intrastelar theory, or are explained only by subsidiary hypotheses. The best support for this theory is of course furnished by the fossil forms, but there is stronger evidence from the same source in favour of the reduction theory. The type of *O. Skidegatensis* is an even more plausible ancestor for the Osmundaceae than is *Thamnopteris*, and has the advantage of being connected with living species by a fairly complete series of forms, whereas no transitional types are known between the protostelic and the medullated Osmundaceae.

One of the most interesting facts about the Osmundaceae as a whole is the extreme slowness with which their structure has been modified. The majority of the Jurassic Osmundaceae are very similar anatomically to modern forms. The New Zealand species of *Osmundites*, particularly, are almost identical with the members of the living genus *Todea*, which now inhabit the same region. There is perhaps no other case among vascular plants where there has been so little change from Mesozoic time to the present.

SUMMARY.

1. Specimens of *Osmundites* were obtained from the Jurassic formations of the North and South Islands of New Zealand, and are considered to belong to the previously described *O. Dunlopi*.

2. They are characterized by a parenchymatous pith, with no internal phloem or endodermis. Leaf-gaps are very narrow and often 'delayed', but seem to have been invariably present.

3. In one specimen typical diarch roots, with a definite cortex, were observed in the pith. Roots often enter the pith of *Osmunda cinnamomea* through the branch gap at a dichotomy, and the same thing probably happened in the case of our fossil.

4. The leaf-trace is monarch, and occasionally mesarch at the base.

It expands into a single endarch arch, which in the base of the petiole is surrounded by a circular ring of sclerenchyma. On either side of this, in the cortex of the stipular wing, is a large island of sclerenchyma, and there is a patch of the same tissue inside each lateral bay of the leaf-trace.

5. The relationship between the Zygopterideae and the Osmundaceae cannot be close, since the anatomy of the leaf-trace and foliar bundle in the two groups is so widely different. The leaf-trace of the Osmundaceae and Ophioglossaceae is typically monarch, whereas that of the Zygopterideae is typically diarch. The simple condition of *Clepsydropsis* probably led rather to the diarch and triarch modern Ferns than to the Osmundaceae.

6. There seem to have been both protostelic and siphonostelic Osmundaceae in ancient times, and there is no convincing evidence that the protostelic members of the family which we know, such as the genus *Thamnopteris*, have given rise to the species with a pith, for no transitional forms have yet been found. Intermediate conditions occur, however, between such a primitive siphonostelic type as *Osmundites Skidegatensis* and the most reduced modern species. The xylem elements described as forming part of a 'mixed pith' in *Osmundites Kolbei* are probably root bundles similar to those here described in *O. Dunlopi.*

7. The theory of the origin of the Osmundaceae, which assumes that they have been reduced from typical siphonostelic forms, has the advantage of explaining very many structural facts in living and fossil members of the family which remain unaccounted for on any other hypothesis.

The writer is under much obligation for material to Professor P. Marshall, of the University of Otago, New Zealand; to Mrs. J. Mackintosh, of Waikawa, New Zealand; and to Professor J. H. Faull, of Toronto.

This work was carried on in the Phanerogamic Laboratories of Harvard University under the direction of Professor E. C. Jeffrey, to whom the writer is much indebted for information and advice.

BIBLIOGRAPHY.

1. KIDSTON, R., and GWYNNE-VAUGHAN, D. T.: On the Fossil Osmundaceae. Parts I–IV. Trans. Roy. Soc. Edin., 1907–10.
2. FAULL, J. H.: The Anatomy of the Osmundaceae. Bot. Gaz., vol. xxxii, 1901, pp. 381–419.
3. JEFFREY, E. C.: The Structure and Development of the Stem in the Pteridophyta and Angiosperms. Phil. Trans. Roy. Soc., B., vol. cxcv, 1902.
4. SINNOTT, E. W.: The Evolution of the Filicinean Leaf-trace. Ann. Bot., vol. xxv, 1911.
5. SCHUSTER, J.: *Osmundites* von Sierra Villa Rica in Paraguay. Ber. Deutsch. Bot. Gesell., xxix, 1911, pp. 534–540.

DESCRIPTION OF FIGURES IN PLATE XXXVII.

Illustrating Dr. Sinnott's paper on Jurassic Osmundaceae from New Zealand.

Fig. 1. Transverse section of the specimen from Kawhia. × 8.

Figs. 2 and 3. Transverse sections of a specimen from Waikawa. Roots may be observed in the pith at the left of the centre in Fig. 2, and at the extreme left and at the right of the centre in Fig. 3. × 12.

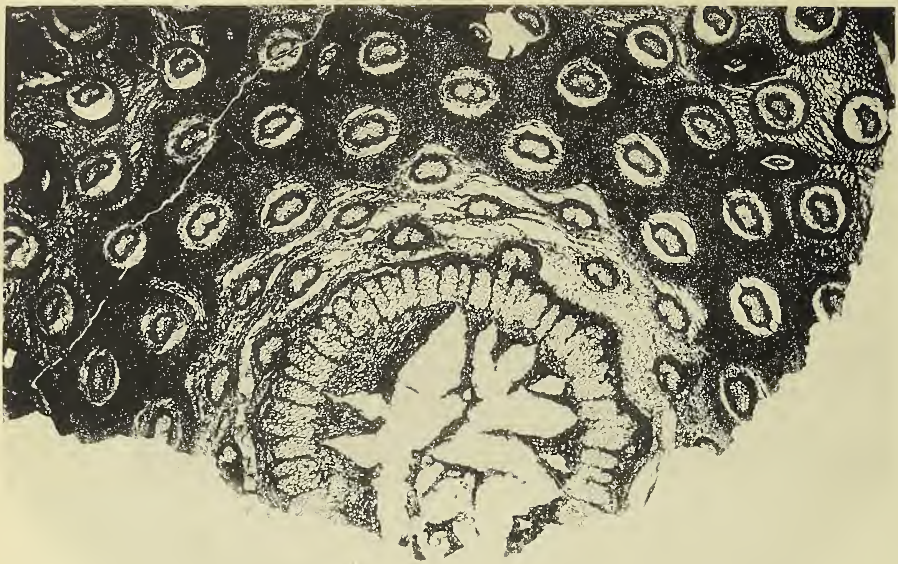
Fig. 4. Base of petiole from Kawhia specimen. × 15.

Fig. 5. Root in the pith, from Fig. 2. × 35.

Fig. 6. Root in the pith, from Fig. 3. × 35.

Fig. 7. Root entering the pith of *Osmunda cinnamomea* through a branch gap. × 10.

Fig. 8. Root enclosed in the pith of *O. cinnamomea*, a short distance behind a dichotomy. × 10.



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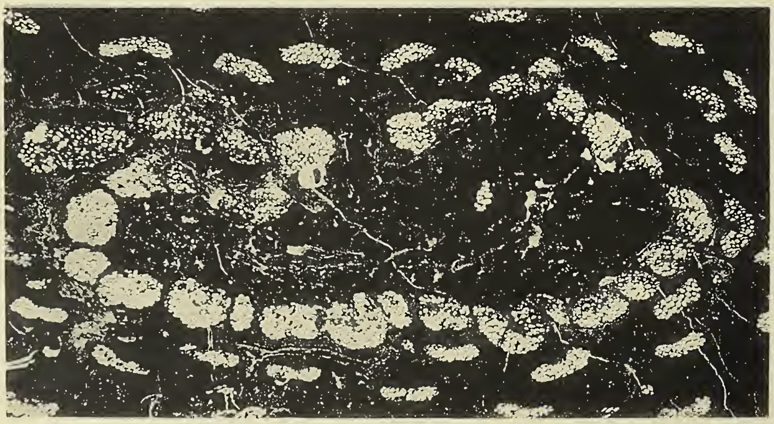

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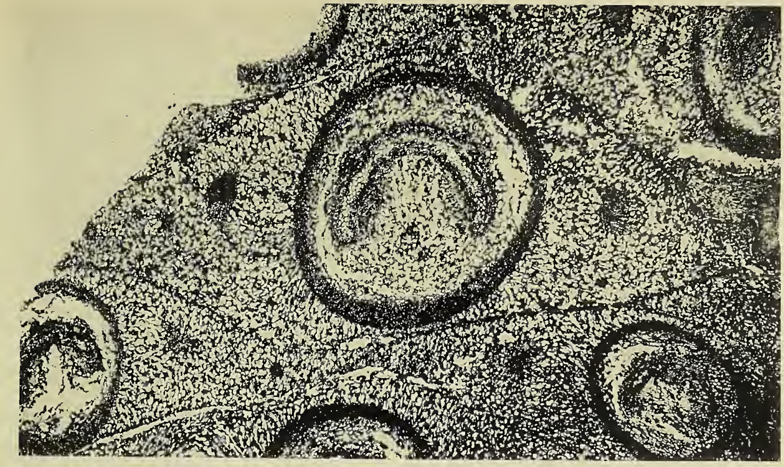
SINNOTT — JURASSIC OSMUNDACEAE.



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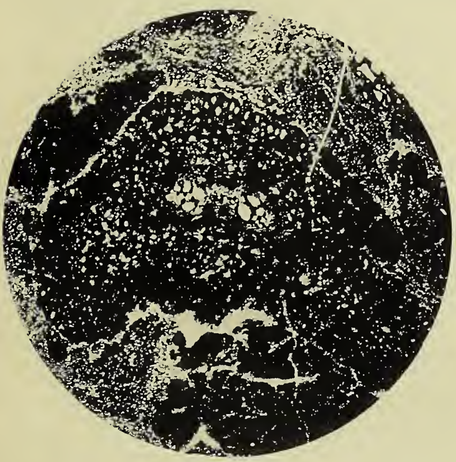

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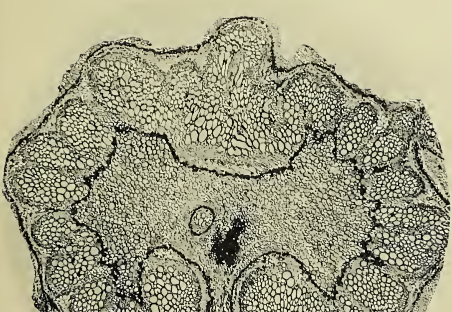

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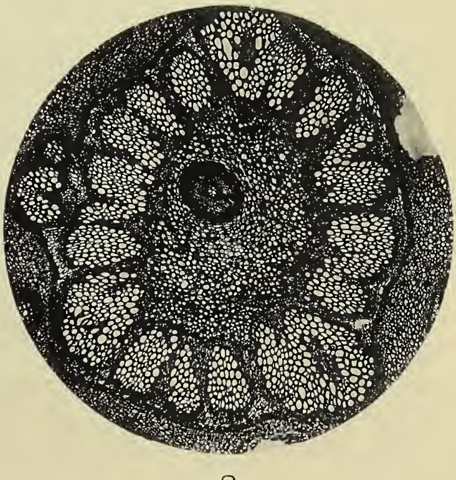

8.

Huth, coll.

On the Structure and Origin of the Ulodendroid Scar.

BY

D. M. S. WATSON, M.Sc.,

Lecturer on Vertebrate Palaeontology in University College, London.

With Plate XXXVIII and two Figures in the Text.

SINCE my first discussion of the problem of the origin of the ulodendroid scar was published,[1] the description by M. A. Renier of a beautiful specimen, showing a branch still connected with a scar, has placed my primary conclusion beyond dispute.[2] M. Renier, however, believes that this branch was only attached to the umbilicus instead of the whole area of the scar, a view which seems to me to be negatived by all we know of the structure of lepidodendroid stems and to be inconsistent with the actual structure of well-preserved specimens of *Ulodendron*.

The only other work of importance which has appeared since is the description by M. P. Bertrand of a *Ulodendron* with horizontally widened scars, each with a double umbilicus.[3]

M. Renier's theory of the origin of ulodendroid scars is as follows: In the young condition a small branch issues laterally from the trunk, in the way which is perfectly familiar in structural as well as in impression material. Subsequently, by the secondary growth of both branch and trunk, the base of the former is included in the latter and swells rapidly, so as to acquire a conical form, where it is included in the cortex of the trunk. The real external surface of a ulodendroid scar on this theory is the inner aspect of the outer cortex of the conical base of the branch.

Text-fig. 1 will, I hope, tend to make this view clearer.

The evidence advanced in support of this theory is of two kinds—first, that drawn from impression material examined by its author, and second, supposed evidence drawn from published descriptions of petrified material: this latter appears to me to be evidence founded on misconceptions which could never have arisen had the author examined the original or other suitable structural material of Lepidodendrons with branches issuing laterally.

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[1] Manchester Memoirs, lii, No. 4, 14 pp., 2 plates.

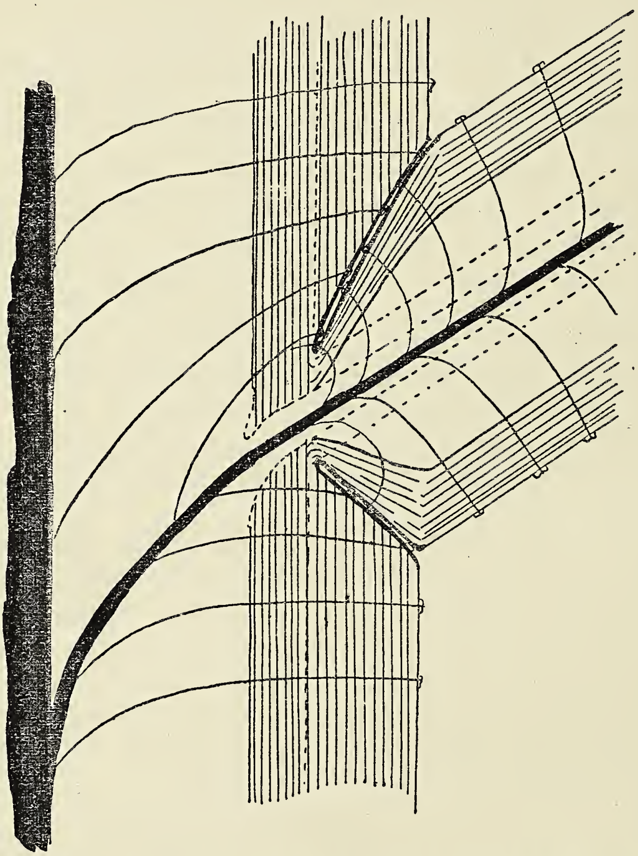
[2] Mém. Soc. Géol. Belgique, t. ii, pp. 37–82, Pl. VII–IX.

[3] Ann. de la Soc. Géol. du Nord, t. xxxix, 1910, pp. 345–61.

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

The important part of M. Renier's evidence is as follows:

1. M. Renier has found a specimen of a type which, although not common, is still not unusual, in which the quincuncial arrangement of the leaf-traces on the trunk is continued on to the area of the scar on an internal cast. This is held to show that the inner surface of the scar is of the same morphological nature as the inner surface of the outer cortex of the trunk.



TEXT-FIG. 1. A diagram of the base of a ulodendroid branch of *Bothrodendron*, prepared to illustrate M. A. Renier's theory of the origin of the ulodendroid scar. The dotted lines show the putative original size of the trunk and branch. Wood, black; middle cortex left blank; outer cortex with ruled lining.

2. In a case where both specimen and counterpart of an excellent specimen of *Ulodendron* are preserved, M. Renier points out that the leaf-trace scars on any ulodendroid scar of the specimen which is a cast of the inner side of the outer cortex do not correspond with those of the reverse of the same scar which is an impression of the external surface. M. Renier claims that this non-correspondence can only be explained by assuming that

the external surface of the scar really represents the internal surface of the conical base of the branch demanded by his theory. This is by far the most important argument advanced by him.

3. M. Renier points out that, in the specimen on which the above observation was made, the leaf-trace sections on a cast of an admittedly subcortical surface of the stem are depressions; the mould of the external surface of the scar also has its leaf-trace sections represented by impressions, and it is reasonable to assume that it is therefore of the same nature as the subcortical cast of the stem.

4. There is usually a raised rim round a ulodendroid scar which can only be a part of the base of the branch.

This is a statement—I believe a fair one—of the whole of the evidence brought forward in support of M. Renier's theory. It will be noticed that none of it is direct; in all cases it depends on the interpretation of certain observed facts.

DISCUSSION OF M. RENIER'S THEORY.

M. Renier's theory is open to many objections from a purely general consideration of our knowledge of lepidodendroid stems; some of these are pointed out below.

The structure of the lepidodendroid stem, as it is now known in about twenty-five species of the genera *Lepidodendron*, *Lepidophloios*, *Sigillaria*, and *Bothrodendron*, is as follows:

1. There is a stele of small size relatively to the diameter of the stem.

2. Surrounding the stele is a broad zone of middle cortex, composed of very soft tissue, usually with intercellular spaces. This tissue decays very readily and is usually lacking in petrified specimens.

3. Surrounding the middle cortex is the outer cortex, composed of hard cells which are proved to be very resistant to decay. This tissue is either uniform throughout or shows a gradual change of character along a radial line.

In the middle of the outer cortex the secondary cortex arises from a cambium which is set up by the division of a cylinder of cells. The secondary cortex seems to resist decay better than any other tissue in the plant: in petrified material, both from the coal balls and such isolated specimens as the types of *L. Wünschianum*, it is always well preserved, and scraps of it are very common objects in coal-ball sections.

4. Outside the secondary cortex is a thin layer of outer cortex carrying the leaf-bases. In really old specimens this seems to have been commonly shed.

The problem of the 'growth and development' of lepidodendroid stems is a difficult one, and for our present purpose it is unnecessary to discuss the whole of it; it is, however, necessary to discover what increase in the diameter of a lepidodendroid stem may be expected as a result of secondary growth.

It must be pointed out at once that the case is quite incomparable with any that we have an opportunity of examining in living material, as, except for the Lycopods themselves, no living plant has an anatomy of the general type of *Lepidodendron*, and all the angiospermous trees differ so absolutely in their structure as to give us no help.

It seems certain that when once the wood and outer cortex of a lepidodendroid stem were laid down and matured, their diameter was fixed, as additional tracheides or cells cannot have been introduced.

It must be noticed that this fact, the fixity in dimensions of the primary outer cortex and wood when once matured, implies an enormous mass of living and dividing tissue at the growing point.

I have seen purely primary stems of the order of 15 cm. in diameter, and much larger examples must occur. The idea of a growing point of the order of 20 cm. in diameter is a somewhat remarkable one, but seems to be supported by impressions. Several specimens are known in which a branch whose diameter is of this order ends suddenly, the leaves forming a bunch all over it. A similar occurrence is known in *Stigmaria*. These cases seem to me to be real growing points, and not to be explicable by any injury.

If we can regard the diameter of the outer cortex as fixed—and, so far as I can see, we must do so—it provides us with a datum by which we can measure the actual increase in diameter of the stem owing to its secondary growth. That we are justified in making this assumption is, I think, rendered very probable by the fact that, whilst, in stems with an appreciable amount of secondary cortex, the cylinder of primary outer cortex which lies outside it and carries the leaf-bases is usually split longitudinally, having been unable to stretch sufficiently to accommodate the growing trunk, the precisely similar ring of primary outer cortex within the periderm is never fissured or in any way injured. This view implies that the secondary growth of the wood has no influence on the diameter of the stem, being allowed for by the crushing up of the middle cortex (the volume of the secondary wood in most cases is so small in comparison with that of the middle cortex as to allow of this taking place).

Accepting the diameter of the primary outer cortex as constant, it is easily seen that the increase in diameter of any lepidodendroid stem is twice the thickness of the secondary cortex. The thickness of the secondary cortex in proportion to the diameter of the stem is always small; in large trunks, in which it is usually greater than in small branches, it may be as much as 9 cm. in a stem of 30 cm. ultimate diameter, as in the Dalmeny tree of *L. Wünschianum*. This implies an increase of only one-half of the original diameter, an amount which seems to be about the maximum in the very large series of sections I have examined.

We are now in a position to examine into the possibility of M. Renier's explanation of the ulodendroid scar.

If his view be correct, it should be possible to find a whole series of specimens of *Ulodendron* in the youngest of which the umbilicus was of nearly the same diameter as the scar, whilst progressively older ones show a gradual increase in the diameter of the scar as compared with the umbilicus. In the whole series of *Ulodendrons* which I have examined, about two hundred in number, none has an umbilicus more than one quarter of the scar's diameter. This fact alone tells strongly against M. Renier's view.

In the great majority of Ulodendrons, such, for example, as the originals of the figures of this paper, the diameter of the umbilicus is about one-fifth of that of the scar. In *Bothrodendron* this ratio often drops to one-eighth. On M. Renier's theory, the diameter of the umbilicus represents the original diameter of the branch, which has increased by secondary growth to the diameter of the scar; that is, the branch must have increased to five or more times its original diameter, but we have seen above that the actual greatest known increase in diameter of lepidodendroid stem observed, in a series which is now very large, is of the order of one-half its original diameter; that is, only one-tenth of the amount required by M. Renier's theory of the ulodendroid scar.

If we suppose, for the sake of argument, that M. Renier's theory is a true one, and that his 'Schema 2' or my Text-fig. 1 represents the condition of affairs at the bottom of a ulodendroid branch, then it appears that the whole of the stele, inner, and middle cortex of a branch 4 cm. in diameter are only in connexion with the corresponding tissues of the trunk by a neck 5 mm. in diameter, i. e. only one-eighth of the diameter of the branch.

In my last paper on the ulodendroid scar I showed that the diameter of the wood alone, in stems comparable to this in size, is of the order of one-eighth of the diameter of the branch; in other words, the actual wood strand of the branch of M. Renier's 'Schema 2' would only just pass through the hole into the trunk without contraction, and the rest of the stele and the very important middle cortex would be completely cut off.[1] If, on the other hand, we imagine the stele of the branch to have retained its putative original diameter, then we must suppose that in the mature condition the area of the living and conducting tissues of the base of the branch were suddenly reduced to $\frac{1}{64}$, i. e. $(\frac{1}{8})$, of their section further out, which is manifestly improbable.

The foregoing discussion will, I think, have shown the improbability of M. Renier's theory from a purely general standpoint. I now propose to discuss very briefly the case of calamitian branches, which he adduces as analogous to his theory.

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[1] Renier, op. cit., p. 49.

On p. 49, M. Renier says:

> 'Les cicatrices ulodendroïdes résultent évidemment de l'accroissement simultané en diamètre de la tige et des rameaux. Ceux-ci acquéraient ainsi une base conique tout comme les rameaux des *Calamites*.'

The structure of calamitian stems is so extremely different from that of those of *Lepidodendron* that comparisons of the two always seem to be very unfortunate, and in the present case rest on a complete misconception of the mode of branching of a calamite.

The branching calamitian specimens to which M. Renier obviously refers are those of which the originals of Figs. 3 and 2 of Scott's 'Studies' are examples.[1] It is true they resemble superficially the D'Arcy Thompson specimen of *Ulodendron*.[2] Specimens of this type, however, are invariably only pith casts. Williamson and Scott have shown that mature calamitian branches show no contraction at their base. Dr. Scott has kindly pointed out to me that in very young primary specimens the attachment of branches was really small, but this contraction is obliterated during the secondary growth, and affords no parallel to M. Renier's theory of the ulodendroid scar.

Before discussing M. Renier's specimens, it is necessary to obtain clear ideas of the meaning of the various surfaces which can be seen in impression material of lepidodendroid stems. As M. Renier correctly points out, there are at least three 'assises cellulaires' which may be preserved in impression material.

The innermost of these is commonly the wood, but in a few cases is really the inner cortex, which is usually preserved in petrified stems of *Lepidodendron vasculare* and *Bothrodendron mundum*. This zone is not of much importance for our present purpose.

The other two zones may give three distinct types of surface:

(1) On the external surface, the leaf-bases.

(2) The outer surface of the secondary cortex within the leaf-bases.

(3) The inner surface of the outer cortex.

It appears to be probable that in some species old trunks of *Lepidodendron* shed their leaf-bases, leaving only the secondary cortex exposed. Such specimens have only two types of cortical surface.

It is of interest to try and discover what thickness of coal film may be expected as the result of the conversion of any given thickness of outer cortex into coal. The average specific gravity of coal is about 1·25; that of dry cork 0·3. This means that, in being converted into coal, cork is reduced to about 0·24 of its volume if we assume, as is of course not the case, that no material is lost in such change. Making a slight allowance for this loss, we find that it is probable that a piece of coal occupies only one-fifth of the

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[1] D. H. Scott, Studies in Fossil Botany, 2nd ed., vol. i, 1908, pp. 17 and 18.

[2] D. W. Thompson, Notes on *Ulodendron* and *Halonia*. Trans. Edin. Geol. Soc., vol. iii, 1880.

volume of the cork from which it is made; that is, that each centimetre of secondary cortex of a tree-trunk will, on fossilization, be only represented by 0·2 cm. of coal film. To take as an example the thickest secondary cortex of any petrified *Lepidodendron* represented in the Manchester collections, we find that the secondary cortex of the Dalmeny tree of *L. Wünschianum*, which is 4·5 cm. through, would be represented by a coal film 1 cm. in thickness. Coal films whose thickness is of this order are often found in connexion with large lepidodendroid trunks.

M. Renier's first specimen is easily explicable on the theory that the branch is attached to the whole area of the scar. The description of it as showing the quincuncial arrangement of the leaf-trace scars of the trunk continued on to the scar is not quite correct. What actually happens is that the multispiral arrangement of the leaf-trace sections on the scar, which are obviously related to the vascular bundle of the branch, is continued on to the ordinary surface of the trunk. This means simply that these latter traces arise from the branch stele after it has parted from that of the trunk. This explanation applies, whichever theory of the scar be held, and the facts are familiar in structural material; cf. Professor Weiss's figures of the 'biserial *Halonia*'.

The suggestion that it necessarily indicates the similar morphological nature of the inner surface of the scar-tissue and the outer cortex of the trunk is not true, for it is obvious that the intersection of the leaf-traces by a thick abscission tissue, such as is postulated on my theory of the scar, will produce results closely resembling their intersection by the secondary outer cortex.

Although I am not quite convinced of the accuracy of M. Renier's statement that the leaf-trace sections of the inner and outer surfaces of the ulodendroid scars of his second specimen do not correspond, I will accept it for the sake of argument.

I find the thickness of the coal film covering a ulodendroid scar, in the comparatively few (about fifteen) specimens still retaining such a film which I have been able to examine, to be about 1 mm.

I have already shown that this must represent a tissue in the plant at least 4 mm., or more probably 5 mm. thick. This would on my theory represent the thickness of the abscission tissue. Except rarely in the lower part of scars, the leaf-traces will never enter this tissue at right angles; they generally do so at a considerable angle. If they do so at 45°, then in passing through the tissue of the scar, which I have shown above to have been in all probability at least 4 mm. in thickness, each will have moved outward a distance of 4 mm., which is ample to explain the differences in position of the sections on the two surfaces of M. Renier's scar.

M. Renier's third piece of evidence is of lesser importance. I shall show later on in this paper that the scars of leaf-traces may be represented either by projections or depressions on the outer side of the same tissue in the same specimen.

M. Renier's fourth piece of evidence does not distinguish between the two theories.

It now remains to examine the two structural specimens to which M. Renier appeals in support of his theory ; both were described by Professor Weiss, and full series of sections of them are in the Manchester Museum.

The first case is the large stem of *Lepidodendron fuliginosum* type, which was described by Weiss as the 'biserial *Halonia*'.[1] This specimen is regarded by Kidston as a *Ulodendron*.

It is unfortunately decorticated to such an extent that in the neighbourhood of the lateral branches little or none of the secondary cortex is preserved, and therefore is not in a condition to show that reduplication of the cortex which M. Renier claims to see in it. The inner part of the outer cortex is, however, very thick and well preserved, and is directly continuous from the trunk into the branch. The middle cortex of the trunk is also directly and widely continuous with that of the branch.

Professor Weiss's account is, of course, both accurate and complete, and renders it unnecessary to discuss the stem further.

The other case, the *Lepidodendron vasculare* figured by Weiss and Lomax,[2] is slightly different, because the branch, instead of leaving the trunk nearly horizontally, inclines strongly upwards.

Examination of Fig. 3 of this paper, which is a median longitudinal section of a precisely similar specimen, will show that there is no contraction at the base of the branch, which is in organic connexion with the trunk over an area of 5 cm., an amount greatly in excess of the diameter of the umbilicus of any known ulodendroid scar.

The longitudinal section figured in Fig. 3 explains the condition observed in *Knorria* by Solms-Laubach :[3]

> 'In the *Knorriae* . . . the thinner lateral branch will often impede the further growth in thickness of the main stem, and then the base of the side branchis seen to be embedded on a lateral groove which forms on the stem.

Knorria is a condition which really represents an internal cast of the outer cortex ; the groove referred to by Solms-Laubach is really only a cast of the tongue of outer cortex coming down between the branch and the stem. This tongue is clearly shown in Fig. 3, and equally well in a series of transverse sections of a similar condition on a smaller scale, in Dr. Scott's collection.

M. Renier's suggestion, on p. 50 of his memoir, that *Lepidodendron*

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[1] Trans. Linn. Soc. London, 2nd ser., Bot., vol. vi, pp. 217–35.

[2] F. E. Weiss and J. Lomax, The Stem and Branches of *Lepodendron selaginoides*. Mem. and Proc. Manchester Lit. and Phil. Soc., vol. xlix, Mem. 17, 1905.

[3] Solms-Laubach, Fossil Botany, Oxford, 1891, p. 205.

vasculare may be a *Bothrodendron*, cannot be upheld, as the work of Hovelacque[1] has made us well acquainted with the characters of the leaf-cushions, which are typically lepidodendroid, and project considerably.

It thus appears that the whole of the evidence that M. Renier has furnished in support of his theory is either incorrectly reported or readily explicable on the abscission layer theory of ulodendroid scars.

DESCRIPTION OF EXCEPTIONALLY WELL-PRESERVED SPECIMENS OF ULODENDRON.

I now propose to describe two exceptionally well-preserved specimens of *Ulodendron* which show that the leaf-traces seen in section on the outer surface of a ulodendroid scar have passed through the tissue of that scar and arisen within the trunk. The proof of this will show that M. Renier's theory, on which the outer surface of the scar is the inner side of the outer cortex of the branch, and should of course receive its leaf-trace bundles from without, is incorrect.

These two specimens belong to the Manchester Museum, and are examples of *Ulodendron*, *sensu strictu*; they are preserved as flattened trunks in shale, and are remarkable because they show the outer face of ulodendroid scars as positives on a coal film.

The finer specimen is a piece of trunk, which in its present flattened state measures 19 cm. across, and is 22 cm. long; in this distance it bears three and a half large ulodendroid scars. The whole of one side is still covered with a coal film, the surface of which is preserved with extraordinary perfection, being almost completely free from slickensides.

The stem surface shows the longitudinal cracks which have been luminously explained by Kidston,[2] as being due to the rupture of the outer layers of the cortex, to accommodate the expanding stem. In the specimen under consideration, these cracks occur also on the scars, showing that considerable secondary growth took place after their formation.

The best-preserved scar is represented by Pl. XXXVIII, Fig. 3.

The general features are well shown in that figure, the sharply marked-off umbilicus, whose gently sloping edges show on the upper half small projections of the same size as the leaf-traces, which can only be the sections of leaf-traces cut during the almost vertical part of their passage, before they turn out into the middle cortex. The whole surface of the umbilicus is covered by a coal film, a fact which is against M. Renier's theory, because on his view it necessarily represents the phloem and middle cortex in addition to the wood of the branch, and these tissues would almost certainly have been defective.

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[1] Mém. Soc. Linn. de Normandie, xvii.

[2] Ann. and Mag. Nat. Hist., ser. v, vol. xvi, pp. 123, 162, 239.

The extra-umbilical part of the scar shows excellently the transition from the short, almost circular, sections of the leaf-traces in the lower part, to the elongated scars of the upper part of the ulodendroid disc.

In the lower part, each leaf-trace section is raised on a little cushion, which presents a quite illusory resemblance to a true leaf-base. Examination of the figure will confirm this.

In the upper part each leaf-trace is seen to cut the surface of the scar as an elongated groove, which dips down into the stem towards the umbilicus. Between the point where the leaf-trace has entered the scar surface and the centre, the coal film forms a low ridge, which can only be due to the persistence of the leaf-trace, either within the scar tissue or below it, from the intersection to the umbilicus. This scar therefore affords absolute proof that the outer surface of a ulodendroid scar has received its leaf-trace bundles from within the trunk.

The fact shown in this scar, and still more clearly in the other two perfect scars of the same specimen, that the leaf-trace sections appear as depressions on the upper, and as raised papillae on the lower, part of the same scar, shows that the third line of evidence does not hold in the present example.

The other *Ulodendron* which I wish to describe is, like the first, a flattened trunk 20 cm. in maximum width, and 66 cm. long, in which distance it bears nine ulodendroid scars on one side, and ten on the other. The coal film has been largely removed from this specimen, leaving a cast of a sub-cortical surface. On this the areas of the scars are faintly marked off in a way not readily intelligible in the light of M. Renier's explanation.

Three ulodendroid scars show a good deal of the coal film. The outer surface of this is well preserved, and confirms the facts observed in the other specimen that I have described. They also show that the leaf-traces seen in section on the outer surface of a ulodendroid scar have sprung from the branch stele whilst still inside the main trunk.

I believe that these two specimens, together with that which I originally described, which is not explained by M. Renier, and seems to be inexplicable on his theory, are sufficient to demonstrate that the branch was attached to the whole area of the scar.

The structure of the scar as seen in the specimens described above, which are probably better preserved than any which have previously been figured, its definiteness, and the smoothness of its surface, can only be explained, if the branch were attached to the whole area of the scar, by calling in the aid of a definite abscission layer comparable to that of leaf-fall. That we are justified in doing so is shown by the description below of petrified specimens showing such a mechanism. Although it is not certain that these actual examples were Ulodendra, the occurrence of cladopsis in a lepidodendroid shows that we are quite justified in supposing that such a process occurred in *Ulodendron*, where we have strong indirect evidence that such was the case.

DESCRIPTION OF AN ABSCISSION LAYER IN STRUCTURAL MATERIAL.

The two structural specimens in which I am able to demonstrate the presence of a cladopsis mechanism are both members of the genus *Lepidophloios*, the first being that described by Williamson in his nineteenth memoir as 'Wild's *Ulodendron*'; though crushed flat, this specimen is very beautifully preserved, and although undoubtedly a *Lepidophloios*, it bore two opposite rows of lateral branches.

The other specimen is an excellently preserved example of a *Lepidophloios* agreeing closely with *L. Harcourtii*; it comes from Shore and is represented by long series of transverse and longitudinal sections in my own collection. The evidence available is not sufficient to determine the arrangement of the lateral branches, which, however, are rather sparsely and apparently irregularly scattered, possibly somewhat as in Lindley and Hutton's *Halonia tortuosa.*

These specimens agree exactly in the structure of the abscission layer which cuts off their lateral branches, but this layer is somewhat younger in 'Wild's *Ulodendron*' than in the other case.

In a transverse section of the specimen of *L. Harcourtii*, No. 203, part of which is shown in Pl. XXXVIII, Fig. 2, a lateral branch, 14 mm. in diameter, is shown in accurate median longitudinal section. Of this branch only a short stump 5 mm. long is preserved, but this shows in excellent preservation the outer, middle, and inner cortices and the stele. In the section figured, the stele is shown in median section, passing inwards till it reaches the level of the periderm of the stem; it then, as is shown by other sections, turns sharply downwards, and passes in gradually until it reaches the stele of the main stem.

The leaf-base-bearing tissue of the stem is directly continuous with that of the branch, and from the periderm of the stem a little upstanding rim of periderm is formed in the base of the branch; this is quite normal and surrounds the branch in the usual position in the primary outer cortex.

Inside the outer cortex the thick and well-preserved middle cortex occurs. It has the ordinary loose tissue full of air spaces, but is not composed of long hyphae-like filaments, as is that of *L. fuliginosum.* Inside this are the inner cortex and stele, which show no unusual features. Such in brief is the structure of the branch. The evidence of other specimens, such as Weiss's biserial *Halonia*, shows that in the young stem all these tissues of the branch are in direct connexion with the corresponding tissues of the stem.

In the present case, however, the whole base of the branch is cut off by a thick layer of secondary tissue. This layer is nearly flat, but possesses a depression, forming a small conical pit on its outer surface round the stele of the branch.

TEXT-FIG. 2. A diagram of the base of a ulodendroid branch, to illustrate the abscission-layer theory of its origin. Wood, black; middle cortex left blank; primary outer cortex stippled; secondary cortex ruled; abscission layer cross-hatched.

This secondary tissue has obviously been formed by a cambium which has arisen in the living tissues of the base of the branch—in the inner layers of the outer cortex, middle cortex, and stele, without any distinction; it cuts right across everything and must have rapidly led to the death of the branch.

In both cases, but particularly in the case under discussion, this layer of secondary tissue becomes continuous with the general periderm of the stem, but it joins this only where the periderm of the branch turns out from it, so as to leave the latter as a low rim round the branch scar.

Finally, in the specimen under discussion, another cambium arising in the deeper layers of the outer cortex has developed a considerable amount of secondary tissue within the main periderm. This cambium appears to have spread to the middle cortex of the base of the branch, where a thick layer of secondary tissue, indistinguishable from that of the main stem, has been formed. In fact, the final result is that the separative tissue across the base of the branch has completely assimilated itself to the secondary cortex of the main stem. This fact explains some of the appearances by M. Renier in impression material of *Ulodendron.*

That the tissue I have just described is really an abscission layer is, I think, certain from its close resemblance to the ordinary mechanism of leaf-fall of dicotyledonous trees, and to the cladopsis of *Agathis.*

It thus seems to be almost certain that the typical ulodendroid scar is a thickened abscission layer at the base of a laterally issuing branch.

GENERAL DISCUSSION OF ULODENDRON.

It will be of interest to examine *Ulodendron*, and also *Halonia*, in the light of this new knowledge.

As Dr. Kidston pointed out many years ago, three types of the ulodendroid condition occur:[1]

1. With the scars oval, well separated, and with a central umbilicus.
2. With the scars oval, well separated, and with an eccentric umbilicus.
3. With the scars circular, close together, and with a central umbilicus.

Specimens at Manchester suggest that there is still a fourth type.

4. With the scars circular, well separated, and with a central umbilicus.

It is useful to consider the meaning of the difference between these types. The difference in the shape of the scar is easily seen to be entirely dependent on the direction of the branch; circular scars can only be produced when the branch issues horizontally and at right angles to the surface of the trunk.

Oval scars will be produced when the branch does not leave the surface at right angles, but inclines upward. Its section by the outer surface will then be nearly an ellipse.

The position of the umbilicus is of more interest; it is certain that in those cases where the scars are oval and the umbilicus eccentric, the surface of the scar was, during life, a deep conical pit. Casts of this pit are very common, and that it was present is always obvious even from the examination of crushed specimens.

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[1] Loc. cit.

The other type of oval scar, with a central umbilicus, can only be produced when the outer surface of the scar was, in life, nearly flat; this is readily obvious from the inspection of hypothetical median longitudinal sections.

It thus appears that ulodendroid scars may be flat or nearly so.

It is almost certain that the scars of *Ulodendron* proper, that is, the type with contiguous circular scars and a central umbilicus, were also usually flat, or at most only slightly depressed.

The proof of this statement is as follows:

1. There is in the Manchester Museum a portion of a round stem of *Ulodendron* preserved in ironstone, which is apparently uncrushed, and in which the scars are essentially flat.

2. If the scar had been at all deeply conical, the tissue composing it would have been puckered when it was crushed flat. Such well-preserved external surfaces as the originals of Pl. XXXVIII, Figs. 1 and 2, never show any sign of such puckering.

At the same time, a well-preserved specimen from Low Moor, in the Bradford Museum, which is preserved in ironstone, and hence only slightly crushed, does show slightly depressed areas. The only specimen which has ever been figured as showing the scars as deep conical pits is that described by Carruthers.[1] This specimen, which I have never seen, has probably been misinterpreted. It is possible that it was so preserved that the leaf-trace bundles going to the scar were interpreted as the scar surface itself; at any rate it seems certain that, in the vast majority of cases, the scars of *Ulodendron*, *sensu strictu*, were flat, or at most but slightly depressed.

The question of the attribution of ulodendroid trunks to different genera presents some difficulties.

It is generally conceded by all palaeobotanists that those specimens with oval, well-separated scars and an eccentric umbilicus belong to *Bothrodendron*.

Those specimens which have the scars oval, well separated, and with a central umbilicus, occur in the Lower Carboniferous as well as in the Coal Measures, where they appear to be very rare.

In all cases that I have seen, or found recorded in the literature, individuals of this type have their leaf-bases higher than they are broad, and apparently of the *Lepidodendron* type. Those from the Lower Carboniferous of Scotland were referred by Dr. Kidston in 1885 to *Lepidodendron veltheimianum*; the very few Coal Measure specimens I have seen are referable to Kidston's *L. Landsburgii*.

These specimens have leaf cushions often well preserved, and strikingly like those of *Lepidodendron*; but in those cases that I have seen the leaf-scar is never visible, and the leaves seem to have remained attached to the stem

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[1] Monthly Microscopical Journal, vol. iii, 1870, p. 144.

for a very long period, and not to have been shed as in an ordinary *Lepidodendron*, a fact which may be held to justify the removal of the species from *Lepidodendron* proper.

The type with contiguous circular scars was referred by Dr. Kidston to *Sigillaria*. Here again, no specimen showing well-preserved leaf-scars has ever been figured, although specimens showing the cushions perfectly preserved are not uncommon. As in the former case, this fact raises a strong suspicion that the leaves were never shed by the intervention of an abscission layer. The specimens I have seen, although not conclusive, suggest that the leaf was only attached to quite a small area at the top of the leaf-cushion, instead of to a very large part of it as in *Sigillaria*.

On the whole it is perhaps best to keep the genus *Ulodendron*, and to include in it both types, that with long leaf-bases, and that with short, square cushions. At the same time it must be understood that the difference between these and *Lepidodendron* is very small, probably consisting solely in the longer retention of the leaves.

This long retention of the leaves is probably due to the fact that, when the whole branch was shed, it was unnecessary to make special arrangements for shedding each individual leaf.

The fact that the specimen of *Lepidodendron fuliginosum* type described by Professor Weiss as a biserial *Halonia* has been identified by Kidston as *Sigillaria discophora*, an identification with which I agree, shows that *Ulodendron*, in its structure, approaches much more nearly to the *Lepidodendron* type than it does to any form of *Sigillaria*.

GENERAL DISCUSSION OF HALONIA.

It is of interest to consider the halonial branch in the light of our knowledge of *Ulodendron*.

As has been recognized for many years, the two forms resemble one another in being lepidodendroid stems which have borne series of lateral appendages. Much confusion between the two types exists in the literature, but when well preserved they are readily distinguished. As Dr. Kidston pointed out many years ago in a classical paper, *Ulodendron* has invariably its scars in two opposite rows, whilst in *Halonia* they are typically in a series of spirals running round the stem. Another difference which seems to me quite typical is that *Halonia* is very often found branching, forming in fact the ultimate branches of the tree, whilst only one branching specimen of *Ulodendron* seems to be known, and the stems always seem to be the main trunks, a conclusion which is proved in the case of the two vertical specimens in the Manchester Museum.

Dr. Kidston has shown that all satisfactory specimens of *Halonia* belong to the genus *Lepidophloios*.

In a typical specimen of *Halonia*, preserved so as to retain the leaf-bases, such for example as the specimen figured by Williamson in his nineteenth memoir, Pl. VI, Fig. 25 A, as a multiseriate *Ulodendron*, the halonial 'tubercles' are seen in the form of large, slightly depressed areas, recalling the scars of *Ulodendron.* They are, however, of very different structure ; each scar is divisible into two concentric areas, of which the inner is usually smooth and shallowly concave, whilst the outer is divided into irregular segments by more or less radial lines. Examination of the outer area in any well-preserved specimen will show that it is solely impressed on the long leaf-bases of the stem, having been formed by the pressure of the equally long leaf-bases of the branch. In the case of any of the scars in Williamson's specimen, the second and third from the top in the centre row being the best preserved, the inner area which is not leaf-base is 0·5 cm. in diameter, whilst the whole area is 1·5 cm. across. These sections should be the diameters of the outer cortex of the branch and of the whole branch. A section of *Lepidophloios macrophyllum* (Q. 418, Cash Coll., Manchester Museum), 1·5 cm. in greatest diameter, has an outer cortex only 0·5 cm. in diameter, and so agrees exactly with the condition which must have occurred in the original of Williamson's figure. The fact that the greater part of the halonial area is made of the leaf-bases shows that this type can only occur in *Lepidophloios*, which genus alone has leaf-bases long enough to form the outer part of the area. What is in essence this explanation has already been given by Dr. Kidston.

One very interesting specimen of *Halonia* from the famous quarry 'Peel Delph', which is in the Manchester Museum, is preserved as a very fine cast in sandstone of the interior of the outer cortex. This cast is still attached to the matrix, and shows that the very prominent tubercles, which occur in all Halonias preserved as Knorrias, do not touch the mould of the outer surface of the stem by nearly 1 mm. This space must represent some tissue in the plant which had not decayed at the time it was buried, and as it cuts completely across the base of the branch, this structure can only be an abscission layer, similar to that which I have demonstrated in structural material. The halonial tubercles themselves obviously represent the casts of the holes in the outer cortex left by the decay of the middle cortex of the base of the branch.

The evidence that Dr. Kidston brought forward in the case of the halonial branches of *Lepidophloios scoticus*, although not quite conclusive, seems to render it very probable that the appendages of the halonial tubercles of that type were cones borne on the end of long peduncles.

The fact that the possible increase in diameter of lepidodendroid stems by secondary growth is very small shows that in such cases as that figured by Williamson (nineteenth memoir, Pl. VIII, Fig. 23), the lateral appendages of halonial tubercles must have been of very large size—at least 2 cm. in diameter in the case cited; this is considerably larger than the axis of any known lepidodendroid cone, and hence probably of any cone peduncle, which suggests that in some cases at any rate the lateral branches were not cones.

The above brief discussion shows that halonial tubercles bore lateral branches (which may in some cases have been the peduncles of cones), which were shed by the intervention of an abscission layer, just as were the branches borne by ulodendroid scars, and that the two cases differ in the arrangement of the branches, in their position on the plant, and in the structure of the scars owing to the presence in *Halonia* of an aureola of leaf-bases pressed down by those of the branch, a condition only possible in *Lepidophloios.*

SUMMARY OF NEW AND DISPUTED FACTS AND CONCLUSIONS.

1. In Lepidodendroids, small branches issuing laterally were sometimes shed by an abscission layer, developed from a cambium which forms across the base of the branch in all its living tissues, except that part of the primary outer cortex which lies outside the secondary cortex. This abscission layer becomes connected with the secondary cortex of the main stem, and in old age entirely takes on the structure of the latter.
2. The ulodendroid scar is such an abscission layer cutting off a branch which was formerly attached to its whole area.
3. *Halonia* is essentially similar to *Ulodendron*, in that it represents a stem from which lateral branches have been cut off by an abscission layer.
4. The most practical distinction between *Halonia* and *Ulodendron* lies in the arrangement of the branches, but the scars themselves are quite different when well preserved.
5. The well-preserved halonial scar is divisible into two areas, of which the inner represents the abscission layer, whilst the outer results from the pressure of the long leaf-bases of the branch on those of the stem; consequently the halonial condition can only occur in *Lepidophloios.*
6. In some cases, at any rate, the lateral branch of *Halonia* was not the peduncle of a cone.
7. No specimen of *Ulodendron* showing a well-preserved leaf-scar has ever been described, except in that type with oval scars and an eccentric umbilicus, which belongs to *Bothrodendron.*
8. Consequently it is best to retain the generic name *Ulodendron*, as is done by most continental authorities.
9. The reason why the leaf-scars are not found is that when whole branches were shed it was unnecessary to shed the individual leaves.

10. The increase in diameter of lepidodendroid stems, owing to secondary growth, is very slight.

11. The growing points of *Lepidodendron* must have been of very great size.

I wish to express my thanks to Professor Sir T. H. Holland, in whose department in Manchester this paper was written some time ago, and to Professor F. E. Weiss and Dr. D. H. Scott, for advice and criticism in relation to it.

EXPLANATION OF PLATE XXXVIII.

Illustrating Mr. Watson's paper on the Structure and Origin of the Ulodendroid Scar.

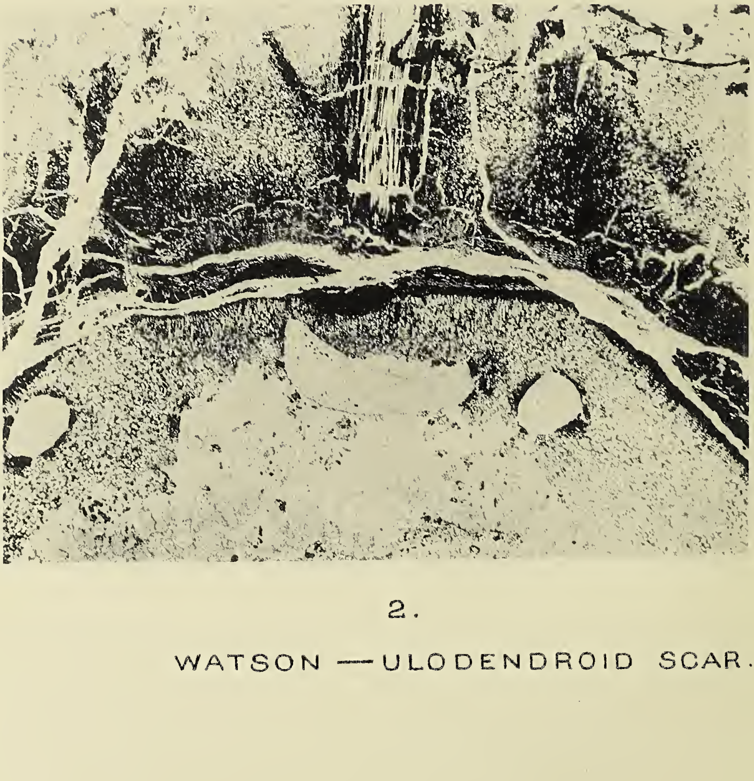
Fig. 1. 'Wild's *Ulodendron*.' Transverse section of a large stem giving off a lateral branch, the stele of which is just cut tangentially at the extreme top of the photograph. The lighter coloured tissue surrounding this stele and the corresponding light area in the main stem are the middle cortex. The separative tissue is clearly seen cutting across this, and at the extreme right it comes in contact with the periderm of the main stem. Specimen in the Manchester Museum.

Fig. 2. Transverse section of a stem of *Lepidophloios Harcourtii*, showing a lateral branch in median longitudinal section. This is fully described in the text, p. 491. A 20, D. M. S. Watson Collection.

Fig. 3. *Ulodendron majus*. Photograph of an exceptionally well-preserved scar; everything visible is on a coal film and hence represents the real exterior surface. Further described at p. 489. X. I., Manchester Museum.

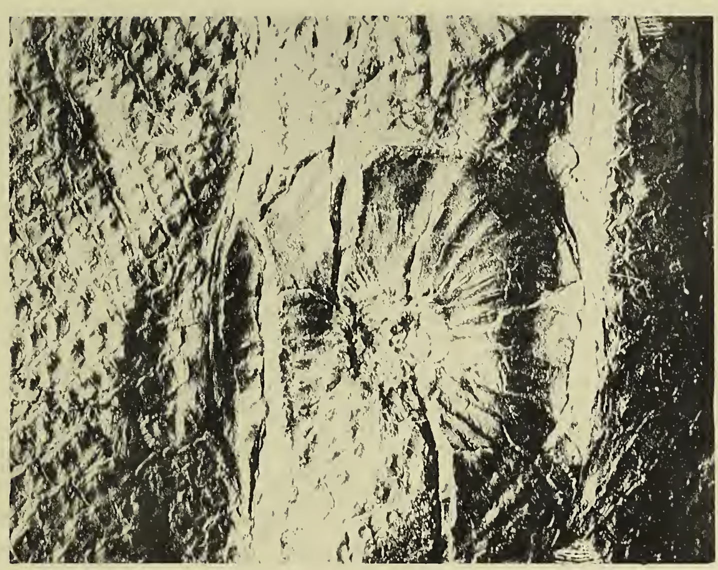
Fig. 4. *Ulodendron*. Photograph of a scar still retaining some of its coal film. Further described at p. 490. X. I., Manchester Museum.

1.



2.

WATSON — ULODENDROID SCAR.



3.

4.

Huth coll.

On a Chemical Peculiarity of the Dimorphic Anthers of Lagerstroemia indica, with a Suggestion as to its Ecological Significance.

BY

J. ARTHUR HARRIS.

With two Figures in the Text.

I. INTRODUCTORY REMARKS.

ABOUT ten years ago I observed certain peculiarities in the dimorphic anthers of the frequently cultivated ornamental shrub, *Lagerstroemia indica*, which seemed worthy of detailed investigation, but the results of studies made in 1903, 1904, and 1905 at the Missouri Botanical Garden have lain among my notes since that time because I have hoped to be able to verify the essential points and to fill in the lacunae. At present I see no prospect of doing anything further with the problem. Nor do I know personally any one who might take my data and complete the work. It seems only right, therefore, to publish the results attained for the benefit of any one who may have the inclination and the opportunity to do the work more thoroughly.

II. FLORAL STRUCTURE AND ANTHESIS.

Briefly described, the structure of the flower is as follows: The petals are unguiculate, with an oval to orbicular crispate limb. The numerous stamens have filiform filaments and elliptical anthers with pulviniform connectives. The greater number of the stamens are bright yellow in colour, but one in front of each sepal is usually larger, both as to filament and anther, and of a reddish instead of a yellow colour. In young buds the anthers are not distinguishable by their colour, both kinds being a light yellow. The characteristic colours are developed as the buds grow older. A detailed study of the numerical relationships of the parts of the flower has been given elsewhere.[1]

The flowers open as a rule about half-past eight o'clock in the morning, although there is great variation in this regard, occasional flowers opening as late as five or six in the evening.

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[1] Harris, J. Arthur: Variation and Correlation in the Flowers of *Lagerstroemia indica*. Ann. Report Mo. Bot. Gard., vol. xx, 1909, pp. 97–104.

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

Upon the opening of the flower the filaments of the smaller stamens turn somewhat upwards, while the pistil is turned in the opposite direction, downwards, for the greater part of the length, but the tip is turned somewhat upward. The larger stamens bend outward proximally but curve in distally, bringing the large anthers towards each other.

These large anthers open by two introrse longitudinal slits soon (perhaps half an hour) after anthesis; their pollen forms two bright green or yellowish-green lines, in sharp contrast to the reddish ground colour of the anther, which darkens with age. The pollen is very freely and readily shed, adhering to any object brushed lightly against the surface of the opened anther.

The smaller anthers do not dehisce until about half an hour after the red ones; their pollen does not leave the cells so rapidly, and when examined microscopically generally does not appear as much shrunken by drying.

The red anthers fall first, generally about eight or ten hours after the flower opens. The yellow anthers often remain on the filaments until the second day. As far as observed, both types fall spontaneously. No insect has been observed eating entirely either type, as has been described for some other forms of pollen flowers.

III. PHYSIOLOGICAL PECULIARITIES OF THE ANTHERS.

The difference in the time of dehiscence of the two types of anthers, the fact that the larger anthers seemed to fall before the smaller, and especially the observation that their pollen seems more dry and powdery, and when microscopically examined appears more shrunken, suggested the desirability of more detailed and accurate observations to determine the nature of these phenomena.

The most plausible explanation for the peculiarities observed would seem to be a difference in the rate of evaporation of water. Two simple experiments proved the correctness of the hypothesis. (*a*) When red and yellow anthers were placed close together on a slide and their pollen crushed out simultaneously and spread out over the glass and examined under the microscope, the pollen from the red anthers dried more rapidly than that from the yellow. (*b*) The necessity of the frequent readjustment of the balance on the pans, of which lots of red and yellow anthers were exposed to the air in watch-glasses, showed that the two lots were decreasing in weight at different rates, and that the red anthers were losing more rapidly than the yellow.

A series of careful parallel weighings was then undertaken to determine as nearly as possible the difference in the rate of evaporation.[1]

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[1] There are two possible sources of error in my work. All my material came from plants kept under glass in winter and transferred to the open for the summer. This might, as Darwin suggested

For these experiments material was collected in the morning between eight and nine o'clock. Buds which were ready to open, or flowers which had just opened, were selected. In no case were anthers which had begun to dehisce employed for weighings.

For each comparison the weighings of the two types of anthers were made at approximately the same intervals of time. Conditions probably differed somewhat from series to series, but since they were the same for both kinds of anthers in any one lot, the comparison may be considered satisfactory. The general qualitative results of every individual experiment were unquestionably the same. Exact comparisons of the curves obtained for the different examinations is a matter of little importance for our immediate purposes.

In the tables[1] are given the actual weights of the masses of red and yellow anthers at different times, the absolute decrease of the weight at the last recording, and the percentage decrease calculated on the basis of the original weight.[2]

It is clear that if the red anthers lose water more rapidly than the yellow, their percentage loss will, in the early weighings, be greater than that of the yellow. If both series are practically completely desiccated, it is also clear that in the later periods the yellow must lose a higher percentage of their original moisture than the red, since the latter must have lost the greater part of their water early in the process of desiccation. A study of the tables shows this to be the case. The exact quantitative relationships vary greatly from table to table, as must be expected from the diversity of method in the carrying out of the weighings. Notwithstanding these inequalities the rule holds without exception for both published and unpublished experiments.

The best method of comparison is to sum the percentage of water lost by each type of anther up to, and including, any given weighing. The results of such summations are shown graphically for series A–C, in which

in his observations on this species, be a factor of some importance. Of course, the Orient would be the best place for such studies, but the Southern States, where the species is hardy out of doors throughout the year, would afford much better material than our northern gardens.

Again, the anthers are minute and the weighings upon which our conclusions rest must be done on very small masses of material, which to be of value must be collected with great rapidity. I am under the greatest obligation to Mr. Dean H. Rose and to Miss Olive Kerley for the exceedingly exacting routine work. If subsequent observers find errors in our work, or conclusions, they are to be attributed to me rather than to my two assistants, who were most conscientious and more deft and rapid in the manipulation of chemical balances than I.

[1] Table A gives the results of one of six series of weighings carried out by Mr. Rose. The remaining five series of comparative weighings made by him led to the same results and are not published. The data of the other tables are due to Miss Kerley.

[2] These tables are of two kinds. In A–C the loss every few minutes (except as noted) for a period of several hours is recorded. In D–G fewer weighings were made over a longer period of time, the anthers being allowed to become nearly or quite air dry. Experiments B and C were also carried over to the second day; hence in these cases, the summed percentage loss up to certain selected intervals is included for the sake of comparison in the second set of tables.

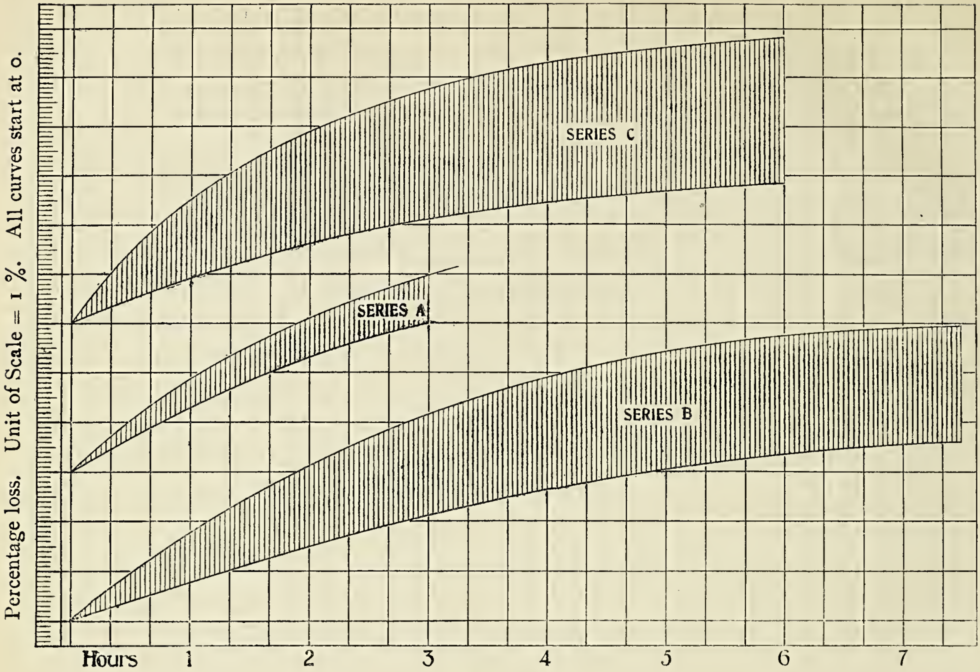
Percentage loss. Unit of Scale = 1 %. All curves start at 0.
SERIES C
SERIES A
SERIES B
Hours
1
2
3
4
5
6
7

FIG. 1.

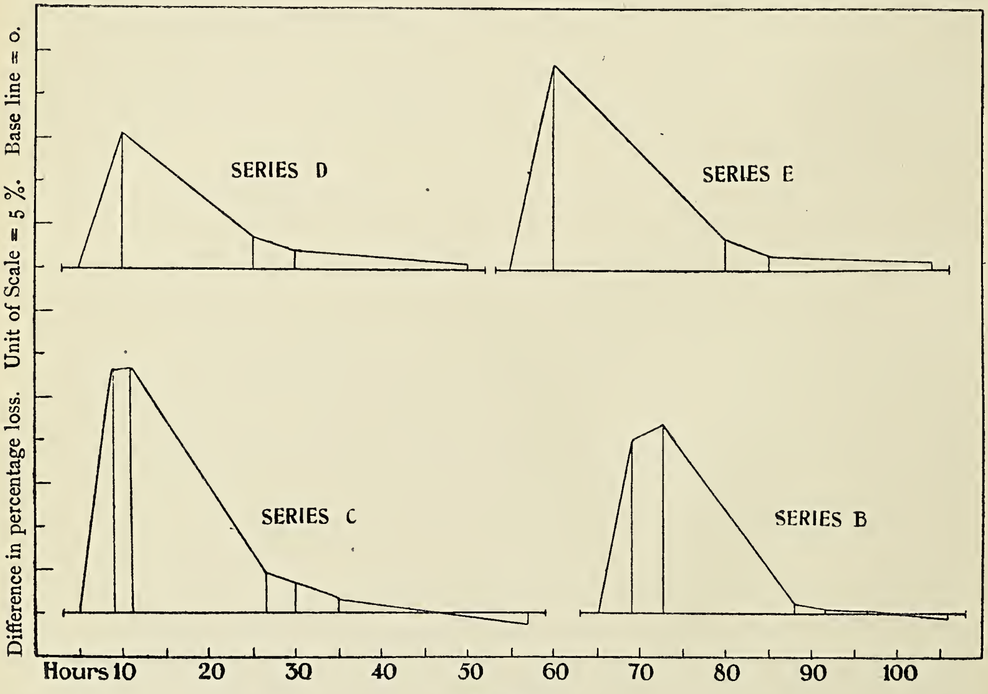
Difference in percentage loss. Unit of Scale = 5 %. Base line = 0.
SERIES D
SERIES E
SERIES C
SERIES B
Hours 10
20
30
40
50
60
70
80
90
100

FIG. 2.

weighings were made every few minutes, in Fig. 1. The rate of loss for the red anthers is represented by the upper curve, while the rate for the yellow anthers is shown by the lower one. The difference in the total relative water loss of the two types of anthers is clearly shown by the (shaded) difference between the two curves.[1] In each case the red anthers lose far more rapidly than the yellow.

Such loss cannot of course continue indefinitely. After the red anthers have lost a considerable part of their water, the rate of loss from the other type must become higher, until finally both types are air dry. This is clearly shown in a second set of diagrams, Fig. 2. In these the abscissae represent hours, while the heights of the ordinates erected at various intervals show *the percentage loss of water by the red anthers* minus *the percentage loss by the yellow anthers.* After a few hours the loss by the red anthers is far in excess of that by the yellow. Finally, the difference becomes practically zero.

IV. NATURE OF THE PHYSIOLOGICAL DIFFERENTIATION OF ANTHERS.

The immediately underlying cause of the physiological peculiarities which have been demonstrated to characterize the morphologically differentiated anthers must be either physical or chemical.

Possible physical factors are dissimilarities in form, resulting in the exposure of widely different surfaces to evaporation, or differences in the histology of the superficial layers, which are capable of modifying the amount of water lost by evaporation. Both types of anthers are of roughly the same form. I have not made critical histological comparisons, but the sections I have seen indicate that there are at least no conspicuous differences between the two types.

Thus, comparatively considered, the evidence for a physical basis of the observed differences is quite negative. Quantitatively examined, the data are strongly opposed to such an assumption. The large anthers are of about the same form as the small ones. The ratio of the surfaces of the two types of anthers will be closely enough

$$Sr : Sy :: (\sqrt[3]{Vr})^2 : (\sqrt[3]{Vy})^2,$$

where Vr, Vy are the volumes of the red and yellow anthers respectively. Volume may for all practical purposes be taken as the equivalent of weight, provided the two types of anthers have the same percentage water content.

In the experiments entered in Tables A–C the anthers were not allowed to dry completely; hence the total water lost by the red is far in

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[1] These are merely draughtsman's curves, secured by fitting a spline as closely as possible to the data given in the tables.

excess of that lost by the yellow. In the illustrative series B–G the anthers were more nearly completely air-dried. The results are:

Experiment.	*Percentage loss of Red.*	*Percentage loss of Yellow.*
B	73·67	74·02
C	73·35	74·55
D	74·00	73·70
E	75·05	74·04
F	74·74	75·16
G	73·29	74·20

In view of the technical difficulties involved, no significance is to be attached to these differences.[1] The density of the two types of anthers may be taken as about the same. Their volume may be regarded as directly proportional to their weight. The average weights are:

Series.	*Mean weight of Red Anthers.*	*Mean weight of Yellow Anthers.*
B	0·000990	0·000705
C	0·000950	0·000740
D	0·000926	0·000743
E	0.000993	0·000723

Thus there is a considerable difference in volume, which means a considerably lower ratio of surface to volume in the red anthers. This should, on purely physical grounds, be followed by a lower rate of evaporation from the large anthers. Instead, a higher initial rate obtains.

Besides these convincing evidences against the physical nature of the physiological peculiarities of the anthers, there are many indications that there is some chemical substance in the small anthers which by its presence (or by its relatively greater abundance as compared with the large anthers) limits the loss of water. The data upon which this statement is based are drawn from experiments in the loss of water, and from those on the reabsorption of water by anthers which had been washed or boiled in water, or extracted with alcohol and ether and dried. These experiments I do not care to describe in further detail, since I believe that the difficulties incurred were beyond our skill, and that the quantitative data we secured are not worth publication. That the results are due to some substance occurring only, or in much greater abundance, in the smallest anthers, I have not the slightest doubt.

V. ECOLOGICAL RELATIONSHIPS OF FLOWER.

Bees were observed as visitors, collecting pollen from the small yellow anthers, but as far as could be observed, almost without exception, taking no notice of the large ones, which are of a colour very similar to that of the unguiculate petals. The following species, kindly determined by Mr. Charles

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[1] Drying on a bath gives a higher percentage loss of water, but there is no clear evidence from several determinations that there are any constant differences in the relative water content of the two types.

Robertson, whose work on floral ecology and on bees is well known, were taken :

Megachile mendica, Cr. ♀
Megachile generosa, Cr. ♀
Bombus scutellaris, Cr. (*B. fraternus*, Sm.) ☿
Bombus americanorum, Cr. (*B. pennsylvanicus*, De G.) ☿

Chloralictus versatus, Rob., seems to visit the larger stamens frequently, but was also observed on the smaller stamens.

Apis mellifera, L. ☿
Agapostemon radiatus, Say. ♀

VI. Summary and Discussion of Observations.

In the Malayan shrub, *Lagerstroemia indica*, the stamens are dimorphic, those of the outer whorl being larger than the more central yellow group, and differing from them not only in colour, but, as Darwin showed, in the pollen which they contain.

Quantitatively, the two types of anthers bear the size (weight) ratio of about 1·3 : 1. The filaments of the large anthers are about 5–6 times as large (heavy) as those of the small ones.

The observations described in this paper show that the differentiation is not merely morphological, but that it is physiological as well. The larger (outer) anthers lose water much more rapidly by evaporation than do the smaller ones. The total loss is finally sensibly the same in the two types.

The underlying cause of the physiological differentiation seems to be chemical rather than physical. The large anthers present, in comparison with their volume, a smaller surface from which evaporation can take place, and should in consequence be expected to show a lower, instead of a higher initial water loss. Furthermore, there is no conspicuous difference in the histology of the superficial layers of cells of the two types of anthers. On the other hand, there appears to be a water-soluble substance occurring only, or in greater abundance, in the smaller anthers which lowers their rate of water loss. The nature of this substance is quite unknown.

The ecological consequence[1] of the physiological differentiation is that the pollen of the larger anthers (which are borne on longer filaments, incurved in a manner to bring them in contact with the body of a visiting insect) is dry and powdery soon after the opening of the flower, and easily scattered over the body of a visiting insect, whereas that of the smaller anthers remains for a much longer time moist, and easily massed by the bees, which collect it in large quantities.

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[1] I have reviewed elsewhere (loc. cit.) the literature of the biology of the flower of this form. It seems unnecessary to go over once more the points in detail.

The morphological and physiological dimorphism is, from the evidences at hand, as clear a case of specialization of parts to facilitate pollination as many others which have been described in the vast literature of floral ecology. The central yellow mass of anthers of the nectarless flowers furnishes the booty to the visiting insects, in a form which does not require moistening (or as much moistening, as in the case of the pollen of many other flowers) in massing for transportation. The outer whorl of anthers which are inconspicuous, because isochromatic with the corolla, furnish the dry powdery pollen for fertilization. Unfortunately for the theory of adaptation, the pollen from both types of anthers will, according to Müller, produce seed. Personally, I feel that this and other interesting problems concerning the anther morphology and physiology of this species must await studies in its natural environment.

COLD SPRING HARBOUR, N.Y.,
Jan. 26, 1914.

TABLE A.

Hour.		*Red Anthers.*		*Hour.*		*Yellow Anthers.*	
	Weight.	*Loss.*	*% Loss.*		*Weight.*	*Loss.*	*% Loss.*
8.40 A.M.	0·0300	—	—	8.40 A.M.	0·0471	—	—
8.55 ,,	0·0285	0·0015	5·00	8.50 ,,	0·0460	0·0011	2·33
9.05 ,,	0·0273	0·0012	4·00	9.05 ,,	0·0450	0·0010	2·12
9.15 ,,	0·0264	0·0009	3·00	9.20 ,,	0·0434	0·0016	3·39
9.25 ,,	0·0256	0·0008	2·66	9.30 ,,	0·0425	0·0009	1·91
9.35 ,,	0·0248	0·0008	2·66	9.40 ,,	0·0415	0·0010	2·11
9.45 ,,	0·0243	0·0005	1·66	9.50 ,,	0·0405	0·0010	2·11
9.55 ,,	0·0232	0·0011	3·66	10.0 ,,	0·0402	0·0003	0·63
10.05 ,,	0·0227	0·0005	1·66	10.10 ,,	0·0392	0·0010	2·11
10.15 ,,	0·0219	0·0008	2·66	10.20 ,,	0·0383	0·0009	1·91
10.25 ,,	0·0214	0·0005	1·66	10.30 ,,	0·0376	0·0007	1·48
10.35 ,,	0·0209	0·0005	1·66	10.40 ,,	0·0368	0·0008	1·69
10.45 ,,	0·0203	0·0006	2·00	10.50 ,,	0·0362	0·0006	1·27
10.55 ,,	0·0196	0·0007	2·33	11.0 ,,	0·0355	0·0007	1·48
11.25 ,,	0·0184	0·0012	4·00	11.20 ,,	0·0346	0·0009	1·91
11.55 ,,	0·0171	0·0013	4·33	11.50 ,,	0·0328	0·0018	3·82

TABLE B.

Hour.		*Red Anthers.*		*Hour.*		*Yellow Anthers.*	
	Weight.	*Loss.*	*% Loss.*		*Weight.*	*Loss.*	*% Loss.*
9.15 A.M.	0·0396	—	—	9.20 A.M.	0·0282	—	—
9.45 ,,	0·0352	0·0044	11·11	9.50 ,,	0·0269	0·0013	4·61
10.15 ,,	0·0320	0·0032	8·08	10.20 ,,	0·0258	0·0011	3·90
10.45 ,,	0·0298	0·0022	5·55	10.50 ,,	0·0250	0·0008	2·83
11.15 ,,	0·0277	0·0021	5·30	11.20 ,,	0·0240	0·0010	3·54
11.45 ,,	0·0254	0·0023	5·80	11.50 ,,	0·0233	0·0007	2·48
1.15 P.M.	0·0214	0·0040	10·10	1.20 P.M.	0·0209	0·0024	8·51
1.45 ,,	0·0204	0·0010	2·52	1.50 ,,	0·0206	0·0003	1·06
2.15 ,,	0·0194	0·0010	2·52	2.20 ,,	0·0200	0·0006	2·12
2.45 ,,	0·0180	0·0014	3·53	2.50 ,,	0·0194	0·0006	2·12
3.15 ,,	0·0177	0·0003	0·75	3.20 ,,	0·0189	0·0005	1·77
3.45 ,,	0·0172	0·0005	1·26	3.50 ,,	0·0183	0·0006	2·12
4.15 ,,	0·0164	0·0008	2·02	4.20 ,,	0·0180	0·0003	1·06
4.45 ,,	0·0160	0·0004	1·01	4.50 ,,	0·0176	0·0004	1·41

TABLE C.

Hour.	*Red Anthers.* *Weight.*	*Loss.*	*% Loss.*	*Hour.*	*Yellow Anthers.* *Weight.*	*Loss.*	*% Loss.*
10.30 A.M.	0·0380	—	—	10.35 A.M.	0·0296	—	—
11.0 ,,	0·0321	0·0059	15·52	11.05 ,,	0·0282	0·0014	4·73
11.30 ,,	0·0286	0·0035	9·21	11.35 ,,	0·0269	0·0013	4·39
1.30 P.M.	0·0206	0·0080	21·05	1.35 P.M.	0·0231	0·0033	11·14
2.0 ,,	0·0194	0·0012	3·15	2.05 ,,	0·0225	0·0006	2·02
2.30 ,,	0·0184	0·0010	2·63	2.35 ,,	0·0220	0·0005	1·67
3.0 ,,	0·0170	0·0014	3·68	3.05 ,,	0·0215	0·0005	1·67
3.30 ,,	0·0164	0·0006	1·58	3.35 ,,	0·0210	0·0005	1·67
4.0 ,,	0·0159	0·0005	1·31	4.05 ,,	0·0206	0·0004	1·35
4.30 ,,	0·0154	0·0005	1·31	4.35 ,,	0·0200	0·0006	2·02

TABLES D–G.

Exp.	*Date and Hour.*	*Red Anthers.* *Weight.*	*Loss.*	*% Loss.*	*Yellow Anthers.* *Weight.*	*Loss.*	*% Loss.*
B	Aug. 10th, 9.15 A.M.	0·0396	—	—	0·0282	—	—
	1.15 P.M.	0·0214	0·0040	10·10	0·0209	0·0024	8·51
	4.47 ,,	0·0160	0·0004	1·01	0·0176	0·0004	1·41
	Aug. 11th, 8.0 A.M.	0·0110	0·0050	12·62	0·0083	0·0093	32·97
	11.40 ,,	0·0107	0·0003	0·75	0·0077	0·0006	2·12
	4.45 P.M.	0·0104	0·0003	0·75	0·0075	0·0002	0·70
	Aug. 12th, 2.0 ,,	0·0104	0·0000	0·00	0·0073	0·0002	0·70
C	Aug. 10th, 10.32 A.M.	0·0380	—	—	0·0296	—	—
	2.33 P.M.	0·0184	0·0010	2·63	0·0220	0·0005	1·67
	4.32 ,,	0·0154	0·0005	1·31	0·0200	0·0006	2·02
	Aug. 11th, 8.05 A.M.	0·0105	0·0049	12·89	0·0090	0·0110	37·16
	11.45 ,,	0·0103	0·0002	0·52	0·0084	0·0006	2·02
	4.50 P.M.	0·0102	0·0001	0·25	0·0079	0·0005	1·67
	Aug. 12th, 2.05 ,,	0·0101	0·0001	0·25	0·0070	0·0009	3·04
D	Aug. 13th, 10.0 A.M.	0·0250	—	—	0·0312	—	—
	— ,,	0·0126	0·0124	49·60	0·0207	0·0105	33·65
	Aug. 14th, 11.0 ,,	0·0072	0·0054	21·60	0·0102	0·0105	33·65
	4.0 P.M.	0·0070	0·0002	0·80	0·0095	0·0007	2·24
	Aug. 15th, 12.0 M.	0·0065	0·0005	2·00	0·0082	0·0013	4·16
E	Aug. 13th, 10.30 A.M.	0·0397	—	—	0·0347	—	—
	3.30 P.M.	0·0175	0·0223	56·17	0·0235	0·0112	32·27
	Aug. 14th, 11.30 A.M.	0·0109	0·0066	16·62	0·0105	0·0130	37·46
	4.30 P.M.	0·0108	0·0001	0·25	0·0098	0·0007	2·01
	Aug. 15th, 11.30 A.M.	0·0100	0·0008	2·01	0·0090	0·0008	2·30
F	Aug. 18th, 10.15 A.M.	0·0444	—	—	0·0411	—	—
	3.0 P.M.	0·0213	0·0231	52·00	0·0284	0·0127	30·90
	Aug. 19th, 11.0 A.M.	0·0112	0·0101	22·74	0·0107	0·0177	43·06
	4.0 P.M.	0·0112	0·0000	0·00	0·0104	0·0003	0·72
	Aug. 20th, 12.0 M.	0·0112	0·0000	0·00	0·0102	0·0002	0·48
G	Aug. 18th, 10.30 A.M.	0·0412	—	—	0·0353	—	—
	3.30 P.M.	0·0218	0·0194	47·08	0·0230	0·0123	34·84
	Aug. 19th, 11.30 A.M.	0·0102	0·0106	25·72	0·0094	0·0136	38·52
	4.30 P.M.	0·0100	0·0002	0·48	0·0092	0·0002	0·56
	Aug. 20th, 12.30 ,,	0·0100	0·0000	0·00	0·0091	0·0001	0·28

The Structure of the Flower of Fagaceae, and its Bearing on the Affinities of the Group.

BY

E. M. BERRIDGE, B.Sc., F.L.S.

With nine Figures in the Text.

OF the families usually classed together under the term Amentiferae, the Fagaceae has received less thorough investigation at the hands of botanists than almost any other division of this great group. The chalazogamic forms have naturally attracted attention, and those with very simple flowers have been carefully examined, in the hope that they would reveal primitive characters. Yet the possibility remains that the Amentiferae, or at all events certain families among them, are not primitive, but reduced forms of some other angiospermic alliance; if this be the case the Fagaceae, including *Fagus*, *Castanea*, *Castanopsis*, and *Quercus*, would be just the division most likely to indicate such an affinity, possessing as they do more complete flowers than the rest of the group.

The opposite view, that the Amentiferae are directly derived from certain gymnospermous families, was held by Eichler (8), and was also brought forward prominently by Treub (23) after the discovery of chalazogamy. Engler (9), accepting Treub's view, and considering this character peculiar to *Casuarina*, placed this genus in a separate group, the Verticillatae, a connecting link between the Angiosperms and Gymnosperms, where it still remains isolated in his system, although more recent work has shown that *Casuarina* differs in no essential manner from other Amentiferae (4, 10), and that chalazogamy prevails among the Coryleae, Betuleae, and Juglandaceae[1] (3, 17, 18).

Nawaschin, in his elaborate study of the Birch and Alder, suggests the derivation of the Betuleae from the Coniferae, and of the Casuarineae from the Gnetales, and arrives at the conclusion that the placentae and ovules arise from the organic axis of the flower independently of the carpels, a view supported by Nicoloff (19) on anatomical grounds. Wettstein, in

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[1] For a concise discussion of the systematic position of *Casuarina* see Moss, C. E.: Modern Systems of Classification. New Phyt., vol. xi, p. 209, 1912.

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

his Systematic Botany, works out this theory in fuller detail, and grapples with the difficulty of deriving the bisexual flowers from the simpler unisexual forms ; his solution, however, is based on the hypothesis that the flower is a reduced inflorescence, and if applied to such a case as *Castanea* would involve a most intricate series of reductions and modifications to account for the varying and elaborate character of the epigynous, multipartite flower.

A modification of this view has been recently brought forward by Lignier (15), who, after giving a minute description of the anatomy of the flower of *Welwitschia*, concludes that the Gnetales are very primitive Angiosperms, and that they form the starting-point of a phylogenetic series which contains the amentiferous alliances, this series being parallel to that to which the main body of Angiosperms belongs.

On the other hand, for morphological reasons, Prantl (20) looks upon the Fagaceae and Betulaceae as derived by reduction from plants bearing bisexual flowers possessing a perianth, multilocular ovary, and suspended ovules, but he considers that the two families have been developed independently, the Fagaceae being the more primitive of the two.

Goebel (11) and Arber and Parkin (1) also regard the Fagales and Juglandales as reduced forms, the latter pointing out that the superior gamophyllous perianth, syncarpous ovary, and complicated inflorescence are not features likely to be characteristic of a primitive family.

Van Tieghem (24, 25) was the first to show that the floral anatomy of *Juglans* and of the Coryleae was similar to that of the higher Angiosperms ; this conclusion was opposed by Nicoloff (10), who emphasized certain details in the structure of the flower of Juglans, on which he based his view that the ovule in this genus was axial and not appendicular ; finally Benson and Welsford (5), in a more recent study of the same and allied genera, confirmed Van Tieghem's view, and showed that the placental bundles arise from the carpellary traces. The observations of Nicoloff, however, are of interest as indicating an essential likeness in the structure of the flower in *Juglans* and in the Fagaceae, which will be more fully dealt with in another part of this paper.

Quite recently the view that the Cupuliferae are a primitive family has received support from the study of the structure of the foliar rays of the wood by several American observers. No high degree of primitiveness, however, is here claimed for the Amentiferae, but only a degree relative to other Angiosperms, which would be quite consistent with the derivation of the group from bisexual flowering plants also possessing somewhat primitive characteristics, for a similar early type of wood structure is said to occur also in the Ericaceae and Platanaceae (22, 2), and in certain shrubby Rosaceae (7).

None of the writers who regard the Amentiferae as derived from angiospermous forms possessing bisexual flowers suggest affinity with any modern group of Archichlamydeae, but seem, rather, to incline to Coulter's (6) conclusion that, 'whether they represent a single genetic stock or several, they appear to be isolated from the higher alliances.'

Hallier has attempted to demonstrate an affinity between the Cupuliferae and one or other of the higher groups of Dicotyledons. In his earlier scheme for the classification of the Angiosperms (12), he derived the Cupuliferae from the Hamamelidaceae, and through this order from the Laurineae, thus connecting them indirectly with rosalian forms allied to the Combretaceae; in later papers, however, he discarded this view in favour of the theory that they were descended from the Anacardiaceae and Burseraceae, chiefly on account of the strong anatomical likeness of *Juliania* to these orders on the one hand, and to *Juglans* on the other (13).

But this view, based on the characteristics of some of the most reduced members of long reduction series, does not seem to take sufficiently into account the more complex members of the group, such as the Fagaceae, and the facts brought forward in the following pages seem to a certain extent to confirm the earlier theory, that the ancestors of the Cupuliferae were nearly allied to the Rosaceae.[1]

MORPHOLOGICAL CHARACTERS OF CASTANOPSIS CHRYSOPHYLLA.

Castanopsis chrysophylla, the Golden-leaved Chestnut, a Californian shrub sometimes grown in English gardens, was selected for special study, as being one of the less well-known members of the Fagaceae. The fact, moreover, that it possesses flowers which, though functionally female, are structurally bisexual, with an androecium of twelve stamens constantly present, seemed to promise that its investigation would throw light on the organization of the more reduced forms in the group.

The genus is very closely allied to *Castanea*, the chief points in which it differs from the latter being the trilocular form of the ovary, and the irregularity of the cupule; the latter is sometimes absent, and sometimes encloses, according to Prantl (20), only one flower. This writer regards the genus *Castanea* as a branch of the large tropical family *Castanopsis*, which has become adapted to northern temperate climates.

The flowering branches of *Castanopsis chrysophylla* bear numerous catkins; the lower ones, growing in the axils of the leaves, are entirely staminate, and though closely resembling those of *Castanea vulgaris*, are shorter and smaller. Only two or three small catkins in the axils of bracts

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[1] In a more recent paper, L'Origine et le Système phylétique des Angiospermes (Archives Néerlandaises des Sci. Nat., sér. 3 B., t. i, 1912, p. 146), Hallier, while still adhering to his later view, admits the possibility of the derivation of the Amentaceae from the Hamamelidaceae or Rosaceae, particularly in view of certain likenesses in the anatomical structure of the wood.

near the apex of the shoot bear fertile pistillate flowers, and of these the upper ones are abortive.

The longer staminate catkins are not infrequently branched at the base, and the tufts of flowers on them also appear to be telescoped branches. These tufts usually contain from four to six dichasia, the secondary flowers of which are well developed; sometimes tertiary flowers are also present. The tufts on the female catkins may contain as many as ten well-developed flowers, at the base of each of which appear two small secondary buds, all densely packed together. As will be shown later on, in a separate section of this paper, these secondary axes give rise to the 'valves' of the cupule; hence, in the later stages, each ripening nut appears to be furnished with a separate cupule.

Each tuft of flowers is surrounded by numerous hairy bracts, which presumably are the subtending bracts of the dichasia, as they correspond in number to the latter, but are frequently somewhat displaced; in addition, two primary lateral bracteoles enclose each dichasium, and are clearly distinguishable even after the appearance of the cupule. Four or five of the ovaries of a single tuft of flowers develop into nuts, but of these only one, or occasionally two, contain embryos; in the others only the dead remains of ovules are to be found; hence it appears that parthenocarpy is prevalent here as in other members of the Amentiferae.

Flowers.

The staminate flowers possess in all cases a rudimentary ovary, and are evidently reduced forms of the perfect flowers from which the nuts develop.

Both male and female flowers possess a gamophyllous perianth of six leaves, the three alternate lobes being larger and overlapping the others. The perianth is thickly covered with simple unbranched hairs, those on the outer surface and edges of the lobes being thick-walled, and growing in tufts of two to four together. Among these simple hairs are scattered multicellular glandular hairs somewhat peltate in form.

All the flowers are completely epigynous, and possess two whorls of six stamens inserted at the junction of the perianth and ovary. The six stamens of the outer whorl, which are opposite to the lobes of the perianth, are slightly adnate to the latter, and develop somewhat earlier than the six stamens which alternate with them. The anthers are introrse and versatile, and the filaments are very long, as in *Castanea*, and strongly incurved in the bud. The pollen of the flowers which are functionally pistillate seems perfectly normal, and like that of the staminate flowers; the grain shows three longitudinal furrows with a pore in the middle of each furrow, and two nuclei can be clearly distinguished within it. The female flowers are strongly protogynous, the stigmas protruding when the stamens are still curled up in the bud; they are, therefore, pollinated by the pollen from the staminate flowers on the lower catkins of the same or other plants. In *Castanea* also, this must necessarily be the case, for the stamens of the female flowers seldom contain any fully developed pollen-grains. Cross fertilization is probably effected, to some extent, by bees, which sometimes visit Sweet Chestnut trees in great numbers. Eichler (8) only mentions the presence of 'six staminodia opposite the perianth lobes' in the female flowers of *Castanea*; but more than six are sometimes to be found, and from their position it is evident that twelve is the original number, arranged as in *C. chrysophylla.*

These genera may be added, therefore, to the numerous cases fully described by Kerner (14), where a diclinous plant shows marked protogyny. It seems obvious that extreme protogyny might in certain cases tend to a separation of the sexes, for the early development of the stigmas would naturally lead to the pollination of the pistils on the younger branches by pollen from flowers on the older ones of the inflorescence. This tendency would render the pistils of the older and earlier flowers and the stamens in the younger later flowers comparatively useless, and prepare the way for their elimination.

That such a process might be much modified by other circumstances, such as the relative positions of the flowers, is evident, when such complex cases of dichogamy as those among the Umbelliferae are taken into consideration, but in the Fagaceae a series of stages in the process appears to be represented, for while in *Castanopsis* we have apparently functional stamens in the female flowers, in *Castanea* we find these stamens containing little or no good pollen; in the Oak they are reduced usually to mere papillae, while in the Beech as a rule they are entirely absent.

The ovary in *Castanopsis*, as in the other Fagaceae, is completely fused with the receptacular cup, and though usually trilocular, four or even more loculi frequently are present, particularly in the flowers growing at the apex of each tuft. The three main loculi occur opposite the larger perianth lobes, the extra one appearing opposite the smaller lobes. The placentation is axile, and the ovules, like those of *Castanea*, are paired, suspended, bitegumentary, and epitrope.

The ovary of the staminate flower at the time of opening is not merely rudimentary as in *Castanea*, but shows an interesting stage of arrested development. The six placental margins can be clearly seen projecting into the cavity of the ovary, but not meeting in the middle. The lower half of each margin is fused with the neighbouring one, but above it becomes free and runs up into the style. Between the style and the stamens the ovary wall grows up into a disc-like ring of tissue, so that the style appears to be inserted nearly half-way down towards the base.

No upgrowth of the apex of the flower axis to meet the ingrowing septa was observed in any young stages of development in *Castanopsis*, but in some hand sections of young ovaries of *Quercus cerris* gathered on January 16 such an upgrowth was found to be present.

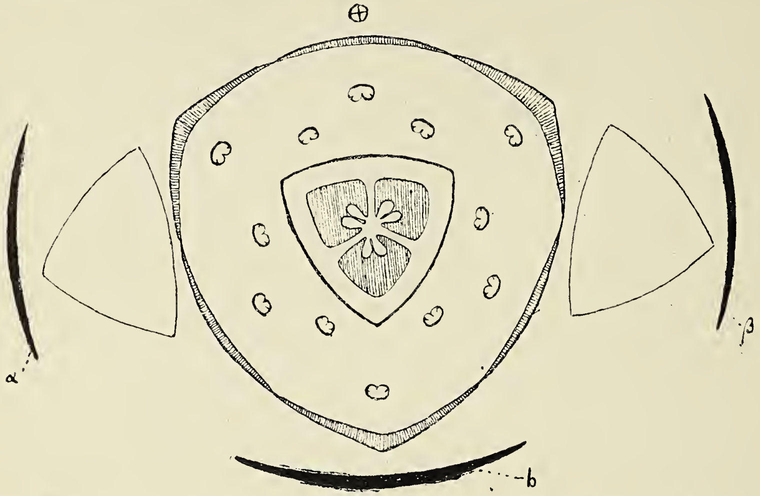

FIG. 1. Floral diagram of *Castanopsis chrysophylla*. *b.*, subtending bract of dicha *a*, *β*, bracteoles of primary flower.

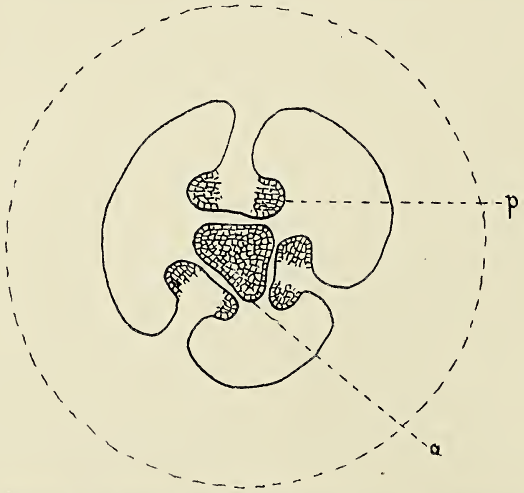

FIG. 2. Cross-section of young ovary of *Quercus cerris*. *a.*, apex of flower axis; *p.*, placenta. × 90.

Fig. 2 is a sketch of one of these sections, showing that this prolongation of the axis is quite independent of the septa, and that the latter alone bear the placentae, on which young ovules are already present. Later the placental tissue seems to fuse with and entirely surround this axial prolongation, thus forming the greater part of the central column of the ovary, including the outer layers in which the ovular bundles run.

The fruit of *C. chrysophylla*, like that of some of the Oaks, takes two years to develop. At the time of pollination, about the middle of July, ovules are present, but the archesporial cells are hardly distinguishable from the rest of the nucellar tissue, and the integuments are only beginning to appear. Little development takes place during the winter months, and fertilization does not occur in England till the latter half of the month of May.

Cupule.

There can be little doubt that in *Castanopsis chrysophylla* each valve of the cupule represents, not a bracteole covered with emergences, as Eichler suggests in his account of the cupule of other members of the Fagaceae, but one of the secondary axes of the dichasium, beset with

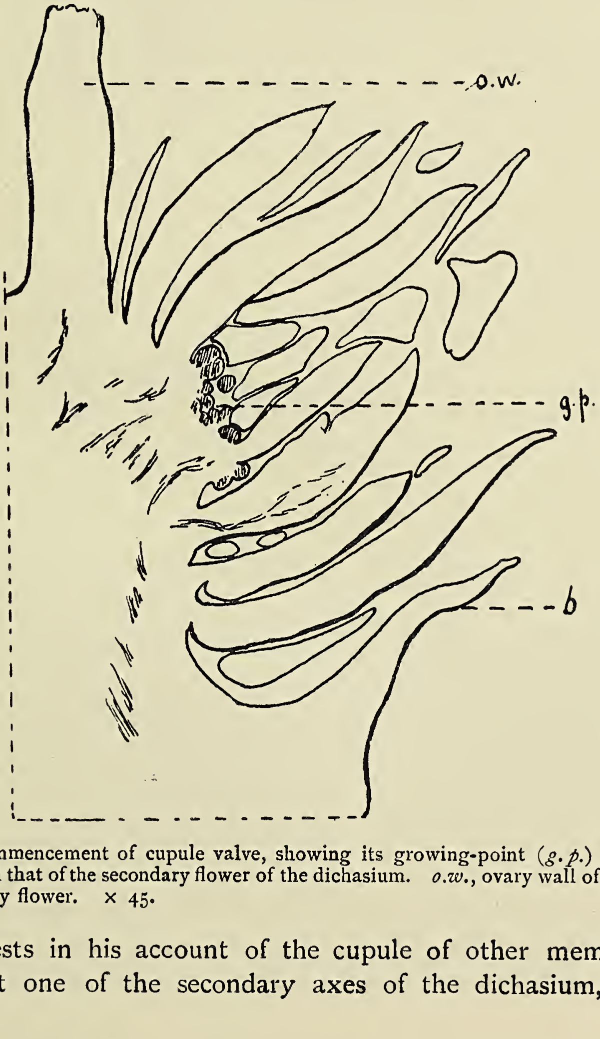

FIG. 3. Commencement of cupule valve, showing its growing-point (*g.p.*) and its position corresponding with that of the secondary flower of the dichasium. *o.w.*, ovary wall of primary flower; *b.*, bract of primary flower. × 45.

spinous bracts. At the time of flowering, that is, about the middle of July, the lower flowers in the tufts borne by the female catkins are flanked by sessile buds with well-defined growing points (Fig. 3). These doubtless represent the secondary flowers of the dichasia. Round the upper terminal flowers of the tufts these outgrowths are so compressed that their nature cannot easily be recognized, but they too appear to have a growing point round which the young spines cluster, and they also therefore are probably axial in nature. A month later these buds have developed into groups of spines as large and conspicuous as the ovaries themselves, and in the axils of many of the spines tiny buds appear (Fig. 4).

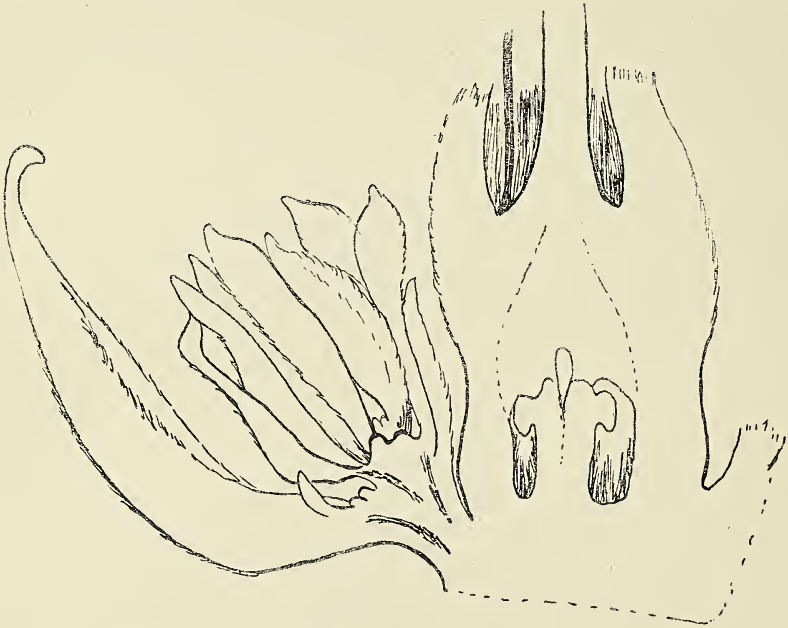

FIG. 4. Longitudinal section through ovary and adjacent group of spines. × 30.

The axial nature of the valve is further indicated by the character of the vascular supply. Near its apex, where it is free from the pressure of the adjacent ovaries, a circle of six to nine bundles is found, and each spine receives a single strand formed by the fusion of two branches from these bundles. Near the base, where the valve is flattened between the growing ovaries, the bundles are arranged, not in a circle, but in a straight line, being evidently displaced by the pressure to which the whole organ is subjected.

Little further development occurs till near the time of fertilization, about the middle of the following May, when the 'cupule valves' begin to grow quickly till they entirely cover the nuts with a mass of sharp branching spines. Three such 'valves' usually separate each nut from its neighbours, but this trimerous arrangement is only an effect of pressure, for, as we have seen, the valves represent axes which form part of dichasial inflorescences.

Prantl (20) was able to distinguish in several species of *Pasania* two bracteoles below the cupule, and hence concluded that it must in these cases be an outgrowth of the axis of the primary flower, and that the many scales on it were metamorphosed leaves; he also extended this view to the cupules of *Quercus* where the primary bracteoles are not clearly distinguishable. He states that the secondary flowers of the dichasia are present in *Pasania*, and therefore regards the cupule as an intercalary growth; he points out that the reduced leaves appear upon it in acropetal succession, but seem to be retarded till after the development of the floral leaves at the apex of the shoot.

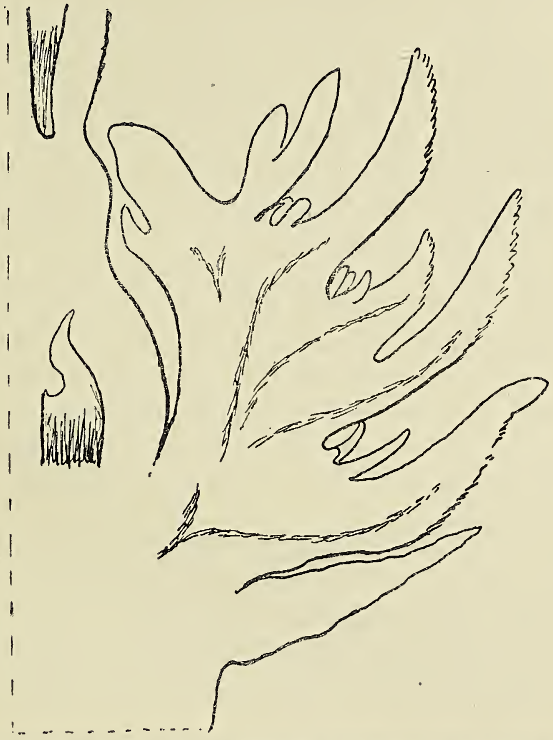

FIG. 5. Longitudinal section through ovary and adjacent cupule valve, just before the time of fertilization. × 44.

If, however, we extend what seems a perfectly obvious interpretation of the cupule in the case of *Castanopsis chrysophylla* to *Quercus*, and regard its cupule also as a modification of the fused secondary axes of the dichasium, it is evident that the scales, i. e. the leaves, on these might naturally develop later than the floral leaves of the primary axis.

All the evidence, in fact, which is brought forward by Prantl in support of his conclusion that the cupule is an intercalary growth of the axis—for instance, the leaf-like nature of the cupular scales in *Fagus Sieboldii*, or the appearance of bracts below and prior to the outgrowth of the valves in *Castanea*—is equally applicable in support of the view that the valves are modified secondary or tertiary axes, and not, as Eichler maintained, merely bracts covered with emergences.

THE ANATOMY OF THE FLOWER.

There is a great similarity in the structure of the flower of the four genera of the Fagaceae, and the following description, which applies primarily to *Castanopsis*, is in the main true of all. In *Fagus* a larger number of bundles is present than in *Castanopsis*, while in *Quercus* they show much irregularity both in number and degree of fusion, but there is no essential difference in their arrangement.

In all the genera the receptacular cup is fused to the ovary wall right up to the base of the styles, and it is only at this level that the traces of the parts of the flower become separate from one another. There is, therefore, much more complete fusion than in most rosalian forms of inferior ovaries, and more resemblance to such advanced types as those of the Philadelpheae and Curcurbitaceae. In the ovary wall, therefore, we find only one series of bundles; in *Castanopsis* they number twelve, and are large and fan-shaped and evidently compound.

Six of these, occupying the angles and middle of the sides of the triangular ovary (Fig. 6, *A*, *B*, *C*, &c.), divide radially at the margin of the receptacle into three branches, which supply the perianth leaves, the outer whorl of six stamens, and the styles; the six intermediate bundles (Fig. 6, *a*, *b*, *c*, &c.) divide into two branches only, these passing to the inner whorl of stamens and to the styles.

In view of the fact that these intermediate bundles (*a*, *b*, *c*, &c.) originate in exactly the same manner as the whorl of six (*A*, *B*, *C*, &c.) from which the perianth bundles spring, it is not unreasonable to suppose that they primitively supplied a whorl of petals, and that the inner whorl of stamens is an antipetalous series, although we have no direct evidence of the previous existence of a corolla in these genera.

In *Castanea* the number of bundles in the ovary wall is often increased by branches from the original twelve, while in *Fagus* a group of strands occurs in each angle of the nut and spreads out into the wing; each seems to be equivalent to three or four wall-bundles with the component traces less closely associated together, and this brings the total number of these main bundles up to 18 or 20.

In addition to the bundles in the wall, the central column of the ovary contains a circle of small vascular strands (Fig. 6, *o.p.b.*) which resolve themselves into six amphiphloic bundles passing into the funicles of the six seeds. Also in *Castanopsis*, but in that genus only, three tiny vascular strands (Fig. 6, *i.p.b.*) occupy the middle of this circle; these only become lignified and therefore easily distinguishable in the course of the second year of development of the ovary; they can be traced above the point where the ovular bundles pass out of the column, and occupy the intermediate positions opposite the septa. Each probably represents two fused marginal bundles of the carpels, or they may possibly have originally supplied additional ovules at a higher level than the two now constantly present in each loculus. They may possibly be axial, but their orientation does not support this view, and in *Castanopsis* no upgrowth of the apex of the flower axis between the carpels was observed, though in *Quercus cerris* such an elongation of the axis occurs.

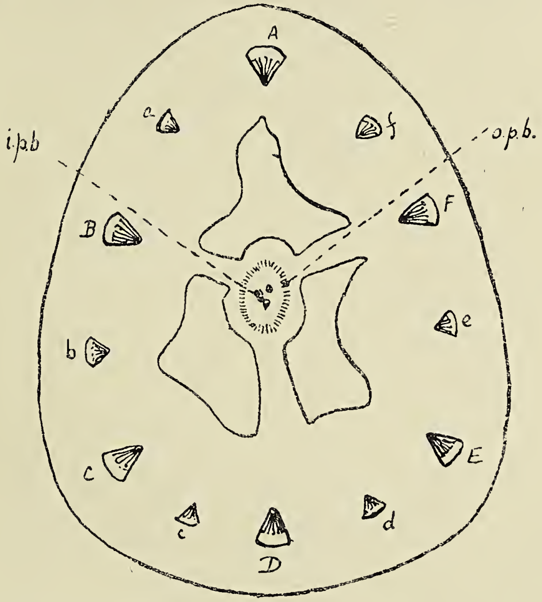

FIG. 6. Transverse section of lower part of ovary. A, B, C, &c., *a*, *b*, *c*, &c., main bundles of the ovary wall; *o.p.b.*, outer placental bundles; *i.p.b.*, inner placental bundles.

In the pedicel of the flower, below the level at which the loculi begin to appear, another set of twelve bundles (Fig. 7, *c.b.*) is present, alternating with the main bundles (Fig. 7, *m.b.*) of the ovary wall. These supply the carpels, and originate as a rule by the fusion of two branches from the neighbouring main bundles. While still in the base of the ovary these carpellary bundles break up into a number of vascular strands. The lowest, running inwards, fuse with similar branches to form the three tiny bundles in the middle of the placental column. At a slightly higher level two more branches pass outwards and become associated with the neighbouring main bundles. These dorsal bundles (Fig. 7, *d.b.*) probably fuse in pairs during the course of the main bundles up the ovary wall, and each fused pair reappears at the base of the styles as a single strand representing the dorsal bundle of the carpel.

In addition to the above, numerous anastomosing strands spring from the carpellary bundles themselves, and from their dorsal branches, and run inwards to form the circle of bundles in the placental column, which at a higher level breaks up into the ovular traces. These anastomosing strands are represented diagrammatically in Fig. 7 by the dotted lines *p.b.* The dorsal branches almost invariably give off one of these placental strands just at the point where they join the main bundles of the wall, so that the latter, i. e. the perianth and stamen traces, appear to contribute to these ovular bundles, but in longitudinal sections it is quite clear that there is no direct union between the two sets of traces.

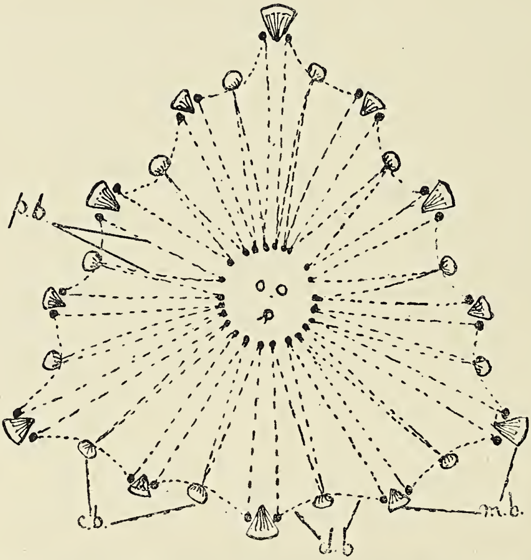

FIG. 7. Diagram representing arrangement of bundles in the base of the ovary of the flower of *Castanopsis*. *m.b.*, main bundles of wall; *c.b.*, carpellary bundles; *d.b.*, branches running outwards to the main bundles; *p.b.*, vascular strands running to the placenta.

Fig. 8 shows the relations between two carpellary bundles (*c.b.*), with their numerous branches, and the neighbouring main bundles supplying perianth leaves and stamens. The common origin of these two sets of bundles, as well as the fusion of the dorsal stylar branches with the main bundles, are facts wholly unfavourable to Wettstein's view that this is one of the most primitive forms of bisexual flowers, and that it has arisen through the association of originally independent male and female flowers within a common perianth.

In *Castanea* the structure of the flower is the same as in *Castanopsis*, and, apart from the greater number of main bundles in the ovary wall, it is the same in *Fagus*.

In *Quercus* the presence of groups of stone cells in the outer layers of the receptacular wall makes it almost impossible to cut thin serial sections of the ovary at the later stages when the bundles are fully lignified, but such sections as were obtained showed the presence of carpellary bundles below the loculi, breaking up into dorsal branches and placental strands just as in *Castanopsis*. The dorsal branches, however, frequently run independently up to the styles, so that the ovary wall often shows twenty or more bundles.

So far, in the examination of the flower, a trimerous structure is apparent throughout; it is only in the pedicel that we come across any indication that this trimery is adaptive, the result of the development of the flowers within a confined space. In *Fagus* the whole vascular supply of the flower springs from fifteen bundles arranged in three groups of five; in *Castanopsis* the same three groups of five main bundles may be traced, and frequently a fourth group is present, corresponding to a fourth loculus in the ovary, which, if fully developed, would bring the number of main bundles up to twenty. These fifteen to twenty bundles combine in various ways to give the twelve which traverse the ovary wall.

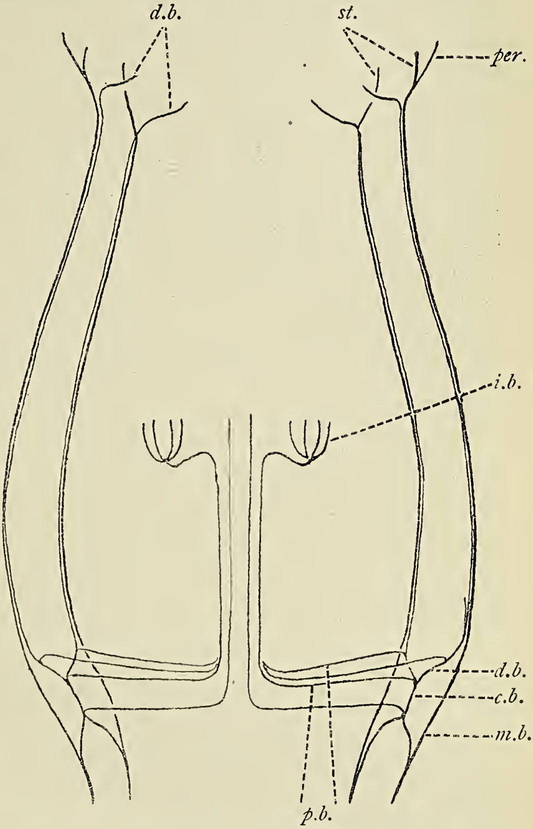

FIG. 8. Diagram of course of bundles in flower of *Castanopsis chrysophylla*. *m.b.*, main bundles; *per.*, branch to perianth; *st.*, branch to stamen; *d.b.*, branch to style; *c.b.*, carpellary bundle; *p.b.*, placental branches; *i.b.*, branches in the outer integument of ovule.

In *Castanea*, on the other hand, the middle flower of the dichasium, which is compressed on both sides, sometimes shows only two groups of five main bundles, the third group being altogether missing.

It seems not improbable, therefore, that the organs of the flower were originally arranged in multiples of five, and the possibility of a relationship with families prevailingly pentamerous must be taken into account.

It would appear to be a point of some significance that the vascular structure of the inferior ovary is practically identical in *Castanea* and *Castanopsis*. That structure corresponds rather with the multilocular condition in the former than with the trilocular form of the ovary in the latter. In *Castanea* six loculi are most commonly present, situated opposite the perianth leaves, and therefore with the six largest main bundles taking the position of dorsal carpellary bundles. From such an arrangement it would appear that the trilocular ovary of *Castanopsis* and *Quercus* is derived by the obliteration of the loculi opposite the smaller perianth leaves, for when a fourth is present it occupies that position. It is very probable also that the 6-locular ovary most usual in *Castanea* is derived in the same manner from ovaries containing many loculi, for 12-locular forms are not infrequent, i. e. forms in which the number of loculi, and therefore of carpels, corresponds with the number of carpellary bundles. The variations, therefore, in the ovary appear to be due to the disappearance of septa only, not of complete whorls of carpels, as Eichler (8) suggests, and the frequent occurrence of partial septa in *Quercus* and *Castanea* support this view.

Such a mode of reduction seems to indicate that the Fagaceae are derived from ancestors which possessed syncarpous and multilocular ovaries; if the latter were also inferior, the union of the outer walls with the receptacular cup would also have tended to preserve the vascular supply of the carpellary wall from complete disappearance, since it becomes merged in the general supply of the whole flower.

COMPARISON WITH JUGLANS REGIA.

No attempt has been made at present by the writer to work out the floral anatomy of other amentiferous forms with a view to a comparison with the Fagaceae. The vascular structure of the flower of *Juglans regia* has, however, been very fully described by Van Tieghem (24) and Nicoloff (19), and also by Benson and Welsford (5), and has been the basis of much discussion concerning the affinities of the group; a comparison, therefore, can be readily made, and, moreover, seems to furnish an explanation of one of the features which led Nicoloff to insist so strongly on the axial nature of its ovule.

His Fig. 15, which represents a transverse section of the pedicel in *Juglans*, and which is here reproduced (Fig. 9), differs from a section taken through the same region in *Quercus Robur* mainly in the fact that, while in the Oak the outer circle of bundles supplies the cupule only, and the inner supplies both perianth and carpels, here the cupule and perianth bundles are associated together in the outer circle, while the inner circle consists of bundles which supply only the ovule and styles. In *Juglans*, four alone of these latter, two anterior and two posterior, behave as do the carpellary traces in the Fagaceae, giving off dorsal branches which fuse in pairs and run up to the stigmas, as well as placental strands which pass first horizontally and then vertically through the septa to the ovule. All the others, according to Nicoloff, contribute placental bundles only. This latter series of vascular strands, converging to the central column and then passing upwards to the ovule, was regarded by this author as belonging to the floral axis, but it seems probable that we have here a network of carpellary strands comparable to that present in the same position in the less reduced Amentiferae such as *Quercus*. Neither Van Tieghem nor Benson and Welsford mention this vascular network, which probably only shows lignification at a late period of development, but include in their description only the four strands springing from the dorsal bundles of the carpels. It is interesting, however, to find the whole network still persisting, and thus linking this reduced form with the more complex multilocular ovary of the Fagaceae.

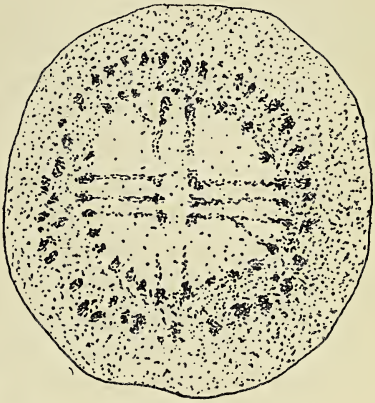

FIG. 9. Transverse section of pedicel in *Juglans regia*. After Nicoloff.

AFFINITY WITH THE ROSALES.

There appears to be nothing in the structure of the flower of the Fagaceae to preclude the view that the family is derived from some rather primitive angiospermic stock possessing bisexual flowers with syncarpous multilocular ovaries; there seem also to be indications that the ancestral flowers may also have been dichlamydeous, pentamerous, and epigynous.

These features, as well as the large number of characteristics common to the Fagaceae and Rosaceae which are summarized below, seem to point to ancestors allied to some of the epigynous types among the Rosales.

Besides the morphological characters already mentioned, we find prevailing in both genera the woody habit, alternate stipulate leaves, cymose or frequently dichasial inflorescences, and stamens, when present, arranged in at least two whorls, introrse, and strongly incurved in the bud. Where the ovary is syncarpous the placentation is axile, the ovules paired, bitegumentary, and epitrope, and the seeds exalbuminous.

The numerous resemblances in the anatomical structure of the vegetative organs can readily be traced in the 'Concluding Remarks' of

Solereder's 'Systematic Anatomy of the Dicotyledons'. Hallier (13) has laid great stress upon these features in his comparison of the Cupuliferae with the Anacardiaceae and Burseraceae, but it may be observed that the Cupuliferae are classed together with the Rosaceae in Solereder's summary quite as frequently as with these members of the Terebinthales. Here we find that the Cupuliferae resemble the Rosaceae in the nature of the epidermis, hypoderma, stomata, and hairs, both ordinary and glandular, in the place of origin of the cork and in the structure of the pericycle; while in respect of many characters of the wood, the secretory system, and the mineral contents of the cells, the Cupuliferae seem to approach the Rosaceae more nearly than they do the Burseraceae and Anacardiaceae.

The special features in the anatomical structure of the flower of the Fagaceae have been shown to be (1) the presence of carpellary bundles originating by fusion of branches from the main bundles supplying the perianth and stamens; (2) the concentration of the placental strands in a definite layer beneath the loculi of the ovary, with a corresponding absence of vascular tissue in the septa; (3) the double nature of the dorsal bundles of the carpels. The carpellary bundles in the Pomeae bear a similar relationship to the perianth and stamen traces, and the placental bundles to which they give rise run inwards horizontally to the middle of the flower; while in *Fuchsia* and *Oenothera*, forms usually regarded as derived from the Pomeae, there is apparently a still closer resemblance in the distribution of the bundles, for Van Tieghem mentions a network of small strands running near the base of the loculi to the ovules, and notes the marked absence of vascular tissue in the septa. The dorsal bundle of the carpel is described by Van Tieghem as arising in the Pomeae by direct radial doubling of the sepal traces, but in *Pyrus Aria*, *P. communis*, *P. Aucuparia*, and *Exochorda grandiflora*, this does not appear to be the case; the dorsal bundle has been found to be of double origin, formed by fusion of two branches from the carpellary bundles, which alternate with the perianth traces. Hence in the details of vascular structure, as well as in general form, the flowers of the Rosaceae and Fagaceae show distinct resemblances.

Little stress can be laid on the occurrence of multicellular archesporia and chalazogamy in both alliances, for these characters appear in such isolated genera throughout the Dicotyledons that it is doubtful whether they are of any phylogenetic value. Aporogamy in particular appears to be due to an adjustment to some change of condition within certain ovaries which have undergone reduction. It seems quite evident, however, that these two characters can no longer be regarded as linking the Cupuliferae closely with the Gymnosperms.

Finally, two of the most marked features which separate the Fagaceae from the higher alliances—the trimerous nature of the flower and the form of the inflorescence—have been shown to be probably the result of a strong tendency in the family to aggregation of the flowers and economy in the parts, and the appearance of a cupule in these forms seems to be due to the same tendencies, since it is simply a modification of certain branches of the inflorescence for purposes of protection.

The numerous resemblances here enumerated between the Rosaceae and allied orders on the one hand, and the Fagaceae and other Cupuliferae on the other, can hardly be fortuitous, but seem to point to some real relationship between the groups. Complex forms like the Fagaceae can hardly have been derived from rosalian forms through the Hamamelidaceae, but this order would probably be included in the same cycle of affinity.

SUMMARY.

The inflorescence, flowers, and cupule of *Castanopsis chrysophylla* are described and the anatomical structure of the flower fully worked out.

The latter is compared with that of *Castanea vulgaris*, *Fagus sylvatica*, *Quercus Robur*, and *Juglans regia*.

The flower is shown to differ in no essential from other epigynous types of angiospermic flowers, and a fairly detailed comparison is drawn between the Rosaceae and the Cupuliferae, since it seems probable that the epigynous Rosaceae or their near descendants are the forms with closest affinity to the ancestors of the Fagaceae.

BIBLIOGRAPHY.

1. ARBER, E. NEWELL, and PARKIN, J.: The Origin of the Angiosperms. Journ. Linn. Soc., Bot., vol. xxxviii, 1907, p. 29.
2. BAILEY, J. W.: The Evolutionary History of the Foliar Ray in the Wood of the Dicotyledons, and its Phylogenetic Significance. Ann. of Bot., vol. xxvi, 1912, p. 647.
3. BENSON, M. J.: A Contribution to the Embryology of the Amentiferae, Pt. I. Trans. Linn. Soc., ser. 2, Bot., vol. iii, 1894, p. 409.
4. BENSON, M. J., SANDAY, E., and BERRIDGE, E. M.: A Contribution to the Embryology of the Amentiferae, Pt. II, *Carpinus*. Trans. Linn. Soc., ser. 2, Bot., vol. vii, 1906, p. 37.
5. BENSON, M. J., and WELSFORD, E. J.: The Morphology of the Ovule and Female Flower of *Juglans regia*, and of a few Allied Genera. Ann. of Bot., vol. xxiii, 1909, p. 623.
6. COULTER, J. M., and CHAMBERLAIN, C. J.: Morphology of the Angiosperms, 1903.
7. EAMES, A. J.: On the Origin of the Herbaceous Type in the Angiosperms. Ann. of Bot., vol. xxv, 1911.
8. EICHLER, A. W.: Blüthendiagramme, 1878, pp. 20–30.
9. ENGLER, A.: Die natürlichen Pflanzenfamilien. Nachtrag, Pts. II–IV, Leipzig, 1897.
10. FRYE, T. C.: The Embryo sac of *Casuarina stricta*. Bot. Gaz., vol. xxxvi, Aug., 1908, p. 101.
11. GOEBEL, K.: Organography of Plants, Pt. II, Engl. ed., 1905.
12. HALLIER, H.: Ueber die Verwandtschaftsverhältnisse der Tubifloren und Ebenalen, den polyphyletischen Ursprung der Sympetalen und Apetalen, und die Anordnung der Angiospermen überhaupt. Abh. naturw. Ver. Hamburg, vol. xvi, 1901, p. 1.
13. ————: Ueber *Juliania* . . . neue Beiträge zur Stammesgeschichte der Dicotyledonen. Beih. z. Bot. Centralbl., Abt. II, vol. xxiii, 1908.

14. KERNER, A.: The Natural History of Plants, vol. ii, Engl. ed., 1895.
15. LIGNIER, O., and TISON, A.: Les Gnétales, leurs fleurs et leur position systématique. Ann. des Sci. Nat., Bot., 9^{e} série, t. xvi, 1912.
16. MOSS, C. E.: Modern Systems of Classification. New Phyt., vol. xi, 1912, p. 209.
17. NAWASCHIN, S.: Zur Embryobildung der Birke. Bull. Acad. Imp. Sci. St. Pétersbourg, vol. xiii, 1892; Bot. Centralbl., vol. liv, 1893, p. 345.
18. —————: Ein neues Beispiel der Chalazogamie. Bot. Centralbl., vol. lxiii, 1895, p. 353.
19. NICOLOFF, TH.: Sur le type floral et le développement du fruit des Juglandées. Journ. de Bot., vol. xviii, 1904, p. 132.
20. PRANTL, K.: Beiträge zur Kenntniss der Cupuliferen. Bot. Jahrb., vol. viii, 1887, p. 321.
21. THOMPSON, W. P.: On the Origin of the Multiseriate Ray of the Dicotyledons. Ann. of Bot., vol. xxv, Oct. 1911, p. 1005.
22. TREUB, M.: Sur les Casuarinées et leur place dans le système naturel. Ann. Jard. Bot. Buitenzorg, vol. x, 1891, pp. 145–231.
23. VAN TIEGHEM, PH.: Anatomie de la fleur femelle et du fruit du noyer. Bull. Soc. Bot. de France, vol. xvi, 1869, p. 412.
24. —————: Recherches sur la structure du pistil et sur l'anatomie comparée de la fleur, 1868, pp. 70, 153.

On the Trifoliolate and other Leaves of the Gorse (Ulex europaeus, L.).

BY

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IN seedlings of gorse the axis usually bears a certain number of trifoliolate leaves after the cotyledons, and before the simple leaves. Of the simple leaves those first formed are nearly always flat, while the later ones are normally spiniform.[1] The production of trifoliolate leaves is to be regarded as an ancestral character.

In a natural habitat the number of trifoliolate leaves may vary considerably, even among seedlings growing close together. Individual diversity with respect to this character is thus suggested, but it seemed probable that experiments would reveal some degree of plasticity. In order to gain some information on the subject, seedlings were grown on sand, and others on a mixture of loam, sand, and leaf-mould. When the seedlings had advanced far enough,[2] they were examined, and the result compared for the two kinds of soil. The result appears to be worth recording, but further experiments require to be carried out in a different way, as indicated by some unsatisfactory features in the present experiment.

Remarks on some characters of the seedlings must now be made. Bifoliolate leaves occur, but are not so numerous as trifoliolate leaves. One or more of them are often produced as a transition from trifoliolate to simple leaves, and vice versa. The seedlings show great diversity as regards their leaves. A few bear simple leaves only, while others produce trifoliolate leaves in various numbers from one up to twenty or more. Then again the trifoliolate leaves may be variously distributed. They may begin directly after the cotyledons, and form an uninterrupted series succeeded by the simple leaves. Or the series of trifoliolate leaves may be preceded, and also interrupted once or many times, by simple and bifoliolate leaves.

Among the flat leaves two-lobed or three-lobed (or more deeply divided) leaves are occasionally present. These as well as the bifoliolate leaves may be regarded as showing an 'attempt' to realize the ancestral

[1] Goebel: Organography of Plants (Engl. ed.), vol. i, p. 168.

[2] Most of the plants were left until they had produced several spiniform leaves; it might then be assumed that no further trifoliolate leaves would be formed.

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trifoliolate type. Hence these are included in the countings, and for convenience the bi- and trifoliolate and lobed leaves will be spoken of together as compound leaves. Among the apparently simple leaves some have an articulation at a distance from the base. This again is an indication of a compound nature, and these leaves would also have been counted with the trifoliolate leaves, but the presence of the articulation was overlooked until many of the plants had been examined and thrown away. Thus one type of approach to the trifoliolate condition has been ignored, but it appears probable that the nature of the result has not been thereby altered.

The experiment was started at the end of March, 1913, when seeds were sown in two boxes of soil, composed of loam, sand, and leaf-mould, and in four boxes of sand, two of the latter being kept drier than the others during part of the experiment. The first examination of seedlings was made in the middle of July. It was then found that, though there was a good crop of seedlings, spiny above, others were still young, and some seeds were only just beginning to germinate. The backward seedlings, which were much more numerous in the cultures on sand, were examined later at intervals when they had progressed far enough. When the experiment was stopped at the beginning of September, some seeds were again just germinating, but the experience gained by that time appeared to show that the rest of the plants could be discarded without altering the direction of the result.[1]

The main result of the experiment is that seedlings of gorse grown on good soil, under the conditions of the experiment, produce a somewhat larger average number of compound leaves than those grown on sand. The seedlings on soil gave an average of 10·79 compound leaves per plant, while those on sand gave an average of 8·27 ; the difference (2·52) amounts to about 23 per cent.

The number of seedlings examined was 2,895, of which 1,094 were on soil, and 1,801 on sand. In counting the leaves, the different boxes were dealt with separately. The values for the average number of compound leaves given by the two boxes of soil came out very close, namely 10·73 and 10·87. The figures for the boxes of sand were 8·60 and 8·43 for the two drier boxes, and 8·49 and 7·51 for the two damper boxes.

The conclusion appears to be justified, that in this case the presentation of an ancestral character is influenced to some extent by the nature of the soil, though at the same time there is great independent diversity in respect of this character.

The very backward seedlings which were not included in the countings from the boxes of soil were comparatively few, and would not have greatly

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[1] See below, p. 529. The soil and sand used in the experiment were not sterilized. Root-tubercles were present on some seedlings. The effect of these would probably be to slightly diminish the difference in nutrition on the two kinds of soil.

affected the average. Those omitted in the boxes of sand were more numerous, but, had they been included, they would no doubt have somewhat increased the average difference between soil and sand, as the later countings (i. e. of backward seedlings) on sand gave low values for the number of compound leaves.

In considering the result that the ancestral character is more pronounced on good soil, the suggestion may be made that this is a case of an ancestral character being favoured by ancestral soil conditions, since the gorse plant may be supposed to be descended from a plant with trifoliolate leaves, and having normal habitats among richer soil than that usually frequented by gorse. Other hypotheses expressed in terms of response to different physical factors might be attempted, but the above point of view should not be lost sight of, and may prove to have some theoretical importance.[1]

Lothelier[2] carried out some experiments in which, when gorse was grown in an atmosphere saturated with moisture, the spiny character of the plant disappeared, and the leaves became flat. Wollny,[3] however, found that only 12·5 per cent. of the plants of gorse with which he experimented gave the reaction described by Lothelier. Thus variation as regards plasticity has been recorded in gorse plants.

It is quite possible that there may be also differences in plasticity as regards the number of compound leaves produced by the seedlings, some of the latter being more easily influenced by soil conditions than others. If this is so, the leaf characters of a seedling may be thought of, for the sake of clearness, in relation to theoretical normal conditions (as giving a fixed point). Then when conditions are other than normal, the actual number of compound leaves formed by a given seedling may be regarded as determined by three factors, viz. (*a*) the number of compound leaves that would be produced by this individual under normal conditions; (*b*) the degree of plasticity of this seedling; (*c*) the deviation from normal conditions.

For comparison with the result obtained with gorse seedlings, reference may be made to an experiment carried out some years ago on the garden wallflower.[4] In this case a presumably ancestral character was recognized in the three-armed hairs found on the early leaves of the seedling. These hairs were more numerous on seedlings grown on sand than on those grown on loam.[5] Thus the ancestral character is here more pronounced on poor

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[1] See F. Darwin, Presidential Address, British Association, Dublin Meeting, 1908.

[2] Lothelier: Recherches sur les plantes à piquants. Revue Générale de Bot., vol. v, p. 519.

[3] Wollny, as quoted by Goebel, Experimentelle Morphologie der Pflanzen (1908), p. 35.

[4] Boodle: On the Occurrence of Different Types of Hair in the Wallflower. Ann. of Bot., vol. xxii, p. 714.

[5] Only forty seedlings were used in this experiment, so the result requires confirmation. Further experiments were begun, but were abandoned on account of the trying nature of extensive hair-counting.

soil. This is the reverse of the result with gorse, but, as the wild form of the wallflower is more or less xerophytic (growing on rocks, sea-cliffs, &c.), this again appears to be a case of an ancestral character being favoured by ancestral soil conditions.

More general theoretical considerations are not included in the present note, as the writer hopes to carry out further experiments, which should yield a better basis for the discussion of the subject.

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The Significance of Certain Food Substances for Plant Growth.

BY

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With two Figures in the Text.

EVIDENCE has been rapidly accumulating during recent years which proves that the soluble humus of the soil is an essential factor of soil fertility, providing not only food and energy for numerous soil bacteria, but also serving as a source of food for plants. Amongst numerous investigators may be mentioned Krzemieniewski (1), who demonstrated that soil humates have a stimulating influence on the fixation of nitrogen by nitrogen-fixing bacteria, and Hutchinson and Miller (2), who have shown that soil humates can also be readily assimilated by plants, and serve as a source of nitrogen.

During a search made during the summer of 1912 to find a material rich in soluble humates, to serve as a medium for the growth and distribution of nitrogen-fixing organisms, it was discovered that when peat is submitted to the action of certain aerobic soil organisms at a temperature of 26° C., it is rapidly decomposed, and a large amount of the humic acid present is converted into soluble ammonium humate.

This 'bacterized' peat, after being sterilized, was found to be an excellent medium in which to grow nitrogen-fixing bacteria and apply them to the soil. Mixed with soil in the proportion of 9 oz. of soil to 1 oz. of bacterized peat, saturated with a mixed culture of nitrogen-fixing organisms, a large increase in the nitrogen content of the soil was obtained after being kept in an incubator for seventeen days at a temperature of 26° C., as shown by the following tables :

Plumstead soil.

Soil plus sterilized bacterized peat	(*a*) 0·717	grm. N per	100 grm. soil.
	(*b*) 0·709	,,	,,
Soil plus active bacterized peat	(*a*) 0·792	,,	,,
	(*b*) 0·789	,,	,,

An average gain of 77 mg. of N per 100 grm. soil.

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Rothamsted soil.

Soil plus sterilized bacterized peat	(*a*) 0·367	grm. N	per 100 grm. soil.
	(*b*) 0·363	,,	,,
Soil plus active bacterized peat	(*a*) 0·421	,,	,,
	(*b*) 0·417	,,	,,

An average gain of 54 mg. of N per 100 grm. soil.

It was also found (3) that an aqueous extract of the bacterized peat (one part peat to 200 partswater) supplied all the plant food necessary for water-cultures with tomato, barley, and buck-wheat seedlings.

Experiments conducted at Kew Gardens and Chelsea Physic Garden during the summer of last year (1913) on various pot plants—wheat, barley, oats, maize, salvia, fuchsia, carnation, primula, &c.—demonstrated that the bacterized peat possessed a certain growth-stimulating property which could not be accounted for by any known manurial constituents present (4). Further experiments showed that this stimulating substance was soluble in water and effective in very minute quantities. Dr. Rosenheim, in an experiment with seedlings of *Primula malacoides* potted up in loam, leaf mould, and sand, found that plants watered twice with the water extract of only 0·18 grm. of bacterized peat were, after six weeks' growth, double the size of similar untreated plants, and it was noted that flower production and root development were promoted equally with increase of foliage.

These results suggested that the growth-stimulating action of the bacterized peat might be due to the presence of substances similar in nature to the accessory food bodies concerned in animal nutrition.

Recent work on animal metabolism has shown that animals cannot live on a diet of pure proteins, carbohydrates, fats, and inorganic salts alone. In addition very small amounts of certain other organic compounds, the so-called accessory food bodies, are absolutely essential for normal nutrition and growth.

These substances were first recognized in connexion with the diseases of beri-beri and scurvy, which are caused by a deficiency in diet of minute amounts of certain nitrogenous substances necessary for normal metabolism. They have been obtained chiefly from vegetable tissues—rice polishings, wheat, barley, lentils, yeast, fresh vegetables, and fruits; amongst animal tissues from milk, egg-yolk, and ox brain.

More recently the researches of Osborn and Mendel (5) and Hopkins (6) have demonstrated the importance of small amounts of similar accessory food substances in the metabolism of growing animals. These investigators have shown that young rats fed on a dietary consisting of a mixture of pure proteins, carbohydrates, fats, and inorganic salts failed to grow, but on the addition of a very small amount of certain substances obtained from milk growth was normal.

Very little is known at present as to the nature and composition of these substances. The all-important practical point is that their existence and action have been proved. Unfortunately the active substance appears to be largely destroyed by chemical manipulations, and it is difficult to obtain sufficient to study its chemical constitution and properties.

Cooper and Funk (7) were the first to show that the curative substance for beri-beri is precipitated by phosphotungstic acid from an aqueous solution of an alcoholic extract of rice polishings. Later Hopkins found that the active growth-substance in his experiments could be obtained from the phosphotungstic acid precipitate of proteid-free milk. Funk (8), by a complex fractionation of the phosphotungstic precipitate of anti-beri-beri substance, succeeded in isolating a substance melting at 233° C., which he called *vitamine*, and which, in amounts of 0·02 to 0·04 grm., cured polyneuritis in pigeons. This substance he considered to be of the nature of a pyrimidine base. Hopkins (9), however, states that the additions in his growth experiments were free from amino acids, purine, and pyrimidine bases.

At present the only definite statement that can be made concerning these bodies is that they are similar in being precipitated by phosphotungstic acid, and in being active in very minute amounts. It has been suggested that they belong to a new group of nitrogenous compounds, which exist only in small amounts in food materials, but are so extremely active that minute quantities are sufficient to supply the needs of the organism.

As previously stated, the growth results obtained with a water extract of bacterized peat suggested that the peat might contain substances similar to these accessory food bodies. Water and sand cultures of wheat seedlings were therefore commenced to test this hypothesis. The first experiment was made to determine how far the phosphotungstic acid fraction of bacterized peat extract was effective in stimulating plant-growth. The bacterized peat was extracted with absolute alcohol in a shaking machine for three hours, and the extract evaporated to dryness *in vacuo*.

The residue was taken up in distilled water, filtered, and to the filtrate sulphuric acid was added until the concentration of the latter reached 5 per cent. A slight precipitate of humic acid was filtered off, and to the filtrate an excess of a 30 per cent. solution of phosphotungstic acid was added. The whole was then left to stand overnight, when the liquid was decanted off through a filter, the precipitate repeatedly washed with a 5 per cent. solution of sulphuric acid, and finally decomposed with an excess of baryta. The liquid was filtered off from the precipitate of barium phosphotungstate, and the filtrate, freed from the last traces of baryta by means of a very dilute solution of sulphuric acid, was evaporated to dryness *in vacuo*. From seven kilos of bacterized peat the amount of dry substance obtained from the phosphotungstic acid fraction amounted to 12·0096 grm., and since this was made up for experimental purposes into a solution containing the fraction from 10 grm. of peat per litre, the proportion of the dry phosphotungstic acid fraction in the final solution employed consisted of seventeen parts per million. This fraction was tested upon wheat seedlings in conjunction with Detmer's complete food solution. Ten seedlings were germinated in clean sand in each of nine pots, which were arranged in three series of three pots each. Series I was treated with complete food solution, Series II with complete food plus alcoholic extract from 10 grm. of peat per litre of solution, and Series III with complete food plus phosphotungstic fraction from 10 grm. of peat per litre of solution. The food solution employed contained nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 400, 200, and 1,220 parts per million respectively, so that in addition Series III had seventeen parts per million of dry substance obtained from the phosphotungstic fraction. Each pot was treated with 100 c.c. of its solution one week after sowing the seed, and the treatment repeated once weekly for five weeks, at the end of which period the plants were uprooted, washed, dried, and weighed. The results were as follows:

TABLE I.

	Series.	*Weight of 30 plants.*	*Increase over Series I.*
I.	Complete food solution	11·94 grm.	
II.	Complete food plus alcoholic extract . . .	14·46 grm.	21·1 %
III.	Complete food plus phosphotungstic fraction	15·45 grm.	29·4 %

The results thus obtained indicate that the substance in bacterized peat which is so effective a stimulant of plant growth is also precipitated by phosphotungstic acid, and that this phosphotungstic fraction is quite as effective as the original alcoholic extract of the peat. Funk found that upon further fractionation of his phosphotungstic acid precipitate with silver nitrate and baryta, and elimination of the reagents, he obtained some relatively pure crystalline substances to which he gave the name 'vitamines', and these he considered to be the specific curative substances. In order to determine how far the growth stimulant in bacterized peat resembled these so-called 'vitamines', a further fractionation was carried out along the lines described in his paper.

The phosphotungstic acid precipitate was decomposed as before described with baryta, and the last traces of baryta eliminated by means of sulphuric acid. To the filtrate from the barium salt, silver nitrate was first added, and then baryta, until no further precipitate was produced. The brownish precipitate was filtered off, well washed, suspended in dilute sulphuric acid, and decomposed with sulphuretted hydrogen. The filtrate from the silver sulphide was then exactly neutralized with baryta, the clear liquid filtered off from the precipitate of barium sulphate, and evaporated to dryness *in vacuo*. The weight of dry substance obtained from the silver fraction from 7 kilos of bacterized peat amounted to 0·2452 grm., and since this also was made up for experiment into a solution containing the silver fraction from 10 grm. of peat per litre, this solution contained the dry substance from the silver fraction in the proportion of 0·35 part per million. This fraction was also tested concurrently with phosphotungstic acid fraction upon wheat seedlings. Fifteen seeds were germinated in each of nine pots, which were arranged in three series of three each. Series I was treated with complete food solution containing nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 400, 200, and 1,220 parts per million respectively. Series II was treated with a similar solution, containing in addition seventeen parts per million of the phosphotungstic fraction, and Series III with the complete food solution plus 0·35 part per million of the silver fraction. The pots were first treated one week after sowing the seed, and after that each pot received once weekly 100 c.c. of its food solution for seven weeks. At the end of that period the plants were washed, dried, and weighed, and after the gross weight had been taken, the plants were all dried in the steam-oven at 100° C. until their weight was constant.

The results are as follows :

TABLE II.

Series.	*Gross weight of 45 plants.*	*Increase over Series I.*	*Dry Weight.*	*Increase over Series I.*
I. Complete food	64·5 grm.		13·3480 grm.	
II. Complete food plus phosphotungstic fraction .	96·8 grm.	50·0 %	16·3818 grm.	22·7 %
III. Complete food plus silver fraction	96·5 grm.	49·6 %	15·7148 grm.	17·7 %

The silver fraction from the peat extract, corresponding with the 'vitamine' fraction of Funk, having thus given results approaching those of the phosphotungstic fraction, a preliminary investigation was made to test its effect on the growth of wheat seedlings in water culture. Two sets each consisting of eighteen similar seedlings were carefully selected, each set being originally of equal weight, viz. 4·73 grm. Each set was divided for purposes of water culture among three similar bottles of 200 c.c. capacity, six plants being inserted through notches in the corks of each bottle, so that the roots dipped into the solution. The three bottles of Set I were filled with Detmer's nutrient solution, made with pure salts in physiologically pure distilled water, in which the proportions of NH_3, P_2O_5, and K_2O were 400, 200, and 1,220 parts per million respectively ; while those of Set II contained a precisely similar solution which had received in addition 0·35 part per million of the silver fraction of peat extract. The bottles were aerated daily, and the solutions changed twice a week, while at the end of every sixteen or seventeen days the plants were removed, dried, and weighed.

The results obtained are shown in the following table:

TABLE III.

Series.	*Weight of set of* 18 *plants.*		*Percentage gain or loss on original weight.*
I. Pure food solution	Original weight	4·73 grm.	
	After 16 days	5·42 grm.	+ 14·7 %
	After further 17 days	5·29 grm.	+ 11·8 %
	After further 17 days	4·33 grm.	− 8·4 %
II. Pure food solution plus silver fraction	Original weight	4·73 grm.	
	After 16 days	5·57 grm.	+ 17·7 %
	After further 17 days	6·65 grm.	+ 40·6 %
	After further 17 days	7·33 grm.	+ 54·9 %

The results are graphically represented in Diagram 1, in which the dotted line represents the changes in weight of the series in pure food, and the unbroken line shows the progressive increase in weight obtained by addition of the silver fraction.

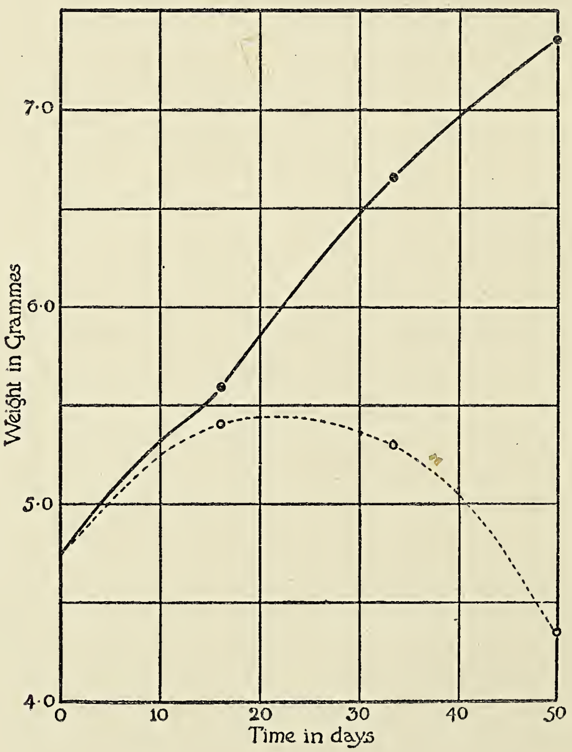

DIAGRAM 1.

The slow growth of the seedlings in this experiment will be noted. This is accounted for by the fact that they were grown in an unheated greenhouse on the roof of King's College from late in February to early in April this year, when there was very little sunshine.

Up to a certain point the two series of plants increased in weight to an almost equal extent. Beyond this point the seedlings growing in pure food solution were unable to utilize the food supplied to them, and gradually withered and died, whilst the seedlings with the silver fraction of bacterized peat extract continued to grow, and were strong and healthy throughout the experiment, their vigour being specially manifest in a well-developed root system. It is evident from this experiment that bacterized peat contains a substance or substances which enable the plant to utilize the normal mineral food constituents supplied to it. In nature doubtless this substance or substances are supplied by 'humus'—the decayed organic matter in the soil.

A series of experiments by Fürst (10) in 1912 on the anti-scorbutic accessory food substances obtained from various seeds is suggestive in connexion with the fact noticed above, that in both series of plants growth was almost equal up to a certain point.

Fürst showed that it was only during germination that the anti-scorbutic substances were developed in seeds of barley, oats, peas, and flax. Guinea-pigs suffering from scurvy when fed on these seeds before germination soon died, but seeds, soaked in water for twenty-four hours, and then kept in a warm room for two or three days until the young roots began to show, were as effective in curing the disease as extracts from green vegetables.

The development of these substances during germination must have a definite relation to the seed itself, and suggests the possibility of the formation during germination of special growth substances, which enable the young embryo to utilize the food material present in the seed. If this is so, the removal of the source of these growth substances by the cutting off of the seed as soon as possible after germination should render the effect of an addition of such substances in the food solution all the more marked.

In order to test this hypothesis, two series of wheat seedlings, similar to those used above, but in a rather younger state, were taken. Before the removal of their seeds these two sets were of equal weight: viz. 3·97 grm. Their seeds were carefully removed, injury to the plants being avoided, and after this process the two sets weighed respectively 3·2 and 3·17 grm. These were treated in precisely the same manner as before: the first being given complete food salts, and the second food salts with the addition of the silver fraction. The weights of the two sets at various dates are shown in the following table:

TABLE IV.

Series.	*Weight of set of* 18 *plants.*		*Percentage gain or loss in weight.*
I. Complete food solution	Original weight	3·20 grm.	
	Weight after 16 days	3·37 grm.	+. 5·3 %
	After further 17 days	3·20 grm.	+ 0·0 %
	After further 17 days	2·85 grm.	− 10·9 %
II. Complete food solution plus silver fraction	Original weight	3·17 grm.	
	After 16 days	3·63 grm.	+ 14·5 %
	After further 17 days	4·29 grm.	+ 35·3 %
	After further 17 days	5·05 grm.	+ 59·3 %

Diagram 2 (below) shows the variation in weight of the seedlings throughout the experiment, the dotted curve representing the series in pure food, while the unbroken curve shows the effect of the addition of the silver fraction.

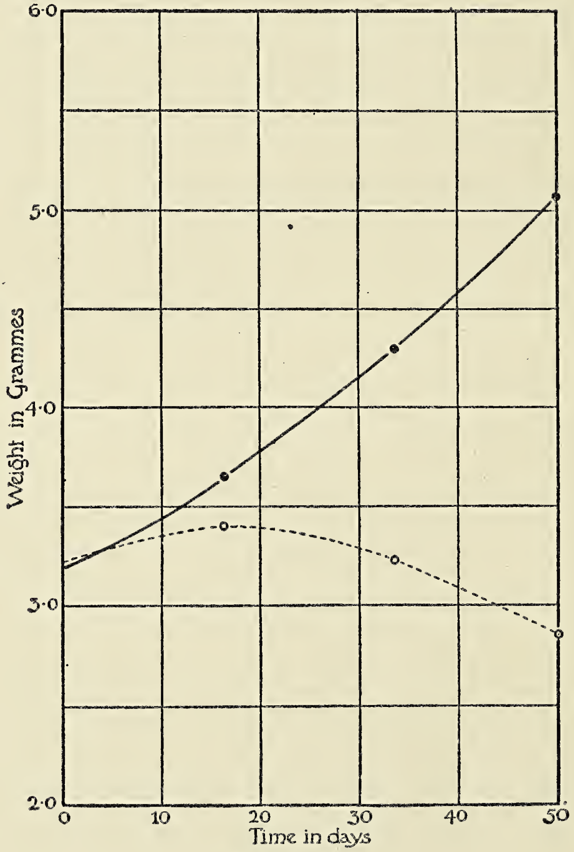

DIAGRAM 2.

In this experiment the plants growing in pure food solution made no growth beyond the small amount which could be accounted for by the traces of the growth substances absorbed by the embryo before the seed was cut off. The plants with the silver fraction, however, show a progressive increase in weight, which was manifest by an increase in size whilst the experiment lasted. The result appears to support the view that during germination certain substances are developed which enable the embryo to utilize the food material present in the seed, and that these substances can be replaced in whole or part by the silver fraction from an extract of bacterized peat.

One striking fact connected with these substances is that they are active in extremely minute quantities, 0·35 part of the silver fraction per million parts of culture solution being sufficient to give the results quoted above.

The chief interest of these experiments centres round the possibility that the nutrition of a plant depends, not only upon the supply of mineral food constituents, but also upon a supply of certain accessory organic food substances, very small amounts of which are sufficient to supply the needs of the plant. During the early stages of growth of the embryo, these substances are supplied by the seed; later when the young plant is able to maintain itself, they are obtained from the humus of the soil.

How far this theory is a correct one can only be decided by further careful experiments which are now in progress. In view, however, of the growth results obtained by ordinary water-culture methods in the laboratory it must be noted that the above results were only obtained by using chemically pure salts and physiologically pure distilled water, thus avoiding any possible inclusion of traces of soluble organic matter. Care was also taken to prevent any bacterial or algal contamination of the culture solutions.

Nothing definite can yet be stated as to the nature or composition of these growth-stimulating substances, but experiments in progress appear to indicate that they resemble more closely the accessory food factors of Hopkins than the vitamines of Funk.

In conclusion, I wish to express my indebtedness to Miss Mockeridge, B.Sc., for her valuable assistance with the chemical part of this investigation; also to thank Mr. Watson of the Royal Gardens, Kew, and Mr. Hales of the Chelsea Physic Garden, for their kindness in carrying out a series of pot experiments.

BIBLIOGRAPHY.

1. KRZEMIENIEWSKI: Bull. Acad. Sci. Cracovie, 1908.
2. HUTCHINSON and MILLER: Centralbl. f. Bakt., vol. xxx, 1911.
3. BOTTOMLEY: Report Brit. Assoc., Trans. Sect. K, 1912.
4. ————: Journ. Roy. Soc. Arts, vol. lxii, 1914.
5. OSBORNE and MENDEL: Carnegie Inst. Publication 156, 1911.
6. HOPKINS: Journ. of Physiol., vol. xliv, 1912.
7. COOPER and FUNK: Lancet, 1911.
8. FUNK: Journ. of Physiol., vol. xlv, 1912.
9. HOPKINS: Brit. Med. Journ., vol. ii, 1913.
10. FÜRST: Zeitschr. f. Hyg. u. Infekt., vol. lxxii, 1912.

The Morphology of the 'Corona' of Narcissus.

BY

W. C. WORSDELL.

With three Figures in the Text.

As a result of the recent investigation of two unusually interesting abnormal flowers [1] of *Narcissus Pseudo-narcissus* var. *tridymus*, De Graaff, I have been able to confirm the conclusions arrived at by Celakovsky [2] (as a result of his study of abnormal flowers of *N. Tazetta*) with regard to the morphological nature of the 'trumpet' or 'corona'. In my flowers the three sepals were normal, save for the complete absence of any corona. The androecium and pistil were also normal. The three petals were all partially transformed into stamens, and it was owing to this fact that, as it so happened, the nature of the corona became revealed in its true light. The corona of each petal was bilobed and was confined to these members of the flower, the sepals, as above stated, being devoid of it. As each petal had its own individual corona, it is obvious that the normal corona had become completely split up. In both flowers the corona of each petal was seen to be an intermediate structure *between a petaloid ligular outgrowth from the upper surface of the petal*, *on the one hand*, *and the basal lobes of the versatile anther on the other*, in just the same way as the organ bearing it was an intermediate structure between a petal and an anther. In the case of one petal the corona lobes were obviously direct downward extensions, in petaloid form, of the anther lobes, while at the same time continuous on their outer margin with the normal ligular outgrowth from the petal; and the presence of this marginal petaloid portion of the corona was a *result* of the presence of the corresponding marginal petaloid portion of the upper portion of the anther (Fig. 1). This was, perhaps, the best case for demonstrating the absolute oneness of the anther lobes with the corona, the texture and colour of the normal corona being always present. In another petal, besides the downwardly directed narrow

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[1] Handed to me by Mr. F. J. Chittenden at the meeting of the Scientific Committee of the Royal Horticultural Society of March 24, 1914.

[2] Ueber die Bedeutung und den Ursprung der Paracorolle der Narcisseen (Bulletin international de l'Académie des Sciences de Bohême, 1898).

[Annals of Botany, Vol. XXVIII. No. CXI. July, 1914.]

anther lobes having the colour and consistence of a corona, there were, in addition, two upwardly directed, broad, petaloid lobes (Fig. 2), so that in this case the corona was divided into two parts, exactly corresponding to the two parts into which the metamorphosed *petal* was divided, viz. upper anther lobes and lateral petaloid portions. In a third petal the basal anther lobes were partly polliniferous and partly corona-like.

With these clear transitional structures before me, there can no longer be any doubt in my own mind as to the true morphological interpretation of the corona. Moreover, this could never have been arrived at without the aid of these teratological data.

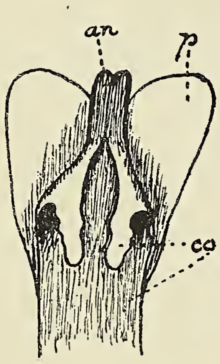

FIG. 1. *Narcissus Pseudo-narcissus* var. Staminoid petal showing 'corona' as a downward prolongation of anther lobes. Lettering as in Fig. 2.

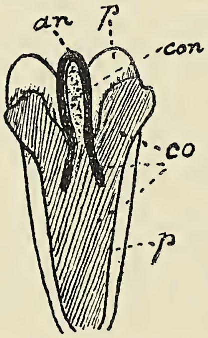

FIG. 2. Ditto; the extreme petaloid portion of 'corona' is upwardly directed. *p.* = petal; *an.* = anther; *con.* = connective: *co.* = corona. (Slightly schematized.)

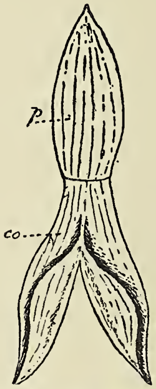

FIG. 3. *Narcissus Pseudo-narcissus* var. Perianth segment (*p.*) with its two-lobed corona segment (*co.*), showing transition to anther structure. (Semi-diagrammatic.)

But the point of chief interest has yet to be mentioned. In the flowers from whose structure Celakovsky deduced his remarkable conclusions, it was the petaloid *stamens* which exhibited the transitional conditions of the corona, and from which he further deduced the staminal origin of the perianth. In my own flowers it was the staminoid *petals* which exhibited the interesting transformations in the corona, which latter is thus seen to be changing *back* again to its original, primitive condition, viz. of basal anther lobes. Celakovsky's view of corona-morphology becomes thus doubly supported, viz. by the occurrence of the same transformations taking place in *both* directions: progressively and retrogressively!

There is one further point of importance to be noted. In the change which is under way, whereby the three petals become partially transformed into stamens, *the entire corona of the flower appears to become involved*, the sepals being left without any trace of this structure; from which it might be reasonably deduced that in *Narcissus* the normal corona is an outgrowth of the corolla only, and that the sepals, although equally derived from stamens possessing versatile anthers, no longer have, if they ever did have, any structure corresponding to the basal lobes of the anther. The *petals* of some genera of Amaryllidaceae, although derived by transformation of versatile anthers, are also devoid of any trace of a ligular outgrowth. On the other hand, Celakovsky, from a study of abnormal flowers of *N. Tazetta*, concluded that the calyx possessed a corona as well as the corolla, but that in some of the double flowers in which the stamens produced corona structures, that of the calyx was either greatly reduced or entirely suppressed. Hence it would be best, on the whole, to regard the calyx corona in the flowers I have been investigating as having also become suppressed as a result of the changes undergone by the corolla.

Since writing the above note another abnormal flower, of the same species, has come into my hands, which shows almost equally important features. The corona has undergone complete dialysis, and *each* segment of the perianth bears a *bilobed* corona segment ; this character in itself represents, in my opinion, one step in the transition to the two basal lobes of the versatile antler (see above). But in the case of one of the six perianth lobes, the two lobes of its ventral corona segment each possessed an *infolded flap on its inner margin*, the two flaps being united below (i. e. towards the base of the corona segment) to form a short enation from the upper surface of the corona (Fig. 3). This structure, in my opinion, undoubtedly represents a still further step in the direction of transformation of the individualized corona segment into the basal lobes of the versatile anther, of which the perianth segment (*p.*) represents the apical portion.

The following conclusions, therefore, are reached as a result of this investigation:

1. Both sepals and petals, in Amaryllidaceae, have been derived by transformation of stamens in an originally achlamydeous flower.
2. The corona, in this order, has been derived by petaloid transformation of the upturned basal lobes of the versatile anthers, and subsequent fusion thereof to form a continuous rim.

KEW,
March 30, 1914.

NOTE.

PRELIMINARY NOTE ON OCCURRENCE OF STOMATA IN HYPOGEAL COTYLEDONS.—It is generally assumed that cotyledons which on the germination of the seed remain within the testa and in the majority of cases, also below ground, have been derived at some time or another from cotyledons that emerge from the testa. Goebel ('Organography of Plants,' Part II, p. 403) states that 'in hypogeous cotyledons the functioning as assimilation organs has only been given up at a late period in connexion with the deposition within them of reserve material, and that in consequence of this they no longer reach the light'.

The striking results obtained by A. W. Hill in his work on the 'Morphology and Seedling Structure of the Geophilous Species of *Peperomia*' (Ann. Bot., vol. xx, 1906) suggested an investigation into the occurrence of stomata on hypogeal cotyledons present in dicotyledonous and in monocotyledonous seeds. If stomata occur on hypogeal as they do on epigeal cotyledons this may be regarded as an instance of the retention of a primitive structural feature that has now become useless.

It has been found very difficult to secure adequate supplies of material, and seeds have therefore been examined just as they could be obtained and not according to any scheme of classification. In the case of large cotyledons the upper and lower epidermis was removed by tangential section. In the case of minute cotyledons the whole leaf, and in some cases the whole seedling, was rendered transparent in caustic potash. If stomata were observed their relative numbers and distribution were noted. If, on the other hand, stoma initials only were found the seeds were germinated in the hope that the initials would develop further. Some few of the seeds were germinated in the normal way, the cotyledons remaining within the seed coat. From other similar seeds the testa was removed so that the cotyledons were freely exposed to light and air, the object being to induce the cotyledons of such seeds to revert to their original habit of developing to a greater or lesser extent, as assimilatory and transpiratory organs. In all cases the seeds were germinated on moist Sphagnum and not in soil.

During the investigation several difficulties were encountered. In the first place, very little literature is available giving accurate data as to the names of plants possessing the hypogeal character in their cotyledons. Lubbock's two volumes 'On Seedlings', Kerner's 'Natural History of Plants', Goebel's 'Organography of Plants', and occasional odd papers on single seedlings have supplied a certain amount of information, but these are by no means exhaustive. To germinate all available seeds for the purpose of observing the nature of their cotyledons seemed an unnecessary undertaking if such germination has already been carried out for other purposes. Miss Blackburn, of Bedford College, London, has kindly communicated a list of seeds with hypogeal cotyledons compiled from her own observations, A further difficulty lay in the germination of the seeds themselves, many of which are tropical. Even when the optimum conditions of heat and moisture were provided, many foreign seeds, for other reasons, did not germinate. It may be that they had been stored for too long a time and had lost the power of germination, or perhaps other and biological conditions necessary to germination were not supplied.

Again, it is not possible in all cases to determine with certainty that what might be regarded as a stoma initial will prove to be so, without following out its development during the further growth of the cotyledon. In several observed cases the supposed stoma initials have not developed into stomata on germination. Presumably this may be either because they were really ordinary epidermal cells with a superficial resemblance to stoma initials or because they have lost the power to undergo further division. This explanation may possibly apply to the case of the genus *Lathyrus*.

According to Lubbock, Goebel, Hildebrandt, Janczewski, and others, there are:

(1) Several *orders* in which all species show epigeal cotyledons, e. g. Cruciferae, Scrophulariaceae, &c.

(2) A few *orders* in which all species possess hypogeal cotyledons, e. g. Nymphaeaceae, Guttiferae, &c. And, on the other hand, there are:

(3) *Genera* with species, some of which have hypogeal and others epigeal cotyledons. This third class is particularly interesting, seeing that one might reasonably expect to find stoma initials present on all such cotyledons before germination. According to the authorities cited, the following are to be included under this head: *Clematis*, *Anona*, *Rhus*, *Trichosanthes*, *Ardisia*, *Jatropha*, *Rhamnus*, *Mercurialis*, *Phaseolus*, *Erythrina*, *Anemone*, *Abrus*. Species of those genera that have so far been examined give evidence in support of this view.

More than forty types of seeds have already been investigated. It is hoped later to give a complete account of these examples and of such others as it may be possible to obtain, both as regards the occurrence of stoma initials or fully developed stomata before and after germination, and also to correlate this anatomical feature with the morphology of the cotyledons.

The hypogeal cotyledons of the seeds examined fall into *three* categories:

(1) Those in which definite stomata are formed within the testa before germination, e. g. *Pachyrhizus angulatus*, *Phaseolus multiflorus*.

(2) Those in which stoma initials are present, which develop into stomata on germination, e. g. *Citrus decumana*.

(3) Those in which no stoma initials are present or, if present, have lost the power of further development, e. g. *Pisum sativum*, *Tropaeolum minus*.

In many such species the first two or three pairs of true leaves are reduced to mere scales, e. g. *Walsura piscidia*, *Calodendron capense*.

The seeds used in this investigation have been provided by the Directors of the Royal Botanic Gardens of Kew, Edinburgh, Calcutta, and Trinidad.

ELSIE M. BLACKWELL.

Hartley Botanical Laboratories,
University of Liverpool,
March, 1914.

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BY

FRANK CAVERS

THE objects of *The Journal of Ecology* are (1) to foster and promote in all ways the study of Ecology in the British Isles, serving as the organ of the British Ecological Society; (2) to present a record of and commentary upon the progress of Ecology throughout the world. Its contents include original articles and notes on ecological topics of current importance, including methods of ecological study and research; reviews and notices of publications of general ecological bearing and of work upon plant and animal communities in all parts of the world; reports and notices of meetings of the British Ecological Society; and the progress of the Nature Protection movement and of ecological work in Nature Reserves.

The Journal of Ecology, being largely a Review, is indispensable to those desiring to keep abreast of the extensive and widely scattered literature of Ecology, of which it will take a comprehensive view, including in its scope not only work dealing with general principles, methods, surveys, and other ecological studies, but also publications dealing with Plant and Animal Ecology and Geography, Planktonology, the science of soils (soil physics, chemistry, and the investigation of soil floras and faunas), and other branches of Natural Science (Geology, Climatology, Agriculture, Forestry, &c.), so far as these have a direct bearing upon Ecology—the relationships of plants to their environment in the widest sense, including the inter-relationships between plants and animals. In brief, it aims at furnishing a comprehensive review of progress in the entire field of Ecology, touching, as this field does, kindred branches of science on various sides.

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Investigations on the Phylogeny of the Angiosperms: No. 4. The Origin and Dispersal of Herbaceous Angiosperms.

BY

EDMUND W. SINNOTT

AND

IRVING W. BAILEY,

Bussey Institution of Harvard University.

With Plates XXXIX and XL and eight Diagrams in the Text.

Contents.

RECENT comparative work on the internal and external structure of vascular plants has been directed mainly toward the determination of lines of evolutionary descent, a knowledge of which shall make possible

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the construction of a natural classification for the higher plants. Comparatively few investigations have as yet been undertaken for the purpose ot discovering not alone the history and evolution of species, but more particularly the origin and development of the different growth-forms or habits which are assumed by plants, and of the various types of internal or external structure produced to meet particular environmental conditions. Such studies, although concerned primarily with phylogeny in its broadest sense, also introduce important problems in ecology, physiology, and experimental morphology, and are therefore worthy of careful attention from students of evolution.

The most fundamental of such differences in general habit of growth among Angiosperms, and the one which is of greatest economic significance, is that between woody and herbaceous plants. The former series comprise trees, shrubs, and undershrubs, and have persistent, woody, aerial stems which increase in size from year to year by the activity of a cambium, and which usually attain considerable height and thickness. Herbaceous plants, on the other hand, are short-lived and limited in growth, at least in their aerial portions, and possess stems which are usually smaller and of softer texture.

These two great groups are approximately equal in number of species, woody forms predominating in the tropics and herbs in more temperate regions. The distinction which separates them, however, is of practically no value in determining natural relationships, for nearly half the families of the Angiosperms include both woody and herbaceous members, and in a large percentage of the genera, as well, there are species from both groups. It is quite evident, therefore, that whichever of these two classes is the more recent must have arisen quite independently many different times, and from numerous ancient stocks. The whole question as to the origin of these plant types, their relative antiquity, and the way in which they have been developed and become dispersed is consequently of great botanical interest.

It is somewhat surprising that there has been so little discussion of the problem among botanists, and that so few hypotheses have been put forward. A rather widely accepted opinion seems to be that ancient Angiosperms were herbaceous, and that the woody and arborescent members of the phylum have been derived from these primitive forms by a decided increase in the extent of the cambium and in the amount of its activity. This theory is presumably based on a comparison between the development of an individual and the history of the race, and also on the general supposition that the complex should be derived from the simple. Such a proposition, however, has very rarely been formulated in a definite manner.

The alternative hypothesis, that woody plants are the more ancient and that herbaceous forms have been derived from these by simplification and reduction, has been generally current among geologists for some time, but the first definite statement of such a view which the writers have found is that made by Hallier (8) in 1905. In discussing the origin of the Angiosperms he states that 'from their arborescent habit (&c.) the Magnoliaceae are, without doubt, the oldest of living Angiosperm families, and down from them, through the Schizandreae, Lardizabaleae, Berberidaceae, Ranunculaceae, and Nymphaeaceae, clear through to the Monocotyledons, there has been a continuous and unbroken line of reduction from the ancient arborescent forms to lianas, shrubs, undershrubs, herbs, and water-plants. . . . In the Berberidaceae, Menispermaceae, and species of *Clematis*, the vascular bundles of the axis are already separated by the widening of the medullary rays; and in the herbaceous Ranunculaceae (as well as in the Piperaceae and Chloranthaceae) by a much greater widening of the medullary rays, by an early cessation of cambial activity, and by the appearance of additional circles of vascular bundles, the type of stem which is characteristic of the Monocotyledons has been developed.'

Such an hypothesis of reduction from primitive arborescent forms has also been worked out under the direction of Professor Jeffrey by several members of his laboratory (12, 1, 2 and 5). Eames (5) in a paper devoted to the subject brings forward evidence that the earliest Dicotyledons possessed a solid tubular woody cylinder of considerable thickness which has gradually been reduced, and finally broken up into a circle of separate strands, the 'typical' herbaceous condition.

A discussion of this general problem is the purpose of the present paper, in which the writers will bring forward evidence from the fields of palaeobotany, anatomy, phylogeny, and phytogeography in an attempt to come to some definite conclusion as to the relative antiquity of these two great types of plants, and as to how they have become dispersed over the world.

I. EVIDENCE FROM PALAEOBOTANY.

One naturally turns at first to geology in an endeavour to settle any phylogenetic problem, but in the question under consideration, as in so many others, evidence from this source, though of value, is not at all conclusive.

The change of growth-habit during evolutionary history by some of the lower orders of vascular plants, as shown in a comparison between their ancient fossil forms and their living members, is perhaps of importance as indicating by analogy what may well have been the course of events among the Angiosperms. The ancient members of the lycopodiaceous and equisetal series, for example, were with few exceptions provided with well-marked secondary wood and in the great majority of cases were decidedly arborescent in habit. Their living representatives, however, the modern Lycopodiaceae, Isoetaceae, and Equisetaceae, are entirely herbaceous but vestiges of a cambium in *Isoetes* and *Equisetum* point to the previous activity of such a tissue. In these orders the herbaceous forms have, without much question, been derived from ancestral woody types.

Among Angiosperms, palaeobotanical evidence seems at first sight to point certainly to the conclusion that the arborescent members of the phylum are more ancient than the herbaceous ones, for the overwhelming majority of fossil Angiosperms are related to forms which to-day are always trees and shrubs. The objection frequently raised to this conclusion, and with good reason, is that the absence of herbaceous leaf-impressions is not due to the absence of herbs in ancient times, but rather to the fact that leaves of such plants are generally much more delicate and less apt to be preserved in a fossil state than are the tougher ones of trees and shrubs.

Herbs, however, are not entirely lacking as fossils. In the middle Cretaceous of the Potomac occurs an impression which has been variously assigned to *Plantago*, the Xyridaceae, and the Cyperaceae, and which is obviously herbaceous. In the Florissant, presumably Eocene, occurs a leaf referred to *Carduus*. From the Miocene onward, especially in the strata from Spitzbergen and Switzerland investigated by Heer (9 and 10), herbaceous forms are increasingly abundant. In the Tertiary flora of Switzerland Heer records 24 per cent. of the Angiosperms as being herbaceous. In the strata which he studied, the number of herbs is much greater in the more recent than in the older formations.

On the whole, therefore, evidence from palaeobotany as to the relative antiquity of herbs and woody plants cannot be called conclusive. It seems to indicate that the earlier Angiosperms were mainly trees and shrubs, and that the number of herbaceous forms has gradually increased. The ease or difficulty of preservation, however, plays too important a part to make such evidence very convincing. It seems strange, nevertheless, that so very few herbs are found from the Cretaceous, and so many, comparatively, from the Tertiary, for conditions which would affect preservation do not seem to have been radically different in the two periods. In general, the evidence from palaeobotany, as far as it goes, seems to favour the theory that woody plants are more ancient than herbs.

II. EVIDENCE FROM ANATOMY.

A comparison between the stem structure of woody plants and herbs provides us with evidence which has a much more direct bearing on the problem of the relative antiquity of the two groups, and anatomical facts have consequently been the main basis for such inferences and conclusions as have heretofore been drawn.

Since the chief difference between woody plants and herbs consists in the presence or absence of an active cambium, the most important anatomical facts for our purpose are those concerned with the development of this meristematic layer, and the character of the tissues which it produces. Practically all herbaceous forms, at least among Dicotyledons, show evidences of cambial activity. This may be confined to the isolated vascular bundles themselves, as in the Monocotyledons and many Dicotyledons, where its activity is shown by the radial arrangement of some or all of the xylem and phloem elements; or it may extend during later development across the interfascicular parenchyma which separates the bundles and cause a slight radial arrangement in this tissue also; or it may form an unbroken ring of vascular elements arising simultaneously about the pith. The origin of secondary growth in most woody Dicotyledons follows the last category, the cambium appearing simultaneously throughout its whole extent. In others, however, especially stems with well-marked primary bundles and wide rays, it belongs to the second type, for the cambium is early active in laying down secondary wood opposite the protoxylem groups, but only later do these isolated cambia become united into a continuous ring by their extension across the gaps between the bundles. It is this irregularity in the ontogenetic development of the vascular ring of herbs and woody plants which has been emphasized by Sachs and his followers, who have applied the term 'fascicular' to that part of the cambium which occurs opposite the primary strands, and 'interfascicular' to that which later arises between them.

It is easy to attribute a phylogenetic importance to these facts of ontogeny, and to infer that the earlier stages in the development of the twig repeat, in a general way, conditions which obtained in ancient forms. To imagine that the stem of the ancient Dicotyledons was herbaceous in texture and supplied with a ring of separate primary bundles; that cambial activity began first in these bundles, increasing them slightly in size, and that finally it became continuous across the intervening spaces and laid down an unbroken ring of secondary wood, thus making possible the development of woody plants, is a perfectly natural conclusion. It should be noted, however, that such a theory of phylogeny was not made by Sachs and his followers, who concerned themselves simply with the development of the individual; nor have the writers been able to find it put forward in any of the common text-books. Doubtless the idea that herbs are more primitive than trees has been in the minds of many people, but the theory has been definitely formulated very rarely indeed.

But do the anatomical facts, when carefully considered, give sound warrant for such a conclusion? The irregular development of the cambium may be regarded with equal logic as a step towards its disruption and disappearance rather than as a primitive feature. On such a theory, herbs are to be regarded as plants which have suffered reduction from a woody condition, and not as the most ancient type of Angiosperms. The whole problem centres about the question as to whether cambial activity has arisen in a continuous or a discontinuous fashion, and it will therefore be worth our while to consider what evidence is presented by a study of the mode of origin of secondary growth in other groups of plants.

In the ancient Lycopodiales from the Palaeozoic we have probably to deal with forms in which cambial activity had but recently made its appearance, for in most species the ring of secondary wood is narrow in proportion to the primary stele, and in many instances it seems not yet to have appeared. Whenever present, it is always perfectly uniform and continuous at its origin. Unlike that of the Lepidodendrids, the stem of the ancient Calamites was provided with a ring of separate primary bundles, and in many cases the cambium laid down xylem opposite these and parenchyma opposite the gaps; but even here there is no evidence that it was not continuous from the first, or that all parts of it were not simultaneous in appearance. *Lyginodendron* and *Cordaites* also possessed distinct and separate primary bundles, but the secondary tissue was in both instances perfectly continuous, as in the previous cases. In some very young stems of *Lyginodendron*, in fact, the very first formed secondary wood has been preserved, and it is evident that the entire ring appeared simultaneously and was not influenced at all by the position of the primary bundles.

In all these groups the primary wood was centripetal in its formation, and was not in intimate contact with the zone of secondary wood. In the Conifers and Angiosperms, however, centripetal wood is either very much reduced in amount or has disappeared, and the protoxylem has become closely attached to the centrifugal primary xylem, which in turn is intimately associated with, and merges gradually into, the secondary wood. It is a noteworthy fact that in such forms the cambium does not arise in the same continuous and uniform line as in the lower groups, but that its appearance in the young twig is much more irregular. This is evident to some extent in the Conifers, but even more in the woody Angiosperms. It is most strikingly apparent, however, in those herbaceous forms where secondary wood is produced long before secondary radial or parenchymatous tissues appear. In view of the evidence from the lower forms which we have discussed, it seems more reasonable to consider this irregularity of the cambium as a recent rather than as a primitive phenomenon, and to regard it as due principally to the close attachment of the primary to the secondary wood and the gradual modification of cambial activity, originally uniform, in conformity to the position of the primary strands.

That a uniform and continuous cambium is a primitive condition is also indicated by the character of the anomalous and extrafascicular cambia which appear in many forms. These are almost always continuous for a considerable distance around the stem and are not limited to the production of small bundles.

From all the evidence, therefore, it seems reasonable to conclude that the occurrence of distinct 'fascicular' and 'interfascicular' cambia in herbaceous plants is the result of reduction and specialization from a primitively uniform and continuous condition of the secondary meristem. Such a conclusion is of course distinctly in favour of the view that herbs have been derived from plants possessing well-developed secondary wood.

Aside from facts concerned with the origin of the cambium, evidence of value may also be derived from a study of the tissues which it lays down. In the secondary wood of very many herbaceous plants uniseriate medullary rays are either absent or represented by rows of vertical parenchyma cells, and in many cases vertical parenchyma of all kinds has entirely disappeared save in the vicinity of the huge vessels, thus leaving the mass of the wood composed simply of fibres. In the secondary xylem of all woody plants, both ancient and modern, radial parenchyma is always well developed; and in the higher Conifers and all woody Dicotyledons vertical parenchyma, too, not associated with the medullary rays, is a characteristic feature. That these tissues should be absent from the secondary wood of so many herbaceous plants, therefore, is much more logically explained, as Eames has pointed out, by the great development of the broad segments of interfascicular parenchyma, which are quite sufficient for the storage and transportation of all food within the woody ring, and render unnecessary a development of smaller groups of radial or vertical parenchyma. Such a conclusion indicates again that the herbaceous type of stem is reduced rather than primitive in its character.

All evidence from the anatomy of the secondary wood, therefore, lends strong support to the theory that woody plants are more ancient than herbs. To make the argument still more convincing, however, it is necessary to demonstrate clearly the manner in which the herbaceous type of structure has been developed from that of a woody stem.

Hallier suggests, as we have noted above, that the many-bundled herbaceous stem has been derived by the increase in width of certain medullary rays, which break up the continuous cylinder into separate strands, but he elaborates no extensive theory.

The only hypothesis which seems to have been formulated in detail is that originating with Professor Jeffrey and worked out under his direction by several students. In brief, this hypothesis explains the segments of interfascicular parenchyma which occur between the bundles of the herbaceous stem as due not to gradual increase in width of medullary rays, but to the transformation into parenchyma of the entire segments of xylem which are directly opposite the outgoing leaf-traces. Evidence in support of this view has been obtained principally from a study of certain woody and herbaceous members of the Rosaceae. Some typically woody forms in this family show signs of 'compounding', or the aggregation and increase in size of medullary rays, in the region just below the departure of the leaf-trace. Good examples of intermediate conditions between such a woody stem and the herbaceous type are not cited from other aerial stems, but almost entirely from the prostrate or subterranean rhizomes of several genera. In the case of the procumbent stem of *Potentilla palustris*, for example, there is (5, p. 217) 'in the segment of the xylem directly below the exit of each leaf-trace a decided lack of vessels and an agglomeration of rays'—the first step in the transformation of woody tissue into parenchyma. In the root-stock of other forms 'a segment of the central cylinder is set off for some distance below the passing out of a leaf-trace by the formation of large rays resulting from xylem parenchymatization. A progressive change of the xylem of this segment into parenchyma occurs upwardly towards the point of exit of the trace; more and more of the secondary xylem being transformed, until only the primary tissue is left. This now subtends pure parenchyma, through which it passes out as the leaf-trace. . . . All that is necessary to produce a typical herbaceous stem from this structure is to reduce the size of the normally lignified xylem segments.' And again, 'whole segments of the central cylinder in relation to the leaf-trace become transformed entirely into parenchyma with the exception of the primary wood (which is the trace in the stem). Thus there is obtained a stem with alternating segments of typical xylem and parenchyma, the latter with tiny groups of typical xylem elements on their centrad sides, or, in other terms, alternating large and very small bundles, the latter being leaf-traces.' Attention is also frequently called to another feature in the development of the herbaceous type, the progressive 'localization of the primary wood', or the breaking up into fewer and fewer separate strands of what was originally a continuous ring of primary elements. It is noted that when the xylem ring is very thin, the mere 'setting off' of the leaf-traces, by conversion of segments on either side into parenchyma, is often sufficient to produce an herbaceous structure.

It is evident that this hypothesis regards as the most important factor in the evolution of the herbaceous type of stem the so-called process of 'compounding' in connexion with the leaf-trace, by which the segments of normal secondary xylem opposite the departing traces are gradually converted into parenchyma, thus producing a stem with a few well-separated vascular bundles. The important steps in the development of an herbaceous stem, according to such a theory, are presented successively in Diagrams 1, 7, and 8.

Although this hypothesis accounts for many of the facts in the Rosaceae, it meets with difficulty when applied to other families and is open to criticism on several counts.

In the first place, the supposed transitional stages from a woody to an herbaceous condition which it cites, and which form indeed the strongest evidence in its support, are found not in the aerial parts of the stem but in its underground portions. In this region, however, the absence of mechanical strain and the necessity for storage operate to modify the vascular structures profoundly. Roots and rhizomes of almost all plants, because of their storage function, show areas in which the number of parenchyma cells has become so very large that whole segments of the woody cylinder have in fact been converted into parenchyma. One would hardly expect intermediate steps between woody plants and herbs to originate in such a specialized underground region or to persist there, but such transitional conditions would naturally be sought rather in the aerial stems themselves, where the actual evolutionary development must have taken place. The fact that intermediate stages in harmony with the theory which we are criticizing are so rare in aerial stems must be regarded as one of its weak points.

That the localization of primary wood has been one of the chief factors in the origin of the herbaceous stem also appears rather doubtful when we observe that so many typically woody plants, such as most Araliaceae, Fagaceae, Betulaceae, Proteaceae, and others, which belong to families containing practically no herbs, possess a ring of primary wood which is composed of very distinct bundles ; and that an enormous number of herbs have a perfectly continuous vascular ring.

The fact, however, which militates most strongly against the validity of the hypothesis under discussion is that, in practically all many-bundled herbaceous stems, the interfascicular parenchyma is *not* subtended by tiny leaf-trace bundles, nor is the stem composed of the presumably typical ‘ alternating large and small bundles, the latter being leaf-traces ’. On the contrary, all the bundles in the aerial stem of a multifasciculate herb tend to be of the same general size, the leaf-traces in the stem usually growing a little smaller as they near their point of departure. At this point, also, the segment of secondary wood which each of them subtends usually grows smaller, too, and may become partially disintegrated into parenchyma. It cannot well be called a compound ray, however, and it most certainly does not correspond to the interfascicular parenchyma, for this latter tissue is not subtended by protoxylem, but seems rather to represent in most cases an ordinary medullary ray which has grown very wide. This failure of the theory of Jeffrey and his school to explain the stem

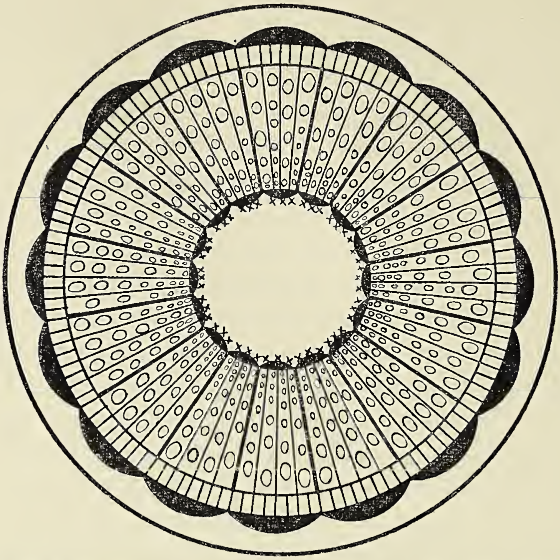

DIAGRAM 1. Woody twig with all rays narrow, the foliar rays being slightly wider than the others.[1]

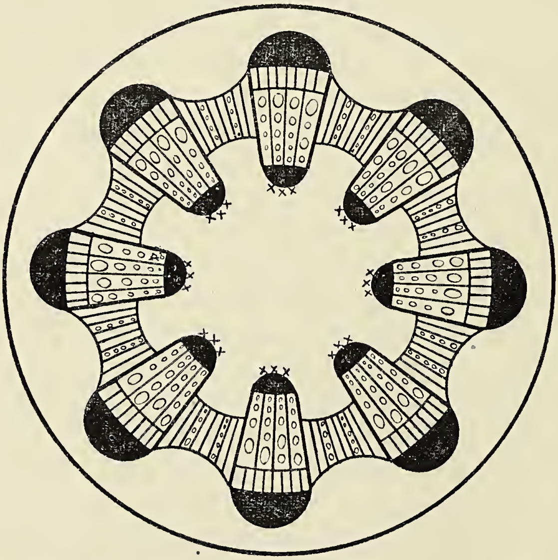

DIAGRAM 2. Herbaceous stem derived from No. 1 through more or less complete conversion into parenchyma of segments *alternate* with the leaf-traces.

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[1] Crosses represent protoxylem. Bundles in solid black next the pith are leaf-traces in the stem.

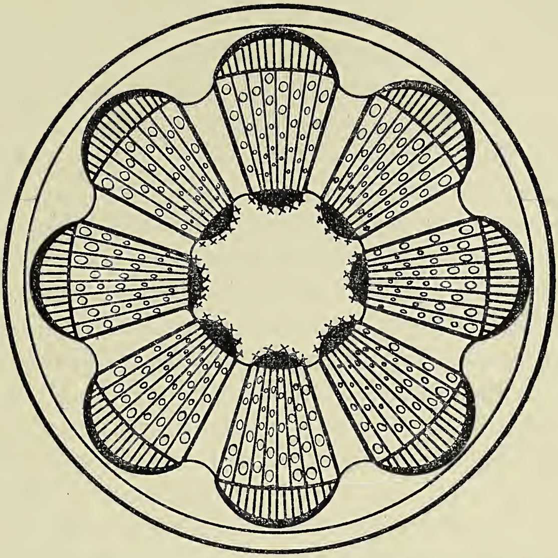


DIAGRAM 3. Woody twig with broad foliar rays which have localized the protoxylem.

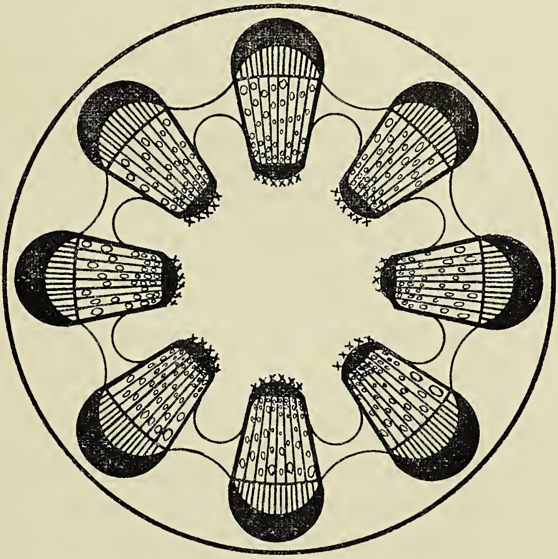


DIAGRAM 4. Herbaceous stem derived from No. 3 through increase in width of broad rays and decrease in their radial extent.

anatomy of such a wide range of plants, together with the other objections which we have cited, must be regarded as casting considerable doubt on its truth.

If this hypothesis is to be rejected, however, we must endeavour to construct one in its place which shall explain the facts before us more satisfactorily and indicate clearly how the transition from woody plants to herbs, which we believe to have taken place, has been brought about.

In the first place, the type of herbaceous stem which possesses many distinct bundles, although somewhat more frequent than any other condition, cannot at all be regarded as 'typical' for all herbs. Many genera and often whole families possess vascular cylinders which are continuous and unbroken. The Caryophyllaceae, Phytolaccaceae, Hypericaceae, Lythraceae, Onagraceae, Ericaceae, and Polemoniaceae may be cited as families in which practically all the herbaceous forms are characterized by such a solid ring of primary and secondary wood. In many other families there are numerous genera where the stem is of this type. It is worthy of note that in all such cases the twigs of related woody forms show in the same way a practically unbroken primary ring and an absence of very wide rays in the secondary wood. The herbaceous stem, therefore, is essentially the first annual ring of the corresponding woody form, with a reduced amount of secondary growth. The manner in which such an herbaceous stem has arisen is indicated in Diagrams 5 and 6; and in Pl. XXXIX, Figs. 3 and 4, 5 and 6, and 8 and 9.

In the case of the many-bundled herbaceous stems we have already noted the resemblance which the interfascicular parenchyma, in less reduced forms, bears to the broad medullary ray of woody plants. The first annual ring of *Fagus* (Pl. XXXIX, Fig. 2), for example, resembles rather closely certain of the stouter herbaceous stems. In a paper contemporaneous with this, the writers (3) bring forward evidence in favour of the theory that the wide ray of Angiosperms has had its origin not in a process of 'compounding' or gradual conversion of vertical tracheides into radial parenchyma, but by a simple increase in width of the primitive uniseriate ray. This widening of the rays is especially apt to occur opposite the gaps in the primary cylinder and causes these gaps to become wider and longer, thus 'localizing' the primary wood even in many woody plants. In the development of the multifasciculate herbaceous type, these rays usually increase still more in width and become the interfascicular parenchyma. Cambial activity tends to become much reduced within them or to disappear there altogether. Such a condition is characteristic of the softer herbaceous stems of Dicotyledons and probably formed the basis on which has been built up the typical stem of the Monocotyledons, which seems clearly to have been primitively herbaceous. The origin of such a many-bundled herbaceous stem from a broad-rayed woody form is shown in Diagrams 3 and 4; and in Pl. XXXIX, Fig. 12, and Pl. XL, Figs. 13, 14 and 15, and 16 and 17.

In certain cases there has indeed been a conversion of portions of the woody ring into parenchymatous or fibrous tissue, as is shown in Fig. 7, a transverse section of an herbaceous stem of *Salvia*; and in Diagram 2. In all such instances, however, the conversion is not opposite the *protoxylem*, as Jeffrey's theory supposes, but opposite the *gap* between the protoxylem clusters. Instead of a single ray becoming very high and wide and constituting the interfascicular parenchyma, as in most multifasciculate herbs, a whole segment, including a number of small rays and their adjacent vertical fibres and vessels, becomes thin-walled and more or less parenchymatous. The vertical elements never tend to become horizontal, however, and the structure is not comparable to the 'aggregation' and 'compounding' of rays observable in certain woody families. In some cases the interfascicular parenchyma seems to owe its origin both to increase in width of rays and to conversion of woody elements into parenchyma (Pl. XXXIX, Figs. 10 and 11).

Most vines have the structure of multifasciculate herbs (Pl. XL, Fig. 18). The rays opposite the gaps become very wide, and those in the fascicular segments usually disappear.

It seems clear, therefore, that in the development of all herbaceous stems a simple reduction in the amount of secondary wood has been the chief factor; and that this has been supplemented by the increase in bulk, to a greater or less degree, of the ordinary parenchymatous tissue. Instead of calling upon a subterranean rhizome to furnish an intermediate condition between related woody and herbaceous stems we can find these transitions clearly shown in different parts of the woody axis. The base of most herbaceous stems is much stouter than the upper portion, and often shows a close resemblance to a woody twig. On passing upward from such a base to the more delicate portions of the stem, we can readily observe the progressive decrease in cambial activity and increase in parenchymatous tissue which have caused the development of the herbaceous type. Pl. XXXIX, Fig. 1, a cross-section of a somewhat decumbent aerial stem of *Arctotis grandis*, shows in one plane the transition from a continuous woody stem to one where the bundles are quite isolated in a true herbaceous fashion.

The main point to be emphasized in this anatomical study of the evolution of herbs is that an herbaceous stem, in all its essentials, is like the first annual ring of its woody relatives. If this has a continuous ring of primary and secondary wood, as do the twigs of woody species of *Nicotiana*, *Hypericum*, and *Hibiscus* (Pl. XXXIX, Figs. 5, 3, and 8), the corresponding herbaceous stem will show the same features (Pl. XXXIX, Figs. 6, 4, and 9). If the woody form possesses wider rays and an interrupted primary cylinder, as does *Xanthorrhiza* and the arborescent species of *Acanthopanax* and

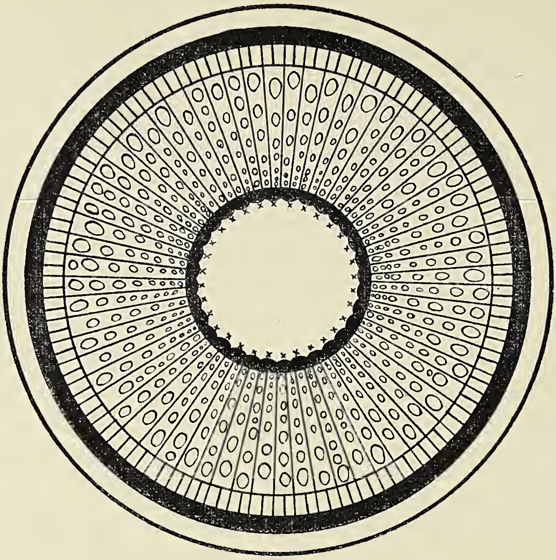


DIAGRAM 5. Woody twig with all rays narrow and protoxylem ring continuous.

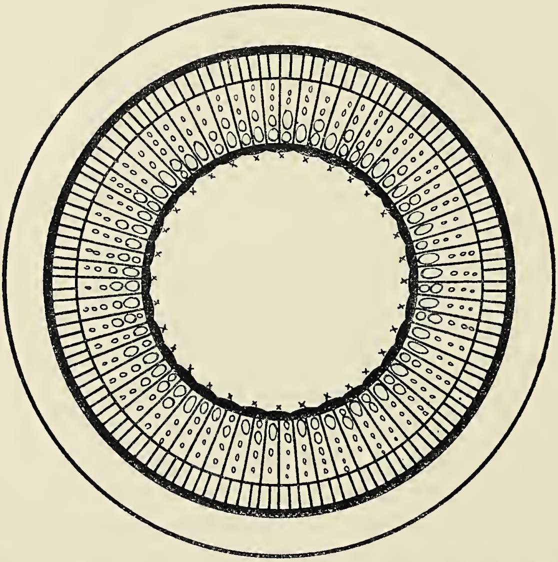
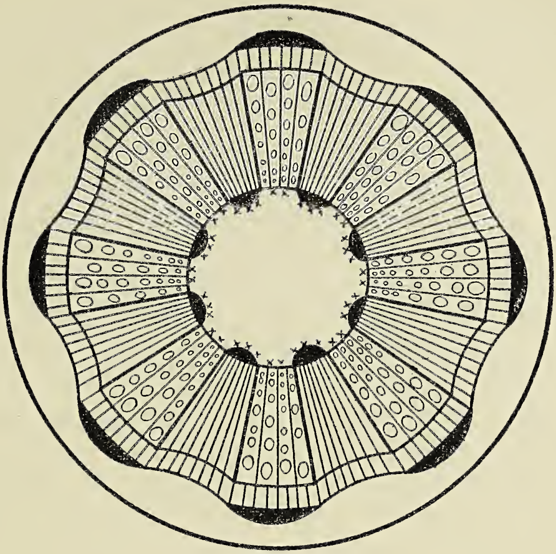
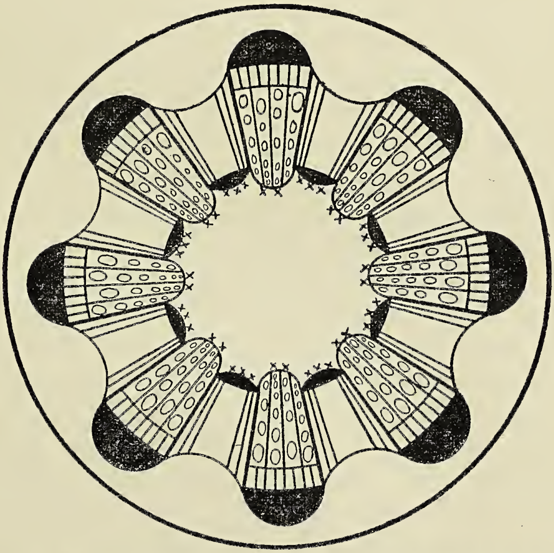


DIAGRAM 6. Herbaceous stem derived from No. 5 through decrease in cambial activity.





DIAGRAMS 7 and 8. A woody stem and an herbaceous one derived from it, showing steps in the development of the herbaceous type in accordance with the hypothesis of Jeffrey and his school. The interfascicular parenchyma originates in the segment *opposite* the outgoing leaf-traces.

Senecio (Pl. XXXIX, Fig. 12, and Pl. XL, Figs. 14 and 16), its herbaceous representative will show but a slight exaggeration of the same thing (Pl. XL, Figs. 13, 15, and 17). The whole process of reduction from a woody to an herbaceous condition is therefore a very simple and natural one.

III. EVIDENCE FROM PHYLOGENY.

Still further evidence as to the relative antiquity of herbs and woody plants is furnished by a study of the distribution of these two types in the general system of Angiosperm classification which has been constructed by phylogenists.

There are two main groups of Gymnosperms from one of which it is generally conceded that the Angiosperms have arisen. These are the Gnetales and the Bennettitales or ancient Cycads. The former are represented to-day by three genera, a shrub, a woody liana, and an anomalous woody desert-plant. The Conifers, from which they seem to have sprung, are without exception woody forms and almost entirely arborescent. The Bennettitales, on the other hand, are typically Cycad-like in habit, and there is no indication that they were ever herbaceous. Primitive Angiosperms, if they approached either of these two groups very closely, were therefore in all probability woody rather than herbaceous in habit.

The history of the Angiosperm flower is as yet so uncertain that there is much difference of opinion as to whether the simple and almost naked-flowered forms, which may be grouped under the inclusive order Amentiferae, or such perfect-flowered types as the Ranales, are the most ancient members of the phylum; but there is very general agreement of opinion that the distinction should belong to one of these two orders. As at present constituted the Amentiferae are composed entirely of woody forms, with the exception of the Saururaceae and certain of the Piperaceae and Chloranthaceae; and the Ranales are also entirely woody, save for the Ranunculaceae, certain of the Berberidaceae, and two aquatic families. If either of these two great orders contain forms which are very close to the original Angiosperms (and if these forms have maintained their ancient habit of growth), the chances seem entirely in favour of the view that the primitive Angiosperms were either arborescent forms or at least possessed well-marked secondary growth, and therefore were not herbaceous.

Evidence in favour of the comparatively recent origin of herbs is also presented by a study of those families which include both woody and herbaceous species, and in which it is also possible to distinguish the primitive from the more recent members.

The Leguminosae are an instance in point. The two sub-families Mimoseae and Caesalpineae, with their regular or nearly regular floral structures and frequently numerous stamens, are without much question more primitive than the Papilionatae, in which the corolla is typically irregular and papilionaceous. It is significant that of the 121 genera of the first two sub-families, 113 are entirely woody, and that the other 8 contain both woody and herbaceous forms. There are no entirely herbaceous genera. Counting the mixed genera as half woody and half herbaceous, this gives a percentage of only 3·3 of the genera which are herbaceous. Out of 304 genera of the Papilionatae, on the other hand, 187 are entirely woody, 55 have both woody and herbaceous forms, and 62 are entirely herbaceous, making 29 per cent. of the genera herbaceous. It is evident that the more primitive members of the Leguminosae are almost all woody, but that there is a much higher percentage of herbaceous forms in the more recent portion of the family.

The Violaceae are another example of the same thing. The Paypayroleae and Rinoreae are almost certainly the most primitive members of the family from the simplicity and regularity of their flowers. The 8 genera which are included under them are all trees or shrubs. The Violeae, which from the irregularity of the corolla seem clearly to be more recent than the others, include the only two genera, *Hybanthus* and *Viola*, which contain herbaceous species.

Of the two great families Araliaceae and Umbelliferae which form the order Umbelliflorae, the former undoubtedly have the more primitive, because the more varied, floral structure, and are probably much more ancient. With very few exceptions they are trees or shrubs. The Umbelliferae, on the other hand, which during the course of evolution have become sharply stereotyped as to floral plan, are almost entirely herbs.

The same fact is noticeable among genera as well. *Potentilla* is divided by Wolf (15) into two sections, the Trichocarpae and the Gymnocarpae, and he considers the former from its floral structure to be clearly more primitive. Of its two subsections, the evidently more ancient Rhopalostyleae are all shrubby, and the more primitive members of the other subsection, the Nematostyleae, are shrubs or undershrubs. The Gymnocarpae are entirely herbaceous.

Other instances of the same sort of thing might be brought forward. Among the Halorrhagaceae, for example, the two genera placed by Engler at the base of the family, *Loudonia* and *Halorrhagis*, are the only ones which ever approach a woody condition, the other genera being mainly aquatic plants. The sub-family Cobaeoideae, of the Polemoniaceae, which includes only trees and shrubs, is considered by Engler, on evidence from the reproductive organs, to be more primitive than the other sub-family, the Polemonioideae, which is entirely herbaceous. The Borraginaceae are divided into four sub-families, the Cordioideae, Ehretioideae, Heliotropioideae, and Borraginoideae ; and the first two of these, which are presumably the most ancient from their floral structures, consist entirely of trees and shrubs. The last two are, with very few exceptions, herbaceous.

It is a noteworthy fact that in practically every instance where there are herbs and woody plants in the same group, and where it is possible to determine from floral evidence their relative antiquity, it is found that the woody plants are more primitive than the herbaceous ones. This is inexplicable on the theory which regards the herb as the most ancient type of Angiosperm vegetation, but is much easier to understand on the alternative hypothesis.

Still further evidence, as to the relative antiquity of the two plant-types under consideration, is furnished by a general survey of the distribution of herbs among the families of the Dicotyledons. Of the 240 families enumerated in Engler's 'Syllabus' (7th edition), 121, or just over 50 per cent., are entirely woody, whereas only 35, or 14 per cent., are entirely herbaceous. 84 families, or 35 per cent., possess both woody and herbaceous forms; of these 14 are rarely herbaceous and 18 rarely woody. The 35 entirely herbaceous families are the following: Saururaceae, Balanophoraceae, Rafflesiaceae, Hydnoraceae, Cynocrambaceae, Basellaceae, Nymphaeaceae, Ceratophyllaceae, Tovariaceae, Resedaceae, Sarraceniaceae, Nepenthaceae, Droseraceae, Podostemonaceae, Hydrostachyaceae, Cephalotaceae, Tropaeolaceae, Callitricaceae, Limnanthaceae, Stackhousiaceae, Balsaminaceae, Hippuridaceae, Cynomoriaceae, Pirolaceae, Lennoaceae, Primulaceae, Pedaliaceae, Martyniaceae, Orobanchaceae, Lentibulariaceae, Globulariaceae, Phrymaceae, Adoxaceae, Brunoniaceae, and Calyceraceae. It is evident that almost all of these families are either parasites, insectivorous plants, water plants, or are monotypic, and that they can lay no particular claim to being primitive. Practically all the typical land herbs belong to families which have woody members as well, but much more than half of the families which have woody plants (121 out of 205) possess no herbaceous forms at all.

It is hard to explain such a fact as this on the hypothesis that herbs are extremely ancient, for if they were there would naturally be many more entirely herbaceous families, and it would be very improbable that over half of all the families should have lost, or at least should be without, herbaceous forms. If we believe that practically all land families originated as woody plants, however, and that the herbaceous type was developed independently in a little less than half of the families, we can understand the facts much more clearly.

A study of the distribution of the two types in the Archichlamydeae and in the Metachlamydeae is also of interest. Of the 3,551 genera enumerated by Engler in the former great group, 2,397 are woody, or 68 per cent., and only 1,154, or 32 per cent., are herbaceous. Among the 3,002 genera in the Metachlamydeae, however, only 1,254, or 42 per cent., are woody, considerably more than half being herbaceous. Trees constitute 53 per cent. of the woody Archichlamydeae, but only 23 per cent. of the woody Metachlamydeae. A great majority of the woody Dicotyledons and an overwhelming proportion (1,281 genera out of 1,569) of the arborescent ones thus occur among the more primitive of the two grand divisions.

The origin of herbs among the Monocotyledons presents a somewhat different problem. The weight of evidence at present available from all sources seems to indicate that Angiosperms are monophyletic, and that the Monocotyledons have arisen as offshoots from an ancient (and therefore presumably woody) dicotyledonous stock, probably as a direct aquatic herbaceous adaptation, through steps similar to those by which the aquatic Halorrhagaceae, for example, seem to have sprung from such a semi-woody form as *Halorrhagis*. It seems clear that the shrubby and arborescent Monocotyledons have been derived secondarily from herbaceous forms, and do not correspond to the primitive woody Dicotyledons. The development of monocotyledonous herbs, however, seems to have taken place very far back under much more equable climatic conditions than those obtaining over most of the earth at present, and therefore in response to a very different climatic environment from that which we shall attempt to show has been the cause for the origin of most herbaceous Dicotyledons. When conditions arrived very favourable for the development of terrestrial herbaceous vegetation, these herbs, long an inconspicuous part of the flora, assumed a much more prominent position ; and especially in the form of grasses, sedges, and rushes became widely distributed in temperate regions. The present paper, however, deals particularly with the apparently much more recent origin of herbs among the Dicotyledons.

IV. EVIDENCE FROM PHYTOGEOGRAPHY.

Finally, evidence as to the relative antiquity of herbs and woody plants may also be obtained from a study of the present distribution over the earth of the members of these two groups. The writers have with some care gone through a large number of published 'Floras' and lists of plants[1] from

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[1] Analyses have been made of the following works:

NORTH AMERICA.—Vascular Plants of Ellesmereland, Simmons; Gray's New Manual of Botany, Robinson and Fernald; Manual of the Flora of the Northern States and Canada, Britton; Flora of the South-Eastern United States, Small; Flora of the Southern United States, Chapman; Flora of the Florida Keys, Small; Flora of the British West Indian Islands, Grisebach; New Manual of Rocky Mountain Botany, Coulter and Nelson; Flora of Los Angeles and Vicinity, Abrams.

SOUTH AMERICA.—Flora Braziliensis, Martius, Eichler, and Urban; Report on the Princeton Expedition to Patagonia: Botany, Macloskie.

EUROPE.—Islands Flora, Stefansson; Botany of the Faroes, Warming and others; Handbog i Norges Flora, Blytt; Flora Rossica, Ledebour; Handbook of the British Flora, Bentham and Hooker; Flora des Nordostdeutschen Flachlands, Ascherson and Graebner; Flora der Schweiz, Schinz and Keller; Flore française, Cusin and Ansberque; Compendio de la Flora española, Ibiza; Description physique de l'Ile de Crète : Botanique, Raulin; Flora Sicula, Tornabene; Flora of Syria, Palestine, and Sinai, Post; Flora Orientalis, Boissier.

ASIA.—Index Plantarum Japonicarum, Matsumura; Flora Hongkongiensis, Bentham; Flora most of the important phytogeographical regions, and have tabulated by families the number of herbaceous and of woody dicotyledonous plants therein recorded. In many cases the number and habit of Monocotyledons have been gone over as well, but, as has been stated above, the problem of the origin of herbs in this group is of less importance, since the weight of evidence seems to point to the conclusion that Monocotyledons arose at first as herbaceous offshoots from some ancient dicotyledonous stock, and that their living woody forms are recent rather than primitive.

In many floras data as to the stature and growth-habit of the plants are not presented, or are incomplete, and in such cases it has been necessary to supply this information as far as possible from other sources. In the tabulation everything recorded as a tree, shrub, half-shrub, undershrub, suffrutex, or perennial suffruticose plant has been counted as woody, and the few species described as both woody and herbaceous have been divided as equally as possible into the two categories.

1. Distribution of Herbs in Temperate and in Tropical Regions.

One of the most conspicuous facts brought out by this study of distribution is the great contrast between temperate and tropical regions in the proportions of their herbaceous flora. The following table presents an analysis of the dicotyledonous floras of various regions in the north temperate zone and in the tropics:

TEMPERATE REGIONS.

Region.	*No. of Species.*	*No. of Herbs.*	*% Herbs.*
North-Eastern United States (Gray) . . .	2,280	1,748	77
Northern United States (Britton and Brown)	2,662	2,089	78
South-Eastern United States (Small) . . .	4,608	3,312	72
Southern United States (Chapman) . . .	2,266	1,666	74
Rocky Mountains (Coulter)	2,206	1,910	87
Los Angeles (Abrams)	802	627	78
Florida Keys (Small)	415	225	54
Great Britain (Hooker)	927	821	89
France (Cusin and Ansberque)	3,924	3,492	89
Germany (Engler)	1,117	947	85
Switzerland (Schinz and Keller)	1,899	1,726	91
Russian Empire (Ledebour)	14,704	12,588	86
Norway (Blytt)	857	741	86
Iceland (Stefansson)	221	200	90
Ellesmereland (Simmons)	76	71	93

of British India, Hooker; Handbook of the Flora of Ceylon, Trimen; Flora of Bombay, Cooke; Flora of the Upper Gangetic Plain, Duthie; Materials for a Malayan Flora, King; Flora van Nederlandsche Indië, Miquel; Excursionsflora von Java, Koorders; Flora of Manila, Merrill.

AFRICA.—Flora of Tropical Africa, Oliver and Thiselton-Dyer; Flora Capensis, Harvey and Sonder, Thiselton-Dyer, and others; The Flora of Madagascar (Journ. Linn. Soc. Bot., xxv, 1890), Baron.

AUSTRALASIA.—Flora Australiensis, Bentham and Mueller; Handbook of the Flora of New South Wales, Moore; Manual of the New Zealand Flora, Cheeseman.

INSULAR FLORAS.—Flora of the Hawaiian Islands, Hillebrand; Flora Vitiensis, Seeman; Flore de la Polynésie française, Castillo; Botany of the Galapagos Islands, Stewart; Report on the 'Challenger' Expedition: Botany, Hemsley; Flora de las Islas de Juan Fernandez, Johow; St. Helena, Melliss; Botanical Observations on the Azores, Trelease; Phytographia Canariensis, Webb and Berthelot; Flore des Iles Canaries, Pitard and Proust; Botany of Socotra, Balfour; Flora of Mauritius and the Seychelles, Baker.

Region.	*No. of Species.*	*No. of Herbs.*	*% Herbs.*
Faroes (Warming et al.)	164	150	91
Spain (Ibiza)	4,481	3,554	79
Crete (Raulin)	1,461	1,161	79
Sicily (Tornabene)	1,697	1,295	76
Syria (Post)	2,949	2,477	84
Flora Orientalis (Boissier)	9,771	8,110	83
Japan (Matsumura)	3,257	1,861	57
TROPICAL REGIONS.			
Brazil (Mueller)	15,981	4,092	26
Ditto, Amazon Valley only	2,209	265	12
British West Indies (Grisebach)	2,249	675	30
Tropical Africa (Oliver and Thiselton-Dyer)	8,577	3,560	42
British India (Hooker)	10,454	4,344	42
Bombay (Cooke), Lowland only	1,249	487	39
Upper Gangetic Plain (Duthie)	1,084	583	54
Ceylon (Trimen)	1,793	670	37
Java (Koorders)	3,188	867	27
Dutch East Indies (Miquel)	6,398	1,599	25
Malay Peninsula (King)	3,252	553	17
Hongkong (Bentham)	728	293	40
Manila (Merrill)	333	106	32

Of course, in all these regions the Monocotyledons, comprising about 30 per cent. of the angiospermous vegetation, are with comparatively few exceptions herbaceous.

A perusal of the foregoing table shows very clearly that in the north temperate zone herbs are the dominant Dicotyledons, but that in the tropics the situation is exactly reversed and woody plants are the prevailing type of vegetation.

This general fact is well shown also by a comparison of the percentage of herbs in various families in their temperate and in their tropical ranges. A few such comparisons are presented in the following table:

Family.	*North Temperate Zone.*	*Brazil.*	*India.*	*Tropical Africa.*
	%	%	%	%
Amarantaceae	100	79	44	84
Leguminosae	90	22	38	41
Euphorbiaceae	95	14	10	29
Violaceae	100	6	52	18
Lythraceae	100	30	42	76
Convolvulaceae	100	42	52	65
Verbenaceae	100	20	4	7
Rubiaceae	100	20	30	19
Compositae	100	39	91	80

An overwhelming majority of arborescent Dicotyledons are confined to the tropics, where they constitute from 25 per cent. to 40 per cent. of the species instead of from 1 to 5 per cent., as in temperate regions.

In view of the palaeobotanical evidence at hand with regard to climatic conditions at the time when the first Angiosperms appeared, these facts as to the present distribution of their woody members relative to climate are of some importance.

The Angiosperms, according to most authorities, had their origin in the late Jurassic or the early Cretaceous. The Jurassic floras of such widely separated regions as the Antarctic continent, England, India, and North America have so very many plants in common that the essential uniformity of the vegetation all over the earth at that period is generally admitted by geologists. The succeeding flora of the Cretaceous displays almost as great a uniformity in its composition. Not only were these floras homogeneous, but they appear to have flourished under much more equable conditions than those now prevailing over most of the earth. The occurrence far north of the Equator of so many warmth-loving plants such as Cycads, Ferns, and Palms, which are not adapted to resist cold, and their association in such regions with other species now characteristic of the temperate zones, indicates that low temperatures were unknown and that the climate was probably devoid of any wide extremes of heat or cold. Very few regions of the earth in modern times, save perhaps certain oceanic islands, exhibit climatic conditions resembling those of the Mesozoic; but the absence of extreme cold, which seems above all else to characterize these ancient climates, is to-day found only within the tropics. If such conditions at present favour the development of woody rather than herbaceous Angiosperms (as our tables have shown), it seems to be a reasonable conclusion that in ancient times as well they would have tended to produce a predominance of ligneous plants, and that the Angiosperms at their inception were in consequence mainly or entirely woody.

If we assume, then, that woody plants were originally the dominant type of dicotyledonous vegetation throughout the world, the question arises as to where herbaceous forms were first developed and what were the causes for their origin. The present distribution of herbs throughout the various regions of the world provides important evidence for the solution of these problems.

2. Development of Herbaceous Forms in the North Temperate Zone.

Since herbaceous plants attain their highest predominance to-day in the great land-mass of the north temperate zone, this region must have seen a more profound change in the growth-habit of its vegetation than any other, if our theory is correct. We should expect, therefore, to find some evidence that in past times woody plants constituted a more important feature of the flora of this region than they do at present, and such evidence is, in fact, forthcoming.

In the first place, it has been shown that during Cretaceous and Tertiary times very many genera and even families of trees flourished in the north temperate zone which are either absent or rare in this area at present, but of which many still occur in warmer regions. The presence of *Ficus*, *Eucalyptus*, *Diospyros*, *Cinnamomum*, *Aralia*, and various Proteaceous genera as fossils in Northern Europe or America may be cited as an example.

Turning to living plants, we find that a comparison of the percentage of herbs in the eastern United States and in Europe north of the Alps (see previous table) shows that in the former region about 24 per cent. of the dicotyledonous flora is woody, but in the latter only about 13 per cent. Professor Gray many years ago called attention to the paucity of trees and shrubs in the European flora compared with the American, and suggested that the difference was due to the fact that the Alps and the Mediterranean prevented the southward escape of the European flora at the advent of the glacial period; but that in America the preglacial flora was able to migrate freely to the south and suffered much less extinction. This conclusion, that the European flora was decimated relatively more in the glacial invasion than was the American, has been suggested by several other writers and seems to be a sound one, since we know from the foregoing fossil evidence that many families of plants existed in Europe in the middle and latter parts of the Tertiary which are now found there no longer. Many of these, however, such as the Ebenaceae, Menispermaceae, and Lauraceae, still exist in the United States. Dr. Gray believed that herbaceous plants in Europe had lost as much by extinction as had woody ones from the ice invasion, but a comparison of the percentage of herbs in the two regions apparently indicates that such has not been the case, but that woody plants have suffered proportionally much the more. This is also suggested by the fact that, although almost all the herbaceous families of Dicotyledons in temperate North America are represented in Europe north of the Alps (the only exceptions being herbaceous members of the Acanthaceae, Capparidaceae, Loasaceae, Melastomaceae, Nyctaginaceae, Passifloraceae, Phytolaccaceae, and Sarraceniaceae), there are sixteen families of trees, shrubs, and woody climbers which are not indigenous to the latter region. These are the Anonaceae, Bignoniaceae, Calycanthaceae, Cyrillaceae, Ebenaceae, Hamamelidaceae, Juglandaceae, Lauraceae, Leitneriaceae, Magnoliaceae, Menispermaceae, Platanaceae, Sapindaceae, Sapotaceae, Styracaceae, Ternstroemiaceae. The only families in Europe which do not occur in America are the Resedaceae and Dipsacaceae, both herbaceous.

It seems quite evident, therefore, that herbs have proven themselves much better able to resist unfavourable conditions, such as enforced migration and an increasingly cold climate, than woody plants. This is, of course, what might naturally be expected from the fact that herbs, which are able to reproduce in a single year from seed, can thus be disseminated much more rapidly, and, above all, can adapt themselves to a cold climate by living over the periods of low temperature underground or in the form of resistant seeds.

This ability of herbaceous plants to thrive in cold climates is also emphasized by a study of alpine and arctic floras. We have already stated the percentage of herbs in the dicotyledonous floras of Switzerland, the

Rocky Mountains, the Faroes and Ellesmereland, and have shown that in all these woody plants constitute only about 10 per cent. of the whole. This 10 per cent. includes mainly such genera as *Salix* and *Betula*, which are very hardy. Many families are represented here as herbs, however, which are quite unable to maintain in such regions representatives with aerial stems which persist through the winter.

The predominance of herbaceous plants in arctic and alpine regions suggests an explanation for the very high percentage of herbs in the flora of northern Europe, and provides important evidence as to the climate of that region during the glacial period. We know that the ice-sheet did not extend over the extreme south of England or over much of northern France and Germany, and it is highly probable that the present flora of countries north of the Alps has been derived from the remnant of the ancient flora which was able to exist on these unglaciated areas. The fact that the percentage of herbaceous plants on the lowlands of northern Europe to-day is so very high (really an arctic or alpine percentage) seems to indicate that the climate during the height of the glacial period was cold enough to kill off all but those hardy trees and shrubs which are now in the flora of northern Europe, but was not cold enough to exterminate the herbaceous vegetation to nearly as great an extent. It was probably very much like that of Switzerland or any similar mountainous region of the present day, and was neither extremely rigorous nor very mild.

This conclusion is strengthened by the remarkable similarity which exists between the flora of northern Europe and that of the Rocky Mountains. The two are composed of almost exactly the same families, and in these are hosts of identical genera and a large number of identical species. Many of these genera and species are found nowhere else in America; in fact, the flora of the Rockies presents a much closer resemblance to that of Europe than does the flora of the eastern part of the continent. This suggests that both in the Rocky Mountains and in northern Europe we have a flora essentially like that which flourished near the ice-front during the glacial invasion, and that it is composed of the survivors (mainly herbs) of the widespread northern preglacial flora which were able to withstand the rigours of a much colder climate. In temperate America the percentage of woody plants has been greatly increased since glacial times by migration from the south, but in Europe this has probably occurred only to a very slight extent owing to mountain ranges; for the climate of northern Europe at present is such as would support a much larger percentage of woody plants than does that of eastern North America, but its actual percentage is only about half as great. Such facts as these point again to the important connexion between the past and present climate and geography of a region, and the size of its herbaceous flora.

It is almost certain, therefore, that the percentage of woody plants in the flora of northern Europe is much smaller than it was before the advent of the glacial invasion, and that the proportion of woody plants to herbs in the United States is a much closer approximation to the preglacial ratio. There is evidence, however, that even in the latter region woody plants suffered heavily during the Ice Age, and that they now form a much smaller part of the whole flora than they did before the glacial period. This evidence is derived from a comparison of the flora of eastern North America and that of eastern Asia. Dr. Gray (6) in 1889 called attention to the fact that the floras of these two regions were strikingly similar in many ways, and that they contained in common a large number of genera and species which were entirely absent in Europe and but scatteringly represented in western North America. Dr. Gray's theory, which has since received further support, regards these two very similar floras as remnants of the preglacial vegetation of the great north temperate land-mass, a vegetation which was pushed south by the advance of the glacier, and which in Europe suffered much by extinction. That part of their flora which eastern America and eastern Asia to-day possess in common may therefore be taken to represent at least a part of that which in ancient times covered the arctic lands. A study of its composition as to percentages of herbs and woody plants is consequently of much importance in throwing light on the composition of the preglacial boreal flora.

Dr. Gray has published a list of the genera and species of eastern North America which are absent in Europe, but which are represented by identical or closely related forms in eastern Asia. This list may therefore serve as a rough approximation to a portion of the ancient preglacial vegetation. It comprises 142 genera of Dicotyledons, of which 70 are woody or predominantly so, and 240 species, of which 128 are woody. The flora is therefore just about equally divided between herbs and woody plants.

An analysis of the present flora of Japan, one which has probably suffered very little, if at all, from glacial invasions and may thus be expected to display a ratio between herbs and woody plants much like that of the preglacial boreal flora, shows that of the Dicotyledons comprised within it only 54 per cent. are herbaceous.

These two pieces of evidence both point to the conclusion that the flora of the great northern land-mass just before the glacial period contained a much greater proportion of woody plants than it does to-day, but that even in these ancient times about 50 per cent. of the dicotyledonous vegetation was herbaceous. We have already brought forward evidence that at the close of the Mesozoic, at any rate, herbaceous vegetation was apparently very inconspicuous. What was the factor, then, which led to the development of such a large number of herbs in so many families of plants during the course of Tertiary time?

Evidence from fossil plants seems to indicate clearly that the climate of Europe, North America, and the Arctic regions, very uniform and equable at the beginning of the Tertiary, grew distinctly colder during the course of that epoch, and also that it became clearly differentiated into zones with decreasing temperature from south to north. This is shown by the occurrence within the Arctic Circle in the later Cretaceous of tropical types, which were succeeded in the middle Tertiary by a distinctly temperate vegetation, this in turn giving way to the impoverished Arctic flora of to-day. Conditions changed less rapidly in more southern latitudes, and the ancient flora became slowly modified into its present temperate form. Throughout the Tertiary the climate of the great northern land-mass seems to have suffered a progressive refrigeration. We have already called attention to the fact that herbs are much better able than woody plants to withstand a climate with extremes of cold, and this suggests the hypothesis that the herbaceous type of vegetation has been developed in the north temperate zone mainly as an adaptation to this progressive refrigeration of the climate. A more detailed discussion of the various factors which have led to the evolution of herbs will be found in the latter part of this paper, where evidence will also be brought forward that the majority of herbaceous genera have arisen in the northern hemisphere and have spread thence over the rest of the world.

3. The Herbaceous Element in various Insular Floras.

A. *Endemism as a Criterion of Antiquity.*

Evidence of much value in helping to solve the problem which confronts us may be drawn from an analysis of the composition of any flora known to be very ancient. In the study of all floras one of the most certain criteria of antiquity to which we may appeal is the extent of endemism displayed. It is generally admitted that the endemic species in a flora, those which are peculiar to it and are found in no other region, are for the most part more ancient than the non-endemic element, for they must either have had their origin in the region—a process usually requiring a long period of time—or else they must be remnants of an older vegetation which has elsewhere become extinct. Endemic genera and finally endemic families are in this way regarded as progressively more ancient portions of the flora. Although this rule is in general a pretty safe one to follow, it cannot be applied too rigidly in an attempt to determine the comparative age of herbs and woody plants, for herbs seem to change much more rapidly, and hence become sooner endemic. We have seen, for example, that the indigenous dicotyledonous flora of the eastern United States is composed of 77 per cent. herbaceous forms and 23 per cent. woody ones. These figures are probably about the average for the combined flora of southern Canada, the United

States, and northern Mexico. In Britton and Brown's 'Flora' there are recorded, as entirely or almost entirely confined to this general region of temperate North America, 182 dicotyledonous genera comprising 1,411 species which, although not including by any means all the genera endemic in this territory, may well be taken as a fair sample of them. Only about 8 per cent. of this presumably ancient portion of the flora, however, is composed of woody plants, in contrast to the 23 per cent. of such forms in the total indigenous flora. In other words, roughly two-thirds of the woody genera of the United States also occur in Europe or some other part of the world and almost all the endemic genera of temperate North America are herbaceous in habit. *Quercus*, *Betula*, *Populus*, *Salix*, and many other woody plants, however, which are common to North America and Europe, and are therefore endemic in neither, and which we know from fossil evidence to be very ancient, are in all probability much older than scores of herbaceous genera which are not common to both regions and which therefore constitute the bulk of the endemic flora of each. It seems much more likely that these plants have developed since an interchange of vegetation between Europe and North America was interrupted by arctic refrigeration, and that they have consequently been limited in their distribution to one hemisphere or the other. In short, new varieties, species, and genera are apparently produced much more rapidly among herbs than among woody plants. This is only natural, since the life-cycle among herbs is annual or at the most biennial, instead of covering a much longer period of years as it does in woody plants. Therefore, if the amount of change in each generation is approximately the same in the two groups, it will accumulate much more rapidly in an herbaceous species than in a woody one. Endemism among herbaceous plants is consequently an important criterion of antiquity when one is considering such plants alone, but is of very much less value in determining the relative ages of herbs and woody plants in a flora composed of both. Endemism among woody forms, however, may almost always be considered an excellent indication of antiquity, because such plants usually change with comparative slowness. With this caution in mind, let us compare the endemic, or presumably ancient, and the non-endemic, or presumably recent, elements of the floras of various regions with regard to the proportion of herbs and woody plants which they contain.

Insular floras are of particular value for such a study, since they have not been exposed freely to a flood of immigration but have developed in comparative isolation and therefore possess a large endemic element. Very many oceanic islands are known to possess faunas and floras which are very ancient in type and which may well be regarded as remnants of the organic life of a much earlier period in the earth's history.

B. *The Hawaian Islands.*

The Hawaian Islands, isolated so completely in the middle of the Pacific Ocean, support a fauna and flora which are recognized by biologists to be very ancient. The flora is particularly rich for an oceanic island and consists of 718 species of Angiosperms, of which 574 are endemic, or 80 per cent. There are 265 genera, and of these 36 are endemic, or 13 per cent. Such a high degree of endemism seems clearly to imply great antiquity. An analysis of the dicotyledonous flora with regard to the relative proportions of herbs and woody plants is presented in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	582	138	24
Non-endemic species	82	62	76
Endemic species of non-endemic genera . .	257	55	21
Species of the endemic genera	243	21	8·5

It will be observed that the species of the endemic genera, that element of the flora which is presumably the most ancient of all, if endemism is a true criterion of antiquity, is overwhelmingly woody; that the next younger element, the endemic species of non-endemic genera, has a considerably higher percentage of herbaceous forms, and that the youngest element, composed of the non-endemic species, is preponderantly herbaceous.

This ancient flora contains many curious plants. Families and genera which in most other parts of the world are entirely or mainly herbaceous are represented here by woody forms. Wallace (14, p. 328) remarks that 'Among the curious features of the Hawaian flora is the extraordinary development of what are usually herbaceous plants into shrubs or trees. Three species of *Viola* are shrubs from three to five feet high. A shrubby *Silene* is nearly as tall; and an allied endemic genus, *Schiedea*, has numerous shrubby species. *Geranium arboreum* is sometimes twelve feet high. The endemic Compositae are mostly shrubs, while several are trees reaching twenty or thirty feet in height. The numerous Lobeliaceae, all endemic, are mostly shrubs or trees, often resembling Palms or Yuccas in habit, and sometimes twenty-five or thirty feet high. The only native genus of Primulaceae—*Lysimachia*—consists mainly of shrubs; and even a plantain has a woody stem sometimes six feet high.'

Guppy (7), who has made a special study of the floras of the Pacific, regards the Compositae with 8 endemic genera, and the Lobeliaceae with 5, as the most ancient portion of the Hawaian flora, since they are strikingly developed here but have almost no near allies elsewhere in the Pacific, a circumstance which he attributes to their establishment on Hawaii during the latter part of the Tertiary when most other Pacific islands were submerged. Of these families the 13 genera, comprising 98 species, are entirely woody save one, *Lipochaeta*, which includes 5 species that are essentially herbaceous.

If the Hawaian flora is really an ancient one, a conclusion to which all the facts at our command seem to point, and if it is truly representative of the flora of the tropics and sub-tropics of late Tertiary time, then we must admit that these ancient floras were composed overwhelmingly of woody plants.

C. *The Fiji Islands.*

The flora of Fiji is also predominantly woody, and an analysis of it (based on Seeman's 'Flora', which is doubtless far from complete but probably representative of the whole) is presented in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	563	81	14
Non-endemic species	292	76	26
Endemic species of non-endemic genera	251	5	2
Species of the endemic genera	20	—	—

The ancient element of the flora here is almost entirely woody and nearly all the herbs are apparently of recent introduction, since so few of them are endemic.

D. *Tahiti.*

Data for a general analysis of the flora of Tahiti were not available, but its herbaceous percentage is doubtless much like that of the rest of Polynesia. There are four endemic dicotyledonous genera (*Fitchia*, *Lepinia*, *Apetahia*, and *Sclerotheca*), none of which possess herbaceous species. *Fitchia* approaches the Hawaian arborescent Compositae more closely than does any other Polynesian plant; and *Apetahia* and *Sclerotheca* may be taken to represent the Hawaian tree-Lobelias. The most ancient element in the flora is entirely woody.

E. *Other Polynesian Islands.*

Detailed figures for other Polynesian islands are not at hand, save for an analysis of the flora of French Polynesia (Castillo). In this group, out of 308 native Dicotyledons, 79, or 23 per cent., are herbaceous. The indigenous flora of the other islands doubtless contains an approximately equal percentage of herbs.

There are a large number of species which, although not endemic in any one island or group of islands, are nevertheless limited or nearly limited to Polynesia. Including such plants with those which are endemic to particular groups, there are thus endemic to Polynesia (exclusive of Hawaii) 32 genera and 452 species. 10 of these species are herbs, or 2·2 per cent., but none of these belong to the endemic genera. It is thus apparent that the element in its flora which Polynesia shares with the rest of the world, the widespread tropical types and the plants which are therefore presumably recent arrivals (since isolation has not yet had time to break them up into new species), includes a fairly large percentage (25 per cent. or so) of herbs. The element of the flora which is endemic in Polynesia or in the separate groups within it, and which is therefore probably much more ancient, is almost entirely composed of woody plants.

F. *The Galapagos Islands.*

Outside of Polynesia there are two groups of islands in the Pacific the flora of which is important for our study—the Galapagos Islands and Juan Fernandez. The former, situated 700 miles off the coast of South America, have a flora of 446 species of Dicotyledons, of which 226, or 50 per cent., are endemic. An analysis of the flora is presented in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	446	270[1]	61
Non-endemic species	220	177[1]	80
Endemic species of non-endemic genera . .	215	93	43
Species of endemic genera	11	—	—

There are but two endemic genera (*Lecocarpus* and *Scalesia*), a fact which indicates that the flora is not a very ancient one, at least when compared with that of Hawaii. These two genera, however, are both arborescent, and apparently represent the most primitive portion of the vegetation.

G. *Juan Fernandez.*

The island of Juan Fernandez (with the islet of Masafuera) lies well outside the tropics, 400 miles off the coast of Chile. It is apparently of great antiquity geologically, and possesses, for so small an island, a rich and peculiar flora. An analysis of it is appended:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	89	41	46
Non-endemic species	33	31	94
Endemic species of non-endemic genera . .	36	10	27
Species of endemic genera	20	—	—

In this island, as in others which we have discussed, the more ancient portion of the flora is woody and the recent element herbaceous. About 70 per cent. of the dicotyledonous flora of adjacent Chile, enjoying a very similar climate, is composed of herbaceous plants. It is significant, however, that large, characteristic Chilean genera of Leguminosae, Compositae, and Orchidaceae are absent. The flora of Juan Fernandez, with its predominance of woody forms, may perhaps be regarded as a remnant of a Chilean flora of much more ancient times, before the development or invasion of such a preponderance of herbs.

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[1] Estimated.

H. *St. Helena.*

The island of St. Helena, in the middle of the South Atlantic Ocean, also supports a fauna and flora of great antiquity. Wallace refers to its primal state, before the advent of man, as (14, p. 308) 'a kind of natural museum or vivarium in which ancient types, perhaps dating back to the Miocene period, or even earlier, had been saved from the destruction which has overtaken their allies on the great continents'.

The native flora of the island is now but a fragment of what it was before the advent of civilization, and comprises only about 62 species of flowering plants (41 Dicotyledons and 21 Monocotyledons), of which 38 are endemic. An analysis of the flora is presented in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	41	15	37
Non-endemic species	11	8	73
Endemic species of non-endemic genera . .	22	7	32
Species of endemic genera	8	—	—

The indigenous flora of the island thus comprises but a small percentage of herbs, and the endemic element a progressively smaller one. Annual herbs compose a large part of the introduced vegetation, but there are no indigenous annuals. The most conspicuous element in the endemic flora is the Compositae with six genera and ten species. All species are trees save one. Other noteworthy plants are a shrubby *Lobelia* and a shrubby *Plantago*, recalling those of Hawaii, and a shrubby species of *Heliotropium*. The general character of the flora of St. Helena is southern extra-tropical, and its present prevailingly woody composition may be taken to indicate that the vegetation of the adjacent continental areas during the Tertiary was made up for the most part of woody plants.

I. *The Canaries.*

The Canary Islands, 200 miles off the coast of northern Africa, support a large flora and one which displays a considerable degree of endemism. According to Pitard and Proust there are 1,303 indigenous species of Angiosperms, of which 459 are endemic; and 39 endemic genera. The growth-habit of the plants is not indicated by these authors, but an analysis (of the Dicotyledons) made from the earlier work of Webb and Berthelot, although based on fewer species, is doubtless very nearly correct. It is presented in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	783	526	67
Species of non-endemic genera	653	492	75
Species of endemic genera	130	34	26
Species of endemic genera (exclusive of the Crassulaceae)	67	6	9

The herbaceous percentage of the indigenous vegetation is but little lower than that of most Mediterranean floras, but the species of the endemic genera show a much smaller percentage of herbs. If we leave out the four endemic genera of Crassulaceae, which are somewhat anomalous and in which it is usually difficult to decide whether a species should be classed as a herb or a woody plant, only 9 per cent. of the species of the endemic genera are herbs. If the endemic genera are to be regarded as the most ancient portion of the vegetation, their present composition points clearly to the conclusion that the Canaries once had a flora composed very largely of woody forms, and that since this flora must have been derived mainly from the adjacent mainland, the vegetation of the mainland must also have been at one time predominantly woody.

J. *The Azores.*

The Azores, although much farther from a continent than the Canaries, have but a very small endemic element in their flora and possess no endemic genera. The indigenous flora is made up largely of European species, though there are a number which exhibit American affinities. Out of 247 native Dicotyledons 217 are herbaceous, or 88 per cent., a proportion much like that of northern Europe. The paucity of endemic species is probably due to the great extinction of the ancient flora during the ice invasion. The present flora, therefore, unlike that of most oceanic islands, has been recently acquired, and consequently displays, as we might expect, a very large percentage of herbs.

K. *Bermuda.*

The flora of Bermuda seems also to be very recent in origin, for there appear to be no undoubtedly endemic species, the indigenous plants being identical with those in the West Indies and the eastern part of the United States. We consequently fail to find the dominance of woody plants which is apparent in oceanic islands that received their flora in more ancient times. No exact statistics for Bermuda are easily available, but herbs predominate in the element which has been derived from the United States, and woody plants in the West Indian portion.

L. *Socotra.*

The island of Socotra, in the Indian Ocean, 150 miles east of Cape Guardafui, possesses a flora which, from the extent of its endemism, must be rather ancient. An analysis of it, based on Balfour's 'Flora', is given below:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	517	325 [1]	63
Non-endemic species	331	280 [1]	85
Endemic species of non-endemic genera . .	164	43	26
Species of endemic genera	22	2	9

[1] Estimated.

Of the nine endemic genera of Dicotyledons, perhaps the most interesting is *Dendrosicyos*, the only arborescent member of the Cucurbitaceae. It is evident that in Socotra, as in all other islands which received a part of their flora from the mainland long ago, the ancient element is almost entirely woody, whereas the more recent part of the vegetation is predominantly herbaceous.

M. *Mauritius and the Seychelles.*

The islands of Mauritius and the Seychelles, with the islets of Rodriguez and Bourbon, are probably not strictly oceanic islands, but were doubtless long ago isolated from any large body of land. Of the 1,058 species of vascular plants in Baker's 'Flora', 536 are endemic to the Mascarene islands (Madagascar and the smaller neighbouring islands), and 304 are endemic to single islands. An analysis of the dicotyledonous flora follows:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	587	194	33
Non-endemic species	254	149	59
Endemic species of non-endemic genera	270	42	16
Species of endemic genera	63	3	5

In this case, as in that of other ancient insular floras, the primitive element is almost entirely woody, but that of more recent origin mainly herbaceous.

N. *Ceylon.*

Ceylon is not an oceanic island as are most of those which we have discussed, but it nevertheless possesses a considerable endemic element in its vegetation, and illustrates the same principle which we have brought out in the case of other ancient insular floras. An analysis of it follows:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	1,793	670	37
Non-endemic species	1,182	528	45
Endemic species of non-endemic genera	569	140	24
Species of endemic genera	42	2	4·7

O. *Discussion of Isolated Insular Floras.*

It is thus very clear that woody plants constitute a more conspicuous element in the flora of isolated oceanic islands than in the flora of the adjacent continental areas from which their vegetation has presumably been derived, and also that the most ancient portion of the island floras, if endemism is to be regarded as a criterion of antiquity, is much more woody than the recently acquired elements. Annual herbs, which seem to be the last step in reduction, are almost entirely absent from insular floras, as has been noted by Darwin, Hooker, and others. Since the vegetation of these isolated oceanic islands is to be regarded as more ancient in its composition than that of larger land areas, it may be looked upon as a vestige of an earlier and much more uniform flora which flourished over the earth during the middle or latter part of the Tertiary, and before the great flood of herbaceous vegetation, developed chiefly in the north temperate lands, had spread far over the globe.

This conclusion is strengthened by the many similarities which these widely separated island floras bear to one another.

The arborescent or shrubby Compositae of Hawaii, Tahiti, the Galapagos, and Juan Fernandez are all related, and those of St. Helena are also characteristically American, rather than African, in type. Woody species of *Plantago* occur only in Hawaii, Juan Fernandez, and St. Helena.

On Juan Fernandez flourish three species of *Wahlenbergia*, a South African genus with several species on St. Helena; *Sophora tetraptera*, growing elsewhere only on Easter Island, New Zealand, and Chile; *Gunnera*, from South America, South Africa, Australasia, and Hawaii; *Coprosma*, found elsewhere only in New Zealand and Hawaii, and one or two other genera and species with very scattered distribution. The endemic arborescent genus of Labiatae, *Cuminia*, is much like the shrubby *Bystropogon* of western South America and the Canary Islands. Woody Borraginaceae occur in the Canaries, St. Helena, and Juan Fernandez; the genus in the latter group, *Selkirkia*, resembling in floral structure *Myosotidium* of the Chatham Islands.

Melhania, of St. Helena, is close to the Mascarene *Trochetia*; *Psiadia*, an otherwise exclusively Mascarene genus, has a species in St. Helena, and there are similar species of *Acalypha* and *Carex* in both places.

Several plants from the Canaries, such as *Prenanthes pendula*, *Carpylanthus salsoloides*, *Euphorbia arbuscula*, and others, approach very closely species from Socotra. There is also a distinct American element in the flora of the Canaries, and a much stronger affinity still with South Africa and the Mascarene region.

The flora of Socotra contains species of *Graderia* and *Camptoloma*, genera otherwise confined to South Africa; and of *Campylanthus*, elsewhere found only in the Cape Verde and Canary Islands and in Baluchistan. A well-marked American affinity is shown by the presence of *Thamnosma*, *Dirachma*, and *Coelocarpus*. The genera *Elaeocarpus* and *Porana* flourish in Madagascar, Australia, and Socotra, but not in Africa.

In the Mascarene region, aside from the forms already mentioned, occur the genera *Labourdonnaisia* (Natal and Cuba), *Ocotea* (Canaries and South Africa), and *Mathurina* (from Rodriguez), which is close to the Central American *Erblichia*.

Almost all the larger oceanic islands, therefore, have floral affinities with several other oceanic islands or with distant continental areas, and this fact strengthens the belief that these highly peculiar insular floras are isolated vestiges of a very ancient vegetation which was much more uniformly distributed over the earth than is the present one, and which possessed very few herbaceous Dicotyledons.

The predominance of woody plants on oceanic islands was apparently noted first by Darwin, but has also been emphasized by Hooker, Hemsley, and others. The only attempt to explain this phenomenon, so far as the writers have noted, is the one made by Darwin. In his discussion of the inhabitants of oceanic islands (4, p. 413) he remarks that 'islands often possess trees or bushes belonging to orders which elsewhere include only herbaceous species; now trees, as Alph. de Candolle has shown, generally have, whatever the cause may be, confined ranges. Hence trees would be little likely to reach distant oceanic islands; and an herbaceous plant, which had no chance of successfully competing with the many fully developed trees growing on a continent, might, when established on an island, gain an advantage over other herbaceous plants by growing taller and taller and overtopping them. In this case, natural selection would tend to add to the stature of the plant, to whatever order it belonged, and thus first convert it into a bush and then into a tree.' But if trees really find it so difficult to reach distant islands, it seems strange that the flora of these regions should so universally be woody in its character. That the difficulty of migration among arborescent forms has been somewhat over-emphasized is indicated by the fact that such islands as the Azores and Bermuda, which we believe to have but recently received their flora, or at least the great bulk of it—and such others as Krakatoa, where we know the vegetation is but newly arrived—are almost or quite as well supplied with woody plants as are the lands from which they have derived their flora. Neither is there anatomical evidence that a tree has ever arisen from an increasingly vigorous herb. Herbaceous plants often grow rankly under very favourable circumstances, and may thus attain a woody zone of considerable width; but although the outer portion of this zone may be composed of wood which is quite normal, the very reduced condition of vessels, rays, and parenchyma, characteristic of the ordinary herbaceous stem, may still be seen in that part of the vascular ring next the pith, and furnishes a clear proof that these plants are indeed overgrown herbs. Such instances, however, are rare, and practically all plants which are typically woody display a normal and unreduced type of xylem structure next the pith. Also, these 'woody herbs' always retain their characteristic ability to produce flowers and seeds in a very short time. The advantages of this ability are so obvious that it is hard to imagine how it could ever have been lost, as lost it must have been if trees, none of which reach maturity so early, even on oceanic islands, have been derived from herbs. There is no information available to the effect that herbs introduced into oceanic islands tend to become more robust. On the contrary, animals and plants in such localities often grow smaller than the continental form. Herbs are the dominant type of vegetation to-day, and more plants have apparently won success through reducing their stature than through adding to it in the way that Darwin suggests.

The flora of oceanic islands is, of course, in a rather unusual environment, and conclusions drawn solely from a study of it cannot be regarded as entirely conclusive. There are other regions, however, with a much more extensive land surface, which have long been more or less isolated, and which, therefore, may be expected to preserve an ancient type of floral composition.

4. The Herbaceous Element in the Temperate Floras of the Southern Hemisphere.

A. *Australia.*

The best examples of such partially isolated continental areas are the temperate regions of the southern hemisphere. Perhaps the most notable of these is the island continent of Australia. This great body of land has been cut off to a large extent from other continental areas apparently since early in the Tertiary, and has consequently received but a scanty number of immigrants in recent times. This is well shown in the character of its fauna, which preserves a comparative abundance of many such types as the marsupials, struthious birds, Dipnoi, &c., which we have every reason to believe were at an earlier period much more widely distributed over the globe. Many recent types of animals have become widely dispersed only since Australia became isolated, and have consequently been unable to enter it, save in rare cases.

From its high degree of endemism (89 per cent.), the flora also appears to be an ancient one, and many of its most characteristic plants seem to have been widely distributed over the earth in Tertiary time. Many of the Myrtaceae inhabited Europe and America in the Miocene. Leaves which have been identified as belonging to the Proteaceae, the most typical Australian family, have been found frequently in the European Tertiaries, and members of several other families which at present reach their greatest development in Australia were formerly much more widespread. We should, therefore, expect to find that in the composition of its vegetation with regard to the relative proportions of herbs and woody plants, the Australian flora would exhibit a more primitive condition than do those of the northern hemisphere.

With this point in view, a careful analysis of the Australian vegetation was undertaken, using Bentham's 'Flora Australiensis' as a basis. Separate counts were made for Queensland, New South Wales, Victoria, Tasmania, and Western Australia. As a check on these counts, Moore's 'Flora of New South Wales' was also gone through. The Northern Territory, of which the flora is largely Malayan and tropical, was left out of consideration. The results of this analysis are briefly set forth in the following table:

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species:			
Australia (excluding Northern Territory)	5,711	1,741	30
Queensland	1,786	624	35
New South Wales	1,833	640	35
New South Wales (Moore)	2,352	843	35
Victoria	1,186	541	45
Tasmania	683	323	47
Western Australia	2,578	614	24
Non-endemic species	586	361	62
Endemic species of non-endemic genera	1,101	703	64
Species of the 398 endemic genera	4,024	677	17

The larger part of Australia, and that which supports by far the greatest number of species, is outside the tropics, and the characteristic vegetation of the continent flourishes under climatic conditions somewhat like those in the southern United States. The climate of Tasmania, on the extreme south, is very similar to that of England. Despite this temperate character of the climate, however, woody plants are very much more abundant than in the corresponding regions of the northern hemisphere, constituting from 50 per cent. to 75 per cent. or more, instead of from 12 per cent. to 25 per cent., of the dicotyledonous vegetation. Tasmania has more than four times as great a proportion of woody plants as Great Britain. The flora of the endemic genera contains a still smaller percentage of herbaceous plants.

The flora of Australia, therefore, strongly resembles that of a typical oceanic island, since woody plants form a comparatively large part of the indigenous vegetation, and an overwhelming part of the endemic. This renders it probable that the predominance of woody forms in both regions is due to the same cause. We have suggested that the greater part of the herbaceous flora of the world has had its origin in the land-mass of the northern hemisphere during Tertiary time, as an adaptation to the progressive refrigeration of the climate, and that subsequently it has spread thence over all the accessible parts of the globe. Oceanic islands or other isolated regions which were not open to copious immigration would thus be expected to show a predominance of the ancient woody vegetation. Such an hypothesis, which easily accounts for the paucity of herbs in the vegetation of oceanic islands, explains the same phenomenon in the flora of the island continent of Australia. All the evidence at hand seems to indicate that there has been no unbroken land connexion between this continent and Asia since early Tertiary times. The immigration of northern vegetation has consequently been greatly hindered; but plants can cross much wider oceanic barriers than can most land animals, and it is therefore not surprising to find a considerable number of genera in Australia which are obviously

European or Asiatic in origin. Many of these are characteristic, not of the warmer parts of Eurasia, as might be expected, but of the distinctly temperate northern regions. Such genera include *Ranunculus*, *Draba*, *Viola*, *Stellaria*, *Rubus*, *Potentilla*, *Daucus*, *Galium*, *Bidens*, *Mentha*, *Rumex*, and many others which are familiar. Hooker has compiled a list of such genera, apparently northern in origin, which have evidently entered Australia from the northern hemisphere. A study of this list shows that 37 of its members are characteristic of tropical or sub-tropical Eurasia, and that 18 of these, or only 50 per cent., are herbs; whereas out of 117 genera from the temperate parts of the Old World, 109, or 93 per cent., are herbaceous. These very numerous 'temperate' genera, which include a large number of species, make up the greater portion of that 30 per cent. of the Dicotyledons of Australia which are herbaceous. The rest of the herbs are either characteristically tropical forms, or are included in the few endemic herbaceous genera. The latter belong for the most part to the Australian families Stylideae and Goodenoviae, which are mainly herbaceous, and to the Compositae. These native herbs, as well as the northern ones, are most abundant in the cooler and more mountainous country in the south-eastern corner of the continent, and many of the native herbaceous genera are strictly alpine forms.

All these facts lead to the conclusion that the ancient flora of Australia, long ago isolated and now represented by the endemic genera, was almost entirely composed of woody plants; and that the bulk of the herbaceous flora of the continent to-day is not primitive, but has entered it in comparatively recent times from the great centre of distribution of herbaceous plants in the north temperate zone. A further discussion of this immigration from the north will be reserved until we have considered the floras of New Zealand, southern South America, and South Africa.

There are affinities between the flora of Australia and those of various oceanic islands which suggest that they may both be fragments of an ancient, more widely dispersed, vegetation. The phyllodineous Acacias, for example, are confined to Australia, with the exception of one species in the Mascarene islands, and another in Hawaii. The genus *Santalum* has its centre of distribution in Australia and the East Indies, but also occurs in Hawaii and Juan Fernandez. *Elaeocarpus*, another Australian and East Indian genus, occurs in New Zealand, the Mascarenes, Socotra, New Caledonia, and Hawaii. There are several other similar cases.

B. *New Zealand.*

New Zealand is neither a true oceanic island nor a typical continental one, for although it was apparently connected with the mainland in very ancient times, it has certainly been isolated for a long period. This isolation has resulted in the development of a highly endemic flora, 1,143 out of 1,571 species of vascular plants being peculiar to the Dominion, and 872 out of 1,026 species of Dicotyledons. An analysis of the various elements in the dicotyledonous flora is set forth in the accompanying table :

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species (both islands)	1,026	569	55
Indigenous species (North Island)	569	297	52
Indigenous species (South Island)	457	272	60
Non-endemic species	154	125	81
Endemic species of non-endemic genera . .	811	432	53
Species of strictly endemic genera	61	12	20
Species of 'nearly endemic' genera . . .	254	97	38
Species of endemic or nearly endemic alpine genera	95	87	92
Species of endemic or nearly endemic lowland genera	156	9	6

The same fact is shown by these figures which we have so often observed in the composition of other ancient floras: that the most ancient element contains an overwhelming proportion of woody plants, that the next younger has a much larger herbaceous representation, and that the most recent portion of all is almost entirely herbaceous. The endemic and nearly endemic genera, therefore, are to be regarded as representatives of that ancient vegetation, woody in character, which seems to have been dominant in early Tertiary times.

The herbaceous portion of the New Zealand flora, like that of Australia, is composed of two distinct elements, one consisting mainly of northern and the other of endemic genera. The north temperate element in the flora is very conspicuous, for 115 New Zealand genera (more than one-third of the total) occur also in Europe, most of them being characteristic northern plants, and even 58 species are identical in the two regions. Of the 72 genera of Dicotyledons which are typically northern in character, 2, or only 3 per cent., are woody. Practically the entire element introduced into the flora from the north temperate zone is therefore herbaceous, in strong contrast to the endemic vegetation. These northern forms are most numerous in the cooler upland districts of the South Island.

There are, however, 23 of the endemic or nearly endemic genera, containing 97 species, which are composed of herbs. These include such typically Antarctic forms as *Colobanthus*, *Donatia*, *Aciphylla*, *Celmisia*, and *Abrotanella*; and others which are confined entirely to New Zealand, as *Pachycladon*, *Notothlaspi*, *Haastia*, *Colensoa*, and others. 18 genera and 88 species, an overwhelming majority, are characteristically alpine, as the table indicates. The concentration of so many herbaceous forms in the mountains makes the percentage of these plants in the South Island, which contains most of the mountains of the Dominion, greater than that in the North Island.

It therefore seems probable, if our thesis is correct, that these endemic genera of herbs have arisen locally and quite independently in adaptation to the progressive refrigeration which has been taking place in the southern hemisphere as well as in the northern. That the climate of New Zealand has actually become much cooler in recent times is clearly shown by a study of the Tertiary fossils of the South Island. These include many sub-tropical plants, such as *Agathis*, which are now restricted entirely to the northern portion of the Dominion. The Antarctic origin of herbs will be discussed more fully later.

The flora of New Zealand presents many interesting affinities with that of Australia and the oceanic islands, regions which we have regarded as retaining a very ancient flora. The intimate relationship to Australia is especially well marked, for a large number of genera (those which we have called 'nearly endemic') occur in both regions and, with few exceptions, nowhere else. Such genera are *Hymenanthera*, *Plagianthus*, *Olearia*, *Scaevola*, *Cyathodes*, *Leucopogon*, *Persoonia*, and others. There are also some remarkable affinities between the flora of New Zealand and those of the Pacific oceanic islands. The genus *Gunnera* is found in New Zealand, Tasmania, Hawaii, Juan Fernandez, South America, and South Africa; *Metrosideros* in New Zealand, Australia, and the Pacific islands; *Sophora tetraptera* in New Zealand, Easter Island, Juan Fernandez, and Chile; *Cyathodes* in New Zealand, Australia, Hawaii, and other Pacific islands; and *Coprosma* in New Zealand, Malaysia, Hawaii, Juan Fernandez, and other islands.

C. *Patagonia and Fuegia.*

Patagonia and Fuegia are not isolated from a large continental area, as are the other regions which we have discussed, and in recent times, at least, they have been very freely open to immigration from the north. It seems probable, however, from geological evidence, that the free entrance of northern animals and plants into South America did not take place till the early Pliocene. Many ancient types are therefore preserved in the continent. We have already seen that herbaceous plants form less than 27 per cent. of the dicotyledonous flora of Brazil, and only 12 per cent. of the flora of the rain forests in the Amazon valley, a proportion which is much lower than that of any other continental area within the tropics. No analysis of the endemic genera is at hand, but practically all the characteristically Brazilian types are woody. It seems reasonable to infer that the ancient flora of tropical South America was almost entirely devoid of herbaceous plants.

A somewhat more complete study was made of the flora of that part of temperate South America included in Patagonia and Fuegia. The following table presents an analysis of the dicotyledonous vegetation of this region. Under 'endemic' genera are included both those which are quite peculiar to the region, and also those which have their centre of distribution here, but a few species running up into Chile.

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	1,587	1,120	71
Species of non-endemic genera	920	800	87
Species of endemic genera	667	320	48

The ancient element here, like that in the other floras which we have studied, is very much more woody than the younger portion. Most of the herbaceous endemic genera, as in New Zealand, are alpine forms and seem to have been developed locally as an adaptation to the increasing refrigeration of the climate. The great bulk of the herbs, however, belong to such genera as *Myosotis*, *Ranunculus*, *Gentiana*, *Anemone*, *Viola*, and many others which are characteristically north temperate in distribution, and which have evidently entered South America along the Andes from their centre of dispersal in the northern hemisphere. 98 out of 515 genera in Macloskie's 'Flora' are typically northern plants, and 93 of these are herbs, or 95 per cent. It is reasonable to infer that if Patagonia had been as widely isolated as New Zealand, it would display the same predominance of woody forms in its vegetation.

D. *South Africa.*

South Africa is also open freely to immigration from a large continental area, but it supports a flora which is very rich and peculiar. The following table is a brief analysis of the dicotyledonous portion. 'Endemic' genera are those which are entirely confined to South Africa, or have the great majority of their species within that region.

	Total Species.	*Herbs.*	*% Herbs.*
Indigenous species	7,984	3,319	42
Species of non-endemic genera	3,298	1,929	58
Species of endemic genera	4,686	1,390	30

The most ancient element in the flora, the species of the endemic genera, thus contains a very much smaller proportion of herbs than does the more recent element, the non-endemic types. It should be stated that many of the 'woody' plants are small, and that there are comparatively few trees.

Africa was in all probability isolated from the north temperate land area during at least the early part of the Tertiary, and it has been suggested that this South African flora is a remnant of the ancient vegetation of the continent, for a distinct South African affinity is evident in the widely distant floras of Mediterranean Africa, the Canaries, Socotra, the Abyssinian and tropical highlands, Angola, the Cameroons, and Fernando Po. But whether or not this typical Cape flora was once dominant throughout Africa, it is at least one of considerable antiquity as its high degree of endemism shows, and the preponderance of woody plants within it is an indication that the ancient vegetation of this part of the world contained comparatively few herbaceous plants.

The endemic genera of herbs seem to have been developed locally, both in adaptation to a temperate climate with increasingly great extremes of temperature, and also to an arid or semi-arid environment. The dwarf succulents among the Asclepiadaceae, and the desert herbs in many other families, are examples of the latter type. There is present, however, as in Australia, New Zealand, and South America, a large element of north temperate genera, such as *Mentha*, *Stachys*, *Rubus*, *Potentilla*, *Sium* and many others; and these consist, with very few exceptions, of herbaceous plants. The majority of South African herbs do not belong to the endemic genera.

E. *Madagascar*.

The island of Madagascar is continental in its origin, but that it is of very great antiquity is shown by the highly peculiar character of its fauna and flora. 75 per cent. of the indigenous species of plants are endemic.

There are no means at hand for ascertaining with any degree of precision the percentage of herbs in the dicotyledonous flora, but it is probably much the same as in the neighbouring Mascarene islands, where we have shown that about one-third of the Dicotyledons are herbs. The endemic element is large, and comprises 148 genera, of which 7 are Monocotyledons. There is also an endemic family, the Chlaenaceae, of 7 genera and 24 species of trees and shrubs. The 141 dicotyledonous genera peculiar to the island contain 296 species, of which 34 are herbs, or only 11 per cent. There are a large number of arborescent Compositae, and many woody members of families which are usually herbaceous.

A few dicotyledonous herbs occur among the endemic genera as in all tropical regions, but the majority of herbaceous plants belong to genera which are characteristic of the north temperate zone, such as *Hieracium*, *Viola*, *Alchemilla*, *Sanicula*, *Geranium*, and many others. Not only are the genera northern, but some of the species are also identical with those in Africa and Europe. These northern plants, with very few exceptions, inhabit only the central mountainous area of the island, which enjoys a fairly temperate climate.

The facts, therefore, seem to indicate that the ancient flora of Madagascar, like that of most regions in the southern hemisphere, was at one time overwhelmingly woody in its composition, and that most of the herbaceous element has been relatively recent in its appearance.

F. *Northern Plants in the Antipodes.*

The occurrence in the floras of Australia, New Zealand, Patagonia, South Africa, and Madagascar of a very large number of genera and species characteristic of the north temperate and even of the arctic zones has been noted by many botanists as one of the most striking features of the vegetation of the Antipodes. Sir Joseph Hooker enumerates 125 genera and even 42 species which are common to Europe (many of them to America also) and to the 'Antarctic' floras, and this list has since been considerably extended, so that at least 217 typically northern genera are now known to occur in the south temperate zone.

The present distribution of these plants and their allies seems to indicate very clearly that they have not had their origin in the southern hemisphere, but have entered it as immigrants from a centre of distribution somewhere in the north. That this immigration was not an ancient one but has been comparatively recent is indicated by the slight degree of endemism appearing in the northern types. Change has evidently not been great enough to cause the creation of distinct genera, for the genera endemic in the Antarctic floras are but rarely related closely enough to the northern types to suggest an origin from them *in situ*. Still more conclusive is the presence of a large number of species, even, which are identical with those in distant regions of the north. It is extremely hard to believe that so many plants could have preserved their identity for a very long period of time.

But in this great invasion which has spread the 'Scandinavian' flora throughout most of the southern lands, it is significant that not all northern types have participated. It has been in reality an invasion of *herbs*, for 198 out of 217 characteristic northern genera, or 96 per cent., including all those rich in species (save *Erica*), are composed of herbaceous plants. The nine woody genera are *Rubus*, *Ribes*, *Berberis*, *Sambucus*, *Salix*, *Myrica*, *Empetrum*, *Erica*, and *Vaccinium*. Of these *Erica* and *Myrica* occur only in South Africa. All the genera save *Erica* are very poor in species, and they compose but an insignificant part of the northern element in the flora. *All* the Antarctic species identical with northern ones are herbs.

All these facts are in harmony with our hypothesis that the majority of herbaceous plants have had their origin in the continental area of the north temperate zone, and that they have developed comparatively recently in adaptation to the progressive refrigeration of the climate. The enormous number of species and genera produced in this great and continuous land mass, in contrast to such regions as Australia, New Zealand, and Patagonia, has probably been due to the opportunities for easy dispersal over a wide area, with the consequently tremendous increase in number of individuals and their exposure to many different environments.

Such conditions would tend not only to produce a very rich and varied flora, but also, as Darwin has pointed out, the great competition involved would make its members hardy and able to thrive under all sorts of conditions. Wallace has remarked upon 'the wonderful aggressive and colonizing power of the Scandinavian flora, as shown by the way in which it establishes itself in any temperate country to which it may gain access'. It should be noted, however, that the members of the northern flora which exhibit this aggressiveness, and which are widely distributed as weeds and common plants all over the world, are all herbs!

Our next task will be to trace, if possible, the dispersal of this dominant herbaceous flora from the northern continental areas in which it arose into the distant southern lands where now it flourishes. These antipodean colonies are at present isolated from their northern congeners by many degrees of latitude, a circumstance for the explanation of which various hypotheses have been put forward.

The theory which regards these plants as representatives of an exceedingly ancient type of vegetation which has long since been exterminated everywhere save near the poles, has not only to meet the botanical evidence which indicates that herbs in general are of recent origin, and that their arrival in the extreme south has occurred in relatively modern times; but also the geological evidence that Australasia, South America, and Southern Africa have been open to immigration for only a comparatively short time.

If we admit that the invasion of the south temperate regions by an herbaceous flora from the north took place at a relatively recent date, geologically speaking, it is evident that this invasion must have crossed the tropics along some range of mountains where approximately temperate conditions would obtain. The question of the routes along which this southward migration occurred has been discussed by Wallace, who brings forward evidence that it has proceeded over three main courses—the central African highlands; the Himalayas, Malay Peninsula, and East Indies; and Central America and the Andes.

Only the last of these now presents an unbroken chain of highlands from north temperate lands to high southern latitudes; and scattered throughout its length are many northern genera and species, although even here there are usually wide gaps between the northern and the southern ranges of the same species. Along this route it is obvious that the northern flora must have reached Chile, Patagonia, and Fuegia. The close resemblance of these northern plants in the Patagonian region to those of New Zealand, Tasmania, the south-eastern portion of Australia and the Antarctic islands—a resemblance often extending to identity in species, and one which is shared by the endemic flora as well—makes it very probable that an interchange of plants between these various regions was possible in former times. In fact, the existence of an Antarctic continent well stocked with plants, and either connecting Australia, New Zealand, and South America directly or providing a much readier means of transit than those available at present, has been appealed to by almost every one who has studied the distribution of the Antarctic flora. It seems more likely, from the absence of any considerable Patagonian element in the Australasian fauna that the Antarctic connexion was rather in the nature of a large archipelago, as Hutton (11) and Macloskie (13) have suggested, than of a continuous bridge. 'Such conditions seem to speak to us', says Macloskie (13, p. 959), 'of a broken chain rather than a continuum of land; of an archipelago on a grand scale, some of its components of the continental kind as to dimensions; and with interruptions which secured animal and vegetable isolation; yet sufficient to permit occasional passage of seeds and of birds, and an occasional transit of a quadruped, a sort of quasi-Fuegian archipelago on a larger scale.'

Recent geological evidence lends strong support to the hypothesis of a temperate Antarctic continent, for remains of Ferns, Conifers, and Angiosperms have been obtained in considerable abundance from the Tertiary deposits of Seymour Island, near Graham Land.

The array of northern herbs in temperate South America, New Zealand, and Tasmania and a part of those in Australia have therefore in all probability reached the Antipodes from the north along the Andes and across Antarctica. The absence of many of these plants from the northern Andes to-day is probably due, as Wallace suggests, to the interruption of such favourable conditions as a lowered snow-line, which very likely assisted the migration of temperate forms in the past. It seems very probable that past glacial periods, even perhaps the last Ice Age in the northern hemisphere, may have aided the invasion of the northern flora by increasing the area of temperate regions in the tropics.

That there has also been immigration along the mountains of south-eastern Asia and the East Indies into the southern hemisphere is indicated by the presence in Australia of many northern genera which could have entered by no other route. Wallace has remarked on the fact that the 'northern' flora of Australia consists of two distinct elements, one which closely resembles that of New Zealand and South America, and which is mainly confined to the south-eastern part of the continent, and includes all the species which are identical with European ones; and another of European genera, 'usually of a somewhat more southern character', which do not occur in New Zealand or South America, and which are represented by very distinct species. The latter portion of the flora presumably reached Australia through southern Asia at a time when immigration from the north was easier than at present, and the presence of many palaearctic forms in the mountains of the East Indies points out the highway which was traversed. That the invasion over this route was interrupted considerably earlier than was that along the Andes is indicated by the fact that the northern genera which are absent in South America (and which therefore must have entered Australia from Asia) have no species identical with those in the north temperate zone.

The European element in the flora of South Africa comprises at least 60 genera of north temperate plants, and has evidently attained its present extension by migration along the African highlands at a time when opportunities for immigration were somewhat better than at present. That this invasion, also, was earlier than that along the Andes is shown by the fact that, although the genera are still European, very few of the species have preserved their identity. The fact that so many northern genera are present in the uplands of Madagascar would seem to prove that this island was recently much more closely connected with the highlands of Africa than at present; but that this connexion was never continuous enough for the migration of land animals is rendered probable by the strict confinement to Madagascar of most of its characteristic fauna.

G. *The Ancient Flora of Antarctica.*

Since the presence of a considerably extended Antarctic continent, able to support a rich vegetation, is necessary to account for the distribution of palaearctic plants in the Antipodes, it is worth our while to discover, as far as we may, the character and composition of its 'endemic' flora. It seems to be a reasonably safe conclusion that all genera commonly designated as 'Antarctic' from their confinement to the temperate regions of the southern hemisphere, were inhabitants of the ancient Antarctic continent. The writers have compiled a list of 36 genera of Dicotyledons which occur or have representative types in the three main Antarctic regions—Australia, New Zealand, and temperate South America—and which possess but very few species outside these two regions. This list may well be regarded as fairly representative of the flora of Antarctica before the advent of the cold period which drove the phanerogamic vegetation northward into South America and Australasia. Of these 36 genera, only 15 are typically herbaceous, or 41 per cent. If we include in the flora of Antarctica the 52 genera which are confined at present only to Australia and New Zealand, and which at one time in all probability flourished on the Polar continent, the list of Antarctic dicotyledonous genera is increased to 88, and of these only 34, or 38 per cent., are typically herbaceous. Such a preponderance of woody forms in a region which was gradually being subjected to refrigeration is remarkable. Practically all the herbaceous plants in this flora are at present alpine forms. It seems altogether probable that they were developed comparatively recently as an adaptation to extreme winter conditions, just as were the herbs in Arctic regions, but that even they were finally forced to leave Antarctica and now constitute part of the endemic herbaceous genera of the south temperate zone. That they have not been derived directly from northern herbs but are related rather to the ancient vegetation, fragments of which are now preserved in oceanic islands, is made clear from their floral structure. *Colobanthus*, for example, has its closest ally in *Alsinodendron*, one of the shrubby Caryophyllaceae of Hawaii ; and *Myosotidium* is nearly related to *Selkirkia*, a woody member of the Borraginaceae from Juan Fernandez.

It is evident, therefore, that the lowland flora of Antarctica, and probably the entire flora up to comparatively recent times, agreed with those of other portions of the earth which have long been isolated from the continental area of the north temperate zone in being composed almost entirely of woody plants ; and that the herbaceous element in the vegetation of this Polar continent was derived partly from recent immigration out of the north and partly from a similarly recent acquisition of the herbaceous habit by certain members of the endemic flora.

The general results of our study of the present distribution of herbs throughout the world indicate that up to the early Tertiary they must have formed an insignificant portion of the vegetation ; that the bulk of herbs originated in the continental area of the north temperate zone in response to the progressive refrigeration of the climate during the Tertiary; that this aggressive herbaceous flora spread at every opportunity into the temperate regions of the southern hemisphere, and that in these Antarctic lands a comparatively small body of herbs had an independent origin.

V. FACTORS IN THE DEVELOPMENT OF HERBS.

We have spoken of the progressive differentiation and refrigeration of climate in the north and south temperate zones as the chief factor in the development of the herbaceous habit. The first effect of such refrigeration was doubtless to stunt and dwarf trees into shrubby plants and thus shorten the time necessary for them to reach maturity. This process we may still see in arctic and alpine regions. This would first affect the more susceptible and warmth-loving plants and would reduce them—if they lived—with considerable rapidity, until finally very dwarf forms were developed which would produce seed in a single year and which would stand being killed back every winter. They would be the primitive perennial herbs, and it is significant that the present arctic and alpine vegetation is composed almost entirely of perennials. The annual herb seems to have been developed from such plants under more favourable environments, where a plant growing from seed could become large enough in a single season to reproduce itself, and did not need a subterranean food-reservoir to give it a rapid start.

It is probable that the earliest herbs were developed in mountainous regions, as it would be here that extremes of climate would first be felt. That decrease in temperature has indeed been the chief factor in the evolution of herbs on the north temperate zone is indicated by the fact that families possessing woody forms which are able to withstand cold almost never include herbs. Thus the Salicaceae, Betulaceae, Fagaceae, Myricaceae, Empetraceae, Aquifoliaceae, Aceraceae, Ericaceae, Rhamnaceae, Tiliaceae, and Thymeleaceae, which comprise almost all the woody plants of the north temperate zone, have practically no herbaceous members. Being naturally hardy to cold, they have not been forced to adopt the herbaceous habit. The more susceptible families, on the other hand, which once flourished in northern regions, have either developed herbaceous representatives or been exterminated.

Refrigeration of climate has doubtless produced herbaceous plants in mountainous regions throughout the globe as well as in the Polar land areas. The Andes, the highlands of Africa, and the Southern Alps of New Zealand have certainly been the centres of origin of many herbs. In the last-named region, the reduction of the genus *Veronica*, for example, from trees down through all degrees of dwarfing to perennial herbs may be very clearly traced. Many herbs of the tropical lowlands have apparently been produced in mountainous regions and then migrated downward.

A very large number of herbs have doubtless arisen in arid regions also, springing up rapidly and producing seed during a rainy season and thus bearing the same relation to extremes of moisture that northern herbs bear to extremes of temperature. Many of the endemic herbs of South Africa, West Australia, and other regions which are subject to dry seasons of considerable length have in all probability been developed in this way. The stunting effect of desert conditions has resulted in an extreme paucity of trees in arid regions and in the reduction of most of the woody species to stunted undershrubs or suffruticose plants, some of which are practically perennial herbs.

There is also a considerable element of characteristically tropical herbs, which seem to have been evolved in very warm regions and not to have come from the mountains. They were probably developed in response to variations in moisture consequent on the alternating wet and dry seasons, or for various other reasons. The genus *Peperomia* is a good example of such tropical herbs.

A great body of herbaceous plants, originating for the most part in the tropics, have apparently not arisen because of extremes of either heat or moisture but from the acquisition of a rapidly climbing habit. The Cucurbitaceae, Convolvulaceae, Asclepiadaceae, and other families and genera are composed mainly of climbing plants, and in them there has been a progressive reduction in the amount of xylem and increase in bulk of parenchyma until typically herbaceous stems have been produced. A large proportion of the species counted as herbs in our tabulations of tropical floras is made up of such scandent types, which are essentially different from the terrestrial herbs characteristic of temperate regions.

Any xerophytic condition tends to dwarf plants and, if it is periodic, to produce herbaceous forms. Desert vegetation is composed largely of shrubs or undershrubs, but there is also a considerable body of herbs, both annuals and perennials, which spring up rapidly and produce seed during any rainy period, and which have the same relations to extremes of moisture that ordinary herbs have to extremes of temperature. Many of the endemic herbs of South Africa, West Australia, and other regions which are subject to dry seasons of considerable length, have in all probability been developed in this way.

It is altogether probable that dicotyledonous herbs were developed in rare instances in the Cretaceous or very early Tertiary, before extremes of climate had been generally produced and in response to local xerophytic conditions, probably for the most part in the mountains; but it seems very probable that the great mass of herbaceous vegetation, certainly among the Dicotyledons, is of much more recent origin.

VI. General Conclusions.

Let us now enumerate briefly some of the general conclusions at which we have arrived as to the origin and dispersal of herbaceous Angiosperms.

That the herbaceous vegetation of to-day should be regarded as of comparatively recent development is indicated by evidence from Palaeobotany, that almost all the earliest Angiosperms of which we have knowledge were woody; from Phylogeny, that herbs tend to occur more frequently in plant-groups which are recent rather than in those which are primitive; from Anatomy, that the ancient type of cambial activity produced a solid ring of secondary wood, and that the herbaceous stem has been reduced from such a condition; and finally from Phytogeography, that herbaceous vegetation, most richly developed in the great land-mass of the north temperate zone, has spread thence over most of the globe; and that regions which have long been isolated consequently possess a flora which, especially in its endemic portion, is very ancient and therefore composed almost entirely of woody plants. The progressively increasing refrigeration of the climate of the north temperate zone has been by far the most important (though not the only) factor in the development of herbs; for these plants, from the brevity of their life-cycle and their ability to survive periods of extreme cold either underground or in the form of resistant seeds, are able to thrive in regions which are subject to winters severe enough to eliminate most plants with perennial aerial stems. Herbaceous plants, having in this way become adapted to adverse conditions, are therefore the most hardy and aggressive types of vegetation and have consequently been able to invade successfully all regions of the globe.

SUMMARY.

1. The problems under discussion in the present paper are the relative antiquity of herbs and woody plants; and the manner in which the more recent of these two types has been developed and become dispersed.

2. Palaeobotanical evidence shows that the ancient representatives of several of the lower orders of vascular plants were woody, although their living members are herbaceous. Almost all the earliest Angiosperm remains are also of woody plants. The geological evidence in this case, however, although seeming to indicate that the primitive members of the phylum were woody, cannot be regarded as proving the point conclusively, for the leaves of trees and shrubs are probably more likely to escape destruction and to be preserved as fossils than are the more tender ones of most herbaceous plants.

3. Evidence from anatomy indicates that in all groups of vascular plants which possess secondary growth the cambium appeared originally as a uniform and continuous layer; and that its distinction into a 'fascicular portion, producing wood, and an 'interfascicular' portion, producing parenchyma, has been of comparatively recent occurrence. The continuous woody ring which characterizes the stems of all trees and shrubs is therefore to be regarded as more primitive than the many-bundled herbaceous type. The structure of woody stems also displays primitive histological characters often absent in those of herbs.

4. Jeffrey and his school have maintained that the herbaceous stem was derived from the woody type by a conversion into parenchyma of whole segments of the central cylinder directly opposite the bundles which were to depart as leaf-traces, and that these segments constitute the interfascicular parenchyma between the bundles of the herbaceous stem. The writers dissent from this theory, both on the ground that evidence in its support is derived not from the aerial parts of the plant but almost entirely from prostrate stems or subterranean rhizomes, which are nearly always much modified in structure, owing to their function as food reservoirs; and, more particularly, on the ground that it is not in harmony with a great body of anatomical facts. In practically all families of herbs, the interfascicular parenchyma is never subtended by a tiny leaf-trace bundle of protoxylem, but always abuts directly on the pith tissue between the strands of primary wood.

5. The chief factor in the development of the herbaceous stem seems to have been a simple decrease in the activity of the cambium. This has

often been supplemenated by widening of the medullary rays and, more rarely, by an increase in the development of secondary parenchymatous tissue between the bundles. In the case of many families, however, the herbaceous members have a perfectly continuous ring of vascular tissue. The herbaceous stem in its general character is very much like the first annual ring of a related woody plant.

6. Evidence from phylogeny shows that the more primitive groups of Angiosperms, and the lower seed-plants from which they have presumably been derived, are composed overwhelmingly of woody plants. In those particular orders or families, also, which comprise both herbs and woody plants, and in which it is possible to determine with reasonable certainty the relative antiquity of the various members, the primitive types are found to be much more woody than the recent ones. In more than half of the families of Dicotyledons, there are no herbaceous species, and the few families which are entirely herbaceous are almost all insectivorous plants, water plants, parasites, or monotypic families, and hence can lay no claim to great antiquity. Woody plants are much more abundant among the Archichlamydeae than among the Metachlamydeae.

7. Since the Monocotyledons probably arose in very ancient times from the primitive Dicotyledons as an aquatic herbaceous adaptation, woody forms in this group are to be considered as recent rather than as primitive.

8. Evidence from phytogeography also supports the contention that the most ancient Angiosperms were woody. There is a great preponderance of herbs in temperate regions and of woody plants in the tropics. The latter climate probably approaches more nearly to that under which Angiosperms first appeared.

9. Herbs have a short life-cycle, and are therefore able to survive periods of cold underground or in the form of seeds. Their great development in temperate regions has probably been in response to the progressive refrigeration of the climate during the course of the Tertiary.

10. A considerable number of plants now occur only in eastern Asia and eastern North America. They form a flora which probably approaches that of the north temperate zone in preglacial time. It is composed of about an equal number of herbs and of woody plants.

11. The advent of the glacial period resulted in the extermination of a large part of the vegetation of the north temperate zone, but this extermination was proportionately much greater among woody plants than among herbs. The present flora of Europe contains a decidedly smaller element of woody plants than does that of corresponding temperate North America because of the inability of the plants in the former region to migrate southward on the approach of the glaciers.

12. The present flora of Northern Europe has apparently received but

little immigration from the south since the retreat of the ice. Its general composition as to proportions of herbs and woody plants is probably much the same as it was during glacial time. It consequently furnishes us with an indication of what the climate of Europe was like during the Ice Age, since there is in general a rather close agreement between the minimum temperature of a region and the percentage of herbs in its flora.

13. The endemic plants in a flora are usually to be regarded as its most ancient element. Herbs, however, from the brevity of their life-cycles, are apt to become modified more rapidly than woody plants, and hence may be developed into endemic types in a comparatively short time. Endemic trees or shrubs are almost always very ancient.

14. In various oceanic and continental islands which are believed to possess faunas and floras of considerable antiquity, the most ancient element in the vegetation—the species of the endemic genera—is almost entirely composed of woody plants; the next younger, the endemic species of non-endemic genera, shows a larger proportion of herbs; and the non-endemic species, presumably the most recent element, are overwhelmingly herbaceous.

15. The continental areas of the southern hemisphere are also believed to retain many ancient types of animals and plants. In these regions, as in isolated islands, the most ancient part of the vegetation is overwhelmingly woody and the more recent mainly herbaceous.

16. These facts point to the conclusion that the ancient flora of the world was for the most part composed of woody plants, and that the majority of herbs, originating comparatively recently in the land-mass of the north temperate zone, have spread thence over most of the earth.

17. This recent entrance of northern plants into the Antipodes has mainly been along the Himalayas, the Andes, and the African highlands. These invasions have been almost entirely of herbs, which from their ability to migrate rapidly and to withstand adverse conditions have become the most ubiquitous and aggressive type of vegetation, and constitute practically all plants known as weeds.

18. The recent date of the invasion of the south temperate zone by these northern herbs has a bearing on various problems of geology and climatology, such as the periods of direct lowland or mountain-chain connexion between the northern continents and Australasia, South America, South Africa, and Madagascar; the climate of these regions during the Tertiary; and the extent and climate of the Antarctic continent. The latter question is perhaps the most important. The distribution of these northern plants and of the 'endemic' Antarctic flora can well be explained only on the assumption of the recent existence of a temperate Antarctic continent which was more closely connected than at present with South America and

Australasia. This connexion seems rather to have been by a series of large islands than by a continuous land bridge.

19. A hypothetical reconstruction of the ancient flora of Antarctica indicates that nearly two-thirds of its 'endemic' dicotyledons were woody plants.

20. The chief factor in the development of herbs has been the progressive refrigeration of climate in the temperate zones during the Tertiary period. This probably was effective in gradually stunting woody plants more and more until their aerial portions persisted only for a single growing season. The annual herb seems to be the most recent type. Most families which are able to withstand cold in the form of trees and shrubs have produced few or no herbaceous members.

21. The great majority of herbs were probably developed in the land-mass of the north temperate zone, and arose first in the mountains. Herbs have also been produced in mountainous regions in the tropics and the southern hemisphere. Many tropical lowland herbs have descended from the mountains. Others have been evolved under tropical conditions in response to the alternation of wet and dry seasons or for other causes. Many herbs in desert or dry regions have likewise arisen in response to discontinuity in the amount of moisture. A large body of plants, particularly in the tropics, have become herbaceous and semi-herbaceous through an assumption of the climbing habit. Most herbs seem to have appeared during the Tertiary, many of them probably not till the latter part of the period, but it is likely that in rare cases, under adverse climatic conditions, they were present in the Cretaceous.

The writers wish to express their sincere thanks to the authorities of the Arnold Arboretum and of the Gray Herbarium, of Harvard University, for frequent use of their libraries and collections; and to Professors H. C. Ernst and S. B. Wolbach, of the Harvard Medical School, for loan of their photomicrographic apparatus.

BIBLIOGRAPHY.

1. ADKINSON, J.: Some Features of the Anatomy of the Vitaceae. Ann. Bot., vol. xxvii, 1913.
2. BAILEY, I. W.: The Relation of the Leaf-trace to the formation of Compound Rays in the Lower Dicotyledons. Ann. Bot., vol. xxv, 1911.
3. ———— and SINNOTT, E. W.: Anatomical Evidences of Reduction in certain of the Amentiferae. Bot. Gazette, vol. lviii, 1914.
4. DARWIN, C.: The Origin of Species. 6th ed.
5. EAMES, A. J.: On the Origin of the Herbaceous Type in the Angiosperms. Ann. Bot., vol. xxv, 1911.

6. Gray, A.: Scientific Papers, vol. ii.
7. Guppy, H. B.: A Naturalist in the Pacific.
8. Hallier, H.: Ein zweiter Entwurf des natürlichen (phylogenetischen) Systems der Blütenpflanzen. Ber. d. Bot. Gesellsch., 23, 1905.
9. Heer, O.: Flora Fossilis Arctica, vol. i.
10. ———: Recherches sur le climat et la végétation du pays tertiaire.
11. Hutton, F. W.: Ancient Antarctica. Nature, vol. lxxii, July 13, 1905.
12. Jeffrey, E. C.: The Relations of Palaeobotany to Botany-Morphology. Amer. Nat., vol. xlvi, 1912.
13. Macloskie, G.: Report on the Princeton Expedition to Patagonia: Botany, vol. ii.
14. Wallace, A. R.: Island Life.
15. Wolf, T.: Monographie der Gattung *Potentilla*.

EXPLANATION OF PLATES XXXIX AND XL.

Illustrating Messrs. Sinnott and Bailey's Paper on *Herbaceous Angiosperms*.

PLATE XXXIX.

Fig. 1. *Arctotis grandis*. Section of semi-decumbent herbaceous stem. Below is a stout zone of wood with wide rays; above, a many-bundled herbaceous structure. The wide rays in the former have given place to the interfascicular parenchyma in the latter. × 7.

Fig. 2. *Fagus Americana*. First annual ring, showing essentially 'herbaceous' structure. × 20.

Fig. 3. Guttiferae. *Hypericum aureum*, shrubby species, first annual ring. × 20.

Fig. 4. Guttiferae. *Hypericum perforatum*, herbaceous species, mature stem. × 16.

Fig. 5. Solanaceae. *Nicotiana glauca*, shrubby species, first annual ring. × 9.

Fig. 6. Solanaceae. *Nicotiana tabacum*, herbaceous species, mature stem. × 9.

Fig. 7. *Salvia sp.* Section of herbaceous stem showing conversion of interfascicular segments of wood into vertical parenchyma. It will be noted that the segments opposite the protoxylem have retained their typical woody structure. × 18.

Fig. 8. Malvaceae. *Hibiscus rosa sinensis*, shrubby species, first annual ring. × 16.

Fig. 9. Malvaceae. *Abutilon* (herbaceous species), mature stem. × 14.

Fig. 10. Rosaceae. *Rosa rugosa*, shrub, first annual ring. × 8.

Fig. 11. Rosaceae. *Sanguisorba*, herb, mature stem. × 15.

Fig. 12. Ranunculaceae. *Xanthorrhiza apiifolia*, shrub, first annual ring. × 30.

PLATE XL.

Fig. 13. Ranunculaceae. *Delphinium formosum*, herb, mature stem. × 16.

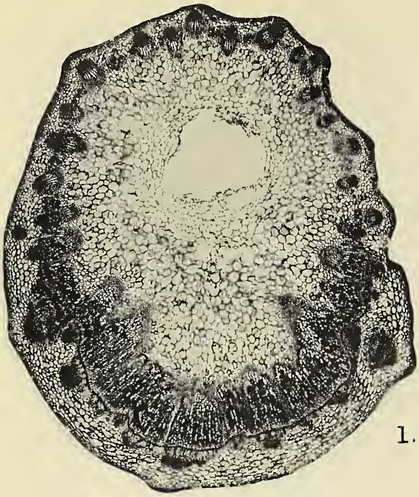
Fig. 14. Araliaceae. *Acanthopanax ricinifolium*, tree, first annual ring. × 8.

Fig. 15. Umbelliferae. *Sium cicutifolium*, herb, mature stem. × 9.

Fig. 16. Compositae. *Senecio Grayii*, shrubby species, first annual ring. × 14.

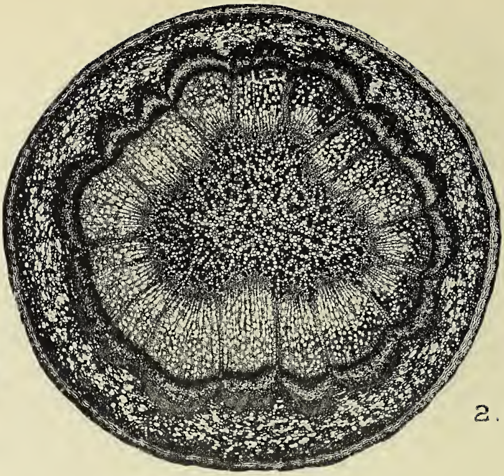
Fig. 17. Compositae. *Solidago rugosa*, herbaceous species, mature stem. × 30.

Fig. 18. *Clematis virginiana*. Section of typical climbing stem. × 14.



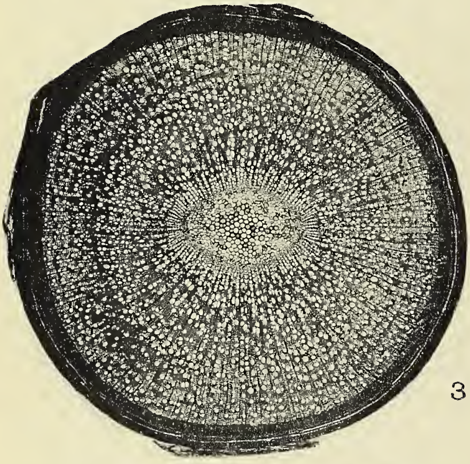
1.

Arctotis grandis.



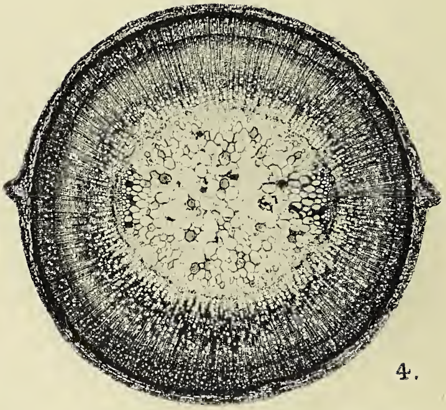
2.

Fagus americana.



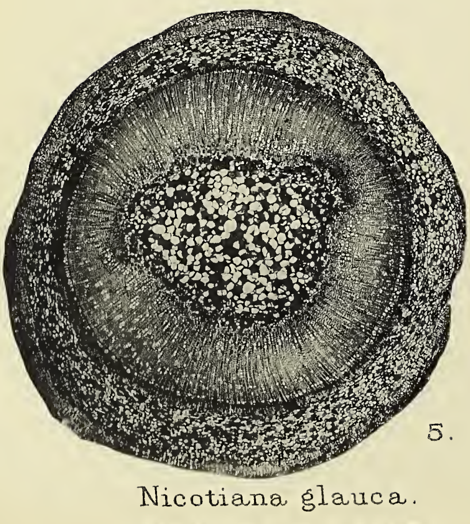
3.

Hypericum aureum.



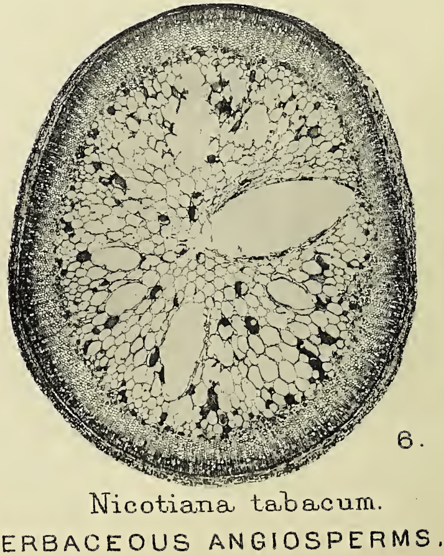
4.

Hypericum perforatum.



5.

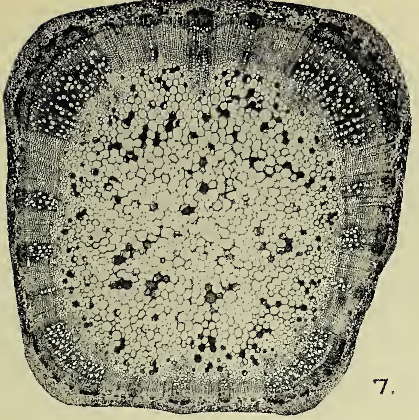
Nicotiana glauca.



6.

Nicotiana tabacum.

SINNOTT & BAILEY—HERBACEOUS ANGIOSPERMS.



7.

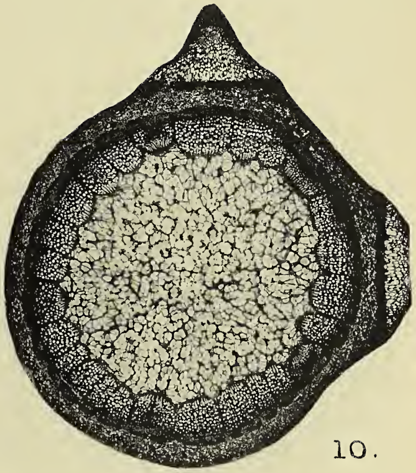
Salvia sp.

8.

Hibiscus rosa sinensis.

9.

Abutilon sp.

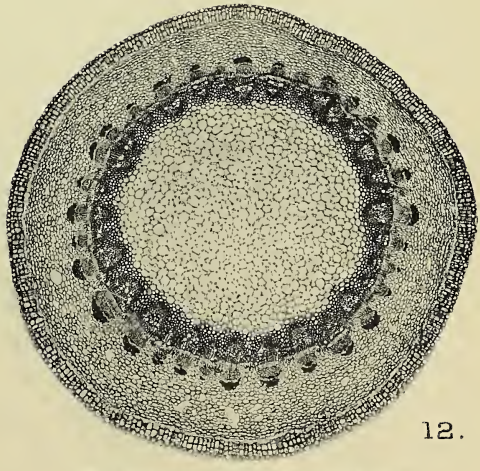


10.

Rosa rugosa.

11.

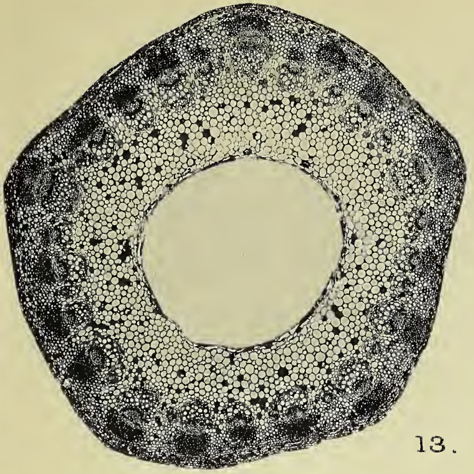
Sanguisorba sp.



12.

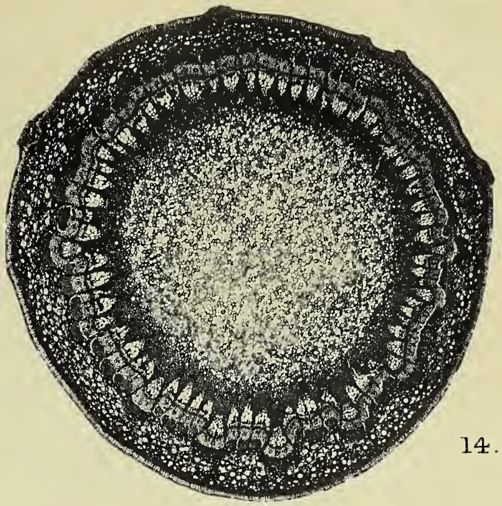
Xanthorrhiza apiifolia.

Huth coll.



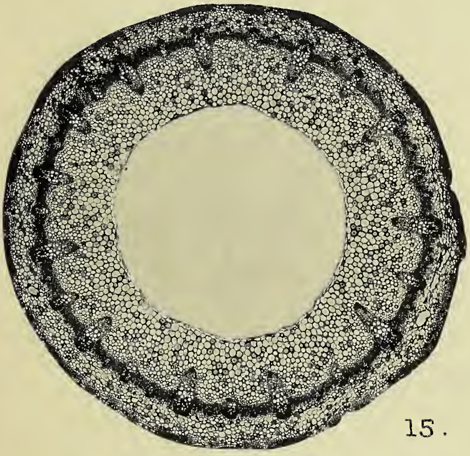
13.

Delphinium formosum.



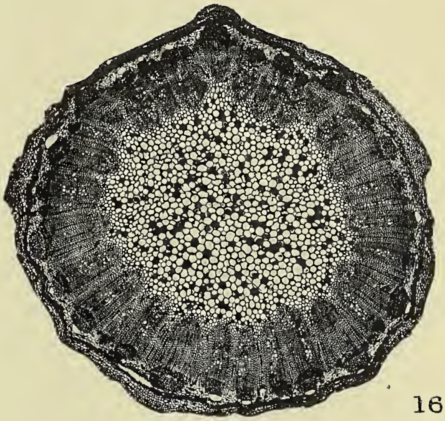
14.

Acanthopanax ricinifolium.



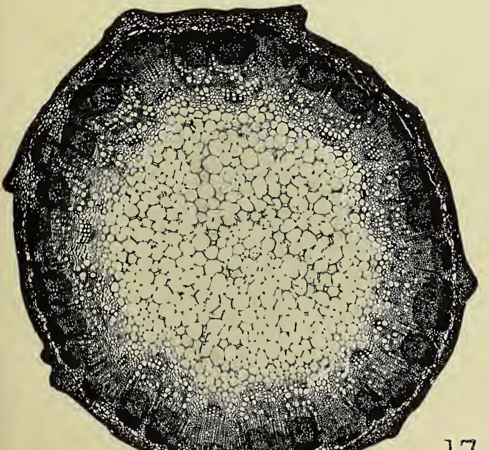
15.

Sium cicutifolium.



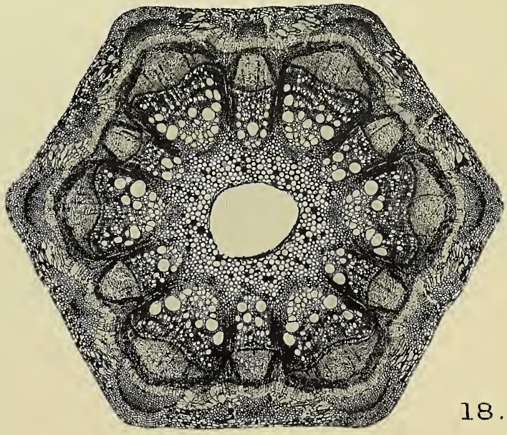
16.

Senecio Grayii.



17.

Solidago rugosa.



18.

Clematis virginiana.

Huth coll.

SINNOTT & BAILEY—HERBACEOUS ANGIOSPERMS.

Some Studies on the Structure of the Plant Cell by the Method of Dark-ground Illumination.

BY

S. REGINALD PRICE, M.A.

With Plates XLI and XLII.

TABLE OF CONTENTS.

§ 1. HISTORICAL AND INTRODUCTORY.

IT may be fairly said that until ten years ago—that is, till the year in which Siedentopf ('03, pp. 1–39) elaborated the method of the ultra-microscope—the method of dark-ground illumination with the microscope was regarded as a means of exhibiting objects with striking and pretty effect, rather than a means of observation of minute structure and of scientific research. Siedentopf and Zsigmondy ('03), by using a powerful beam of light projected perpendicularly to the axis of the microscope, showed that particles could be exhibited which were beyond the range of possible resolution with the ordinary transmitted light. No direct light enters the microscope; the small particles scatter the light, and by reason of the diffraction images so produced, they appear as though they were self-luminous against a black background. There is no need in this place to deal with the development of the method of dark-ground illumination for high power work since that date, but reference may be made to several published accounts of the subject (Thirkill, '09; Spitta, '09, pp. 172 sqq.; Price, '13, and so on).

The study of colloidal hydrosols by this method was first carried out by Zsigmondy ('09), and since then the method has become a great factor in the development of the study of colloids, a study which has made enormous progress during the last decade. In the field of biological thought this 'Kolloidchemie' has had a very great influence, and it is leading to a better understanding of many reactions and processes.

It had become increasingly evident that the substance protoplasm, both of animal and vegetable life, was of a colloid nature (Price, l. c., '13; Czapek, '05, pp. 34 sqq., and references there cited), and so, as was to be expected, an investigation of protoplasmic structures by dark-ground illumination methods produced some highly interesting results.

The first examinations of the structure of the plant cell by the method were made by Dr. Gaidukov ('06, four refs.) and published in 1906. The greater part of the published work in this direction has been performed by this investigator, whose results and conclusions will be found in his work, 'Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin' ('10), especially in Sections VI and VII. No detailed account of his work will be given in this place. A short outline of this has been published (Price, l. c., '13), and constant reference will be made to his results throughout this account and in the general discussion.

It will be enough to state here that he showed how the method could be applied to the observation of the living contents of the plant cells, especially of filamentous Algae, plant hairs, and such few-celled structures. He established, as a main result, the fact that the protoplasm is a hydrosol complex (in the terminology of the colloid chemists) in most cases, which on the death of the cell is coagulated and converted into the hydrogel state (Gaidukov, '10, p. 61; see also Bechold, '11). In most cases the hydrosol is seen to consist of a heterogeneous complex—a large number of particles of usually submicroscopic size (ultra-microns) rapidly oscillating in a fluid homogeneous substratum—the so-called disperse and continuous phases respectively. This, as has been shown by Zsigmondy and many other investigators, is the general character of a liquid colloid or hydrosol 'solution', though the size and number of particles present may vary enormously (Zsigmondy, l. c., '09, p. 211, Plate I, &c.). In certain cases no such rapid movement of the particles was made out, and Gaidukov inclines to the view that this was due to the fact that the microns were so small in size and so closely packed that no definite particles could be distinguished ('10, p. 61). As is well known, such hydrosol solutions do exist; in fact, there must be supposed to be a complete range in size of the particles from molecular to microscopic dimensions. The coagulation of the hydrosol results in the cessation of the motion of the particles, with the production of an appearance of large overlapping diffraction images, a frequent character of gel structure when observed by dark-ground methods (ib., p. 61).

The movement of the particles above mentioned is of the nature of a Brownian movement—a phenomenon previously well known for small particles of microscopic size in suspension (Perrin, '13), and shown by Zsigmondy to extend to these ultramicroscopic particles, where the phenomenon is manifested as a much more vigorous motion—'like a swarm of dancing gnats in a sunbeam' ('09, p. 134). The phenomenon has been the subject of some fine researches by Perrin (l. c., '13), who has shown, almost beyond doubt, that the movement is to be regarded as an outcome or manifestation of the activity of the ultimate molecules of the continuous phase. It has been suggested that, on account of the great activity of the movement for ultra-microns, the phenomenon should in this case be termed the Brownian Zsigmondy movement (Bechold, '11, p. 43).

Besides the work of Gaidukov, comparatively little has been done with the method in the particular direction of the study of the plant cell. A short preliminary note (Price, '12) was published some time ago containing an account of a few of my observations with the method; most of these are amplified in the present paper.

Ruhland ('12, p. 376), in his researches on the behaviour of the plasmatic membrane as an 'ultra-filter' (Bechold,' 11, p. 86), has made use of the method to a certain extent, chiefly in the study of the size of particles in certain dyes or stains, and to examine the process of the taking up of these through the plasmatic membrane.

Other observations have been made which are somewhat akin but are not actually botanical in nature. Thus the observations of Fauré-Frémiet ('10) on the nucleus of certain Protozoa is of interest and is referred to below.

§ 2. THE PRESENT WORK.

The work which is at present described was undertaken with the idea of testing the possibility, by the use of the method, of getting some more facts concerning the colloid structure of the living and dead cell, of studying the reactions of the colloid, and so on. There is undoubtedly a great deal of work to be done in the study of the physiology and properties of the cell itself, as this is, after all, the unit which has to be considered in general physiological processes, and, following the work of Gaidukov, it seemed that something in this direction might be expected of the method of dark-ground illumination.

As the method is a comparatively new one, some of the results here described are to be taken as special cases indicating lines of study which might be increasingly productive in the future, and so in many cases it is impossible to draw general conclusions from them.

Practically the whole of the work has been done in the Botany School, Cambridge, during the tenure of the Frank Smart Studentship. I should like to take this opportunity of thanking Prof. Seward for granting me all facilities for the work and for encouragement during its progress. To all those who have taken an interest in the work and often helped with discussion and hints I desire to express my best thanks, and also to Mr. F. F. Blackman, who kindly read the manuscript.

Observations have chiefly been made with a simple type of dark-ground illuminator—the Zeiss paraboloid (Zeiss pamphlets)—used in conjunction with various lenses. Although dry lenses are recommended, the best results have usually been obtained with the 3 mm. Zeiss immersion apochromat and suitable stop. A clear image with few diffraction colour fringes is obtained by use of this, chiefly with the oculars 6 and 8. As illuminators, a Nernst lamp, sunlight, and a small arc lamp have been used. The last of these, a semi-automatic pattern, is most satisfactory for use in most cases, although the intense brilliancy of sunlight renders visible even smaller particles. The light is concentrated on to the plane mirror of the microscope by means of a spherical glass flask of water, which serves to absorb much of the heat and prevent undue heating of the object on the stage.

Object-slides and cover-glasses must be flawless and of good quality; they are specially cleaned and kept in alcohol. The object, mounted in water in the ordinary way, is first viewed with a low power and the illumination is adjusted, the final refinement under the high power being best performed by trial. In this way certain structures of the cell can often be more clearly brought out.

Considerable difficulty is experienced in selecting material suitable for observation and experiments in various directions. Gaidukov showed that only in certain cases was good observation possible. The considerations affecting the suitability of material are roughly as follows: the tissues or structures must generally be only one cell in thickness; the walls of the cells must be optically homogeneous; the diameter of the cell or cell-filament must not be so small that the diffraction image of the walls interferes with observation of the cell contents; and the cells must not contain a large number of bodies of the nature of chromoplasts, as these, by scattering the light, interfere with good observation of the more minute structures. All these criteria must be taken into account, and it may be seen at once that the field of choice is comparatively limited. This is obviously a barrier to the extended use of the method, and one which in many cases must apparently prove insurmountable.

Only a small quantity of material must be taken—a few filaments of Algae, a single small leaf, and so on—and this is mounted so as carefully to exclude air bubbles.

In experiments dealing with the action of reagents in the cell, in most cases considerable difficulty has been experienced, chiefly as to the possibility of observing the first action of the reagent. Two methods were adopted; in that generally employed, the solution was added to the edge of the cover-slip and the liquid drawn under by placing a piece of filter-paper along the other edge. The method necessarily brings about a dilution of the solution, so that no critical knowledge of the concentration of the reacting solution is possible. The other method is to mount the objects directly in the solution and to observe the effects as soon as possible. This is suitable for actions with a considerable time factor, such as plasmolysis, but is useless in studying the first stage of fixation and coagulation.

The figures are drawn to illustrate as nearly as possible the appearance of the objects. The main outlines were sketched with a camera lucida, the smaller details being put in as accurately as possible. In many cases it is impossible to represent the smaller particles which are present, and in any case the drawing, lacking animation, gives no real idea of the activity of the particles of the hydrosols.

§ 3. OBSERVATIONS OF LIVING CELL STRUCTURES.

A description will first of all be given of some of the material which has been examined in the normal living state by this method and subsequently used for other experiments. Some of the objects here described were observed by Gaidukov ('10), but in these cases further facts as to structure have been made out, while the others are here described for the first time.

Spirogyra.

Spirogyra has been carefully observed and described by Gaidukov, and furnished one of the most favourable objects. This is fortunate, as it has so often been used by investigators on plasmolysis and the physiology of the single cell. A few further facts as to its colloid structure may be added.

According to the nature of the study, types with closer or more distant chloroplast spirals may be chosen. By focusing outside the chloroplast, it is clearly seen that comparatively large microsomes exist in the protoplasmic hydrosol—at least these appear large with this method of illumination. In certain cases some of these microsomes can be made out in direct illumination (*Hellfeldbeleuchtung*). As will be shown more fully below, a large number of much smaller particles occur in the protoplasm, which can often be made out by careful focusing and manipulation of the light. From these careful focusing experiments, it appears that the smaller particles tend to occur more especially towards the outside and inside of the plasma layer (Price, '12). It may be mentioned here also, that the chloroplast has a structure very distinct from that of the cytoplasm. No distinct microsomes appear in it, and on the whole it seems to have a fairly homogeneous gel-like structure. In reaction, as shown below, it differs quite markedly; the subject will be referred to again (see § 7).

As Gaidukov has already shown, particles of a larger order of magnitude frequently occur in the sap vacuole. In species of *Spirogyra* with a loose type of spiral these are usually very conspicuous, and to avoid their interference with observation a close spiral type is useful. These 'sap particles' are found to be of very general distribution in plant cells (see § 5).

In many cases it was possible to observe the nucleus, especially in starved or poorly nourished material of a large species, which was used for a long time for observation. As is well known, the nucleus is suspended in the cell-cavity by cytoplasmic threads; these threads were clearly seen as slightly heterogeneous processes, stretching from the cytoplasmic layer of the cell to the cytoplasm surrounding the nucleus. They seem to be viscous in nature and show the larger microsomes clearly present. The microsomes here exhibit an oscillating movement and also a movement of translation, gradually passing one another in different directions, and giving an appearance of moving in a viscous medium. The appearance of the nucleus itself is described elsewhere; it is differentiated in structure from the cytoplasmic mass which surrounds it. The microsomes move more especially on the outside of the cytoplasm which envelops the nucleus (see Pl. XLII, Fig. 1).

Elodea canadensis.

At the edge, the leaf of this water-plant is only one cell thick, and these edge cells have proved quite useful for observation (Price, '12). The young leaves are removed from the stem, carefully brushed to remove epiphytic growths and mounted in water. In these edge cells the wall is optically clear, and the chloroplasts are few and inconspicuous under dark-ground illumination (Pl. XLI, Fig. 6).

The protoplasm forms a general layer on the inside of the wall, surrounding a central vacuole, but threads and strands of protoplasm frequently cross the vacuole, the conformation of these constantly altering during the circulation of the protoplasm. The protoplasm is seen to be filled with large numbers of particles (submicrons), which appear as glittering points of light and are in a state of active Brownian movement. If circulation and streaming is not taking place, this generally soon begins, possibly under the slight warming which the leaf undergoes on the stage. The protoplasm flows on as a viscous liquid, carrying the particles with it, which retain their Brownian oscillations (Gaidukov, '10, p. 52). The particles in the stream seem to move with considerable rapidity. After a time the chloroplasts begin to be carried forward, as though the circulating stream took some time to acquire sufficient energy to do this. It is a significant fact that the chloroplasts never move as rapidly as the protoplasmic submicrons, so that their motion does not give an accurate measure of the rate of movement of the protoplasm (Pfeffer, '06, p. 289). No definite differentiation into endoplasm and ectoplasm was made out.

The nucleus has been observed in some cases in the living cell, a description of this being given below. No very definite membrane bounding the vacuole could be made out in this case, nothing more than would be expected from the appearance of the surface of a viscous fluid.

The vacuole itself contains numerous 'sap particles', which are undoubtedly of two kinds and which vary in number with the vitality of the cell. In some cases these particles aggregate together and form a mass, which lies in the vacuole and is slowly rolled about by the circulating protoplasm. No account of any observations on such a body could be found. Generally, the sap particles have a Brownian movement quite independent of the protoplasmic movement, though at times some of the particles become attached for a time to the protoplasmic stream, and are carried along as though adhering to a viscous surface. This independence of motion is perhaps one of the best tests of the identity of the sap particles.

Elodea densa.

The leaf-edge cells of this species are rather larger than those of *E. canadensis* and provide good material. The phenomena shown are much the same as for *E. canadensis.* The sap particles are very distinct and of a crystalline character, clearly seen in direct illumination. As, however, the material is more difficult to obtain and keep healthy, it was not used extensively.

Saprolegnia.

Many fungal hyphae seemed to promise good material for observation. The rather large coenocytic hyphae of *Saprolegnia* are quite suitable and revealed some interesting features.

In many cases a most active oscillating movement of the protoplasmic particles occurs, but in certain regions 'plugs' are present in the hyphae, with bright particles and no apparent motion, while in the case of older material very little movement of particles was made out.

At the ends of the hyphae, the protoplasm is generally densely packed, and it is to be expected that here the greatest activity of the protoplasm would be manifested. In some cases, no motion at all could be observed, but in others a very rapid movement as of very fine closely aggregated particles was clearly seen. The appearance of fixity may of course be elusive, as Gaidukov has pointed out, but there is here no direct evidence for the presence of amicrons; in fact, the appearance of closely packed particles rather controverts this.

In the clearer parts of the hyphae—for example, below a sporangium, or between the protoplasmic plugs—larger vesicular 'particles' are usually clearly seen, with an oscillating and wandering motion. These are clearly visible in direct illumination, but there is considerable difference in size, so that possibly only the larger ones are then seen. In any case, they are much more clearly seen by dark-ground methods. The 'plugs' of protoplasm in the older parts of the hyphae have the appearance of being made up of close aggregations of these vesicular bodies, so that these older plugs may be really accumulations of waste or excreted material. The finer plasma particles with their rapid motion are quite indistinguishable in direct illumination.

The study of this fungus gave the idea that particles of graded size from the smallest to the large vesicles existed in the colloid complex here, and it also suggested the possibility of a spontaneous reversibility between the sol and the gel states of the protoplasm. This idea was followed further, and is discussed again below.

No sign of any structures which could be identified as nuclei was made out.

Pythium.

The hyphae of the species used (*P. Debaryanum*), although much smaller, showed much the same phenomena as those of *Saprolegnia.* Protoplasmic plugs are frequently present, and it was found that at the basal end, below these, there was a general thinning out, and that in this region protoplasmic movement frequently occurred. Sometimes this oscillation of particles was present throughout the length of the hypha. Particles seemed to be graded in size much as in *Saprolegnia.*

Mucor.

Cultures were made by dropping the spores into sterilized liquid media, so that the hyphae were obtained free from air bubbles (Andrews, '12). The hyphae again exhibited characters of the same nature as those just described, but interesting results were obtained by a study of spore germination.

Movement of very fine particles occurred, and 'plugs' composed of larger motionless particles were also present, with much the appearance of coagulated protoplasm. These were thus judged to be in the hydrogel state. On fixation with glacial acetic acid added to the edge of the cover-glass, little change took place in these plugs, but after the complete fixation of the whole of the protoplasm, the plugs were no longer to be distinguished from the rest of the contents of the hypha. This seems to indicate that the plugs are certainly to be regarded as protoplasmic, but that they represent a different state of aggregation, or a different condition of the protoplasm. Further evidence was derived from germination of the spores (Pl. XLI, Fig. 1).

It should be mentioned that the so-called plugs are not to be regarded as solid plugs, but as peripheral band-shaped layers of protoplasm lining the inside of the wall. There is a clear longitudinal passage through them, as sap particles were observed to pass them.

Plant Hairs. (*a*) **From stem and leaves.**

Cucurbita.

The large multicellular serial hairs which occur on the stem and leaves of *Cucurbita spp.* provéd to be good and interesting objects (Heidenhain, '07, Fig. 251, p. 458, and Fig. 276, p. 487), and useful for the study of plasmolysis.

The hairs employed were taken from growing seedlings. The cell-wall of the larger cells is very clearly optically homogeneous, and the cell includes only a few inconspicuous chloroplasts. The protoplast consists of a layer lining the cell-wall and a number of strands crossing the cell lumen, the conformation and direction of these strands constantly changing. The general protoplast, with the strings, contains a large number of microsomes, which have rather a vesicular appearance under dark-ground illumination. These microsomes, or many of them, are visible with transmitted light, but, as in all cases with these minute particles near the limits of resolution or visibility, they show much more clearly by dark-ground methods; in fact, they are generally then the most conspicuous features of the cell structures. Moreover, with this method of illumination, the Brownian movement of the microns in *Cucurbita* (and other cases) is very obvious and can be studied much more readily.

As in the case of *Elodea*, the microsomes are carried along by the protoplasmic stream, while they also execute Brownian oscillations. It is possible to observe also the presence of much smaller particles in the protoplasm. There is what appears to be a slight membrane limiting the protoplast towards the vacuole, and also forming the surfaces of the strands.

In no case yet has the nucleus been clearly made out for study.

The sap particles are as a rule very few, and do not interfere to any extent with observations of the protoplast, so that for this, as well as other reasons mentioned, these hairs have been useful for the study of plasmolysis.

The streaming in these hairs is, according to Pfeffer, a normal phenomenon ('06, p. 284), but the wounding effect consequent on their removal may stimulate them to greater activity in this respect.

Hairs of Tomato.

The hairs from the stem of the Tomato are treated in much the same way, being removed from the plant and mounted in water. They are serial hairs, but only one cell thick (Price, '12).

The wall is not quite homogeneous, however, as fairly numerous bright spots appear on its surface with clear spaces between. Observations in this case were made with sunlight, a Nernst lamp, which was first used, showing little but sap particles and indications of a protoplasmic movement. In sunlight the protoplasmic particles were generally rather small and arranged in different layers. On focusing at the outside, there appeared first within the wall very minute particles with a rapid Brownian movement and a large path of motion. Within this layer, forming presumably the chief mass of the protoplasm, are larger and more numerous particles also with a strong Brownian movement. The sap particles come into focus in the lower plane, and these are seen to be of quite another order of magnitude, with a more sluggish Brownian movement. They are generally elliptical or rod-shaped, much as in *Elodea.*

The dead hairs show the protoplasm quite motionless, and coagulated into a mass, containing large relatively bright particles.

Salvinia.

The hairs which clothe the filamentous segments of the submerged leaves show a slightly heterogeneous wall, while the protoplasm contains particles which have an oscillating movement. The material is fairly suitable for plasmolysis experiments.

(*b*) **Root Hairs.**

Root hairs have been successfully used for experiments on plasmolysis, and attempts have been made to see whether they would be suitable for work with this method. Generally, however, they are difficult to mount satisfactorily, and the torn or crushed tissues to which they are attached often interfere with observation, while colloid particles escape into the liquid and fill the field.

Mustard.

Mustard seeds were germinated on damp blotting-paper, and the root hairs were mounted with a small piece of the root, with as little injury to the hairs as possible (Price, '12). The protoplasm is abundant towards the tip of the root hair, and 'plugs' of various sizes occur along its length. The distal end of each plug generally shows small particles in motion, while motionless particles occur towards the basal end.

The appearance is on the whole very similar to that already described for fungal hyphae. Only a few particles of the nature of sap inclusions occur in these hairs.

§ 4. SOME FUNGUS SPORES AND THEIR GERMINATION.

It has been already indicated that some observations have been made on the changes which occur in the protoplasm, as revealed by dark-ground methods, when fungus spores germinate, and it is hoped to extend these to other cases. The results so far obtained seem to have a direct bearing on the discussion of the nature of the protoplasm, especially as regards the dormant state, and its reawakening to activity, and also the spontaneous reversibility of state already mentioned.

With the exception of spores of Myxomycetes (Gaidukov, '10, p. 57), spores have apparently not been studied by this method before.

Spores generally will not germinate in the absence of oxygen, and so it was not found possible to keep a spore under observation during germination. The method used was to take a series of cover-glasses, and place a few spores in a drop of liquid in the centre of each. These cover-glasses were supported over a water surface, and the whole covered with a bell-jar. A cover-glass with its germinating spores can then be taken at any time and gently lowered on to an object-slide. In this way an almost continuous series of observations can be made, although the method is rather more laborious than would at first seem necessary.

The best series of observations has been made on the spores of a species of *Mucor*, and these will first be described (Pl. XLI, Fig. 1).

The spores when freshly mounted appear comparatively clear, with almost homogeneous contents, and no sign of movement of protoplasmic particles was seen at all. For some hours a swelling of the spore takes place, due of course to the taking up of water by the spore contents. After this the protoplasm forms a peripheral layer, with the appearance usually attributed to a gel, and some fairly large scattered 'nodes' represented by brighter diffraction images. Later, the contents of the spores become more opaque, and assume an appearance still more typical of the hydrogel state, as occurring in coagulated plant cells. Before germination, apparently as a normal succession, the protoplasm passes into a typical hydrosol with rapidly oscillating minute particles. This state is, I think, to be regarded as that occurring just prior to the actual production of a germ tube—the protoplasm has just entered into the state of vital activity (Pl. XLI, Fig. 1).

The spore then begins to put out a germ tube, the protoplasm being entirely, as far as can be seen, in the active hydrosol condition. The tube grows very rapidly at first, and the greater part of the protoplasm passes into it. The old spore-wall generally retains a little protoplasm, or possibly some of the reversible protoplasmic colloids which have produced a new sol with the absorbed watery liquid. It appears as a complex of very fine particles with a rapid Brownian movement, and a very considerable free path of motion. It may be mentioned here that the protoplasm escaping from a broken hypha showed particles of two orders of mean magnitude—the larger particles, which seem to build up the hydrogel plugs of the hyphae, and extremely minute ones, which in contrast appear as fine as dust, but which are very like those remaining in the old spore-case just described. Both types, however, seem to produce liquid sols with the mounting medium.

The formation of the 'plugs' occurs as the hyphae increase in length—the 'active protoplasm' occupies less and less of the total volume. They are composed (Pl. XLI, Fig. 1) of aggregations of motionless particles, forming more or less opaque band-shaped plugs to the hyphae. Several of these occur in the length of the hypha, and, especially in the older ones, considerable lengths may be thus occupied. In some cases a motionless aggregation of this nature occurs right at the end of an apparently growing hypha, so that it appears as though this state must represent an active condition of the protoplasm for growth. The action of a fixative such as glacial acetic acid has already been described, the contents of the fixed hypha failing to show any such differentiation.

Streaming movements (Andrews, '12) were also seen to take place in the hyphae. The smaller particles were carried along in a rapid stream, as though by an invisible flowing fluid. The larger particles may also be carried, and seem to collect to a certain extent at the plugs. Larger vesicles (of oil or glycogen?) also occur, but are generally not numerous.

The aecidiospores of *Melampsora Rostrupii*, Wagner (*Caeoma mercurialis*), *Triphragmium Ulmariae*,[1] and *Phragmidium disciflorum*, James, when fresh and mounted in water, all show much the same appearance under dark-ground illumination. The spore-wall is generally slightly marked or papillose on the outside, and thus rather interferes with the critical observation of the contents. The latter have a striking appearance of intense activity due to Brownian movement, so that the internal condition of the spore is quite different from that of the *Mucor* spore just described. The appearance as of a boiling mass is due to a number of yellow particles, or rather little globules, as these can be seen to escape on crushing the spore and so liberating its contents. On germination of the spores, these globules pass into the germ tube, and the phenomena here observed are somewhat as already described for fungal hyphae.

The globules may represent some reserve substance of the nature of glycogen, or oil suspended in the protoplasm. The latter, since it allows of the free movement of these, must be in the fluid or hydrosol state, but must apparently contain extremely minute particles as the disperse phase.

It seems more logical, perhaps, and more in accordance with the facts,

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[1] Aecidiospores or primary uredospores of *T. Ulmariae*.

to regard the whole mass as protoplasmic, with the nature of an emulsion colloid, the globules, of whatever nature they may be, forming the disperse phase, in a possibly complex hydrosol basis—probably not a true continuous phase.

§ 5. THE SAP PARTICLES OR INCLUSIONS.

Solid particles have long been known to occur in the cell sap in certain cases, where they execute an oscillating Brownian movement, the best-known case being the particles of calcium sulphate and so on, which are present in the terminal vacuoles of some Desmids (West, W. and G. S., '04). They have also been frequently noticed, but not very definitely recorded, as occurring in the cells of many Green Algae. Professor West kindly informs me that he has observed such small particles in the cells of *Spirogyra*, *Zygnema*, *Microspora*, *Tribonema*, *Ulothrix*, and many Desmids and others, and he regards them rather as a sign of approaching death, or an indication of bad health of the filament.

As already stated, sap particles have been observed by the dark-ground method in many, or indeed most, of the cells so far examined. Any small particles of a solid nature free in the cell sap will perform a Brownian movement as these particles do.

They are generally rendered conspicuous as bright specks or definite outlines, of various sizes, with an oscillatory and translatory motion certainly of a Brownian nature, the rate of this varying generally with the size of the particles. The particles are almost certainly to be regarded as of different nature in different cases; they certainly vary in form and appearance. Generally, those so far encountered seem to fall into two categories of form as follows:

(*a*) Structures or particles of a vesicular or drop-like nature, with a higher refractive index than that of the cell sap.

(*b*) Particles with a regular and apparently prismatic crystalline form; at least this is the idea given by them as they pass through different positions. Pl. XLII, Fig. 6, indicates a series of diagrammatic representations of such particles from the leaf cells of *Elodea* drawn in different positions. They thus appear to be flat rectangular plates of a transparent crystalline nature. Little idea of their shape could be gathered in direct illumination, and no results were obtained by the use of polarized light or crossed nicols.

With regard to class (*a*), probably different forms are included here, as there seem to be particles with a solid structure, as well as others of the nature of bubbles or vesicles in the liquid. It is generally very difficult to distinguish the two, however.

It seems probable that they have been noticed before only in the more obvious cases, and when fairly abundantly present. Also it appears certain that they increase greatly in numbers and in size as the cell approaches its death point, and so they may have been definitely associated with this unhealthy condition.

In the case of the crystalline particles of the *Elodea* leaf, there seems to be no doubt that they increase in number with decreasing vitality of the plant, and experiments were made to observe this. In one series of experiments, shoots of *Elodea* were placed in water in the dark, and the leaves examined from time to time with dark-ground illumination.

Nov. 30, 1912. Sap particles few and of typical form.

Dec. 2. Sap particles increasing in number; small vesicular inclusions also appeared, especially in the edge cells.

Dec. 3. A large increase in number. The particles quite obvious under low powers.

Dec. 6. A still further increase, nearly all the particles being of crystalline form. Under a low power the leaf seemed full of glittering, dancing points of light.

During this observation the leaf cells became poorly nourished, the chloroplasts became pale and often retreated to the lateral walls, thus rendering observation clearer. There is no doubt that the particles increased greatly in number.

A similar phenomenon seems to occur as the leaves get older—the old leaves of a shoot showing many more sap inclusions than the young leaves.

In *Elodea densa* under somewhat the same conditions, the crystalline particles become comparatively large and ultimately cease movement, falling apparently to the bottom of the cell, where they appear as aggregations of needle-shaped crystals.

Particles or corpuscles which apparently correspond in some respects with these have been observed in the Fungi by previous investigators, notably by Guilliermond ('13, and references there cited), and also by this method in *Saprolegnia* and other cases described above. These 'metachromatic corpuscles' are frequently present in the vacuoles of the hyphae. They are extremely variable in size and animated by Brownian movements. They are stated to be here of the nature of reserve material (Maire, '03), and probably play an important part in the life of these Fungi. To quote Guilliermond—' they have been identified in most of the Algae and Protozoa, but they do not seem to be represented in the higher plants.'

This rather seems to identify them from appearance with the sap inclusions described, but if they are part of the nutritive apparatus in this case, it seems very doubtful whether this identity is more than superficial.

The method of dark-ground illumination, providing as it does an efficient means for the study of these corpuscles, should help to decide their nature, and it is hoped to carry out more work in this direction.

These sap particles provide an interesting case for the study of Brownian movement; they are graded in size, and their rate of movement varies inversely as this. It appears also that particles of apparently the same size in the protoplasm move more slowly than those in the cell-sap, probably on account of the greater viscosity of the continuous phase of the protoplasm.

§ 6. THE NUCLEUS.

Only in certain favourable cases has it been possible to study the structure of the nucleus by this method, which from the point of view of cytological questions connected with the nucleus is certainly disappointing. There is, however, still room for much work in this direction with really suitable material. The selection of this is even more difficult than of material for general observation, but in a few cases moderately clear analysis of the resting nucleus has been possible.

The nucleus has been frequently observed in *Spirogyra*, *Elodea canadensis*, and *E. densa*, and some other cases. A species of *Spirogyra* with a loose spiral and narrow type of chloroplast furnishes the best material, but in no case as yet, with this or any other material, has any success been obtained in observing the processes of division by this method. Attempts have been made to observe the nucleus in pollen mother-cells, but generally in the few cases so far tested the cytoplasm is rather dense. Darling ('12) has shown that it is possible to observe stages of nuclear division in the pollen mother-cells of *Acer*. Root hairs have also been tried, especially those of *Trianaea* and Mustard. These would not be expected to show division stages, but generally nuclei are very difficult to see at all well with the method. The nuclei of most Fungi are extremely small, and as yet no very clear observations have been obtained, using fungus hyphae.

The appearance presented by the nuclei of *Spirogyra* and *Elodea* will be described in some detail.

The nucleus of *Spirogyra* (cf. Gaidukov) is suspended at the middle of the cell vacuole by cytoplasmic strands, as already described, which merge into a thin layer of cytoplasm surrounding the nucleus. As already mentioned, microsomes are present in this thin layer of cytoplasm on the outside (Pl. XLII, Figs. 1 and 2). There seems to be no very definite limiting layer to the nucleus here, but the cytoplasmic covering is very thin (cf. Lawson, '11): no movement of particles can be detected inside this layer, and the inner portion is nearly clear. The nucleolus, which in some cases was first mistaken for the nucleus, appears as a slightly milky looking body, sharply marked off apparently by a definite membrane from the general nuclear plasma. This boundary has a bright appearance under dark-ground illumination, indicating that a really definite membrane is present here. The nucleolus seems to contain a gel of very fine structure. This is probably to be regarded as chromatin material, and the appearance of the membrane may be due to the difference in refractive index between this and the nuclear plasma.

On fixation with glacial acetic acid the whole nuclear mass becomes milky at first, while the nucleolus is more rapidly affected and becomes slightly more distinct. The movement of particles on the outside of the nucleus ceases almost at once. After complete fixation the nucleolar membrane becomes even more distinct.

In the cells of *Elodea* the nucleus is present generally as a rather flattened ellipsoidal mass in the layer of protoplasm which lines the cell-wall. It is best observed in the edge cells, but when against the dorsal or ventral walls of the cell (according as one side or the other is under observation) it may often be quite well seen as a circular mass in practically any cell of the leaf. Starvation of the leaves, with the consequent diminution in size of the chloroplasts and their migration to the side walls already described, often facilitates the observation of the nucleus in this case.

The nucleus here, whether ellipsoidal or spherical in form, is only slightly milky in appearance, with apparently a hydrogel structure as before (see Pl. XLI, Fig. 8). The overlying cytoplasm is often small in amount, but the minute particles in the protoplasmic stream can be seen constantly flowing past the nucleus. As far as could be made out, these nuclei in the resting stage showed no further sign of organized structure, not even a nucleolus, and were almost homogeneous throughout.

The effect of fixation is very marked. The nuclear substance, although with the appearance of a hydrogel before, undergoes a marked change. It rapidly becomes almost opaque (Pl. XLI, Fig. 9), and assumes a mottled structure with fairly large diffraction images. This is, in fact, the general type of image presented by a regularly coagulated gel of fairly coarse structure. A further discussion of the nature of the change between the two gel states will be given when dealing with the fixation of the cell colloids. The nuclear colloid is much more opaque and of coarser structure than the cytoplasm after fixation.

In some cases *after* fixation, a structure which corresponds to a nucleolus can be seen.

In some respects the nuclear structures described agree to a certain extent with those observed by Fauré-Frémiet ('10) in the nuclei of certain Protozoa, an almost homogeneous but nebulous structure in the living state, with, however, in his case a nucleolus and some 'globules basophiles'. Action of dilute alkalis causes the structure to clear, and the nucleolus and globules become more distinct, and this condition is to a certain extent reversible.

No such changes have, however, been observed in the plant cell.

On the whole, the facts brought forward hardly throw any new light on the structure of the nucleus from the general cytological point of view.

What is indicated is that the colloid state of the nucleus differs from that of the cytoplasm, and that the so-called nuclear membrane represents possibly nothing more than the surface which is formed between the hydrosol complex of the cytoplasm and the gel structure of the nuclear plasm.

It is hoped that some further studies of nuclear structure may be possible.

§ 7. THE CHLOROPLAST.

A few observations and notes on the structure and behaviour of the chloroplast have been collected, and these indicate rather clearly that this structure is to be considered, in cases examined, as rather definitely differentiated from the rest of the cytoplasm.

The chloroplast has usually been observed as slightly opaque and heterogeneous, with a motionless gel structure, containing the green pigment which is probably in definite solution or absorbed by the gel. Even when the cytoplasm is filled with active particles, as in *Spirogyra*, *Elodea*, *Mougeotia*, the chloroplast shows a fixed structure.

The chloroplasts in *Elodea* moved by the protoplasmic stream do not move as rapidly as the stream, indicating that they are rather separated or isolated from it.

Where there is a large chloroplast, as in *Spirogyra*, it generally behaves towards reagents as if it were a structure more or less separate, and differing in its attributes. On plasmolysis here, it often remains coiled round the outside of the contracted protoplast, and suffers no apparent change in organization. Fixing agents, although coagulating the hydrosols and producing a general heterogeneity in the cytoplasm and even the gel of the nucleus, do not seem to change the structure of the chloroplast to any marked extent. After prolonged action of the agent, and after the colour has been removed from the chloroplast, a greater opacity is sometimes apparent. The most striking case of distinction was in the action of dilute potash on the cell, especially in the plasmolysed state,—the cytoplasm becomes extremely white and dispersive of light (Pl. XLI, Fig. 4), while the walls of the vesicles become almost or quite opaque. This change takes place whether the potash is added to the living cell or to the cell after fixation. The chloroplast, however, remains quite clear green, and presumably almost unaffected by the reagent. It appears as a dull green band, wound round the intensely white mass of the rest of the protoplast. No explanation of the action of potash on the protoplast to account for this has been found. A similar effect is produced by several other reagents, generally of an alkaline character—potassium carbonate, ammonia solution, slightly by potassium hydrogen carbonate, and so on.

It seems quite probable that some sort of membrane or bounding surface exists between the chloroplast and the cytoplasmic hydrosol, much in the same way as between the gel of the nucleus and the cytoplasm, and if this is so, the chloroplast may be supposed to act as an osmotically discrete structure.

§ 8. SOME STUDIES OF PLASMOLYSIS.

The phenomena revealed in the study of the plasmolysed cell by the dark-ground method are interesting, and certainly furnish some new ideas of the process of plasmolysis in general, although the interpretation of the structures observed is often very puzzling. Before any general discussion can be made, the empirical results in the best cases studied will be described. The ordinary appearances of plasmolysis will be assumed in most cases.

(*a*) **Appearance of Plasmolysed Cells in Various Cases.**

It may be noted first that Gaidukov has already described the effect of the entrance of electrolytes ('10) into the cell in causing the coagulation of the hydrosol, where it comes into contact with the protoplast, and he supposes that in this way a definite 'Plasmahaut' or plasmatic membrane may be produced on the outside.

To study this phenomenon various types of plasmolysing agents and of various strengths have been used. The results are, as will be seen, rather various, and require very considerable amplification, but, on the whole, they indicate a definite formation (or previous existence) of layers in the protoplast on plasmolysis, and also that a differentiated surface layer of the protoplast exists apart from the action of electrolytes upon it.

As plasmolysing agents, both electrolytes and non-electrolytes have been compared. Attempts have been made to represent roughly some of the more typical cases in the figures, and these will be referred to in the description.

Spirogyra.

As *Spirogyra* is a good object for dark-ground study, and has often been used for plasmolysis experiments, it was used very largely in the study of plasmolysis in this work. Its behaviour under plasmolysis is in some respects rather peculiar, and is rather variable under different conditions, so that various special cases will be described.

Pl. XLI, Fig. 2, represents a cell of a filament plasmolysed with dilute glycerine, which is to be regarded as an imperfect plasmolysing agent (since it enters the vacuole after a time), and also a non-electrolyte. As in practically every case of plasmolysis with dilute glycerine, there is what may be called a layering of the protoplast; there is a distinct differentiation into an external layer composed of extremely fine particles only and an inner portion of the protoplast which contains the larger microsomes, so conspicuous in the unplasmolysed cell. The chloroplast is practically unaltered in structural appearance. The protoplasmic threads or fibrils, which in the figure drawn are of fairly large size, seem to be formed by the pulling out of the outer layer of the protoplast, the fine colloid structure of this layer being very well seen at the bases of any of these threads which could be observed in optical section in this region. The nature and structure of the fibrils are further described below (sect. *b*). The particles in the outer layer were in rapid movement, and nothing could be seen of a hydrogel layer (*Schicht*) bounding this on the outside. Some definite boundary, however, whether a mere surface maintained by surface tension (molecular attraction) or a definite membrane, must be assumed as the colloid particles do not escape into the surrounding liquid.

Pl. XLII, Fig. 5, shows much the same general features. The cell was here plasmolysed with an electrolyte, a 10 per cent. solution of potassium nitrate. The layering of the protoplast is much the same as before. Large processes are drawn out in the case shown, which pass into coarse fibrils at the end and there generally branch repeatedly. The fine structured surface layer appears much as before, but the fibrils are much thicker than in Pl. XLI, Fig. 2. On the whole no marked difference can be said to occur in the appearance of the two cases, typical examples of many, one plasmolysed with a non-electrolyte and the other with an electrolyte.

Pl. XLI, Fig. 3, shows a type of plasmolysis frequently obtained with practically any of the agents used—glycerine, potassium nitrate, cane sugar, sodium chloride, and so on. The formation of vesicles of the nature shown is well known in *Spirogyra*, and has been fully described by de Vries ('85) and others. The vesicles are thin-walled 'bladders' of protoplasm containing presumably cell sap. The figure is drawn to show, as nearly as possible, the different appearances observed in three depths of focus. (*a*) is focused so that the surface and edge of the vesicle wall are seen; (*b*) shows the contents of the vesicle and the general mass of the protoplast; while (*c*) is in the upper focus, just within the wall of the cell.

The wall of the vesicle has the appearance of a slightly milky or opalescent elastic membrane, with some extremely fine particles in its substance which oscillate rapidly with a large free path when seen in surface view. At the edge, as seen in optical section, these particles naturally appear more crowded. If the wall of the vesicle contains any protoplasm in the hydrogel state it must be imagined as a very thin layer (too thin to show the usual hydrogel structure) both on the outside and inside of the vesicle. The layer between remains in the hydrosol state. It is certain that when the vesicle undergoes fixation the whole is converted into a gel, with the usual opaque and heterogeneous appearance. The vacuole then seems to emerge from the inner portion of the protoplast, and there is thus evidence to show that the vesicle is produced from the layer with fine particle structure, which almost certainly normally exists on the inside of the protoplast—the vacuole wall layer. These vesicles, then, probably represent the sort of structure which must be attributed to the vacuole wall.

The vesicle generally encloses numerous sap particles, which are very conspicuous and show their usual Brownian movement. The general mass of the protoplast, which is also shown in this focus, is as already described, the larger microsomes showing their accustomed oscillation, and the outer layer being somewhat differentiated.

Focus *c* (Pl. XLI, Fig. 3), just inside the wall, gives the appearance of a very large number of closely packed fine particles in very rapid oscillation, the whole producing quite a cloudy appearance. It is very difficult to decide whether this represents a layer lining the inside of the wall, or whether the particles are in the space lying between the wall and the protoplast, but I am rather of the opinion that the former is the case. If this is so it must represent a very thin layer of protoplasm with a fine structure left attached (by viscosity?) to the outer wall. It may be a fine layer connected by fibrils with the main protoplast (see (*b*) below). This layer was not made out in all cases of plasmolysis, but the fact that it sometimes occurs, whatever its nature may be, seems worth recording.

In *Mougeotia* somewhat similar phenomena have been observed. The protoplast usually contracts, leaving a vesicular structure at each end, the wall of this being practically transparent. This vesicle wall seems to be rather in a gel condition, and not as the vesicle wall in *Spirogyra*. No movement of particles could be observed in this wall, and also, in a case of fragmentation of the protoplast of the type shown, no movement in the colourless membrane connecting the two portions could be seen. This finally became a thread with no visible structure. It seems rather surprising that the structure of this membrane and the protoplast generally should be so different in two genera of Algae so closely allied.

The material of *Cucurbita* hairs also furnished some good cases for the study of plasmolysis, especially as there are practically no chloroplasts to interfere with observation. Plasmolysis was effected with potassium nitrate 5–10 per cent., cane sugar 20 per cent. and 30 per cent., and other agents.

The simplest case observed was probably that in which the protoplast gradually contracts from the wall and masses itself towards the middle of the cell. In this process fine protoplasmic fibrils are usually produced, showing the typical features of these. As the protoplast slowly comes away from the wall the outside is seen to be bounded by what seems to be a definite membrane of a transparent nature as shown by the bounding line in Pl. XLII, Fig. 4. The finer particles of the protoplast are present towards the outside and seem continuously to impinge on this transparent membrane or surface layer. It is quite possible that a distinct boundary between a slightly viscous colloid in the sol state and a watery liquid would possess this appearance, but the probability of the presence of a definite membrane is strengthened by the fact that the colloid particles, although bombarding the surface, do not escape and produce a new colloid sol with the water. It is a significant fact that the figure is drawn from a case of plasmolysis in cane sugar, and unless water is to be regarded as sufficiently dissociated to cause coagulation of the sol on the surface, the presence of this membrane or 'Schicht' seems to be normal, and not produced by plasmolysis.

Pl. XLII, Fig. 3, shows another case of plasmolysis of *Cucurbita* with 30 per cent. cane sugar; vesicular structures have been produced somewhat like those in *Spirogyra*. These vesicles are composed of a very thin membrane, in which very fine scattered particles are seen to be moving. There seem to be vesicles within a larger one. Sap particles are enclosed, but are in this type of material comparatively few in number.

The characters of the membrane rather distinguish it from the general mass of the protoplast. The plasmolysis here was performed with a non-electrolyte, and the membrane of the vesicle seems to be produced just as in the case of plasmolysis by electrolytes.

The phenomena of plasmolysis have been briefly described for some of the more typical cases. No marked difference can be said to have been observed with different types of reagents, electrolytes, and non-electrolytes, those which penetrate the protoplast slowly, and those which cause more perfect plasmolysis.

The description of the formation of the protoplasmic fibrils, already casually mentioned, is reserved for another section.

(*b*) The Protoplasmic Fibrils.

In certain cases of plasmolysis, described by numerous investigators, Bower ('83), Gardiner ('84), Chodat et Bourbier ('98), and more recently studied by Hecht ('12), the contracted protoplast remains attached to the cell-wall by fine threads or fibrils of protoplasm. These fibrils vary in size and number, and were at one time thought to be continuous with the protoplasmic connexions through the cell-wall, and especially were they thought (Kohl, '91) to indicate the presence of connexions through the cross walls of certain filamentous Algae. This view was, however, disproved, in part at any rate, by Chodat et Bourbier (see also Strasburger, '01), and it appears that these fibrils represent nothing more than fine strings adhering to the cell-wall by their viscosity.

These fibrils have been well observed by methods of dark-ground illumination, and some further facts concerning their nature and structure have been noted. They appear, when thus illuminated, as bright lines stretching from the cell-wall to the contracted protoplast. They are often extremely fine, so much so that at times they are not distinguishable in illumination by transmitted light. It thus seems probable that previous workers have observed only the coarser threads (de Vries, '85) which are generally present, but the difference is not very material, being probably one of size only.

Observations of them have been made in most cases, but they have been studied more especially in *Spirogyra*, *Mougeotia*, *Elodea*, *Cucurbita*, *Vaucheria*. Plasmolysis, usually for a short period (10–15 minutes) in the agents and of the strengths described, generally produces the fibrils, and after a little experimentation they can be produced almost with certainty in any given material.

The fibrils are often very distinct and numerous in *Spirogyra*. They appear sometimes quite straight as though stretched, but more often branched towards the wall and loose. Under these conditions the threads are seen to have a constant and fairly rapid waving motion, as though swayed by a gusty breeze (cf. Hecht, '12). This movement of the fibrils is possibly to be attributed to the Brownian movement of the cell contents; it may be impressed on the fibrils by the oscillations of the particles of the protoplast at the base. It may, however, be a manifestation of direct molecular movement, the same phenomenon as the Brownian movement manifested in these fine fibrils. The idea that the fibrils are moved by diffusion or by osmotic currents is hardly feasible on account of the rapidity and oscillating nature of the motion.

The fibrils themselves seem to be homogeneous in structure, but often carry particles in their substance which are identical with the protoplasmic particles; they are often much finer than the diameter of the larger protoplasmic microsomes. The particles seem to show some movement of translation of their own, and their presence may in some cases help to produce the Brownian oscillation of the whole fibril. Ultimately the fibrils often break, and their free ends can be seen waving in the liquid, and they are often so numerous as to give a distinctly cloudy appearance to the space between the cell-wall and protoplast. In this way also it seems that particles may be liberated from these fibrils into the liquid.

In some cases the fibrils appeared very clearly as though pulled out from the outer layer of the protoplast (Pl. XLI, Fig. 2), which contains the finer particles. In some cases also, little attaching discs of protoplasm appeared on the cellulose wall of the cell. In one case especially of plasmolysis with glycerine, very numerous threads were produced. These could be seen to pass to a wall which lay slightly obliquely with the vertical plane. A little patch or disc of protoplasm, composed of very fine particles in rapid motion, was present here at the end of one of the fibrils (Pl. XLI, Fig. 2), and pulled out into a little V-shaped piece as though slightly viscous. Such protoplasmic discs seem to correspond to the protoplasmic network on the cell-wall described recently by Hecht ('12). It seems possible that such a disc may really represent the end of a protoplasmic connexion passing through the wall. In no case with this method have intercellular connexions been made out, however, although numerous attempts have been made to observe them.

As described by others, the threads pass to the side as well as the end walls, and can be produced in coeonocytic material such as *Vaucheria.* In this case the threads always seem to be stretched, and to show no motion whatever.

It is somewhat difficult to relate these fibrils in structure to the general colloid complex of the protoplasm. If a boundary layer of gel is produced by the action of the plasmolysing agent, or the salts in the escaping cell-sap, these fibrils should be thus bounded, and apparently should consist entirely of a thread of hydrogel, on account of their small size. They may certainly have this nature, and may be regarded as fine strings pulled out from the outer layer of the protoplast, like fine strings from a coagulating jelly. The activity of the particles, both at the walls and at the junction of the thread with the contracted protoplast, is, however, hardly accounted for on this hypothesis, unless we regard the gel layer to be so thin as to be formed only at the outside of these little processes, and to be quite invisible.

After fixation of the protoplast, the threads usually continue their oscillating motion. This rather indicates that this is to be regarded as Brownian movement set up by the molecules and microns in the liquid which surrounds the fibrils.

It seems impossible as yet to decide whether these fibrils are really functionally concerned in the process of plasmolysis, or whether they are to be regarded as artifacts due to the contraction of a viscous coagulable fluid.

§ 9. THE ACTION OF FIXING AND OTHER REAGENTS ON THE PLANT CELL.

Since it is possible by this method to determine very clearly the point of coagulation of the protoplasmic hydrosol, it seemed that it would be of interest to compare by this the relative and absolute effects of various fixing agents and other reagents on the plant cell. Such a study might do something to decide as to the relative merits of fixing and killing agents, and to the possible extent and nature of the artifacts, which are presumably produced to a greater or less extent by all these agents. In such an observation the time factor as well as the resulting structure must be taken into account; and in this direction was encountered an experimental difficulty which has not as yet been completely overcome. In spite of this, the rough results show some features of interest and seem quite worth recording although necessarily incomplete. The difficulty is that of allowing the fixing fluid to flow quickly on the object, while the latter is under observation on the stage of the microscope, and in optical continuity with the sub-stage illuminator. The fresh objects have to be mounted in water and adjustments made, and then some means is required of irrigating them quickly. The only method which was used to any extent was to take the reagents in a more concentrated form than is required for fixation, and to draw them under the edge of the cover-glass by means of a small piece of filter-paper placed at the opposite edge. The reagent is thus considerably diluted, and in most cases only watery solutions could be used. Also it is almost impossible to know at what instant the fixative reaches the object in question, practically the only test being the observation of the first reaction on the cell-contents. Thus, no comparisons of the rates of coagulation by the same reagent in different concentration were obtained, nor of accurate comparisons of the rates of fixation by different reagents. It is hoped that this may, however, be possible at some future time. The actions described are merely general statements of the actions of the reagents and the resulting structures of the protoplasts.

The material used has been very largely *Spirogyra* and *Elodea*; both are obtained easily, and both have proved very good objects for dark-ground work in most directions.

The general reaction which occurs on fixation is of course a coagulation of the hydrosol, but as the gel appearances produced by different fixatives differ quite markedly, it must be concluded that the process is something more than a mere coagulation (Fischer, '99, pp. 1–71). The change is indicated in dark-ground illumination by a complete alteration in structure; the hydrogel produced has generally the appearance of a large number of overlapping diffraction images, rather bright and milky and with no movement of the particles. Like the various sol states, the gels differ considerably in character and general appearance.

A short description of the action of some fixatives on various types of material will now be given.

Osmic Acid. A 1 per cent. solution was run under the edge of the cover-slip. *Spirogyra* was chiefly studied. In this case the first action is to produce an extremely fine precipitate in the cell-sap, which under the lower powers was first mistaken for the milkiness of fixation. This precipitate is very probably produced by the presence of tannins in the cell-sap (Tunmann, '13, p. 255), and with this type of illumination very much resembles the precipitate which caffeine and antipyrin give with tannins in the same way (ibid., p. 258; Wisselingh, van, '10). The precipitate gradually aggregates into larger particles which eventually become motionless. The protoplasm assumes a coarsely granular milky appearance, but where observation is good (where the precipitate is less) the microsomes of the protoplast can be seen, apparently fixed as such, and lying in the coagulated substratum (Pl. XLI, Fig. 5). Thus really comparatively little change occurs except the cessation of the motion of the colloid particles and the formation of a gel network.

In a cell plasmolysed with 30 per cent. cane sugar, and fixed with osmic acid, the larger microsomes also remained as 'nodes' in the gel. The bases of the fibrils also were fixed, and still give the appearance of being continuous with the outer skin of the protoplast. The broken fibrils retain their oscillating movements, indicating that this movement is a direct Brownian effect on them *per se.*

In the case of *Cucurbita* hairs the penetration and fixation were very slow.

Acetic Acid. Glacial acetic acid run under the cover-slip furnishes a rapid coagulating agent, which, however, in the conditions used, often causes considerable alteration of form and contractions of the protoplast. The hydrogel produced is rather opaque, and shows no microsome structure but a collection of overlapping diffraction images.

In *Elodea* the first action was observed to take place on the nucleus. Almost instantly this begins to become highly dispersive, the light being nearly all scattered by the surface layer. It soon shows a coarse gel structure as in Pl. XLI, Fig. 9. On the cytoplasm the action is not nearly so marked, but a rather finer structured opalescent gel is produced.

On *Spirogyra*, the hydrogel produced is rather milky, and of a fine structure, but fixation is apparently fairly good. The walls of the vesicles of cells, plasmolysed to show them, usually altered in shape, as though they were elastic and subjected to sudden changes in pressure. The wall was fixed as a rule without any escape of the contents of the vesicle, and was converted into a membrane composed of a fine structured gel, which is practically opaque to oblique light.

In the hyphae of *Mucor* the effect was almost instantaneous, the protoplasm, sol and gel alike, being converted into an opaque gel composed of overlapping diffraction images of the nodes.

Mercuric Chloride. This was used in 0·5 per cent. solution, which of course became diluted before its action. In *Spirogyra* the effect was first observed on the nucleus and its surrounding cytoplasm. The cytoplasm coagulates and rounds off slightly; the suspending threads themselves are fixed and still show the larger microsomes after fixation. The nucleus becomes more dispersive, and the nucleolar membrane more distinct.

Chrom-acetic Fixing Agent. The fixation with this solution seems to produce much the same type of gel as that formed by acetic acid, differing from the microsome-fixation type described for osmic acid.

Absolute Alcohol. This reagent used as described, that is, diluted with water before reaching the object, produces considerable deformation of the protoplast, breaking of the suspending threads of the nucleus, and so on, and a comparatively slow coagulation of the hydrosol to a milky gel. Thus used it is undoubtedly a bad type of fixing agent.

§ 10. DISCUSSION AND CONCLUSIONS.

It is obvious, from even the restricted amount of cell material examined, that our conception of the structure and constitution of the plant cell, and its reactions to plasmolysis, must be one of considerable elasticity, so that bearing this in mind no attempt will be made to dogmatize and apply the results to cell structure in general. Some short survey of the cells examined, and of the general properties of the colloid complexes involved, will, however, be attempted, bearing in mind the possibility of the extension of the facts and theories to other cells which have not yet been examined.

It has become increasingly evident during the last few years that the protoplasm of different plant cells possesses different properties, and from the methods of colloid analysis by dark-ground and other methods, it seems highly probable that, in many cases at any rate, these differences are to be partially attributed to the differences in nature of the colloids, quite as much as to differences in chemical constitution.

Gaidukov's work and that set out in the present account show that there are certainly big differences between the colloids of the cells of *Spirogyra*, *Elodea*, *Vaucheria*, *Mucor*, and so on. In general, as Gaidukov has stated ('10, p. 60 sqq.), the protoplasm is in many cases to be regarded as a complex hydrosol, containing reversible and irreversible portions, but in other cases the normal structure seems to be rather that of a gel, or at any rate departs strongly from that of the typical hydrosol. It is true that some of these cases, in which the protoplasm appears to be an almost transparent liquid (or viscous solid), may be really resolvable into an extremely fine colloid sol—with particles too small to be rendered apparent by this method—almost approximating to a true solution. It would be thought, however, that judging from the size of the particles in the other cases examined, such a solution would show at least a strong translucence.

Some evidence has been brought forward to show that in some cases the protoplasm can almost undoubtedly exist in the gel state—as judged by its appearance and structure—and that this state is to be regarded as a more or less active one. This hydrogel seems capable of undergoing a spontaneous transformation, spontaneous, that is, with regard to the cell, into a normal sol state, either by the direct absorption of water, or probably by some more obscure process. The change described for the germination of the spores of *Mucor*, for instance, rather indicates a change from the gel to the sol state, by an absorption or an adsorption of water. The gel condition not irreversibly coagulated is possibly more capable of undergoing long periods of rest than the active sol. Other fungus spores examined in a resting state seem to show a similar gel structure.

In the case of hyphae and root hairs with the so-called hydrogel plugs, it has been shown that a slight change occurs on fixation, although the gel structure here very nearly approximates to that presented by the coagulated protoplast. Hence it seems probable that this gel condition is not like that of the partially irreversible coagulated sol, but is somehow a condition intermediate between the sol and the true gel—a condition which is in the main reversible. As a mere suggestion, it may be that the reversible portion only forms a gel network, enclosing the non-coagulated irreversible portion in its meshes, or holding it as an absorption compound, so that the whole mass is reversible.

It seems probable that this state is also to be attributed to the protoplasm of those cells which show none of the sol characters—it is really in this intermediate gel condition. In fact, as has been already mentioned, from various considerations there is reason to suppose that the gel is a really active vital state, capable of performing the functions of growth and nutrition.

Even in the hydrosol condition, it has been seen that very marked variations in the structure exist, both in the nature of the colloid and the organization of the protoplast. The colloid particles vary in size from microsomes just visible in transmitted light, to sub-microns which probably grade into amicrons. Also, although the complex is possibly always more or less emulsoid in character, yet some cells, as for example the *Cucurbita* hairs, show a much more marked emulsoid character than such a type as *Elodea*.

Taking all these different states of the protoplasm into account, it is clear that 'das Protoplasma sehr polymorph ist' (Gaidukov, '10, p. 61).

The plasma of the nucleus, and, in the green cell, of the chloroplast, seems to differ considerably in properties from the general cytoplasm in those cases so far examined. The nucleus seems to consist of a nearly clear fine structured gel, which is considerably altered in structure by the action of the usual coagulation or fixing agents. The gel of the nucleus must undoubtedly be regarded as an active state of the protoplasm. The chloroplast also has generally the appearance of a slightly translucent gel with very little heterogeneous structure. Hence both nucleus and chloroplast are to be considered as fairly sharply differentiated from the ordinary plasma. It seems also quite probable that a definite surface membrane is organized at the boundary of these two colloid systems in each case. Whether this membrane is to be regarded as a mere boundary surface, with the properties of the colloid surface, or as a coagulation membrane produced on the inside of the cytoplasm complex in each case, can hardly be for the present determined.

The effect of fixing agents on the colloid complexes requires much further study, when it seems possible that some definite basis may be found for the selection of an agent in any special case. From the effects produced in the experiments described above, it is clear that something more than the mere rate of coagulation must be considered in the action of these agents (Lee, A. B., '13, pp. 20, 21). The appearance of the gel produced, as well, of course, as the rate of formation of the gel, differs considerably with the agents employed. In some cases, as with that of fixation by osmic acid described, the larger microsomes of the protoplast may remain present in the fixed colloid, while in other cases all identity of the microsomes is lost. The action may be one of absorption of the fixing agent by the colloid as formed. This seems to be indicated by the well-known fact that certain fixing agents are best followed by certain stains. It may be repeated here that in all cases observed the fixative has also an action on the protoplasmic complex in the normal and vital gel state. As fixation in most cases implies coagulation of a mobile hydrosol, it seems hardly possible to imagine a fixing agent which shall be even approximately perfect in its action. The resulting gel must be regarded as a complex of artifacts in the strict sense of the term, and whether these artifacts shall be small enough to imitate closely the pre-existing conformation, or shall be larger, depends on the perfection of the fixative. The most perfect agent is that which causes the least change on coagulation, and it seems that this can best be judged by dark-ground methods.

There seems in many cases to be fairly good evidence to regard the continuous phase of the colloid complex as more or less viscous in character; certainly the whole complex is in some cases distinctly viscous. This viscosity may be used to explain the fact that the protoplast or plasmolysis is often pulled out into fine fibrils connecting with the cell-wall, or adhering as a fine thin layer. The evidence of the appearance presented by the plasmatic surface or membrane and the vacuole wall has been given. There is no structural contradiction to Czapek's hypothesis of the membrane containing a small quantity of fatty emulsion suspended in the colloid surface film ('11). The argument of Lepeschkin ('11), that as the outer surface of the colloid protoplast does not mix with water the membrane cannot consist of colloid particles distributed in a watery medium, bears considerable weight—there must be a continuous membrane of some sort apparently to prevent the escape of colloid particles. As has been shown, this membrane may be the hydrogel layer produced by coagulation. It seems hardly probable, or even possible, that pure water could be ionized sufficiently to produce a gel layer at the outside of a watery sol—such an action would mean that no ionic dissociation whatever occurred in the colloid. The very dilute solutions which enter the cell continuously may serve to produce and maintain such a layer. It is an interesting fact that *pure* distilled water is often toxic to a cell (Osterhout, '13), producing pathological states similar to plasmolysis. In this case there may be a slight breaking down of the plasmatic surface, and a failure on the part of the pure water to produce a new membrane at every surface; permeability would thus be produced, and the cell-sap partially liberated, through the pressure set up by the state of tension of the protoplast; this would consequently contract. It may be argued against this that the cell-sap contains enough electrolytes to maintain the vacuole wall, but it may be that both membranes together are required to maintain the cell in its normal state of turgor. The action of chemically pure distilled water has not been examined as yet by dark-ground methods. Hence practically all the argument just given is rather a surmise. There remains of course an explanation to be made of the action of non-electrolytes such as the sugars and so on in producing normal plasmolysis.

The above short conclusion, although in part quite theoretical, may serve to indicate some of the general lines along which progress may be made by the study of the plant cell by such methods. Micro-chemistry in the plant cell has always necessarily lagged behind chemistry in the laboratory, and this must undoubtedly be the case in dealing with micro-colloid chemistry.

It is, however, undoubtedly the case that the method of dark-ground illumination permits of a study of certain aspects of the structure of the cell, which is hardly possible by other methods. There is no idea that the method will ever displace the usual staining processes in cytological analysis, but, as has been partially indicated above, it should be extremely useful as a supplement to these, to examine the nature and value of fixation, the comparative modifications of the hydrosols, and the processes of the living cell.

SUMMARY.

The method of dark-ground illumination, which has been only sparingly used in botanical work, has now been applied to a more detailed study of the plant cell, under various conditions. The method often reveals new structural features, and is useful in establishing the presence of minute particles which are difficult to see, or are unresolved in direct illumination. Some of the results indicate possible lines of work rather than completed results.

The method is very restricted in application, on account of the difficulty of selecting suitable material for examination. An account of the methods used is given, and also descriptions of the structure of various types of material studied by the method, and used in further investigations.

The protoplasm, as is generally recognized, is a colloid complex; this seems to exist both in the hydrosol and hydrogel state, and to a certain extent these states appear to be spontaneously reversible. The gel in this state differs from that produced by the coagulation of the hydrosol, or by the action of a fixing agent on the hydrogel.

The process of germination of certain Fungus spores, followed by the method, showed the gradual conversion of the gel contents of the spore into a hydrosol (on absorption of water): later a formation of a gel may occur again.

Of the structures present in the cell, the nucleus and chloroplast are probably to be regarded as specialized parts of the plasma, with a hydrogel structure, and a limiting membrane of a certain type. Only in very favourable cases can the nucleus be studied, however.

Particles and vesicular bodies—here called 'sap particles'—are usually present in the cell-sap, and exhibit a continuous Brownian movement. They generally increase in number with decreasing vitality of the cell.

The effects of plasmolysis were studied in various cases with different reagents—electrolytes and non-electrolytes, and so on, and some features of considerable interest were made out. An outer layer with a much finer structure than the general parts of the protoplast was usually evident on plasmolysis, and this layer seemed to be the part concerned in the formation of the fine protoplasmic fibrils, which often connect the plasmolysed protoplast with the wall of the cell. The presence of a 'Hautschicht' seems to be quite definite. Vesicular plasmolysis also indicates that a similar layer exists on the inside of the protoplast, against the vacuole.

The fibrils were also studied in detail, and indicate some interesting structures.

An attempt was made to study the action of fixing and coagulating agents from a comparative point of view, but here there were difficulties in application, so that the results are by no means complete. Comparatively little is revealed as to the general action during fixation, except that a change to an opalescent hydrogel occurs. The rate of formation and the structure of the gel differ in different material, and with different coagulating agents.

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LITERATURE.

ANDREWS, F. M.: Protoplasmic Streaming in *Mucor*. Bull. Torrey Bot. Club, vol. xxxix, Oct. 1912, p. 455.

BECHOLD: Die Kolloide in Biologie und Medizin. Steinkopff, Dresden, 1911.

BOWER, F. O.: On Plasmolysis and its Bearings upon Relations between Cell-wall and Protoplasm. Quart. Journ. Micros. Science, New Series, vol. xxiii, 1883, pp. 151–68.

CHODAT, R., et BOURBIER, A. M.: Sur la Plasmolyse et la Membrane plasmique. Journal de Botanique, t. xii, 1898, No. 8, pp. 118–32.

CZAPEK, F.: Biochemie der Pflanzen. Vol. i, Gustav Fischer, Jena, 1905.

————: Über eine Methode zur direkten Bestimmung der Oberflächenspannungen der Plasmahaut von Pflanzenzellen. G. Fisher, Jena, 1911.

DARLING, C. A.: Mitosis in Living Cells. Bull. Torrey Bot. Club, vol. xxxix, 1912, p. 407.

FAURÉ-FRÉMIET, E.: Étude physico-chimique sur la structure de noyaux du type granuleux. Comptes Rend., t. cl, 1910, p. 1355.

FISCHER, A.: Fixirung, Färbung und Bau des Protoplasmas. Jena, 1899, pp. 1–71.

GAIDUKOV, N.: Über Untersuchungen mit Hilfe des Ultramikroskops nach Siedentopf. Berichte d. d. Bot. Ges., Bd. xxiv, 1906, p. 107.

————: Weitere Untersuchungen mit Hilfe des Ultramikroskops nach Siedentopf. Ibid., p. 155.

————: Über die ultramikroskopischen Eigenschaften der Protoplasten. Ibid., p. 580.

————: Ultramikroskopische Untersuchungen der Stärkekörner, Zellmembranen und Protoplasten. Ibid., p. 581.

————: Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin. Gustav Fischer, Jena, 1910.

GUILLIERMOND, A.: Les Progrès de la cytologie des champignons. Prog. Rei Bot., vol. iv, 1913, pp. 389–542.

HEIDENHAIN, M.: Plasma und Zelle. Gustav Fischer, Jena, 1907.

HECHT, K.: Studien über den Vorgang der Plasmolyse. Cohn's Beiträge z. Biologie der Pflanzen, Bd. xi, Heft 1, 1912.

KOHL, F. G.: Protoplasmaverbindungen bei Algen. Ber. d. d. bot. Ges., Bd. ix, 1891, pp. 9–17.

LAWSON, A. A.: Nuclear Osmosis as a Factor in Mitosis. Trans. Roy. Soc. Edin., vol. xlviii, Pt. 1, No. 7.

LEPESCHKIN, W. W.: Zur Kenntniss der Plasmamembran. Ber. d. d. Bot. Ges., Bd. xxviii, 1910, pp. 91–103.

————: Zur Kenntniss der Plasmamembran, II. Ibid., pp. 383–93.

————: Zur Kenntniss der chemischen Zusammensetzung der Plasmamembran. Ber. d. d. bot. Ges., Bd. xxix, 1911, pp. 247–60.

————: Über die Einwirkung anaesthesierender Stoffe auf die osmotischen Eigenschaften der Plasmamembran. Ibid., pp. 349–54.

For short summary of these see BLACKMAN, F. F.: The Plasmatic Membrane. New Phyt., 1912, pp. 180–95.

MAIRE, R.: Remarques taxonomiques et cytologiques sur le *Botryosporum pulchellum*. Ann. Mycologici, t. i, 1903, pp. 335–40.

OSTERHOUT, W. J. W.: Protoplasmic Contractions resembling Plasmolysis, which are caused by Pure Distilled Water. Bot. Gaz., 1913, vol. lv, p. 446. See also Science, N.S., vol. xxxv, p. 112, and vol. xxxvi, p. 350.

PERRIN, J.: Les Atomes. Librairie Félix Alcan, Paris, 1913.

PFEFFER, W.: Physiology of Plants. Eng. trans., A. J. Ewart, Oxford, Clarendon Press, 1906, vol. iii.

PRICE, S. R.: The Method of Dark-ground Illumination in Botanical Research. Science Progress, 1913, pp. 343–54.

————: Some Observations with Dark-ground Illumination on Plant Cells. Proc. Camb. Phil. Soc., vol. xvi, 1912, pp. 481–5.

RUHLAND, W.: Studien über die Aufnahme von Kolloiden durch die pflanzliche Plasmahaut. Prings. Jahrb., vol. li, 1912, pp. 376–431.

SIEDENTOPF, H., and ZSIGMONDY, R.: Über Sichtbarmachung und Grössenbestimmung ultramikroskopischer Teilchen mit besond. Anwendung auf Goldrubingläser. Ann. der Physik, 1903, vierte Folge, Bd. x, pp. 1–39.

SPITTA, E. J.: Microscopy. Murray, London, 1909.

STRASBURGER, E.: Ueber Plasmaverbindungen pflanzlicher Zellen. Prings. Jahrb., vol. xxxvi, 1901, pp. 493–610.

THIRKILL, H.: Ultramicroscopy and Ultramicroscopic Particles. Science Progress, 1909, pp. 55–89.

TUNMANN, O.: Pflanzenmikrochemie. Gebrüder Bornträger, Berlin, 1913.

DE VRIES, H.: Plasmolytische Studien über die Wand der Vakuolen. Prings. Jahrb. f. wiss. Bot., vol. xvi, 1885, pp. 465–598.

WEST, W. and G. S.: British Desmidiaceae. Vol. i, p. 5, Ray Society, 1904.

WISSELINGH, C. VAN: Tests for Tannin in *Spirogyra.* Proc. Konink. Akad. van Wetenschappen, vol. xii, 2nd part, July, 1910, pp. 684–705.
ZEISS, CARL: Pamphlets on Dark-ground Illumination.
ZSIGMONDY: Colloids and the Ultramicroscope. Eng. trans. Wiley and Sons, New York, 1909.

EXPLANATION OF PLATES XLI AND XLII.

Illustrating Mr. S. R. Price's paper on Dark-ground Illumination.

PLATE XLI.

Fig. 1. Spores of *Mucor* in various stages. (*a*) Fresh spores showing slightly granular structure. (*b*) Spores after swelling in water for a few hours, with colloid hydrosol contents. (*c*) Early stage of germination. (*d*) Later stage, showing the fine colloid particles in the spore cavity and the protoplasmic plugs in the hypha.

Fig. 2. Cell of *Spirogyra* plasmolysed with dilute glycerine, showing fine fibrils. Note the structure at the base (*a*) and the minute attaching disc (*b*) as well as the larger microsomes.

Fig. 3. Portion of plasmolysed cell viewed in three depths of focus. (*a*) The outer wall of the vesicle, showing fine particles which exhibit an oscillating movement. (*b*) The interior of the vesicle with enclosed sap particles, also the general structure of the protoplast. (*c*) Outer focus, showing extremely fine particles (for description see text).

Fig. 4. Cell of *Spirogyra,* showing a vesicular plasmolysis treated with dilute potash. The thin walls of the vesicles (cf. Fig. 3) become coarse structured and opaque, while the whole protoplast, except the chloroplast, becomes somewhat milky.

Fig. 5. Portions of two cells of *Spirogyra* fixed with osmic acid. (*a*) Outer focus; the microsomes are still evident and the gel is fairly clear. (*b*) Inner focus, showing the fine precipitate produced by the tannins.

Fig. 6. Single cell from leaf-edge of *Elodea canadensis,* showing chloroplasts (*c*), protoplasmic microns (*p*), and sap particles (*s*).

Fig. 7. Cell from leaf-edge of *Elodea canadensis* plasmolysed with 10 per cent. potassium nitrate. Note the fine protoplasmic threads connecting the balled-up portions of the protoplast to one another and to the walls; also the crystalline sap particles inside the vacuoles.

Fig. 8. Living nucleus of cell of *Elodea canadensis* with appearance of translucent 'hydrogel' microsomes of protoplasmic stream passing over nucleus.

Fig. 9. Nucleus of same, fixed with glacial acetic acid, showing coarse hydrogel structure producing overlapping diffraction images.

PLATE XLII.

Fig. 1. Nucleus of *Spirogyra* with surrounding cytoplasm and suspending threads. The almost clear hydrogel of the nucleus, and the nucleolus, are shown. The microsomes of the cytoplasm are very distinct.

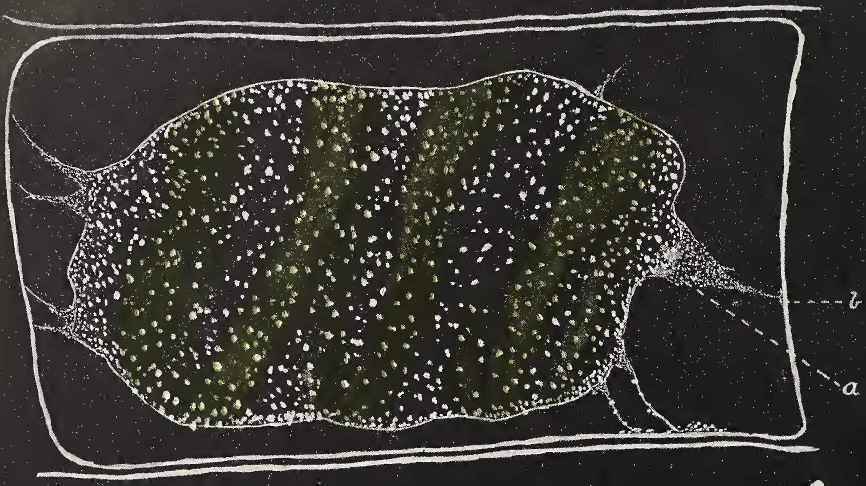
Fig. 2. Suspending threads and cytoplasm surrounding the nucleus in a species of *Spirogyra.* Nucleolus not distinguished.

Fig. 3. Portion of hair of *Cucurbita* plasmolysed with 30 per cent. cane sugar. Three vesicles produced enclosing some sap particles; vesicle walls of thin membrane with scattered very fine particles.

Fig. 4. Portion of hair of *Cucurbita* plasmolysed with 30 per cent. cane sugar. Note the structure of the protoplast with external layer and fine particles below it, coarser particles in the interior.

Fig. 5. End of cell of *Spirogyra* plasmolysed in 10 per cent. potassium nitrate solution, showing coarser threads than in Pl. XLI, Fig. 2.

Fig. 6. Diagrammatic drawings of sap particles from *Elodea* in different positions. (*a*) Crystalline. (*b*) Vesicular.



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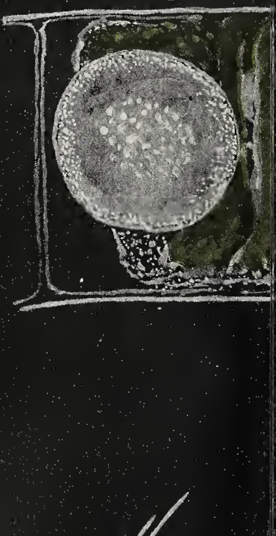

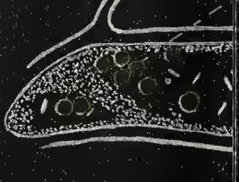

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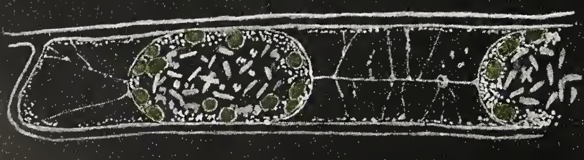

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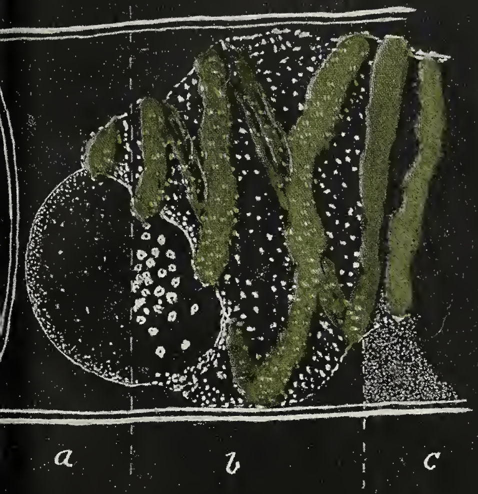
PRICE—DARK-GROUND ILLUMINATION



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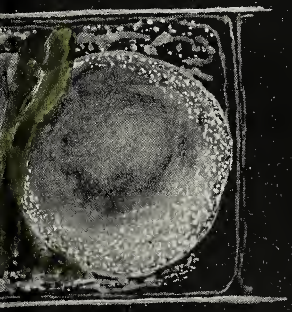

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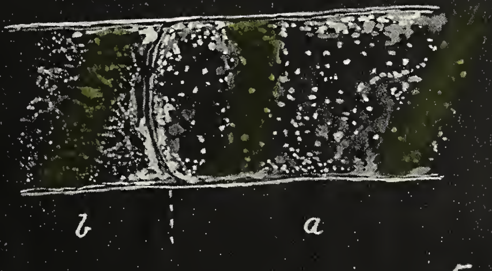

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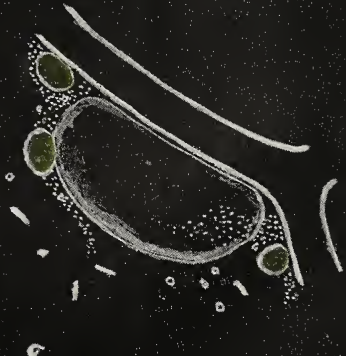

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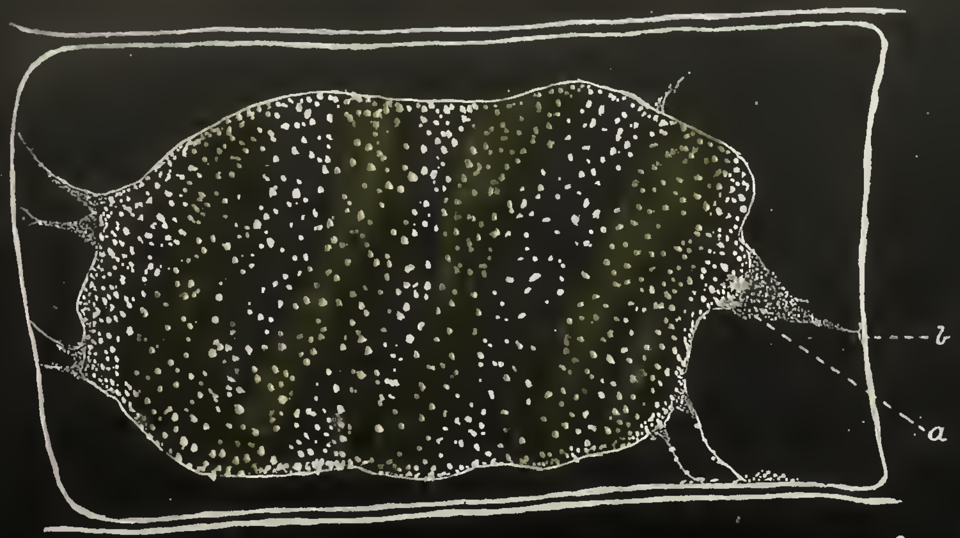

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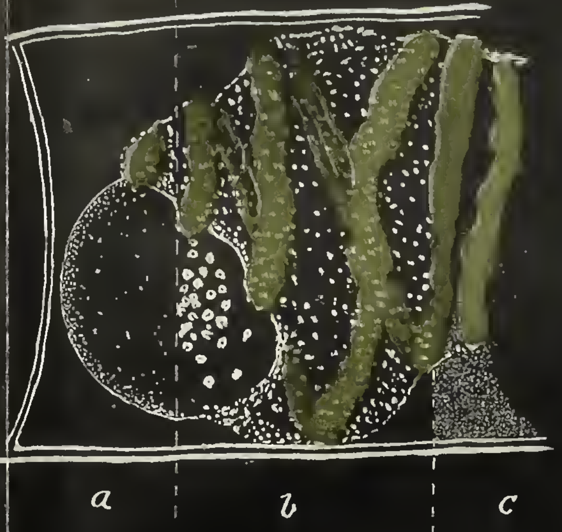

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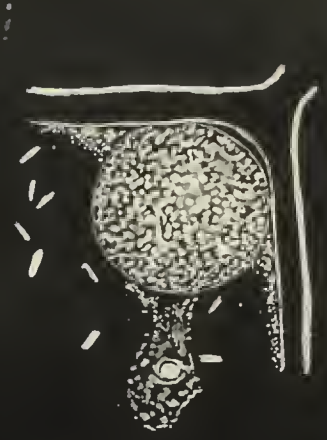

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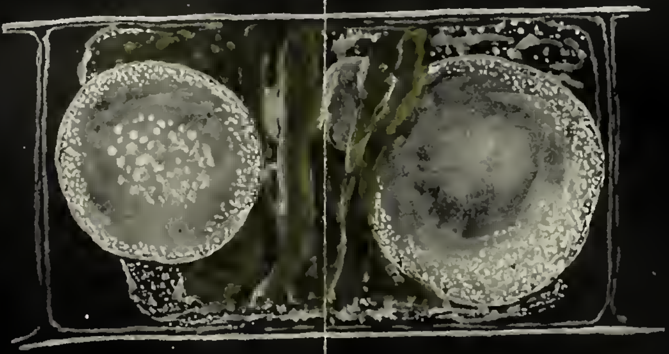

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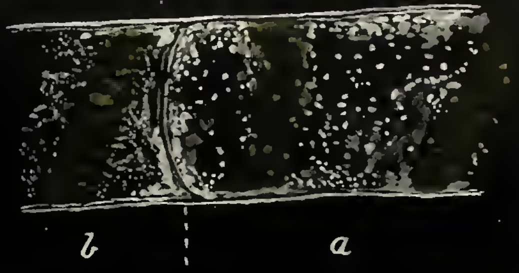

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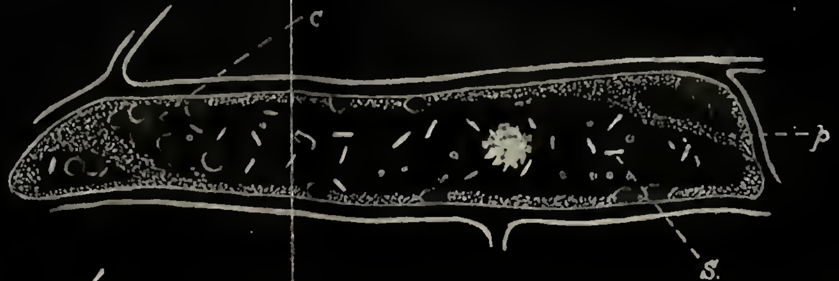

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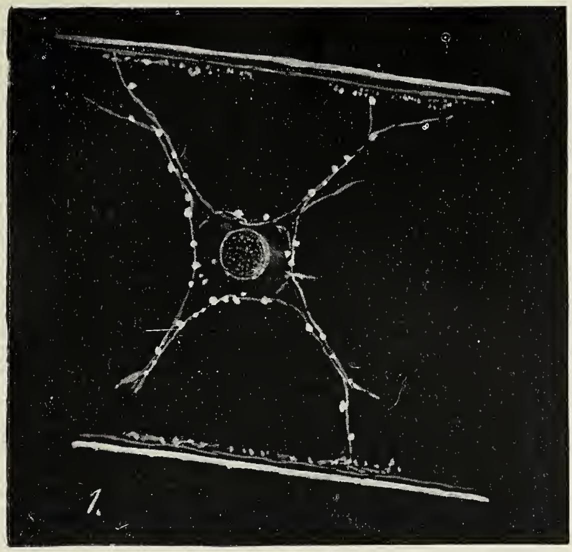

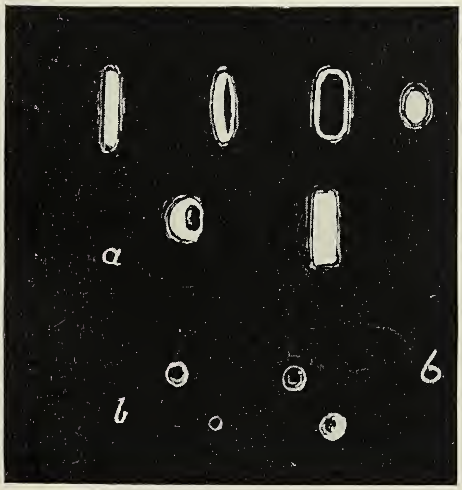

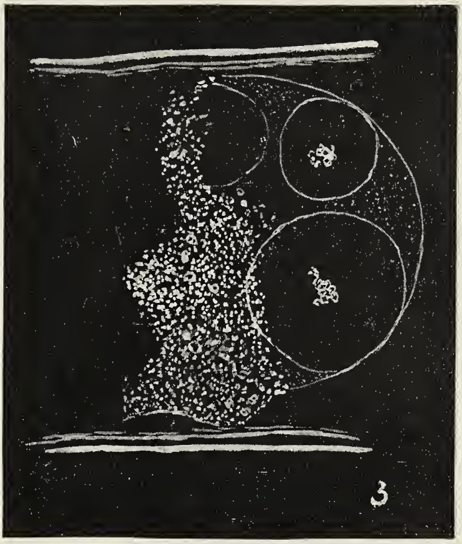

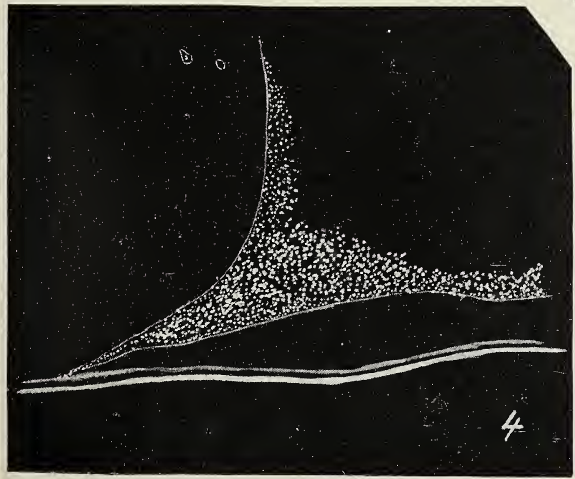

S. R. P. del

PRICE—DARK-GROUND ILLUMINATION

The Behaviour of the Chromatin in the Meiotic Divisions of Vicia Faba.

BY

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With Plates XLIII and XLIV.

IN 1911 an account was published of the vegetative mitoses in *Vicia Faba* (4), in which it was shown that, alike in the sporophytic and gametophytic divisions, longitudinal fission takes place in the telophase and persists through the resting stages till it is completed on the spindle of the following division. In the last premeiotic telophase (that is to say, in the initiation of the spore mother nucleus), as in those of other vegetative divisions, longitudinal fission of the daughter chromosomes occurs, and it has appeared worth while to connect this division with the mitoses already studied in the pollen grain, by tracing the longitudinal fission through the meiotic phase. The great simplicity of some of the stages in the Bean, serving, as it does, as clear and easily obtainable demonstration material, may further justify an account of this often investigated stage.

Plants of Carter's 'Monarch' were grown in the summer of 1910 by my former colleague, Mr. Snell, in a garden at Penge, and in 1911 at the Chelsea Physic Garden, under the direction of Mr. W. Hales, whom I desire to thank for his care in this connexion.

The buds were fixed between 11 a.m. and 2.30 p.m., mainly on sunny days. Various media were used, of which Flemming's strong fluid, diluted with an equal quantity of water, proved the most successful. Sections were cut from 5 μ to 15 μ in thickness, and were stained for the most part with the combination of Breinl, which gives excellent results on this material.

Development was studied both in the micro- and in the megasporangium, and while, for the sake of uniformity, it has seemed preferable to restrict the figures and description to the former, it may be noted that the main facts have been confirmed in the latter also.

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EARLY PROPHASES.

The pollen mother-cells, before meiosis begins, are packed with a dense, finely granular cytoplasm. The nuclei are large (Pl. XLIII, Fig. 1); they generally contain a single nucleolus, lying in a more or less central position, and the reticulum is spread just inside the membrane, so that it forms a hollow sphere. It consists of an irregularly diamond-shaped mesh, the origin of which may be traced to the longitudinal fission and cross connexion of the chromosomes of the preceding telophase. The spaces in the network are thus alternatively due to the pulling apart of the sides of the chromosome where fission has taken place, and to cross attachment between neighbouring chromosomes which, it must be kept in mind, are viscous structures.

The first evidence that reduction is about to take place is given by the partial separation of the reticulum from the nuclear membrane (Fig. 2). The enlargement of the membrane has been recorded at different times by a number of authors, and has been specially emphasized by Lawson (5) in 1911. While it is taking place the chromatin mass in *Vicia* contracts (Figs. 2, 3), still retaining its reticulate character, till it closes in about the nucleolus and the synaptic stage is entered. The nucleolus is sometimes squeezed out (Fig. 4) and lies at the periphery of the nuclear vacuole, where it may be recognized during the succeeding stages.

It is very difficult to gauge the amount of contraction occurring at this period, since nuclei at the same stage of development are by no means necessarily of the same size, but measurements of neighbouring nuclei show that whereas, in a particular case, the diameter of the presynaptic nucleus in which the chromatin lay in contact with the membrane was $12{\cdot}5\,\mu$, the membrane after synapsis had begun was $15\,\mu$ in diameter, and the contracting reticulum 9 by $10\,\mu$, while the chromatin mass in full synapsis, in a vacuole of similar dimensions, measured only $7{\cdot}5\,\mu$ across. These differences are too constant to be accounted for by the inadvertent selection of tangential sections, too great, in otherwise uncontracted material, to be due to artifact, and it seems impossible to doubt that in *Vicia Faba* a definite first or synaptic contraction of the chromatin occurs, in addition to the enlargement of the nuclear membrane.

During the earlier stages of synapsis fine threads may be observed bridging across the space between the nuclear membrane and the reticulum (Pl. XLIII, Fig. 2). As contraction increases these are broken and finally disappear, being no doubt absorbed either into the chromatin network or into the cytoplasm. This connexion between the membrane and the reticulum accounts for the fact that the presynaptic chromatin is held around the outer limits of the nuclear vacuole, and it may be surmised that a similar provision exists in vegetative nuclei.

The synaptic mass is dense and impenetrable when ordinarily stained, but a light stain reveals that the chromatin retains its reticulate arrangement during the period of maximum contraction (Fig. 3). Later the cross connexions begin to break down (Fig. 4), and the formation of the free spireme is initiated.

POST-SYNAPTIC PROPHASES.

As the nucleus passes out of synapsis loops of free spireme are thrown into the nuclear vacuole (Figs. 5–9), the whole area of which, as the loosening proceeds, becomes occupied by the chromatin thread; this is not always wholly in the form of a simple spireme, but may still show the diamond-shaped mesh of the reticulum (Fig. 10) to a greater or less extent. We have thus clear evidence of the persistence of a longitudinal split, which can be traced back through synapsis to the preceding telophase; quite similar fissions may be recognized (Fig. 11) even where the cross attachments have already disappeared.

Subsequently, after the chromatin has been spread through the nuclear vacuole, a second contraction begins, and, with the shortening and thickening of the thread, the longitudinal fission becomes difficult or impossible to trace. It is rendered clearly recognizable again only with the onset of the metaphase.

The synaptic stages are frequently found, and usually extend throughout the whole anther; they may be judged, therefore, to be somewhat prolonged. The same criteria indicate that the loosening and formation of the spireme take place slowly, but that the second contraction is a much more rapid process. The subsequent metaphases and anaphases, as well as the homotype division, appear to be swiftly accomplished.

At all stages in meiosis, disintegration of the contents of the anther may set in. This is a not uncommon phenomenon among cultivated plants, and is indicated by contraction and abnormal appearance both of the cytoplasm and the nuclear contents. At an early stage, when the cell as a whole still presents a healthy appearance, globules of chromatin are extruded from the nucleus and pass into the cytoplasm, frequently invading a neighbouring cell (Pl. XLIV, Fig. 33). They are for a time attached by fine threads to the parent nucleus, and closely resemble the 'chromatic bodies' described by Digby (1) as normally present in *Galtonia candicans*. The bodies observed in *Vicia Faba*, however, seem clearly related to the incidence of an abnormal condition.

THE FORMATION OF THE GEMINI.

The first indications of pairing appear among the free strands of spireme which have already been described as thrown out across the nuclear vacuole when the loosening of the synaptic mass begins. This occurs prior to the temporary obscuration of the longitudinal split, which is still quite clear some time after pairing has begun. Loops of similar form are observed lying side by side in pairs (Pl. XLIII, Figs. 5, 7, 8) in more or less close relation. At other points isolated loops occur with markedly similar sides, united by a swollen dot (Figs. 6, 8). A little later straight segments of the spireme may be found lying parallel one to another (Fig. 8), or again curved filaments connected by their longer limbs while their shorter limbs lie free (Fig. 9). These and other figures, in which the associated strands become intertwined and more closely approximated, are found after the loosening of synapsis is complete (Fig. 11) and, still more frequently, throughout the second contraction phase (Figs. 12–16), until the appearance of the mature gemini. As development proceeds, one end of a double loop is frequently set free (Fig. 13). The long twisted structures thus produced are the precursors of the figure-of-eight-shaped gemini on the heterotype spindle.

The earliest recognizable approximations thus occur immediately on the loosening of synapsis, when portions of the spireme (which is not necessarily continuous) are first set free from the reticulum. The threads, paired and unpaired, soon extend through the whole of the nuclear area, but the increasing association between separate filaments no doubt entails some pulling about and rearrangement of the whole, till, as pairing becomes more frequent and the thread begins to thicken, the chromatin is drawn into the loose, irregular knot which constitutes the second contraction. The chromosomes, or their precursors on the spireme, run out to the limits of the nuclear vacuole but merge into a central tangle, so that each chromosome is pretty sure to lie in contact with several others, and attractions between allelomorphs as yet unpaired are thus facilitated. A similar arrangement has been observed by several investigators, and its interest lies in the comparatively simple opportunity it affords for the association between homologous paternal and maternal segments.

The spireme of the second contraction breaks up to form seven gemini; some of these are at first of considerable length (Pl. XLIV, Fig. 16), and are twisted hither and thither in the nuclear areas; they undergo the usual shortening and thickening (Fig. 17), and, by the time that they are arranged on the spindle, they are relatively small and stout, and of more or less uniform size (Fig. 18).

THE NUCLEAR MEMBRANE AND NUCLEOLUS.

Until the formation of the gemini the nuclear membrane shows no marked alteration in appearance, while the cytoplasm, which is at first a uniform, finely granular mass, increases in density around the nucleus and leaves the periphery of the cell relatively empty. By these changes, and by the subsequent rounding off of the pollen mother-cell, the succession of stages in the nuclear history can be roughly gauged.

As the gemini shorten and thicken, the cytoplasm encroaches on the nuclear area and the limiting layer loses its definite character, till, on the equatorial plate, a nuclear membrane cannot be identified, and the vacuole is represented only by a light area around the chromosomes (Fig. 18). Meantime the encroaching cytoplasm gives rise to the spindle.

Lawson (6, 7) has regarded similar phenomena as evidence that the nuclear membrane becomes 'closely applied'[1] to each of the gemini, so that there are formed 'as many osmotic systems as there are bivalent chromosomes'.[2] For him the nuclear membrane never disappears, and the spindle fibres do not intrude into the nuclear system but are attached to the nuclear membrane where it is wrapped around each chromosome.

In *Vicia Faba* no evidence could be obtained that the nuclear membrane as a definite entity 'completely envelops'[3] each of the gemini. If we choose to define the nuclear membrane as the inner limit of the cytoplasm wherever it abuts on nuclear material, we may be justified in using this term to indicate the area of contact between the surrounding cytoplasm and each individual chromosome, when at last the nuclear vacuole has begun to disappear. On such an interpretation, Lawson's description may be applied to this plant, but it is impossible to regard the area of contact of the chromosomes and cytoplasm as in any special way related to the limiting layer of the vacuole, much less as identical with it. The term nuclear membrane seems more appropriately restricted to the latter.

At about this time also the nucleolus disappears. In the early prophase it is in close relation to the developing chromosomes, to which it apparently gives up a part of its contents. On the heterotype spindle, and during the homotype division, a number of deeply staining granules are present, and these, no doubt, represent the remains of the nucleolus. A nucleolus is not formed in the brief resting stage between the heterotype and the homotype divisions, but nucleoli again appear (Pl. XLIV, Fig. 31) as soon as the latter mitosis is complete.

THE CHROMOSOMES ON THE SPINDLE.

The gemini, after they are set free, assume the familiar oval and twisted forms. Single and double figures of eight apparently occur in every nucleus (e. g. Figs. 18, 19), and various more or less elongated loops may also be observed. There is considerable regularity in the appearance of the same figures in different nuclei, but there is not enough variety of form to repay a special study of this point.

The gemini are always attached to the spindle by one end (never by the middle), in such a way that the two sides of the loop or twist—that is to say, the two homologous chromosomes—are both in contact with the spindle and both stand right out from it more or less coiled round one another (Fig. 19). Very soon they begin to move apart, producing a U-shaped

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[1] Lawson (7), p. 616.

[2] Lawson (6), p. 144.

[3] Lawson (7), p. 616.

figure (Fig. 20); and as they move, each arm of the U is seen to be longitudinally split (Fig. 21), so that the bivalent chromosome, which, viewed laterally, appears as a U form, is revealed when examined from the front, as two V's set base to base. Thus the longitudinal fission, lost sight of at the beginning of the second contraction, reappears upon the spindle. The chromosomes are attached to the spindle by their apices, and somewhat bulged out from it; where they are much twisted, figures somewhat more complicated than those described above may be observed.

The next stage is the separation of the two V-shaped sister chromosomes. Generally one pair of ends is freed before the other (Fig. 22); the free limbs appear to contract somewhat, suggesting that the chromosomes are being pulled apart. Later, in the anaphase (Fig. 23), the limbs are usually of the same length. The longitudinal fission is always recognizable first at the end of the chromosome not attached to the spindle, and is never seen, at this stage, to reach quite to the attached end.

It is instructive to compare the state of affairs on the heterotype spindle with that in the vegetative division. There also in *Vicia Faba* the chromosomes of the metaphase are attached to the spindle by one end, and there also the two halves of the chromosome move apart, so that a U-shaped or penthouse-shaped figure is formed. Only, in the vegetative division, this arrangement is due to the longitudinal split, and not, as in the heterotype, to the separation of whole premeiotic chromosomes. Consequently no further fission takes place on the vegetative spindle, and the chromosomes of the anaphase have the form of rods and not of V's.[1] Otherwise the mechanism of the two types of division is strikingly similar.

THE HOMOTYPE DIVISION.

When the chromosomes reach the pole of the spindle they become united one to another laterally (Figs. 25, 26), and sometimes also by their ends (Fig. 26), as in the somatic telophases, but without losing their V-shape. They do not produce a reticulum, and no satisfactory evidence of a new fission in their limbs could be obtained. After a brief resting period the cross attachments disappear, the chromosomes elongate considerably (Fig. 27), extending through the vacuole which has developed about them, and reach a stage which may be compared to the spireme of the vegetative prophases. Contraction then takes place, and they pass on to the homotype spindle, still retaining their individuality and their characteristic form (Fig. 28).

The two homotype spindles may lie parallel (Fig. 29) or at an angle (Fig. 30) one to another, often one or both are curved (Fig. 30), and they

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[1] A V-shaped chromosome in vegetative mitosis is due to the attachment of a bent rod by its middle, and is represented in the meiotic anaphase by a double V such as is found in *Lilium*.

may be laterally compressed so that one diameter of the equatorial plate is much longer than the other. The broad aspect of such a spindle is shown in Fig. 28.

On the spindle the two arms of the V separate, so that the longitudinal fission, begun in the last premeiotic telophase, is here completed, and the daughter chromosomes pass as rods (Fig. 29) to the poles of the spindle (Fig. 30). Cross connexions appear between them, longitudinal fission in preparation for the first mitosis in the pollen-grain occurs (Fig. 31), and a spireme is produced (Fig. 32), the diamond-shaped mesh of which, as already shown (Fraser and Snell, '11), is quite similar to that of the diploid nuclei.

CONCLUSIONS.

The points of special importance emerging from this series of events are the persistence in *Vicia Faba* of the longitudinal fission from the last sporophytic telophase to the metaphase of the homotype division, and the fact that, as far as any indication can be obtained, the association of the allelomorphs begins after synapsis, taking place in the course of the formation of the heterotype spireme, when the cross connexions of the reticulum break down.

The relative unimportance of the position which the chromosomes of a pair assume in relation one to another has been recently emphasized by Farmer (1912), and attention may be called to the fact that in *Vicia Faba* lateral and end-to-end approximations take place in the same nucleus, and become recognizable at the same late stage of development, nor is either type related to the appearance of a double thread, either in the presynaptic prophases or in the premeiotic divisions.

The chromosomes of the vegetative division are seen to be double structures, not only in the premeiotic telophases, but in the postmeiotic also (Fraser and Snell, '11), so that, unless a different interpretation be placed upon the duplication at different stages, it is clear that it cannot be ascribed in the former, since it is impossible to ascribe it in the latter, to an approximation of paternal and maternal segments. Rather it is due in both alike to longitudinal fission or vacuolization. As cross connexions appear, and as the nuclear area enlarges, the diamond-shaped mesh of the reticulum is formed by the pulling apart of the split portions of the chromosomes. In the meiotic prophase this mesh can be traced unbroken into full synapsis, and may be recognized in places at a considerably later stage. After the synaptic phase, as the cross connexions break down, the sides of the split strands tend to approximate, and the fission is only made clear here and there by fortunate accident.

Having regard to the origin of the duplication, it is impossible to relate it to the formation of the gemini, and the confusion which has so frequently arisen is probably due to the fact that the very severance of cross connexions which tends to obscure the split, makes possible the approximation of the first strands of the now free spireme, and the consequent pulling apart and rearrangement by which fresh attractions come into play, and new pairs are produced.

Where a presynaptic association of paternal and maternal segments occurs, evidence of its existence must be looked for, not in the double thread, but rather in the pairs of chromosomes of similar form which have been recorded by Müller (8, 9) for the sporophyte of *Yucca* and other plants, and by Reed (10) for that of *Allium*. Pairing of this type has not been recognized in *Vicia*.

SUMMARY.

1. The spore mother-cell shows a dense, finely granular cytoplasm, and a large nucleus. The nuclear reticulum is spread just inside the membrane, and shows an irregularly diamond-shaped mesh such as is found, both in other diploid, and in haploid nuclei (Pl. XLIII, Fig. 1).

2. The first indication that reduction is about to take place, consists in the separation of the reticulum from the nuclear membrane (Fig. 2). The membrane enlarges and a considerable contraction of the chromatin mass occurs. During synapsis the chromatin retains its reticulate character (Fig. 3).

3. In the loosening of synapsis some of the cross connexions of the reticulum break down (Fig. 4), and free lengths of spireme appear (Figs. 5–8). Some of these form pairs of similar loops, and other paired figures between the sides of which approximation takes place (Figs. 5–9).

4. The loosened spireme extends throughout the nuclear vacuole; in its threads the longitudinal fission, initiated in the preceding telophase, and recognizable in the diamond-shaped mesh of the reticulum, can still be traced (Figs. 10, 11).

5. Pairing between independent portions of the spireme becomes more frequent, and the rearrangement entailed pulls the thread into a loose knot (Fig. 12).

6. The thread becomes shorter and thicker, and the second contraction figure is produced (Figs. 13, 14).

7. The spireme breaks up into seven paired segments, the gemini (Pl. XLIV, Figs. 16, 17), which are thus produced by the association of different parts of the spireme. It is indifferent whether this association takes place laterally (Pl. XLIII, Figs. 7, 12) or end to end (Figs. 6, 8, 14).

8. The nuclear membrane disappears, the cytoplasm invades the nuclear area, forming the spindle, the gemini shorten and thicken, and are arranged on the equatorial plate (Pl. XLIV, Fig. 18).

9. Each of the gemini is attached to the spindle by one end (Fig. 19). The two constituent chromosomes move apart along the spindle fibres, but remain for a while in contact at their free end (Figs. 19, 20).

10. As they separate the longitudinal fission in each becomes distinct (Figs. 21, 22). The chromosomes travel up the spindle as V-shaped structures attached by the apex of the V (Figs. 23, 24).

11. At the pole of the heterotype spindle the chromosomes become united laterally, and sometimes end to end, by cross connexions (Figs. 25, 26).

12. When these break down they elongate; they retain their individuality and their V-form (Fig. 27).

13. They pass as V's on to the homotype spindle (Fig. 28), and there the limbs of the V separate, going to different poles, so that the longitudinal fission, begun in the last premeiotic telophase, is at last completed; the daughter chromosomes have the form of rods as in a vegetative division (Fig. 29).

14. At the pole of the homotype spindle the chromosomes become united (Figs. 30, 31), both laterally and end to end. Longitudinal fission takes place in them, and the reticulum of the spore nucleus is produced, and shows a diamond-shaped mesh similar to that of other vegetative nuclei (Fig. 32).

BIBLIOGRAPHY.

1. DIGBY, L. ('09): Observations on Chromatin Bodies. Ann. Bot., xxiii, p. 491.
2. FARMER, J. B., and MOORE, J. E. S. ('05): On the Meiotic Phase (Reduction Division) in Animals and Plants. Q. J. M. S., vol. xlviii, p. 489.
3. FARMER, J. B. ('12): Telosynapsis and Parasynapsis. Ann. Bot., vol. xxvi, p. 623.
4. FRASER, H. C. I., and SNELL, J. ('11): The Vegetative Divisions in *Vicia Faba.* Ann. Bot., xxv, p. 845.
5. LAWSON, A. A. ('11): The Phase of the Nucleus known as Synapsis. Trans. Roy. Soc. Ed., vol. xlvii, p. 591.
6. ——— ('11): Nuclear Osmosis as a Factor in Mitosis. Trans. Roy. Soc. Ed., vol. xlviii, p. 137.
7. ——— ('12): A Study in Chromosome Reduction. Trans. Roy. Soc. Ed., vol. xlviii, p. 601.
8. MÜLLER, H. A. C. ('09): Ueber karyokinetische Bilder in der Wurzelspitze von *Yucca.* Jahrb. f. wiss. Bot., vol. xlvii, p. 103.
9. ——— ('12): Kernstudien in Pflanzen. Archiv für Zellforschung, vol. viii, p. 1.
10. REED, T. ('14): The Nature of the Double Spireme in *Allium Cepa.* Ann. Bot., vol. xxviii, p. 271.

EXPLANATION OF PLATES XLIII AND XLIV.

Illustrating Dr. H. I. C. Gwynne-Vaughan's paper on the Meiotic Divisions in *Vicia Faba.*

Magnification × 2,000 throughout.

PLATE XLIII.

Fig. 1. Pollen mother-cell with resting nucleus showing diamond-shaped mesh of reticulum.

Fig. 2. Three stages in the separation of the reticulum and nuclear membrane.

Fig. 3. Full synapsis; surface view.

Fig. 4. Rather later stage, showing extrusion of nucleolus and beginning of formation of spireme.

Fig. 5. Loosening of synapsis; two similar loops thrown out.

Fig. 6. Same; threads united end to end.

Fig. 7. Same; parallel association of threads.

Fig 8. Same, rather later; three distinct pairs.

Fig. 9. Same.

Fig. 10. Nuclear area filled by postsynaptic spireme showing fission; cross connexions have in this case persisted longer than usual.

Fig. 11. Postsynaptic spireme; some paired strands recognizable, also fission of chromatin thread.

Fig. 12. Second contraction.

Fig. 13. Later stage of same, showing a conspicuous double loop, one end of which has been set free.

Fig. 14. Second contraction; several pairs of associated chromosomes may be recognized.

Fig. 15. Same; longitudinal fission indicated in places.

PLATE XLIV.

Fig. 16. Five newly separated gemini.

Fig. 17. Gemini, rather later stage.

Fig. 18. The seven gemini on the equatorial plate.

Fig. 19. Metaphase of first meiotic division.

Fig. 20. U-shaped gemini of metaphase.

Fig. 21. First meiotic division, showing longitudinal fission in the sister chromosomes.

Fig. 22. Same; separation of the V-shaped sister chromosomes.

Fig. 23. First meiotic division; anaphase.

Fig. 24. Same; telophase.

Fig. 25. Cross attachment of chromosomes after first meiotic division.

Fig. 26. Same; showing both lateral and terminal attachments.

Fig. 27. Elongation of V-shaped chromosomes.

Fig. 28. V-shaped chromosomes on second meiotic spindle.

Fig 29. Second meiotic division; anaphase.

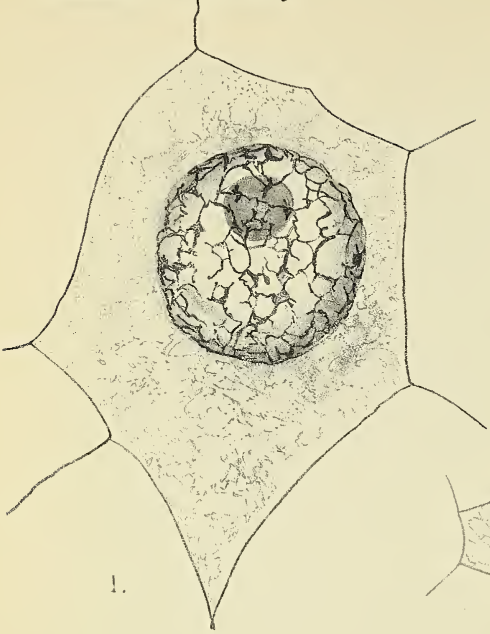
Fig. 30. Same; telophase.

Fig. 31. Very late telophase, showing fission and attachment of chromosomes.

Fig. 32. Resting nuclei of young pollen grains, showing diamond-shaped mesh of reticulum.

Fig. 33. Extrusion of chromatic bodies from nucleus of pollen mother-cell.

Annals of Botany.



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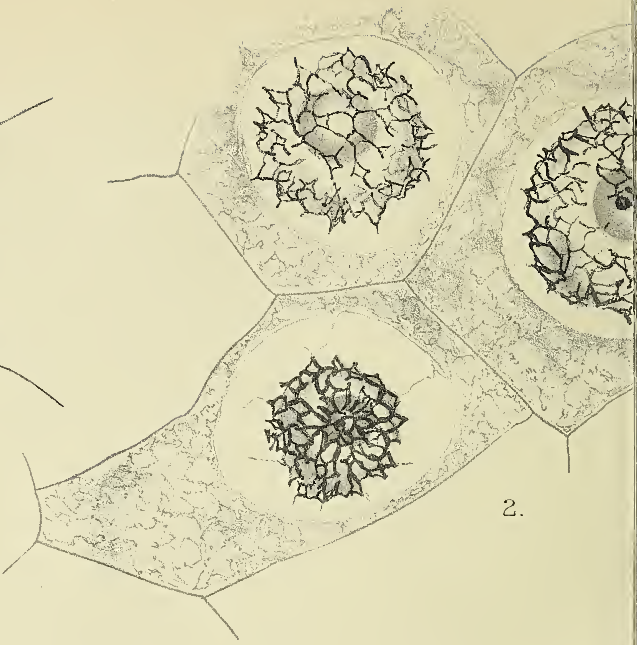

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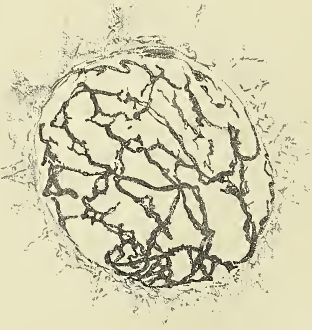

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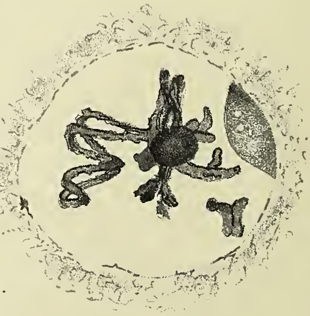

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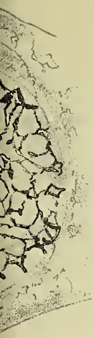
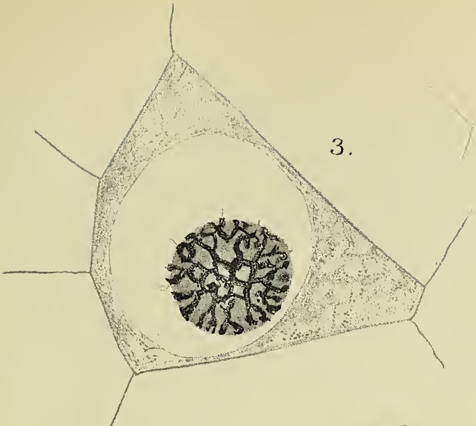
FRASER—MEIOSIS IN VICIA FABA.





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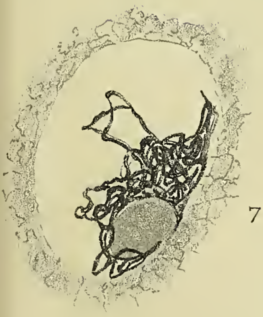

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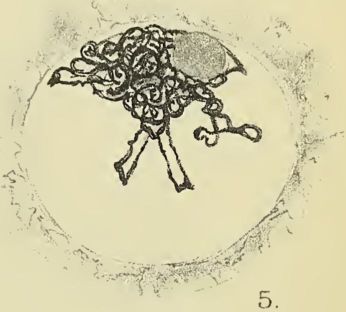

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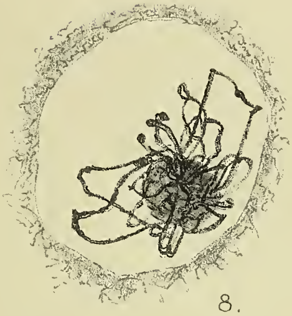

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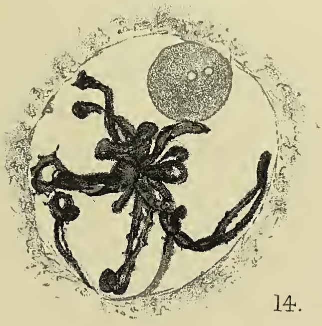

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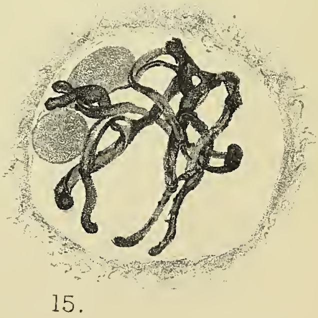

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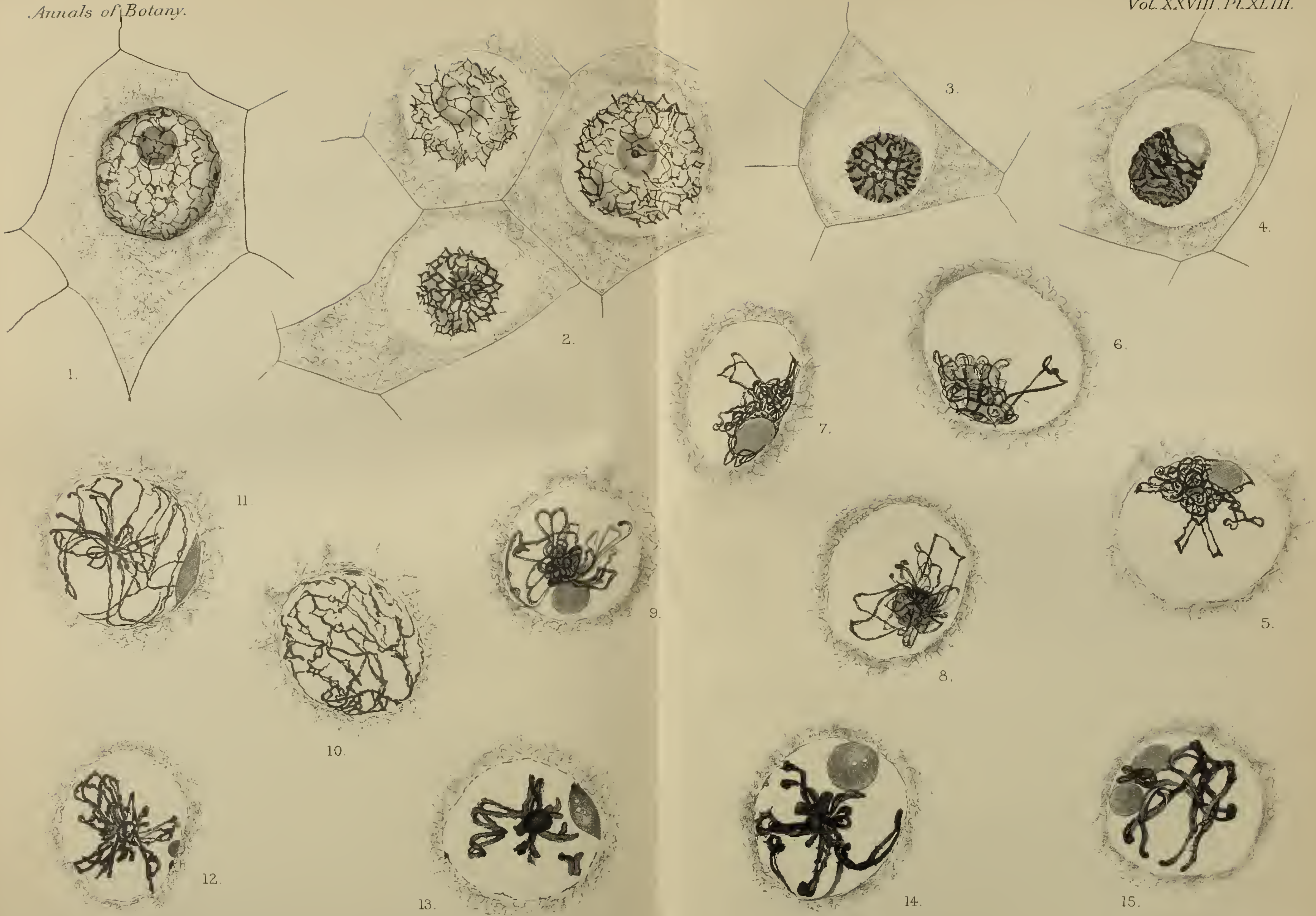

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FRASER—MEIOSIS IN VICIA FABA.

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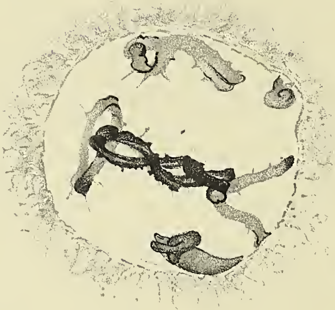

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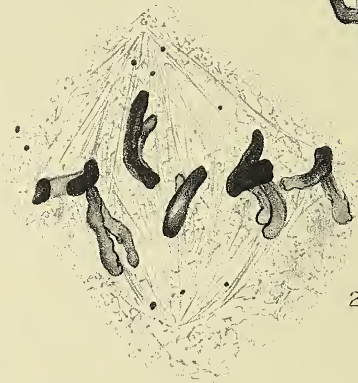

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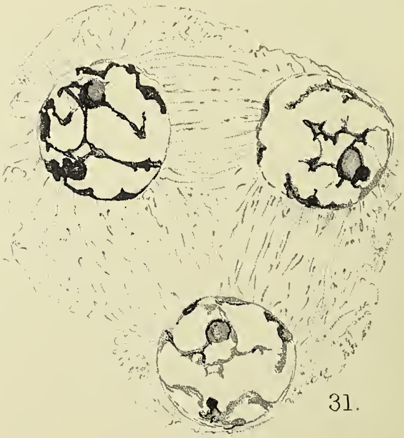

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FRASER—MEIOSIS IN VICIA FABA.


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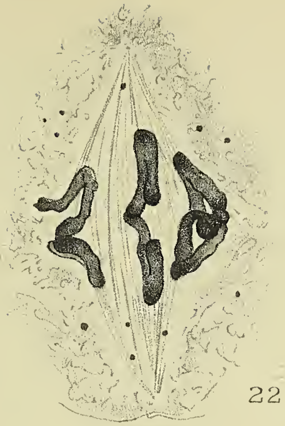
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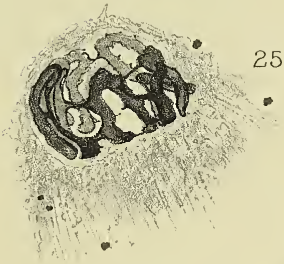
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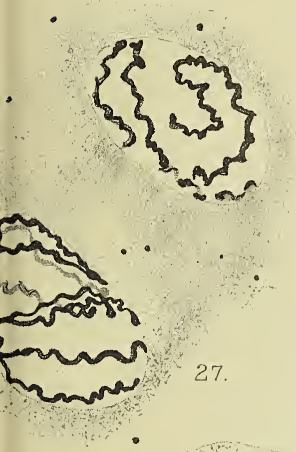
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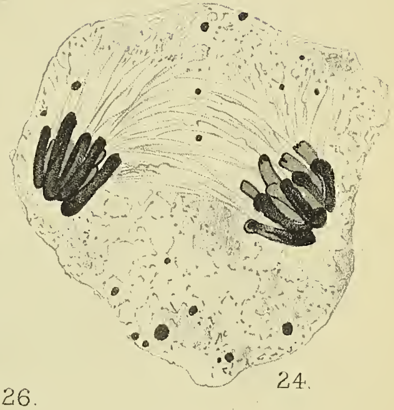
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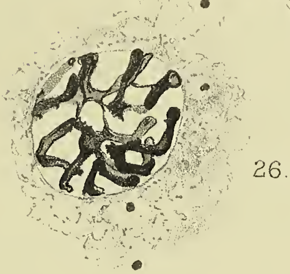
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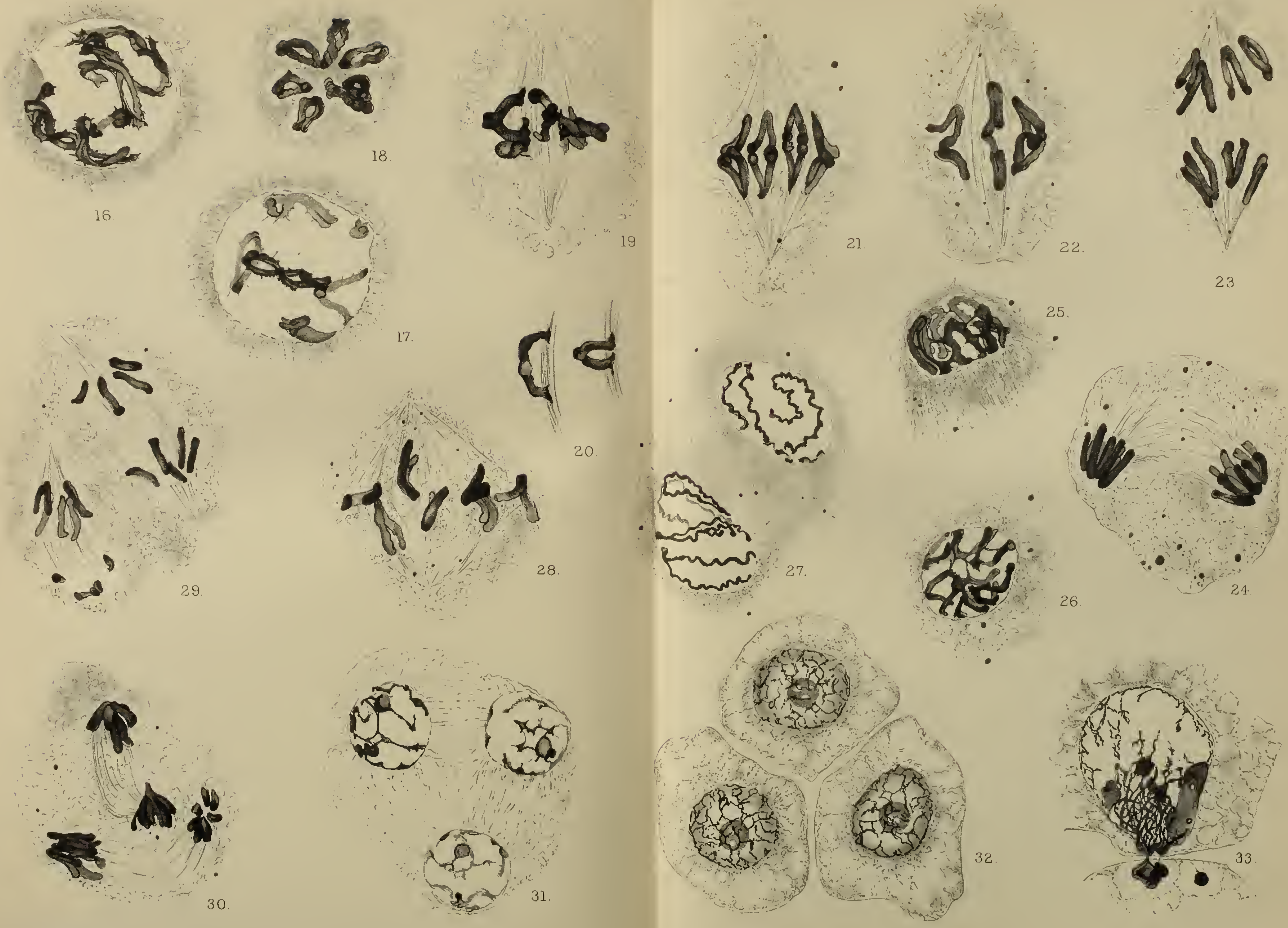

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FRASER—MEIOSIS IN VICIA FABA.

Huth, lith et imp.

Ostenfeldiella, a New Genus of Plasmodiophoraceae.

BY

C. FERDINANDSEN

AND

Ö. WINGE.

With Plate XLV and four Figures in the Text.

DURING a stay in the Danish West Indies in the winter 1913–14 Dr. C. H. Ostenfeld was studying *Diplanthera Wrightii*, Aschers., a Potamogetonacea, growing in shallow water on muddy soil on the coast of the island of St. Croix. This plant has a horizontal monopodial rhizome with partly stretched, partly shortened internodes, some of the latter being barely half a millimetre in length. From the rooting nodes branches are sent out in horizontal and upward directions. Dr. Ostenfeld noticed that sometimes the shortened internodes of the ascending branches, which are clothed with the sheaths of the linear alternating leaves, were conspicuously thickened, their diameter being about two to three times that of the normal ones, and the whole branch thereby bearing a certain resemblance to a string of pearls (Text-fig. 1). Dr. Ostenfeld found the swellings to be due to a Plasmodiophoracea, and after his return from the West Indies he placed his material at our disposal for closer investigation.

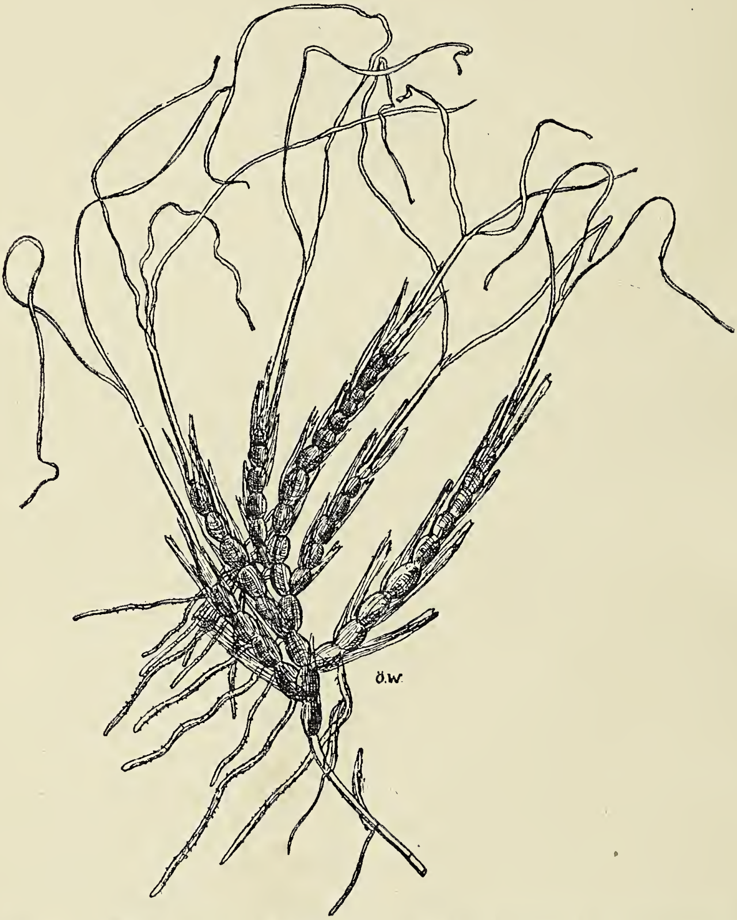
We regret not to have been able entirely to elucidate the cytology of the fungus, the material being fixed in alcohol only. On the other hand, the preservation was good enough to allow a thorough investigation of the ordinary biology of the parasite, and its relation to the host plant.

THE ANATOMY OF THE NORMAL DIPLANTHERA STEM.

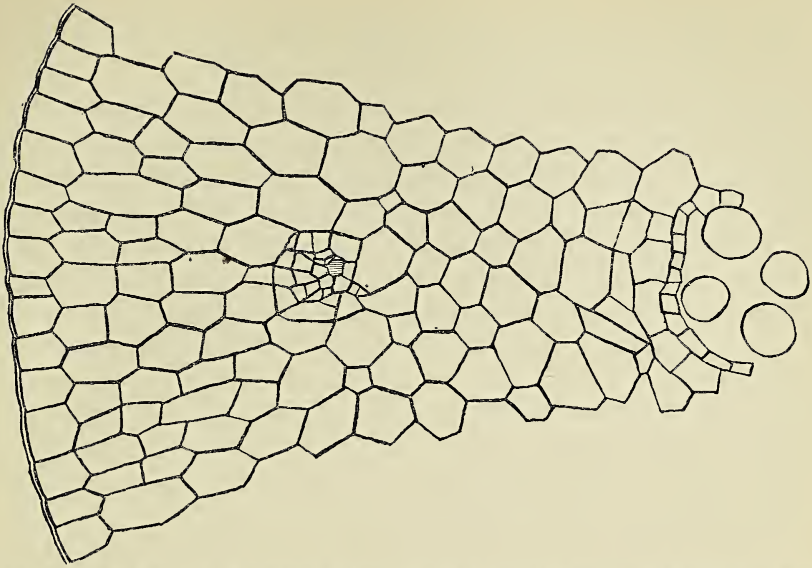
In order to determine the anatomical changes called forth by the fungus, we first studied sections of the normal stem. Text-fig. 2 shows a part of a transverse section through one of the shortened internodes of an ascending normal axis. In the middle of the stem is seen the central cylinder surrounded by the endodermis. The relations are diagrammatically represented

[Annals of Botany, Vol. XXVIII. No. CXII. October, 1914.]

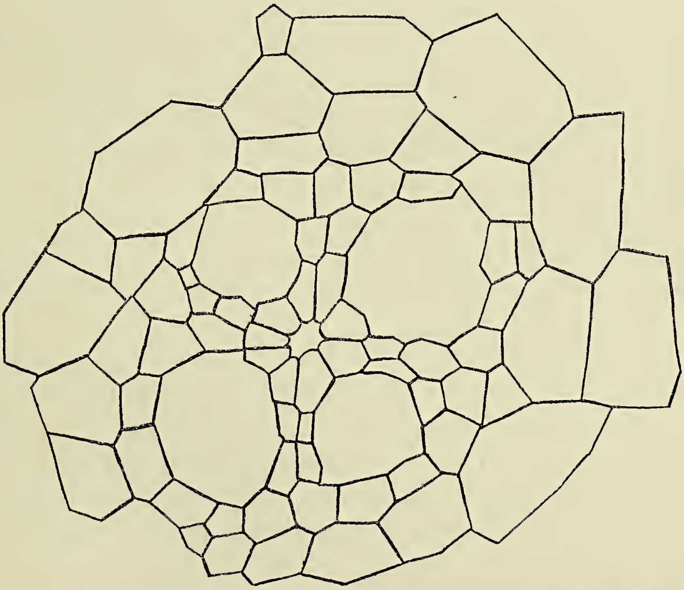
in Text-fig. 3 (the vascular tissue bundles are not filled in), where four phloem groups are seen round a narrow central lacuna. In the cortex is seen a difference between the inner and the outer part. The inner cortex has polyhedral, nearly isodiametric cells, about 35 μ across, while the cells of the outer cortex are radially stretched. On the limit between the two tissues is seen a small vascular bundle (Text-fig. 2), whereof a few may be found scattered in the stem. Intercellular spaces are lacking both in the cross-section (Text-fig. 2) and in the longitudinal section (Text-fig. 4), which is cut just under the apex of the stem. We have observed, however, narrow intercellular spaces measuring only the diameter of a single cell on the limit between the inner and the outer cortex ; they are also present in the leaves, where they are interrupted by narrow cell-bridges.



TEXT-FIG. I. Habit sketch of *Diplanthera Wrightii*, infected by *Ostenfeldiella Diplantherae*. Natural size.



Text-fig. 2. Part of a transverse section of a normal stem of *Diplanthera Wrightii.* To the right the central cylinder with four phloem groups, surrounded by the endodermis. In the cortex two layers can be distinguished, the inner having nearly isodiametric, the outer radially elongated cells. On the limit of the two tissues is seen a cortical strand. × 200.



Text-fig. 3. Transverse section through the central cylinder of the stem of *Diplanthera Wrightii.* Four phloem groups (not filled in) are seen surrounding a small central channel. Outside, the endodermis cells of the inner cortex. × 435.

THE BIOLOGY OF THE FUNGUS AND THE ANATOMY OF THE INFECTED DIPLANTHERA STEM.

As may be seen from Text-fig. 1, a long row of internodes is infected by the fungus, but the uppermost one is still fresh and living; it is thus evident that the shoots continue to grow in length in spite of the presence of the parasite. It was therefore to be expected that the youngest stages of our fungus might be found in the top of the stem, for which we prepared some slides with longitudinal sections through the growing apex of a shoot. The sections were stained with safranin-anilin-blue-goldorange. Text-fig. 4 shows a part of such a section, only the right side being entirely represented. Near the left side lies the central cylinder of the stem (*a*), and in the cortex to the right (*b* and *c*) are seen the two tissues previously mentioned, viz. the inner and the outer cortex. The nuclei of the host plant are shaded. In some cells are seen very small circles, one or more in each cell, representing uninucleate amoebae of the parasite, and these occur also in the very top of the growing zone. In the meristematic parts of the stem one always finds these amoebae uninucleate, hence it becomes evident that the nuclear divisions of the parasite are always followed by division of the myxoplasm itself—a fact that seems to be very rare in the Plasmodiophoraceae, these organisms having commonly a tendency to become plurinucleate very soon after infection.[1] Further, we must suppose that this occurrence of a single or a few uninucleate amoebae in the cells of the meristematic tissues of the host plant must be due to the fact that the divisions of the parasite are keeping pace with those of the host cells.

The uppermost embryonic internode, which is not yet deformed contains only uninucleate myxamoebae; the next, slightly swollen, but yet light-coloured internode, has in the lower part already pluri- to many-nucleate myxoplasms; while the sporogenous plasms and the spore-balls are, as a rule, found only in the third conspicuously swollen and brown-coloured internode (Pl. XLV, Fig. 2). The succeeding internodes are completely filled with mature spore-balls (Text-fig. 4).

As seen from Text-fig. 4 and Pl. XLV, Fig. 4, the parasite only infects the inner cortex of the stem,[2] no myxamoebae being met with in the central cylinder nor in the cells of the outer cortex. The vascular bundles in the cortex seem always to be free from myxamoebae. The outer cortex, which, as stated, is free from the fungus, is filled with starch grains, these latter not being developed in the tissue attacked by the parasite (Pl. XLV, Fig. 3).

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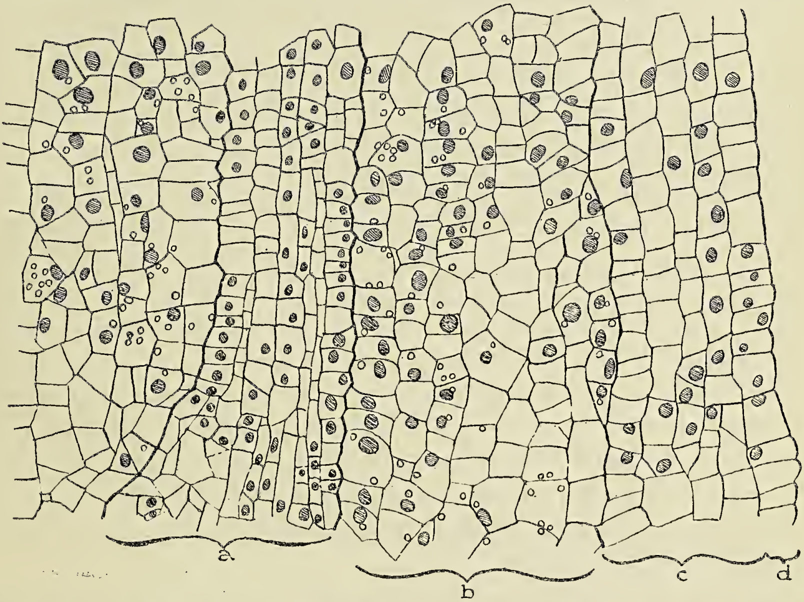
[1] The same is stated for *Sorosphaera Veronicae*, Schroet., by R. Maire and A. Tison: La Cytologie des Plasmodiophoracées et la classe des Phytomyxinées (Ann. Myc., 1909).

[2] Also in the host plants of *Sorosphaera Veronicae*, Schroet., and *Sorodiscus Callitrichis*, Lagerh. et Winge, the epidermis and the outermost layers of the cortex are free from the parasite, the central cylinder however being sometimes infected. Ö. Winge: Cytological Studies in the Plasmodiophoraceae. Archiv f. Botanik, vol. xii, no. 9, 1912, p. 18.

The infected cells grow to an abnormal size (Pl. XLV, Fig. 4), reaching about 125–200 μ across, the normal ones measuring only 35 μ, and their nuclei are dissolved towards the beginning of the sporogonic stage (Pl. XLV, Fig. 3). The mature spores have a brown and rather thick membrane, their diameter being about 4 μ. The spore masses entirely fill every infected cell as in the case of *Plasmodiophora* (Pl. XLV, Figs. 3, 4). By the pressure of the giant cells the elements of the surrounding outer cortex become stretched tangentially (Pl. XLV, Fig. 4), whereas normally they are radially elongated (Text-fig. 2). Unilateral attacks may occur, the central cylinder thereby being laterally displaced—in extreme cases almost to the periphery of the stem. As a rule the diameter of the infected internodes is enlarged to two to three times that of the normal ones, these latter being commonly about 1 mm. across.



TEXT-FIG. 4. Part of a median longitudinal section just under the growing apex of an infected *Diplanthera* stem. *a*, the central cylinder; *b*, the inner cortex; *c*, the outer cortex; *d*, the epidermis. The nuclei of the host plant are shaded. Uninucleate amoebae of *Ostenfeldiella* (represented by small circles) are seen only in the cells of the inner cortex. × 300.

The above-mentioned characteristic localization of our fungus in the tissues of *Diplanthera Wrightii* explains the fact that an infected stem can keep on growing in spite of the parasite. The central cylinder, as well as the outer cortex, escapes the attack; hence the transport of nourishment, assimilation, and respiration are not disturbed by the parasite.

The common picture of the attack, we suppose, must be painted as follows: Zoospores are swarming in the mud and penetrate the young apex of a *Diplanthera* stem, until the initials of the inner cortex are reached.

Here they divide, spreading only in the daughter-tissue of these initials, and being unable to reach either the outer cortex or the central cylinder. A rich intravaginal branching of the stem taking place near the growing apex, the branches naturally are in danger of being infected, and as Text-fig. 1 shows, this condition occurs, and may be supposed to be the rule.

Finally we give a diagnosis of the parasite, which is, no doubt, a real Plasmodiophoracea. In honour of Dr. C. H. Ostenfeld, who discovered the fungus, and who also on former occasions has contributed to the knowledge of these organisms,[1] we associate his name with our fungus, naming it:

Ostenfeldiella, Ferd. et Wge. gen. nov.

Genus caulicolum, submarinum Plasmodiophoracearum, *Plasmodiophorae* propius accedens, colore autem saturato sporarum nec non modo crescendi proprio satis distinctum. Etymologia a cl. doctore C. H. Ostenfeld, fungi inventore, de studio plantarum maris vascularium optime merito.

Ostenfeldiella Diplantherae, Ferd. et Wge. sp. nov.

Myxamoebae uninucleatae in partibus merismaticis caulium hospitis nunc singulatim, nunc plures in cellula singula inventae, corticem interiorem solum infestantes. Ad basim internodii secundi myxamoebae plurinucleatae sese formare incipiunt, et in internodiis sequentibus sporosori, cellulas pluries auctas, 125–200 μ diametro, totum implentes, iacent. Sporae globosae, $4–4\frac{1}{2}$ μ diametro, siccitate collabescentes, singulatim brunneolae, gregatim saturate brunneae, membrano satis crasso indutae, plasmate oleoso, flavescenti, refringenti farctae.

In internodiis brevibus caulium ascendentium limo sepultorum *Diplantherae Wrightii* Aschers., qui aggressu fungi ad modum siliquae *Ornithopodis sativi* usque ad 3 mm. diametro intumescunt, ad litus insulae St. Crucis Indiae occidentalis (Leg. C. H. Ostenfeld).

COPENHAGEN,
May, 1914.

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[1] See Ö. Winge, l. c., p. 16, and C. Ferdinandsen et Ö. Winge: *Plasmodiophora Halophilae* sp. nov. Centralbl. f. Bakt., Parasitenk. u. Infektionskr., Bd. xxxvii, 1913, p. 167.

EXPLANATION OF PLATE XLV.

Illustrating Messrs. Ferdinandsen and Winge's paper on Ostenfeldiella, a New Genus of Plasmodiophoraceae.

The figures are all microphotographs.

Fig. 1. Part of a median, longitudinal section just below the growing apex of a *Diplanthera* stem infected with *Ostenfeldiella Diplantherae*. Cf. Text-fig. 4. To the left the central cylinder; to the right the inner cortex, where the small amoebae of the parasite are to be seen. × 600.

Fig. 2. Longitudinal section on the limit between the second and the third internode (from the apex) of an infected *Diplanthera* stem. In the second internode (above) are seen only plurinucleate myxoplasms, whereas in the third internode (below) sporogenous plasms and spore masses have been developed. × 40.

Fig. 3. Part of a longitudinal section through an infected *Diplanthera* stem. Giant cells from the inner cortex with sporogenous plasms or spore masses. The nuclei of the host plant are about to be dissolved. To the left the unaffected cells of the outer cortex with starch grains. × 325.

Fig. 4. Part of a transverse section through the infected *Diplanthera* stem. The giant cells of the inner cortex are filled with spore masses of *Ostenfeldiella* (in some cells dropped out), whereas the central cylinder, as well as the outer cortex and the epidermis, is free from the parasite. × 50.

Fig. 5. Mature spores of *Ostenfeldiella Diplantherae*, partly collapsed from the action of the fixing agent, 90 per cent. alcohol. × 950.

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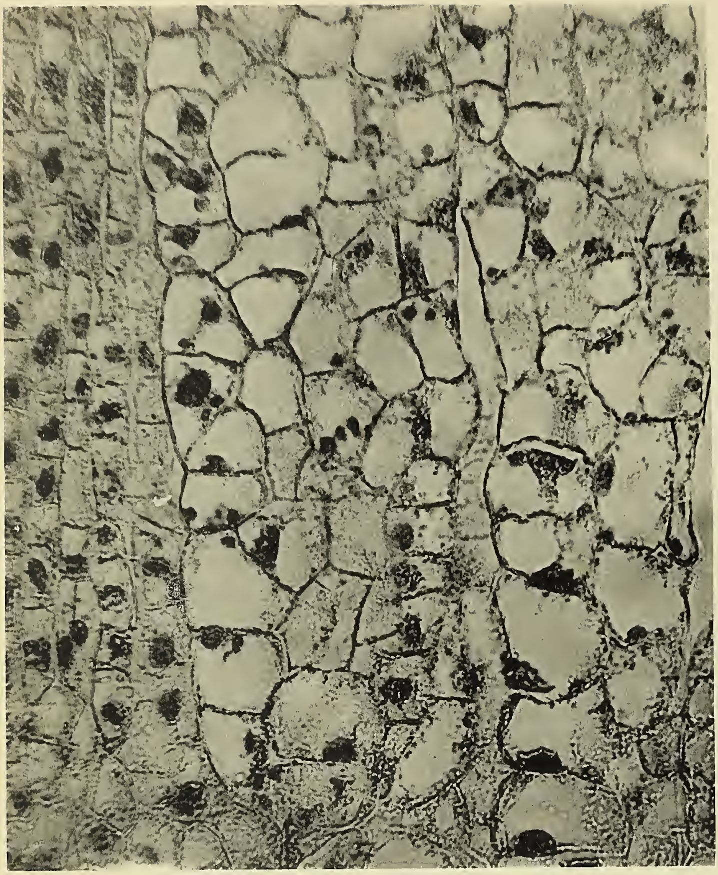

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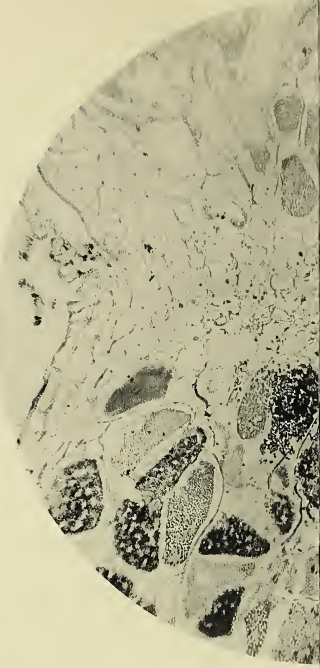

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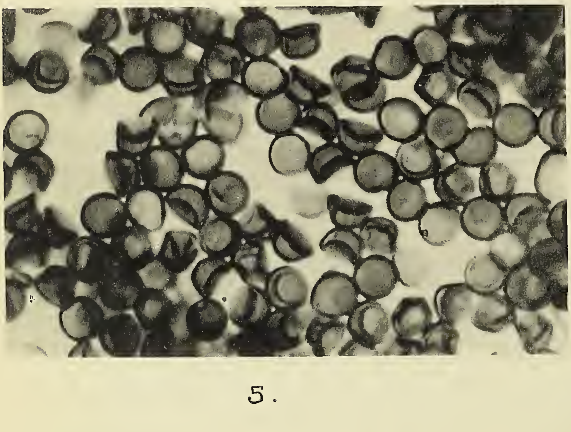

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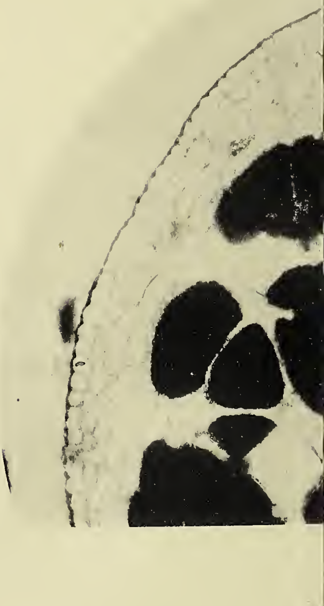
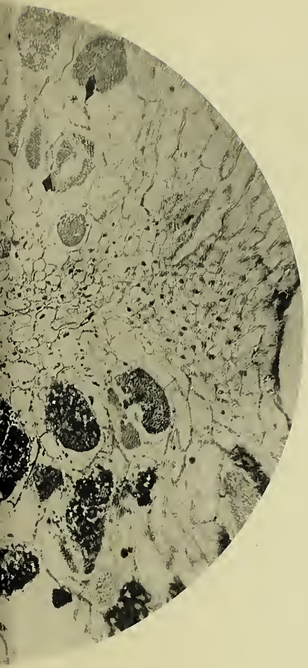
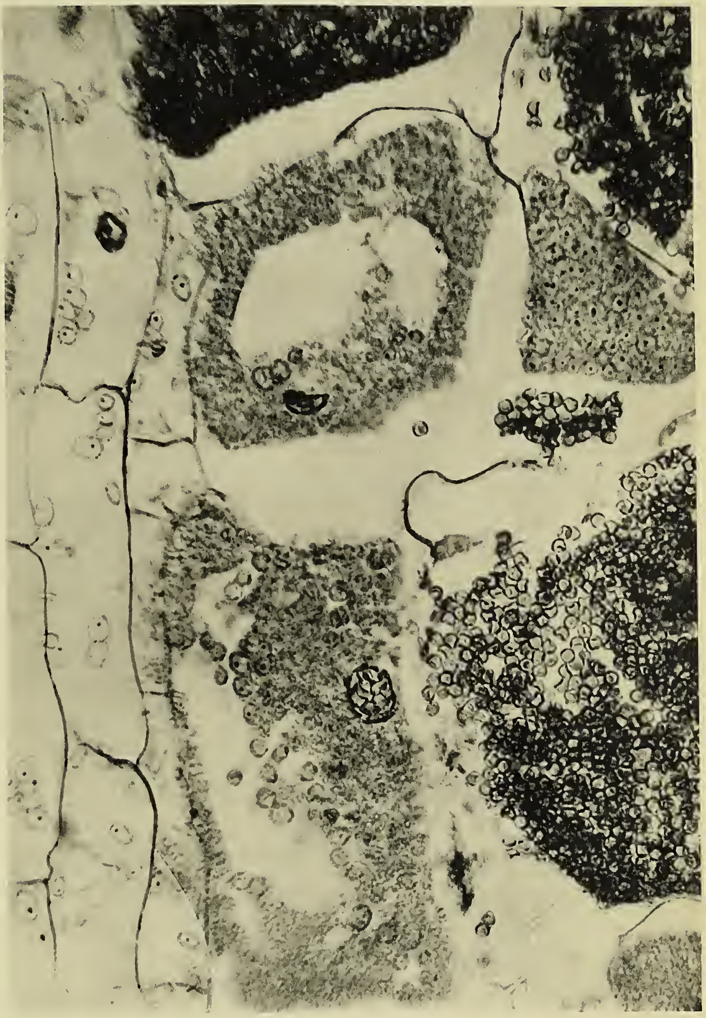


FERDINANDSEN & WINGE—OSTENFELDIELLA.

Vol. XXVIII, Pl. XLV.





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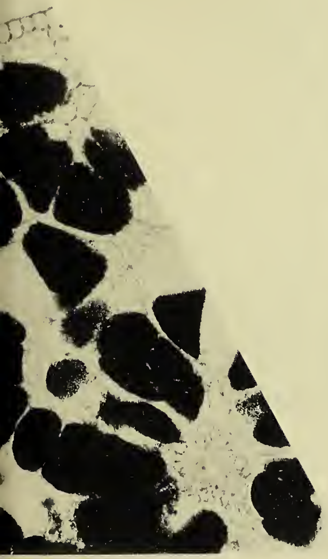

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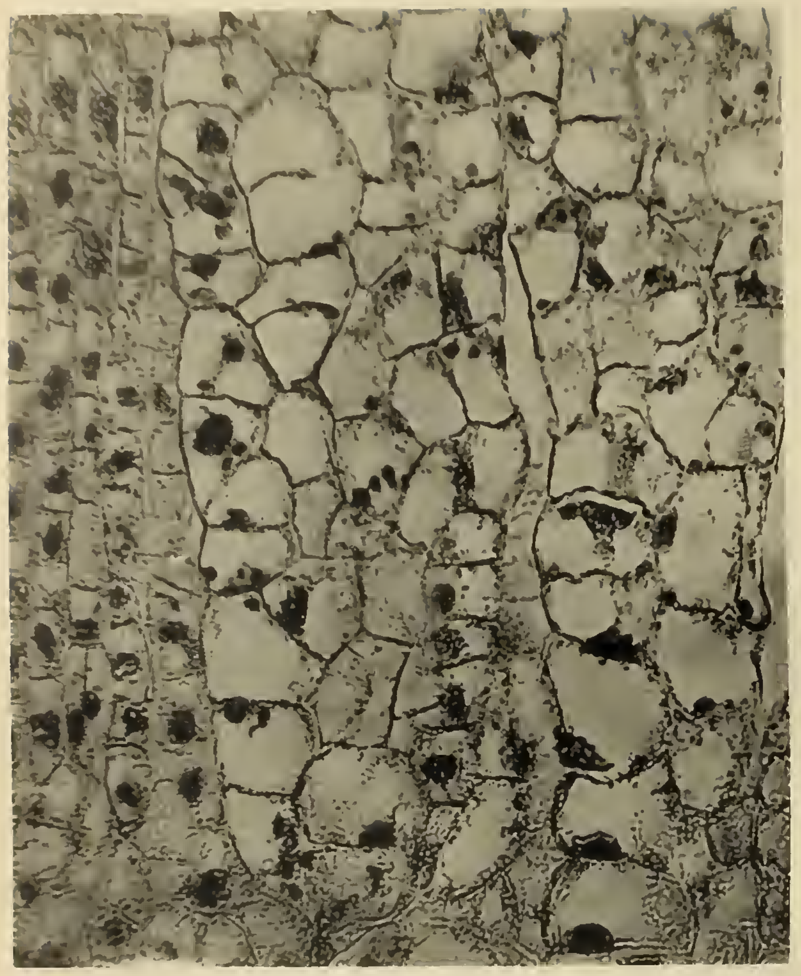

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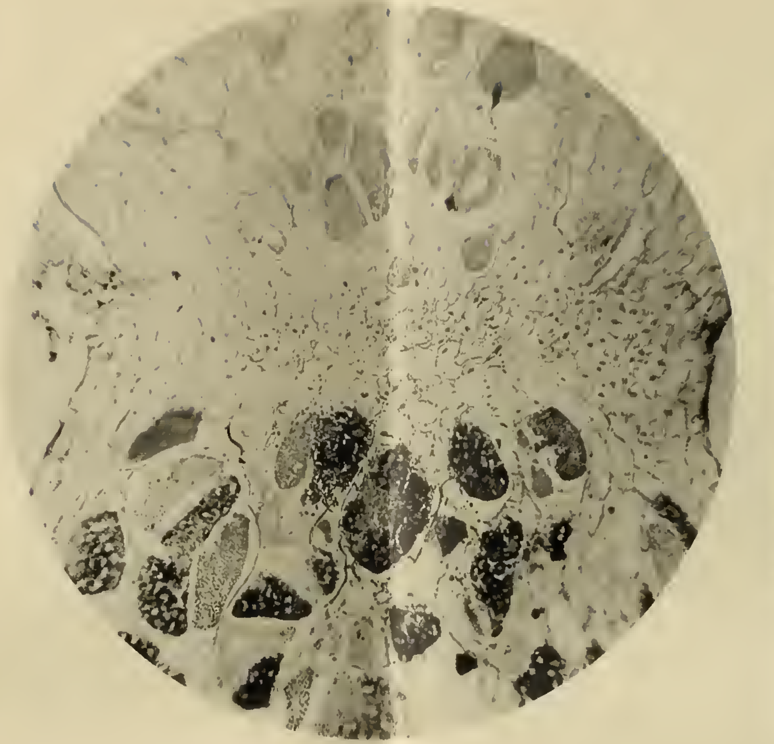

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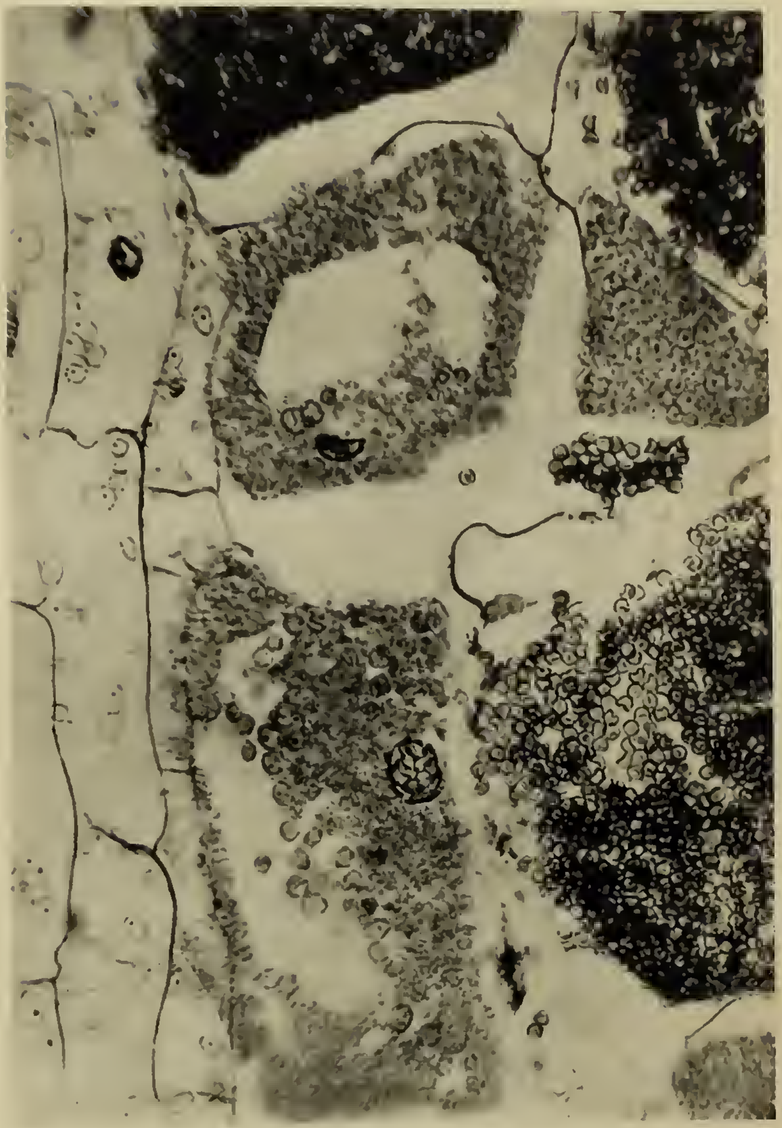

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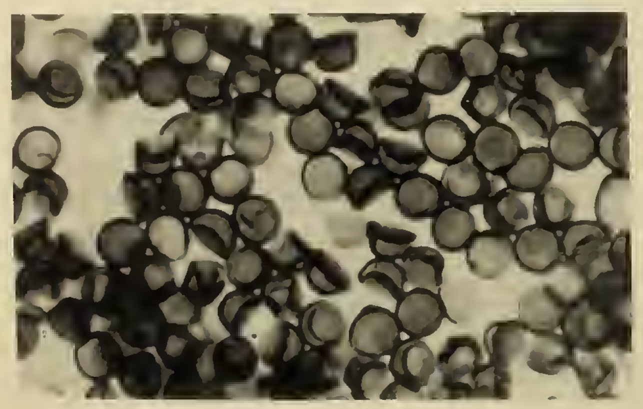

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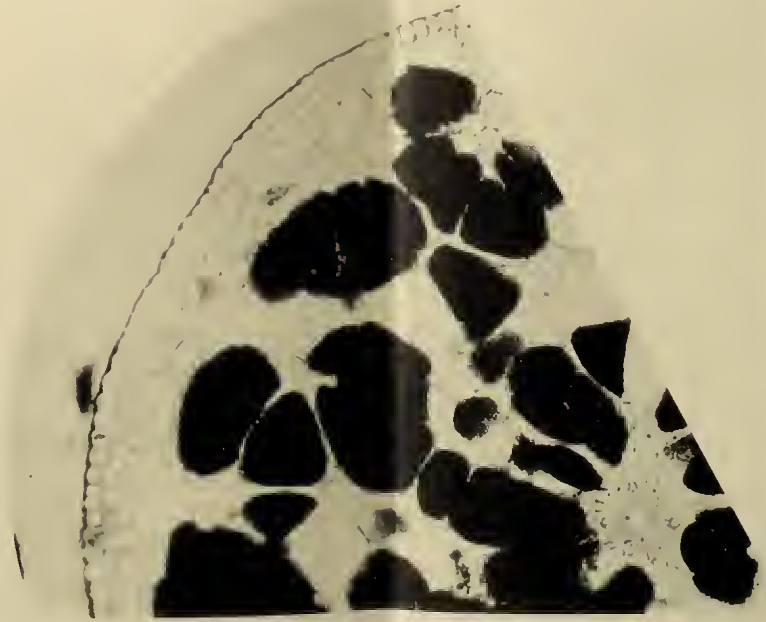

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FERDINANDSEN & WINGE—OSTENFELDIELLA.

The Structure and Affinities of Macroglossum Alidae, Copeland.

BY

DOUGLAS HOUGHTON CAMPBELL,

Leland Stanford Jr. University, California.

With Plates XLVI–XLVIII and eight Figures in the Text.

THE discovery of a new generic type in such a group as the Marattiales is an event of more than ordinary interest. Such a type is the recently established genus *Macroglossum*,[1] based upon a remarkable Fern sent from Sarawak, in Borneo.

A recent visit to the East Indies enabled the writer to visit Sarawak, and to collect material of this Fern at the original station, near Bau. Through the kindness of Mr. J. C. Moulton, Director of the Sarawak Museum, and Mr. Young, the head of the Gold Mining Works at Bau, the writer secured a supply of material, including a number of prothalli and young sporophytes as well as specimens of the adult plant.

The plants were found to be pretty abundant at the place where they were first collected, and ranged in size from the original specimen, a magnificent plant with fronds nearly four metres in length, to very young specimens just emerging from the prothallus.

Soon after the plant was first collected at Bau, Mr. Moulton found it near Mount Penrissen, also in Sarawak. The writer collected a single specimen of *Macroglossum* at the foot of Mount Mattang, about ten miles from Kuching, but the sporangia were very young, and it is possible that it was not the same species and may have been the same as a plant grown in the Botanical Gardens at Buitenzorg in Java under the name *Angiopteris Smithii*. This plant was only about half the size of the Bau specimens, and, to judge from the immature sori, they were more like *M. Smithii* and so perhaps identical with it.

After leaving Sarawak the writer visited Java, and in the Botanical Gardens at Buitenzorg a single fruiting specimen of *Macroglossum* was found. The plant had been in the gardens for many years, but its origin is unknown. Presumably it had been sent with other plants from Borneo by collectors, but there was no record of it, so it is impossible to say

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[1] Copeland, E. B.: Ferns of the Asiatic-Malay Region. Philippine Journal of Science, Botany, vol. iv, 1909.

[Annals of Botany, Vol. XXVIII. No. CXII. October, 1914.]

whether it came from Sarawak or from Dutch Borneo. The plant was somewhat smaller than the specimens of *M. Alidae* from Bau, but it was assumed to be the same species. A further examination, however, has shown a number of differences, especially in the sporangium, and it should probably be considered specifically distinct. The plant at Buitenzorg is the type of Raciborski's *Angiopteris Smithii*,[1] but there is no question that it should be transferred to the genus *Macroglossum*, and would therefore become *Macroglossum Smithii*.

The full-grown plant is very striking in appearance. From the short caudex arise the closely set, simply pinnate, massive leaves, which attain a length of nearly four metres. They stand almost vertical, and the appearance of the plant is very different from that of any species of *Angiopteris*, whose bipinnate leaves are much less closely set, and usually spread out widely from the caudex. The leaves are arranged spirally about the stem, which is apparently always radial in structure. The leaf-base is much enlarged and the stipules very conspicuous. The latter are united in front by a broad commissure (Pl. XLVIII, Fig. C). The leaves are simply pinnate, the pinnae, which are closely set, being sometimes more than 50 cm. in length. They are very thick, with quite smooth margins in the adult plant.

In general habit the plant more nearly resembles a large *Danaea* than it does *Angiopteris*. It also is superficially in habit like some of the large species of *Zamia*. This was remarked when the plant in the Buitenzorg gardens was compared with a *Zamia* growing not far away from it.

Plate XLVIII, Fig. B shows the caudex of a small individual with the leaf bases and the thick, much-branched roots.

The plants grew on a wooded bank above a small stream, and by examining the earth below the larger plants a number of prothalli were secured. As is usual with the Marattiaceae, the prothalli were most abundant where the earth had fallen away leaving freshly exposed surfaces. They are easily distinguished from the prothalli of ordinary Ferns by the more massive structure and the emergence of the primary leaf upon the upper surface of the gametophyte. Gametophytes of different ages were secured, some of unusually large size; and a number of young sporophytes of different ages were also collected.

THE GAMETOPHYTE.

As in all the Marattiales, the gametophyte of *Macroglossum* is a dark-green thallus with a very thick midrib which projects strongly on the lower surface (Pl. XLVI, Fig. 4). The wings of the thallus, also, are comparatively thick and never composed of more than a single layer of cells, as

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[1] Raciborski, M.: Über einige unbekannte Farne des Malayischen Archipels. Bull. Acad. d. Sciences, Cracovie, 1902.

in the leptosporangiate Ferns. The larger prothalli (e.g. Pl. XLVI, Fig. 5) are so much like a thallose Liverwort in appearance that it is necessary to make a close examination before their real nature is apparent. The presence of the old archegonia upon the ventral surface, however, can usually be easily demonstrated.

The younger prothalli show the familiar heart-shaped form, with a deep sinus, which becomes much less evident in the older stages and may become quite obsolete.

There was no opportunity for studying the germination of the spores and the early stages of the gametophyte, but it is highly probable that they would agree closely with those of *Marattia* and *Angiopteris*, described in detail by Jonkman.[1]

Pl. XLVI, Fig. 1 shows the youngest stage collected by the writer. This was 5 mm. in length, with a deep sinus in front. The posterior part was much narrower and the thickened midrib was confined to the anterior broader portion. The bottom of the sinus was occupied by a row of marginal initials of the usual type, and the first antheridia and archegonia had already been formed. The antheridia are developed first and are borne both upon the upper and lower surfaces of the thallus. On the upper side they form a median group a short distance behind the growing point of the prothallus, while on the lower surface they form two small groups on either side of the midrib. The group of young archegonia occupies the centre of the midrib between the two ventral groups of antheridia and the apex of the thallus (Fig. 2).

The older prothalli (Pl. XLVI, Figs. 3–5) show a less conspicuous sinus and may be almost orbicular in outline. In these older gametophytes the midrib is extremely conspicuous and its surface covered with great numbers of old archegonia which are visible to the naked eye as dark-brown dots. Numerous rhizoids are present in the basal region, but they are much less developed in the younger parts of the gametophyte.

The gametophytes are evidently long-lived and may reach a very considerable size. The specimen shown in Fig. 5 was nearly three centimetres in length. The apex had been injured and from the broken surface adventitious buds were forming. This multiplication of the prothallus by budding is not at all a rare phenomenon among the Marattiaceae.[2] A true dichotomy of the thallus apex probably also occurs, as it does in some other Marattiaceae. The gametophyte shown in Pl. XLVI, Fig. 7, with two nearly equal branches, probably was the result of the dichotomy of the original thallus apex.

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[1] Jonkman, H. F.: La Génération sexuée des Marattiacées. Arch. Néerlandaises, etc., t. xv, 1880.

[2] Campbell, D. H.: The Eusporangiatae. Carnegie Institution of Washington, Publication No. 140, 1911.

The apex of the gametophyte is occupied by a row of similar marginal cells, no one of which can be certainly identified as a single apical cell (Pl. XLVI, Fig. 12), although it is possible that the central cell of the marginal row may be a definite apical cell. In horizontal section the marginal cells are oblong, and narrower in front. A vertical section shows the usual form found in Fern prothalli, i.e. it appears semicircular with single basal segments extending the whole depth of the thallus (Pl. XLVI, Fig. 13).

THE ANTHERIDIUM.

The antheridium in *Macroglossum* is very much like that of the other Marattiaceae in its development. Antheridia are produced abundantly both on the dorsal and ventral surfaces of the prothallus. As in all of the eusporangiate Ferns, the mother-cell first divides into an inner and an outer cell, the former by subsequent divisions giving rise to the mass of spermatocytes; the outer one, by a series of intersecting walls, forming the cover. There is a more or less regular quadrant division of the inner cell (Pl. XLVI, Fig. 15), beyond which it is not possible to trace any regular sequence in the divisions of the sperm-cells.

Surrounding the mass of sperm-cells there are later cut off from the adjacent tissue the characteristic narrow, mantle cells (Figs. 18, 19, *m.*).

In the cover cells, as in other Marattiaceae[1] and in *Ophioglossum*, a series of intersecting walls is formed, the last division cutting out a small triangular opercular cell, which is destroyed at the time of dehiscence (Figs. 20, 21).

The number of spermatocytes is very large in *Macroglossum*, probably exceeding that of any other of the Marattiaceae. In the larger antheridia as many as a hundred spermatocytes may be seen in a single section. The spermatozoids, however, are smaller than is usual in the Marattiaceae.

The development of the spermatozoid is entirely like those of other Marattiaceae. The nucleus of the spermatocyte has no nucleolus and soon begins to assume the characteristic curved form (Pl. XLVI, Fig. 22), finally becoming much elongated. The blepharoplast, which at first is globular, very soon becomes elongated and forms a slender coiled body lying along the curve of the nucleus. From the blepharoplast the cilia are developed, but the details were not followed, as there seemed to be no peculiar features present. The free spermatozoids were not seen.

On comparing the spermatozoids with those of *Angiopteris* (Pl. XLVI, Fig. 24) they are found to be slightly smaller and the nucleus rather less elongated. In the latter respect *Macroglossum* perhaps more nearly resembles *Danaea* than it does *Angiopteris*.

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[1] See Campbell, loc. cit.

THE ARCHEGONIUM.

The archegonium of *Macroglossum* does not differ essentially from that of the other Marattiaceae, perhaps being most like that of *Angiopteris*. The mother-cell is divided into two by a transverse wall, the outer cell giving rise to the short neck, the inner one either first dividing into a basal cell and a central cell, or giving rise directly to the egg-cell and canal cells, the basal cell being suppressed (Pl. XLVII, Figs. 25, 26). The cover-cell, as usual, divides first by intersecting walls into four primary neck-cells, each of which undergoes division once or twice, so that each of the four series of neck-cells contains two or three cells (Figs. 29–31). It is possible that occasionally a third division occurs, but this is not usually the case.

The central cell, as in other Ferns, pushes up between the neck-cells, and this narrow upper part is separated from the lower broad portion as the primary neck canal cell, which subsequently divides into two either by the formation of a cross-wall or simply by a division of the nucleus. From the central cell a conspicuous ventral canal cell is cut off, as in *Marattia* and *Angiopteris* (Pl. XLVII, Figs. 30–32).

Where a basal cell is present it undergoes several divisions, but there seems to be no definite sequence of cell-divisions, and the basal cell is not always recognizable. Compared with *Angiopteris* (Fig. 33) the neck canal cells seem to be rather narrower in *Macroglossum*, otherwise the archegonia are very similar.

THE EMBRYO.

Only a few of the young embryos were found, but these showed a very notable difference when compared with the embryo of *Angiopteris*. In *Macroglossum* there is a very large suspensor developed, and in this respect it resembles *Danaea*,[1] but the suspensor is much larger.

Several one-celled embryos were seen. These filled up the venter of the archegonium and varied somewhat in shape (Pl. XLVII, Figs. 34, 35). In one case a two-celled embryo was seen (Fig. 36) which closely resembled a corresponding stage in *Danaea*. The upper part was somewhat pointed and formed the beginning of the suspensor. Another stage is shown in Fig. 39.

The suspensor was extraordinarily developed in the older embryos that were found, but differed in position from the suspensor of *Danaea*. In the latter, the suspensor, which is quite short, pushes the embryo downward, so that the long axes of the suspensor and embryo coincide. To judge from the few specimens secured in *Macroglossum* this is not the case. The suspensor grows laterally, pushing the embryo a long distance sideways from the archegonium (Pl. XLVII, Fig. 37). How far this large

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[1] See Campbell, loc. cit.

suspensor is derived from the original suspensor cell, and how much, if any, is derived from the hypobasal cell, must for the present remain doubtful.

The embryo, except for the suspensor, is much like that of *Angiopteris* at the same stage. It is much flattened, and near the centre of the upper surface could be seen a cell which may possibly represent the stem-initial (Fig. 38). The root-initial had not yet been differentiated, but it probably arises from the central region of the embryo, as it does in the other Marattiaceae.

Pl. XLVII, Figs. 40–42 show three transverse sections of an embryo in which the root is just differentiated. Fig. 42 passes through the foot, and shows part of the thick lateral suspensor (*sus.*). Fig. 41 is a nearly median section, and passes through the root-initial (*r.*), from which the first segment has been cut off. As in the other Marattiaceae, the root is a strictly endogenous structure, the initial cell arising near the centre of the embryo, probably from the lower part of the epibasal region. Fig. 40 shows the upper surface of the embryo. The large cell (*st.*) perhaps is the apical cell of the stem, but this is not certain.

No large embryos were found, the youngest sporophytes obtained having the cotyledon well developed and the root penetrating the ground. Sections of such sporophytes (Pl. XLVII, Fig. 43) show the same arrangement of the organs as in other Marattiaceae.[1] As is usual in these Ferns, the young sporophyte has the axis of the cotyledon coincident with that of the root, a common vascular bundle extending without interruption from the leaf into the root. The stem-apex is inconspicuous and consists of a shallow group of large cells, so much alike, that it is not easy to distinguish the actual apical cell. In Pl. XLVII, Fig. 44, it is probable that the large cell (*st.*) is the initial cell of the stem-apex, but this is not certain. In the few cases found, the initial cell in longitudinal section appeared oblong in form.

The second leaf makes its appearance very early, and there is soon discernible a strand of procambium cells, representing the second leaf-trace, connecting the leaf rudiment with the common bundle of the primary leaf and root. As in the other Marattiaceae there is no evidence at this stage of any true cauline bundle or stele.[2] The cotyledon, like that of *Angiopteris*, shows a good deal of variation. It is usually fan-shaped, with a more or less marked dichotomous venation, but there is frequently a more or less distinct midrib, and sometimes the cotyledon is almost lanceolate in outline, with a conspicuous midrib (Pl. XLVI, Fig. 6) and lateral veins.

Text-fig. 1 shows a series of cross-sections from a young sporophyte in which the second leaf was just discernible. The stem-apex is occupied by a group of meristematic cells, among which it is not easy to recognize with certainty a single initial cell.

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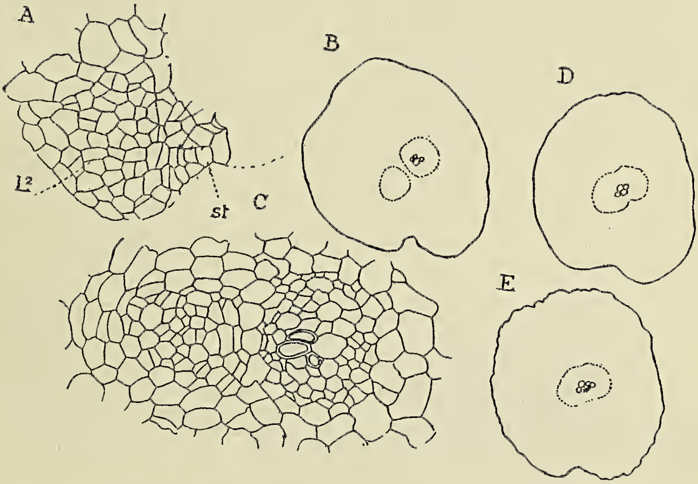
[1] See Campbell, loc. cit.

[2] See Campbell, loc. cit.

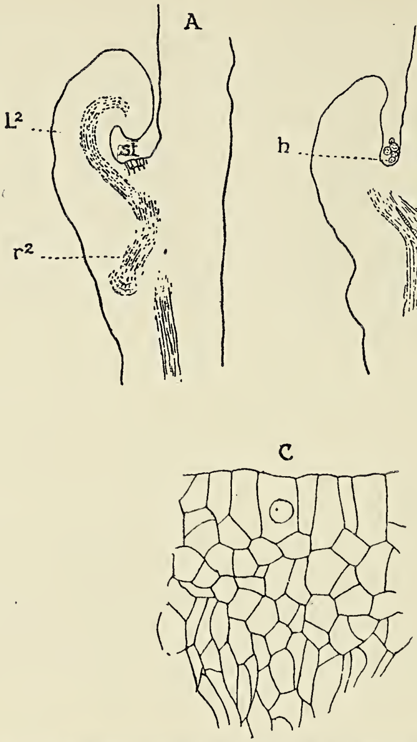
A section taken a short distance below the apex (B) shows the leaf-trace belonging to the cotyledon, in which the first tracheary tissue can be seen, and near it a second group of cells forming the trace for the second leaf.

The primary leaf-trace shows a small group of reticulate tracheides near its inner side and may be described as collateral, in this respect resembling *Danaea.* Sections taken still lower down show the coalescence of the two leaf-traces into a single strand, which is continued directly into the primary root. This axial bundle, after the fusion of the leaf-traces is complete, has the tracheary tissue forming a short band in cross-section occupying the middle of the bundle (E). The stele of the primary root is, usually at least, diarch, and probably derives its two xylems from the first and second leaf-traces respectively.



Text-fig. 1. A, Transverse section passing through the apical region of a young sporophyte, in which the second leaf (L^2) was just recognizable. *st.*, stem. × 180. B, Section taken a short distance below the apex, showing the separate traces of the first and second leaves. × 50. C, Central region of B. × 180. D, E, Sections taken further down, showing the union of the leaf-traces. × 50.

Text-fig. 2 shows two longitudinal sections of a sporophyte in which the second leaf was already pretty well advanced, and the third could also be seen. In A the second leaf is cut almost in the median plane, and the single vascular bundle is clearly seen. In B the section traverses the cotyledon, and shows the continuity of the vascular bundles of the cotyledon primary root. The junction of the second leaf-trace with the primary vascular strand is also shown. At this stage the second root is evident, arising close to the base of the second leaf-trace, with which its vascular cylinder is united. C shows the stem-apex of this sporophyte. The large oblong cell in which the nucleus is shown is probably the single initial, but the segmentation appears to be very irregular. Below the apex there is a mass of irregular isodiametric cells, and no evidence of a procambium cylinder, the elongated cells lower down being assignable to the leaf-traces.



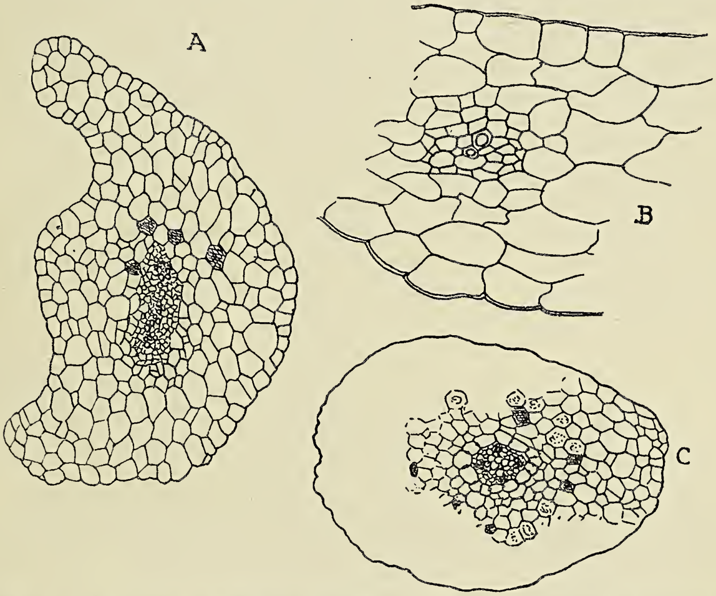
TEXT-FIG. 2. A, B, Two nearly median sections of a young sporophyte in which the second leaf, l^2, was well advanced; the stem-apex, *st.*, and second root, r^2, are shown in A. *h*, tannin hairs. × about 35. C, Stem-apex, more highly magnified.

THE COTYLEDON.

As has already been indicated, the form of the cotyledon is somewhat variable. The petiole is more or less strongly winged, but no stipules are developed. Text-fig. 3, A, shows a section of the petiole. The conspicuous vascular bundle is elongated in cross-section and is concentric in structure, the phloem being of nearly equal width all round, although somewhat less developed on the ventral side. No definite endodermis can be recognized. No mucilage ducts are present, but there are a number of tannin cells, filled with opaque dark contents. These form an irregular ring about the vascular bundle.

The epidermis is much alike on the two sides of the leaf, except that stomata are developed only upon the lower surface. The mesophyll does not show any development of palisade cells, but is composed of about half a dozen layers of nearly uniform cells with irregular intercellular spaces, which are somewhat more conspicuous upon the lower side of the leaf. The bundles of the delicate veins approach the collateral form, the small group of tracheary tissue being nearer the ventral side of the bundle and separated from it by about two layers of cells.

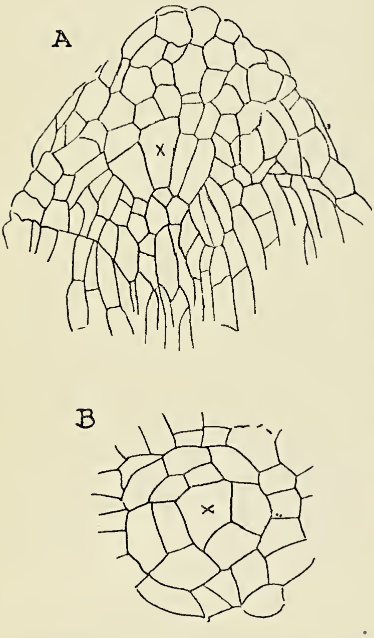
New leaves are formed in rapid succession. The second or third are much like the cotyledon, except that they are slightly larger. These early leaves have finely but distinctly serrated margins. The next leaves are lanceolate in outline, and in the later leaves the margins are almost entire except at the apex, which becomes much attenuated and is coarsely serrate. A considerable number, ten or more, of simple leaves are developed before any division of the lamina occurs. These simple leaves are much larger than has been observed in *Angiopteris*, and a greater number are formed. In this respect *Macroglossum* more nearly resembles *Danaea*, but these early leaves are much larger. Indeed, such a plant as that shown in Pl. XLVIII, Fig. A, much resembles the adult plant of *D. simplicifolia*. All of the early leaves have a conspicuous midrib from which the lateral veins extend. These veins usually fork close to the midrib, and the branches may extend to the margin, or they may fork once more. As in *Angiopteris* and *Marattia*, the first two leaves are destitute of stipules, which are first developed in the third leaf (Pl. XLVI, Figs. 8, 9). For a time, at least, a root is formed corresponding to each leaf, but whether this holds for the old sporophyte was not investigated.



TEXT-FIG. 3. A, Section of petiole of cotyledon. × 25. B, Section of lamina of cotyledon. × 180. C, Section of tetrarch root. × 50.

THE PRIMARY ROOT.

The primary root in *Macroglossum*, as in the other Marattiaceae, has a single initial cell. This appears in longitudinal section nearly triangular in outline, but is sometimes more or less truncate below (Text-fig. 4, A). The cross-section also is irregularly triangular in outline (Text-fig. 4, B). There was not available sufficient material to make a complete study of the apical growth of the primary root, and it is probable that an examination of a considerable number would show some variation in the form and segmentation of the apical cell. The root-cap, as in the other Marattiaceae, is derived in part from lateral segments of the apical cell, and the arrangement of the cells of the root-cap is quite irregular.



TEXT-FIG. 4. A, Longitudinal section of apex of first root, showing the single initial cell (*x*). × 250. B, Transverse section of root-apex. × about 250.

In the later roots, as in other Marattiaceae, the single initial cell is replaced by a group of similar initial cells, but no detailed study was made of these larger roots, which do not apparently differ essentially from the other forms that have been studied.

The primary root in all cases observed was diarch. The endodermis is not as conspicuous as is often the case, and its cells were not of uniform size. Outside the endodermis was an irregular zone of cells infested by a mycorrhiza similar to that in the prothallium. This occurrence of a mycorrhiza in the primary root has been noted in other Marattiaceae.[1]

The second root may show a triarch bundle (Text-fig. 5, D), and as the roots become larger the number of xylems increases.

The first and second leaves of the young sporophytes are destitute of stipules, which first make their appearance in the third leaf (Pl. XLVI, Fig. 9). These first stipules are small, with somewhat serrate margins. The stipules become large in the later leaves, and soon are very conspicuous (Pl. XLVI, Fig. 11). These large stipules are joined in front by a commissure, and completely enclose the next younger leaf.

The nature of the vascular system in the stem of the Marattiaceae has been examined critically by the writer,[2] and the conclusion was reached that no true cauline stele was present in the young sporophyte, whose

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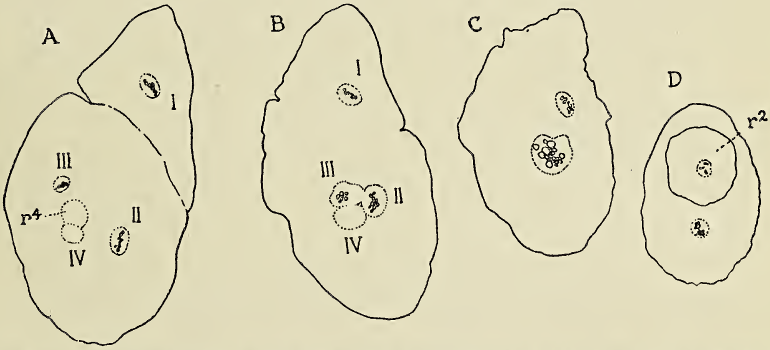
[1] See Campbell, loc. cit.

[2] See Campbell, loc. cit.

vascular system was composed entirely of leaf-traces. Several of the young sporophytes of *Macroglossum* were cut transversely for comparison with the other Marattiaceae, and, as in these, it was clear that the vascular system of the stem is at first composed of the leaf-traces alone. Text-fig. 5 shows several sections taken from such series. These sections resemble more nearly corresponding ones of *Danaea* than of *Angiopteris*, the leaf-traces remaining separate for a longer time than in *Angiopteris*. In the sporophyte figured below the fourth leaf-trace was just visible. A is taken a short distance below the stem-apex, and shows the four leaf-traces completely separated. Close to the fourth leaf-trace is the beginning of the fourth root (r^4). The leaf-traces in all cases show a concentric structure, and as the sections from the older part of the plant are examined, the leaf-traces are seen to converge, and finally unite into a central strand. In C the second and third traces are completely united, but the primary leaf-trace is still free. In D is shown a section in the transition region between the cotyledon and primary root. The triarch second root (r^2) is shown also in this section.



TEXT-FIG. 5. Four sections from a series of a sporophyte in which the fourth leaf was still very young. × 25. The roman numbers show the leaf-traces; r^4, the fourth root; r^2, section of the second root.

Sections from the middle and basal regions of an older sporophyte confirm the impression that the central vascular strand or 'stele' is the result simply of the fusion of independent leaf-traces, and there is no evidence of its being continued as an independent strand into the stem-apex. It is likely, however, as happens in the other genera, that later truly cauline bundles make their appearance, but this point was not investigated.

While *Macroglossum* resembles *Danaea* in the arrangement of the vascular bundles in the very young sporophyte, in other respects it is more like *Angiopteris*. In *Danaea* and *Kaulfussia*, mucilage ducts are formed at an early period, while they seem to be quite absent from the young sporophytes of *Macroglossum* and *Angiopteris*. In both of these, however, tannin sacs are present in the cotyledon and in the later leaves, as well as in the stem and roots. The solid stele formed by the fusion of the early leaf-traces is retained longer in *Macroglossum* than in *Danaea*, and in this respect it also shows unmistakable likeness to *Angiopteris*.

THE ADULT SPOROPHYTE.

The stem of the adult plant is a nearly globular caudex, a foot or more in diameter, completely covered by the closely set leaves. It appears to be strictly radial in structure.

The leaves are more numerous than in *Angiopteris*, and the habit of the plant more compact. About a dozen leaves are present in the full-grown individual.

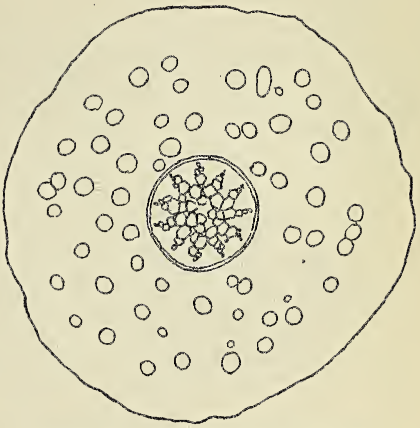
The leaves have a relatively short stipe and the numerous pinnae closely set, giving the leaf much the aspect of a large *Zamia*. The leaflets are almost 50 cm. in length, with a maximum width of about 7 cm. The margin is quite entire, and the apex is sharply acuminate. The closely set veins usually branch close to the midrib, and each branch forks again, each vein in the fertile leaves having above its extremity the very much elongated sorus. The sori are closely set, and form a broad solid band separated from the margin of the leaflet by a narrow border. The sporangia are so crowded as to be scarcely distinguishable, and the appearance of the elongated sorus is very much like the synangium of *Danaea*, this resemblance being increased by the fact that the sorus is sunk in a sort of trough, and only the upper part of the sporangia appears above the surface of the leaf. Along each side of the sorus is a fringe of fine hairs, the indusium, which is much better developed than it is in *Angiopteris*. The leaf-base is much enlarged and the stipules extremely conspicuous (Pl. XLVIII, Fig. C). The stipules are connected by a large commissure in front.

Pl. XLVIII, Fig. B shows the base of a small plant which, except for its size, is much like the fully grown one. The numerous stout roots are extensively branched, more so than is the case in *Angiopteris*.

Pl. XLVII, Fig. 46 shows a section of the petiole of a leaf from this plant. There were eighteen vascular bundles arranged in a circle inside the margin, and four bundles towards the centre of the section, one of the latter being much larger than the others. Three mucilage ducts (*m.*) were present. Separated from the epidermis by three or four layers of parenchyma cells, there is a broad band of sclerenchyma.

Sections of the base of the stem of this same plant (Pl. XLVII, Fig. 45) showed a structure very much like that of the younger sporophyte. In the centre was a single large bundle, somewhat crescentic in section, and four bundles of smaller size, arranged in pairs, and probably representing double leaf-traces. Several mucilage ducts (*m.*) were present in the neighbourhood of the vascular bundles, but none were seen in the cortical region.

THE ROOTS.



TEXT-FIG. 6. Section of root of an older sporophyte. × 16.

The roots (Text-fig. 6) differ from the other Marattiaceae that have been examined in the complete absence of mucilage ducts. There are numerous large tannin sacs, some of which approach the size of mucilage ducts, but the latter seem to be quite lacking. In the outer part of the cortex a few scattered sclerenchyma cells were seen, but there is no distinct ring of sclerenchyma such as occurs in the roots of *Danaea*. The stele of the root is much like that of other Marattiaceae, but the xylems are less distinct than is usually the case, and extend almost to the centre of the cylinder. The specimen figured had eleven xylems, but there is doubtless some variation in this respect.

THE SPORANGIA.

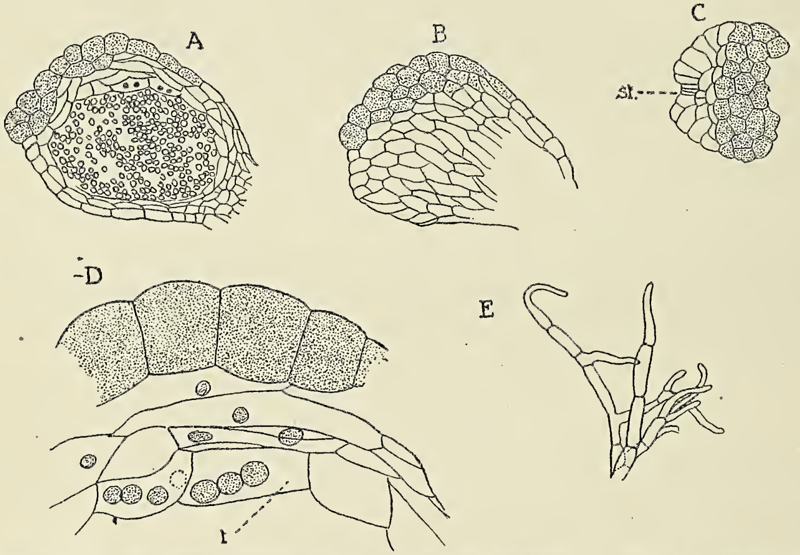
The sorus in *Macroglossum* is very much longer than in any species of *Angiopteris*, and much more nearly resembles that of *Archangiopteris*. It may reach a length of 8 mm., and the number of sporangia in *M. Alidae* about 70. This is something more than half the maximum number in *Archangiopteris*.[1] The appearance of the sorus is very different from that of *Angiopteris*, as the individual sporangia are less evident, and they are very closely crowded, so that the elongated sorus, as we have seen, closely resembles a true synangium like that of *Danaea*. In reality, however, the sporangia are quite separate, as in *Angiopteris*. The resemblance of the sorus to the synangium of *Danaea* is increased by the development of an elevated ridge between the sori, which are thus partially sunk below the surface of the leaf. The sorus is surrounded by a conspicuous fringe of indusial hairs, which arise from the base of the sporangia and reach to the top. These indusial hairs, which branch repeatedly, are much more evident than in any species of *Angiopteris*, and are very similar to those found in *Archangiopteris*.

The sporangia (Text-fig. 7) also are more like those of *Archangiopteris* than they are like those of *Angiopteris*. Owing to their position they are

[1] Christ, H., and Giesenhagen, K.: Pteridographische Notizen. Flora, lxxxvi, 1899.

more compressed laterally than in *Angiopteris*, and are also much less convex on the dorsal surface. In *Angiopteris* the whole dorsal surface of the sporangium is composed of cells with dense brown contents, probably tannin. These tannin cells in *Macroglossum* are confined to the upper free portion of the sporangium, and are also much less developed on the inner or ventral face.

The annulus, which is so conspicuous in *Angiopteris*, is almost entirely absent in *Macroglossum Alidae*, being represented by a single transverse row of about half a dozen cells (Text-fig. 7, C), which are hardly at all thickened even in the ripe sporangium.



TEXT-FIG. 7. A, Sporangium, median longitudinal section. × 75. B, Surface view of sporangium. C, Apex of sporangium, showing place of dehiscence, *st.* The shaded cells are tannin (?) cells. D, Wall of sporangium, showing the multinucleate tapetal cells, *t*. × about 250. E, Indusial hair. × 75.

As in other Marattiaceae, the tapetal cells remain intact until a late period and become multinucleate (Text-fig. 7, D, *t*). Between the tapetum and the surface of the sporangium there are two or three layers of cells. The dehiscence is by a cleft along the inner face, on either side of which the cells are narrower, but the band of narrow cells bounding the cleft is less conspicuous than in *Angiopteris*.

Text-fig. 7, E, shows one of the much-branched indusial hairs.

The sorus, as already stated, lies above the end of a vein, and between the sori lies the conspicuous ridge which reaches about half-way up the sporangia, so that their position recalls that of the synangia of *Danaea*, where, however, the ridge between the synangia is narrow and reaches above the level of the synangium.

A section of the leaf passing through the sorus (Text-fig. 8) shows the conspicuous bundle belonging to the vein above which the sorus lies; and below the vein is a large mucilage duct. Occupying the centre of the ridge between the sori is a group of sclerenchyma cells, which form a pseudo-vein, which is very evident in horizontal sections. These sclerenchyma cells stain strongly with safranin. Very large intercellular spaces are developed in the mesophyll, whose cells are very much elongated. A very conspicuous palisade tissue is present below the ventral surface of the leaf, and this palisade tissue is separated from the epidermis by a single layer of colourless hypodermal cells. Stomata were observed only on the dorsal epidermis.

TEXT-FIG. 8. Cross-section of a sporophyll passing through a sorus. × about 35. *m.*, mucilage duct; *in.*, indusial hairs.

THE SYSTEMATIC POSITION OF MACROGLOSSUM.

In view of the fact that Raciborski considered the specimen of *Macroglossum* growing at Buitenzorg to be a species of *Angiopteris*, it may be well to summarize the points of difference between *Macroglossum* and *Angiopteris*.

The gametophytes are very similar, but the antheridia of *Macroglossum* develop a much greater number of spermatozoids than are found in *Angiopteris*. The spermatozoids are somewhat smaller and relatively shorter, resembling in the latter respect those of *Danaea*.

The embryo differs from that of *Angiopteris* in the very important fact that it develops a large suspensor, thus again suggesting a remote relationship with *Danaea*. The later development of the embryo, while perhaps most nearly approximating *Angiopteris*, nevertheless, is in some respects intermediate between that of *Angiopteris* and the other Marattiaceae in having the early leaf-traces remain separate longer than is the case in *Angiopteris*.

The adult sporophyte is like *Danaea* and *Archangiopteris* in the simply pinnate leaves. The sorus is made up of separate sporangia as in *Angiopteris*, but the sporangia are much more crowded and very much more numerous, and in this latter respect it is most like *Archangiopteris*. The number of sporangia in the sorus of *Angiopteris* usually ranges from about 7 to 15; the maximum number seen by the writer was about 25 in *A. Brooksii*, Copeland. In *Macroglossum Alidae* the maximum exceeded 70; in *Archangiopteris*, according to Christ and Giesenhagen, it may reach 120. The sporangia themselves, to judge from the figures of *Archangiopteris*, are more like the latter than they are like *Angiopteris*. From the latter they differ markedly in shape, and in the much less developed annulus. The difference in shape is correlated with the sinking of the sorus between the ridges and by the greater crowding. The indusial hairs are much better developed in *Macroglossum*, and also are much more like those of *Archangiopteris* than *Angiopteris*. In the latter, the conspicuous ridges between the sori are quite wanting and the sporangia stand quite free above the leaf; the sori are usually separated from each other by a considerable interval. A section of the leaf in *Angiopteris* shows no trace of the large mucilage ducts found in *Macroglossum*, and the palisade tissue is not so well marked, although it is well developed in some species.

While it is evident that *Macroglossum* should be included in the Angiopterideae, its affinities seem to be with *Archangiopteris* rather than with *Angiopteris*. The resemblances to *Danaea* are interesting, but it is doubtful whether they indicate anything more than analogies. It is of course possible that the elongate sorus with its individual sporangia may have been derived from the solid synangium of such a form as *Danaea*, but there is no positive evidence that such has been the case.

Of the two species examined, *M. Smithii*, the plant growing at Buitenzorg, is more like *Angiopteris* than is *M. Alidae*. The sori are less elongated, and the small ones are not very unlike those of *Angiopteris*. Moreover, the sporangia themselves have the annulus somewhat better developed than is the case in *M. Alidae*. However, the differences between this species and *M. Alidae* are not very marked, and it is evident that the two are closely related, although probably specifically distinct.

SUMMARY.

1. The genus *Macroglossum* comprises two known species, *M. Alidae*, Copeland, first collected at Bau in Sarawak, Borneo, and afterwards at Mount Penrissen, Sarawak; *M. Smithii* (*Angiopteris Smithii*, Raciborski),

growing in the Botanical Gardens at Buitenzorg, Java, but of unknown origin.

2. The full-grown plant approaches in size the larger species of *Angiopteris* ; the leaves may reach a length of 4 metres. The plant differs much in appearance from *Angiopteris*, the leaves being more numerous and upright, and simply pinnate. The very much elongated and partially immersed sori are also strikingly different from those of *Angiopteris*.

3. The gametophyte is much like that of *Angiopteris*. It may reach a length of 3 centimetres. Branching is not uncommon, either by adventitious buds, or occasionally by a dichotomy of the growing point.

4. The antheridia occur both on the dorsal and ventral surfaces. They are of the usual type, but the number of sperm-cells is probably greater than in any other Marattiaceae. The spermatozoids are relatively small and less elongated than those of *Angiopteris*.

5. The archegonia are much like those of *Angiopteris*.

6. The embryo develops a conspicuous suspensor and thus resembles the embryo of *Danaea*.

7. The later development of the embryo seems to correspond to that of the other Marattiaceae. The apical cell of the stem is not always clearly demonstrable.

8. As in the other Marattiaceae, no true cauline stele is developed in the young sporophyte, and the vascular system of the axis is composed entirely of leaf-traces.

9. The structure of the axis in the young sporophyte is to a certain extent intermediate between that of *Danaea* and *Angiopteris*.

10. The cotyledon is much like that of *Angiopteris* ; the first leaves, about ten, remain simple. Stipules first appear in the third leaf. The first leaf-trace shows a collateral structure.

11. The primary root is usually diarch. A single root is formed for each of the early leaves. The primary root has a single initial cell.

12. The leaf of the adult sporophyte differs from that of *Angiopteris* in its simply pinnate form, as well as in the anatomy of the leaflets. The latter show a conspicuous mucilage duct below the sorus, and a better developed palisade tissue.

13. The sporangia of *Macroglossum*, especially *M. Alidae*, are smaller than those of *Angiopteris*, and very much more numerous. The sorus is much more compact, and partially sunk in a trough formed by elevated ridges between the sori, while in *Angiopteris* no such ridges are present. The annulus is very slightly developed in *Macroglossum*, and the indusial hairs very much more conspicuous than in *Angiopteris*.

14. The roots of the adult plant have no mucilage ducts, thus differing from the other Marattiaceae that have been described.

15. *Macroglossum* should undoubtedly be placed in the Angiopteridiae,

but its affinities are with *Archangiopteris* rather than with *Angiopteris*. Of the two species examined, *M. Smithii* is nearer to *Angiopteris* than is *M. Alidae*.

EXPLANATION OF PLATES XLVI–XLVIII.

Illustrating Professor Douglas H. Campbell's paper on the Structure and Affinities of *Macroglossum Alidae*, Copeland.

PLATE XLVI.

Fig. 1. Young gametophyte seen from below, showing the first antheridia. × 2.

Fig. 2. The apical region of the same. × 20. Young archegonia.

Figs. 3, 4. Older gametophytes. × 2. Fig. 4 seen from below, showing the thick midrib covered with old archegonia.

Fig. 5. An old gametophyte, showing the formation of adventitious buds, *b*. × 2.

Fig. 6. Gametophyte, with very young sporophyte attached. × 2.

Fig. 7. A large gametophyte which has branched dichotomously; each branch has a sporophyte. × 2.

Fig. 8. Sporophyte with three fully developed leaves, still attached to the gametophyte, *pr*. × 2.

Fig. 9. Base of the third leaf, showing the stipules, *st*. × 4.

Fig. 10. Young fifth leaf. × 2.

Fig. 11. Base of a young sporophyte, with eight fully developed leaves; *st*., stipules. × 2.

Fig. 12. Surface view of the apical region of the gametophyte. × 365.

Fig. 13. Longitudinal section passing through the apical region of the gametophyte. *x*, one of the marginal initial cells; ♀, young archegonium. × 365.

Fig. 14. Cross-section of the gametophyte, showing the position of the mycorrhiza, *m*. × 90.

Figs. 15–19. Stages in the development of the antheridium, seen in longitudinal section; Figs. 15–17, × 600; Figs. 18, 19, × 365. *m*., mantle cells.

Fig. 20. Surface view of a young antheridium.

Fig. 21. Surface view of an older antheridium, showing the triangular opercular cell.

Figs. 22, 23. Spermatocytes, showing the development of the spermatozoid. *bl*., blepharoplast. × about 900.

Fig. 24. Spermatozoid of *Angiopteris*. × about 900.

PLATE XLVII.

Figs. 25–31. Development of the archegonium, seen in longitudinal section. × 600. *o*., egg-cell; *v.c*., ventral canal cell.

Fig. 32. Full-grown archegonium, showing two distinct neck canal cells. × 365.

Fig. 33. Two archegonia of *Angiopteris*. × 365.

Fig. 34. Archegonium containing a one-celled embryo. × 600.

Fig. 35. One-celled embryo, elongated laterally.

Fig. 36. Two-celled embryo; the upper (hypobasal) cell becomes the suspensor.

Fig. 37. Median section of an older embryo, showing the very large suspensor, *sus*. × about 90.

Fig. 38. The same embryo more highly magnified; the large shaded cell is perhaps the initial cell for the stem-apex.

Fig. 39. A young embryo which was arrested in its development.

Figs. 40–42. Three horizontal sections of an embryo somewhat older than that shown in Fig. 38. The initial of the root, *r*., is shown in the middle section. *st*., stem-initial (?); *sus*., base of the suspensor.

Fig. 43. Longitudinal section of a sporophyte which has broken through the gametophyte. × 35. The vascular strand of the cotyledon and primary root are continuous: the second leaf (L^2) was just visible, and also the beginning of the second root (not visible in this section).

Fig. 44. The apical region of the same. × 365. *st.*, stem-initial (?); L^3, initial for third leaf (?); *tr.*, tracheide from the central region of the common primary vascular bundle.

Fig. 45. Cross-section of the axis of an older sphorophyte. × 2. Five vascular bundles and as many mucilage ducts are visible. *r.*, *r.*, roots.

Fig. 46. Cross-section of the petiole of a leaf from the same plant. × 2.

Fig. 47. Margin of the leaf of a mature sporophyte, showing the much elongated sori. × 3.

PLATE XLVIII.

Fig. A. Young sporophyte of *Macroglossum Alidae*. × $\frac{2}{3}$.

Fig. B. Caudex of a young sporophyte, showing the leaf-bases and the much-branched roots.

Fig. C. Leaf-base from a somewhat older specimen.

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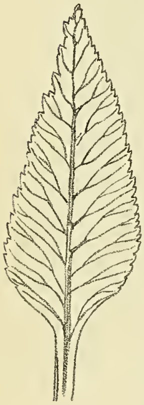

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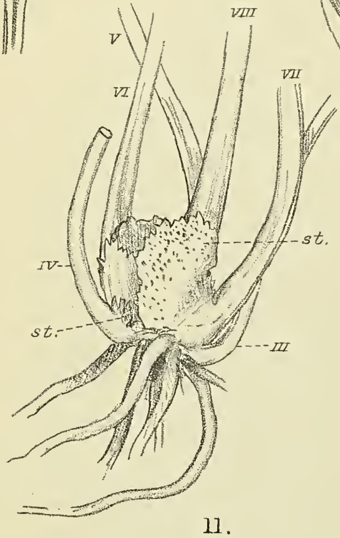

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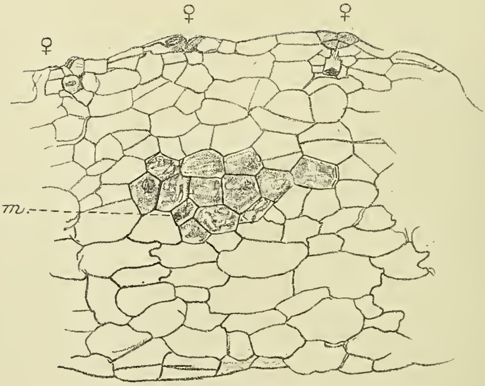

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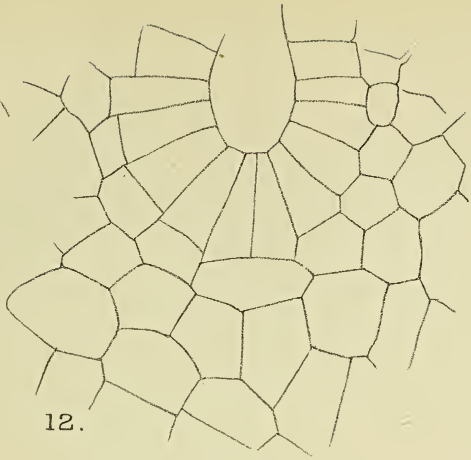
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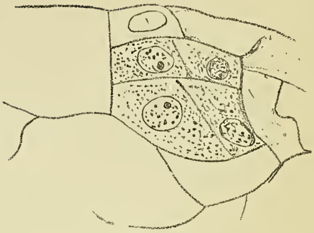
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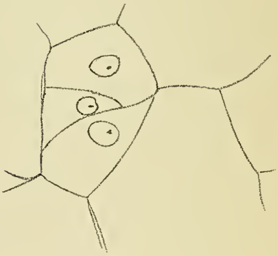
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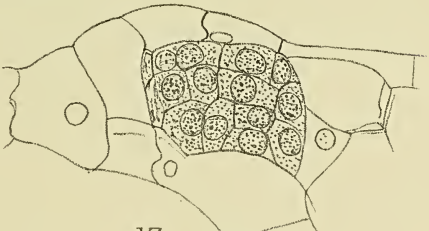
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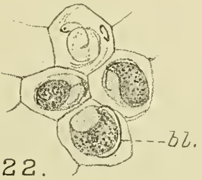

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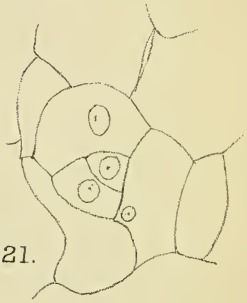
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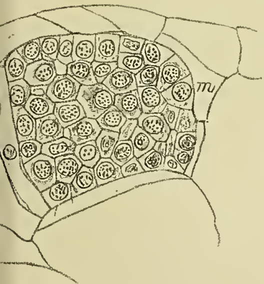

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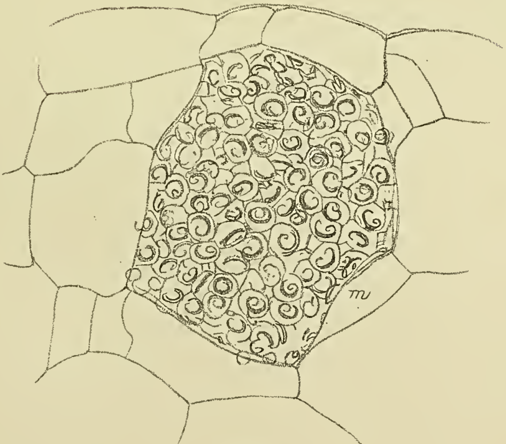

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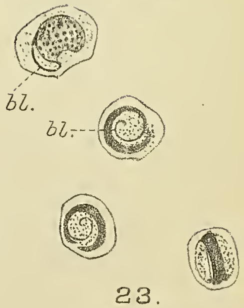

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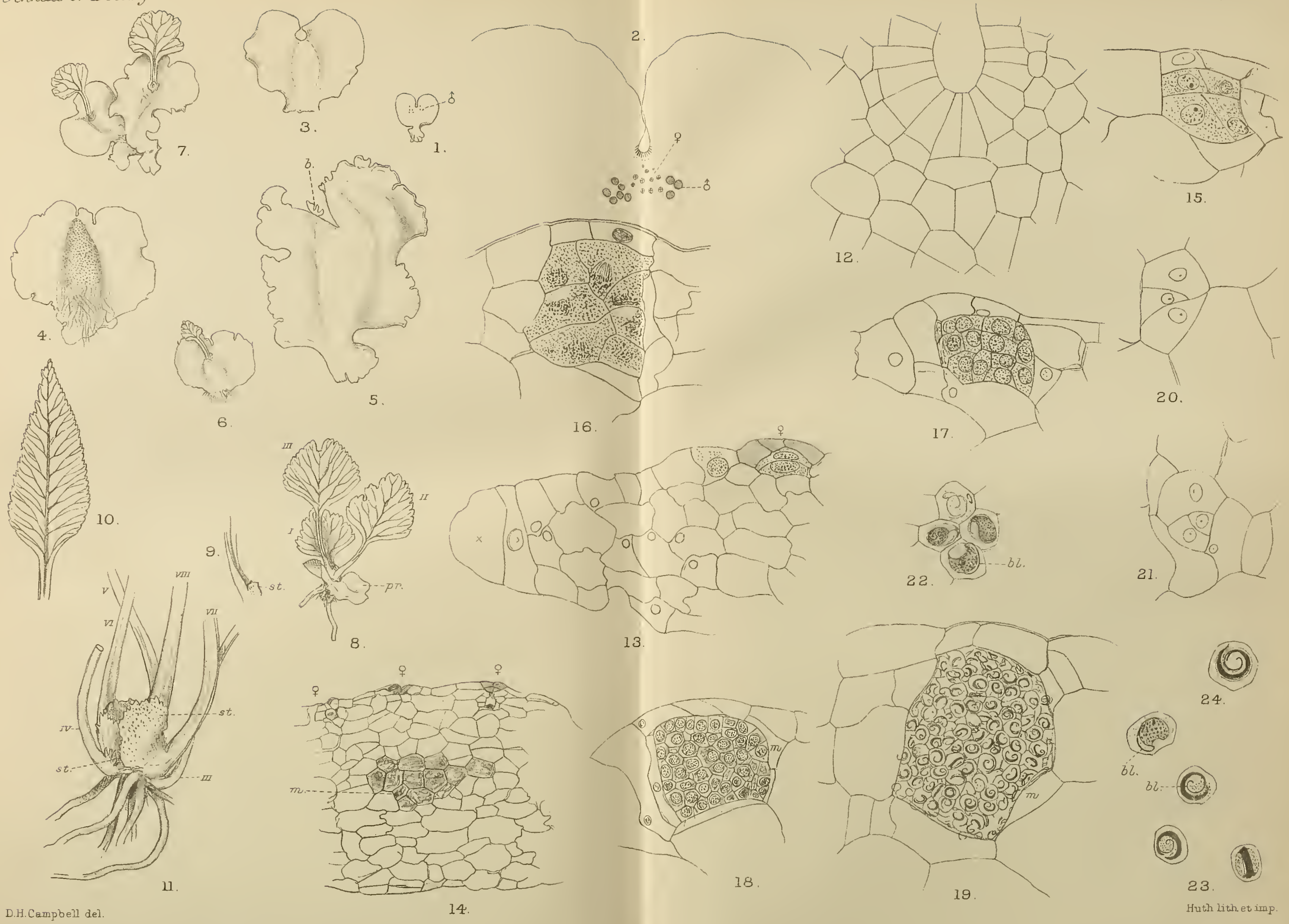

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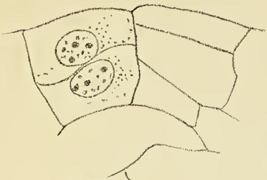

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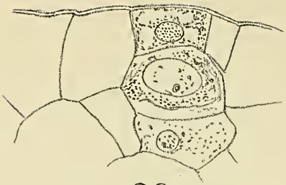

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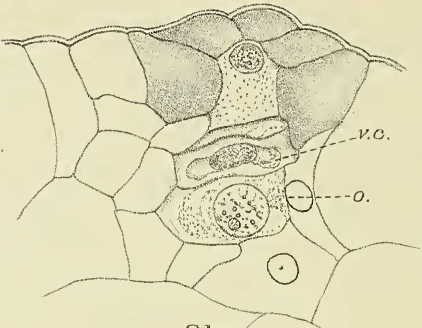

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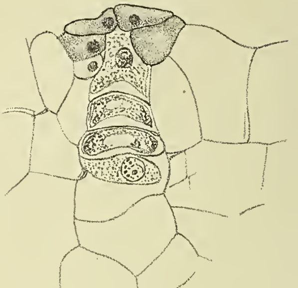

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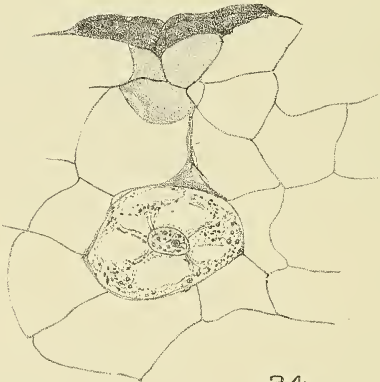

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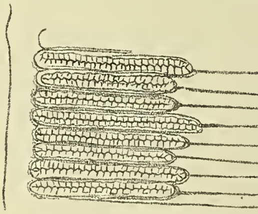

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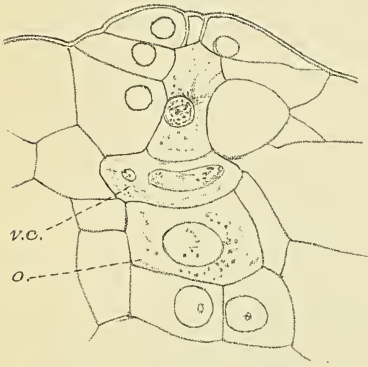

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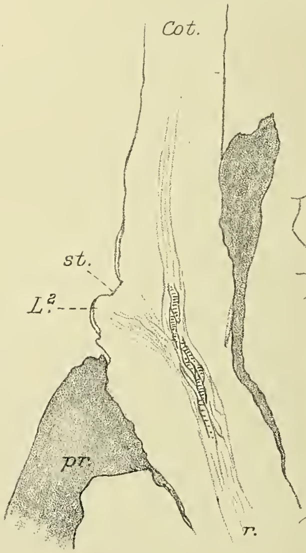

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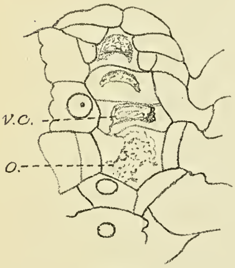

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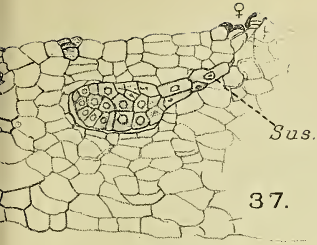

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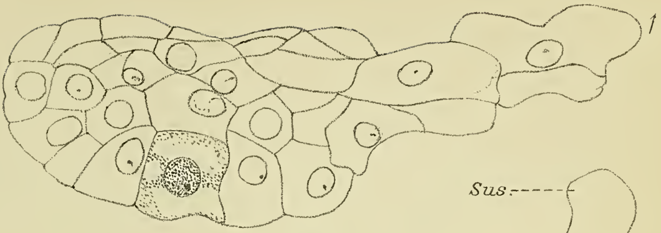

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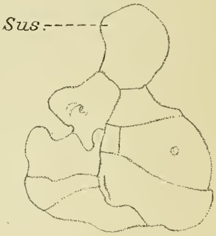

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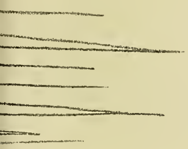

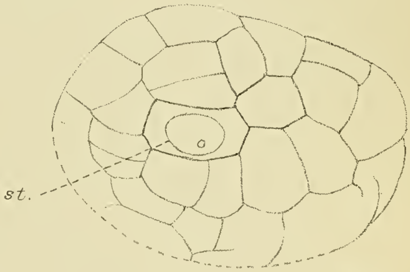

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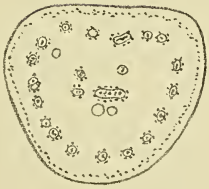

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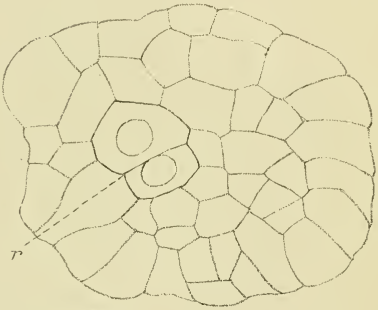

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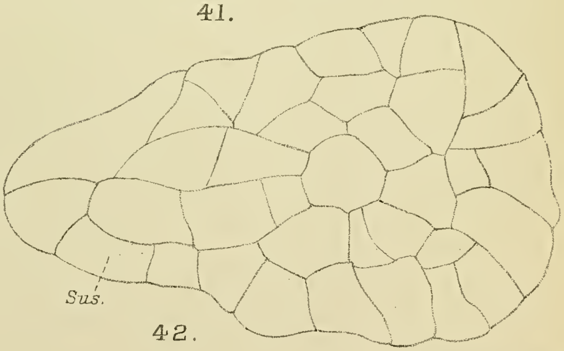

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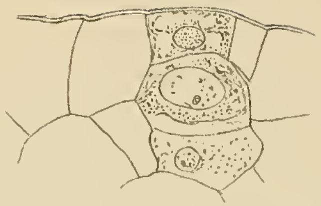

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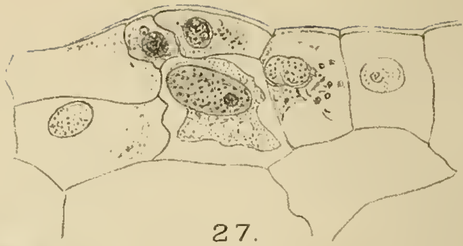

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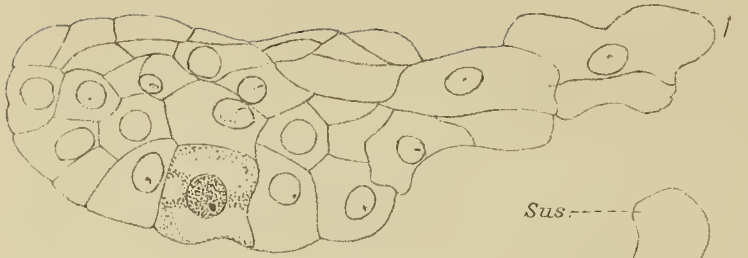

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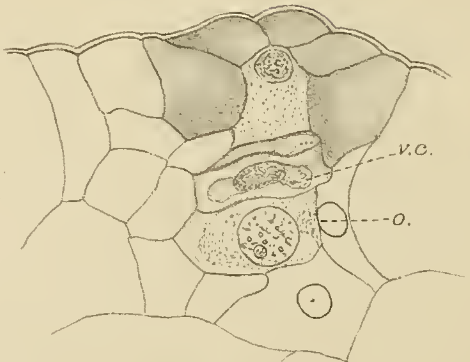

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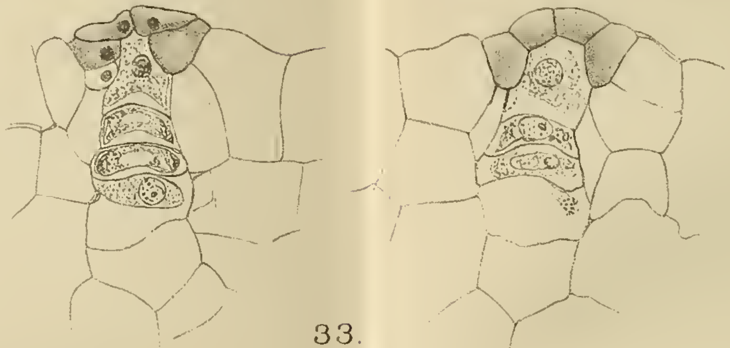

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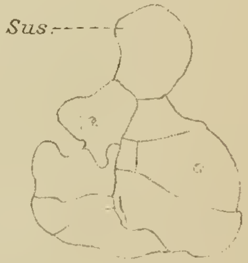

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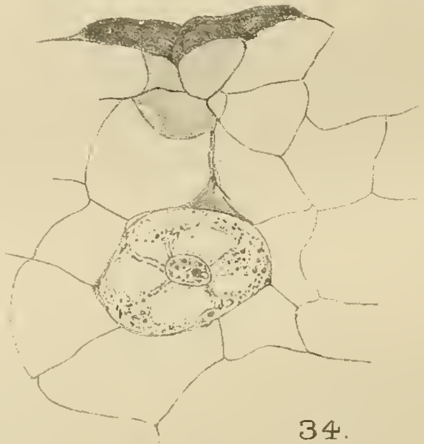

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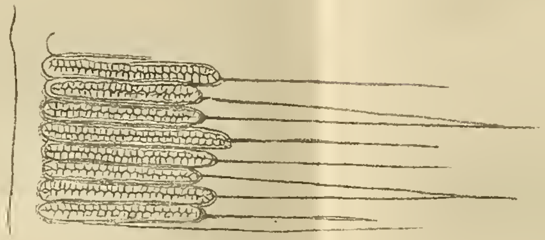

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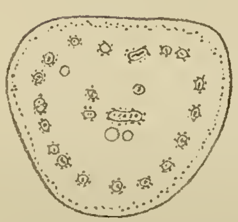

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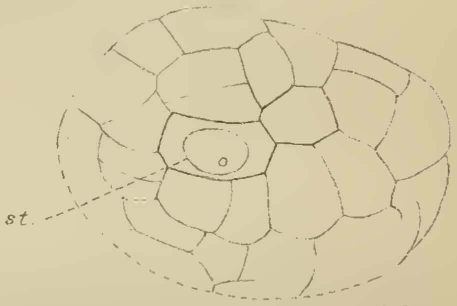

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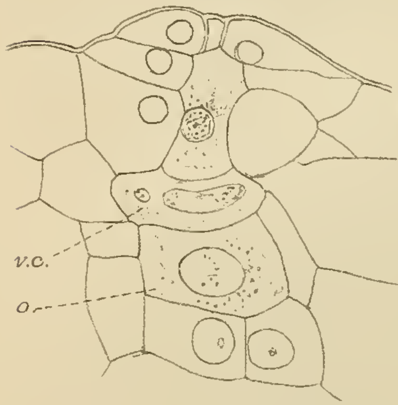

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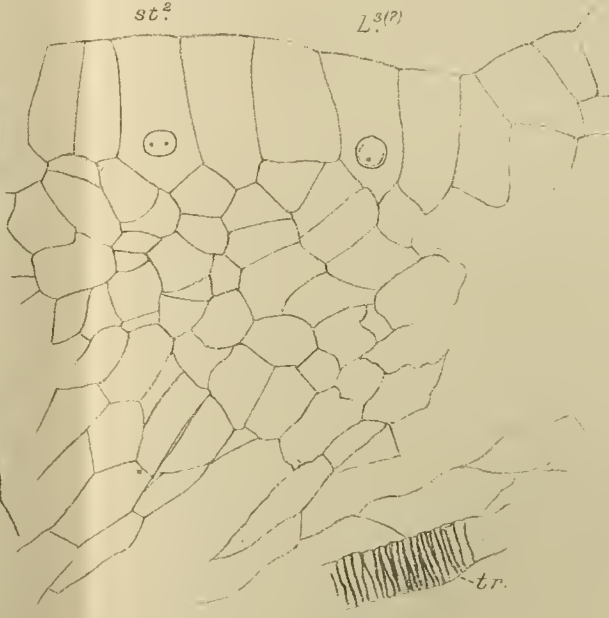

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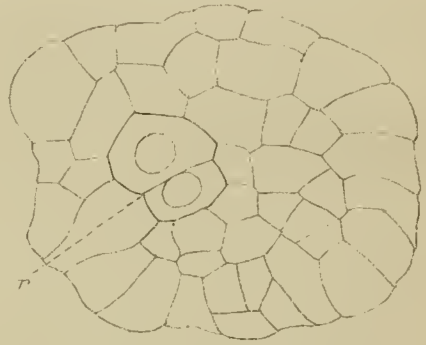

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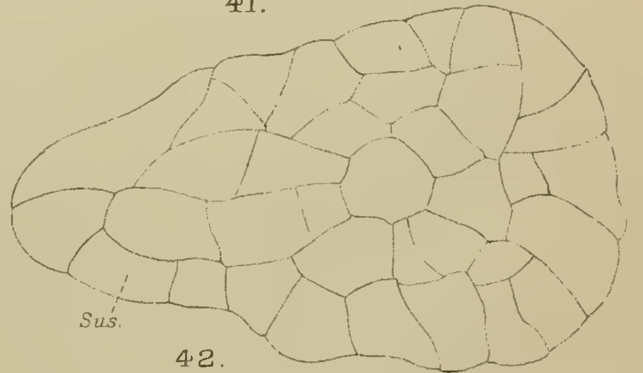

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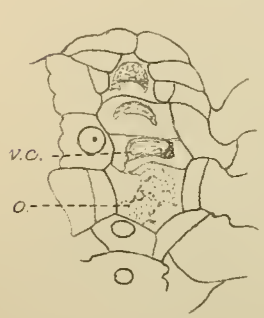

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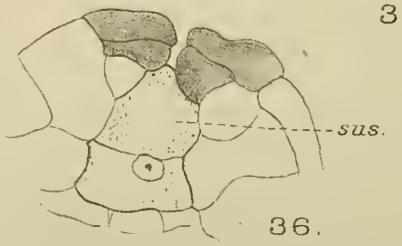

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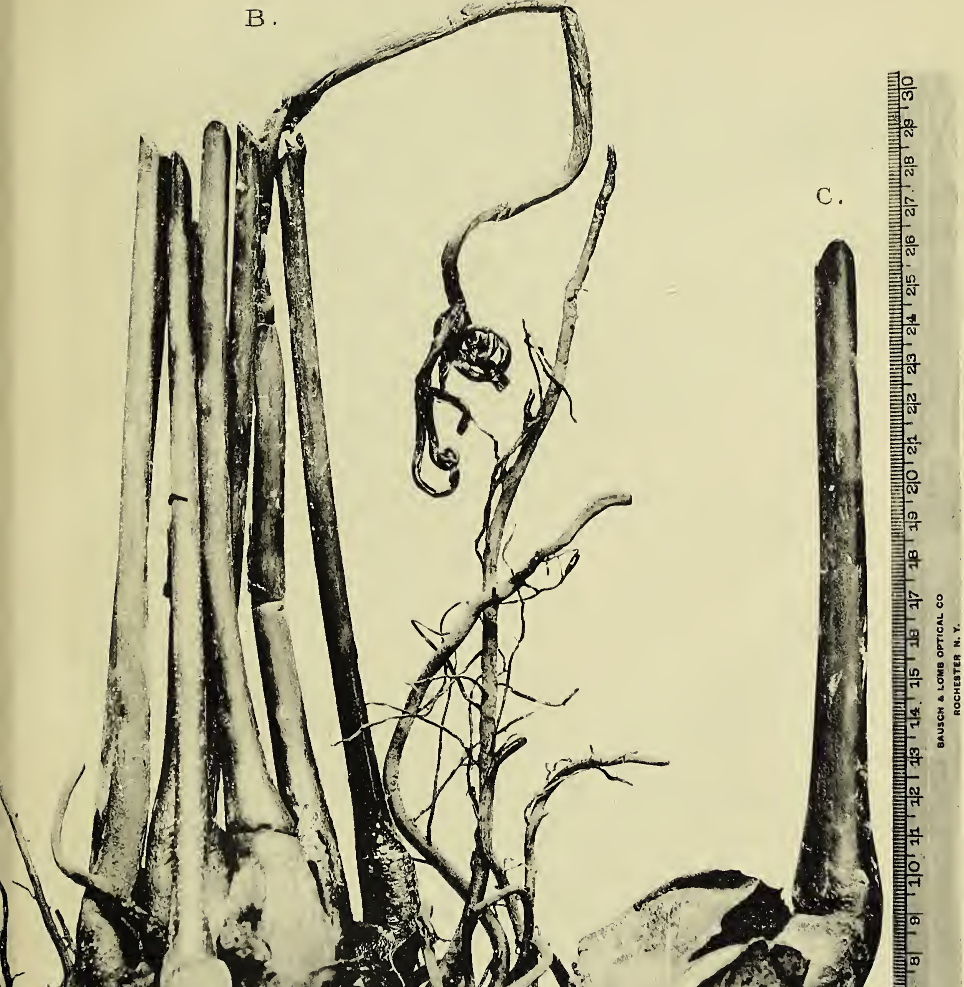

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The Anatomy of some Xerophilous Species of Cheilanthes and Pellaea.

BY

A. S. MARSH, B.A.

With eleven Figures in the Text.

MATERIAL.

THE species examined were all collected by Mr. A. G. Tansley either in America or in Algeria, and to him I am indebted for my material, and for his very great assistance throughout the investigation.

The plants were *Cheilanthes persica,* gathered in 1910 at Djebel Murdjadjo, Oran; *Cheilanthes Fendleri*, from Santa Catalina, California; *Ch. lanuginosa*, from Manitou, California; *Ch. gracillima*, from Crater Lake, Oregon; and *Pellaea andromedaefolia*, from Santa Catalina, California. The four last-mentioned species were all obtained in the Western United States of America in the summer of 1913.

For purposes of comparison, *Pellaea falcata*, a New Zealand mesophytic Fern, was also investigated to some extent.

EXTERNAL MORPHOLOGY AND XEROPHILOUS ADAPTATIONS IN THE LEAVES.

The fronds of all four species show well-marked xerophilous characteristics, which have been concisely expressed as follows in the 'Synopsis Filicum':

'*Cheilanthes lanuginosa*, Nuttall. Frond bipinnatifid, rachis tomentose, texture subcoriaceous; upper surface grass green, slightly tomentose; lower surface densely matted with a coat of pale brown woolly tomentum; the margin of the segment much incurved.

'*Cheilanthes persica*, Mett. (= *Ch. Szovitzii*, Fisch. & Meyer). Frond bipinnatifid, rachis scaly and tomentose, texture subcoriaceous; upper surface green, slightly tomentose; lower surface densely matted with a coat of pale brown woolly hairs; the margin of the segment much incurved.

[Annals of Botany, Vol. XXVIII. No. CXII. October 1914.]

'*Cheilanthes gracillima*, Eaton. Frond bi- or tripinnatifid, rachis slightly fibrillose, texture subcoriaceous; upper surface naked, lower densely matted with pale brown woolly tomentum; the margin of the segment much incurved.

'*Cheilanthes Fendleri*, Hk. Frond tripinnatifid, rachis densely clothed above with lanceolate acuminate entire scales; upper surface quite naked, under surface scaly only, not matted; texture subcoriaceous, margin much incurved.

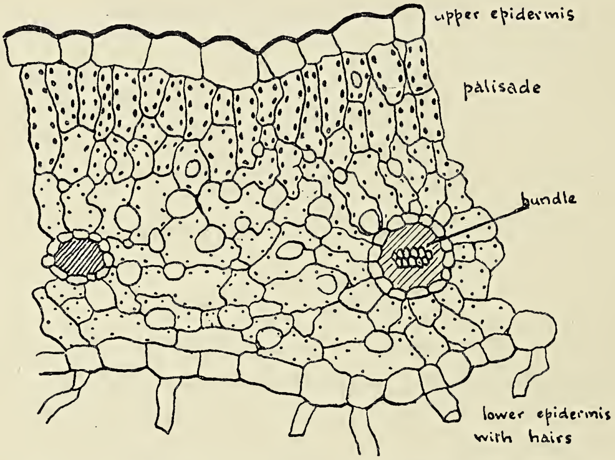

FIG. 1. *Cheilanthes lanuginosa.* Transverse section of leaf.

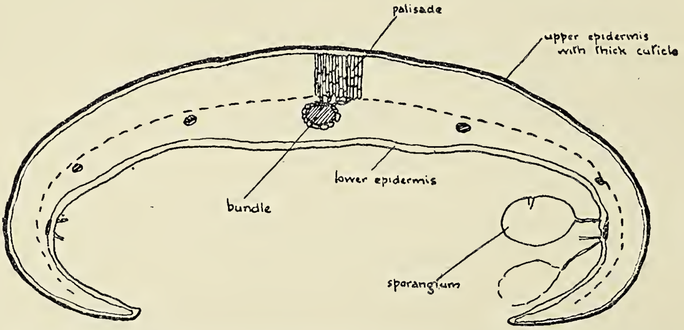

FIG. 2. Diagram of transverse section of leat of *Pellaea anaromedaefolia.*

'*Pellaea andromedaefolia*, Fée. Texture subcoriaceous, rachis and both surfaces naked, involucre scarcely changed from the substance of the frond, and sometimes nearly meeting edge to edge.'

LEAF ANATOMY.

In addition to these characteristics, the minute anatomy of the leaf shows in each case a reduced lacunar system, the greater or less development of a palisade, and the localization of the stomata on the lower surface, where they are protected by the inrolled margins alone (*P. andromedaefolia*), or by inrolled margins plus a covering of scales (*Ch. Fendleri*) or hairs (the other *Cheilanthes* species). These characters are shown in the figures of transverse sections of leaves (Figs. 1, 2). The most strongly marked xerophily is shown in the *Pellaea*, which possesses a very thick cuticle on its upper surface, and has also a palisade, which in the middle of the leaf is three cells deep. The lacunar tissue is reduced, and the margins are much inrolled.

STEM ANATOMY.

The stelar anatomy of *Cheilanthes* and *Pellaea* is of interest in that each genus contains both solenostelic and dictyostelic forms, together with others that are intermediate between these types. The four species of *Cheilanthes* investigated are placed next one another in the 'Synopsis Filicum'. They are all in § *Physapteris*, and Hooker and Baker, from a study of their external morphology, must have considered them nearly related one to another. The stelar anatomy, however, is different in each species. In the investigation of this feature series of hand sections were cut, and in all except *Cheilanthes Fendleri* these were drawn, forming Figs. 3–6.

In *Ch. Fendleri* we have a true solenostele. The leaf-gaps are long but fail to overlap.

In *Ch. gracillima* (Fig. 3) the stele over the main portion of its course behaves as a simple solenostele. At certain points, apparently not in accordance with any phyllotactic rule, extra leaves are inserted in such a way that two leaves are given off almost exactly together. The result is that the two leaf-gaps are at the same level, the stele therefore at such points being dictyostelic. Such pairs of leaves are seen in sections 8–10 and 15–16 in the diagram, Fig. 3. In section 15 there is also a 'perforation' or gap not formed by the giving off of a leaf-trace. Most of the leaves are given off dorsally, but in sections 8, 25, and 32 this rule is seen not to apply.

In *Cheilanthes lanuginosa* (Fig. 4) the departure from solenostely is still greater, though at intervals the transverse section may be a complete ring (sections 15, 16). The leaf-traces may be given off singly (sections 4, 7, 21), in pairs (section 10), or even in threes (section 17), and the leaf-gaps of one series may remain open till the departure of the next series (section 5). The stem, as in the two species preceding, is distinctly dorsiventral, a definite cauline ventral portion of the stele persisting throughout the stem.

In *Cheilanthes persica* (Fig. 5), however, the arrangement of the leaves on the stem is radial. The leaf-gaps are also very long, so that this is the most pronouncedly dictyostelic form investigated.

A further link in this chain of variation in stelar anatomy in § *Physapteris* is provided by *Cheilanthes lendigera*, another species of this section which is described by Gwynne-Vaughan (2). In this, so far as the endodermis and pericycle are concerned, each leaf-gap in the stele is closed up before the next above is formed. The leaf-gap in the xylem, however, remains open until it overlaps the gap formed in the xylem by the departure of the next leaf-trace above. In this manner a small separate xylem strand is formed within the stele, which crosses over at each leaf-insertion in just the same way as does the free dorsal meristele of so many of the simpler dorsiventral dictyosteles.

FIG. 3. *Cheilanthes gracillima.* Series of transverse sections of stem in acropetal order. In this and in Figs. 4, 5, 6, leaf-traces are represented in solid black.

In the genus *Pellaea* considerable variation in stelar anatomy also takes place. *Pellaea falcata* is a simple solenostele, and is mentioned as such by Gwynne-Vaughan, who also describes *P. rotundifolia* and *P. andromedaefolia* as examples of the simpler form of transition from solenostely

FIG. 4. *Cheilanthes lanuginosa.* Series of sections of stem in acropetal order.

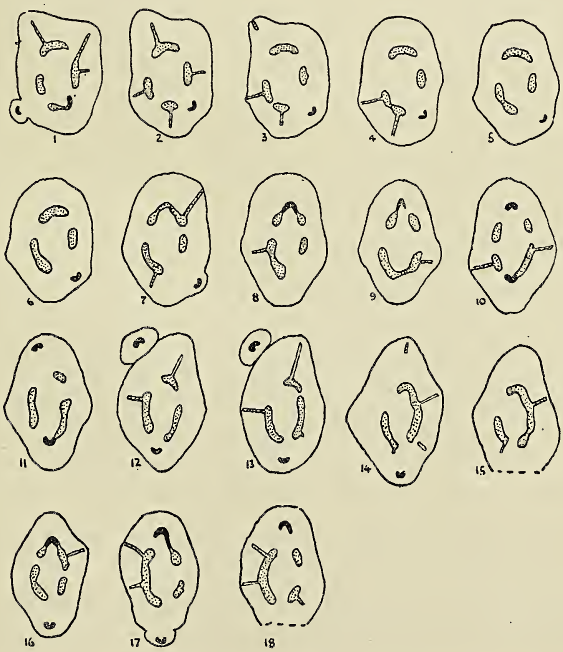

FIG. 5. *Cheilanthes persica.* Series of sections in acropetal order.

to dictyostely, viz. by overlapping of leaf-gaps formed in two rows on the dorsal surface. The *P. andromedaefolia* which I examined may perhaps have been older material than that used by Gwynne-Vaughan, for though

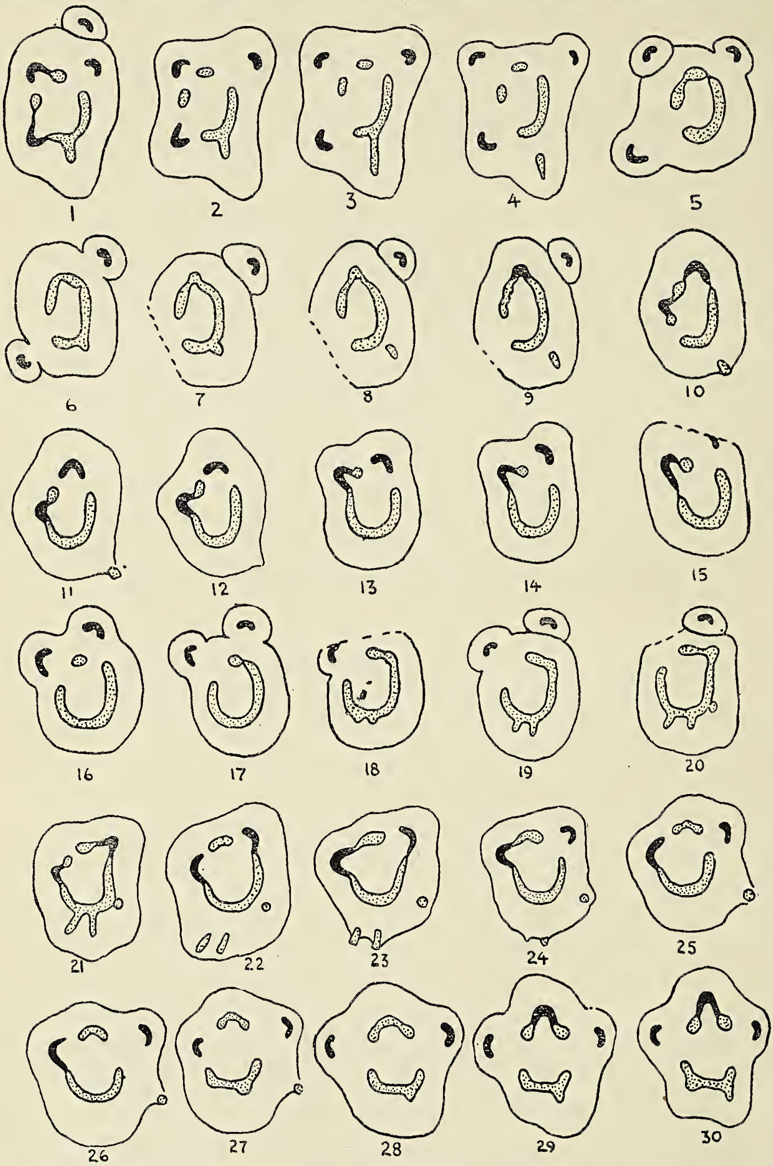

FIG. 6. *Pellaea andromedaefolia.* Series of sections of stem in acropetal order.

it showed a dorsiventral dictyostele, its structure was more complicated than that which he describes. The leaves come off at approximately the same level in threes or in pairs (Fig. 6). From the appearance of section 29 in my series it seems that the arrangement in threes may be produced by the interpolation of an extra leaf at a place where primitively two are given off, an arrangement still maintained in sections 10–19.

The detailed anatomy of the stelar tissues furnished results of some interest. In none of the five xerophilous Ferns was there to be seen any protoxylem, though search was made in both transverse and longitudinal sections. In *Cheilanthes Fendleri* and *Pellaea andromedaefolia* the apical region was thoroughly examined, and it was clear in these cases that there was no special protoxylem, the tracheides to be first lignified being large scalariform elements scattered irregularly in the xylem.

There was found to be a close correlation between the stelar structure and the amount and arrangement of the wood within the stele. *Cheilanthes Fendleri* and *Ch. gracillima*, of which the former is perfectly solenostelic and the latter scarcely departs from this type, both have xylem forming a narrow continuous band of tracheides, not broken up by parenchyma (Fig. 7). *Cheilanthes lanuginosa* is intermediate between the simple types and the large-stemmed dictyosteles. It has a wider band of wood somewhat broken up by parenchyma (Fig. 8). *Cheilanthes persica* and *Pellaea andromedaefolia* are large-stemmed dictyostelic forms with a broad band of wood, among which a good deal of parenchyma occurs (Fig. 9).

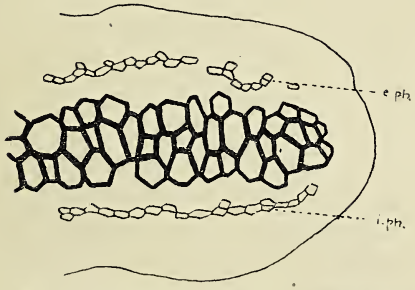

FIG. 7. *Cheilanthes Fendleri.* Transverse section of stele of stem at edge of leaf-gap. *e.ph.* = external phloem; *i.ph.* = internal phloem.

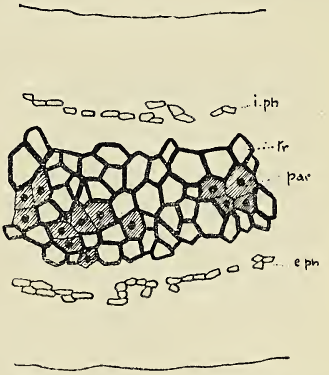

FIG. 8. Portion of transverse section of stele of stem of *Cheilanthes lanuginosa*. *i.ph.* = internal phloem; *e.ph.* = external phloem; *tr.* = tracheide; *par.* = parenchyma.

The phloem of these forms is remarkable for the absence of differentiation into protophloem and metaphloem. The total number of sieve-tubes is in no case very large. These elements are narrow and arranged in a single line on each side of the xylem ring. The whole of the phloem in these Ferns may fairly be said to correspond with the protophloem of mesophytic species, the slow growth necessitated by a xerophilous habitat rendering unnecessary those facilities for active translocation which would be given by large metaphloem elements.

There was, however, in all five species, just as great a development of phloem and endodermis on the inside of the stele as on the outside, contrasting in this respect with the reduced types of stele described by Jeffrey (5) in some species of *Antrophyum* and *Vittaria*. But at the leaf-gap the phloem was found to be absent in every case, so that there was no continuity of internal and external phloem (Fig. 7). In fact, in the gap xylem and endodermis are constantly separated by only two or three layers of parenchyma. Even *Pellaea falcata* shows reduction of the phloem in the leaf-gap, though its stele has no other reduced characters.

PETIOLES.

The anatomy of the petiole was carefully investigated in each species. The petiolar stele forms a single strand in every case, lying surrounded by a sclerenchymatous cortex, which can be fairly easily separated, leaving only the central trace. The stelar strand thus obtained was embedded and microtomed. By cutting at 10 μ and keeping every tenth section, I obtained a series of sections at 100 μ intervals, from the base upwards. At the very base in each species there is a simple adaxially curved anarch leaf-trace, but the changes in structure found on following the petiolar strand upwards vary considerably, so that for each species it is best to have a separate description. Diagrams of the various stages are given in Fig. 11.

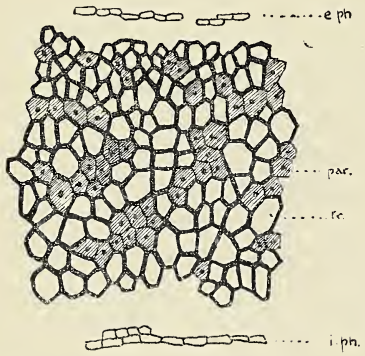

FIG. 9. Portion of transverse section of *Pellaea andromedaefolia*. *i.ph.* = internal phloem ; *e.ph.* = external phloem ; *tr.* = tracheide ; *par.* = parenchyma.

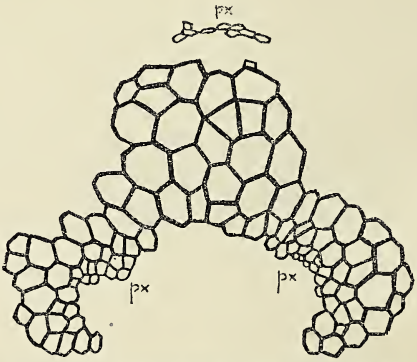

FIG. 10. Transverse section of xylem of stele of *Cheilanthes Fendleri* petiole. *px.* = protoxylem.

Cheilanthes Fendleri. The anarch C-shaped strand soon develops three protoxylems, all of which are endarch. The metaxylem immediately exterior to the median protoxylem dies out at a higher level, but the two portions of the stele thus left separate (except for the median protoxylem connexion) soon rejoin. This junction, however, is effected in such a way that our protoxylem is now in an exarch position. This protoxylem next becomes completely detached and runs through the major portion of the petiole as a single separate group (Fig. 10). At a higher level, a little below the insertion of the first pinna, it splits into three or four smaller protoxylem groups, though the pinna trace is given off entirely from the lateral portions of the stele. The other two protoxylems become rather broader in the higher regions of the petiole, but otherwise they remain unchanged throughout. All three protoxylems were seen in longitudinal section to consist of spiral and annular elements, while the main mass of the tracheides has scalariform thickening.

Ch. lanuginosa. The series in this species is very easily derived from that in *Ch. Fendleri.* Three protoxylems appear simultaneously just above the base, but at this point the centre of the trace is so narrow that the median protoxylem occupies the whole thickness of the stele. The stele next shows a definite separation into two masses joined only by the protoxylem. As in *Ch. Fendleri*, these two masses rejoin and the median protoxlem, now exarch, soon becomes separated from the main stele. This stage is reached more quickly than in the former species, and there is the important difference that the median protoxylem does not persist. It is found for some distance as a line of thickening among the parenchyma (diagram 5 in the *Ch. lanuginosa* series, Fig. 11), but in the higher regions of the petiole no trace of it can be recognized.

Ch. gracillima. In this small plant the two lateral protoxylems, occupying an endarch position, are the only ones ever definitely organized. There is no trace of a median splitting, and only a slight and transitory indication (in the presence of somewhat smaller elements at the position shown by the dots in diagram 3) of a median protoxylem. True protoxylem elements, however, are not present, but this condition is clearly only slightly removed from that represented by diagram 5 in the *Ch. Fendleri* series. The higher regions of the petiole in *Ch. gracillima* show no further change in the number or position of the protoxylem groups.

Ch. persica. In petiolar structure this species is rather different from the American forms, but this is only what one would expect from the divergences in the stem anatomy, and from the totally dissimilar geographical distribution. The petiolar stele has the shape, in transverse section, of an obtuse-angled isosceles triangle, the base of which shows a slight adaxial curve. At each angle a protoxylem gradually becomes distinct as we examine the series of sections from the base upwards, and, once established, this structure continues throughout the length of the petiole. This type is probably related to that of *Ch. Fendleri*, being modified from some structure similar to that shown in diagram 5 of the *Ch. Fendleri* series. The loss of the two lateral hooks would easily give the type with the three protoxylem angles.

Pellaea andromedaefolia develops three endarch protoxylem groups, the median one of which, in the main part of the petiole, is located in the position shown in diagram 3 of the series in Fig. 8. This is a condition which perhaps shows an unfulfilled tendency towards exarchy of the central protoxylem, an end which has become realized in the species of *Cheilanthes.*

These facts admit of only one simple explanation, viz. that the base of the leaf preserves primitive characters. This is the position which has been taken up by Sinnott (7). He has pointed out that the leaf-trace of all Ferns except Osmundaceae, Ophioglossaceae, and Marattiaceae can be derived from a triarch-mesarch trace, and that the nearest approach to this condition is usually found at the leaf-base. The mesarch protoxylems have in the course of evolution generally become endarch, and in very many forms all trace of mesarchy is lost, even at the leaf-base. This is the condition found at the base of the petiole of *Ch. Fendleri*, and it is only by assuming that the acropetal succession recapitulates the evolutionary succession that we can reasonably bring the peculiar petiole of *Ch. Fendleri* into line with the normal type of filicinean leaf-trace. Though it is difficult to imagine what could be the actual cause of the exarchy and detachment of the median protoxylem, it is simple, from a study of the leaf-base transition, to

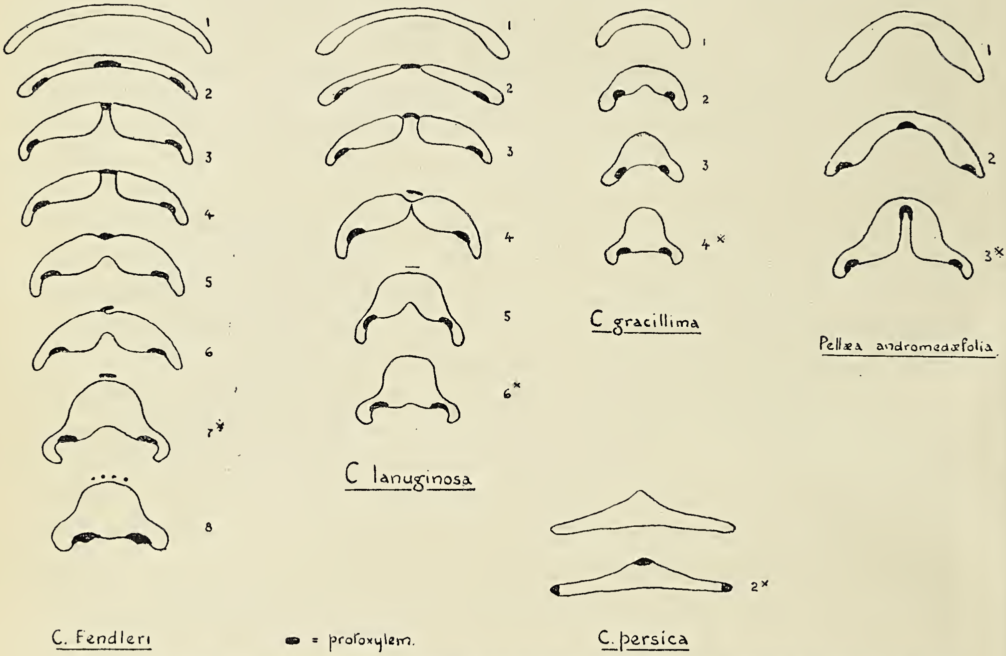

FIG. 11. Series of transverse sections of the xylem of the petiolar traces of the five Ferns. Each series is numbered in acropetal order. * represents in each case the structure prevalent throughout the main part of the petiole. No. 8 in the *Ch. Fendleri* series shows the arrangement just below the first pinna.

derive *Ch. Fendleri* from the normal type and the other *Cheilanthes* petioles from *Ch. Fendleri.* In *Ch. lanuginosa* the series is essentially that of *Ch. Fendleri,* except that the primitive endarch condition of the median protoxylem is not represented, and that this protoxylem lasts only over a limited distance at the base of the petiole. In *Ch. gracillima* there is omission of the early stages ; there is no sign of the median splitting of the trace, of the central protoxylem there is only an indication, and even that in an exarch position. Taken alone it would be difficult to fit this Fern into the Sinnott type, but in conjunction with *Ch. lanuginosa* and *Ch. Fendleri* the connexion becomes clear.

The derivation of *Ch. persica* was mentioned when dealing with that species. In the case of *Ch. persica* especially, as also in that of the two first-mentioned species, one sees that Sinnott rather underestimated the plasticity of the Fern petiole when he spoke of the 'striking constancy' of the endarchy of the filicinean petiolar bundle. While acquiescing in his general fundamental classification and derivation of Fern leaf-traces, one sees in studying these five xerophilous Ferns that the anatomy of the leaf-base, as of the stem, may furnish a sound guide to relationships, but that it is not to single species but to groups of allied species that the test should be applied. Within quite narrow circles of affinity certain forms may slip ahead of the general evolutionary march. This holds good for the petiole of *Cheilanthes gracillima* or *Ch. persica,* which have diverged from the closely related *Ch. Fendleri,* just as for a solenostelic species occurring in a genus otherwise haplostelic (e. g. *Gleichenia pectinata*).

Another point of interest is that petiolar structure may show a secondary simplicity. Thus in *Cheilanthes Fendleri* and *Ch. lanuginosa* the ordinary transverse section of the petiole shows a single compact mass of xylem. Nearer the base we find a stage at which, but for the protoxylem forming a connexion, the xylem is split medianly into two masses. This may give place at a still lower level to a single C-shaped strand. We have seen in deriving *Ch. lanuginosa* and *Ch. gracillima* from *Ch. Fendleri* that the earlier stages may be dropped out at the base of the petiole. Such a dropping out of early stages at the base, together with a secondary simplicity at a higher level, gives the state of affairs found in many Ferns (e. g. *Onoclea sensibilis, Peranema,* and *Diacalpe*), where a trace, double below, unites to give a single strand at a higher level. This renders advisable a relaxation of the inflexibility of Sinnott's assertions as to the constancy of the characters of the leaf-base, but such a relaxation really adds to the strength of the theory, and disposes of the objections raised by Davie (1), who claimed that his petioles in *Peranema* and *Diacalpe* were incapable of plausible explanation on Sinnott's theory. The accumulation of this additional evidence for the theory of the primitiveness of structures found at the leaf-base, entails no necessity that one should accept all

Sinnott's corollaries to his general proposition. Especially should this be remembered before adopting Sinnott's sceptical attitude towards the theory of Tansley and Gwynne-Vaughan, 'who consider the siphonostele to have been produced by the influence of an arched trace on a protostele'.

That the two theories are not so incompatible has been recently established by Salisbury (6), who has shown that narrowing of the base of the leaf-trace, found so frequently in Ferns, has no appreciable influence in checking the free supply of water to the leaf. The transpiration current being practically as great as if there were no basal narrowing of the petiolar stele, the effect of the leaf-trace on the conducting tissues of the stem, which supply the water necessary for this transpiration current, will be the effect of the full current in the main part of the petiole.

SPORE COUNTS.

All the species except *Cheilanthes persica* were bearing ripe sporangia, and counts were made of the spores per sporangium, the results being as follows:

Pellaea andromedaefolia	30, 31.
P. falcata	60, 56.
Cheilanthes Fendleri	53, 57.
Ch. gracillima	30, 31.
Ch. lanuginosa	32, 30.

The results show that the purely solenostelic form has in each genus more spores per sporangium than the dictyostelic species. Among the *Cheilanthes Ch. Fendleri* has the larger number, and this corroborates the evidence for the primitiveness of this species derived from the anatomy of the stem and petiole.

SUMMARY.

1. The xerophilous species of *Pellaea* and *Cheilanthes* show well-marked leaf adaptations. Hairs or scales on the lower surface, inrolled margins, thick cuticle, and palisade parenchyma are the chief characters observed.

2. The *Cheilanthes* species, all belonging to § *Physapteris*, show a wide range of stelar anatomy in their stems. *Ch. Fendleri* has a simple solenostele. *Ch. gracillima* has a solenostele becoming occasionally dictyostelic by the interpolation of an extra leaf, and *Ch. lanuginosa*, bearing its leaves much more closely on the stem, only rarely shows a complete ring in cross-section. These three have dorsiventral rhizomes, while *Ch. persica* has a radial dictyostele which is much more dissected than that of any of the three other species.

3. Of the genus *Pellaea*, *P. andromedaefolia* was the only xerophytic

species examined. It possesses a dorsiventral dictyostele with leaves given off approximately in pairs, though by interpolation of extra leaves these pairs are often converted into groups of three. Among other species of *Pellaea*, *P. falcata* has a typical solenostele, and Gwynne-Vaughan describes *P. rotundifolia* as a dictyostele with two dorsal ranks of leaves.

4. Parallel with the advance towards dictyostely is an increase in the thickness of the band of xylem, and in the extent to which the xylem is broken up by parenchyma. Thus *Cheilanthes Fendleri* is solenostelic, and has a narrow compact band of tracheides, while *Ch. persica* is a dictyostele with a broad band of mixed tracheides and xylem parenchyma. Intermediate between these comes *Ch. lanuginosa*.

5. There is no protoxylem in the stems of any of the five species investigated. The earliest tracheides to be organized are large and scalariform, and have no constant position in the stele.

6. The internal and external phloems are equally well developed, each consisting of a single line of small elements, corresponding in size and position with the protophloem of mesophytic Ferns. The internal and external phloems are not continuous with one another through the leaf-gaps.

7. The petioles may all be derived from a type with three endarch protoxylems such as has been described by Sinnott. This relatively primitive condition is found in *Pellaea andromedaefolia*, and at the very base of the petiole in *Cheilanthes Fendleri*. *Ch. Fendleri* at higher level shows in successive sections its median protoxylem passing through the stele, becoming exarch, and then completely detached and isolated. The other *Cheilanthes* species all show a secondary simplicity of petiolar structure in the main part of the petiole. This can be in every case derived from the *Ch. Fendleri* type, the connexion being clearest in *Ch. lanuginosa*, where the *Ch. Fendleri* peculiarities appear at the base of the petiole, but die out at higher levels.

Pellaea andromedaefolia retains its three endarch protoxylems throughout the whole length of its petiole, but the median one is situated at the end of a narrow groove, and only a short distance from the abaxial surface of the xylem mass.

8. The petiolar structure, the stem anatomy, and the greater output of spores per sporangium all point to *Ch. Fendleri* as a near approximation to an ancestral type from which *Ch. gracillima* and *Ch. lanuginosa* have been derived.

BOTANY SCHOOL, CAMBRIDGE.
May, 1914.

LITERATURE.

1. DAVIE: The Structure and Affinities of *Peranema* and *Diacalpe*. Ann. of Bot., vol. xxvi, 1912.
2. GWYNNE-VAUGHAN: Observations on the Anatomy of Solenostelic Ferns. i. Ann. of Bot., vol. xv, 1901. ii. Ann. of Bot., vol. xvii, 1903.
3. HOOKER and BAKER: Synopsis Filicum, 1874.
4. JEFFREY: The Morphology of the Central Cylinder of Angiosperms. Transactions of the Canadian Institute, vol. vi, 1899.
5. ———: The Structure and Development of the Stem in the Pteridophyta and Gymnosperms. Phil. Trans. Roy. Soc., Series B, vol. cxcv, 1902.
6. SALISBURY: The Determining Factors in Petiolar Structure. New Phytologist, vol. xii, 1913.
7. SINNOTT: The Evolution of the Filicinean Leaf-Trace. Ann. of Bot., vol. xxv, 1911.
8. TANSLEY: Lectures on the Evolution of the Filicinean Vascular System. New Phytologist Reprint No. 2, 1908.

Note on Vegetative Reproduction in some Indian Selaginellas.

BY

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1851 *Exhibition Research Scholar, Newnham College, Cambridge.*

With Plate XLIX and seven Figures in the Text.

THE two species of *Selaginella* which form the subject of this note were sent as herbarium specimens by Professor Kashyap, of the Lahore Government College, to Professor Seward, who has very kindly handed them to the writer for investigation. Professor Kashyap collected them in the neighbourhood of Rajpur and Mussoorie, India, in August and September, 1913, and they have been identified by Mr. C. H. Wright, of Kew, as *Selaginella chrysocaulos*, Spring, and *S. chrysorrhizos*, Spring.[1]

According to Hieronymus,[2] both species belong to the group of *Selaginella suberosa*,[3] a subdivision of the pleiomacrosporangiate and monostelic Heterophylleae. In his diagnosis of the group, Hieronymus notes that apparently vegetative reproduction is not normally present, and the representative species reproduce themselves only by spores; Spring does not especially mention any methods of vegetative reproduction, though the presence of stolons at the base of the vegetative stems seems to be a characteristic of *S. chrysocaulos*.[4]

The chief interest of the two specimens under consideration lies in the fact that they show vegetative reproduction, this being normally unrepresented in the group to which they belong, although similar types of reproduction regularly occur in other forms.

The specimens of *S. chrysocaulos* are characterized by the possession of bud-like structures at the tips of some of the vegetative branches (Pl. XLIX, Fig. 1, and Text-fig. 1). Professor Kashyap refers to them as 'surface tubers', their function being propagative. Hieronymus[5] notes that in *Selaginella Lyallii*, *S. Vogelii*, and related species, a transformation of

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[1] For the localities of these two species see Spring's Monographie de la famille des Lycopodiacées, Pt. II. Mém. de l'Acad. Roy. de Belgique, t. xxiv, 1850, pp. 250, 251.

[2] Hieronymus, G. Selaginellaceae (in Engler and Prantl's Pflanzenfamilien, I, 4, ii, p. 621, 1900), p. 626.

[3] Spring considers the two species as closely related to one another and to *S. suberosa.*

[4] Spring, l. c., p. 250.

[5] Hieronymus, l. c., p. 666.

the apex of a vegetative shoot may take place. The observations of Bruchmann[1] show that in *S. Lyallii* the branches of the older shoot-systems close to the ground, or branches of the ordinarily erect system accidentally in contact with the ground, may become enlarged at the apex and form leaves which differ considerably from the leaves of the ordinary branches, resembling the scales on the rhizome and on the lower unbranched parts of the erect system.[2]

TEXT-FIG. 1. *Selaginella chrysocaulos.* A 'surface tuber', showing the dichopodial branching, the occurrence of the ventral rhizophores, and the arrangement and form of the leaves. The enlargement of the end of a rhizophore before branching, with a considerable development of root-hairs, is indicated at A. × 3.

In *S. chrysocaulos* the stem-apices forming the 'buds' or 'tubers' show close and repeated dichotomous ('dichopodial'[3]) branching; a rhizophore often occurs in the fork between two branches, arising in the axil of a leaf (Pl. XLIX, Fig. 1, and Text-figs. 1 and 2).[4] The rhizophores are themselves frequently dichotomously branched (Text-fig. 1), and serve to attach the 'tubers' firmly to the substratum. In several cases the commencement of branching is indicated by the enlargement of the end of the rhizophore[5] (Text-fig. 1, at A). Particularly at such enlargements, the tips of the rhizophores develop very numerous root-hairs, the epidermal cells growing out to some length. This also is indicated at A in Text-fig. 1.

The leaves of the tubers are very closely packed and uniform in size; they are relatively broader and shorter than the leaves of the erect system, and possess a very distinct awn, about one-quarter to one-third of the

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[1] Bruchmann, H.: Die vegetativen Verhältnisse der Selaginellen (Giebel's Zeitschr. f. d. gesamt. Naturwissensch., 1877, i, pp. 524–6), p. 526.

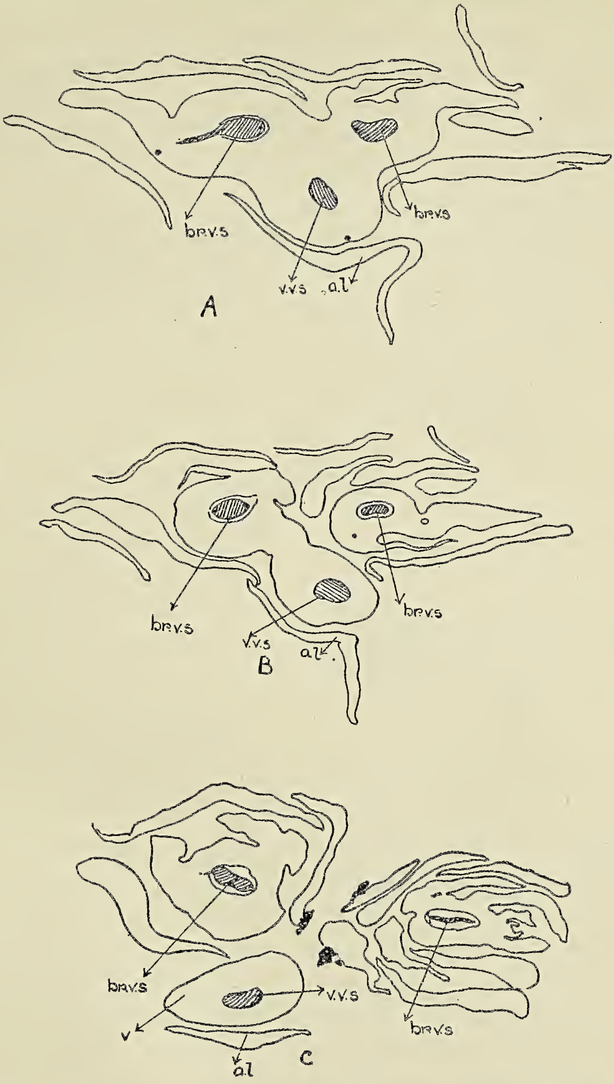
[2] Bruchmann also showed that cones may proliferate in the same way; and Behrens (Über die Regeneration bei den Selaginellen, Flora, lxxxiv, 1897, p. 159) produced proliferated apices artificially in various species, obtaining new plants from them.

[3] Cf. Velenovský, J.: Vergleichende Morphologie der Pflanzen, 1905–13, p. 246 and p. 259, Fig. 177.

[4] Hofmeister, W.: On the Higher Cryptogamia (trans. by F. Currey; Ray. Soc., 1862), p. 383.

[5] Ibid., p. 384.

length of the leaf, this being absent in the ordinary lateral or ventral leaves, though present in the dorsal leaves. The margins of the tuber leaves are also very minutely ciliate,[1] ciliation being present elsewhere only on the upper basal margin of the ventral leaves.



TEXT-FIG. 2. *S. chrysocaulos.* A, B, and C show diagrammatically a series of stages in the branching of a 'surface tuber', with the separation of the ventral rhizophore (*v.*), subtended by its axillary leaf (*a.l*). *br.v.s.*, vascular supply of the vegetative branches; *v.v.s.*, vascular supply of the ventral rhizophore. × 30.

The dried material was prepared for sectioning by treatment with lactic acid. Microtome sections (10 μ thick) were cut in the case of four tubers,

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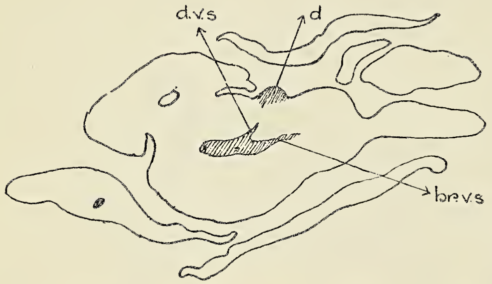
[1] This is not shown in the figure.

but the anatomy was not found to be in any way unusual, the distribution of the vascular system being the same as in ordinary branching of the stem in monostelic forms.[1]

Text-fig. 2, A, B, and C, from a series of transverse sections through a tuber, shows the forking of the axis and the formation of the ventral rhizophore with its subtending 'axillary' leaf. The rhizophore is well developed and separates immediately after the branching of the axis (Text-fig. 2, B).

Text-fig. 3 shows one of a series of sections in which a small dorsal protuberance appears just before the dichotomy of the axis; this is the undeveloped rudiment of the dorsal rhizophore.[2] A slender vascular trace is given off towards it immediately before the bifurcation of the stele (Text-fig. 3). Thus the order of appearance of the vascular supplies at the bifurcation of the stem is as follows: dorsal rhizophore, branch, ventral rhizophore.[3]



Text-fig. 3. *S. chrysocaulos*: Diagrammatic transverse section of a 'surface tuber', just below the separation of a branch. *d.*, the dorsal rhizophore; *d.v.s.*, the vascular supply to the dorsal rhizophore; *br.v.s.*, the vascular supply to the branch. × 30.

The specimens of *Selaginella chrysorrhizos* exhibit a type of vegetative reproduction which is apparently typical for *S. bulbilifera*. Hieronymus[4] remarks that bulbil structures have only been observed as a regular phenomenon in *S. bulbilifera*, and are found both at the apices of cones and of vegetative branches, the latter sometimes becoming elongated and filamentous. They are small bulb-like organs of propagation which consist of a so-called 'lecus' or corm—a swelling of the apex of the shoot caused by hypertrophy of the cortical parenchyma. This tissue is filled with reserve materials, and the whole structure is closely surrounded by numerous crowded leaves, spirally developed and somewhat swollen at the base, but otherwise very similar to the early leaves of many species. The vegetative apex of the bulbils divides once or twice while these are still on the plant, in the same direction as dichotomies take place in the rest of the plant.

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[1] Cf. Harvey Gibson, R. J.: Contributions towards a Knowledge of the Anatomy of the Genus *Selaginella*, Pt. I, The Stem. Ann. of Bot., vol. viii, 1894, p. 133.

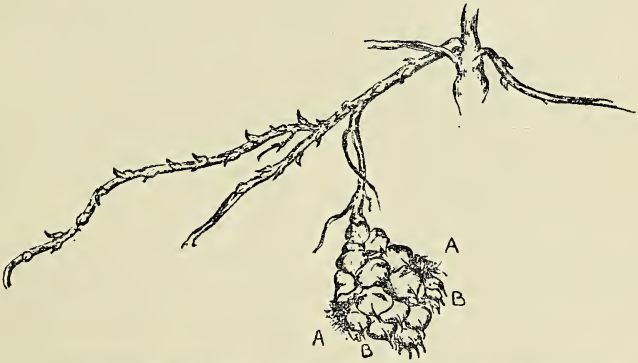
[2] Treub, M.: Recherches sur les organes de la végétation du *Selaginella Martensii*, Spring, 1877, p. 12.

[3] Harvey Gibson, l. c., Fig. 53. This figure of *Selaginella inaequalifolia* shows the axillary leaf subtending the ventral rhizophore. It also shows that the dorsal rhizophore arises slightly before the ventral rhizophore.

[4] Hieronymus, l. c., p. 666.

Vegetative branches of *S. chrysorrhizos* show this type of bulbil formation. Some of the lower branch-systems of the plants become elongated and filamentous, their leaves being much reduced, scale-like, and scattered (Plate XLIX, Fig. 2, and Text-fig. 4). In some cases the ends of these branches penetrate underground and become enlarged, developing numerous closely crowded, colourless scale leaves (Pl. XLIX, Fig. 2, and Text-fig. 4; also Text-fig. 6). Professor Kashyap refers to the structures so formed as 'underground tubers', in contradistinction to the 'surface tubers' of *S. chrysocaulos.*

Sections (10 μ thick) were cut of tubers prepared with lactic acid, as in the case of *S. chrysocaulos.* These showed a large increase in the parenchymatous tissue at the ends of the branches, the cells usually being densely filled with reserve food materials. The vascular tissue is comparatively small in amount, and in many cases lignification is not very strong, the two lateral protoxylems only being well differentiated. Text-figs. 5 and 6 indicate the relatively small amount of vascular tissue present. The characteristic trabecular endodermis of the stem-stele may be recognized in the tubers (Text-figs. 5 and 6), as well as in the filamentous vegetative branches. The scale leaves of the stem-apex overlap closely (Text-figs. 4 and 6), their bases often being rather enlarged and containing reserve materials.



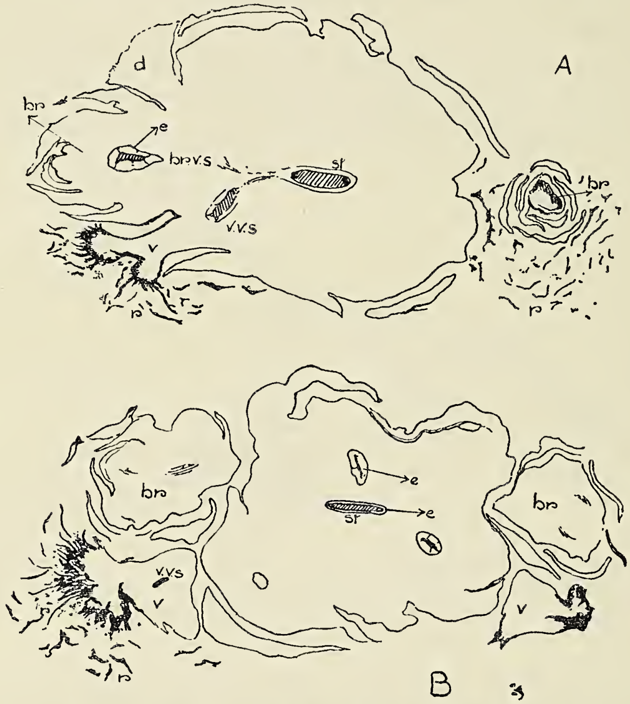
TEXT-FIG. 4. *Selaginella chrysorrhizos.* An 'underground tuber' showing the crowded scale leaves. Branches are indicated at B, and root-hairs developed from rudimentary rhizophores at A. The position of the tuber with regard to the elongated, filamentous vegetative branches springing from the base of the plant is also shown. × 4.

The tubers are dorsiventral in organization, and branching takes place repeatedly as in the surface tubers. A dichopodium is formed, the branches on alternate sides being more strongly developed;[1] the 'lateral branches' thus formed are very short (Text-fig. 5, A and B, from a series of transverse sections of the same tuber; also Text-fig. 6, A and B, from another tuber).

[1] Cf. Hofmeister, l. c., p. 382.

At each dichotomy of the tuber axis, and in the plane at right angles to it,[1] arise two very short branches, which are really exogenous in origin but present a semi-endogenous appearance due to the general enlargement and hypertrophy of the tissues of the tuber (Text-figs. 5 and 6).

The vascular supply to the dorsal branch separates from the stele of the tuber immediately before its bifurcation to supply the two vegetative branches. The ventral branch is subtended by a leaf, and its vascular supply arises from one branch of the stele immediately after bifurcation has taken place. These short branches are undoubtedly the rudimentary dorsal and ventral rhizophores, which remain short and undeveloped since the tubers are already embedded in the ground. The vascular supply, in its passage to the rhizophores, has the characteristic endodermis of the stem-stele.



TEXT-FIG. 5. *S. chrysorrhizos.* A and B show diagrammatically two transverse sections of an 'underground tuber', A near the base, and B near the apex. *br.*, branch; *d.*, dorsal rhizophore; *v.*, ventral rhizophore; *st.*, stele of axis; *br.v.s.*, vascular supply to branch; *v.v.s.*, vascular supply to ventral rhizophore; *r.*, root-hairs; *e.*, trabecular endodermis. In B the dorsal rhizophores accompanying both branches do not appear at this level. × 32.

The rhizophores have somewhat enlarged rounded ends; a similar enlargement in ordinary rhizophores is, according to Hofmeister,[2] due to

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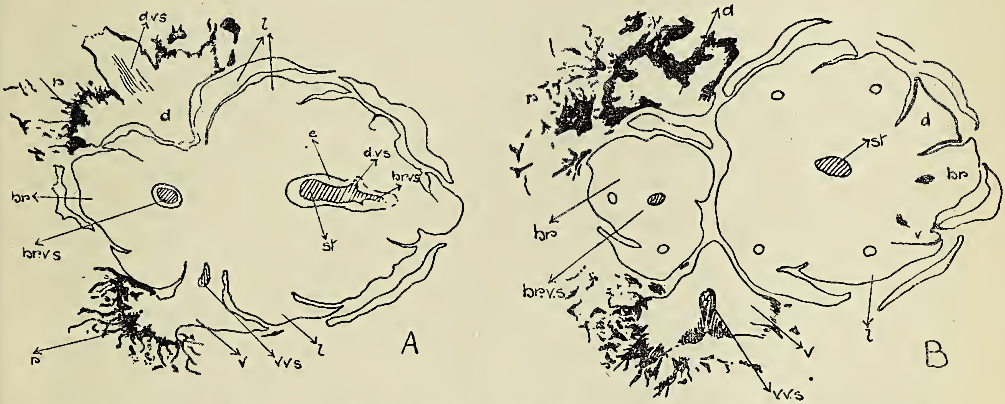
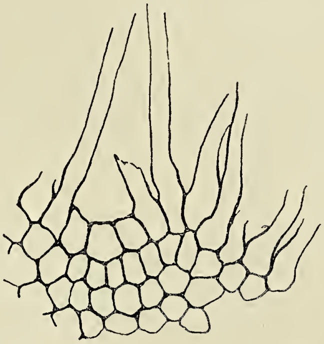
[1] Cf. Velenovský's description of 'tetrachotomy', l. c., p. 260.

[2] Hofmeister, l. c., p. 384.

the commencement of furcation and was noted in *S. chrysocaulos.* Text-fig. 5, A, shows a distinct appearance of dichotomy of the rhizophore heads. The epidermal cells of the ends of the rhizophores grow out into long root-hairs (Text-figs. 5, 6, and 7), which are often so much developed as to appear to the naked eye as masses of hyphae (Text-fig. 4, at A; cf. the development of root-hairs at the ends of rhizophores in *S. chrysocaulos*, Text-fig. 1).



TEXT-FIG. 6. *S. chrysorrhizos.* A and B show diagrammatically two transverse sections of an 'underground tuber'. The lettering is the same as in Text-fig. 5. This tuber shows very clearly the closely-overlapping scale leaves (*l.*). In A continued growth of the dorsal rhizophore is indicated. × 32.



TEXT-FIG. 7. *S. chrysorrhizos.* Longitudinal section of the end of a rhizophore, showing the development of the epidermal cells forming root-hairs. The central area, in which the root-hairs are not developed, is clearly indicated. × 275.

A similar formation of root-hairs was noted by Sarauw,[1] who grew species of *Selaginella* over distilled water. The swollen ends of the rhizophores produced root-hairs about 3 mm. long, forming at first a little disc and later appearing as a short brush, the hairs of which were arranged

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[1] Sarauw, G. F. L.: Versuche über die Verzweigungs-Bedingungen der Stützwurzeln von *Selaginella.* Ber. der deutsch. bot. Ges., Bd. ix, 1891, p. 63.

something like the sterigmata of an *Aspergillus* conidiophore head. Sarauw mentions that there was possibly a small area in the centre of the enlarged rhizophore heads without root-hairs; Text-fig. 7 shows this in one of the tubers of *S. chrysorrhizos*. In one case the rhizophore seems to have continued its growth for a short distance from this area, leaving the root-hairs as a lateral flange or collar near its base (Text-fig. 6, A).

CONCLUSION.

The reproductive 'tubers' in these two species of *Selaginella* are fundamentally similar, the differences between them being dependent upon physiological causes, arising from the position of the tubers on the plant.

In *S. chrysocaulos* the tubers are at the ends of ordinary vegetative branches and remain at the surface of the ground, the development of ventral rhizophores being necessary for their attachment to the substratum. The leaves of these tubers, though differing in form and size from those of the ordinary branches, contain abundant chlorophyll. In the case of *S. chrysorrhizos*, on the other hand, the tubers are developed underground at the ends of filamentous, modified vegetative branches. The leaves are scale-like and without chlorophyll, their bases and the tissues of the tuber containing reserve materials, the storage of which is unnecessary in the chlorophyll-containing surface tubers.

Further, in the case of *S. chrysorrhizos*, both dorsal and ventral rhizophores are developed, but they remain very short, since the tubers are already embedded in the ground and elongation of rhizophores is not needed for purposes of attachment. They are fairly equally developed, being subjected to the same developmental conditions, and both are apparently functional in obtaining nutritive elements from the soil by means of the root-hairs developed from their enlarged ends, probably as a response to nutritive stimuli.[1]

The dorsal and ventral rhizophores of *S. chrysocaulos*, on the other hand, encounter different conditions from the commencement of their development; for instance, the ventral rhizophores are subjected to less light intensity and greater humidity than the dorsal rhizophores, owing to the position of the tubers on the surface of the ground. The ventral rhizophores alone are functional in attaching the tubers and for nutritive purposes; they attain a considerable development, while the functionless dorsal rhizophores are very much reduced, more so even than in *S. chrysorrhizos*.

The writer desires to express gratitude to Professor Seward for per-

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[1] Sarauw, l. c., p. 65; the branching of the rhizophores is said to be due to the dampness of the ground, while the nutritive content of the medium is responsible for their further development.

mission to carry out this investigation at the Botany School; to Mr. Tansley for helpful advice; and to Mr. C. H. Wright for the identification of the material described above.

BOTANY SCHOOL, CAMBRIDGE,
May, 1914.

LITERATURE CITED.

BEHRENS, J.: Über die Regeneration bei den Selaginellen. Flora, vol. lxxxiv, 1897, p. 159.

BRUCHMANN, H.: Die vegetativen Verhältnisse der Selaginellen. Giebel's Zeitschrift f. d. gesamt. Naturwissensch., i, 1877, p. 524.

HARVEY GIBSON, R. J.: Contributions towards a Knowledge of the Anatomy of the Genus *Selaginella*, Spr., Pt. I, The Stem. Ann. of Bot., vol. viii, 1894, p. 133.

HIERONYMUS, G.: Selaginellaceae, in Engler and Prantl's Pflanzenfamilien, I, 4, ii, 1900, p. 621.

HOFMEISTER, W.: On the Higher Cryptogamia. Translation by F. Currey; Ray Soc., 1862.

SARAUW, G. F. L.: Versuche über die Verzweigungs-Bedingungen der Stützwurzeln von *Selaginella*. Ber. d. deutsch. bot. Ges., Bd. ix, 1891, p. 51.

SPRING, A.: Monographie de la famille des Lycopodiacées, Pt. II. Mém. de l'Acad. Roy. de Belgique, t. xxiv, 1850.

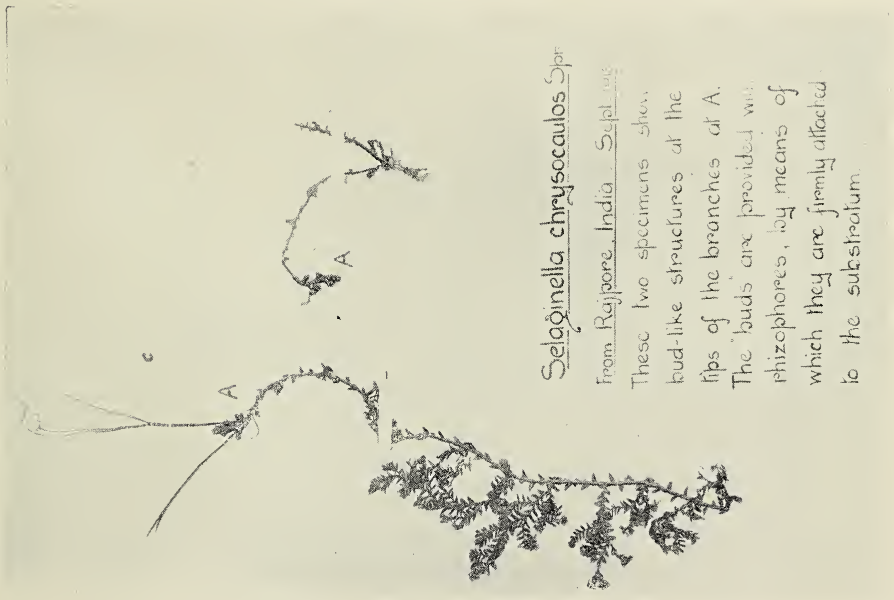
TREUB, M.: Recherches sur les organes de la végétation du *Selaginella Martensii*, Spring, 1877.

VELENOVSKÝ, J.: Vergleichende Morphologie der Pflanzen, 1905–13.

EXPLANATION OF PLATE XLIX.

Illustrating Miss Bancroft's paper on Vegetative Reproduction in some Indian Selaginellas.

Fig. 1. *Selaginella chrysocaulos*. Habit photograph.
Fig. 2. *Selaginella chrysorrhizos*. Habit photograph.
Both slightly reduced.



Selaginella chrysocaulos Spr

From Rajpore, India. Sept 1913

These two specimens show bud-like structures at the tips of the branches at A. The "buds" are provided with rhizophores, by means of which they are firmly attached to the substratum.

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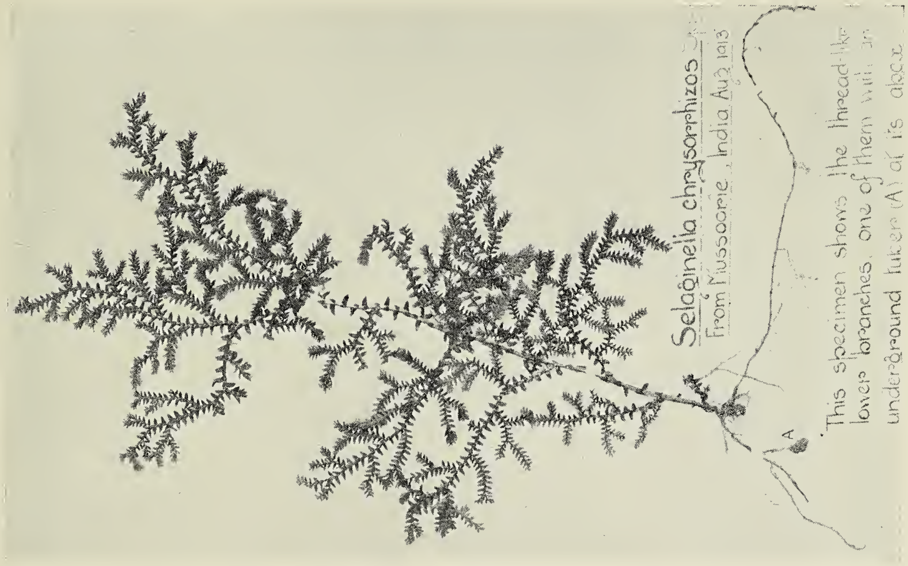

Selaginella chrysorrhizos Spr

From Mussoorie, India Aug 1913

This specimen shows the thread-like lower branches, one of them with an underground tuber (A) at its apex.

2.

BANCROFT—INDIAN SELAGINELLAS

Seedling Anatomy of Ranales, Rhoeadales, and Rosales.[1]

BY

E. N. THOMAS.

Reader in Botany in the University of London.

With Plates L and LI and forty-three Figures in the Text.

THE present century has seen a very vigorous output of work dealing with the anatomical features of the so-called transition between root and shoot structure in Phanerogams. This has, to a large extent, only been possible since the everyday use of the microtome has rendered serial examination of the youngest and slenderest seedlings a matter of ease. Even the most skilful manipulator of hand sections must fail signally to obtain much insight into the anatomical relationships of nine-tenths of the forms during their most illuminating stages.

In comparison with the work of the earlier anatomists in this field (Van Tieghem,[2] Gérard,[3] Dangeard,[4] &c.) the investigators of the twentieth century have all felt it incumbent upon them to deal with very young seedlings, but some have not been so rigorous as others in this respect, and I wish to associate myself with M. Chauveaud in emphasizing the extreme importance of this precaution. Primary structures are not merely obscured at a later date, but may be obliterated. Chauveaud has laid great stress upon this phenomenon and demonstrated it in many forms, notably in *Mercurialis annua*,[5] while I discovered it to be the case so early as 1902 in *Cleome sesquiorgialis*, and subsequently in many other species, including the common Wallflower.[6] Serial sections running through cotyledons, hypocotyl, and primary root of seedlings at different ages prove conclusively the extraordinary changes which take place with age, leading to the disappearance of groups of protoxylem elements. This point has

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[1] Thesis approved for the Degree of Doctor of Science in the University of London.

[2] Van Tieghem, Ph.: Traité de Botanique. (Paris, 1891.)

[3] Gérard, R.: Recherches sur le passage de la racine à la tige. Ann. Sci. Nat., Bot., sér. vi, t. xi, 1881.

[4] Dangeard, P.: Recherches sur le mode d'union de la tige et de la racine chez les Dicotylédones. Le Botaniste, sér. i, 1889.

[5] Chauveaud, G.: L'appareil conducteur des plantes vasculaires et les phases principales de son évolution. Ann. Sci. Nat., Bot., sér. ix, t. xiii, 1911.

[6] Cf. account of transition in Wallflower in 7th and 8th eds. of Scott's Structural Botany—Flowering Plants.

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been exhaustively treated by Chauveaud[1] in his 1911 paper, which brings together his work on seedling anatomy, and it forms the pivot of his thesis on the transitional phenomena. Inasmuch as at an early age exarch or, at any rate, 'alterne' protoxylem elements are present throughout the whole seedling, transition, according to this author, is an appearance largely due to subsequent obliteration of these elements in the upper part.

The British work of the last ten years, following upon the presentment by Miss Sargant[2] of a theory of the origin of Monocotyledons founded upon seedling anatomy, has all had a more or less well-marked phylogenetic aim. In marked contrast to this is the attitude of the earlier anatomists. Thus, in accordance with his bundle concept, de Bary, in the classic 'Comparative Anatomy of Phanerogams and Ferns', treats the hypocotyl merely as the basal internode in which the bundles of the lowest leaves, viz. the cotyledons, fuse and pectinate in a, definite manner. Van Tieghem, on the other hand, is chiefly interested in the hypocotyl as the region in which the arrangements characteristic of the root-stele become modified so as to pass into the arrangements characteristic of the stem.

Van Tieghem's investigations led him to lay stress upon two processes, (*a*) that of the so-called 'rotation' of the protoxylem, which produces the change from endarchy to exarchy, or vice versa, according as the vascular strands are followed from stem to root or root to stem, and (*b*) that of the splitting and fusion which, together with an alteration in position, produce the change from collateral to radial or radial to collateral. These internal changes correspond to external changes in epidermal characters which take place in a well-defined region termed the *collet*, which is the plane of division between hypocotyl and root. Van Tieghem, however, states that the anatomical transition is frequently drawn out by intercalary growth of hypocotyl, of root, or of both.

Rotation of protoxylem is assumed to take place in all cases, but the methods of change observed under (*b*) are classified in the 'Traité de Botanique' under three types, depending upon the nature of the strands concerned (i. e. whether xylem or phloem, or both) in the fusions or splittings, and the relative number of such strands as between stem (= hypocotyl) and root.

Thus in Type 1 the xylem changes position; in Type 3 the phloem changes position; while in Type 2 both do so.

In Type 1 the number of collateral strands in the upper part of the hypocotyl is the same as the number of phloem groups in the root; in Type 3 the number is the same as the number of phloem or xylem groups

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[1] Loc. cit.

[2] Sargant, E.: A Theory of the Origin of Monocotyledons founded on the Structure of their Seedlings. Ann. Bot., vol. xvii, 1903.

in the root; while in Type 2 the number of collateral strands in the upper part of the hypocotyl is *twice* the number of phloem or xylem strands in the root.

Thus the nature of the 'active' strand (xylem or phloem, or both) and the relation in number of stem to root strands are the distinguishing characters between the types. The determination of these characters, as well as the observation of 'rotation', is bound up with the identification of the first-formed protoxylem elements. Chauveaud[1] maintains that the phenomenon of 'rotation' is an illusion produced by early obliteration of protoxylem in the upper part of the seedling, or, as he terms it, 'accélération basifuge'. However this may be, much confusion has arisen through failure to identify the first-formed protoxylem elements with consequent variations in description of transition phenomena, due largely to difference in age of seedlings examined.

Two changes due to age may be noted now: (*a*) obliteration of primary tissues, one effect of which is to make a single structure appear to be two (see Scott [2]); (*b*) production of secondary tissues, one effect of which is to unify separate strands. As these changes go on together, the net result at any one age will depend upon the relative rates of change of these phenomena. Difference in the level at which the cotyledons were examined has probably also played a considerable part in the divergence of accounts. It is sufficient now to draw attention to one or two concrete instances of discrepancy. Thus Dr. Scott[2] describes the cotyledons of the Wallflower as having two bundles, while the precisely similar vascular system met with in the cotyledons of many Piperales,[3] Centrospermae,[4] Cactaceae,[5] and Tubiflorae,[6] is treated as single by the respective authors, who in consequence regard these as cases of Van Tieghem's Type 3, while Dr. Scott's description would make them examples of Type 2.

The practically identical transitional phenomena seen in *Dipsacus* is instanced by Van Tieghem as an example of Type 1. *Phaseolus* and *Medicago* furnish Van Tieghem with examples of Type 2 and Type 3, respectively, though the course of events is very similar in both.

My own observations on Dicotyledons, begun in 1902, very soon convinced me that there is a common ground plan, if not absolutely for all, for the majority of dicotyledonous species.

(*a*) The feature of greatest constancy is the existence of two root poles

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[1] Loc. cit., 1911.

[2] Scott, D. H.: Structural Botany, 7th ed., Part I, p. 78.

[3] Hill, T. G.: On the Seedling Structure of Certain Piperales. Ann. Bot., vol. xx, 1906.

[4] Hill, T. G., and de Fraine, E.: On the Seedling Structure of Certain Centrospermae. Ann. Bot., vol. xxvi, 1912.

[5] De Fraine, E.: Seedling Structure of Certain Cactaceae. Ann. Bot., vol. xxiv, 1910.

[6] Lee, E.: Seedling Anatomy of Certain Sympetalae. Pt. I, Ann. Bot., vol. xxvi, 1912. Pt. II, id., vol. xxviii, 1914.

in the plane joining the centres of the two cotyledons, which will be referred to as the *cotyledonary plane.* In rather less than one-third of the species chronicled (including those described in this paper) there are two other poles in the plane passing between the cotyledons—the *intercotyledonary plane.*

(*b*) In a very few cases the poles of the tetrarch root lie in the diagonal planes.

I shall refer to (*a*) as the *Cruciform* arrangement, of which there are the diarch and the tetrarch varieties, and to (*b*) as the *Diagonal*, of which there are at least the tetrarch and octarch varieties.

The Cruciform type is accompanied by the peculiar vascular arrangement in the base of the cotyledons, which has been variously interpreted as one bundle or two, and to which the name of 'double bundle' has been applied.

The double bundle essentially consists of two groups of phloem, with one group of protoxylem placed between them. The three groups are always on different radii, so that M. Chauveaud[1] well describes the relative positions as 'alterne'. While maintaining this general arrangement, the details of the strand at any one level are frequently characteristic of systematic groups of greater or less magnitude. The protoxylem groups are continuous with the poles of a diarch root, the xylem plate of which is thus always formed in the planes passing through the centre of the cotyledons. When the primary root is tetrarch, the intercotyledonary poles are represented in the cotyledons by groups of protoxylem, which may be found on the flanks of the 'double bundle' (see *Althaea rosea*, Pl. LI, Figs. 23, 24, 25), or may be more or less detached from it as lateral strands (see *Magnolia tripetala*, Pl. L, Figs. 9–12).

The Diagonal type, judging from my own observations and from the published work of others, is far less common, and may indeed perhaps be regarded as rare. I have observed it in Calycanthaceae, Fagaceae, Euphorbiaceae, and it has been described by Miss W. Smith[2] in the Sapotaceae and by Mr. Wright[3] in the Ebenaceae. Its relation to the Cruciform type is not obvious, but there are some significant features which will be considered later. It may be combined with the Cruciform type to form an octarch root if four of these poles are present, or a hexarch root if only the cotyledonary poles of the cross be present (e.g. *Pyrus communis*, &c.). It is usually accompanied by that wide separation of the halves of the 'double bundle', together with early obliteration of the central

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[1] Loc. cit.

[2] Smith, W.: The Anatomy of some Sapotaceous Seedlings. Trans. Linn. Soc., ser. ii, Bot., vol. vii, 1909.

[3] Wright, H.: The Genus *Diospyrus* in Ceylon. Ann. Roy. Bot. Gard., Peradeniya, vol. ii, 1904.

protoxylem group, which gives the most complete appearance of independence at the base. Thus, chance sections of the cotyledonary petiole of *Ricinus communis* certainly do not suggest any connexion between its four widely separated strands and a 'double bundle'; nevertheless, the two central bundles unite higher up, and careful examination at a suitable age reveals the presence of the isolated protoxylem elements in the petiolar base.

The anatomical arrangements found in hypocotyl, primary leaves (cotyledonary and plumular), and primary root, together with their interrelationships, have proved very difficult of description, and there is not a little danger of being led astray by our own conventional even though quite necessary terminology.

Perhaps the greater number of investigators, beginning with Van Tieghem, and including Sargant, Wright, Tansley and Thomas, Hill and de Fraine, and Lee, have described the transition features from above downwards, but not a few, including Smith, Chauveaud, and Compton, have described them in the reverse direction. Although I have been in the habit of describing the course of the vascular strands proceeding from leaves to root, in view of the many pitfalls involved, the reverse procedure is perhaps less open to objection. In both cases the hypocotyl is treated solely as a 'transitional'[1] region, and the inevitable impression of motion or change of position is exaggerated. From many points of view I am inclined to think that a truer concept is obtained by considering the hypocotyl as an axis with an anatomical structure of its own, having relation to that of leaves on the one hand and primary root on the other.

The hypocotyl may be taken to be the region between the *collet* and the cotyledonary node. This region may be short or long, and at either end it will be influenced by its relationships with the other members. The most independent and uninfluenced part will be roughly half-way between these points. The cotyledonary node is always well defined morphologically, the collet far less so. The internal changes do not necessarily correspond in level with the external changes. This is true in general of the insertion of leaf-traces for fossil (e. g. *Lyginodendron* and *Palaeostachya*), and recent forms, as witnessed by de Bary's twenty-three types of leaf-trace insertion, varying in distance from point of entry (i. e. node) and mode of union.

In 1904 Mr. Tansley[2] and I drew attention to the phenomenon of root structure in the central cylinder of the hypocotyl of a number of forms. I think it is safe to say that a more or less *root-like* anatomy is characteristic of the hypocotyl of by far the great majority of the forms now known to us, that in short it is the rule and not the exception. Nevertheless, the

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[1] This finds its most extreme expression in the work of Sterckx.

[2] Tansley, A. G., and Thomas, E. N.: Root Structure in the Central Cylinder of the Hypocotyl. New Phytol., vol. iii, 1904.

structure is by no means the same as that of the root. When the hypocotyledonary region is long enough for its structure to appear uninfluenced by the changes associated with the cotyledonary node and collet respectively, it is seen to consist essentially of a more or less pithed xylem mass with a varying number of practically exarch protoxylem groups, the whole sometimes almost completely surrounded by phloem, but more often with the phloem broken into groups alternating with the protoxylem groups.

All the conducting tissue present at a very early age is directly continuous with that in the cotyledons in an upward direction, and with that in the primary root in a downward direction.

In Text-fig. 42 (p. 730) the phloem breaks away along the line indicated, and a xylem group, together with the two phloem groups thus produced, passes into the base of one cotyledon (*a*, *b*), constituting the curious 'double bundle' described above, the other cotyledon being supplied in a similar manner. At the other end of the hypocotyl the extended phloem groups of the latter are continued by the much smaller and more compact ones of the root (*c*), while the rather scattered xylem is continuous with the definite diarch plate of the same, the pith having disappeared. The ill-defined endodermis of the hypocotyl passes into the well-defined endodermis of the root.

In Text-fig. 43 (p. 731) a similar process takes place, but in addition to the central protoxylem of the 'double bundle', groups of protoxylem on the flanks continue the intercotyledonary groups of the hypocotyl (*a*, *b*). (See also Pl. LI, Figs. 24, 25 of *Althaea rosea.*) In other forms these groups are separated from the double strand as distinct laterals, e. g. *Magnolia tripetala*, and the double bundle itself may be represented by what appears at first sight, particularly at some ages and at some levels, to be two perfectly distinct strands. (See Text-fig. 38, p. 722, of *Prunus persica.*) Following the strands downwards through the hypocotyl, the intercotyledonary protoxylem groups are seen to pass into the intercotyledonary poles of the tetrarch root (*c*).

The most marked 'transition' takes place somewhere in the cotyledon, where the double bundle becomes continued upwards as a single collateral strand. Most authors speak of this as the delayed fusion or precocious splitting of phloem groups, according to direction of description, but none, so far as I am aware, have suggested any reason for the delay, or, in other words, for preparation for root formation in the cotyledons. The double bundle, moreover, is met with in its most extreme form in those species in which root structure is furthest from the cotyledonary node (Euphorbiaceae, Calycanthaceae). The homology with the double bundle of the widely separated central strands of these forms is proved by their union in the upper part of the cotyledon, and by the identification of isolated central protoxylem elements in the lower part.

THE FORMS DESCRIBED.

The species to be described in this paper form part of an investigation on seedling anatomy which has been proceeding for some years. It was begun in 1902 at the instigation of Mr. A. G. Tansley, and some of the work was carried on while acting as his research assistant. I take pleasure in expressing my extreme indebtedness to him for the very kind permission to include the work done at that period.

Quite recently I have been greatly assisted in the matter of preparations, &c., by my Demonstrator and former student, Miss A. J. Davey, B.Sc. Some of the data included have been taken from preparations made by Miss K. Blackburn, B.Sc., in connexion with another investigation now in progress.

In all, some 150 species belonging to the cohorts Ranales, Rhoeadales, and Rosales have been examined, about half of which are Ranalean forms.

Much of the material has been grown at Bedford College, a little at University College, while seedlings for this investigation were raised some years ago at University College, Reading, and also by Miss Strudwick at Cambridge.

RANALES.

More than seventy species have been examined, fifty-two of which are members of the Natural Order Ranunculaceae, seven of Berberidaceae, and one of Lardizabalaceae. In the Magnoliaceae six species have been examined, in the Calycanthaceae four, in Anonaceae two, and in Lauraceae two.

The Ranunculaceae are very uniformly diarch; the Magnoliaceae, Calycanthaceae, and Lauraceae are very largely tetrarch; while the Berberidaceae have tetrarch species. The Calycanthaceae present the most outstanding features, and show the rare Diagonal type in a very interesting form.

Ranunculaceae.

The Ranunculaceae are characterized by a tendency to form cotyledonary tubes, which, as was noted by Darwin, seems to be associated with a tendency to form tubers. The lateral bundles of the cotyledon usually unite with the central high up, so that the base of the cotyledon shows only one bundle which is not usually markedly double. These bundles connect in many forms with the vascular system of the hypocotyl through a very sharp angle, rearrangements in the tissues taking place at the same time, so that the hypocotyl has a root-like structure from very near the node.

Anemoneae.

Anemone coronaria. This species has a marked cotyledonary tube in which two small collateral strands, corresponding to the midribs of the respective cotyledons, face each other across the central cavity. Immediately below the cotyledonary node the strands close into the centre of the axis through a sharp bend, so that the tissues are almost longitudinal in the transverse section, and this may be sometimes more marked on one side than on the other. As the strands enter the axis, the readjustments take place which produce a diarch root very near the top of the hypocotyl. These readjustments are obscured by the fact that, to a large extent, they take place in the bend, but in the section shown in Pl. L, Fig. 3, the bifurcation of the phloem can be seen very plainly in the strand to the south. The aggregation of the phloem in two alternating patches is the root character which appears highest in the hypocotyl, while the absence of pith and formation of a definite xylem plate with exarch protoxylem groups are characters only to be found at a lower level. Although each cotyledonary bundle consists of only three or four xylem elements, one or two of these are usually secondary in formation, judging from the radial arrangement found.

Anemone fulgens forms a cotyledonary tube of considerable length, which in the upper part becomes a solid cylindrical structure through the interlocking of cells of the cavity (Text-fig. 1). The cotyledon strands pass more steeply into the hypocotyl, so that the transition can be followed with greater ease than in *A. coronaria.*

Anemone rivularis is very similar to *A. fulgens.*

Anemone sylvestris. In this a very short cotyledonary tube or sheath is found, which in some individuals is asymmetrical through union of the cotyledons at a slightly higher level on the south side away from the first leaf, and through displacement of the cotyledonary strands to this side.

The transition features are similar to those of *A. coronaria.*

Anemone virginiana. In this species the cotyledons fuse quite at the base as in *A. sylvestris* (Text-fig. 2). In this region the cotyledon strands are very slightly double groups. The features of transition are like those of the above-described species.

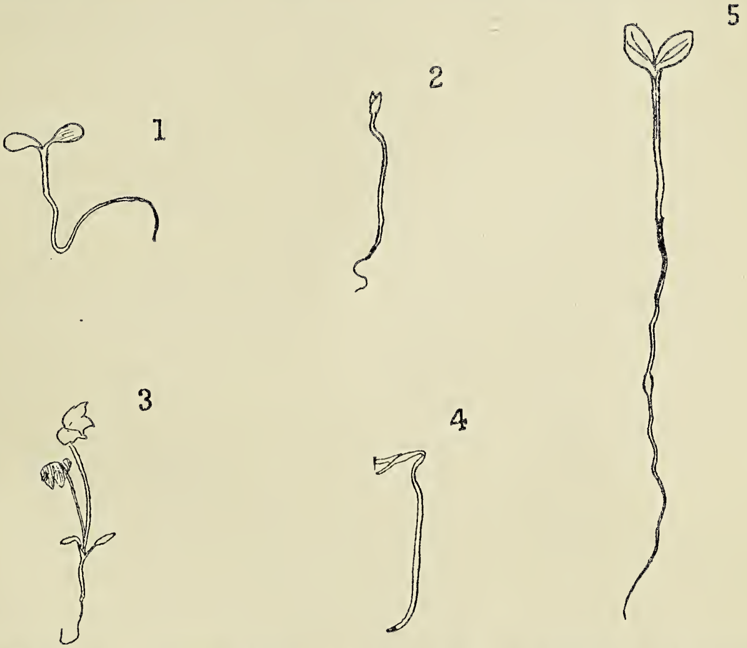
Anemone pulsatilla. There is a very slight cotyledonary tube at the base, in which the cotyledon bundles show a double structure.

In one individual the most remarkable asymmetry was found, resulting in the formation of a diarch root, one of the poles of which is continuous with the vascular system of the first plumular leaf, while the other is continuous with the two asymmetrically placed cotyledonary strands.

Anemone vitifolia also possesses a short cotyledonary tube, which is somewhat asymmetrical through union at a higher level on one side than on the other. The vascular bundles are slightly displaced towards this side, but the transition is normal.

Anemone vernalis has a distinct cotyledonary tube, in which union of the cotyledons takes place on one side first, and rearrangements occur as the bundles turn inwards at the node. This species shows very markedly the early (i. e. high) union of the phloem groups, together with the wide and indeterminate grouping of the protoxylem elements in the upper part of the hypocotyl.

Anemone montana shows the usual features very distinctly. The early phloem grouping and late protoxylem aggregation of *A. vernalis* is seen in this species also.



TEXT-FIGS. 1–5. 1, *Anemone fulgens*; 2, *A. virginiana*; 3, *A. alpina*; 4, *A. nemorosa*; 5, *A. apennina*. All nat. size.

Anemone alpina possesses a short cotyledonary tube or sheath, in which the strands are markedly double (Text-fig. 3). As the individual examined was rather older than the members of other species, it is possible that the doubleness may be, to some extent, an age phenomenon. The result is a much closer resemblance to members of the Rhoeadales cohort than is seen in other species.

Anemone nemorosa. In the quite young stage the seedlings of this species are normal (Text-fig. 4), but very soon become swollen up in a tuberous manner, so that the conducting tissues in some cases become separated by parenchyma. Thus the two phloem groups are separated from the somewhat distorted diarch plate by parenchymatous cells. In the middle line, however, of these cells, at right angles to the plate, there is to be found one row of secondary xylem elements, giving a curious tetrarch appearance. (Cf. *Eranthis.*)

Anemone apennina is a pseudo-monocotyledonous form in which the single cotyledonary member has a long petiolar region, bearing a more or less bifid blade. The root has the customary diarch structure; this structure is not only found throughout the hypocotyl, but also above the cotyledonary node in the lower part of the cotyledonary petiole. Thus the transition takes place *wholly* in the cotyledon, and more or less in the region marked by a collet (Text-fig. 5), half-way between blade and insertion. This species will be more fully described in a subsequent paper.

The twelve species of Anemone examined indicate the prevalence of cotyledonary tubes within the genus and the tendency to form tubers. *A. apennina* is extreme in both characters. The cotyledonary member appears to be absolutely single, and none of the individuals examined show the bisymmetrical anatomy figured by Sterckx.[1]

Twelve species of the genus *Ranunculus* have been examined. With the exception of *R. Ficaria*, which is a pseudo-Monocotyledon and wholly peculiar, the species do not differ from one another in any important respect, and are generally characterized by the sharp angle of entry of the cotyledon strands, rearrangements occurring at the entry, so that a diarch structure is met with just below the node. Secondary roots are produced almost at the node, and in some species, e. g. in *R. sceleratus*, there is a tendency for the primary root to abort as in Monocotyledons. Most of the species have a slight cotyledonary tube, and some form tubers.

Ranunculus acris, *R. gramineus*, *R. parviflorus* show very perfectly the features of the genus. They are slender forms, but with a tendency to the suppression of internodes. The small diarch root structure is found just below the cotyledonary node. As the strands pass from the hypocotyl into the base of the cotyledonary tube, they usually become single and collateral. Each is continued into the free portion of a cotyledon as its single central collateral strand, which only gives off laterals in the lamina (Text-figs. 6 and 7).

R. bulbosus, *R. arvensis*, *R. auricomus*, and *R. repens* are quite similar, and call for no comment.

R. hederaceus shows great development of secondary roots at the node (Text-fig. 8).

R. sceleratus has not only plentiful secondary roots at the node, but the primary root dies out at an early age.

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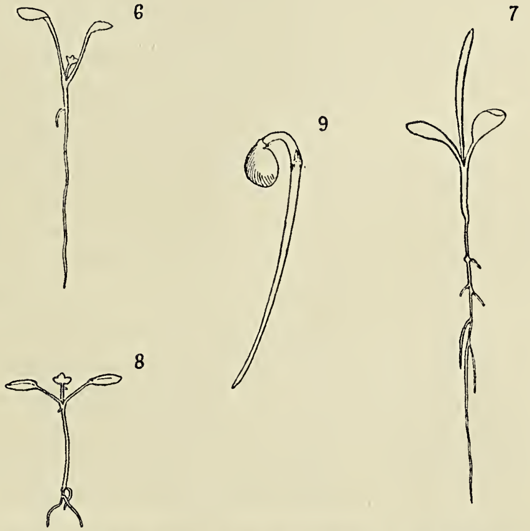
[1] Sterckx, R.: Recherches anatomiques sur l'embryon et les plantules dans la famille des Rénonculacées. Mém. Soc. roy. Sci., Liège, sér. iii, t. ii, 1900.

R. flammula conforms to the general features of the genus, with some peculiarities obviously due to the water habit.

Chauveaud has described half a dozen additional species which do not appear to differ from those described above.

R. Ficaria (Text-fig. 9). The morphology of this species has given rise to much discussion. The cotyledonary member appears to be quite single, and it is traversed by a single strand. As in the other species the primary root is diarch. At the cotyledonary node the greater part of the vascular tissue passes upwards into the cotyledon and is continued as its central strand. From the same region the vascular strand of the first leaf has its connexion. The exact relationship of these vascular strands will be discussed in a subsequent paper.



Text-figs. 6–9. 6, *Ranunculus acris*; 7, *R. gramineus*; 8, *R. hederaceus*; 9, *R. Ficaria*. 6, 7, and 8 $\frac{2}{3}$ nat. size; 9 × $2\frac{1}{2}$.

The seedlings of the genus *Clematis* are distinctly more robust than those of *Ranunculus* and *Anemone*. Nine species have been examined, and the chief interest lies in the two species *C. viticella* and *C. Hendersoni*, in which there are vascular strands in the intercotyledonary plane of the hypocotyl, which are continued upwards as the lateral bundles of the cotyledon. (See Pl. L, Fig. 6, *Liriodendron tulipifera*.)

Clematis vitalba. The transition is high, and the vascular bundle of the cotyledon is double at the node. The diarch plate is found at a short distance below the node, and consists of a radial file of vessels.

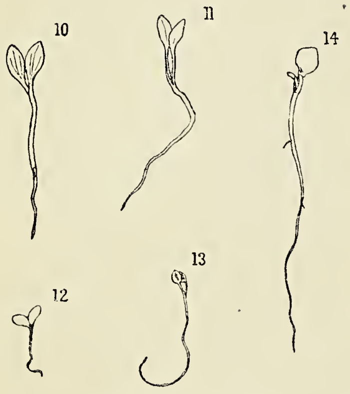
Clematis flammula (Text-fig. 10). This species forms a short cotyledonary tube. The bundles of the cotyledons are double for some distance above the cotyledonary node, and connect with the diarch root in the usual manner. The plumule is noticeably late in differentiation. In seedlings which show plentiful metaxylem and crushed protoxylem, there is no sign of lignification in the plumular rudiments.[1]

Clematis recta possesses a short cotyledonary tube, and presents the usual type of anatomy.

Clematis orientalis (Text-fig. 11). The cotyledonary strands are double in the cotyledons of this form, as in *C. flammula*. The transition is quite normal; the xylem of the root does not meet in the centre of the diarch plate but is present in two pegtop-shaped clumps, the broad ends of which are directed towards one another, but do not touch.

Clematis glauca and *Clematis alpina* call for no remark.

Clematis Davidiana. An interesting peculiarity was met with in one individual of this species (Text-fig. 12), which showed a single cotyledonary member evidently produced by marginal fusion of the pair. The two vascular strands of this member approached each other at the node, and it would seem that the diarch plate is formed in the normal plane, i. e. that passing through the supposed centres of the cotyledons. Comparison with similar anomalies in *Anemone sylvestris* and *A. pulsatilla* adds interest to this occurrence.



TEXT-FIGS. 10–14. 10, *Clematis flammula*; 11, *C. orientalis*; 12, *C. Davidiana*; 13, *C. Hendersoni*; 14, *C. viticella*. All reduced about ½.

C. Hendersoni and *C. viticella* (Text-figs. 13 and 14). These species stand out from the others by virtue of the possession of lateral strands in the base of the cotyledon. These strands pass into the hypocotyl independently of the double bundle and unite with the corresponding bundle from the other cotyledon. There may be said to be practically a tetrarch stage, inasmuch as the small phloem groups of the laterals divide in the hypocotyl, so that the intercotyledonary protoxylem elements are exarch, although through their disappearance the root in its final form is diarch. The tetrarch appearance is enhanced in older seedlings by the production of four masses of secondary xylem, leaving rays in the intercotyledonary plane which are almost as marked as those in the cotyledonary plane.

Thalictrum flavum, *T. glaucum*, *T. javanicum*, and *T. adiantifolium*. This genus presents many histological peculiarities which will not be considered in this paper. The transitional features of the four species examined are quite alike, and similar to those of the genera described above.

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[1] The terms protoxylem and metaxylem are applied in a purely relative sense throughout this paper and no precise or histological meaning is to be attached to them.

Adonis annua. The seedling anatomy is of the ordinary type, but the doubleness is more marked in the cotyledon than in many of the Ranunculaceous forms.

Myosurus minimus, although absurdly reduced, seems to form its minute diarch root in the usual way.

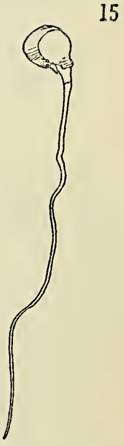
Helleboreae.

Nigella aristata and *N. hispanica.* The hypocotyl has the usual four phloem groups, with the four metaxylems connected in the cotyledonary plane by a thin bridge of crushed protoxylem. At the node the xylem and two phloem groups pass out to form the double bundle of the cotyledon, which gives off laterals at this point. Further down the hypocotyl the fusion of the phloem groups and approximation of metaxylem to form a plate results in the usual diarch root.

Nigella sativa, described by Gérard,[1] and *N. damascena*, described by Chauveaud, appear to resemble the above species.

Helleborus foetidus shows similar features.

Aquilegia vulgaris, *A. alpina*, and *A. canadensis.* This genus very closely resembles *Thalictrum*, and has the same histological peculiarities. The transition features seem to be quite normal, but the seedlings examined were rather old. As in *Thalictrum*, the cotyledonary bundle is markedly double.



Text-fig. 15. *Paeonia herbacea.* $\frac{2}{3}$ nat. size.

Delphinium formosum, *D. luzulinum*, and *D. sp.* (annual form). The seedling anatomy is quite normal. The double bundle is extremely marked, and the genus resembles *Aquilegia* even in some of the histological details.

Trollius asiaticus and *Caltha palustris.* These species conform in some measure to the general type, but present special features which will be dealt with in a subsequent paper.

Paeonieae.

Paeonia herbacea and *P. arborea* show two Y-shaped xylem groups in the cotyledonary plane of the hypocotyl, alternating with two extended phloem groups in the hypocotyledonary plane, which, in combination with the lateral extension of the xylem arms, is slightly suggestive of the tetrarch condition. At the node the half of each phloem group passes out with a xylem Y into each cotyledon, where they form a large extended double bundle, which in *P. arborea* gives off laterals at the base of the petiole. When the strands are followed downwards, it is seen that as the diarch root structure is attained by aggregation of phloem

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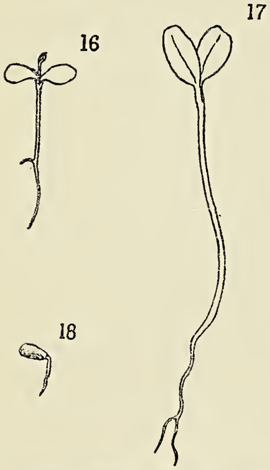
[1] Loc. cit.

groups, &c., the xylem decreases in quantity until only the two small protoxylem strands remain.

Berberidaceae.

The genus *Berberis* is of great interest, inasmuch as both the tetrarch and the diarch condition are to be found within the genus, and even within the species, as in *B. macrophylla.*

Berberis Lycium. The hypocotyl shows four diagonal strands of phloem with some metaxylem to the inside, and with obvious exarch protoxylem elements in the cotyledonary plane, and somewhat less obvious ones in the intercotyledonary plane. At the node the usual double strand passes out into the base of the cotyledon, and is continued up the petiole. At the base of the hypocotyl the slight adjustments take place which convert the structure into that of a typical tetrarch root. (See Pl. LI, Figs. 21–6 of *Althaea rosea.*)



Text-figs. 16–18. 16, *Berberis Lycium*; 17, *B. aristata*; 18, *B. vulgaris*. All $\frac{2}{3}$ nat. size.

B. aristata shows similar features, but may reduce to triarch, while *B. macrophylla* reduces rapidly to a triarch, and sometimes later to a diarch condition.

B. vulgaris, *B. aquifolium*, *B. nervosa*, *B. heteropoda* show early fusion of the phloem groups and a diarch root.

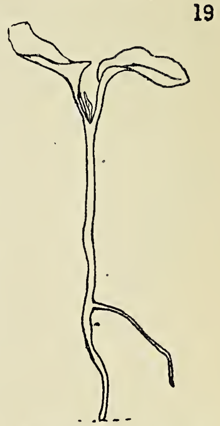
Comparison of seedlings at different stages in these species shows very clearly the changes produced by age. Thus in *B. aquifolium*, at an age when the first leaf is just visible, the halves of the double bundle appear to be very widely separated at the cotyledonary node on account of the obliteration of the central protoxylem group and the semi-obliteration of the two tangentially produced wings, while inside the four phloem groups are to be found masses of metaxylem not to be found at an earlier age.

Podophyllum Emodi. This species has a very long cotyledonary tube with a small central cavity, which becomes occluded in the upper part and widens out round the plumule near the base. The root is typically diarch, but the plate in the hypocotyledonary region develops intercotyledonary xylem arms between the phloem groups, which produce a tetrarch appearance reminiscent of the tetrarch species of *Berberis.* These groups are continued upwards into the plumular leaves, and the main strands are continuous above with the double bundles of the cotyledons in the usual manner. The double bundles are remarkable for the extremely exarch position of the xylem, which constitutes the apex of an outwardly directed V throughout the petiole. Lateral strands are given off as these enter the free laminae. Apart from this special feature *P. Emodi* would seem to resemble *Eranthis hiemalis*, judging from Miss Sargant's [1] description and figures of that species.

Lardizabalaceae.

Decaisnea Fargesii. The hypocotyl of this species is long and stem-like. It shows the usual four groups of phloem with metaxylem within, and somewhat crushed protoxylem outlining the inner border. At the cotyledonary node four small strands are detached which pass independently into the base of the cotyledons. The main portion of the four diagonal bundles of the hypocotyl is continued upwards as the very widely divergent halves of the double bundle, only identifiable as such by the sporadic appearance of isolated central protoxylem elements. (Cf. Calycanthaceae below.) The four apparently collateral strands of the lower part of the hypocotyl gradually pass into the tissues of a diarch root by approximation of the phloems in the intercotyledonary and of the xylems in the cotyledonary planes, respectively.

The behaviour of the lateral strands is somewhat similar to that of the corresponding bundles of *Liriodendron tulipifera* (Pl. L, Fig. 6), while the central double bundle is very like that of *Calycanthus* (Text-fig. 22).



TEXT-FIG. 19. *Decaisnea Fargesii.* $\frac{2}{3}$ nat. size.

Magnoliaceae.

Magnolia tripetala (see Pl. L, Figs. 9–12). The hypocotyl shows as its most characteristic arrangement eight groups of phloem with metaxylem within each, and four groups of protoxylem situated in the cotyledonary and intercotyledonary planes, so that phloem and metaxylem are found in twin pairs in the diagonal planes. The aggregation first of the phloem pairs to form four masses in the diagonal planes, and then of the metaxylem so as to form a quadrangular mass of wood which is nearly solid (see Pl. L, Figs. 10–12), gradually changes the hypocotyledonary arrangement to that of a root as we follow it downwards. In passing upwards from the structure described for the hypocotyl, the phloem and xylem groups nearest to the intercotyledonary plane are seen to fuse a short distance below the node, so that a single collateral strand is formed in this plane, thus sharply distinguishing it from the double bundles in the cotyledonary plane which are continued as such into the petioles of the cotyledons. The collateral intercotyledonary strands bifurcate at the node just after leaving

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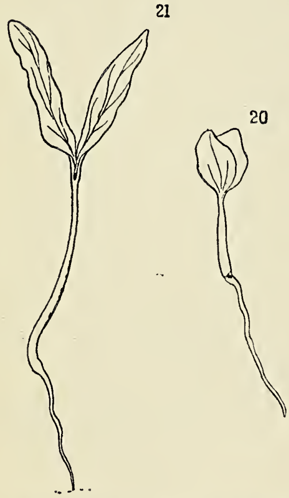
[1] Loc. cit.

the central cylinder, and the halves thus produced pass into the cotyledons as the lateral bundles of the petiole. Thus a tetrarch root is formed from the 'doubles' and two laterals of the cotyledons.

It is to be noted that if the 'double' be regarded as one bundle, the laterals, coming as they do one from each cotyledon, cannot but be considered as two strands, and we have therefore a condition which does not fall into either of Van Tieghem's types, but is partly conformable with Type 3, and partly with Type 2.

Magnolia Soulangeana (Text-fig. 20) has the same mode of transition and general features as *M. tripetala*, with the exception that the lateral bundles do not form a collateral strand just below the node, but pass out as two separate bundles, one going to each cotyledon as in *M. tripetala.*



TEXT-FIGS. 20, 21. 20, *Magnolia Soulangeana*; 21, *M. acuminata.* ½ nat. size.

Magnolia acuminata (Text-fig. 21) resembles *M. Soulangeana*, and forms a tetrarch root in a similar manner. Sections from an older seedling of this species show how completely age masks the primary structure. In this individual a complete ring of secondary tissues is found just below the cotyledonary node, which take the place of the obliterated protoxylem groups of the 'doubles' so obvious at an earlier age.

The transition phenomena of these three species are thus precisely similar.

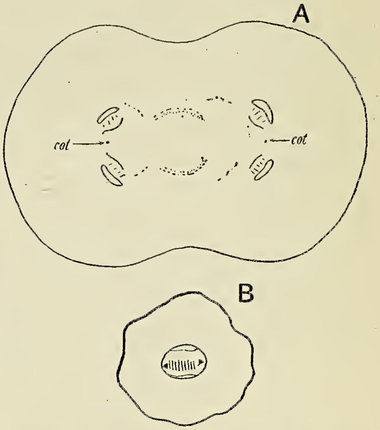
Magnolia Yulan differs markedly from the preceding species in the possession of a diarch primary root, and also in the absence of free lateral strands in the base of the cotyledons.

Liriodendron tulipifera (Pl. L, Figs. 5–8). The upper part of the hypocotyl resembles that of *M. tripetala* in essential features, but the vascular system in the intercotyledonary plane is smaller, so that the stele forms a rectangle of which the intercotyledonary sides are shorter than the cotyledonary sides. As the intercotyledonary strands pass upwards to the cotyledonary node, they unite to form a single collateral strand as in *M. tripetala*, which only bifurcates to form the laterals of the cotyledon petioles as the latter diverge from the axis. As the strands of the hypocotyl are followed downwards towards the root, the intercotyledonary ones gradually disappear, inserting themselves on the more robust cotyledonary strands. This leads to the formation of a diarch root.

Illicium religiosum. This species has not been fully worked out, but it would seem to resemble *Magnolia Yulan* in the absence of lateral bundles and formation of a diarch root.

Calycanthaceae.

Calycanthus floridus, *C. praecox*, *C. occidentalis*, and *C.* (*Chimonanthus*) *fragrans.* The very long hypocotyl shows an elliptical stele with four collateral bundles at the corners. At the cotyledonary node the two at the same end of the ellipse pass out into the base of a cotyledonary petiole, where they approach each other slowly, to form a very widely separated 'double bundle', the halves of which are, as it were, linked together by a few scattered and disorganizing protoxylem elements (Text-fig. 22, A). These protoxylem elements can be identified occasionally here and there in the hypocotyl isolated in the cotyledonary plane. (Cf. *Decaisnea*, described above.) A short distance from the base of the petiole lateral bundles become distinct from the central 'double', but remain in close connexion nearly to the top of the petiole.



Text-fig. 22. *Calycanthus floridus.* A, Cotyledonary node; B, Root of abnormal specimen. Diagrammatic.

Outlining the inner margin of the four cotyledonary strands of the hypocotyl is a fringe of crushed protoxylem elements at an age when no plumular tissue is lignified. At the base of the hypocotyl the protoxylem in the four strands becomes exarch, and with the exception of certain individuals of *C. floridus*, a tetrarch root is formed through the production of a phloem group in the cotyledonary plane. I have not satisfied myself with regard to the origin of this phloem strand, but Chauveaud[1] describes it as taking its origin in the hypocotyl, or, considering the strand from root upwards as ending blindly in the hypocotyl, having no connexion with the upper part of the seedling. Older seedlings of *C. praecox* show small 'secondary' bundles between the halves of the double bundle, as the result of cambial activity near the cotyledonary node. It is possible that these may connect with the cotyledonary phloem groups just described.

The exceptional individuals of *C. floridus* possessed a diarch root with the poles in the customary cotyledonary planes (Text-fig. 22, B). (Cf. *Decaisnea.*)

Anonaceae.

Anona triloba. The stele of the hypocotyl is somewhat similar to that of *Calycanthus* in the upper part, but the double bundle forms much more of a unit with central exarch protoxylem obvious up to the cotyledonary node and present in a rather more mesarch position in the base of the

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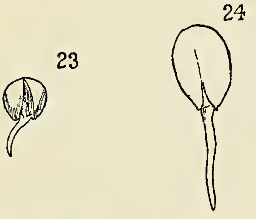
[1] Loc. cit.

petioles. Proceeding downwards, root structure which in *Anona* is diarch is attained at a higher level than in *Calycanthus.*

A. reticulata presents similar features.

Lauraceae.

Laurus Sassafras. The top of the hypocotyl shows four groups of phloem and xylem in the diagonal planes, with two or three very small crushed and almost obliterated protoxylem elements isolated in the cotyledonary plane. As these tissues are followed upwards they are seen to pass into the cotyledons, as in the previous families forming a double bundle which at the base of the petiole detaches two main laterals. In other words, the main lateral strands which in *Magnolia tripetala*, *M. acuminata*, and *M. Soulangeana* pass into the hypocotyledonary axis independently, sweeping round so as to enter the stele in the intercotyledonary plane, in *L. Sassafras* merge with the double bundle at the cotyledonary node and thus enter the stele as one unit. Downwards the hypocotyledonary arrangements merge into those of a tetrach root as the intercotyledonary protoxylem poles become defined.



TEXT-FIGS. 23, 24. 23, *Laurus Sassafras*; 24, *L. nobilis.* $\frac{2}{3}$ nat. size.

Laurus nobilis presents an octarch stage in the seedlings examined, which were, however, too old.

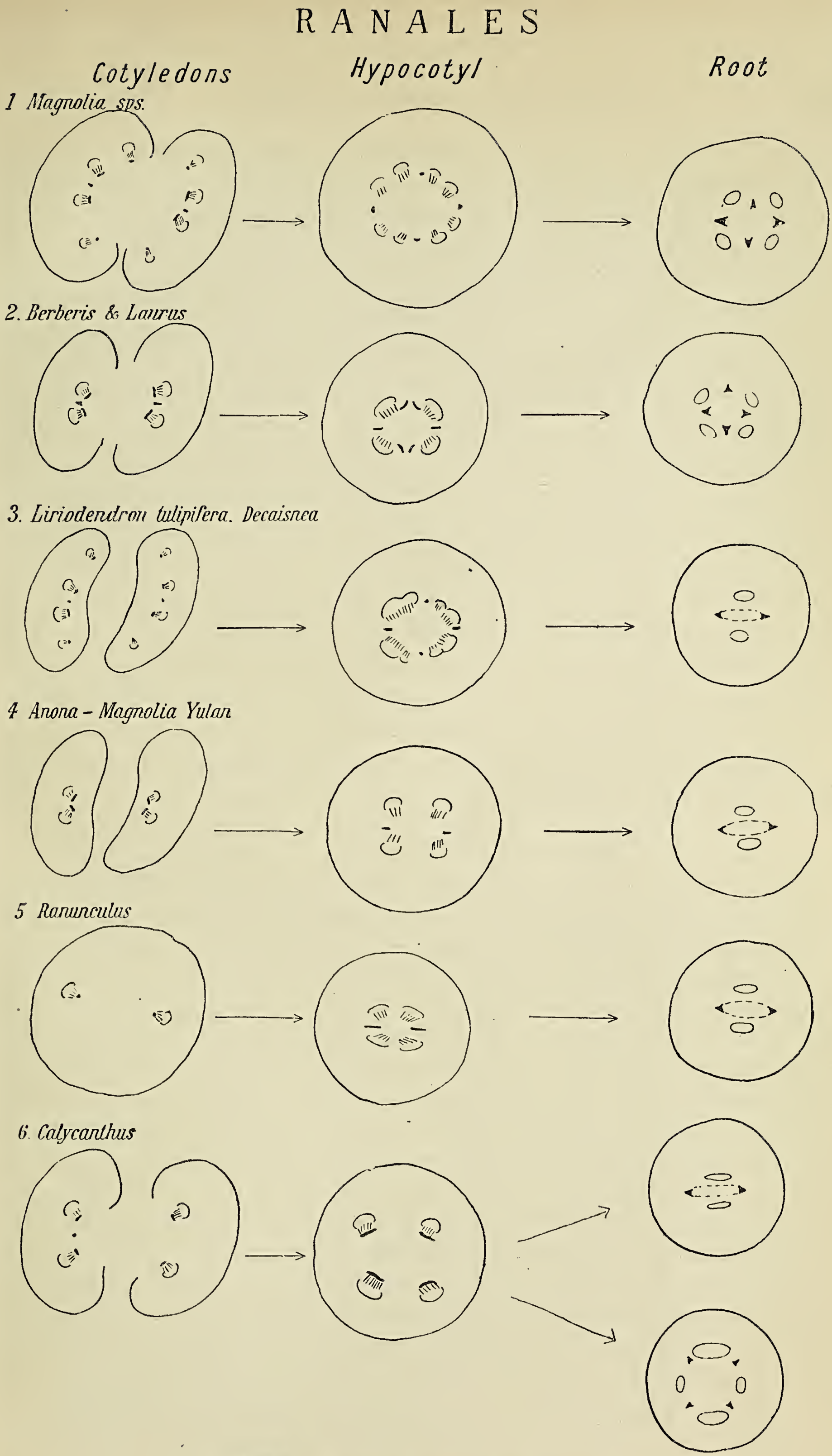
Cassytha is described by Mirande as tetrarch.

Laurus and the species *Lycium* and *aristata* of *Berberis* present the so-called 'Anemarrhena' type reported by de Fraine as occurring in Cactaceae and by Lee in Compositae, in which it may be said that the xylem of the cotyledonary strand divides into three and the phloem into two to form a tetrarch root.

I have been familiar with this type for many years and *Althaea Rosea* (Pl. LI, Figs. 23–6) presents a very good instance of it. In this case the lateral strands are only independent in the lamina and are merged with the central one throughout the petiole, which thus possesses an extended kidney-bean-shaped 'double' bundle, the central protoxylem of which is external at the cotyledonary node (Pl. LI, Fig. 24). *Althaea* differs from *Laurus Sassafras* only with regard to the degree of independence of the laterals, or, in other words, with regard to the exact point of approximation with the double bundle in the cotyledon.

The constancy of the Ranunculaceous type is in contrast to the variation met with in the few members examined of the other Ranalean families.

They furnish, in addition to the 'Anemarrhena' type of *Berberis* and *Laurus*, the series in the Magnoliaceae (Text-fig. 25, 1, 3, and 4) in which *Liriodendron* links the tetrarch and diarch forms. *Liriodendron* and *Decaisnea* couple presence of laterals with a diarch root (Text-fig. 25, 3), while



TEXT-FIG. 25.

Diagrams illustrating principal types of vascular arrangement in cotyledons, hypocotyl, and root of Ranalean forms.

Berberis and *Laurus* form a tetrarch root in the absence of laterals (Text-fig. 25, 2). *Calycanthus* presents that wide separation of the halves of the double bundle with almost complete obliteration of the central protoxylem which is one of the most puzzling aspects of seedling anatomy, and it is coupled with two forms of root.

RHOEADALES.

About thirty species have been examined belonging to the Rhoeadales cohort. They show very great constancy of feature and present good typical examples of the diarch type. They include members of the Cruciferae, Papaveraceae, Resedaceae, and Capparidaceae. They are for the most part herbaceous forms, but the shrubby *Capparis* and *Cleome* are very similar in their anatomy. The species here described include *Papaver*, instanced by de Bary[1] as a form with one bundle to the cotyledonary trace, and *Cheiranthus*, stated by Scott[2] to have two. *Corydalis* and *Fumaria*, in common with the other members of the group, present the features which have been described by all recent authors as illustrative of Van Tieghem's Type 3, but Van Tieghem[3] himself gives *Fumaria* as an example of Type 1.

Cruciferae.

This order shows very uniform structure and it is only in the neighbourhood of *Lunaria* that there is any departure from type.

Hesperideae.

Cheiranthus Cheiri. The hypocotyl shows a somewhat elliptical vascular cylinder consisting of the usual diagonal strands of phloem and metaxylem. The pairs at each end of the ellipse are connected by the more or less crushed protoxylem of the cotyledonary plane. Near the cotyledonary node the ellipse widens out and the vascular tissue at each end is continued into the cotyledons as the double bundles of the petioles, while the tissue in the intercotyledonary plane ultimately connects with the vascular system of the first plumular leaves. Proceeding towards the collet, the hypocotyledonary tissues become continuous with those of a diarch root (similar to that shown in Pl. LI, Fig. 16) by the following stages: (1) disappearance of the pith, (2) aggregation of the alternate phloem masses, and (3) reduction of the xylem to a single file of elements constituting the diarch plate. The epidermis and endodermis also change in character, but usually at a lower level. In the upper part of the cotyledon the double bundle is continued by a single strand. This is the now familiar diarch type (Pl. LI, Figs. 17–20). The examination of a form with three cotyledons revealed a triarch root.

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[1] de Bary, A.: Comparative Anatomy of the Phanerogams and Ferns, p. 236, (Oxford) 1884.

[2] Loc. cit.

[3] Loc. cit., p. 782.

The extreme simplicity of this arrangement is due to the continuity of two strands of alternately placed exarch protoxylem throughout the seedling from the diarch root to some region in the cotyledons where more or less gradually the collateral arrangement is assumed. These strands are continuous with the central bundle of the series of collateral strands of the lamina, all of which are massed with the central at some point usually in the blade, but one independent lateral bundle may be met with in the petiole on each side of the central strand (e. g. *Lunaria biennis*). The existence of the alternate exarch or mesarch protoxylem group is frequently almost or quite non-identifiable on account of the degeneration changes which take place in these elements, leading to their obliteration in cotyledons and top of hypocotyl at an early age.

Cheiranthus maritimus. The structure is in essentials quite similar to that of *C. Cheiri.* No laterals were seen in the petiole.

At an early age the primary protoxylem is degenerated but usually still discernible as crushed streaks, while a few cambial divisions are to be observed. The first plumular leaves are opposite each other and alternate with the cotyledons. Their single central strands are essentially similar to those of the cotyledons, in that they are markedly double, consisting of a central group of protoxylem alternating with two centres of phloem formation. One lignified element which is continuous with the strand of the rudimentary first leaf may be found in the upper part of the hypocotyl at an age when the plumule is not obvious to the naked eye.

Erysimum pulchellum. The transition features in this species are very similar to those in *Cheiranthus.*

Alyssum rostratum. In the seedlings examined there are slight deviations from type in detail. Thus the protoxylem is scattered and not very obvious in the upper part of the hypocotyl, and even in the diarch plate it is crushed and degenerate-looking. This is no doubt only an extreme case of early degeneration of the protoxylem, as it is more marked in the older seedlings and is correlated with very precocious development of the cambium, the activities of which are very manifest even in the youngest seedlings.

Alyssum maritimum, as described by Chauveaud,[1] seems to be quite similar.

Anastatica hierochuntina. The anatomical arrangements are those characteristic of the group, and show the usual features of the diarch type.

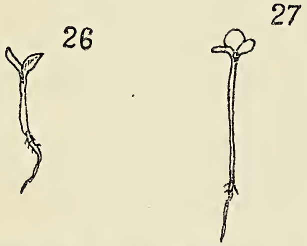
Malcolmia littorea presents a good example of the common arrangement. Degeneration of the protoxylem takes place at an extremely early age, as was far advanced in the seedlings examined, which were still very young as judged by internal as well as external marks.

Matthiola tricuspidata (Text-fig. 26). Normal seedlings show the

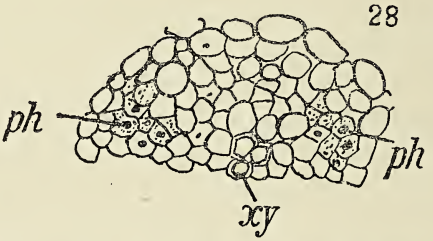
[1] Loc. cit., 1911.

usual structure. An individual having three cotyledons was examined (Text-fig. 27). Two of these cotyledons united near the base and their confluent vascular systems became continuous in the hypocotyl with one of the groups forming the quite normal elliptical stele, and through this with a pole of the diarch root. Thus, contrary to what was recorded of a tricotyledonary specimen of *Cheiranthus Cheiri*, two cotyledons form between them only one pole, so that the root is diarch and not triarch. It is of interest to note that in the free portion of these cotyledons the vascular strand is quite distinctly double, and this not in preparation for the root arrangement but contrary to it, as in the lower united portion the strand becomes single so as to form with that of the other cotyledon or half-cotyledon a double bundle. This would seem to have some bearing on our conception of the meaning of ‘ doubleness ’.

Hesperis tristis. The cotyledons insert themselves by broad flat bases which unite on one side for a very short distance before fusing with the hypocotyl. One minute lateral on either side of the central double bundle remains free until quite near the cotyledonary node, when it unites with the central. The protoxylem is in a somewhat degenerate condition throughout, but persistent and obvious.



Text-figs. 26, 27. *Matthiola tricuspidata.* 26, Normal seedling; 27, Seedling with three cotyledons. Nat. size.



Text-fig. 28. *Draba Aizoon*: vascular system of plumular leaf. *ph*, phloem; *xy*, xylem. × 800.

Draba Aizoon. While the main features are entirely according to type there are several distinctive subordinate characteristics in this species. Thus the hypocotyl shows a very definite central region that may perhaps be termed a ‘ stele ’, which approaches protostelic appearance by early (i. e. high up) loss of pith and somewhat cylindrical disposition of phloem.

This appearance is enhanced by the extremely early activity of the cambium, which is perhaps most active in the position corresponding to the two protoxylem poles of the root. The appearance suggests that there is probably no interval of time, i. e. stage of quiescence, between the cell-divisions of the primary desmogen and those more definite radial divisions termed ‘ secondary ’.

While the cotyledon strands are not markedly double above the cotyledonary node the first plumular leaves show very well the 'doubleness' often seen in the leaves immediately succeeding the cotyledons (Text-fig. 28).

Arabis rosea presents a very perfect example of the diarch type in which complete root structure is present very high up in the hypocotyl. This structure bears no relation to the apparent collet.

Aubretia Antilibani shows a detailed likeness to *Arabis rosea.*

Sinapeae.

Teesdalia nudicaulis is very similar to the preceding genera.

Iberis tenereana (Pl. LI, Figs. 13–16) is a good example of the general features of the type. One seedling shows particularly well the way in which the doubleness of the cotyledonary strand may be masked by early appearance of cambium, which by its activity between the two primary groups of phloem links these together with secondary phloem, with the result that the two original centres of phloem production are so obscured that they may be overlooked at this stage. At the base of the cotyledons the cambium is confined to the wings—that is, to the inside of the phloem groups, so that the bundle is obviously double. At this level in still older seedlings a further age effect is very well seen, namely, the obliteration of the central alternate protoxylem group, which gives the appearance of two separate bundles described by many writers (see de Bary and more recently Scott).

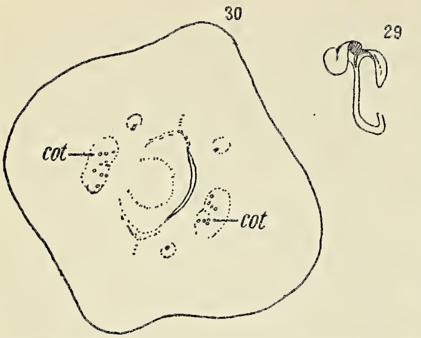
Iberis sempervirens resembles *Iberis tenereana* very closely.

Aethionema persicum. The transitional phenomena are similar to those of the preceding genera. The top of the hypocotyl shows very definite strands in the intercotyledonary plane, each having two or three lignified elements. These are continuous with the plumular midribs, which however are as yet in the undifferentiated desmogen state. The central protoxylem of the cotyledonary strands is obviously degenerating in the cotyledons, hypocotyl, and root. Secondary thickening has begun early, and partly on this account a rectangular-looking stele is found in the top of the hypocotyl which is slightly more elongated in the intercotyledonary than in the cotyledonary direction, and has four phloem groups arranged in pairs.

Peltaria alliacea is characterized by the presence of root structure very close to the cotyledonary node. Very early production of cambium masks the degenerating protoxylem elements of the central position in the cotyledons except quite at the base, where cambium is not formed in the middle line. (Compare *Iberis*, &c.)

Sisymbrium carpaticum conforms completely to the general type. Degenerating protoxylem is obvious throughout the seedling. One tricotylous specimen was examined which formed a triarch root as in *Cheiranthus Cheiri.*

Lunaria biennis is of interest on account of the very different habit of the seedling with its comparatively large thick cotyledons. The base of the cotyledons shows in addition to the central double strand a small lateral on each side. These maintain their independence above the cotyledonary node, indeed in some cases may be said to pass into the hypocotyl independently. There are no differentiated plumular strands as in *Aethionema*, &c., although the presence of a robust cambium and the half-obliterated condition of the protoxylem show that the seedling is past the primary stage. The most noticeable feature of the transition is the presence of four phloem groups throughout the hypocotyl arranged so as to form two elongated bands of phloem, the middle of which is probably chiefly secondary.



Text-figs. 29, 30. 29, *Lunaria biennis.* ½ nat. size. 30. Outline of cotyledonary node of same.

Lunaria annua is described by Chauveaud[1] as showing free laterals in the upper part of the hypocotyl.

Cardamine hirsuta seems to be quite normal, but only a rather old stage has been examined.

Barbarea vulgaris. It is interesting to note in this form, which is placed by Engler not far from *Lunaria*, that there seems to be the same somewhat leisurely manner of completing root structure, although the habit of the seedling is quite different and laterals are not found so low. It differs from *Lunaria* in the presence of a strand already lignified in the hypocotyl, which corresponds to the midrib of the first leaf. In Bismarck brown and gentian violet preparations the red-brown half-obliterated protoxylem elements, which are quite exarch in the cotyledonary node, present a marked contrast to the brilliant blue of the later-formed xylem elements.

Crambe tartarica differs from the other members of the Cruciferae examined in the wide separation of the halves of the double bundle in the hypocotyl. The laterals are free near the base of the cotyledon, and as in *Lunaria* may enter the hypocotyl independently.

Chauveaud[2] reports lateral strands in the hypocotyl of *Brassica oleracea*, and at the base of the cotyledons in *Ricotia lunaria.*

Raphanus sativa is the species in which Chauveaud[3] first observed 'resorption' of protoxylem.

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[1] Loc. cit., 1911.

[2] Loc. cit., 1911.

[3] Chauveaud, G.: Compt. Rend. Acad. des Sc., 1901.

Braya supina and *Biscutella ciliata* are described by Chauveaud as having the same general features.

Resedaceae.

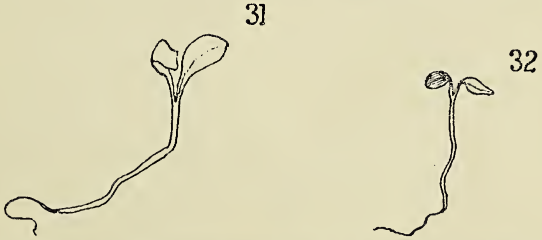
Reseda alba presents a good example of the familiar diarch type of the Rhoeadales.

Reseda odorata and *Caylussa abyssinica*, according to Chauveaud's [2] descriptions, are similar, except that he comments upon the separateness of the halves of the double bundle in the latter.

Papaveraceae.

Papaver paeniflorum and *P. Dan.* The transition features are quite characteristic of the cohort. Older seedlings of *P. Dan* show semi-obliteration of all the primary xylem, including that of the diarch plate. The cotyledons in *P. paeniflorum* are nearly cylindrical, and the small central bundle is not markedly double in the upper part.

Glaucium serpieri. The comparison of seedlings of different ages shows that this is a form in which age causes the almost complete disappearance of the primary elements, which are resorbed at an early stage, only a few isolated rings and portions of rings of wall thickening occasionally remaining. It is therefore very easy to overlook the central exarch protoxylem elements in the upper part of the seedling. The plumular strands lignify late.



TEXT-FIGS. 31, 32. 31, *Glaucium serpieri*; 32, *Chelidonium majus*. Nat. size.

Glaucium flavum, described by Chauveaud, seems to be quite similar.

Chelidonium majus. The cotyledons form a well-marked tube. The double bundle is of the extended secant form at the base of the cotyledons, as in *Glaucium*, &c. The primary elements disappear later, and a fragmentary appearance is given by the scattered rings of thickening. The transition phenomena are quite normal.

Bocconia japonica is a slender seedling showing the same general features as the preceding genera. Evidences of resorption were seen.

Platystemon californicus. The cotyledons are centric in structure. At

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[1] Loc. cit., 1911.

[2] Loc. cit., 1911.

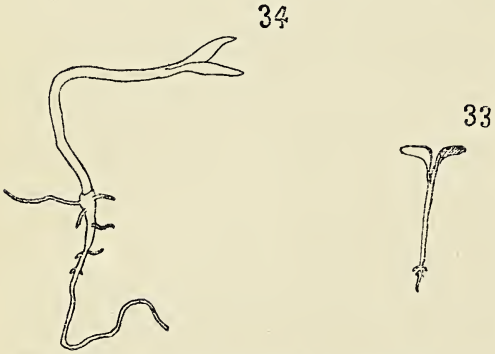
the apex they possess a large double bundle which lower down gives off a lateral on each side. The laterals unite with the central strand again some distance above the base of the cotyledon. The minute diarch root is formed very high up in the hypocotyl.

Corydalis aura and *C. lutea.* The anatomical arrangements are very typical and call for no comment. *C. lutea* shows slight asymmetry in the fusion of its cotyledons.

Capparidaceae.

Cleome sesquiorgialis. This is an interesting form because it shows a much slower root formation than is characteristic of the cohort, or, in other words, complete root structure is only found lower in the hypocotyl than in most of the members of the Rhoeadales, and this is particularly true of the phloem arrangements—four phloem groups existing quite low in the hypocotyl. Examination of this species at different ages is very instructive as it shows that persistence of the degenerate resinous-looking remains of the protoxylem which enables the observer to determine its primary position in a comparatively old seedling.

Cleome arborea is described by Chauveaud[1] as having *fused* phloem groups high in the hypocotyl. His figures show that the double bundle possesses three groups of primary xylem at an early age—the central alternate group and a group at the most remote point of each wing. This feature of *C. arborea* and the persistence of the four phloem groups in *C. sesquiorgialis* suggest the possibility of tetrarch allies.



TEXT-FIGS. 33, 34. 33, *Cleome sesquiorgialis.* Nat. size. 34, *Capparis inermis.* × 2.

Capparis inermis and *C. rupestris.* These species are good examples of the type.

The cohort of the Rhoeadales as judged by some thirty-five species thus presents very uniform features in its seedling anatomy. The only suggestions met with of departure from this uniformity are in the neighbourhood of the Brassicas and in the Cleomes. In the first a tendency to the formation of lateral strands in the hypocotyl recalls forms in which a tetrarch root obtains (e. g. *Magnolia*), as also does the long separation of the phloem groups in the hypocotyl of *Cleome sesquiorgialis* and the marked production of protoxylem groups on the flanks of the double bundles of *Cleome arborea.*

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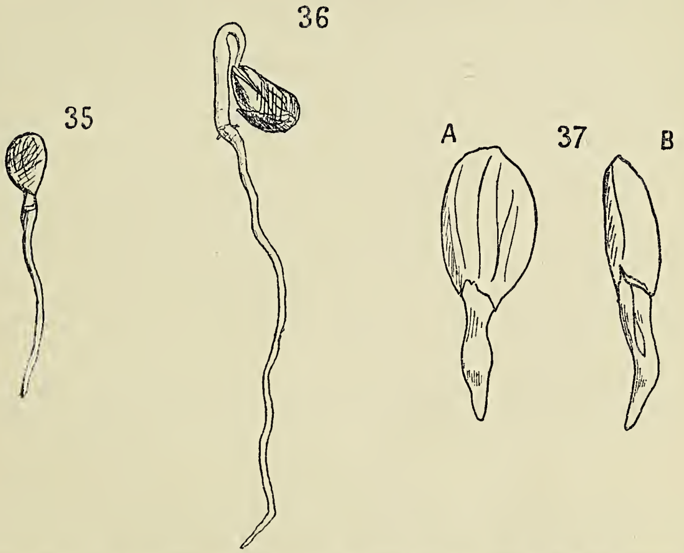
[1] Loc. cit., 1911.

ROSALES.

The seedling anatomy of some forty species has been worked out, of which more than half belong to the Natural Order Rosaceae, while members of the Crassulaceae, Saxifragaceae, Pittosporaceae, Hamamelidaceae, and Platanaceae are included, together with a few species of the Leguminoseae not recorded by Compton.

So far as our information goes, the Crassulaceae and Saxifragaceae are diarch, the Pittosporaceae and Hamamelidaceae tetrarch, while the Rosaceae and Leguminoseae show both varieties of the cruciform type. The cohort also includes forms which are suggestive with regard to the possible relationships of the cruciform and diagonal types.



Text-figs. 35–37. 35, *Prunus spinosa*; 36, *Prunus avium*; 37, A and B, *Prunus persica*. All nat. size.

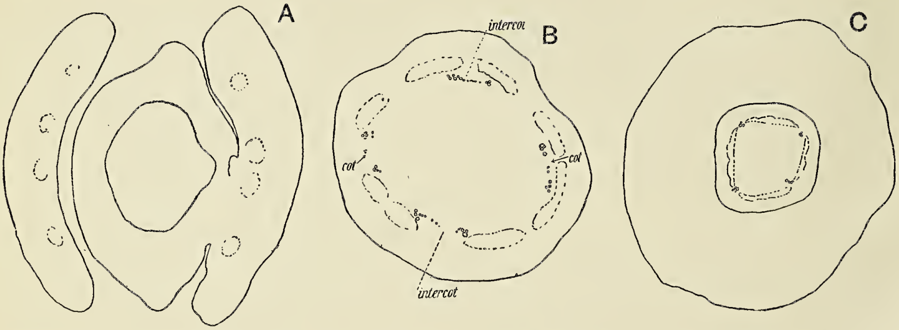
Rosaceae.

The members of the Spiraeoideae and Rosoideae, so far as examined, are diarch, the Prunoideae tetrarch, while within the Pomoideae both tetrarch and diarch forms are found, as well as hexarch and even octarch arrangements, which suggest a possible connexion with the diagonal type.

Prunoideae.

Prunus persica, *P. spinosa*, *P. Cerasus*, *P. avium*, and *P. amygdalus* var. *amara*. The five species of *Prunus* examined show the same hypocotyledonary structure, but with an interesting variant in connexion with the cotyledon strands. The hypocotyl presents four protoxylem groups with or without tangentially developed metaxylem wings, according to age.

Alternating with the four protoxylem groups are four extended phloem masses, which in the upper part of the hypocotyl are twin groups. At the node the usual triad of xylem and two phloem groups pass out to form the double bundle of the cotyledon. In *Prunus amygdalus*, *P. amara*, *P. persica* (see Text-fig. 38), and *P. spinosa*, the intercotyledonary strands are continued upwards as the independent lateral bundles of the cotyledons (see Pl. L, Fig. 11 of *Magnolia*), but in *P. Cerasus* these are continuous with the flanks of the double bundle (see Pl. LI, Fig. 23 of *Althaea*).



TEXT-FIG. 38. *Prunus persica.* A, cotyledonary node; B, hypocotyl; C, root, enlarged. *cot*, *intercot* = protoxylem in cotyledonary and intercotyledonary planes, respectively.

Rosoideae.

Rosa rubiginosa and *R. polyantha.* The transition in these forms is of the simple diarch type. The insertion of the cotyledons at the node is slightly asymmetrical, being delayed on the side of the production of the first leaf. This asymmetry is reflected in that of the hypocotyledonary stele, which shows vascular elements continuous with those of the first leaf on one side of the emerging diarch plate. Complete root structure is present a short distance below the node, as in the members of the Rhoeadales and Ranunculaceae.

Rubus vestitus and *R. xanthocarpus.* The two species of *Rubus* examined show the usual formation of a diarch plate, with rather low insertion of plumular traces. The diarch plate has a characteristic form, being lignified in two bowed lines connected by the protoxylem groups. Lower down in the older seedlings the centre lignifies, so that the stele presents a curious protostelic xylem body, upon the periphery of which the two small protoxylem groups are scarcely visible. One individual of *R. vestitus* possessed intercotyledonary strands in the upper part of the hypocotyl which were continued into the cotyledons as single strands, one passing into the left side of one cotyledon, and the other into the right side of the opposite cotyledon. That is to say, each cotyledon has, in addition to the central double bundle, *one* lateral which is on opposite sides in the two cotyledons. These laterals pass into the hypocotyl, where they persist lower down than the plumular tissue, but ultimately die out, and the root is diarch. This phenomenon is suggestive of tetrarch relationship.

Potentilla chrysantha and *P. fruticosa.* These species show the usual arrangement associated with the diarch type. The plumular traces, which are distinctly double, are inserted rather low down, and in *P. chrysantha* the cotyledon strands pass out asymmetrically into the base of the fused cotyledons.

Fragaria indica. The very slender seedlings of this species show similar features to those in the Potentillas described.

Geum canadense, G. urbanum, G. coccineum, G. japonicum, and *G. montanum.* Very slender seedlings possessing high transition and diarch root. In one individual of *G. canadense* an interesting variant was provided by the presence of a small strand in the intercotyledonary plane, which passed out as a single cotyledonary lateral.

Rhodotypus kerrioides. This seedling has a more robust habit, but it conforms in a general way to the diarch type. The process of root formation is, however, very much delayed throughout the long hypocotyl, and even much below the collet the root has a large central pith. The hypocotyl is characterized by four widely separated metaxylem masses lightly bridged in the cotyledonary plane by the protoxylem strands, and the general form is reminiscent of *Rubus.*

Spiraeoideae.

Spiraea callosa is an extremely minute form, showing the simplest possible production of a diarch root by the fusion of the double bundles at the cotyledonary node.

Exochorda grandiflora and *E. Albertii* possess far more robust seedlings than the Spiraeas, but the transition features are essentially the same. The details, however, recall slightly those of *Rubus* and *Rhodotypus.*

Pomoideae.

Pyrus communis, P. Malus, P. Aria, P. Aucuparia. These species present considerable variation, and furnish proof of the unimportance of the independence or non-independence of the laterals, since not only do both conditions occur within the genus as in *Prunus*, but even within the species, e. g. *P. communis.* The hypocotyl possesses four or six protoxylem groups with alternating phloem strands arranged in the usual triad manner. The group in the cotyledonary plane is continuous with the double bundle of the petiole as always, and the one intercotyledonary or two diagonal groups are continuous either with the flanks of the double bundle (e. g. *P. Malus*, *P. Aucuparia*, and some individuals of *P. communis*), as in Pl. LI, Fig. 25 of *Althaea rosea*, or with independent laterals (e.g. *P. Aria*, and some individuals of *P. communis*), as in Pl. L, Fig. 10 of *Magnolia tripetala*.

The forms which have four groups of protoxylem in the hypocotyl possess a tetrarch root, e. g. *P. Aria*, while those which show six protoxylem groups in the hypocotyl may, or may not, reduce lower down. In one individual of *P. communis* both the intercotyledonary protoxylem group and the diagonal groups were present, and the root was heptarch, obviously through suppression of one diagonal pole of an octarch condition. It is noteworthy that these differences bear no obvious relation to habit of seedling, but would appear to depend upon some inner cause as to the nature of which one can, in the present state of knowledge, only speculate. *Pyrus communis* would seem to be by far the most variable species, but fewer individuals of the other forms have been examined.

The difference between tetrarchy and hexarchy is of the same order as the difference between fused and free laterals in the base of the cotyledon, since in the first case the lateral strands unite to form one pole of the root, as in *P. Aria* and some individuals of *P. communis* (cf. Pl. LI, Fig. 26 of *Althaea rosea*), or remain apart, as in other individuals of *P. communis*. This union or separation in the root, however, is not correlated with the union or separation of the strands in the base of the cotyledons, since all combinations occur, as will be seen by reference to examples and diagrams (Text-fig. 41).

Cotoneaster affinis. The hypocotyl shows the four groups of phloem with more or less metaxylem within, according to age. In the cotyledonary plane are the protoxylem strands. The usual triad passes out to the cotyledons, constituting the double bundle which at the node differentiates lateral strands on the flanks, that in some cases do not separate from the central bundle until some distance up the cotyledons. The diarch root is produced in a somewhat leisurely manner.

Crataegus Oxyacantha is very similar to *Cotoneaster*.

Saxifragaceae.

Saxifraga muscoides, S. Aizoon, Heuchera alba, Philadelphus grandiflorus. These are very slender seedlings with a very high transition to a diarch root.

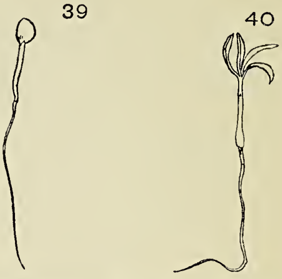
Crassulaceae.

Sedum glaucum is a very swollen seedling with high transition to a diarch root.

Cotyledon orbiculare is another very globose form with extremely minute vascular strands which form a diarch root just below the node.

Pittosporaceae.

Sollya heterophylla presents in the main the usual features of the diarch form, but with very characteristic details. Thus the primary xylem is distributed in the double bundle at the base of the cotyledons in an unusually definite T-shaped manner. The protoxylem is completely exarch, and the arm of the T composed of a single file of centripetally developed elements which spread out left and right inside the phloem groups.



Text-figs. 39, 40. 39, *Sollya heterophylla.* $1\frac{1}{3}$ nat. size. 40, *Pittosporum crassifolium.* $\frac{1}{2}$ nat. size.

Pittosporum crassifolium. This species is noted for its polycotyledony. In the lower part of the hypocotyl are to be found four triad groups, which in the upper part pass out into the four cotyledons as their respective double bundles. The radial files of primary xylem are noticeable, but are not so marked as in *Sollya heterophylla.* The hypocotyledonary strands are continued downwards into those of a tetrarch root. On the periphery of the bundles opposite the protoxylems large secretory sacs are to be found.

In the tricotyledonary forms there is apparently fusion between two of the four vascular triads in the upper part of the hypocotyl, so that three double bundles pass out into the three cotyledons.

Hamamelidaceae.

Liquidambar styraciflua. The hypocotyl possesses the customary four strands. In the position usually occupied by the central protoxylem of the double bundle there is to be found a large secretory canal, so that the halves are completely separated from one another. In each cotyledon they form a double bundle only, differing from the usual arrangement in the presence of the large secretory canal in its centre, on either side of which a streak of crushed protoxylem is obvious. In the root the four xylem strands assume an even greater independence, each with a well-defined completely exarch protoxylem pole. Two phloem groups are present in the customary position, viz. in the intercotyledonary plane, and if it were not for the secretory canal in the cotyledonary plane the root would doubtless be diarch.

Platanaceae.

Platanus occidentalis and *P. orientalis.* The seedlings are remarkable for their slightness, in view of the habit of the adult plant. Although the root is ultimately diarch, the hypocotyl is characterized by the presence of four phloem groups, which may probably be taken as an indication of tetrarch relationship.

Leguminosae.

Ceratonia siliqua, *Acacia dealbata* (see Compton[1]), *Medicago marginata*, and *M. rutitexta*. These species show hypocotyledonary structure which is essentially similar to that of *Prunus persica*, that is to say, four protoxylem strands are present alternating with four phloem groups with metaxylem to the inside. These strands of phloem and metaxylem are more or less twin structures according to the individual. This difference corresponds to some extent to the degree of freedom of the lateral strands in the base of the cotyledon. Thus in *Ceratonia siliqua* they become independent at the node, while in the other species they are massed together at the node, so that these species conform to the 'Althaea' or 'Anemarrhena' type (see Pl. LI, Fig. 23). The root is tetrarch, as in so many Leguminosae.

The cohort of the Rosales may perhaps be said to present the widest range of seedling anatomy of the three cohorts considered in this paper.

It is impossible to assign a position to the tetrarchy of the Pittosporaceae without some knowledge of the origin of the four cotyledons.

If they are derived from the dicotyledonous condition, two methods are possible: (1) bifurcation of two original cotyledons; (2) suppression of internode between cotyledons and plumular leaves which, in the language of Hill and de Fraine,[2] have been thus 'promoted' to be cotyledons.

In the first case the tetrarchy would necessarily be a modification of diarchy, in the second case probably of tetrarchy.

I know of no morphological evidence bearing upon this question. So far as collateral evidence goes, the only other member of the Pittosporaceae examined, namely *Sollya*, is diarch, and the neighbouring order of the Hamamelidaceae shows in *Liquidambar* the curious separation of the halves of the cotyledonary strand by means of a secretory canal; it is probable therefore that *Pittosporum* shows that modification which leads to the production of poles in the diagonal planes (see *Calycanthus*).

Within the Rosaceae it is the Pomoideae section which includes the greatest range of type, the diarch forms of which might be accountable for on the habit hypothesis, but the variations in the genus *Pyrus* do not lend themselves to this interpretation. They illustrate very well the comparative constancy of the hypocotyl, which may be associated with very different arrangements in cotyledons and root.

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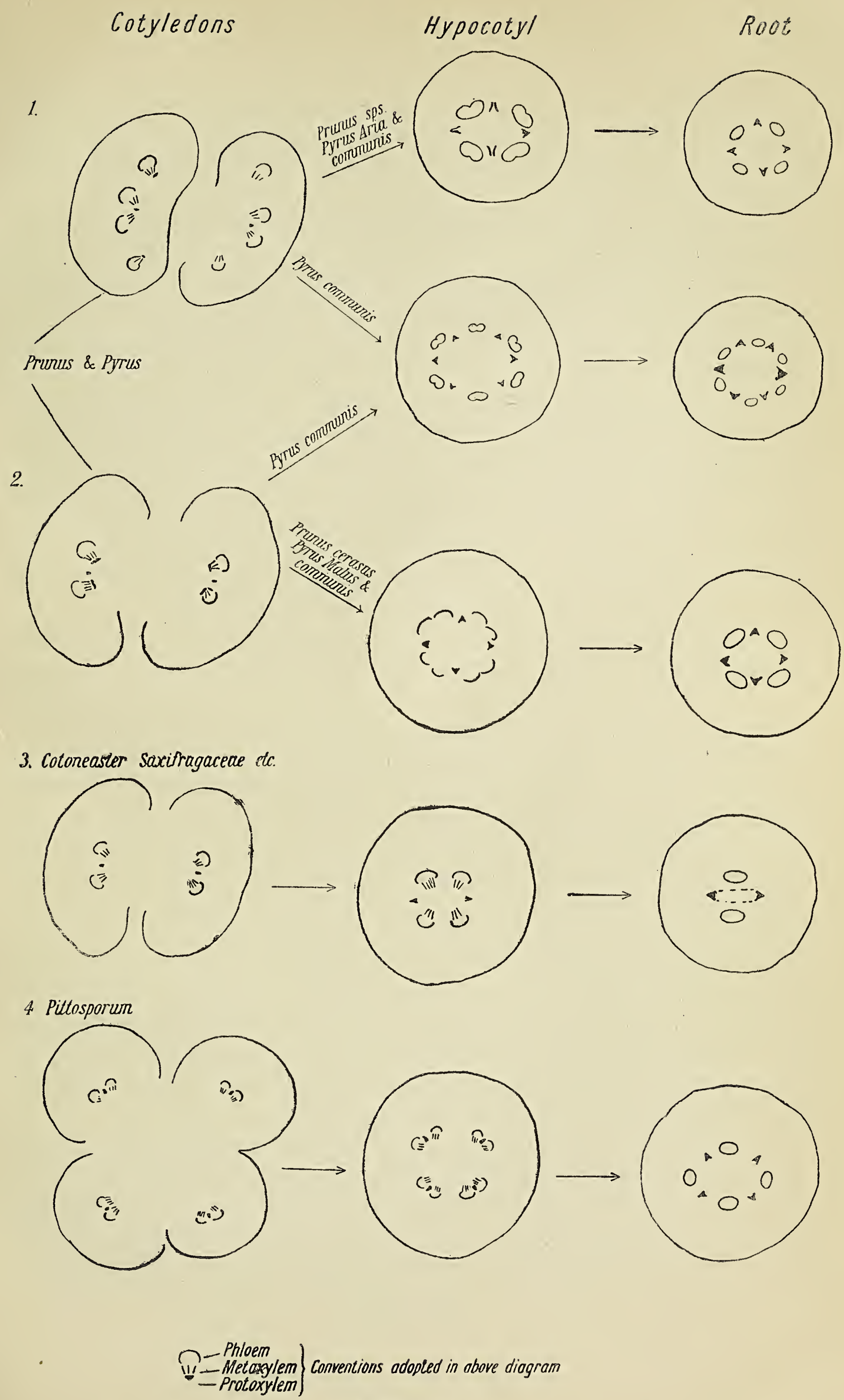
[1] Compton, R. H.: An Investigation of the Seedling Structure in the *Leguminosae*. Journ. Linn. Soc. Bot., vol. xli, 1912.

[2] Hill and de Fraine: On the Seedling Structure of Gymnosperms, II. Ann. Bot., 1909, vol. xxiii.

ROSALES



TEXT-FIG. 41.
Diagrams illustrating principal types of vascular arrangement in cotyledons, hypocotyl, and root of Rosalean forms.

DISCUSSION OF RESULTS.

No attempt will be made in this paper to discuss at length the various aspects of seedling anatomy, but I shall rather concern myself with pointing out the immediate problems suggested by a consideration of the forms herein described.

A review of these species alone would of necessity raise several very pertinent questions.

The universality of the vascular unit of the hypocotyl, which in the cotyledonary plane is continued upwards as the 'double bundle' and in the intercotyledonary plane as two separate strands, forces itself upon one's attention.

If the position in the hypocotyl be regarded as an inevitable stage of transition from leaf to root, to which view the examination of the strands from above downwards predisposes us, we are confronted with the necessity of accounting for the difference in behaviour between the central and lateral strands. Why do not the lateral strands of the cotyledons behave in the same way as the central strand, i. e. each 'form' or relate to a root pole, instead of combining with another to do so, and if root structure is 'carried up' in the central bundle, why not in the lateral strands also?

If with Chauveaud we regard the root as of paramount importance, to which I cannot but think the examination from root upwards inclines us, we tend to become overpowered by conceptions gained from a consideration of root structure, and while these offer a very plausible explanation of the arrangements met with in the diarch type, many difficulties arise in connexion with the tetrarch type. For instance, M. Chauveaud's[1] very happy suggestion (abundantly supported in his own work) that there is a primary phase of the young seedling characterized by alternate or, as he regards it, 'root' structure throughout the plant, while doubtless largely true of diarch forms, breaks down according to his own showing in connexion with tetrarch forms, the intercotyledonary root poles of which are, according to M. Chauveaud, continuous with the 'éléments superposés' of the lateral strands of the cotyledon. M. Chauveaud does not discuss this type in relation to his interesting hypothesis, but it presents the stumbling-block here, as also in connexion with the views of Lee, Hill and de Fraine, &c. By implication Chauveaud would seem to agree with these latter authors in regarding the diarch condition as the more primitive.

Sargant and Compton, on the other hand, as the result of their investigations of monocotyledonous and dicotyledonous forms respectively, sum up in favour of the tetrarch condition as being the more primitive, at any rate for the forms examined. As Compton associates tetrarchy with the arboreal and diarchy with the herbaceous habit, and holds that the arboreal

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[1] Loc. cit., 1911.

habit is primitive for Angiosperms, he may be said to be in favour of tetrarchy in general as being more primitive than diarchy. Miss Sargant[1] in 1903 presented a well-reasoned statement in support of her view that the varieties of seedling anatomy met with in the Monocotyledons were derived from a tetrarch condition, and pointed out that there were suggestions of this condition in the Ranunculaceae, which also furnished so many other points of similarity with the Monocotyledons. I agree with Mr. Tansley, who pointed this out in his criticism of the hypothesis that the elements instanced in *Eranthis* in support of the presence of tetrarchy are probably secondary. Nevertheless the work of the last ten years has shown that the tetrarch condition is to be found very widely in the Dicotyledons, forming about 50 per cent. of the Leguminosae and about the same of all known forms of the Rosales. Similar very rough calculations reveal some 30 per cent. in the Cactaceae and in the Compositae, 15 per cent. in the Ranales, 11 per cent. in the Tubiflorae, 6 per cent. in the Centrospermae, while none at all have been recorded either in the Piperales or in Rhoeadales.

As the total percentage of tetrarch forms recorded in these groups taken together is about 30 per cent., we see that the Cactaceae and Compositae present about the average distribution of the types, the Rosales have an excess of tetrarch forms, the Ranales, Tubiflorae, and Centrospermae an excess of diarch, while the Piperales and Rhoeadales so far as known are exclusively diarch.

These figures of course count for very little in view of the comparatively few forms described (about five hundred in the cohorts enumerated) and their unequal representation of groups, due to the practical difficulties of obtaining the most desirable forms. Thus the preponderance of Ranunculaceae in the Ranalean species recorded in this paper and the comparative fewness of the representatives of the other families probably account for the low percentage of tetrarch forms.

The existence of the tetrarch condition within the Ranales—as recorded in this paper—as well as the prevalence of the type in other parts of the Natural System, notably in the Rosales, removes any special importance that may seem to have attached to *Eranthis* and places the connexion between the seedling anatomy of Monocotyledons and Dicotyledons on a broader basis. Furthermore, the 'Anemarrhena' variety of the tetrarch form is quite common in Dicotyledons (witness *Berberis Lycium* and *B. aristata*, *Laurus*, *Prunus Cerasus*, *Pyrus Malus*, *Medicago*, &c., described in this paper), and no special significance such as has been claimed by de Fraine and Lee attaches to its existence in the Cactaceae and Tubiflorae.

In addition to these definitely diarch and tetrarch forms there are a number of intermediate conditions recognized as such by Compton in the

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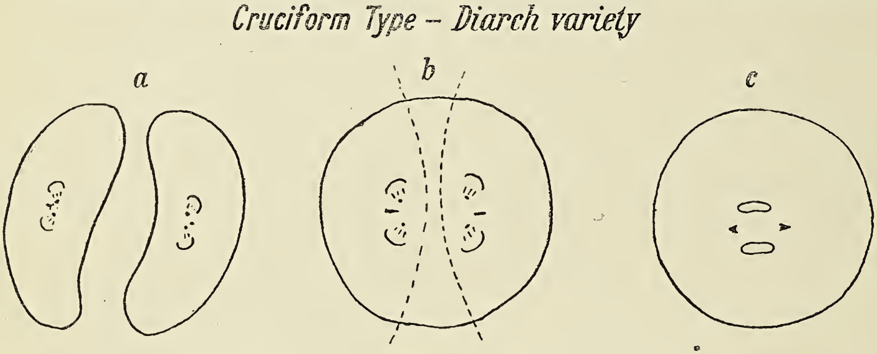
[1] Loc. cit.

Leguminosae and by Lee in the Compositae. Such forms are to be found in the groups described in this paper (e. g. *Liriodendron*, *Decaisnea*, &c.), even in the Ranunculaceae, and I am in complete agreement with Lee when he says, 'Although there are two extremes—diarch and tetrarch—all stages from the one to the other have been described in the preceding pages,'[1] and what is true of the Compositae is true, I think, in general. It is interesting, however, to note that while Compton and Lee agree as to the close connexion between what I have termed the diarch and tetrarch varieties of the cruciform type, they differ fundamentally as to the relative primitiveness of these conditions. While Compton pronounces in favour of the primitiveness of tetrarchy, Lee regards the seedling anatomy of the Composites as founded upon the diarch condition, the tetrarch and the intermediate forms being produced in relation to size of seedling and other factors.

It should be clearly borne in mind that the intermediate cases instanced by me in 1907—of which Mr. Lee says, 'If there are cases, as Miss Thomas assumes, where the tetrarch structure is becoming reduced to diarch'—fall within precisely the same category of phenomena as those of which he remarks, 'There are as certainly seedlings in which the diarch structure is giving place to tetrarch'.[2] It is important that it should be fully realized that there is only one set of phenomena in question, but of these there are two distinct interpretations.



TEXT-FIG. 42.

Mr. Lee's conclusion that 'tetrarchy and diarchy have probably been interchanged several times during the evolution of Angiosperms' is quite unnecessary if, as seems indicated, he suggests it to account for different phenomena, but it may well take its place as a useful and suggestive contribution to the theory of the subject. His view would seem to be that the process of evolution of the diarch and tetrarch forms is a 'reversible one'. This is a tenable hypothesis if we can with any measure of plausibility deduce a reasonable concept of the components, even though the factors directing the action → or ← await discovery. One thing is certain, that we

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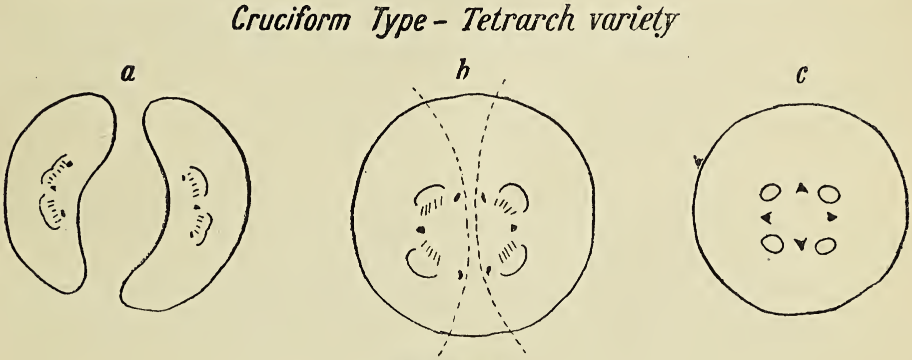
[1] Loc. cit., p. 323.

[2] Loc. cit., p. 325.

cannot regard the cotyledonary strands as the fixed quantity which, when present, will produce a certain type of root. That is to say, there is no inevitable connexion between the presence or absence of lateral strands and the possession of a tetrarch or diarch root respectively. The figures and diagrams in this paper demonstrate this point. When absence of free laterals is correlated with tetrarchy we have the so-called 'Anemarrhena type; when laterals are present, although the root is diarch, they may perhaps be regarded as an indication of tetrarch affinity, since this feature is most commonly found in the allies of tetrarch species.

The factors which control the freedom or aggregation of the lateral strands of the cotyledon petiole are probably capable of elucidation, and there is a fairly obvious correlation between this anatomical feature and the morphology of the cotyledons, particularly with regard to their insertion. The factors which control the production of a tetrarch or of a diarch root are more difficult of analysis. It is probably true that there is a broad correlation between habit of seedling and root anatomy, but the connexion is by no means close or universal. Thus comparison of the habit of the seedlings of the tetrarch and diarch species of *Berberis* and of the Magnolias reveals no obvious difference and it is interesting to note that among the Cactaceae the normal and comparatively slender seedlings are tetrarch, while the swollen much modified forms are diarch. Lee remarks upon this lack of direct correlation in the Compositae, and numerous other instances could be furnished.



TEXT-FIG. 43.

The root symmetry is undoubtedly affected by local as well as by more fundamental causes. Thus many authors have recorded cases of reduction from the tetrarch to the diarch condition in the apical region of the individual, and an increase in poles may also occur, but not so commonly. (See Hill and de Fraine on Classification of Seed-leaves.[1])

The anatomy of the hypocotyl, however, is frequently practically

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[1] Ann. Bot., vol. xxviii, 1914.

identical in closely allied tetrarch and diarch forms, and has more in common in the majority of seedlings than has the anatomy of the cotyledons or of the roots (compare Magnolias and *Liriodendron*, *Berberis* sps.). The hypocotyl shows a varying number of primary centres of xylem which alternate with two phloem groups and from which differentiation proceeds in a more or less tangential direction on either side. These triads[1] recall the 'divergeant' of Bertrand and Cornaille,[2] and they seem to constitute the units upon which the vascular system of hypocotyl, cotyledons, and root (perhaps also that of the plumular leaves, example *Draba*) is built up. They may pass out into the leaves practically unaltered and downwards into the root with very little change, but they may be very seriously modified by more or less unknown causes. The arrangement in the hypocotyl does not suggest to my mind *root* anatomy as it does apparently to M. Chauveaud, but rather a stele of a less specialized character more comparable to the structure of the stem axis of primitive cryptogams both living and extinct. Thus it has the essentials of the exarch to mesarch solid or hollow steles of the Osmundaceae, &c., and the leaf-traces passing off from it are also suggestive of those of the primitive forms. The three centres of xylem differentiation so often seen in the 'double bundle' of Phanerogams render this leaf-trace remarkably like the leaf-trace of *Osmunda regalis* (see Bertrand),[3] if we neglect the internal phloem of the latter.

Our knowledge of the more primitive Spermophytes, living and extinct, points to the probability of the ancestral Spermophyte having been a plant of robust organization with large seeds.

This inclines one to the view that not only is the tetrarch condition probably relatively primitive, but that it is very possibly itself derived from a larger plan such as we see in some of the *Pyrus* forms, in *Laurus nobilis*, and in certain Amentiferae. The suggestive Cryptogamic characters of the hypocotyl are, however, independent of number and size, and are seen in the slenderest forms, the larger ones possessing a greater number of units which are the triads similar to the 'divergeants' of Bertrand and Cornaille.[4]

Several authors have decided that seedling anatomy is of no phylogenetic importance, by which apparently they mean that it does not furnish sharp taxonomic characters. The phenomena upon which they have fixed their attention, namely, the broad features of transition and particularly the polarity of the root, are not, I think, likely to do so; but there are small characters indicative of relationship which become familiar to the worker,

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[1] The presence of isolated protoxylem elements in the upper part of the hypocotyl of *Calycanthus*, *Ricinus*, and other forms in which the hypocotyl appears to have a modern stem structure points to the derivation of this form from the structure described.

[2] Bertrand, E. C., and Cornaille, F.: La masse libéro-ligneuse élémentaire des Filicinées actuelles. Trav. et Mém. de l'Univ. de Lille, vol. lx, 1902.

[3] Bertrand, P.: Progressus, Bd. 4, Heft 2, 1912.

[4] Loc. cit.

and which, when subjected to a careful analysis, might prove of some taxonomic value. The phylogenetic aim of recent work I conceive to be something much broader, and I think the results already obtained, which I will not attempt now to summarize or analyse, fully justify the labours and the hopes of the earlier workers. It seems possible that data such as that supplied by Lee on the Composites may one day be enhanced in value by statistical and experimental work, the need for which is so rightly laid stress upon by Compton and by Hill and de Fraine.

BEDFORD COLLEGE,
LONDON.

EXPLANATION OF PLATES L AND LI.

Illustrating Miss E. N. Thomas's paper on Seedling Anatomy of Ranales, Rhoeadales, and Rosales.

PLATE L.

Anemone coronaria.

Fig. 1. Outline of seedling. Natural size.
Fig. 2. Base of cotyledonary tube of same. × 167.
Fig. 3. Hypocotyl. × 427.
Fig. 4. Root. × 427.

Liriodendron tulipifera.

Fig. 5. Outline of seedling. Natural size.
Fig. 6. Outline of transverse section of base of cotyledons. × 50.
Fig. 7. Hypocotyl. × 300.
Fig. 8. Root. × 427.

Magnolia tripetala.

Fig. 9. Outline of seedling. × $\frac{2}{3}$.
Fig. 10. Outline of transverse section of cotyledonary node. × 30.
Fig. 11. Hypocotyl showing crushed protoxylem. × 167.
Fig. 12. Root. × 200.

PLATE LI.

Iberis tenereana.

Fig. 13. Outlines of bases of cotyledons. × 50.
Fig. 14. Details of bundle of 'north' cotyledon of same. × 253.
Fig. 15. Hypocotyl showing disorganizing protoxylem. × 253.
Fig. 16. Root of same. × 253.

Cheiranthus Cheiri.

Fig. 17. Seedling. Natural size.
Fig. 18. Outlines of cotyledon bases. × 50.
Fig. 19. Bundle of 'south' cotyledon showing crushed protoxylem. × 427.
Fig. 20. Hypocotyl with crushed protoxylem. On the left is one lignified plumular element (*p.*). × 427.

Althaea rosea.

Figs. 21 and 22. Seedlings. Natural size.
Fig. 23. Outline of cotyledon bases. × 30.
Fig. 24. Details of bundle of 'north' cotyledon of same. × 253.
Fig. 25. Hypocotyl with median (*cot.*) and lateral (*int.*) protoxylem groups. × 253.
Fig. 26. Root. × 253.

Annals of Botany,

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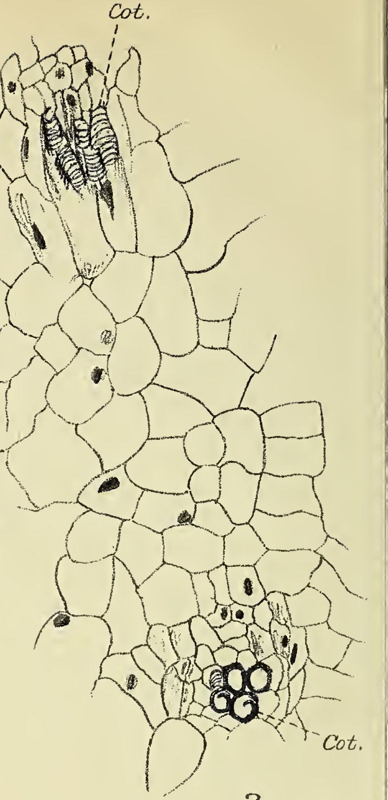

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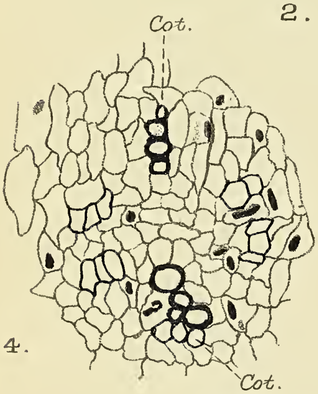

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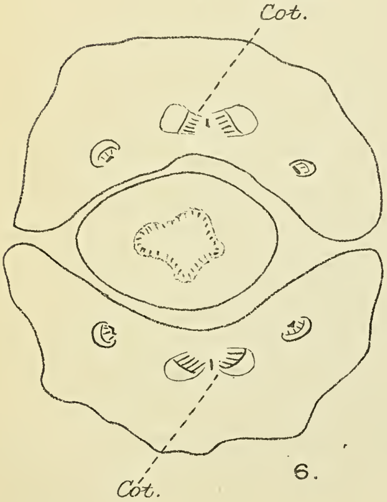

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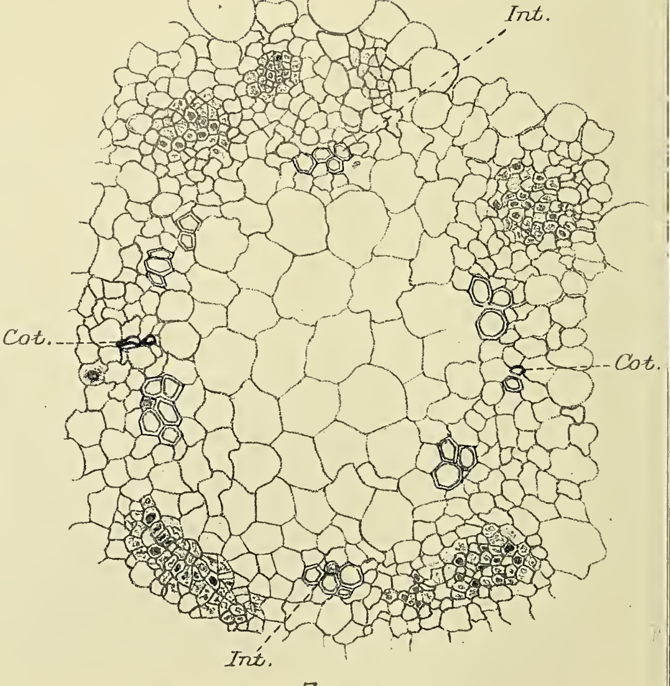

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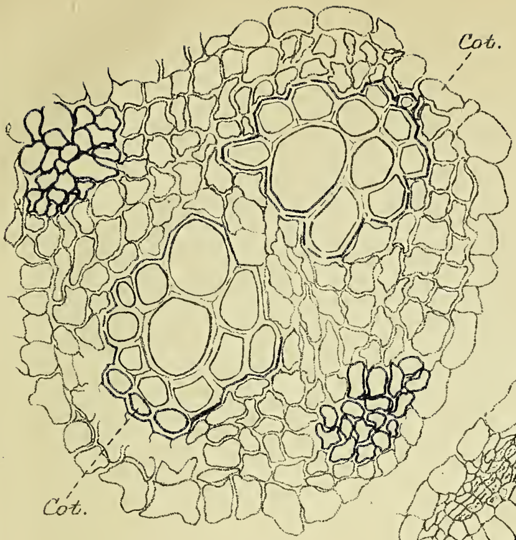
Fig^s 1–4, Anemone coronaria: 5–8, Lirioc

E.N. Thomas del.

E.N. THOMAS—SEEDLING ANATOMY.



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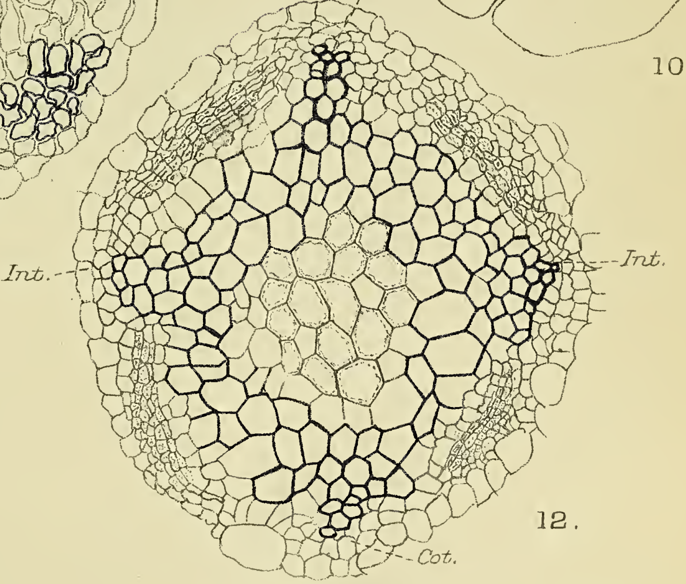

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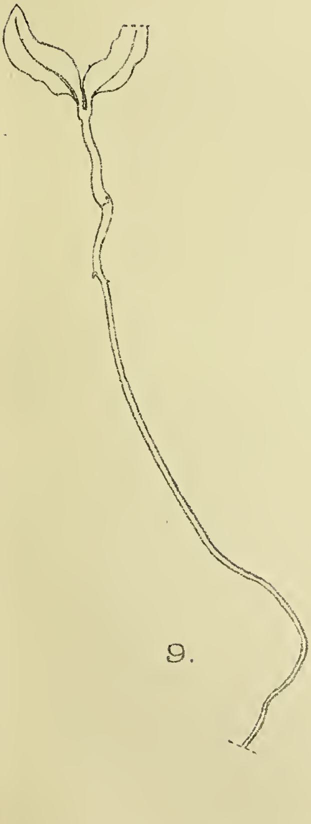

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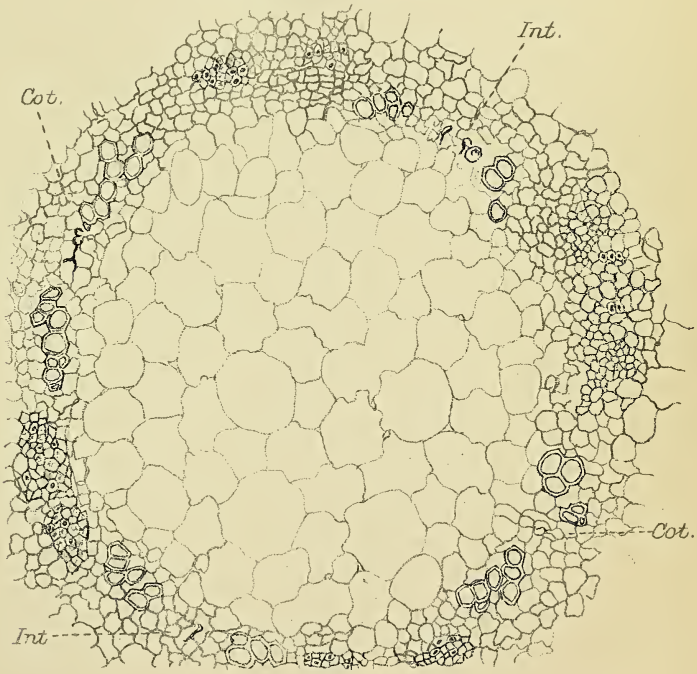

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ndron tulipifera: 9–12, Magnolia tripetala.

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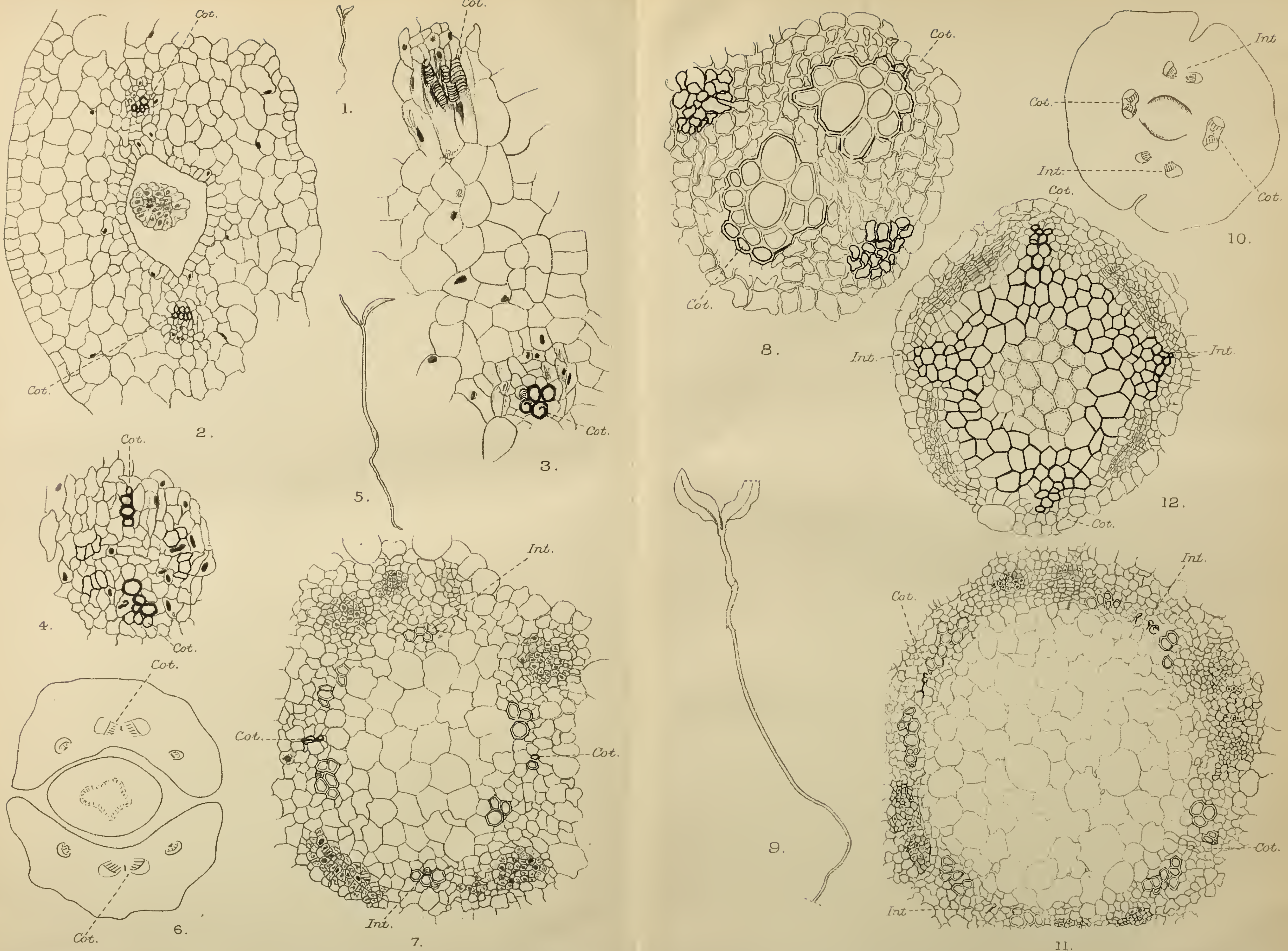

E.N.Thomas del.

Fig^s. 1–4, Anemone coronaria: 5–8, Liriodendron tulipifera: 9–12, Magnolia tripetala.

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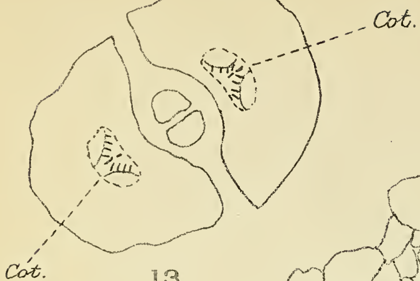
E. N. THOMAS—SEEDLING ANATOMY.



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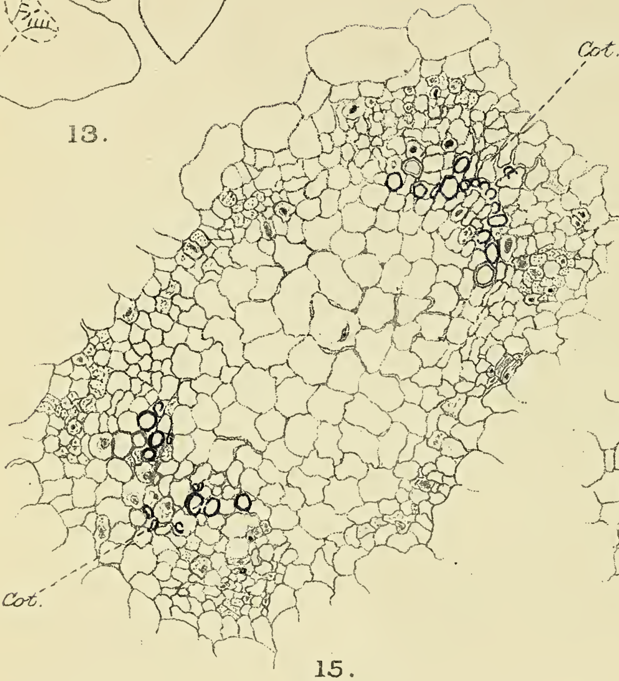

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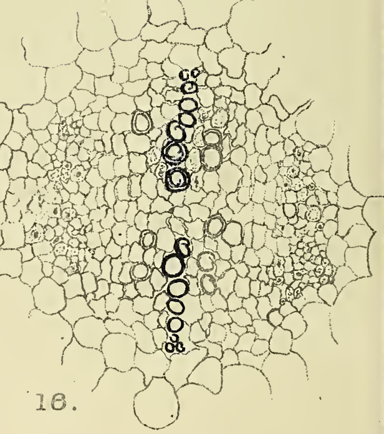

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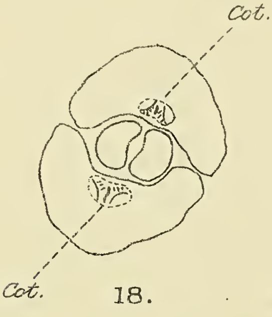

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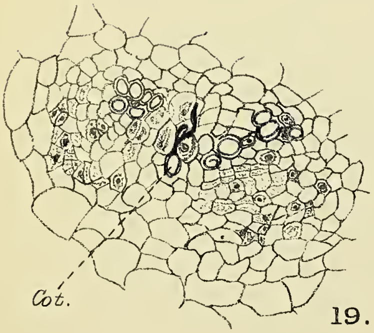

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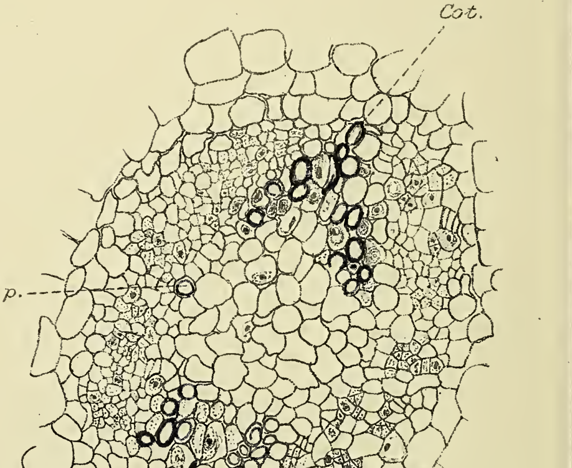

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Figs 13-16, Iberis tenereana: 17-20,

E. N. THOMAS — SEEDLING ANATOMY.


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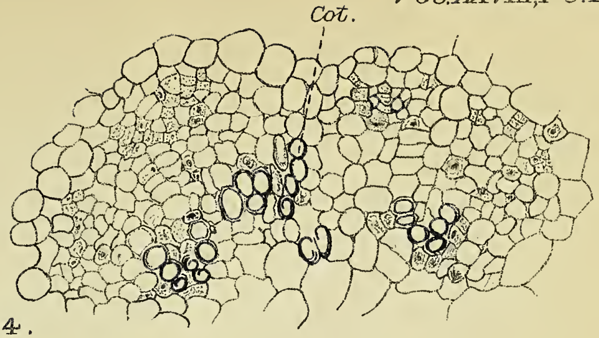

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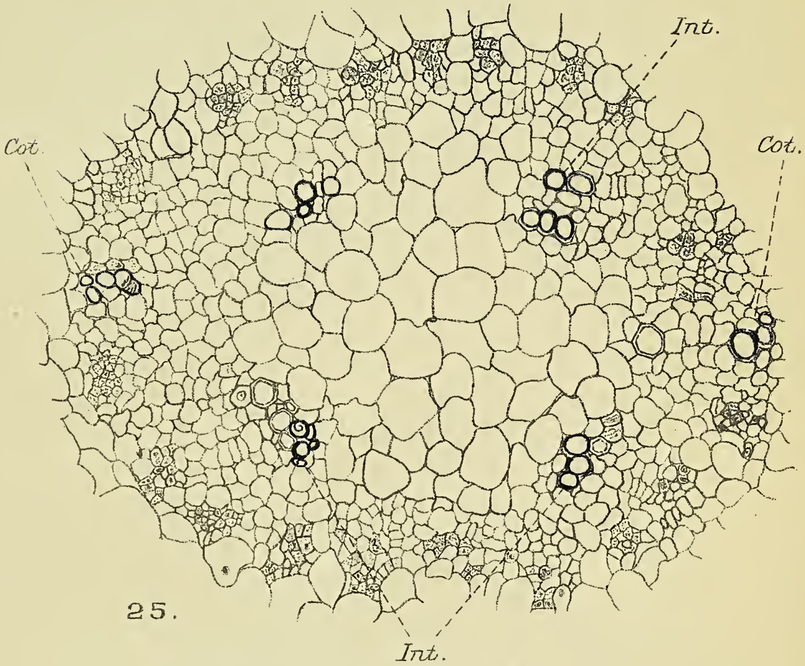

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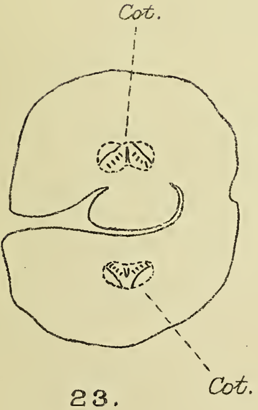

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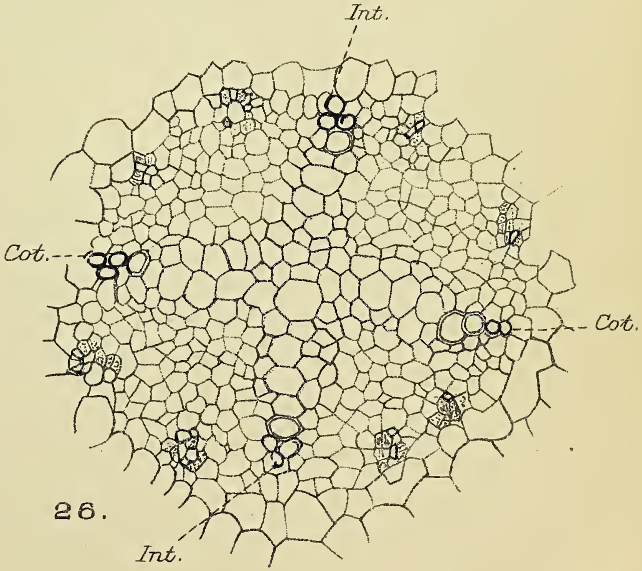

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eiranthus cheiri: 21–26, Althaea rosea.

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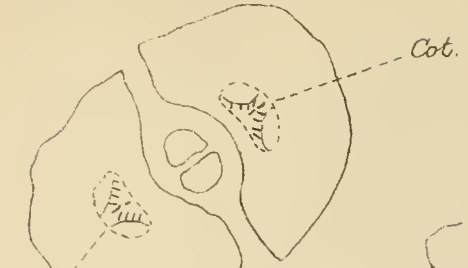

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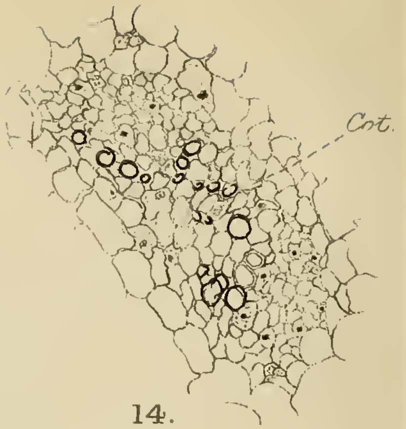

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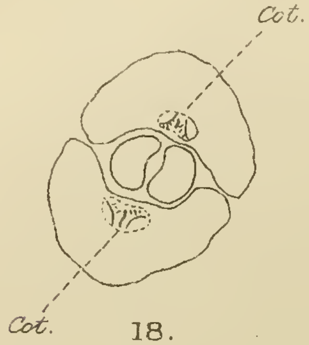

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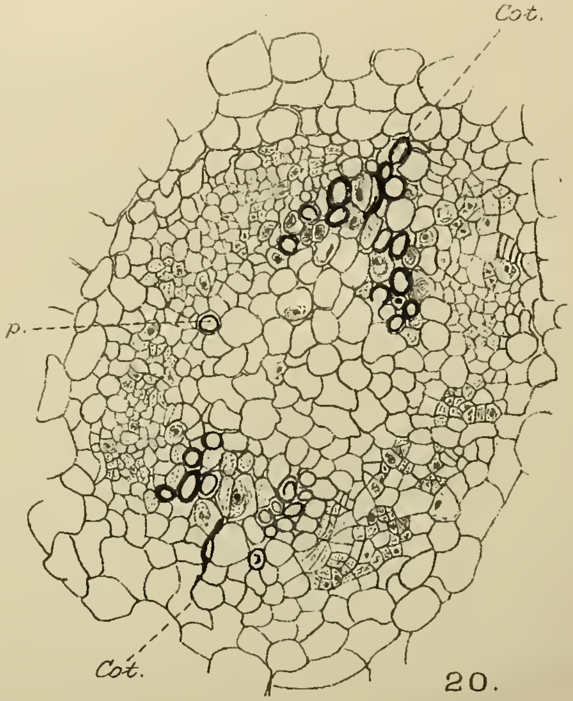

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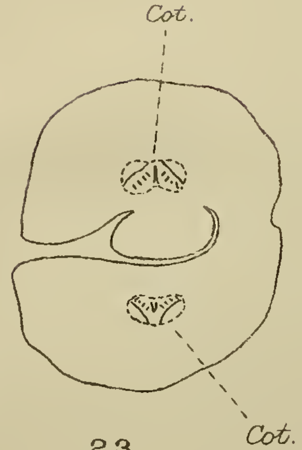

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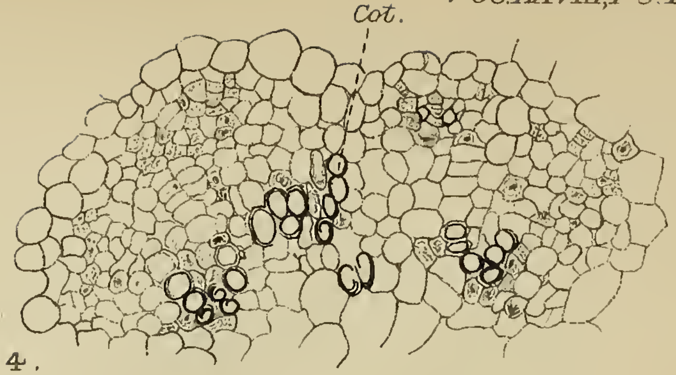

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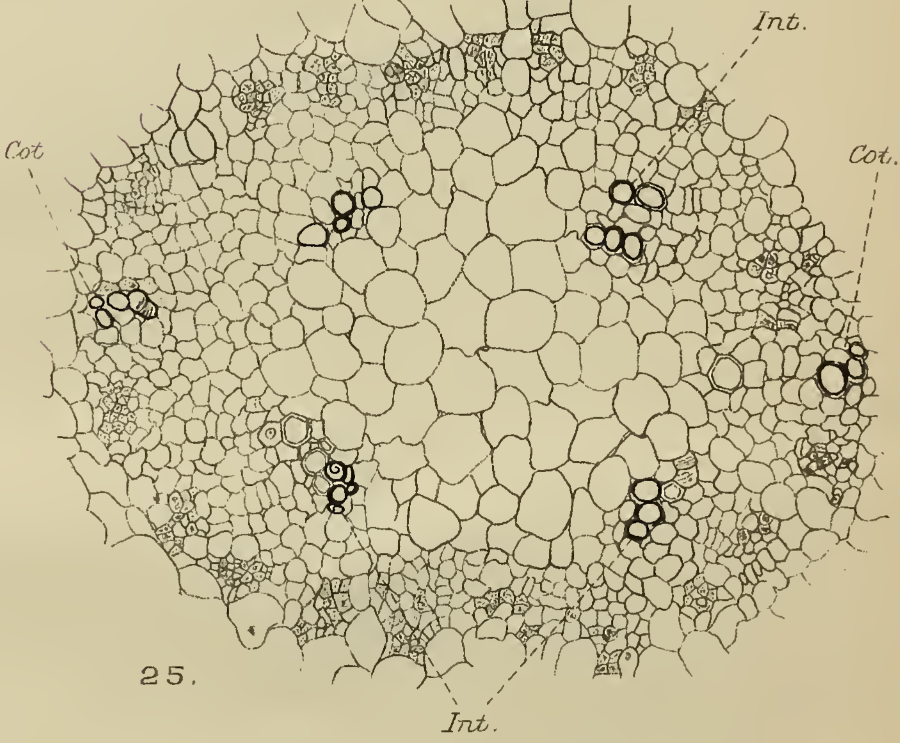

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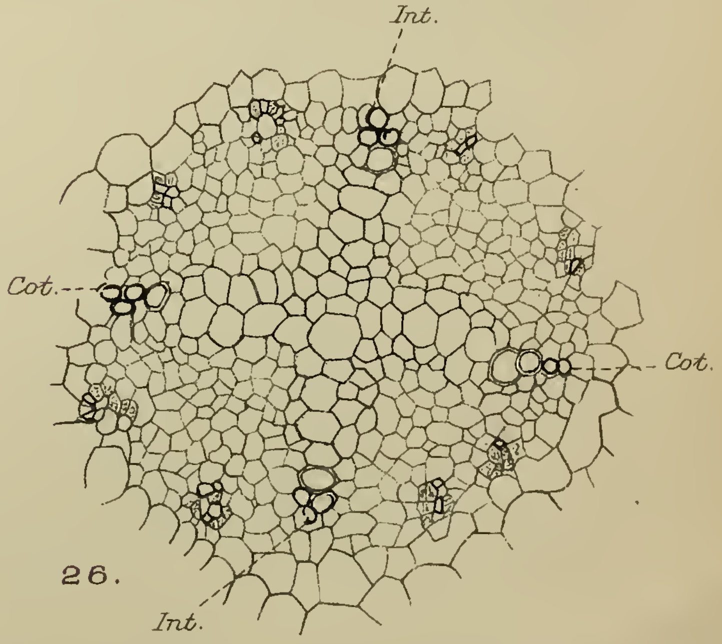

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E.N.Thomas del.

Figs 13-16, Iberis tenereana: 17-20, Cheiranthus cheiri: 21-26, Althaea rosea.

Huth lith. et imp.

E. N. THOMAS — SEEDLING ANATOMY.

NOTE.

MORPHOLOGY AND CYTOLOGY OF THE SEXUAL ORGANS OF PHYTOPHTHORA ERYTHROSEPTICA, PETHYB. (PRELIMINARY NOTE). —The Fungus which forms the subject of this study, as first recorded and described by Pethybridge[1] (1913), has a peculiar arrangement of the sexual organs in that the antheridium, instead of growing up by and becoming laterally adpressed to the oogonium, is pierced by the developing female organ, which then grows through it and, rupturing the wall at the opposite side, emerges and assumes a spherical form.

These observations have been confirmed and amplified, and the cytology has been worked out.

The antheridia arise first as intercalary swellings, rarely terminally, and they are cut off by septa. The hypha in the act of giving rise to an oogonium may be seen not infrequently while still within the antheridium, having pierced one wall, but before it emerges at the opposite side. When it emerges it is at first club-shaped, but it quickly becomes spherical and grows to full size. In every case in which the connexions could be traced the male and female organs are borne on separate hyphae but the Fungus is homothallic.

When the organs are full grown the stalk or lower part of the oogonium lies within the antheridium. A section in the direction of the long axis of the stalk shows the latter in or on the antheridium, and a section at right angles to this direction shows the stalk lying free within it—a small circle within a larger one. There is no trace of the antheridium growing around the base of the female organ when the latter is fully grown or nearly so.

The oogonium, when mature, is filled with dense protoplasm in which are distributed a number of nuclei, which are first elongated and irregular in shape, but afterwards round off and appear to increase in size. About two-thirds of them then gradually disintegrate and disappear. At this time a thick plug is formed in the stalk of the oogonium.

Meantime the protoplasm of the oogonium has become less dense and less deeply staining, and the remaining nuclei, now larger and in the prophase of division, arrange themselves in the form of a hollow sphere with one in the centre. In this position all pass through a normal mitosis. The stages of the prophase, origin of the spireme, chromosome formation, and migration to the nuclear plate, are very clear. The spindle is intranuclear, but the membrane soon disappears. Centrosomes are present. The chromosome number is small but is very difficult to estimate with certainty, though it is probably about five. The oogonial nuclei undergo but a single simultaneous division.

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[1] Pethybridge, G. H.: On the Rotting of Potato Tubers by a New Species of *Phytophthora* having a Method of Sexual Reproduction hitherto undescribed. Sc. Proc. R. Dublin Soc., vol. xiii (N.S.), 1913, No. 35, p. 529.

[Annals of Botany, Vol. XXVIII. No. CXII. October, 1914.]

During and after the division there is no differentiation of the protoplasm within and without the sphere of nuclei into oospheric and periplasmic regions respectively. As soon as the daughter nuclei are reformed all but one in the centre wander out towards the periphery, beginning to disintegrate rapidly at the same time; and simultaneously practically the whole of the protoplasm contracts away from the oogonium wall and forms the uninucleate oosphere. The remaining nuclei, now scarcely recognizable as such, lie just outside the oosphere, scattered over its periphery. Between them and the oogonium wall is an empty space. The nucleus of the oosphere is still small and stains very faintly. It lies in the centre in an irregular mass of cytoplasm which stains somewhat deeply with orange G.

The nuclei of the antheridium divide once, but whether this is preceded by a disintegration of a portion of them is not certain. A very large outgrowth pushes its way from the upper part of the stalk of the oogonium into the antheridium (the so-called 'receptive papilla'). At about the period when mitosis is complete this has been entirely withdrawn, and a movement in the opposite direction begins, the fertilization tube, with a nucleus at the tip, growing into the oogonium. The tube may pursue a somewhat devious course before penetrating the oosphere, and it may contain more than one nucleus. Only one, however, passes into the oosphere, which then ejects the tube and surrounds itself with a wall. The sexual nuclei, having increased enormously in size, appear to come together at first and then separate widely. When the spore wall is mature they approach again and fuse.

The further stages have not been followed. The spore appears to rest in a uninucleate condition, and the behaviour on germination is not yet known.

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DAHLEM, BERLIN.

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