ANNALS OF BOTANY

EDITED BY

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ASSISTED BY OTHER BOTANISTS

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Studies in the Phylogeny of the Filicales.

VI. Ferns showing the 'Acrostichoid' Condition, with Special Reference to Dipterid Derivatives.

BY

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

Regius Professor of Botany in the University of Glasgow.

With Plates I and II and fifteen Figures in the Text.

THE old comprehensive genus, *Acrostichum*, was based upon the fact of the exposed sporangia being spread uniformly over a considerable area of the sporophyll, and not grouped in distinct sori. It will not be necessary here to enter into the history of this genus, or the group of genera, based on this feature, nor to trace their various treatment by different authors: since this has been recently done by Frau Eva Schumann, in 'Flora'.[1] Certain of the earlier writers appear to have taken this single character alone into account in their grouping, with the result that a large number of Ferns of very various types were associated together. An extreme position was taken up in the 'Synopsis Filicum', where, as in some other systematic works, the genus *Acrostichum* was made very comprehensive. A natural consequence was that the genus had to be segregated into numerous sub-genera according to their secondary features. But since the time of publication of the 'Synopsis Filicum' evidence has been steadily accumulating, which shows that the divergence in the features, then held as secondary, is so great as to make it improbable that there is any near relationship of the plants which bear them. The question is freely canvassed now, whether the non-soral character itself has not been acquired along a number of phyletic lines. In fact, it is becoming evident that the 'Acrostichoid' condition is not in itself a sign of affinity at all; but a state or condition, which may have been attained by Ferns of quite distinct evolutionary history. If this be accepted, then *Acrostichum* is not really a genus of common descent, nor even a natural group. But it expresses merely that condition or state of soral development in which freely exposed sporangia spring from a considerable area of leaf-surface.

[1] 1915, pp. 201–7.

[Annals of Botany, Vol. XXXI. No. CXXI. January, 1917.]

The case of *Acrostichum* is parallel with that of *Polypodium*. This comprehensive genus was based upon the definite sorus, consisting of numerous sporangia, without any protective indusium. It is obvious that such a condition may have been primitive in types which never had an indusium at all, such as *Gleichenia*, *Matonia*, *Dipteris*, *Metaxya*, &c.: or it may have resulted from abortion of an indusium previously present, as in *Dryopteris*: there is reason to hold that this latter state may have arisen along more than one phyletic line. Such single characters as those defining *Acrostichum*, or *Polypodium* in the old sense, are in fact too wide to be of service in a system which aims at a true phyletic grouping. Moreover these, though the most prominent of the 'false' genera, are by no means the only ones that will have to be broken up as the basis of comparison is extended. In proportion as a larger number of characters are used in the comparisons, the relations of the plants to their phyletic sources will become clearer, and the classification will be a more natural one. To this end the definite intention should be to bring a larger number of characters into our comparisons. Among these, anatomical data will be of the first importance.

In the present memoir the observations relate chiefly to representatives of the old comprehensive genus, *Acrostichum*. The attempt will be made to refer certain of its forms to their phyletic source, and to suggest their inter-relations. It will then become apparent that while certain Acrostichoid Ferns may have sprung from an indusiate ancestry, others have sprung from a primitively Polypodioid or Matonioid-Dipterid source, where no indusium was ever present, by a simple spread of the soral area from the vascular receptacle to the non-vascular surface of the sporophyll. This source has already been suggested for *Cheiropleuria*.[1]

Gymnopteris (*Leptochilus*) *tricuspis* (Hook.), C. Chr.

In No. III of these Studies [2] it was suggested that a phyletic relation exists between *Metaxya* and certain types of the comprehensive but false genus *Polypodium*. Reference was also made to *Neocheiropteris*, but the relation of this plant to the Ferns named was held to be obscure and in need of further inquiry. Since then opportunity has been found for the investigation of *Cheiropleuria*,[3] and this led to a suggestion of its affinity on the one hand to *Dipteris*, on the other to *Platycerium*. All of these are Ferns with superficial, non-indusiate sori. This character, as well as some others, indicates relationship downwards with Gleicheniaceous and Matonioid types, though such relationship can hardly be regarded as one of near affinity.

In resolving such questions of affinity an increase in the number of the

[1] No. V of these Studies. Ann. of Bot., vol. xxix, p. 475.

[2] On *Metaxya* and certain other relatively Primitive Ferns. Ann. of Bot., vol. xxvii, 1913, p. 473.

[3] Studies, V. Ann. of Bot., vol. xxix, 1915, p. 495.

subjects of comparison materially helps. Since the time of writing the previous memoirs, I have received from Mr. Cave of Darjeeling, through the kindness of the Director of the Calcutta Garden, a supply of a Fern at present known as *Leptochilus tricuspis*, which has been closely related by the older systematists with *Cheiropleuria*. While examining its anatomy for the first time, opportunity has been taken to investigate certain other Ferns also, with a view to testing relationships. This may help to trace the probable affinities of these relatively isolated, but apparently kindred forms. All of them have superficial sori, without indusia; and accordingly they may be expected to show some degree of relationship downwards to a Dipterid-Matonioid, and finally a Gleichenioid source. On the other hand, Sir William Hooker had already suggested for *Gymnopteris* (the genus to which he referred *Leptochilus tricuspis*) a relationship with *Polypodium*.[1] Speaking of the *Gymnopteris* section of *Acrostichum*, he remarks: 'This corresponds in venation and a good deal in habit with *Phymatodes* among Polypodieae.' Thus further inquiry may tend to substantiate affinities in more than one direction.

The Fern now styled *Leptochilus tricuspis* (Hook.), C. Chr., has undergone several synonyms, and has been placed in different systematic relations by various writers. It was first described by Sir William Hooker, from specimens collected by Mrs. Atkinson in hot valleys of Sikkim-Himalaya, under the name of *Acrostichum* (*Gymnopteris*) *tricuspe*, Hook. He placed it next to *Acrostichum* (*Gymnopteris*) *bicuspe*, Hook.—now called *Cheiropleuria bicuspis* (Bl.), Presl—and he remarks of it that 'this very fine and new species, with not a little of the habit and venulation of *A. bicuspe*, differs remarkably in being trilobed or tripartite, and it has always a solitary central costa to each lobe'.[2] It will be seen that, notwithstanding these and other points of difference, its true relation is that indicated by Sir William Hooker: while it serves, as also does *Cheiropleuria*, as a synthetic link between forms often placed apart in the systematic arrangements of various writers.

John Smith[3] followed Hooker in placing it in close relation to *Cheiropleuria bicuspis* (Hook.) and *C. vespertilio* (Hook.), and he remarks: 'It is probable that the above three species are different forms of one only.' This is a position which it will be impossible to maintain.

On the other hand, Diels[4] places the Fern which he names *Gymnopteris tricuspis* (Hook.), Bedd., far away from *Cheiropleuria* in his system (p. 336), and gives no reference of relationship between them. He does, however, compare the latter with the *Pleopeltis* section of *Polypodium*: and this line of comparison will be seen to be materially strengthened by the facts relating to *Leptochilus tricuspis* to be detailed below. Christ also separates

[1] Hooker: Sp. Fil. v, p. 270.
[2] Species Filicum, vol. v, p. 272, Tab. CCCIV.
[3] History of Ferns, 1875, p. 139.
[4] Engler und Prantl, i, 4, p. 199.

this Fern[1] widely from *Cheiropleuria* (p. 128), and does not establish any comparison between them. Similarly Christensen, in the systematic grouping introductory to his 'Index Filicum', places *Leptochilus tricuspis*, C. Chr., with the Dipteridinae (p. 26), while *Cheiropleuria* is placed far away with the Platyceriinae (p. 53). Thus the more modern writers appear to agree in ignoring the comparison which Sir William Hooker established between *Cheiropleuria* and the Fern now before us. Though I had long known of the existence of this Fern, my attention was first definitely drawn to it by Dr. R. C. Davie. I then asked the Director of the Calcutta Garden to send home material. Through his kindness I have received from Mr. Cave of Darjeeling an ample supply of specimens, both dry and preserved, in formaline, upon which the following observations are based. My best thanks are due to the Director, and to Mr. Cave for the selection and preservation of the material.

External Characters.

Leptochilus tricuspis is a fine Fern, with upstanding fronds rising to a height of nearly three feet. They spring from a stout creeping rhizome rather over $\frac{1}{4}$ inch in diameter, of rather fleshy character. It is densely covered while young by brown scales, which fall away from the older parts. From this the leaves arise roughly in alternate order, right and left; but the sequence does not appear to be always strictly maintained. The leaf-stalk is stout, and naked when mature, excepting the last quarter of an inch at the base, where it enlarges into a mammillary swelling, which persists with a terminal scar after the old leaf itself separates by an almost smooth abscission. It is in fact a typically 'Eremobryoid' type, after the terminology of John Smith.[2] Frequently the rhizome may grow to a length of six inches or more without any branching. But occasionally lateral branches are formed, apparently without any near relation to a leaf; their appearance suggests that they arise by some terminal branching, as in *Dipteris*, rather than as appendages to a leaf, as in *Cheiropleuria*.[3]

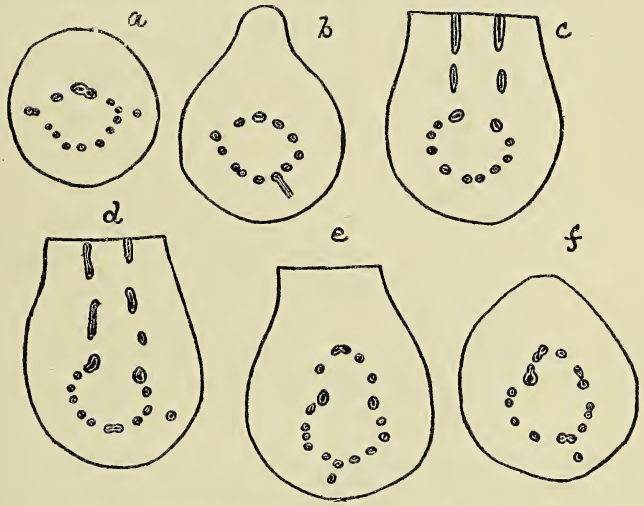
The leaves are strongly dimorphic, though Sir William Hooker mentions a specimen which 'has the upper half of two out of the three lobes contracted and soriferous, thus connecting this group with *Hymenolepis*'.[4] Both sterile and fertile leaves have long smooth petioles, but the fertile are the taller. Both are ternate, with the terminal lobe the longest, and this is an almost constant character. But the specimen from India, in the hands of Dr. Davie, shows a rudimentary fourth lobe (Plate I, Fig. 1), reminiscent of a katadromic helicoid system, as in *Matonia*. The barren leaves are firm and naked, with the three entire lobes of coriaceous texture. Each has a well-marked midrib, the lateral venation being of the type

[1] Farnkräuter, p. 49.
[2] Historia Filicum, p. 66.
[3] Seward: Phil. Trans., B, vol. 194, p. 494.
[4] Syn. Fil., p. 422.

Venatio Anaxeti. The fertile leaves have narrower lobes, but still with the well-marked midrib. Their lower surfaces are covered right and left of the midrib by a dense and continuous sorus of Acrostichoid character. The drawings published by Sir William Hooker [1] adequately represent the main characters; but in order to show the chief habit-features of the plant, a photograph has been made of the finest specimen sent from Darjeeling, the plant being about three feet in height (Plate I, Fig. 2).

Anatomy.

The adult leaf-trace of *Leptochilus tricuspis* consists of five, or sometimes of six, strands, arranged in a horseshoe, with the open side facing directly towards the apex of the axis. The two marginal strands are



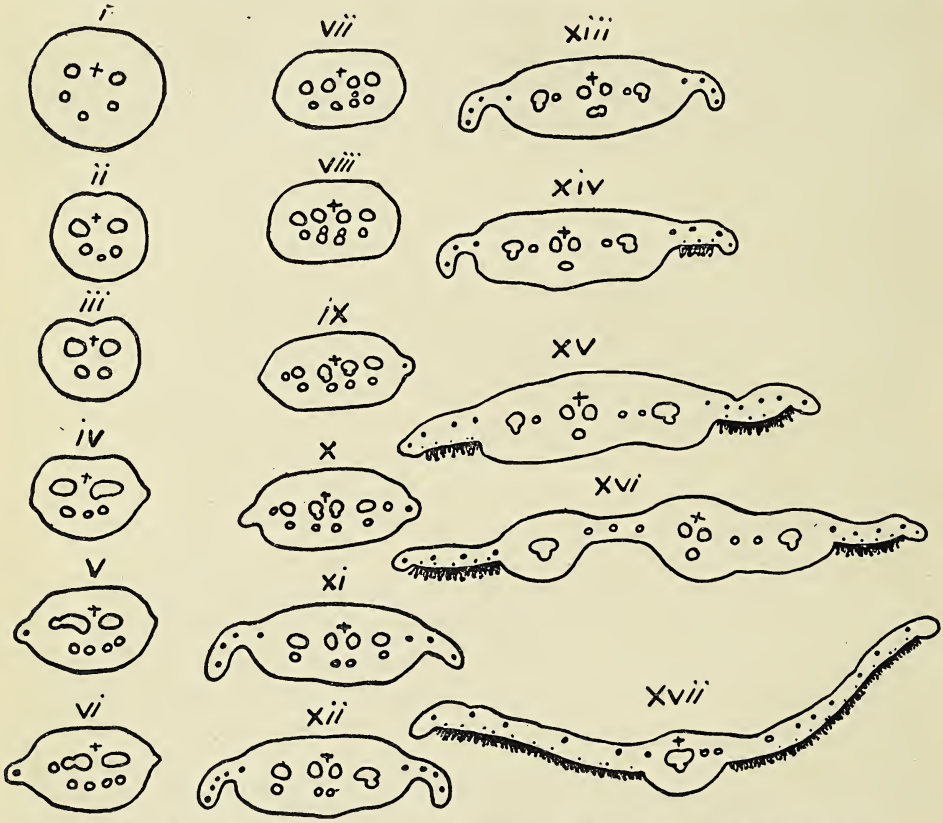
Text-fig. 1. Series of transverse sections of the rhizome of *Leptochilus tricuspis*, including a leaf-insertion. × 4.

larger than the rest, and show indications of the usual recurved hooks. The vascular system of the axis is in the internodes disposed in a ring of strands of unequal size, circular or slightly oval in outline, with occasional fusions, and here and there insertions of root-strands directed laterally or downwards (Text-fig. 1, *a*, *b*). When a leaf-trace is inserted, the distance between the median strands on the upper side increases, and these strands, which are usually of large size, rotate on their axes, so that the edges of their xylem-plates are directed towards the incoming trace (*c*, *d*). Upon these the marginal strands of the leaf-trace insert themselves (*d*, *e*, *f*), while the smaller median strands fusing irregularly (*e*, *f*) enter the vascular ring. The whole construction is such as would naturally follow if a solenostelic axis and a united leaf-trace, such as is seen in *Dipteris* or *Metaxya*, were

[1] Sp. Fil., vol. v, Tab. CCCIV.

subject to profuse perforation. The parenchyma of the rhizome is crowded with nests of black sclerenchyma. Sometimes isolated cells are indurated: more frequently groups of them. The nests are not continuous longitudinally, and they extend only a short way up the petiole. They give a hard, gritty texture to the whole tissue, as in *Neocheiropteris*.

The leaf-trace a little above the base of the petiole consists usually of five strands, the two adaxial being the largest, and occupying the ends of



TEXT-FIG. 2. *Leptochilus tricuspis* series illustrating the vascular system of sporophyll. Adaxial surface uppermost. ×4.

the horseshoe curve. In all the modifications which follow in the upward course it appears that these strands maintain their independence of one another; that is, there is no fusion between the two ends of the curve, but the gap (+) remains constantly open. All the changes are in fact variants on the curve itself, which is not obliterated by them. This is important for comparison on the one hand with *Cheiropleuria*, and on the other with *Platycerium*. The upward course has been followed in the fertile leaf, and the results are shown in Text-fig. 2, i–xvii, all of which are orientated with the adaxial face uppermost. Nos. i–vi illustrate the course up the petiole,

where by fusion the number of strands may be reduced to four (iii); but afterwards the number increases again as the blade is approached (iv), while strands may be given off to supply the lateral wings (v). The origin of these is extra-marginal, for the actual margin of the vascular horseshoe faces inwards. The small strand in the wing seen in v is not formed as a direct branch from the petiolar strands, but is one of those small fibrils which form the ultimate reticulations of the network in the blade. In vi the petiolar strands are seen further subdivided, and ranged apparently in two strata, the one adaxial, the other abaxial.

Nos. vii–xvii are from a different leaf, but they take up the details from the condition seen in vi. Each of the two strata is composed of four strands, while the gap (+) between the two marginal strands is still present. Small fusion-strands are then formed, which pass from the abaxial to the adaxial strands (viii–x), and meanwhile, by extra-marginal branching, strands are passed off laterally into the wings (x), which now assume considerable dimensions. After settling down again to two groups of four (xi), the strands begin to segregate into three groups, destined for the three lobes of the blade (xii–xv). Fusions occur in the lateral groups, so that in each of them the main supply is a strand which appears three-armed in transverse section (xiii–xvi). But still the strands of the central lobe remain distinct, the two adaxial still representing the margins of the original horseshoe, and the space between them (+) having been open throughout the whole development. Finally, however, they fuse into a single strand, as in xvii, which represents one lobe in section. The origin of the vascular supply to the wings, and the sori which soon appear upon them, calls for no special description.

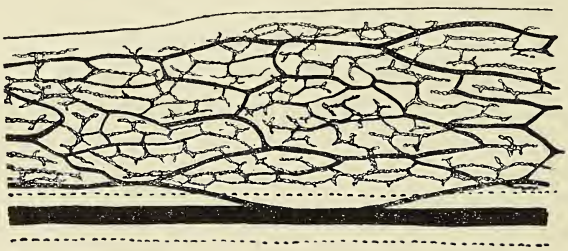
It is thus seen that the whole arrangement is a modification of the fundamental horseshoe, which is spread out laterally and compressed in an antero-posterior direction, so that it apparently forms two strata. Fusions then appear between the members of the two strata, which have approached near to one another by reason of the compression of the horseshoe (viii–ix). Lastly, the flattened series divides up into the three groups which supply the lobes of the blade. Comparing with what is seen in *Cheiropleuria*,[1] the condition is distinctly more complex here, though in both the horseshoe is maintained with free margins. But in *Platycerium*[2] there are fusions across the adaxial face of the leaf, that is between the margins of the horseshoe and antero-posterior fusions as well. Thus it has departed farther than *Leptochilus tricuspis* from the primitive horseshoe. Accordingly, *Leptochilus* takes a middle position between the two structurally, as regards the petiole.

Passing to the sterile blade, the venation corresponds to that of *Dipteris* and *Cheiropleuria*, and it is, like theirs, expanded in a single plane.

[1] Ann. of Bot., 1915, p. 506, Fig. 8.

[2] l. c., p. 509, Fig. 10.

But the special interest attaches to the fertile blade. Here the soral areas occupy the whole lower surface between the midrib and the slightly enlarged margin, as shown in Text-fig. 2, xvii. The rather fleshy expanse is traversed towards its upper surface by a reticulum of stronger strands, corresponding to that of the sterile lamina. But in addition to this there is a second system connected with it, which ramifies in a plane below, but parallel to it, spreading immediately below the soral surface. The appearance as seen in transverse section is suggested by Text-fig. 2, xvii, which further indicates that the sub-soral system consists of very minute strands. The relation of these two systems is indicated in Text-fig. 3, which represents part of a fertile lamina made transparent, and stained so as to bring into prominence both series. The main reticulum of the lamina is represented by heavy lines; this indicates the coarser texture of its strands,



TEXT-FIG. 3. Portion of the soral region of the sporophyll of *Leptochilus tricuspis*. The heavier and continuous lines represent the normal venation of the leaf, which is nearer the upper surface. The lighter and broken lines represent the receptacular system extended in a plane nearer the lower surface. × 5.

which are connected directly with the midrib. The sub-soral system arises from the main reticulum, and is connected with it at many points by strands which run obliquely downwards from it to the sub-soral level. Sometimes such a strand develops only a few ramifications, and the whole falls within a single areola of the main reticulum. But more frequently the branching is more elaborate, and the resulting system, fusing with like strands originating elsewhere, constitutes the connected sub-soral network. Frequently strands of this network cross those of the main network, but at a lower level and without actual junction at the point of crossing; and this is seen with special frequency towards the margin.

The structure thus described, which may be designated as *diplodesmic*, corresponds to what has been seen in *Platycerium*, and in less degree in *Cheiropleuria*. In the latter it has been shown[1] that while the vascular supply to the sorus is the same in principle as that in *Dipteris*,[2] it is not limited to each areola of the main venation, but is liable to extend in a lower

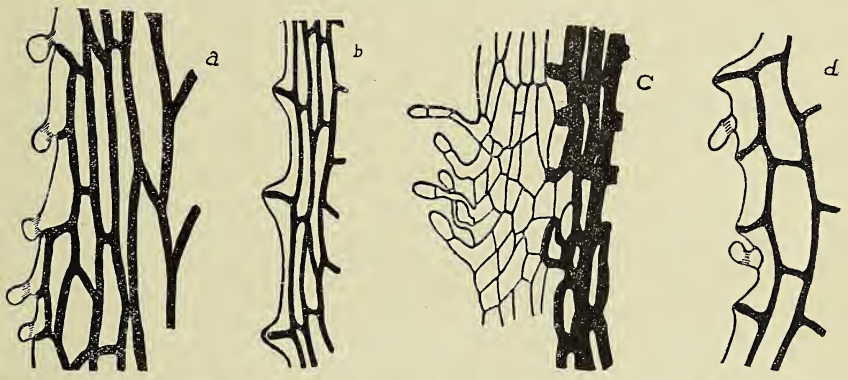
[1] Ann. of Bot., 1915, p. 512, Text-fig. 12.

[2] l. c., p. 499, Text-fig. 2.

plane across the veins limiting those areolae. It had long been known that in the sporophylls of *Platycerium* a sub-soral vascular system existed. It was further shown [1] that if semifertile areas of *Platycerium* be examined, a condition is found more advanced than in *Cheiropleuria*, and that the extension of the soral vascular system, which is seen occasionally in the latter, is the rule in *Platycerium*; it leads in fully fertile leaves to the sub-soral system, which ramifies extensively in a different plane from the main reticulum. This is what is now seen also in *Leptochilus tricuspis*, in which it corresponds more nearly to what is seen in *Platycerium* than in the simpler *Cheiropleuria*, though it still falls short of the complexity seen in the most elaborate examples of the former genus.

Dermal Appendages.

The dermal appendages offer features useful for comparison. Both on the axis and on the young leaves of *Leptochilus tricuspis* they are present as



Text-fig. 4. *a* = margin of scale of *Leptochilus tricuspis*; *b* = ditto of *Neocheiropteris*; *c* = ditto of *Platycerium* sp.; *d* = ditto of *Goniophlebium* sp. All are magnified 85.

scales. The largest have a broad shield-like base, with the short stalk of attachment intra-marginal, so that the scale is peltate. The scale narrows off upwards, and terminates in a very long hair, formed of a single row of cells. The terminal cell remains usually thin-walled, but is not enlarged as a gland. The greater part of the scale is only one layer of cells in thickness, but the stalk is more massive. The whole scale is composed of cells with thick chestnut-brown walls, but the marginal cells have their marginal wall thin. Many of these cells give rise to shortly-stalked glands, which are not partitioned by septa from the cells which bear them (Text-fig. 4, *a*). The scale in development originates from a single row of cells, of which those of the basal region soon undergo longitudinal division, widening out laterally above the short stalk to form the peltate expansion. The enlarged region thus formed continues intercalary growth near its base, and the

[1] l. c., p. 513, Text-figs. 13, 14.

intercalary zone remains thin-walled long after the distal region has become mature and indurated. The insertion of the scale upon the rhizome is at the base of a funnel-like pit in the surface of the rhizome, two or three cells deep. The stalk of the scale fills this, but as it emerges from the pit it expands right and left at once, so that the peltate scale is in close relation to the surface of the rhizome.

The interest of these details lies in the comparison on the one hand with *Dipteris* and *Cheiropleuria*, on the other with *Platycerium* and some other Ferns. Comparing with the *Matonia-Dipteris* series, the hairs of *Matonia* consist of a single row of cells, with a basal intercalary zone, while distally the cells have brown indurated walls, and run out to a pointed tip.[1] In *Dipteris conjugata* the smaller hairs may have the same structure; but various stages of increasing complexity are seen, which are initiated by longitudinal divisions of the basal cells. This region widens into a massive structure with greatly indurated walls.[2] In both of these Ferns the hairs are inserted on the ordinary surface of the rhizome. In *Leptochilus tricuspis* the construction is essentially of the same type, but with the addition of the peltate expansion at the base and the sessile marginal glands: moreover the stalks of the scales are sunk in pits. Thus all of these are essentially of the same type, but show successive steps of increasing complexity.

Comparing on the other hand with *Platycerium*, the scales of the rhizome there found are of the same type as regards their peltate form and the intercalary zone at the base. They vary in size in different species: in *P. alcicorne* they are smaller and narrower. They are, however, inserted not in an involution of surface of the rhizome but upon a convex emergence: so that the scale is not in such close relation to the surface as in *Leptochilus tricuspis*. This is a state similar to that seen in many Cyatheaceae, where the emergences mature into the thorns about the leaf-base. A like condition is seen in many species of *Gleichenia*, at the base of the leaf-stalk.[3] In *Platycerium* the induration is restricted to the central region of the scale, but it is heavier than in *Leptochilus*, and darker in colour. The most marked feature of resemblance is in the glands at the margin. Many of the marginal cells give rise to a hair, composed of two or more cells, of which the last is a gland (Text-fig. 4, *c*). It may thus be held that the scales of the rhizome of *Platycerium* correspond in form, development, and glandular appendages to those of *Leptochilus*; but they are more specialized in their details, and are borne on projecting emergences instead of being sunk in pits.

For further comparison a drawing is given of the margin of a scale of

[1] Seward: Phil. Trans., B, vol. 191, Pl. 19, Fig. 32.
[2] Seward: Phil. Trans., B, vol. 194, Pl. 49, Figs. 29, 30, 36.
[3] Ann. of Bot., vol. xxvi, 1912, pp. 272–3.

Goniophlebium sp. (Text-fig. 4, *d*). This section of *Polypodium* corresponds in its venation to *Leptochilus*, and it is seen that glandular hairs are borne on the margins of its scales ; but here they are partitioned off by septa, a point in advance of *Leptochilus tricuspis*.

Sorus and Sporangium.

The fertile leaf of *Leptochilus tricuspis* was described by Sir William Hooker as 'much elongated but contracted, tripartite nearly to the base, segments scarcely ½ an inch wide, linear strap-shaped acuminate, lateral ones 9 to 10 inches long erecto-subpatent, intermediate one a foot or more long, sori universal except on the costa'.[1] These general features are represented on Hooker's Tab. CCCIV. Part of a very large specimen is represented natural size in Plate I, Fig. 3, as seen from below. The dark-coloured soral areas occupy the whole of the lower surface except the midrib : that on the inner side of each lateral lobe is continuous to the median lobe, while those on the outer side extend some distance downwards below the point of junction of the three lobes. They appear quite uniformly spread over the surfaces, and give no indication of their resulting from a fusion of independent sori. It was natural that Sir William Hooker should place the Fern in the genus *Acrostichum*, as then defined, and in the section *Gymnopteris*, in close juxtaposition with his *Acrostichum* (*Gymnopteris*) *bicuspe* (=*Cheiropleuria bicuspis* (Bl.), Presl). But since his time it has become apparent that the main character upon which the genus *Acrostichum* was based, viz. 'sori spread over the whole surface of the frond or upper pinnae',[2] is one which has been arrived at phyletically from a plurality of sources. Accordingly it becomes necessary to examine the details of the sorus in each case, as well as other characters from the vegetative region, with a view to forming an opinion as to the true affinity.

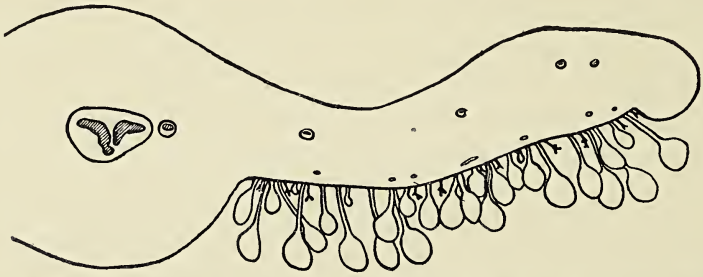
Unfortunately the material of *L. tricuspe* sent from India did not suffice for the observation of the earliest stages of development of the sorus, while its condition, probably as the result of overheating in transit, was not suitable for working out fully the sporangial segmentation. Still the essential points have been observed. The double vascular system of the sub-soral areas of the fertile leaf has already been described. In order to estimate this at its proper diagnostic value it may be stated that the following Acrostichoid Ferns have been examined, and in them the vascular system of the fertile tract has been found to be not double, but simple, viz. *Acrostichum* (*Chrysodium*) *aureum*, L.; *Leptochilus heteroclitus* (Pr.), C. Chr.; *L. nicotianaefolius* (Sw.), C. Chr.; *L. alienus* (Sw.), C. Chr.; *L. cuspidatus* (Pr.), C. Chr. Also *Elaphoglossum tectum* (H. B. Willd.), Moore; *E. petiolatum* (Sw.), C. Chr.; *E. crinitum* (L.), Christ; and *E. latifolium* (Sw.), J. Sm. These results are in accord with the observations of Frau Schumann,[3] which relate

[1] Sp. Fil., vol. v, p. 272. [2] Syn. Fil., p. 399. [3] Flora, 1915, p. 236, &c.

to several of the same species. The absence of a double vascular system in them shows that there is nothing inherent in the Acrostichoid condition leading to that diplodesmic state; and it throws into the greater relief what is seen in *Leptochilus tricuspis*, and accentuates its resemblance in this respect to *Cheiropleuria* and to *Platycerium*.

Passing to the sorus itself, sections show that it is uniformly spread over the whole lower surface, excepting the midrib and the extreme margins. Its constituents are sporangia and hairs: the latter are relatively inconspicuous, and not nearly so numerous as those of *Cheiropleuria*. They are septate and branched (Text-fig. 5), but the branches are few and short, consisting usually of a single cell of somewhat glandular appearance. They

TEXT-FIG. 5. Hairs associated with the sporangia of *Leptochilus tricuspis*. × 125.



TEXT-FIG. 6. Part of transverse section of a fertile leaf of *Leptochilus tricuspis*, showing the diplodesmic structure and hairs associated with the sporangia. × 16.

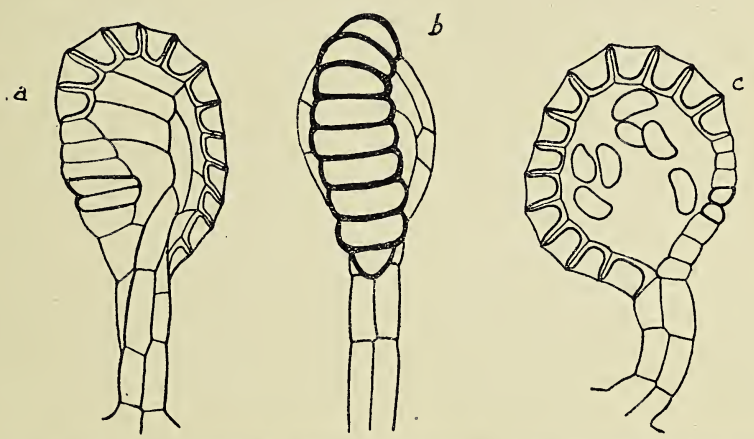
are barely one-third the height of the mature sporangium. The sporangia thus form the chief constituent of the sorus, and their closely serried heads are what are seen from without. They are found of various ages in juxtaposition, and there is no grouping of the older sporangia which suggests any primitive soral relation (Text-fig. 6): their orientation is usually so that the annulus lies in a plane transverse to the leaf.

Comparing this with what is seen in *Cheiropleuria*, the hairs there are very numerous,[1] and are septate, but unbranched, and as long as the sporangia. Thus, as seen from without, the sporangial heads appear isolated, and packed in by the enlarged distal cells of the much more numerous hairs. Comparing, on the other hand, with *Platycerium*, the sori are there distinctly marked, as in *P. Willinkii*, or even isolated, as in some cases of

[1] Ann. of Bot., 1915, pp. 515 and 518, Figs. 15, 17.

P. oethiopicum and *angolense*,[1] and sections show even more decisively that the sporangia are not spread continuously over the whole surface; on the other hand, the sporangia are usually orientated as in *L. tricuspis*, with the annulus lying in a plane transverse to the leaf. Comparing with species of *Leptochilus*, as shown by Frau Schumann,[2] while there also the sporangia of different ages are intermixed, the first formed are seated upon the veins, thus indicating a soral origin. Hairs are present, but only occasionally, here and there, and are unbranched.

The sporangium of *L. tricuspis* is of an ordinary Polypodiaceous type, with a stalk about twice as long, when fully extended, as the capsule itself. It is composed of three rows of cells, and each row consists, as a rule, of three



TEXT-FIG. 7. *a*, *b*, *c*. Sporangia of *Leptochilus tricuspis*. *a*, seen obliquely from the side of the stomium; *b*, from the opposite direction; *c*, sporangium cut in longitudinal section. × 125.

cells. The segmentation of the young sporangium has been seen to be three-sided, according to the usual Polypodiaceous type, a character which it shares with *Platycerium*. The annulus of the mature sporangium consists of about thirteen indurated and eight non-indurated cells, including the stomium (Text-fig. 7, *a*, *b*, *c*). It is not quite definitely interrupted at the stalk, for, as clearly shown in Text-fig. 7, *c*, which is drawn from a longitudinal section, the lowest cells on either side are actually in contact. The stomial cells are slightly thickened and rounded, so as to locate the rupture, and three thin-walled cells adjoin it on either side. These characters are what are commonly regarded as 'Polypodioid'. The sporangia correspond more closely to those of *Platycerium*[3] than of *Dipteris* or *Cheiropleuria*, for in the latter the stalk is four-rowed and the annulus is oblique and continuous, though not fully indurated.[4] But in *Platycerium* the stalk is three-

[1] l. c., p. 513, Figs. 13, 14.
[2] Flora, 1915, p. 237, &c., Figs. 26–32.
[3] Studies, V, Text-fig. 18.
[4] Studies, V, Text-fig. 17.

rowed, and the state of the annulus is essentially the same, though the number of the indurated cells is larger.

In her memoir on the Acrosticheae, Frau Eva Schumann has constructed a conspectus of the genus *Leptochilus*, using among other characters those of the spore.[1] The spores of *L. tricuspis* (and *L. varians*) are bilateral, with smooth walls and no epispore. In these characters they differ from those of other pinnate species of *Leptochilus*. But if comparison in this feature be made with the Ferns to which a relation has been suggested on other grounds, a correspondence is found. The naked bilateral spores of *L. tricuspis* are matched by those of *Platycerium*, of *Neocheiropteris*, and of *Dipteris*. These are significant facts, which suggest that *L. tricuspis* does not find its natural place in the genus *Leptochilus*. But before its actual probable position can be assigned, it will be necessary to examine certain other, presumably allied Ferns.

Neocheiropteris.

The Fern named *Neocheiropteris palmatopedata* (Bak.), Christ, was found by Abbé Delavay, in 1883, near Tapintze. It was again collected by Henry in Yunnan, in 1898, and later by other collectors, but always in sparing numbers. It was originally named *Polypodium palmatopedatum* by Baker.[2] It was described briefly by Christ,[3] and subsequently in more detail and with drawings [4] under the name *Cheiropteris*, which was subsequently changed to *Neocheiropteris*, for reasons of nomenclature. It was found to have a hypogean rhizome, bearing ovate scales, ciliate reticulate, and acuminate: a pedatifid leaf, with middle lobe erect, and two lateral lobes, patent and pedate, the venation of the pinnae reticulate. The Fern is homophyllous. Its large naked sori were described as being on the enlarged end of a vein; the sporangia as having short stalks with vertical annulus, incomplete, of about seventeen cells: the spores bilateral, pellucid, and yellow. Christ drew particular attention to the elongation of the sori parallel to the costa, so as almost to form an elongated sorus following the foliar axes. He suggested a place for the Fern between *Dipteris Horsfieldi* and *Hemionitis elegans*.[5]

A single specimen collected by Henry in Yunnan is in the Glasgow University Herbarium (Plate I, Fig. 4). Upon this, together with the examination of a specimen in the Herbarium at Kew, the following statements are based. The rhizome is rather thick and fleshy. The scales which cover it are peltate, and they are borne on emergences, so that they project slightly

[1] Flora, 1915, p. 250.

[2] Kew Bull., 1898, 232.

[3] Bull. Herb. Boiss., 1898, p. 876.

[4] Bull. Herb. Boiss., 1899, pp. 21–22, Pl. I; see also Diels, Engler und Prantl, i, 4, p. 188, where the figure is reproduced.

[5] A very good photograph on a reduced scale is published in Christ's Geographie der Farne, p. 197, Fig. 87.

from the surface. They consist for the most part of a single layer of cells, with dark-brown walls, except near the point of attachment, where the walls remain thin. Christ mentions their ciliate margin. This appearance is due to the thickening of the walls being continued to the septa dividing the marginal cells, but not to their outer walls (Text-fig. 4, *b*). These remaining very thin and becoming concave, the thicker septa appear under a low power as ciliar projections from the margin. The scales correspond nearly in structure to those of *Leptochilus tricuspis*, but the marginal glands were not found on the old scales available, though occasional appearances could readily be interpreted as collapsed glands. In *Leptochilus* they were not constantly present.

I have already described the vascular arrangements in the stock of *Neocheiropteris*.[1] It corresponds in all essentials to that of *L. tricuspis*: moreover the same gritty nests of sclerotic tissue are present, especially in the cortex. The stele is highly perforated in both cases, and opens on the obliquely upper side to receive the highly divided leaf-trace. The mature petiole of *Neocheiropteris* shows in transverse section a horseshoe of about six strands, of which the two adaxial are the largest. In fact, the plan of vascular construction of the two Ferns is practically identical.

The lamina of *Neocheiropteris* helps to an understanding of that of *L. tricuspis*. Christ compared it with *Dipteris*; but the branching which it shows is more nearly of the type of *Matonia pectinata*. At the end of the long and slender petiole the lamina widens out broadly, with pedate lobation right and left (Plate I, Fig. 4). The midribs of the successive lobes are related as a katadromic helicoid system. It may be interpreted as a sympodial development of dichotomy, that shank of each dichotomy which is not then branched again being directed to the anadromic side: while the katadromic shank undergoes further branching. The whole lamina may be represented as composed of the two branches resulting from an initial dichotomy, as in *Dipteris conjugata*, where the two are very exactly equal, though the further branching in that Fern shows anadromic helicoid development. But in *Neocheiropteris* the two shanks of the first dichotomy do not develop equally. In Plate I, Fig. 4, the two are laid out slightly apart, each having five lobes; and the sinus between them is very slightly deeper than any of the others. The sinus to the left of it is almost as deep, and the result of this is that the first lobe of the left-hand shank takes an almost exactly median position. It is also slightly the longest, and so appears as a distal and median lobe, while it is really the equivalent by branching of the left-hand lobe of the right-hand shank. This is precisely the condition already recognized for *Matonia pectinata*, and the remaining lobes of *Neocheiropteris* follow according to the same plan.

The leaf of *Leptochilus tricuspis* is open to the same interpretation,

[1] Ann. of Bot., vol. xxvii, 1913, p. 473, Text-fig. B.

supposing that the later helicoid branchings are omitted. The three-lobed condition seen in Plate I, Fig. 3 of the base of a fertile leaf would result from a first dichotomy, of which the left-hand shank becomes the left-hand lobe. The right-hand shank branching again would give the right-hand lobe and the apparently central or terminal lobe. Here the latter is more distinctively marked from the other two than it is in *Neocheiropteris* or *Matonia*. But this may be held as an indication, or as a consequence of its further divergence from the phyletic source. The case is, in point of branching, parallel to that of the three-lobed juvenile leaves of *Marsilia*, noted by Goebel.[1]

As bearing on this interpretation of the three-lobed leaf of *Leptochilus tricuspis*, a very interesting example was pointed out to me by Dr. R. C. Davie, of which a photograph supplied by the kindness of the Director of the Royal Botanic Garden in Edinburgh is shown in Plate I, Fig. 2. Here a fourth lobe is present on the left-hand side, and a very rudimentary fifth lobe on the right. This leaf is thus two steps nearer the condition seen in *Neocheiropteris* or *Matonia* than the normal for the species. Such cases show that the dichotomous branching of the lamina, even where there is helicoid development, may grade into the pinnatifid, or pinnate with a definitely terminal lobe. It will be seen below that similar examples are not uncommon in this affinity of Ferns with superficial non-indusiate sori.

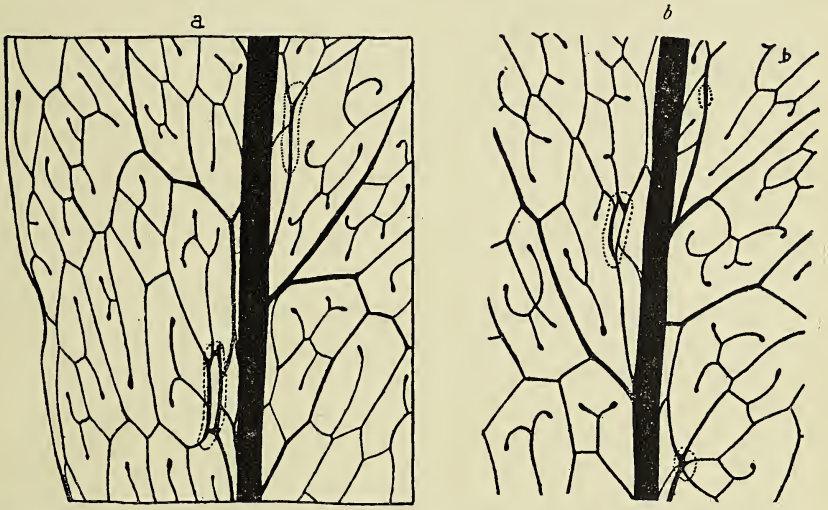
Christ's description of the sori of *Neocheiropteris* has already been cited. These are of very unequal size, being sometimes relatively small and circular, sometimes half an inch long or more, and the largest are usually near the base. They are disposed in two rows, one on either side of the midrib of the lobe. But where the lobe merges in the helicoid curve at the base, the sori are only continued on the acroscopic side (Plate I, Fig. 5). The relations of the sori are closest near to the base of the lamina, where, being of elongated form, they constitute in some specimens an almost continuous soral tract. With a limited supply of dried material it was impossible to make a detailed examination; but the relation of the sori to the venation has been observed. Christ states that the sorus is seated on the enlarged end of a vein. Sori of various sizes have been examined, and this has never been found to be the case. They are always seated on a continuous vein, or on a plexus of veins; and there seems to be no definite relation of each sorus to the venation. For instance, in the two large sori shown in Text-fig. 8, *a*, the upper one is seated on a single large vein, with branches; the lower is inserted on a large vascular loop, from which numerous strands radiate. Similarly in Text-fig. 8, *b*, the largest is again seated above a vascular loop: the smaller sori may be on a plexus of radiating veins, or upon a single vein, which then continues its course. This indefiniteness of vascular supply is exceptional among Ferns. It probably points to a

[1] See Trans. Roy. Soc. Edin., vol. li, pt. 3, No. 21, 1916, 'On leaf-architecture', p. 675.

spread of the sorus over an enlarged area. Perhaps it may be matched among some of the other related ' Polypodioid ' types.

The sporangia are very numerous. The sori observed were quite mature, but nevertheless there are indications that it was of the mixed type. The sporangia have long stalks, not short as stated by Christ ; they are about twice or thrice the length of the sporangial head, and they are composed of three rows of cells. The head itself is flattened, with an interrupted vertical annulus, having from 14 to 17 indurated cells. The spores are bilateral, without perispore, and appear to be 48 to 64 in each sporangium.

These facts relating to the sorus do not supply any points of exact importance for comparison. But they suggest that *Neocheiropteris* is definitely



TEXT-FIG. 8. *a*, *b*. Portions of fertile pinna of *Neocheiropteris*, showing the venation and the outlines of the large sori. × 4.

advanced from a Matonioid-Dipterid state to that which is characteristic of certain sections of *Polypodium* ; while they distinctly favour comparison with *Leptochilus tricuspis*. Both of these Ferns show an interrupted annulus and a three-rowed stalk, as against the oblique annulus and four-rowed stalk of *Dipteris* and *Cheiropleuria*. Incidentally they correspond in these features with *Platycerium*, though they differ in the mixed character of their sori.

Comparison has been made by Hooker and others between *Leptochilus tricuspis* and *Polypodium*, § *Phymatodes*. A similar comparison appears probable for *Neocheiropteris*, which was in fact first named *Polypodium palmatopedatum* by Baker. The resemblance here is perhaps as much to § *Phlebodium*, while, again, comparisons may extend also to various Acrostichoid Ferns. Such comparisons may be followed out with prospect

of success on the basis of habit of vascular anatomy, of dermal appendages, and of sori and sporangia. Unfortunately it cannot be extended to the gametophyte, since this is not yet known either in *L. tricuspis* or in *Neocheiropteris*. As regards habit, the sections *Phlebodium* and *Phymatodes* are, like *Neocheiropteris* and *L. tricuspis*, rhizomatous, with usually a fleshy texture of the stock and broad dermal scales. The leaves are arranged in alternate sequence and long-stalked. The lamina is not finely divided, but shows few lobes or graduates by omission of the lobing to the entire condition, which is the case for many of the species of *Phymatodes*. These steps, seen either on comparison of individuals or species, present interesting features. A good case is seen in *Polypodium* (*Phymatodes*) *decumanum*. Plate I, Fig. 6 shows a three-lobed leaf, readily referable to the same analysis of branching as that given above for *L. tricuspis*, but here the interpretation is more obvious, since the forking to form the left-hand pinna is distinctly below the second forking which gives the median and right-hand lobes. A simpler state in the same species is seen in Plate I, Fig. 7, where only two lobes are developed, the third being rudimentary, but still recognizable at the base of the right-hand lobe. Such examples support the interpretation of the ternate leaf of *L. tricuspis* as derived by two successive dichotomies. They may readily be multiplied by examination of herbarium series of the species of *Phymatodes*.

On the other hand, the number of the lobes is often greater than three, as in *P. aureum* and in many species of *Leptochilus*. But these more complex leaves are merely the result of repetition of that same branching which gives the ternate leaf, so as to constitute a scorpioid sympodium. The result may be the pinnatifid or pinnate leaf typical of so many species of *Phlebodium*, *Phymatodes*, or *Leptochilus*. An interesting feature which they show not unfrequently is a helicoid branching in the basal pinnae. This is illustrated in Plate II, Fig. 8, for *Leptochilus latifolius* (Meyen), C. Chr. It is shown even more prominently in pinnae of the fertile leaf, where a third helicoid branching has been observed. Such branchings, when compared with those of *Neocheiropteris* or *L. tricuspis*, may be held as reminiscent of the helicoid sympodial branching, as seen in the leaf of *Matonia*. Thus it is possible to refer the construction of the leaves of *Phymatodes*, *Phlebodium*, and *Leptochilus* to the modification of a type of construction such as is seen in the *Matonia-Dipteris* series, the modification being in the direction of simplification. *Neocheiropteris* provides an important intermediate type. The chief modifying factors appear to have been reduction in the number of forkings of the adult leaf, and the substitution of a scorpioid for a helicoid development of them.

Comparison on the basis of vascular anatomy presents certain difficulties which had already become apparent in the case of *Cheiropleuria*.[1] This

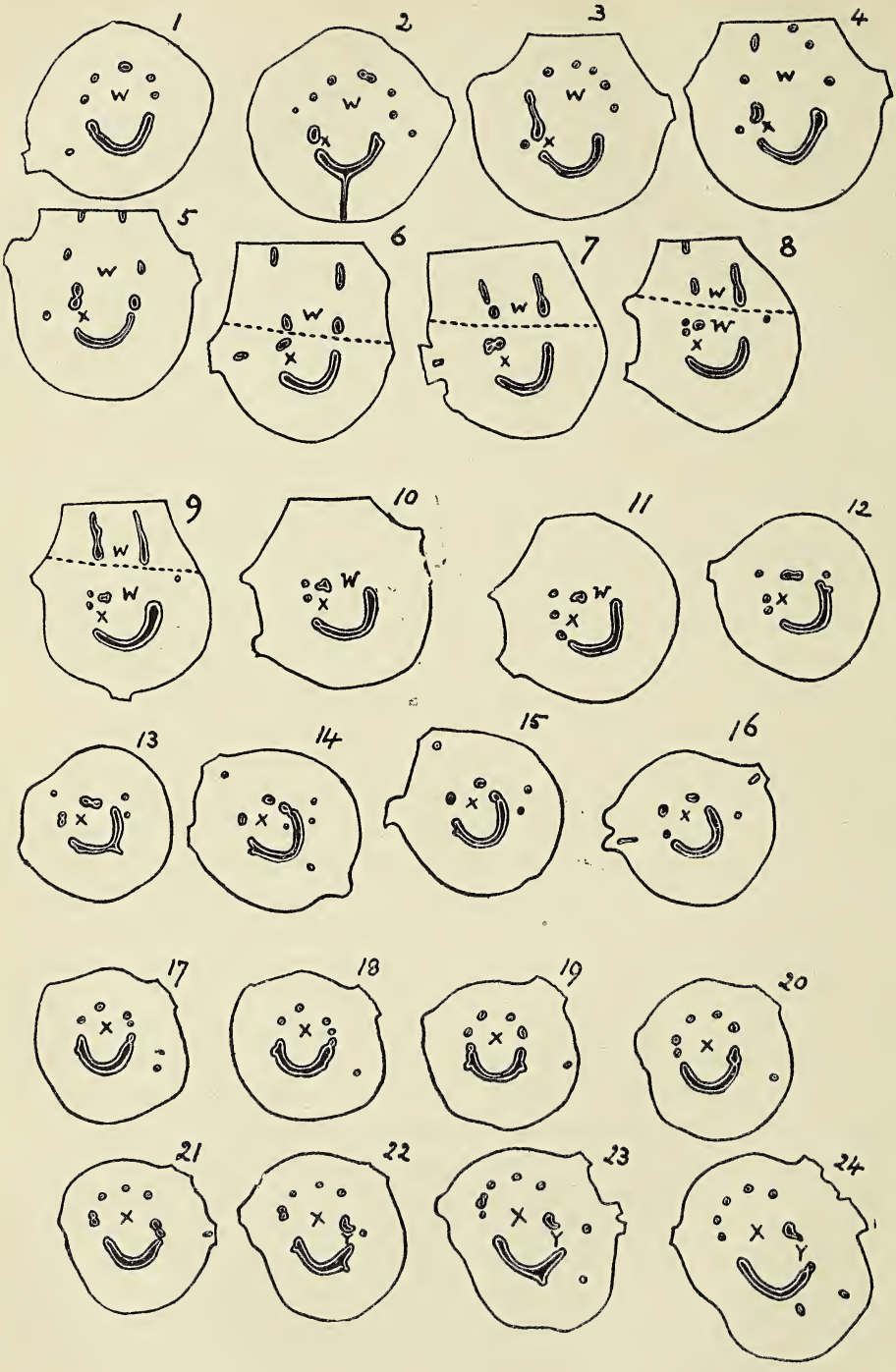
[1] See Ann. of Bot., vol. xxix, p. 497, &c.

Fern, though it has an advanced sorus of 'mixed' Acrostichoid type, is protostelic, like *Gleichenia*, but with a leaf-trace which forks at once after departure. The Matonioid-Dipterid alliance is solenostelic with undivided leaf-trace. But we find *Neocheiropteris* to resemble *Leptochilus tricuspis* in having its vascular system much broken up by 'perforations', both in leaf-stalk and in axis; and this advanced vascular state accords with their advanced type of sorus. But the facts for *Cheiropleuria* show that such a parallel march of characters is not obligatory. With these facts before us, and with already some doubt whether *L. tricuspis* fits naturally into this genus to which it is usually referred, it becomes necessary to know the anatomical details of other species of the genus *Leptochilus*. Mettenius has already examined the vascular system of *L. axillare*, a widely scandent species with entire leaves.[1] He found the leaf-trace to consist of two strands, and the stele of the axis broken up into isolated strands connected in elongated meshes ('perforations'). This is the only statement I have found relating to the stelar anatomy of the genus, though he describes the structure for four species now ranked under *Elaphoglossum*.[2]

Among material of several species of *Leptochilus* which was available for investigation that of *L. nicotianaefolius*, collected in Jamaica, was selected as having a robust rhizome, with leaves alternating in two rows, separated by relatively long internodes, and inserted obliquely on its upper surface. Irregularly disposed on its flanks are slightly projecting pneumatophores with spongy tissue, which lie along the margins of the sections. The base of the petiole is traversed by about eight vascular strands, arranged in a horse-shoe, with the gap facing acroscopically, and on each side of it a strand of larger size. In this it corresponds to *L. tricuspis* and to *Neocheiropteris*, allowance being made for variation in number of the strands. But transverse sections of the stock show constantly present a broad and continuous vascular strap on the lower side of the ring, which varies slightly in its position and in its relations to the other strands. Less distinctly a narrower strap is seen on the upper side. To ascertain the relations of these the vascular system has been followed through two successive leaf-insertions, and the internode between them: and its successive phases are represented in Text-fig. 9, 1–32, which show the relations of the leaf-gaps, and of the strands of the leaf-traces to these two larger vascular tracts.

It will be seen on reading the series in succession that three leaf-gaps are involved. These have been identified by the letters W, X, Y, in the successive sections, and where the completed leaf-trace is fully separated from the stelar tracts, a dotted line of demarcation has been drawn, as in Nos. 6–9 and 26–29. These marks help the identification of the vascular tracts. It will be seen that the details are not uniform, a fact that makes some

[1] Abhandl. K. Sächs. Ges. d. Wiss., 1863, p. 554, Taf. IX, Fig. III.
[2] l. c., pp. 546–7.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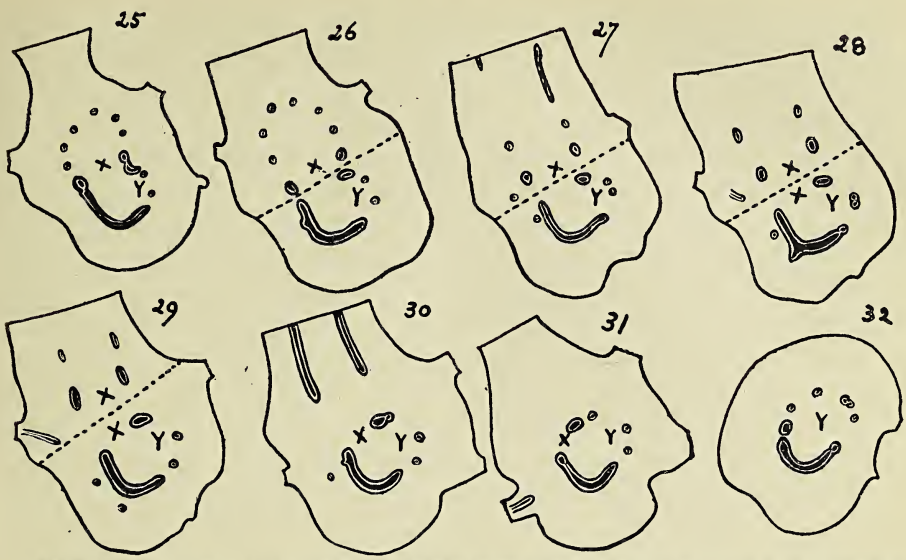


TEXT-FIG. 9. Nos. 1 to 32. Series of transverse sections through the rhizome of *Leptochilus nicotianaefolius*, in order from the base upwards, showing the vascular system, but omitting the sclerenchyma; W, X, Y represent successive leaf-gaps. For details see text. × 4.

difficulty in identification. This is minimized by these conventional signs. The series reads from below upwards.

Starting from Text-fig. 9, 1, which comes from an internode just below a leaf-insertion, there is an obvious leaf-gap (W) with five strands of the leaf-trace already detached. Below is a continuous semicircular vascular tract. In No. 2 the left-hand end of this has separated as a relatively large strand, and the space between is the lowermost limit of the next higher leaf-gap (X). From this it follows that the leaf-gaps overlap, and technically the structure is dictyostelic. Also it follows that at certain points the upper and lower vascular tracts may be fused. The series 1 to 9 show the gradual separation of the leaf-trace (W), the last step being the giving off of the two large marginal strands (4–6). Meanwhile the leaf-gap X widens, and at section 6, where the leaf-trace W has passed off, the stele consists of two meristeles, the larger being the lower, the smaller the upper. The latter soon divides (7–9), to form small leaf-trace strands, and does not appear to maintain its identity throughout. Additional strands may be given off from the lower meristele (10–11 and 14–16), while the foliar strands undergo irregular branchings and fusions among themselves (20–23), forming a semicircle of 5 or 6, to which finally the marginal strands are added (25–27). This completes the leaf-trace of leaf-gap X. But again the leaf-gap Y is already formed in section 22, and widens in successive sections with formation first of the median strands of the trace, with irregular fusions of them as before, but not corresponding exactly in number or position (26–32).

If the vascular system thus observed in *Leptochilus nicotianaefolius* be described in the usual terms, it would be a dictyostele, with leaf-gaps greatly elongated downwards, so as to overlap. The leaf-trace is much divided ('perforated'), and the separate strands are inserted on the margin of the gap. The median strands spring somewhat irregularly from the very narrow lower end of the leaf-gap, while the larger marginal strands are given off near to the upper and wider end of it. The leaf-gaps alternate right and left. Thus the vascular skeleton resembles the type shown in any solenostelic Fern with overlapping leaf-gaps; for instance, as shown in *Pellaea rotundifolia* by Gwynne-Vaughan,[1] but with this difference: that whereas in such a case the leaf-trace is an undivided horseshoe, here it is broken up into a number of strands. Clearly it is a type not far removed from solenostely, as shown by the gutter-shaped lower vascular tract. All that differentiates it from a type like *Metaxya* or *Dipteris* is the slight overlapping of the elongated leaf-gaps, and the subdivision of the leaf-trace by numerous perforations. This subdivision resembles that in *Dicksonia* (*Cibotium*) *Barometz*, as shown by Gwynne-Vaughan;[2] but here it is continued down to the very base of the leaf.

Other species of *Leptochilus* show a similar structure, though varying in the proportion and number of the strands and the overlapping of the leaf-gaps.[3] But the existence of the smaller (upper) and larger (lower) meristeles in the axis is the same. This has been seen in such smaller species as *L. zeylanicus* (Houtt), C. Chr., *L. alienus* (Sw.), C. Chr., and *L. heteroclitus* (Pr.), C. Chr., and it may be held as probably characteristic for the genus. In general their vascular condition differs from that seen in *L. tricuspis* in showing a less advanced state. But this may find its natural explanation in their smaller size. It will not be necessary to enter into their details.

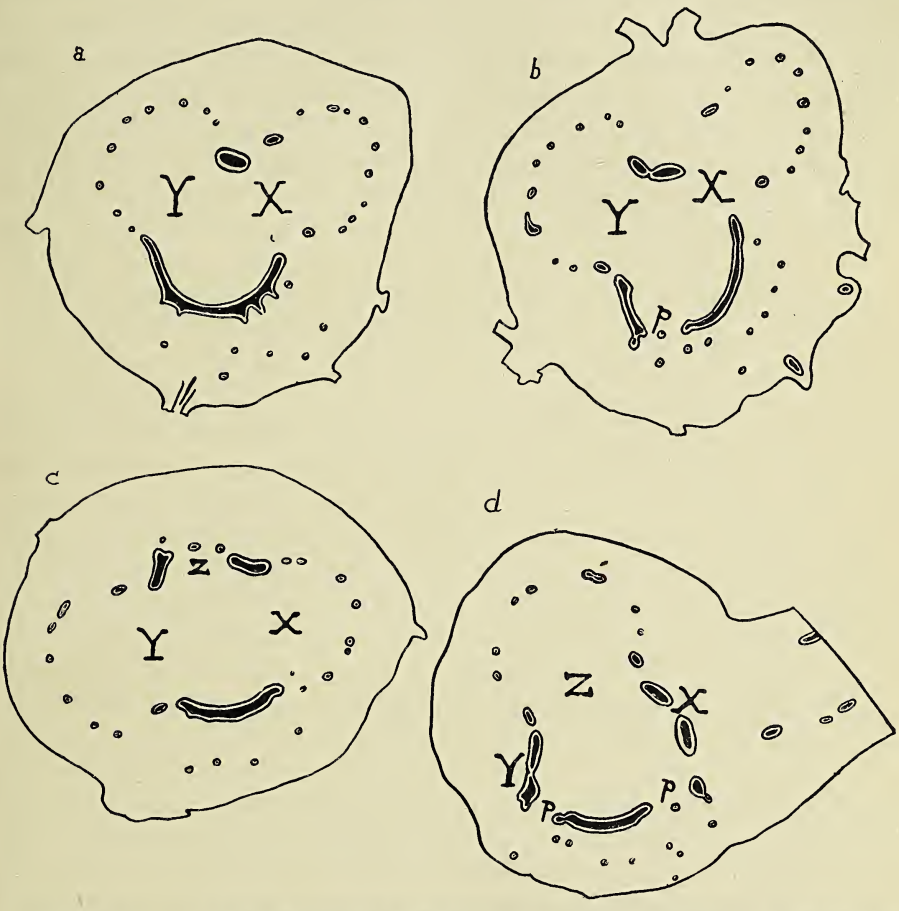
On the other hand, *L. cuspidatus* (Pr.), C. Chr., which has a much more massive rhizome, shows the lower strap large and continuous, and it is thus reminiscent of the primitive solenostele. Its leaves are borne on the massive creeping rhizome, not in two simple lateral rows but upon a more complex and seemingly less regular plan, so that some leaves are seated obliquely on the upper surface. This makes the vascular arrangements more complex than in some of the smaller species. In the case where two lateral leaf-bases are traversed—which is the more frequent—the structure is as in Text-fig. 10, *a*, where there is substantial agreement with what has been seen in *L. nicotianaefolius*: there is a small upper strap, with a much larger lower strap, forming almost half of a solenostele, from which root-strands arise. Right and left are the leaf-

[1] Solenostelic Ferns, II. Ann. of Bot., vol. xvii, Pl. XXIII, Fig. 8.

[2] See Land Flora, Fig. 331.

[3] *Leptochilus axillaris* (Cav.), Klf., has been examined by Mettenius (Ueber *Angiopteris*, p. 554): he found only two strands of the leaf-trace, while the stele was found to be much perforated, with elongated meshes, but a continuous upper strap clearly marked (Pl. IX, Fig. 3).

gaps, with the much-divided leaf-traces passing obliquely outwards. The right-hand trace is complete ; the left-hand has not yet formed its larger adaxial strands. In Text-fig. 10, *b*, these are being separated off ; but besides, there is a gap in the lower strap, with several root-strands close by. This appears not to be a leaf-gap, but of the nature of a perforation.

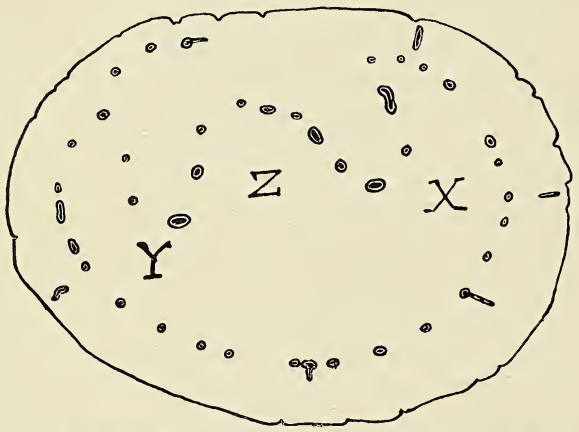


TEXT-FIG. 10. *a–d*. Sections through the rhizome of *Leptochilus cuspidatus*. *a*, showing two leaf-gaps X, Y, without any perforation ; *b*, showing the two leaf-gaps, with a perforation (*p*) ; *c* shows three leaf-gaps, X, Y, Z, of which X, Y are lateral and Z upon the upper surface; *d* shows the leaf-gap Z fully developed, X and Y are almost closed, *p*, *p* are perforations of the lower meristele. × 4.

On the other hand, (*c*) shows an arrangement similar to (*a*), but with the addition of a gap opening on the upper side, and with already two separate strands within it. This is a leaf-gap (Z), as a section higher up shows (*d*) ; for there it has opened out, and from its margins the complete series of leaf-trace strands have been given off. Meanwhile the lateral leaf-gaps of the lower leaves (X, Y) have almost closed. But there are two other

gaps in the lower strap (p, p) which are not related to leaf-traces, and appear to be of the nature of perforations.

The additional complications thus seen in *L. cuspidatus* bear a comparative interest. The presence of occasional perforations, not constant in position or in number, gives an intermediate step between the more primitive non-perforated state, as seen in *Metaxya* or *Dipteris*, and the more regularly perforated, as in *Neocheiropteris* or *L. tricuspis*. The latter is the general condition in the advanced Polypodioid Ferns. It is interesting in this connexion to recall the occasional presence of a leaf-gap in *Metaxya*, as shown in Ann. of Bot., vol. xxvii, Pl. XXXII, Fig. 3, v. These differences are then of degree, not of kind. The Ferns above named, whether or not they be really related, thus provide one of the best examples of transition from the non-perforated to the perforated condition, both in leaf-trace and in the axial stele.



TEXT-FIG. 11. Transverse section of rhizome of *Dryostachyum* (*Photinopteris*) *drynarioides*. X, Y, Z represent leaf-gaps. × 4.

The anatomical details of *L. cuspidatus* help to explain the otherwise difficult case of another Acrostichoid Fern, *Dryostachyum* (*Photinopteris*) *drynarioides* (Hk.), Kühn. It is a Fern of peculiar habit of its leaves, but with Drynarioid venation, and probably closely related to *Drynaria* itself. In transverse section the stock shows very numerous meristeles, arranged in a manner which may be explained by comparison with *L. cuspidatus*. It is shown in Text-fig. 11, in which the letters X, Y, Z indicate leaf-gaps, each with an arch of highly subdivided meristeles. These correspond in position and nature to the similarly marked gaps in Text-fig. 10, *c*, of *L. cuspidatus*. In fact, all that is required for essential similarity of the structure of the stock is the higher subdivision of the solenostele itself by more numerous perforations than in *L. cuspidatus*. These are present in *D. drynarioides*, which is in this respect a more advanced type.

With the exception of *Metaxya*, the Ferns which enter into the above comparisons, though they may vary in leaf-outline, correspond in their venation. They are reticulate, and fall under either *Venatio Anaxeti* or *Drynariae*, two schemes which are very closely related. *They share the character of having small, rather irregular, areolae, with one or more*

vascular twigs ending blindly within them. They are all rhizomatous, creeping or climbing forms, often with a thick fleshy axis. Their mature leaf architecture shows more or less clear reference to a dichotomous origin, with sympodial development, often with a helicoid element in it. It is seen that anatomically they all may be derived from the solenostelic type, by varying degrees of breaking up of the leaf-trace, and of the axial stele by perforations as distinct from the leaf-gaps. Their dermal appendages are often scales, but these are referable in origin to simple hairs, such as are actually seen in *Dipteris* and *Cheiropleuria*. They all have superficial sori, but according to the extent of their spread from the definite vascular receptacle over the leaf-surface, these Ferns have been segregated as Polypodioid or Acrostichoid. The view here advanced is that they constitute a natural phyletic group of Dipterid derivatives which might be styled the Dipteroideae.

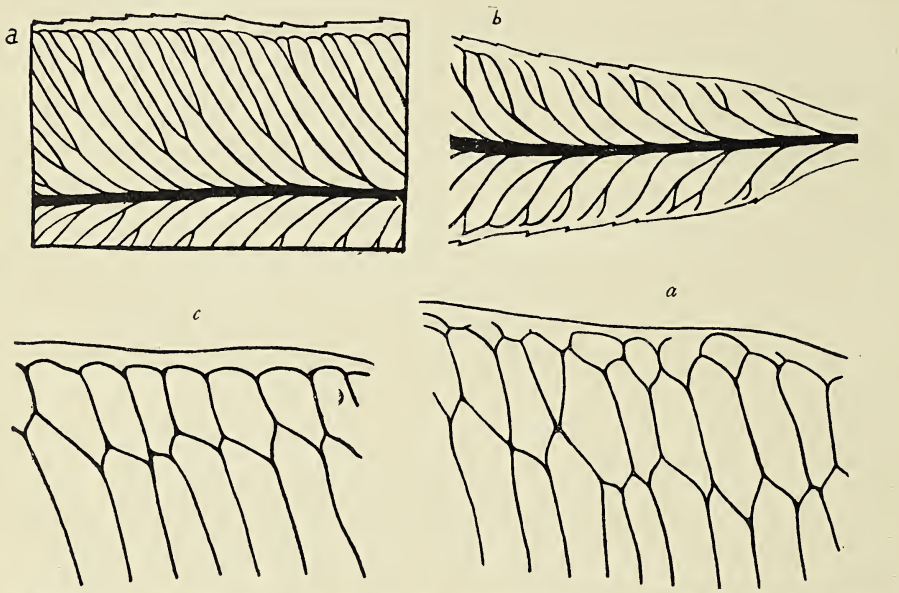
A second series, which probably took a more or less parallel, but phyletically distinct line of progression, is found to be related to *Metaxya* as its approximate source. It includes *Syngramme* and *Elaphoglossum*, and it also involves a progression from a circumscribed sorus to an Acrostichoid state. An obvious character marking off these Ferns from the Dipteroideae is their venation, which is simply pinnate, either without fusions (*Metaxya*), or with few or many of them, but *always without any free-ending twigs within the areolae thus formed. Metaxya* has already been described at length.[1] Its relatively primitive characters are the unbranched hairs, solenostelic axis, undivided leaf-trace, flat receptacle, and simultaneous origin of its sporangia, which have an almost vertical annulus, interrupted at the insertion of the stalk. These characters keep it apart from *Alsophila*, with which it has commonly been classed. Presl established it as a distinct genus. He also noted [2] that not uncommonly more than one sorus may be borne upon each vein, which is exceptional among Ferns. This state is exemplified by Plate II, Figs. 9, 10, which show also the free Pecopterid venation, and the way in which the sori may be extended along the vein into oblong form. From this condition it is but a slight step to that seen in *Syngramme*, which was included as a sub-genus in *Gymnogramme*, in the 'Synopsis Filicum' (p. 386), but is retained as a substantive genus by Diels, and ranked in his Pterideae-Gymnogramminae.[3] Its position as a substantive genus is now generally accepted.

Syngramme, J. Sm.

The genus includes about twelve species of rare and local Ferns from the Malayan region. Hooker remarks that they have the habit and mode of growth of *Eu-polypodium*. They have mostly a short creeping rhizome

[1] Studies, III. Ann. of Bot., vol. xxvii, 1913, p. 443.
[2] Tentamen, p. 59.
[3] Engler u. Prantl, i, 4, p. 257.

bearing stalked, often coriaceous leaves. These were undivided in most species, but *S. quinata* differs from the rest in having five pinnae, one being large and terminal. The appearance is like the leaf of a Horse-chestnut.[1] This species supplies an obvious link in leaf-habit between the simply pinnate *Metaxya* and the entire-leaved species of *Syngramme*. The venation in *S. borneensis*, Hk., is identical with that of *Metaxya*, except for the fact that the veins are united at their margins (Text-fig. 12, *a*). But this is not constant at the base of the lamina (Text-fig. 12, *b*), where numerous free veins occur. In other species the fusions may be more numerous, so as to constitute a marginal reticulum, as in *S. Wallichii*, Hk. (Text-fig. 12, *c*), but



TEXT-FIG. 12. *a*, venation of part of leaf of *Syngramme borneensis*, showing marginal fusions; *b*, basal region of a similar leaf showing fusions often omitted; *c*, venation of leaf of *Syngramme* (*Gymnogramme*) *Wallichii*, Hk., herb. spec. from Singapore; *d*, ditto, but showing more complex reticulation. × 4.

it is irregular, and may show free marginal twigs, or closed loops (Text-fig. 12, *d*). All these appear as natural and slight steps of progression from an open Pecopterid venation, and it is noteworthy that *in none of them are there intra-areolar free-ending vascular twigs, as in the Dipteroideae.*

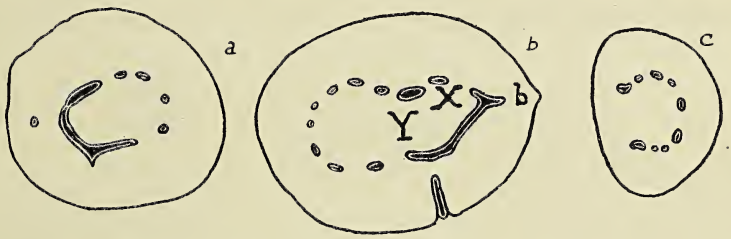
The sori follow the veins, but they stop short of the margin.[2] If the plural sori on the veins of *Metaxya* were linked together into a continuous sorus—and this is even suggested by the elongated form which they often show in *Metaxya*—the result would be what is seen in *Syngramme*. In both the sporangia are accompanied by paraphyses: the sporangia have the induration of the annulus interrupted at the insertion of the stalk, and

[1] See Hooker, Sp. Fil., vol. v, p. 152, Tab. CCXCVII. [2] Engler und Prantl, i, 4, Fig. 135, p. 256.

but little removed from the oblique, and the spores of both are tetrahedral. But the sporangium of *Syngramme* has a three-rowed stalk, as against the four-rowed stalk of *Metaxya*. Both genera have hairs, and not scales, as dermal appendages. Unfortunately the comparison could not be tested by vascular anatomy, but from herbarium material it appears that *Syngramme* has three separate strands in the base of the petiole, which is a structural advance on what is seen in *Metaxya*. Thus the facts appear to support the suggested affinity, the points of difference being those characteristic of a phyletic progression parallel to what has been repeatedly seen in other phyla. In subdivision of the leaf-trace, the anastomoses of venation, extension of the sorus, and in the three-rowed stalk of the sporangium *Syngramme* shows characters of advance on *Metaxya*.

Elaphoglossum.

In his monograph of the genus *Elaphoglossum*[1] Christ plainly states his opinion that this genus is far removed from other Acrosticheae. The only Ferns which he recognizes as resembling them are those of the genus



TEXT-FIG. 13. *Elaphoglossum latifolium*. *a*, Rhizome in transverse section, showing only one leaf-gap; *b*, ditto, showing two leaf-gaps X, Y, while from the largest meristele the vascular supply is passing off to a bud (*b*); *c*, transverse section of base of petiole. × 4.

Syngramme. Their habit is the same, as also their venation. The chief difference consists in the sporangia of *Syngramme* forming elongated sori following the veins. If in Ferns of the type of *Syngramme* the sporangia were spread over the whole surface, then such types would rank as *Elaphoglossum*. The comparison of these genera may be extended to other characters besides those named. Transverse sections of the rhizome of *E. latifolium* (Sw.), J. Sm., collected in Jamaica, are represented in Text-fig. 13. It shows vascular structure nearly approaching a solenostele (*a*), but with leaf-gaps sometimes overlapping (*b*). The leaf-trace (*c*) is much subdivided, and it is so from the first, being given off as distinct strands (*a*, *b*), of which the median separate first. Their arrangement suggests comparison with a fully solenostelic type, such as *Metaxya*, from which the structure could readily be derived by perforation of the leaf-trace and continuation of the perforations downwards to the rhizome. Like *Metaxya*, *Lophosoria*, and

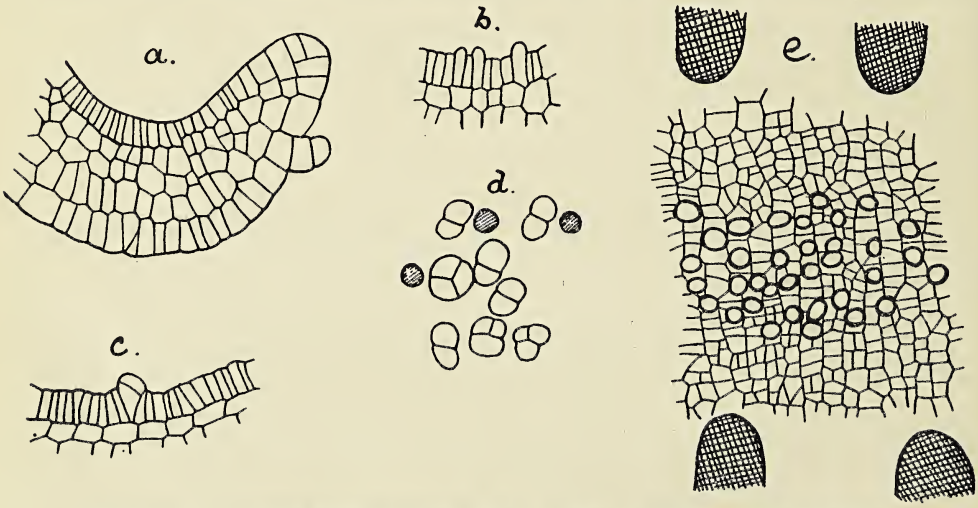
[1] Denkschr. d. Schweiz. Naturf. Ges., Basel, 1899, pp. 14-18.

other Ferns of Cyatheoid affinity, a bud may be formed in *E. latifolium* at the leaf-base on the abaxial side. Its vascular supply comes off from the stele below the leaf-trace itself. Its position is indicated in (*b*), in which the gap of the next higher leaf (x) has just been formed.

The venation of certain species of *Elaphoglossum* is without any anastomoses.[1] In most species the veins fork irregularly near to the midrib, and towards the margin they show anastomoses (Text-fig. 14). In some species there may be two or more rows of meshes of great irregularity.[2] Diels[3] has tabulated the genus *Syngramme* on the basis of venation. *Elaphoglossum* shows similar variations in this respect, its simplest types corresponding to the state of *Metaxya*.

TEXT-FIG. 14. Venation of margin of leaf of *Elaphoglossum latifolium*. × 4.

While *Metaxya* and *Syngramme* have hairs only, *Elaphoglossum* bears dermal scales, often of very elaborate structure, which are used in specific



TEXT-FIG. 15. *Elaphoglossum latifolium* (Sw.), J. Sm. *a*, vertical section of young lamina; *b*, *c*, origin of sporangia; *d*, transverse sections of sporangial stalks and hairs; *e*, tangential section of young soral area, showing the sporangia originating between the veins. × 75.

diagnosis. Hairs are said to be absent: but they exist in the sori. In this respect it is in advance of the other genera.

The sorus in *E. latifolium* covers the whole lower surface of the lamina with a uniform coating of sporangia, with a few hairs. In the young leaf the soral surface bears a deep epithelium (Text-fig. 15, *a*), from which

[1] *E. heliconiaefolium*, Christ, l. c., p. 133. [2] Christ, l. c., p. 50. [3] l. c., p. 257.

single cells grow out (*b*), enlarge (*c*), and undergo the usual segmentation. Transverse sections through the young sorus show that the stalk is composed of three rows of cells; but as one of these does not always extend to the base of the stalk, there often appear to be only two (*d*). As regards the distribution of the sporangia relatively to the veins, it is shown in tangential sections of the young curved leaf (Text-fig. 15, *e*) that their distribution is very uniform and bears no relation to the veins. By later addition of younger sporangia to those first formed, the sorus becomes a 'mixed' one, the later sporangia arising commonly between the veins. The spores have one longitudinal angle and two flattened sides, with prickly epispore, as against the tetrahedral smooth spores of *Syngramme* and *Metaxya*.

The sum of these characters is consistent with the suggested relation of the genera named. The conclusion which may be drawn is that they form a phyletic series, of which *Metaxya* holds the most primitive position, *Syngramme* an intermediate place, and *Elaphoglossum* is the most advanced. They illustrate progress in the substitution of scales for hairs; in the subdivision of the leaf-trace; in the increasing fusion of the veins of the lamina, while the blade itself is simplified in outline; in the extension and fusion of the plural sori on the veins in *Metaxya* into the elongated sori of *Syngramme*, and the spread of the sori over the region between the veins, as seen in *Elaphoglossum*; and finally in the introduction of the mixed sorus in the last-named genus. All of these progressions are such as are seen in other distinct phyla. They are shown in the Dipteroideae. *But as they occur here in genera in which there is a consistent absence of vascular twigs ending freely in the areolae* (a character which is constant for the Dipteroideae), *these genera may rightly be held as constituting a phyletic progression of* Metaxya, i. e. *derivatives parallel with, but distinct from them, to be designated the Metaxyoidae.*

The Systematic Treatment of Acrostichoid Ferns.

The facts and the reasoning contained in the preceding pages have demonstrated with a reasonable probability two distinct progressions of Ferns, which have led from those with circumscribed sori to Ferns with the 'Acrostichoid' condition. It will be well next to consider more generally the various origins of that condition which we are now able to trace. As a consequence of the phyletic grouping of Ferns (based not on this one character of the sorus, but on the sum of many characters) there is reason to believe that they are numerous. Sir William Hooker, using the features of the sorus only, adopted an extreme position.[1] His genus *Acrostichum* included very diverse types. In explanation he remarks: 'Many have been the attempts to divide the species into a number of distinct genera, but, as will be seen by the synonyms I have quoted, not

[1] Species Filicum, vol. v, p. 194, 1864.

in a manner to give general satisfaction. Indeed the passages from one to another group or genus are too apparent to escape notice, and I have thought it better, with the single exception of *Platycerium*, to consider the groups of sectional than of generic value.' Nevertheless his subdivision of the genus into numerous sub-genera shows his clear realization of the heterogeneity of the Ferns included. Naturally his method was pre-evolutionary. His object in adopting an extended limit of the genus was perfectly logical according to the views of the time and the limited facts which he possessed. To him species were definite units. His object was to group these units according to characters readily grasped. In vol. i, p. 3 of the 'Species Filicum' he explains that the main object of the work is 'to assist the tyro in the verification of genera and species'. From this point of view the extension of the generic conception was justifiable. Nor ought we to denounce as unscientific a method so direct and consequent as this because the premise of immutability of species, generally held at the time, underlies it. None the less, the method and the conclusions fall away so soon as the premise itself breaks down. Our duty is then to substitute some phyletic grouping in accord with the current belief in mutability and progression. This may be done by comparison along lines of as many characters as possible. These will, however, be very largely those of the sporophyte. For not only are the gametophyte characters in many cases insufficiently known, but also such characters are apt to be less conclusive than those of the sporophyte.

Proceeding on such lines it has already been shown by various writers that Ferns of Acrostichoid character are referable to types with distinct sori. Indeed the exaggerated genus of Hooker has been so whittled away that in Christensen's Index only three names remain. All the rest are now referred to other genera. The attempt may now be made to summarize those references with a view to realizing how widespread has been the origin of the Acrostichoid character.

The sub-genera included under *Acrostichum* in the 'Synopsis Filicum' are as follows, while noted after each is its designation according to Christensen's Index:

Elaphoglossum—a substantive genus retaining its name.
Stenochloena—a substantive genus retaining its name.
Polybotrya—a substantive genus retaining its name.
Egenolfia—included in *Polybotrya*.
Rhipidopteris—included in *Elaphoglossum*.
Aconiopteris—included in *Elaphoglossum*.
Stenosemia—a substantive genus retaining its name.
Soromanes—referred to *Polybotrya*.
Gymnopteris—included in *Leptochilus*.
Chrysodium—the species all referred to other genera, and the genus is thus sunk. Part of it constitutes the surviving genus *Acrostichum*.

Hymenolepis—upheld under the same name.

Photinopteris—upheld under the same name.

Thus eight substantive genera now represent the old comprehensive genus. They are *Acrostichum*, *Elaphoglossum*, *Stenochloena*, *Polybotrya*, *Stenosemia*, *Hymenolepis*, *Photinopteris*, and *Leptochilus*. To these should be added *Platycerium*, which was placed in an independent position by Hooker: *Trismeria*, which though placed by Hooker in *Gymnogramme* is actually 'Acrostichoid': and *Cheiropleuria*.[1] Note should also be taken of the genera *Gymnogramme*, *Hemionitis*, and *Meniscium*, as showing a soral construction not far removed from the 'Acrostichoid', and liable to merge in it.

These genera have been variously distributed by recent writers, notably by Diels ('Engler und Prantl,' i, 4), Christ ('Farnkräuter'), and Frau Eva Schumann ('Flora,' 1915, p. 201). The opinions which they have expressed and the results arrived at by them will be here placed in relation with the conclusions derived from my own comparative studies. It will be best at first to account for the more outlying plants of Acrostichoid habit, referring them to their probable phyletic affinities.

Taking first what remains of the old genus *Acrostichum*, there are three species—*A. aureum*, L., *A. praestantissimum*, Bory, and *A. fasciculatum* (Fourn.), C. Chr. To the latter the note '(an *A. aureum*?)' is attached in Christensen's Index. These Ferns have been held to be of very doubtful position. Diels (l. c., p. 336) remarks that 'hardly anything can be made out as to the nearest affinity of this isolated type'. This admission is remarkable in view of Sir William Hooker's description of *A. praestantissimum*.[2] In the specific diagnosis he describes the sori as 'covering the whole back of the pinnae, except the costa, and at other times confined to a narrow line at the margin as in *Pteris*, and then closely covered with a narrow pteridioid involucre'. This comparison is amplified in the text, and illustrated by Figs. 3, 4 on Plate LVIII, which also shows the venation. This sixty years old comparison has lately been elaborated by Frau Schumann, with valuable added detail.[3] It may be accepted that a highly probable phyletic sequence led from *Pteris* (*Litobrochia*) *splendens*, Klf., to *A. praestantissimum*, Bory, and on to *A. aureum*, L. The general habit, form of the leaf, and the venation are alike in them all, but the sorus spreads from the marginal commissure, as in *Pteris*, inwards upon the lower surface, giving the Acrostichoid character. This matter will be taken up again in the next memoir of this series.

The case for *Stenochlaena* has been worked out developmentally in these studies.[4] Frau Schumann does not appear to have been aware of this (l. c., p. 245), but she arrives at the same conclusion from examination

[1] Studies, V. Ann. of Bot., 1915, p. 495.

[2] Garden Ferns, 1862, Text to Plate LVIII.

[3] l. c., pp. 208–21, also p. 242.

[4] No. IV, Ann. of Bot., vol. xxviii, p. 391.

of more mature states, referring to Mettenius,[1] and giving a series of diagrammatic drawings illustrating the spread of the sorus over the leaf-surface. The origin of this Acrostichoid type is clearly from *Blechnum*: the habit, anatomy, and corresponding glandular hairs, as well as the soral characters and form of the spores, show this. A phyletic series based on such characters was shown by me to lead from a type like *Matteuccia intermedia*, with separate sori, to *Blechnum* with sori fused above a vascular commissure, and then on to the Acrostichoid state as seen in *Stenochlaena*. Another example of a similar progression is seen in *Brainea*, but this has probably been along a distinct line from *Stenochlaena*. *Brainea* is in fact a small tree-Blechnoid, in which after abortion of the indusium (phyletic margin of the leaf) the sorus had spread outwards over the leaf-surface. The genus *Hymenolepis* is still very uncertain in character and affinities, but probably it also is of Blechnoid origin, related through *Dicranoglossum* and *Taenitis*. The conclusion is then that among the Blechnoid Ferns a passage to an Acrostichoid state has probably occurred along more than one phyletic line.

Another isolated case, which has been regarded as related to the Gymnogrammineae and Cheilanthinae, is the genus *Trismeria* (perhaps also *Microstaphyla*, Presl.). With vascular anatomy, which indicates a similar grade to that of *Llavea* or *Plagiogyria*, it shows an Acrostichoid condition of the sorus. This might readily have been produced from the state seen in the plants named, by spread of the sorus on to the leaf-surface intervening between the veins.

The genera *Polybotrya* and *Stenosemia* are placed by Diels with his Aspidiinae. Frau Schumann (l. c., pp. 252–8) concludes that these genera are akin to one another, and gives interesting figures illustrating successive steps of spread of the sporangia over the extended surface of the sporophyll. Though these do not demonstrate the affinity of these Ferns, still the habit of the sterile leaf, as well as the vascular anatomy, indicates an Aspidioid relation. The latter has been examined in *P. osmundacea*, H. B. Willd., which has its axis greatly elongated, with long internodes, in accordance with the climbing habit. About six meristeles are arranged in a ring in the transverse section. The leaf-gaps are drawn out to great length. The leaf-traces enter the axis as numerous separate strands, and pursue a course downwards for a distance of several internodes through the cortex, thus giving the appearance of a cortical series. Ultimately they insert themselves on the sides or base of the leaf-gaps. *P. cervina* (L.), Klf., shows similar characters, but only three meristeles appear in the transverse section. The leaf-traces behave in the same way as in *P. osmundacea*, but, the axis being much less elongated, the appearance more nearly resembles that of *Nephrodium*. In fact, the vascular system is a dictyostele with sub-

[1] Fil. Hort. Lips., p. 61.

divided leaf-traces, like *N. Filix mas*, but greatly extended. Taking the characters generally into consideration, the near relation of these Ferns to one another, and their reference to an Aspidioid affinity, are probably correct.

All the Acrostichoid types so far considered (excepting *Trismeria*) may thus be referred in origin to some *indusiate* source. There remain for consideration *Elaphoglossum*, *Leptochilus*, *Photinopteris*, *Platycerium*, and *Cheiropleuria*. These were probably derived from *non-indusiate* sources, which will now be discussed, together with their relations one to another. In the first place, *Elaphoglossum* may be segregated from the rest on the point of its venation, as already described above (p. 28). For reasons there given the genus may be held as distal in a series connected with such a form as *Metaxya*. The ultimate origin would probably be from some type like *Gleichenia*, with protostelic rhizome, highly branched leaves with undivided leaf-trace and open venation, dermal hairs, and relatively few large sporangia grouped in superficial sori. The next step is illustrated by *Metaxya*, with solenostelic rhizome, simply pinnate leaves, undivided leaf-trace and open venation, dermal hairs, and more numerous, smaller sporangia in sori sometimes elongated or plural upon the vein. Next *Syngramme*, with leaf rarely branched, usually quite simple, divided leaf-trace, and venation showing irregular fusions, dermal hairs, and the sori continuous along the veins. Finally, *Elaphoglossum*, with rhizome containing a solenostele with occasional perforations, leaves entire, highly divided leaf-trace and reticulate venation without blind twigs, dermal scales, and numerous Polypodioid sporangia extended over the whole lower surface. The parallelism of the progressive characters confirms the relations suggested, some of which had already been indicated by other writers on general systematic grounds. The genera named may be held to indicate a phyletic progression, and they may be designated the Metaxyoideae.

The remaining Acrostichoid genera are *Cheiropleuria*, *Platycerium*, *Leptochilus*, and *Photinopteris*. They have in common a reticulate venation with blind vascular twigs ending within the relatively small areolae. The *Venatio Anaxeti*[1] is the central type for them all, though they differ in detail. There is no indication of any abortive indusium in relation to their superficial soral areas. Of relatively primitive Ferns with such characters the living genus *Dipteris* provides the obvious line of comparison. It is recognized as the surviving representative of the family of the Dipteridinae, which included many forms of the Mesozoic period, characterized by various and elaborate leaf-construction, but still with the same type of venation, and with superficial, non-indusiate sori.[2] It is now suggested that the living genera above named, together with certain Polypodioid genera, such as

[1] Luerssen, Rab. Kryp.-Fl., Band iii, Fig. 22, p. 18.

[2] See Seward, Fossil Plants, vol. ii, pp. 380–94.

Phlebodium, *Phymatodes*, *Niphobolus*, *Drynaria*, and also *Neocheiropteris*, represent the modern derivatives of the phylum of the Dipteridinae. According to the spread of the sori, and their flowing together to form extensive soral areas, these derivatives would be ranked as Polypodioid or Acrostichoid. There is no reason to assume that any limit should exist between these types, and the general similarity of their habit supports their near relation.

Photinopteris and *Dryostachya* are clearly referable as Acrostichoid derivatives from *Drynaria*.[1] The stelar structure (Text-fig. 11) accords with this, though not distinctive evidence, and for *Drynaria* itself the Dipterid affinity may be suggested with as much force as for *Phlebodium* or *Phymatodes*.

The case for a Dipterid relation of *Cheiropleuria* has already been argued at length in a previous memoir of this series,[2] and the conclusion was drawn (p. 524) that it is a Fern of Matonioid-Dipterid affinity, which has, however, retained its primitive dermal hairs, and the protostelic structure resembling that of the still more archaic Gleicheniaceae, while it had adopted a type of leaf and of sorus essentially resembling the relatively advanced species of *Dipteris*, but carried to a still more advanced state; for the sorus is mixed and Acrostichoid, with initiation of a diplodesmic structure of the fertile areas.

In the same memoir the position of *Platycerium* was also discussed. It was concluded that it also is a Dipterid derivative, specialized for an epiphytic habit. This conclusion may be further tested in view of data since come to hand. A memoir by Heinrich Ritter von Straszewski has dealt with 'Die Farngattung *Platycerium*'.[3] He describes its prothallus, noting especially the multicellular glandular hairs on its under surface, comparable with those of the Cyatheaceae and *Diacalpe*. The antheridia have a divided cap-cell. In writing on the vascular structure he does not seem to have been aware of the work of Miss Allison.[4] His observations on *P. stemaria* (Beauv.), Desv. (= *P. Aethiopicum*, Hk.), correspond to hers in showing a medullary system within an outer ring of meristeles and a much-divided leaf-trace. But he is in error when he attempts to correct the observations of Hofmeister on *P. alcicorne* by the suggestion that he had worked on young material. As Miss Allison has shown on quite mature stems, the meristeles are disposed in a single circle in that species. He notes that lateral buds spring from the axis below the leaf-insertions, an arrangement which has its counterpart in many Ferns of Cyatheoid affinity. He advances reasons for the belief that the humus leaf was phyletically prior to the sporophyll type, and that *Platycerium* originally had only

[1] Compare Diels, l. c., p. 328; Christ, l. c., pp. 117, 121; Frau Schumann, l. c., p. 245.
[2] No. V, Ann. of Bot., vol. xxix, p. 495.
[3] Flora, 1915, p. 271, &c.
[4] New Phytologist, vol. xii, 1913, p. 311.

that form of leaf. But is there any need for such a conclusion from the facts? It seems more probable that both were differentiated from a common type, more nearly resembling the leaves of the less specialized Ferns.

In systematic position he holds that *Platycerium* is not to be referred to *Acrostichum*; nor is it near to *Dipteris* or *Cheiropleuria*. He notes similarity to *Niphobolus*, but considers that the prothallus points to the Cyatheaceae, and inclines to the view that it should form a special tribe linking the Polypodiaceae and Cyatheaceae.

In the main this conclusion is in accord with my own view as expressed in Studies, No. V, excepting in the negation of the affinity with *Cheiropleuria*, and the erection of a special tribe. But as von Straszewski wrote without knowledge of the detailed facts which were there brought forward, he may revise his conclusion in view of them. Moreover, the additional observations on forms probably related, which are given above, will be helpful in placing this peculiar genus phyletically. It also is regarded as a Dipterid derivative, specialized for an epiphytic habit. The leaf-trace is subdivided, and the solenostele broken up by profuse perforation into a simple ring of meristeles in *P. alcicorne*, or with addition of medullary strands in *P. stemaria*. This arrangement finds its counterpart in the polycycle of the *Matonia-Dipteris* series. It is true this stat eseems far removed from the protostely of *Cheiropleuria*, but the difference is bridged over by the solenostely of *Dipteris* itself, and the profuse perforation now seen in *Neocheiropteris* and *Leptochilus tricuspis*. In view of the other similarities this obvious point of vascular difference must be discounted.

It has been seen that in *Dipteris* each separate sorus is seated on the end of a blind intra-areolar vascular twig, which may be enlarged; that in *Cheiropleuria* the enlarged vascular receptacle may extend in a lower plane beyond the limits of its own areola[1]: and that this extension carried to a greater extent gives rise to the well-known second vascular system of the fertile region of *Platycerium*.[2] This structure, which is here described as *diplodesmic*, is found also in *Leptochilus tricuspis*. It is significant that it appears in no other type of Acrostichoid Fern examined. The addition of this third example of a peculiar and rare structure, in a Fern less specialized than *Platycerium*, but showing in anatomy, in sporangium, and in spore-formation similarities to *Platycerium*, brings the latter into nearer relations to ordinary types, and tends to minimize its special peculiarities. On the other hand, the phyletic relation of the Dipterids to the Cyatheoids was probably a close one, both having sprung from a Gleicheniaceous source.[3] This would sufficiently account for the character of the prothallial hairs noted by von Straszewski. Thus the additional facts accord with the reference of *Platycerium* as a Dipterid derivative.

[1] Ann. of Bot., vol. xxix, p. 512, Text-fig. 12.
[2] l. c., p. 512.
[3] Studies, II, Ann. of Bot., 1912. Also V, l. c., 1915.

It has been shown how *Neocheiropteris* has a thick rhizome, covered by ciliated scales, and containing a highly perforated solenostele, with divided leaf-trace and gritty sclerotic nests. The stalked pedatifid leaf is a modification of the Dipterid type, but related in point of its simplified helicoid development to *Leptochilus tricuspis* and with similar venation. The sori, seated often on irregular vascular loops, are elongated parallel to the costa, forming at the base an almost continuous elongated soral area (Plate I, Fig. 5). The sporangia resemble those of *Platycerium* and *L. tricuspis*, while all of these have smooth bilateral spores. Remembering the original reference of this Fern by Christ to a Dipterid affinity, it is not difficult to see in it another example of that alliance, showing a spread of the sorus over the leaf-surface.

It is in relation to these Ferns that the plant designated in Christensen's Index as *Leptochilus tricuspis* (Hk.), C. Chr., finds its natural place. It shares with *Neocheiropteris* the rhizomatous habit, long naked leaf-stalk, protecting scales, highly divided leaf-trace, and perforated stele, with gritty sclerotic nests. The lamina is referable, as has been seen, to the same type of branching. The vascular system of the upper leaf is particularly distinctive (Text-fig. 2, p. 6): the modification of the horseshoe as it passes upwards resembles that of *Platycerium*, taking, as we have seen, a middle position between that genus as the extreme type and *Cheiropleuria*. The venation is essentially similar in them all. Further, in the fertile region it has been seen that *Cheiropleuria* has occasional slight extensions of the receptacular strands beyond the single areola: this is the first step towards a 'diplodesmic' state. *L. tricuspis* has a well-developed diplodesmic system, but not so complete or extensive as that well known in *Platycerium*. Hitherto these are the only three genera which show this condition. It has not been possible to make a complete examination of *Neocheiropteris* on this point. Finally the sporangia and spores of *Neocheiropteris*, *L. tricuspis*, and *Platycerium* are very similar. Even the branched soral hairs are present, though those of *Platycerium* are more elaborate, in accordance no doubt with the epiphytic habit. It is concluded that *Neocheiropteris*, *L. tricuspis*, and *Platycerium* are phyletically related Ferns, while *Cheiropleuria* is also related, but rather more aloof; and that *all of them are Dipterid derivatives.*

On the other hand, *Leptochilus tricuspis* differs from all investigated species of *Leptochilus* in the fact that in them no diplodesmic structure has been found, while they show also a less advanced perforation of the solenostele and absence of the sclerotic nests. These and other distinctive features are of sufficient importance to justify its separation from the genus, which may be effected without any introduction of a new name. It was named by Hooker *Acrostichum* (*Gymnopteris*) *tricuspe*, Hook. Beddome later named it *Gymnopteris tricuspis*, Hook.[1] Meanwhile the genus

[1] Ferns of British India, Text to Plate LIII.

Gymnopteris has been sunk in *Leptochilus*.[1] All that is necessary is to restore Beddome's designation for this Fern, thus reviving the genus *Gymnopteris* to receive it. This appears to be an appropriate method for marking the distinction from *Leptochilus*, and the designation will then be *Gymnopteris tricuspis* (Hook.), Bedd.

The genus *Leptochilus* itself remains to be considered. It has recently been revised by Frau Schumann.[2] She segregates it into two main divisions, which she regards as naturally apart. They are distinguished on the basis of simple as against compound leaves. The former she regards as Polypodioid derivatives, the latter as Dryopterid. This, on the whole, I should be prepared to accept, as a first recognition of the polyphyletic origin of the Ferns grouped as *Leptochilus*. But it seems not improbable that the sources of the species so designated may have actually been more than two. In particular, for the reasons given above, *Leptochilus tricuspis* should be referred more directly to a Dipterid origin, as indicated by comparison with *Neocheiropteris* and *Cheiropleuria*. It is possible that investigation of *L. varians* may lead to a similar conclusion for it also. In fact it seems likely that *Leptochilus*, like *Polypodium*, *Acrostichum*, and *Gymnogramme*, may not be a phyletic genus at all. Such genera have been convenient groupings for the classification and recognition of species. Probably none of them are pure genera in the phyletic sense, but mere assemblages of forms having some obvious characteristic in common, which may itself have been evolved along more than one phyletic line.

Note on Sporangial Segmentation.

It has been found that in *Cheiropleuria*,[3] in *Dipteris*,[4] and in *Metaxya*,[5] the segmentation of the primordium of the sporangium is a two-sided one, and the segments themselves are arranged in two rows, while the sporogenous cell itself is two-sided, like half of a biconvex lens. In the large majority of Ferns the segmentation of the sporangial head is three-sided, and the shape of the sporogenous cell is tetrahedral. Among the Ferns here discussed the latter type is found in *Platycerium* and in *Gymnopteris tricuspis*, and it is almost certainly so in *Neocheiropteris*. In the former type the stalk of the mature sporangium is four-rowed, in the latter it is three-rowed or less. The question may be raised whether this difference of segmentation does not militate against the phyletic relations which have been suggested.

The view which may be held as probable is that the two-sided segmentation, though apparently simpler, is really the more primitive. It readily gives the four-rowed stalk, which is a more massive construction than the three-rowed. And thus those sporangia which have the two-sided segmentation are nearer in the character of their stalk to the relatively

[1] See Christensen's Index, p. 342. [2] Flora, 1915, p. 250.
[3] Studies, V, Pl. XXV, Fig. 17. [4] l. c., Fig. 18. [5] l. c., Text-fig. 19, p. 521.

primitive types. This is further illustrated by the structure of the sporangial head in *Metaxya*, *Dipteris*, and *Cheiropleuria*, which all show with more or less plainness the oblique and continuous annulus, while *Neocheiropteris*, *Gymnopteris*, and *Platycerium* have the annulus vertical and interrupted. The conclusion is that the former group, notwithstanding the apparently more simple segmentation, represent in truth a more primitive type, while the latter are derivative. A phyletic transition from the one to the other is no less easy to comprehend than a transition from a two-sided to a three-sided segmentation of the apex of axis or leaf in a Fern, or in *Selaginella*. Accordingly the difference of segmentation of the sporangium appears to be no serious obstacle to the comparisons instituted above, and *Dipteris*, *Metaxya*, and *Cheiropleuria* take their place as more primitive in sporangial construction than *Gymnopteris*, *Neocheiropteris*, or *Platycerium*.

Summary of the Chief Results.

1. *Cheiropleuria*, *Platycerium*, and perhaps *Neocheiropteris* share with the Fern hitherto styled *Leptochilus tricuspis* (Hook.), C. Chr., an extension of the sorus, with a special vascular supply, spreading in a plane below and parallel with the venation of the sporophyll. This is most extensive in *Platycerium* and *L. tricuspis*, and the condition may be described as *diplodesmic*.

2. These Ferns in external morphology, venation, anatomy, sorus, and sporangia are regarded as Dipterid derivatives, and may be grouped phyletically as Dipteroideae. To these may probably be added, later, many Polypodioid Ferns, especially *Phlebodium*, *Phymatodes*, *Niphobolus*, and *Drynaria*, and some simple-leaved species of *Leptochilus*.

3. *L. tricuspis* stands alone in the latter genus in various features, but especially in the diplodesmic character. It should, therefore, be removed, and by reviving its old generic name, now merged in *Leptochilus*, it may be styled *Gymnopteris tricuspis* (Hook.), Bedd. Of that genus it will be at present the only species.

4. A parallel series to the Dipterioideae, but differing in venation, and probably distinct phyletically, is related to *Metaxya* as its probable source. It includes *Syngramme* and *Elaphoglossum*. These may be styled the Metaxyoideae.

5. Both of these progressions illustrate advance from circumscribed sori to an 'Acrostichoid' spread of the sporangia over the leaf-surface. This runs parallel with changes of leaf-form, disintegration of the vascular tracts, passage from dermal hairs to scales, increasing areolation of veins, and changes of sporangia from the continuous oblique to the interrupted vertical annulus. Since there is substantial parallelism in these various characters of advance, the progressions are firmly established: but they are constantly distinguished from one another by their venation.

6. The genus *Leptochilus*, as at present defined, may probably be a composite genus, not a phyletic unity.

7. The Acrostichoid condition has been acquired along a very considerable number of distinct phyletic lines. Accordingly *Acrostichum* connotes not a genus in the sense of a phyletic unity, but a condition or state, which has been arrived at from various distinct sources.

DESCRIPTION OF FIGURES ON PLATES I AND II.

Illustrating Professor Bower's paper on Ferns showing the 'Acrostichoid' Condition, &c.

PLATE I.

Fig. 1. Whole plant of *Gymnopteris tricuspis* (Hook.) Bedd., one-sixth of the natural size.

Fig. 2. Whole plant of the same, showing additional helicoid branching of the sterile leaf. From the Edinburgh Botanic Garden. Photograph supplied by the Director, through Dr. R. C. Davie.

Fig. 3. Base of the fertile lamina, natural size, showing the branching and the soral areas.

Fig. 4. *Neocheiropteris palmatopedata* (Bak.), Christ. Specimen in Glasgow University Herbarium, showing helicoid construction. Half natural size.

Fig. 5. Base of the fertile lamina of *Neocheiropteris*, showing the branching and sori. × 2.

Figs. 6, 7. Leaves of *Polypodium* (*Phymatodes*) *decumanum*, Willd., for comparison of branching with *Gymnopteris tricuspis*. Half natural size.

PLATE II.

Fig. 8. *Leptochilus latifolius* (Meyen), C. Chr. Sterile and fertile leaves, half natural size, for comparison of the branching with *Gymnopteris tricuspis*.

Fig. 9. Part of an old fertile pinna of *Metaxya*, showing the venation and the sori (from which the sporangia have mostly fallen away), more than one sorus being on the single vein. × 2.

Fig. 10. Part of a pinna of *Metaxya*, with mature sori. × 2.

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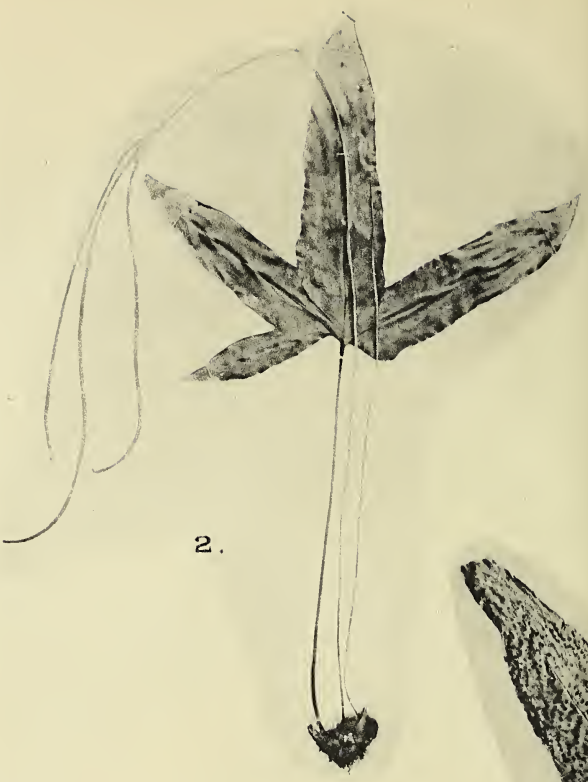

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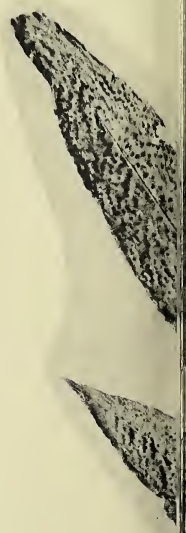

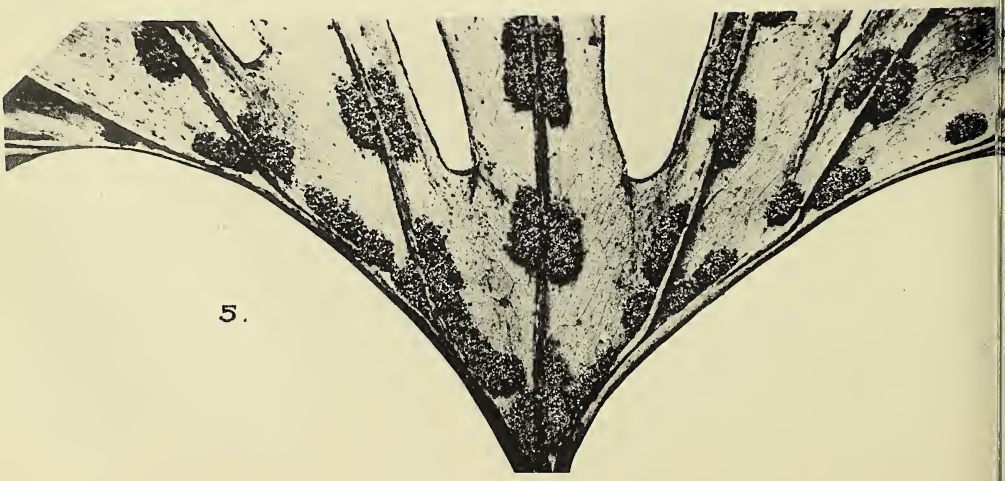

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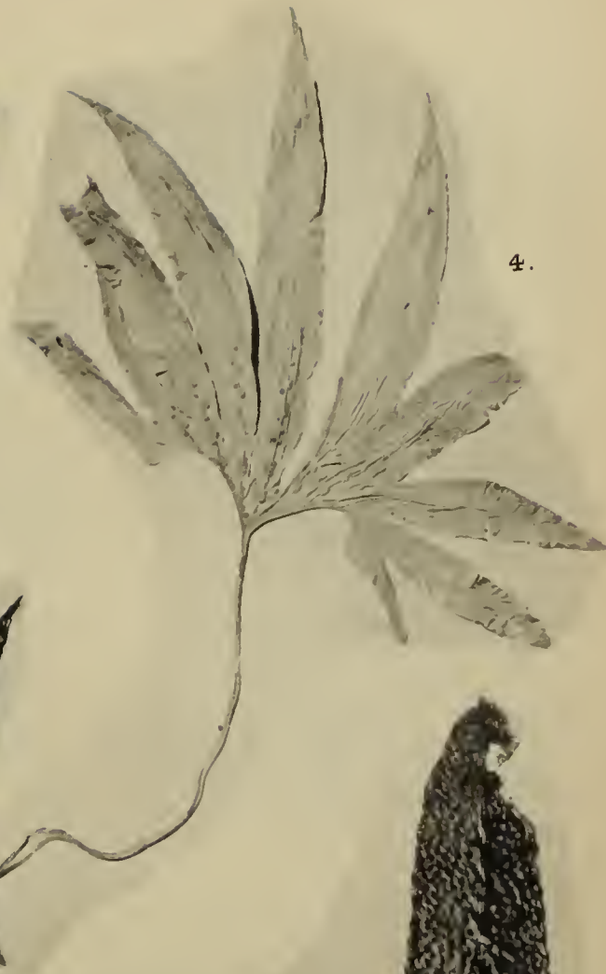

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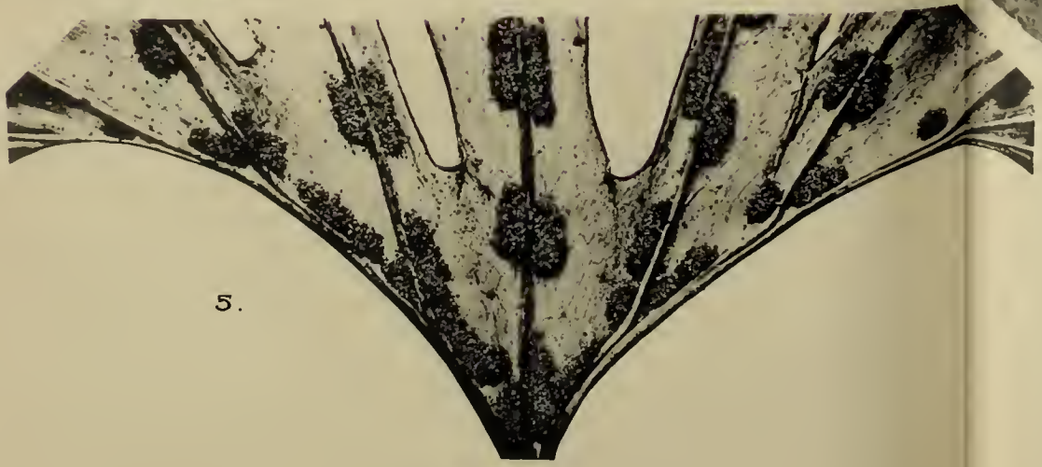

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BOWER—ACROSTICHOID FERNS

FIG. 8.

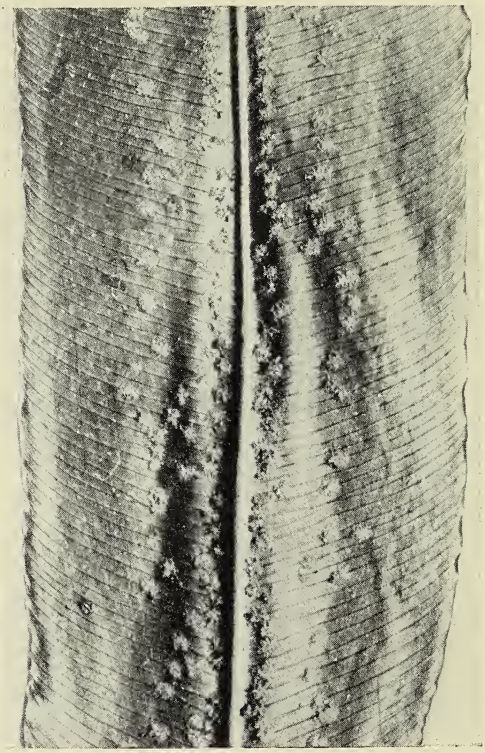

FIG. 9.

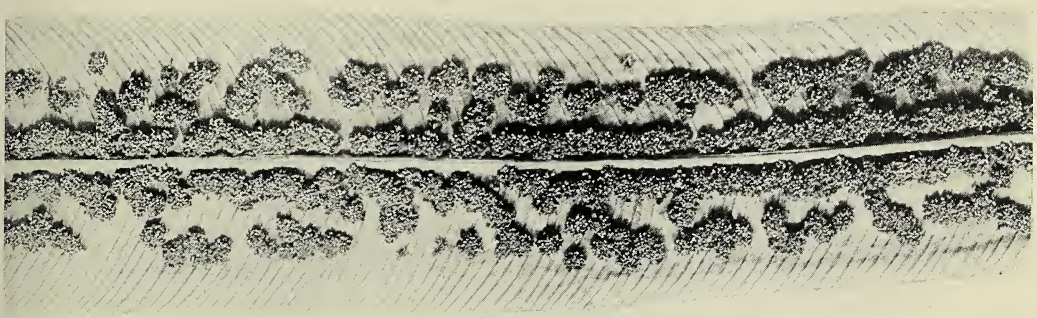

FIG. 10.

On the Occurrence of Intrafascicular Cambium in Monocotyledons.

BY

AGNES ARBER.

With three Figures in the Text.

Introduction.

AN impression seems to prevail among botanists that the occurrence of intrafascicular cambium in Monocotyledons is extremely rare, except perhaps in seedlings. It may therefore be worth while to collect together some of the scattered observations on this subject, which tend to show that an ephemeral cambium is, in reality, a widespread anatomical feature of this group, and also to record a few fresh examples and to figure and describe briefly a case in which the cambium can be particularly well observed.

I am indebted to Miss Saunders, Director of the Balfour Laboratory, for facilities for anatomical work, and to the Newnham College Fellowship Committee for a grant towards the expenses of this and other researches. I wish also to express my thanks to Mr. Boodle, for his help in connexion with the literature, and to Mr. Lynch, of the Cambridge Botanic Garden, for his kindness in supplying me with material.

Review of the Literature.

The earliest observation on the occurrence of intrafascicular cambium in Monocotyledons appears to be that of Russow,[1] who, in 1875, described, in the vascular bundle of *Hemerocallis fulva*, 'eine Andeutung eines Cambiumstreifens'. This was followed in 1884 by Godfrin's [2] record that in the veins of the cotyledon of *Latania borbonica* (Palmae) an active cambial zone occurred. Two years later Möbius [3] observed traces of a cambium in the stems of *Listera ovata* and *Orchis maculata*, and found that *Limodorum abortivum* was distinguished by somewhat more prolonged cambial activity.

In 1888 the subject was placed on a much broader basis by Fröken Sigrid Andersson's [4] researches, which, owing to their publication in

[1] Russow, F. (1875).
[2] Godfrin, J. (1884), p. 47 and Pl. III, Fig. 43.
[3] Möbius, M. (1886).
[4] Andersson, S. (1888).

Swedish, have perhaps scarcely received the attention they deserve. This author found that intrafascicular cambium was extremely widespread in the young tissues of Monocotyledons, and that the development of the bundle in this group was, in this respect, not nearly so divergent from that in the Dicotyledons as had previously been supposed. Her work was not confined to seedlings, as some citations from it might seem to indicate. She figured the following cases, some of which showed a fair development of cambium, while in others merely a trace of meristematic activity occurred: *Triglochin maritimum* (stem), *Cyperus alternifolius* (leaf), *Zea Mays* (leaf-sheath and stem), *Amomum* sp. (stem), *Typha* sp. (leaf), *Brahea filamentosa* (petiole), *Platanthera bifolia* (stem), *Allium senescens* (leaf), *A. nutans* (leaf), *Lilium Martagon* (stem), *L. candidum* (stem), *L. japonicum* (stem), and *Dracaena* sp. (stem). She drew attention to the similarity of the bundles of *Ranunculus repens* and those of *Lilium*, and figured them side by side for comparison.

Ten years later Gravis[1] recorded the occurrence of intrafascicular cambium in the stem and leaf of *Tradescantia virginica*. The cambium, which is extremely clear in the young stem bundle, leaves no trace when the adult state is reached.

In 1899 Queva[2] described the most remarkable instance of intrafascicular cambial activity yet recorded for Monocotyledons. In *Gloriosa superba* tubers are formed and filled with starch in one season and depleted during the next year. The bundles thus pass through two periods of activity separated by a period of rest. In the second period of activity, xylem and phloem elements are formed from the cambium. This resumption of cambial activity, after an interval during which cell division has been in abeyance, is, so far as our present knowledge goes, unique among Monocotyledons.

T. G. Hill,[3] in 1900, rediscovered and figured the intrafascicular cambium of the stem of *Triglochin maritimum*—one of the cases which Andersson had described and illustrated at an earlier date.

The Cyperaceae and Gramineae—families in which Andersson had already recorded the existence of cambium—received further attention from this point of view in 1906. Plowman[4] noted, in this year, that he had observed evidence of cambial activity in the internodal bundles of practically all the examples of the Cyperaceae which he studied. In the same year Chrysler[5] drew attention to its occurrence in the stem and leaves of more than twenty species of grasses. The development of cambium just above the nodes is associated by this author with the power, which the grasses possess, of bending upwards when the stem is laid horizontally.

[1] Gravis, A. (1898).
[2] Queva, C. (1899).
[3] Hill, T. G. (1900), Pl. IV, Fig. 5.
[4] Plowman, A. B. (1906).
[5] Chrysler, M. A. (1906).

Two years later Miss Sargant[1] added to the records already made by Fröken Andersson for the Liliaceae and Scitamineae, by describing cases of cambial development in certain seedlings belonging to these orders. She observed intrafascicular cambium in the hypocotyl of *Yucca arborescens*, *Y. gloriosa*, and *Y. aloifolia*, the cotyledonary bundles of *Milla*, *Dipcadi*, *Galtonia*, *Albuca*, and *Fritillaria*, and the plumular leaves of *Elettaria* and *Musa*.

Observations.

The bundles of the young inflorescence axis of *Eremurus himalaicus*, Baker, one of the Asphodelinae, show cambial activity very clearly. In an extremely young inflorescence axis, less than 5 cm. long, dissected out of the

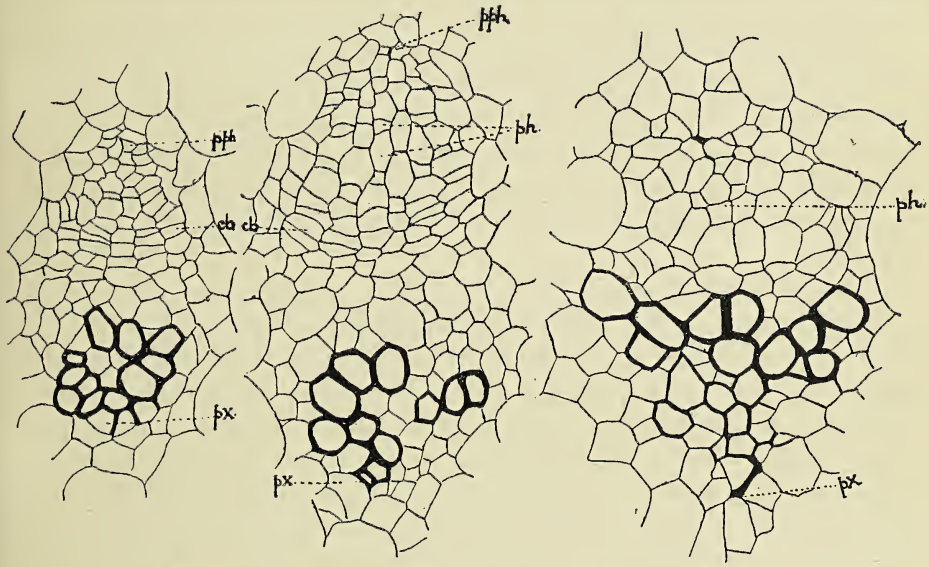

Fig. 1. Fig. 2. Fig. 3.

Figs. 1–3. Transverse sections of vascular bundles from inflorescence axes of *Eremurus himalaicus*, Baker. 1. A bundle from the flowering region of an axis gathered May 7, showing cambium. The phloem is not fully differentiated. 2. A bundle from the region below the lowest flowers of the same axis, showing secondary phloem in process of differentiation into sieve-tubes and companion cells. 3. A mature bundle, from the flowering region of an axis gathered on May 28, in which cambial activity has practically ceased. *cb*, cambium; *ph*, phloem; *pph*, protophloem; *px*, protoxylem. × 275.

terminal bud in February, cambium was recognized, but the most favourable time for observing it seemed to be at the end of April or the beginning of May, when many of the axes exceeded half a metre in height. Fig. 1 shows a bundle from the flowering region of an inflorescence gathered on May 7. Here cells of a meristematic appearance, arranged in radial rows, intervene between the xylem and phloem, while the phloem is not yet differentiated into companion cells and sieve-tubes. In bundles from

[1] Sargant, E. (1908).

another inflorescence axis, gathered on April 27, as many as ten undifferentiated elements have been traced in a single radial row. In Fig. 2 a bundle is represented from a lower level in the axis gathered on May 7. Cambial activity is still in progress, but part of the secondary phloem has become modified into large sieve-tubes and small companion cells, thus somewhat obliterating the radial arrangement. Fig. 3 shows a bundle from the flowering region of an older inflorescence, gathered on May 28. Here cambial activity seems to have completely, or almost completely ceased. In these figures it will be observed that little or no secondary xylem is formed; the cambium seems to confine its activities, mainly if not entirely, to the production of secondary phloem. The same peculiarity has already been recorded for other members of the Liliaceae by Andersson.

Meristematic activity is not rigidly restricted to a single layer of initial cells. Dividing nuclei seem most frequently to occur near the innermost or xylem end of the radial files; they have been noticed in different cases in the first, second, third, fourth and sixth cells, counting from the inner end. In one case nuclei were seen dividing simultaneously in the fourth and sixth cells of the same file, and, in another case, in the first and third.

In addition to *Eremurus*, a few other genera among the Liliaceae have been examined. In the case of shoots of *Asparagus officinalis*, L., at the stage at which they are gathered for market, an ephemeral cambium was detected in the apical region. In such a shoot, gathered on May 1, transverse sections across the 'head', at a distance of 1 cm. from the apex, showed the metaphloem to consist of radial rows of undifferentiated cells, uniformly filled with cytoplasm. Traces of the same arrangement were visible at the base of the 'head', but, 2·5 cm. lower, the differentiation into sieve-tubes and companion cells had completely masked all indications of radial grouping.

Distinct traces of cambium have also been observed in a young inflorescence axis of *Nothoscordum fragrans*, Kunth, gathered on May 18. The bundles are more or less Y-shaped, and the cambium follows a curved line.

In the young inflorescence axis of *Hemerocallis fulva*, L., cambium has also been recognized. Russow,[1] as has already been mentioned, noted more than forty years ago the occurrence of cambium in another species, *H. flava*, L., so the present observation merely confirms its existence in this genus.

Summary.

In this paper the literature on intrafascicular cambium in Monocotyledons is briefly reviewed, and it is recorded that, in addition to the cases

[1] Russow, E. (1875).

already known, cambial activity occurs in the bundles of the young inflorescence axes of *Eremurus himalaicus*, Baker, and *Nothoscordum fragrans*, Kunth, while an ephemeral cambium occurs in the young shoots of *Asparagus officinalis*, L. The fact that cambial activity in Monocotyledons is, actually, more widespread than is generally assumed, offers a slight additional confirmation of the view, already expressed by Anderson, Queva, Chrysler, and Sargant, that the existence of this vestigial, intrafascicular cambium indicates that Monocotyledons have been derived from a dicotyledonous stock.

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Studies in Permeability.

IV. The Action of Various Organic Substances on the Permeability of the Plant Cell, and its Bearing on Czapek's Theory of the Plasma Membrane.

BY

WALTER STILES

AND

INGVAR JØRGENSEN.

With fifteen Figures in the Text.

Introduction.

When plant tissue is immersed in aqueous solutions of various substances part of the contents of the cells diffuses out into the external liquid as a result of changes produced in the permeability of the cell. Czapek (1, 2, 3) in an investigation of the question comes to the conclusion that an important relation exists between the surface tension of aqueous solutions in contact with air and their power of producing exosmosis of the cell contents, and from his conclusions in this respect builds up a theory of the plasma membrane. He alleges that a solution will not produce exosmosis from a plant cell unless its surface tension against air is reduced to a certain definite value. Czapek determined the limiting concentration which is just capable of producing exosmosis from plant cells of certain cell substances whose presence can be easily demonstrated. The solution in this limiting concentration should have the same critical surface tension independent of the substance, and in most of Czapek's experiments he concluded that this was the case. He says, 'Surface active substances do not alter the diosmotic properties of the plasma membrane before they act injuriously on the cell on account of their surface tension' (Czapek 3, p. 1).

Several authors have already subjected Czapek's work to considerable criticism, and indeed it seems strange to attempt to explain the properties of a highly complex organization like the plasma membrane in terms of one physical property.

A very brief consideration is required to make it clear that in the system produced by immersing plant tissue in an aqueous solution of a substance several factors must necessarily come into play. For instance, if we make the assumption that the change in permeability of the cell is due

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to chemical combination between the solute in the external medium and some substance of the plasma membrane, the rate at which the permeability changes will depend upon the active masses of the reacting substances. We know also that according to the principle of Willard Gibbs the lower the surface tension at the interface, the greater will be the concentration of solutes there. The actual rate of the reaction will nevertheless be controlled by the laws of mass action, although the lowering of the surface tension may increase the active mass of the dissolved substance at the interface and so increase the rate of reaction and, in consequence, the rate of change of permeability. On the other hand, if the surface tension of the solution is raised, chemical action will still take place, though it may be at a reduced rate. But other physical factors may be of importance in determining the rate of reaction besides surface tension. Thus, if the reacting substance is removed by chemical action faster than it can be replaced by diffusion, this latter factor will assume greater prominence in determining the rate of reaction.

However, on account of its undoubted complexity the plasma membrane cannot be assumed to be merely a system in which permeability alters simply as a result of chemical action. So, for instance, Schryver (8) thinks of the substances as cemented together in a gel, on the alteration of which the phenomena of permeability changes mainly depend, and in a later paper (9) he points out how many physical factors influence the state of aggregation of the gel.

Czapek investigated the exosmosis of tannin substances from the 'subepidermal mesophyll' cells of *Echeveria* leaves and from other objects. When treated with various reagents, notably with an aqueous solution of caffeine, the tannin in cells is precipitated in a characteristic and easily recognizable manner. Sections of *Echeveria* leaves, for example, are placed in 50 c.c. of the solution under investigation, and removed when equilibrium has been attained and tested for the presence of tannin. If tannin is absent it is assumed that exosmosis has taken place. There is no definite time in which equilibrium is attained; this has to be found in each case. 'Bei fortgesetzter Beobachtung lässt sich die Zeit leicht ermitteln, in welcher ein länger dauernder Gleichgewichtszustand hinsichtlich der Exosmosegrenze besteht, und diese Grenze stimmt mit der theoretischen Tensionsgrenze in der Regel befriedigend überein.'

Plant organs containing coloured cell-sap were also tried, for the exosmosis of anthocyan can be observed without the aid of a microscope. But most of these objects had the disadvantage that they were damaged after less than 24 hours' immersion in solutions of higher surface tension than the critical value. So 'resistant objects' such as *Echeveria* leaves, in which 'secondary damage' does not occur, were chiefly used by Czapek throughout the investigation. In some cases these were left for 24 hours, and even for 48 hours, for the equilibrium condition to be reached.

The surface tension of the various solutions used was measured by means of a capillary manometer, the essential feature of which is the measurement of the pressure just required to force a bubble of air through a capillary tube containing the liquid under examination. From this may be calculated the surface tension of the solution in contact with air. Of course what we are actually concerned with in exosmosis is the surface tension between the solution and the protoplast, which may be a very different thing. But because 'all the experience we possess' indicates that increase in concentration of the solution external to the protoplast causes a lowering of surface tension, and since increase of concentration of these same solutions also brings about a lowering of their surface tension against air, Czapek neglects the essential difference between the conditions in his experiments with the plant and the conditions under which the surface tension is measured.

A large variety of organic substances was used by Czapek in these experiments, including a series of monohydric alcohols, glycol, glycerine, esters, &c. The general conclusion is reached that exosmosis only takes place from the cell when the surface tension of the external liquid when in contact with air is reduced to 0·68 times the surface tension of pure water against air. From this rather remarkable conclusion the surprising deduction is made that the surface tension of the outer layer of the protoplast in contact with air must also have this value. Since Czapek found by means of his capillary manometer that emulsions of various fatty substances occurring in plants, unsaturated triglycerides such as castor oil, linseed oil, and triolein, have the same surface tension, and that the surface tension can never be reduced below this value of about 0·68, he further concludes that the surface-tension properties of the plasma membrane are chiefly due to the presence in the outer layer of the protoplast of such unsaturated triglycerides. These neutral fats are supposed to be present in the form of an emulsion with water, the globules being coated with a layer of soap which acts as a protective colloid. Czapek admits that he has not disproved Overton's view that lecithin and cholesterine may be the essential substances in the plasma membrane, and he is also prepared to agree that there may be some protein present as well. The important substances, however, in his opinion are the neutral fats.

Czapek's theoretical considerations have already been subjected to severe criticism, to which, as we shall draw attention later, he has been unable to make any adequate reply. But, apart from his theory, it becomes of interest to examine whether it is permissible for him to draw the conclusion he does from his experiments. The method he used was laborious and crude, and he regards the measurements he made as referring to equilibrium conditions which he supposed to have become established in the systems he investigated. It is necessary, therefore, to reinvestigate the

problem, and to repeat some of his experiments with more exact methods, which will at the same time enable one to obtain an insight into the kinetics of the changes taking place. This we have done in the experiments described in this paper, and we shall show as a result of them, that not only is the theory founded by Czapek upon his experimental results untenable, but that the facts on which he bases his theory are merely illusions of experimentation due partly to the crudity of the method employed, and partly to a selection and arbitrary arrangement of experiments.

We do not intend in this paper to enter into a general discussion of the permeability of the cell, nor to differentiate between the permeability properties of different tissues. Nevertheless we would point out that the chemical composition and physical properties of membranes in different tissues are not likely to be identical, and their permeability properties are consequently not likely to be expressed by one general law.

In order to avoid discussion which available information does not justify, we use the generally accepted nomenclature, including the term 'plasma membrane'. By this latter expression we mean that part of the cell which is concerned in the phenomena of permeability, without reference to its actual location in the cell.

Methods used in the Present Investigation.

In this investigation we have employed a method for studying permeability changes which is more sensitive and of more general application than the methods of earlier workers, and which at the same time allows the changes taking place to be followed easily from time to time. This method consists essentially of measuring the rate of exosmosis of electrolytes from plant tissue by means of measurements of the electrical conductivity of the solution in which the tissue is placed. In former investigations in which we have used this method the solution external to the tissue has been an electrolyte, and so changes in the conductivity of the external solution have been the resultant of diffusion of electrolytes into the tissue from the solution and diffusion of electrolytes out of the tissue into the solution. In the present investigation the substances examined are all practically non-electrolytes, and the electrical conductivities of solutions of them are almost identical with that of the conductivity of the water used in preparing them. Hence the increase in conductivity will give a much surer measure of the extent of exosmosis than when the effect of acids or salts is under investigation. It is, however, necessary to make allowance for the lowering of conductivity due to the presence of non-electrolytes, and this has been done in the cases cited in the following pages.

A brief account of the method has been given in a previous paper (10), but we may here take the opportunity of describing the method in more detail.

A suitable quantity of the solution under investigation is placed in

a stoppered vessel and a definite amount of plant tissue is added. The vessel is kept at a constant temperature in a thermostat, and the electrical conductivity of the liquid measured at suitable intervals by Kohlrausch's method. From the numbers obtained in this way the relation between time and exosmosis of electrolytes may be exhibited graphically in a curve. In the majority of our experiments we have used potato tissue. Cylinders of potato were cut out from the tuber by means of a cork-borer, and these were cut up into discs of a definite thickness. These discs had a diameter of 17 mm., a thickness of about 1·75 mm., and a weight of about 0·45 grm. After they were prepared they were washed for an hour in running tap-water, rinsed rapidly in distilled water, and then dried with blotting-paper. In each experiment twenty discs were immersed in 50 c.c. of solution. The experiments were carried out at a constant temperature of 20° C., and the conductivity measurements were all made at the same temperature.

In order to correct the results obtained for the depression in conductivity due to the presence of non-electrolytes in the solution, the following method was adopted. Thirty-six grm. of potato discs were boiled in about 95 c.c. of water and the extract so obtained made up to 100 c.c. This should give a solution of approximately double the strength of the external solutions when equilibrium is attained. Quantities of 10 c.c. of this were then taken and made up with either pure water or a solution of the non-electrolyte to 20 c.c. The conductivity of the solutions so obtained was measured, and by this means curves were obtained showing the depression of the conductivity brought about by various concentrations of the non-electrolytes employed. With lower concentrations of cell exudate the absolute decrease in conductivity resulting from the presence of non-electrolytes is smaller, the lowering being approximately proportional to the concentration of electrolyte. These results agree with the extensive observations of Jones and his collaborators (5). By means of these curves the numbers actually obtained in the experiments were corrected before plotting the curves between the time and the exosmosis of electrolytes. In the case of methyl, ethyl, and to a less extent propyl alcohol, where high concentrations of alcohol were used, the correction that has to be applied in this way is large (see Figs. 4 and 5), but in other cases, though it is much smaller, it is as well not to neglect it.

As the discs are liable to vary in thickness and weight some experiments were made in which discs of various thickness and weight were used with the same quantity of external solution in each case. The liquid used was an aqueous molecular solution of pyridine; in each case 50 c.c. of this solution were employed with twenty discs. The weights of the twenty discs in five cases were 3·8 grm., 4·1 grm., 5·8 grm., 9·4 grm., 14·74 grm. The curves given in Fig. 1 show the relation between the time and the exosmosis of electrolytes in the five cases. It will be noticed that in each case there is a rapid exosmosis of electrolytes lasting over the first few

hours, and then the rate of exosmosis rapidly falls off until the quantity of electrolytic exudate remains constant, which means that equilibrium has become established between electrolytes in the external solution and the plant tissue. The rate of exosmosis during the first period does not vary greatly with the weight of tissue, being practically proportional to the

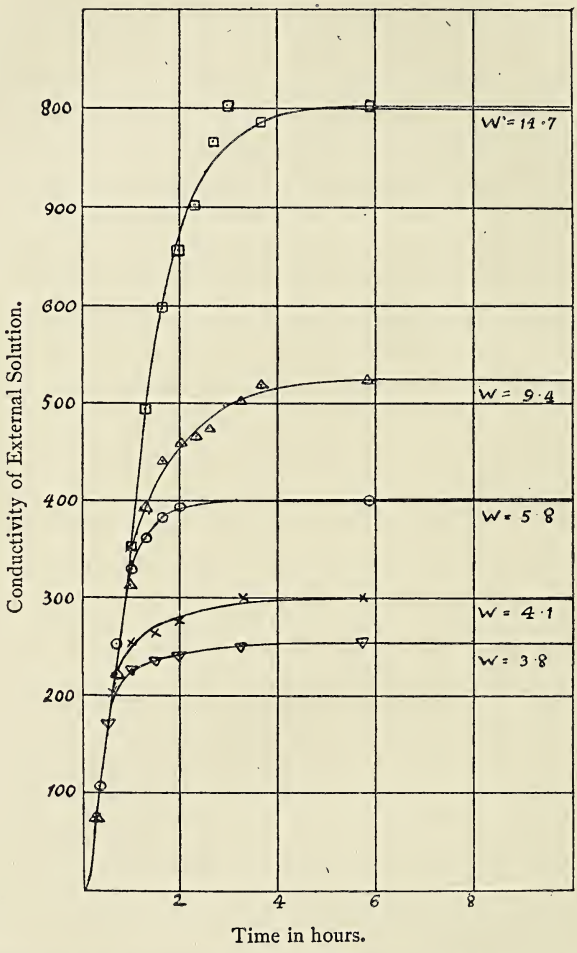

FIG. 1. The exosmosis of electrolytes from various quantities of potato in molecular solutions of pyridine.

surface. There is, however, a very great difference in the final value of the conductivity when different weights of tissue are used. The following table shows the relation between the final value of the conductivity and the weight of tissue used. The numbers in the third column have been obtained by assuming that when equilibrium is obtained there is the same concentration of electrolytes in the solution and the tissue.

Weight of tissue.	*Final value of conductivity.*	*Total quantity of electrolytes in the system.*	$\frac{E}{W}$.
(W)		(E)	
3·8	258	275	72
4·1	300	322	79
5·8	399	440	76
9·4	520	605	64
14·74	802	1027	70

Thus the total exosmosis varies with the weight of tissue used. In the experiments to be described the weight of discs varied somewhat, and in some cases the deviation from the mean was as much as 5 per cent. of the mean value. Hence variations in the values for the final conductivity of the solutions are no doubt due to variations in the weight of tissue employed. It is, however, only the final value of electrolytic exudate which is affected; slight variations in the weight of discs used will scarcely affect the quantity of exosmosis of electrolytes before the final condition is reached, and any differences in the earlier part of the process must be due to other causes.

We have used potato in our experiments, as potato tubers yield a very uniform tissue, and individual variations are likely to be less than with most other plant organs. But the method can be used for almost any plant tissue. We ourselves have used discs of beetroot and other fleshy organs similar to those of potato, and we have also employed leaves of various kinds, as well as roots of living plants, and we have found the method to answer as well in these cases as with potato.

In the cases described in this paper we have chiefly used concentrations of reagents which will produce irreversible changes in the permeability of the cell, i. e. lead to the death of the tissue. In these cases the morphological structure of the tissue employed does not introduce much variation in the results, but if very low concentrations are used, such as would not for a long time lead to irreversible permeability changes, then the morphological structure and chemical composition become of importance. This is easily observed by comparison of potato and carrot. With these reversible changes of permeability and their bearings on theories of permeability we hope to deal more in detail in a later paper.

It is interesting to compare results obtained by this method with some others that have been employed in work on permeability. Two methods that have found favour earlier we have tried in this investigation. The first of these is the exosmosis of the red pigment from anthocyan-containing cells. For this purpose the root of the red beet has been much used in the past. As the cell membranes become more permeable the red-coloured substance diffuses out, and the external solution becomes coloured with it. It is possible to follow the rate of exosmosis by measuring the colour intensity at different times by means of a colorimeter. By following both the increase in colour intensity of the external solution and its increase in conductivity

it can be readily shown that there is a rough parallelism between the two. But the measurement of the electrical conductivity can be made much more accurately than colorimetric measurements.

In the following experiment twenty discs of red beetroot, weighing altogether 10 grm., were placed in 50 c.c. of solutions of isoamyl alcohol (Kahlbaum) of various strengths. The conductivity was measured at various intervals of time and the colour intensity estimated. The colour intensity was compared with that of an extract obtained by boiling 10 grm. of beetroot with water and diluting down to 50 c.c. with slightly acid water. The following results were obtained, which are shown graphically in Fig. 2.

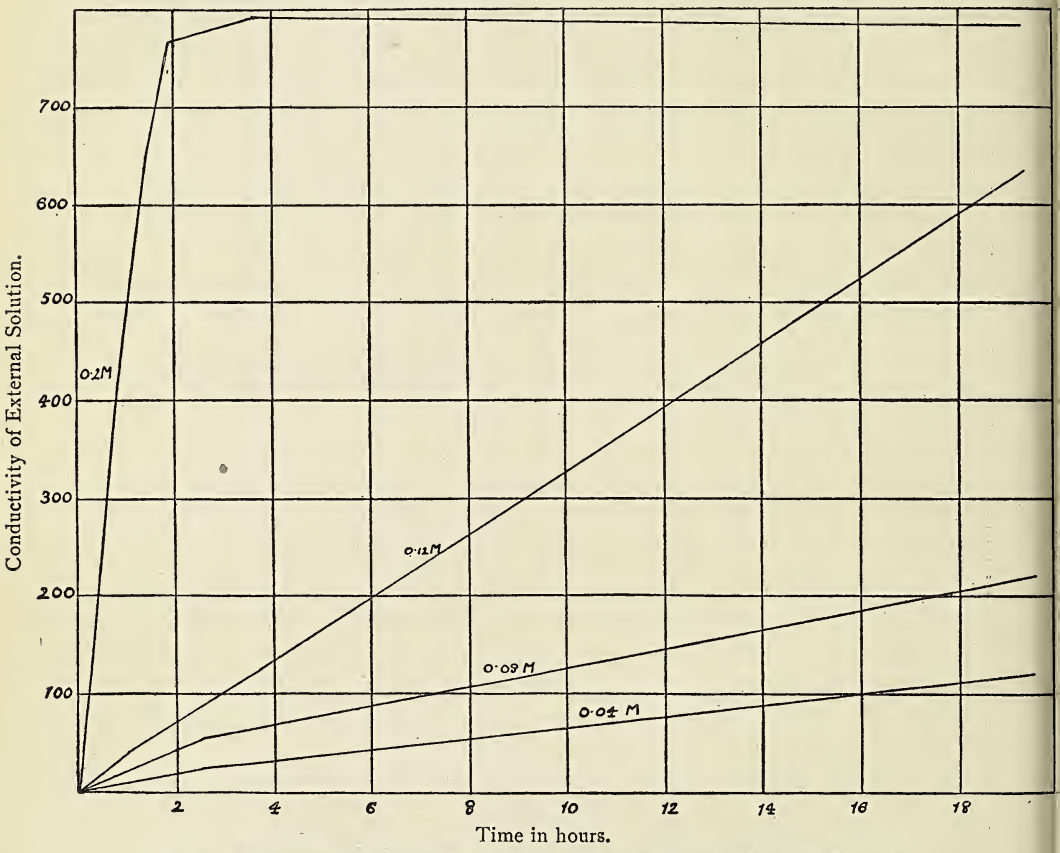

FIG. 2. Exosmosis of electrolytes from beetroot in solutions of isoamyl alcohol of various concentrations.

Ten grm. beetroot (twenty discs) in 50 c.c. 0·2 m. isoamyl alcohol.

Time in hrs. and minutes	*Conductivity.* (Sap extract = 900)	*Colour.* (Sap extract = 1)
0.15	120·8	0·5
0.50	412·4	1·0
1.25	649·5	1·0
1.55	766·0	1·0
3.35	795·5	1·0
19.15	782·0	1·0

0·12 m. isoamyl alcohol.

Time.	*Conductivity.*	*Colour.*
1.0	40·8	less than 0·05
3 45	128·8	0·1
19.15	636·5	1·0
48.0	750·0	1·0

0·08 m. isoamyl alcohol.

Time.	*Conductivity.*	*Colour.*
2.30	55·2	very slight
19.30	220·2	0·2
45.0	578·0	0·33

0·04 m. isoamyl alcohol.

Time.	*Conductivity.*	*Colour.*
2.30	23·5	almost colourless.
19.30	120·0	0·10
43.30	484·0	0·14
67.30	719·5	

It is clear that there is a rough parallelism between the exosmosis of electrolytes and of the sap pigment, but the measurement of the electrolytes can be accomplished much more easily and exactly than that of the pigment.[1]

Moreover, certain substances may react with the pigment and so change the colour, when colorimetric measurements would be rendered difficult or impossible.

Incidentally it appears from a comparison of the results obtained with potato and beet that the latter contains somewhat more electrolytes than the potato.

Another method which may be used depends upon the change of colour of the chlorophyll in leaves following on permeability changes in the cell which bring acids in contact with the chlorophyll. Chlorophyll when acted upon by weak acids gives derivatives of a yellowish or yellowish-green colour. The leaf of the Wood Sorrel (*Oxalis acetosella*) is especially useful in this respect, as the colour change from a bright green to a bright yellow is particularly easily observed.

The method has the disadvantage that it cannot be used kinetically. The only method of comparing the effects of different conditions is to note the time taken for the colour change to take place. It is, again, of very limited application as regards the tissue employed for experimentation. Further, any substance which neutralizes the acid in the cell-sap will prevent the colour change.

[1] Nevertheless, in the lower strengths of alcohol the pigment obviously diffuses out much less rapidly than the electrolytes. The question may arise here of differential permeability of the cell to different substances. With this question we may have occasion to deal later.

Thus, a molecular solution of pyridine and an 0·2 m. solution of isoamyl alcohol both produce rapid exosmosis of electrolytes from the cell; but, owing no doubt to the basic character of pyridine, leaves of *Oxalis* in the pyridine solution do not turn yellow, even when the greater part of the electrolytes have passed out of the cell. In the solution of amyl alcohol, on the other hand, the leaves turn yellow almost immediately after immersion in the solution.

The numbers obtained in the experiment described below show this.

2·5 grm. of leaves of *Oxalis acetosella* were placed in 50 c.c. of the following solutions:

Pyridine, m.

Isobutyl alcohol, 0·5 m.

Isoamyl alcohol, 0·2 m.

The conductivity of the external solution was measured from time to time and colour change in the leaves noted. The results obtained are given in the following table, and are also shown graphically in Fig. 3.

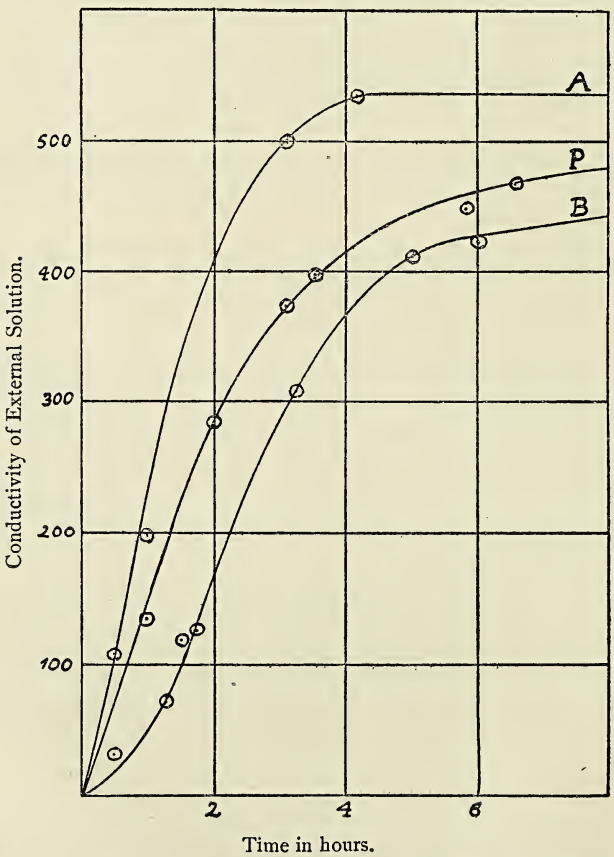

FIG 3. Exosmosis of electrolytes from leaves of *Oxalis acetosella*. A, in 0·2 m. isoamyl alcohol; B, in 0·5 m. isobutyl alcohol; P, in m. pyridine.

Pyridine, m.

Time. hrs. mins.	*Conductivity.*	*Colour.*
1.0	133·6	
2.0	284·2	
3.05	379·8	
3.30	397·6	
4.50	400·9	
5.50	448·8	
6.35	465·3	no general yellowing

Isobutyl alcohol, 0·5 m.

Time. hrs. mins.	*Conductivity.*	*Colour.*
0.30	33·8	
1.15	72·1	leaves turning yellow
1.35	119·8	leaves strongly yellow
2.35	127·3	
3.15	309·0	
5.0	412·6	

Isoamyl alcohol, 0·2 m.

Time. hrs. mins.	*Conductivity.*	*Colour.*
0.30	108·3	leaves strongly yellow
1.0	198·4	
1.25	242·4	
2.20	298·8	
3.0	500·0	
4.10	548·0	

We have also tried leaves of *Pelargonium*, in which a change to olive-green occurs with similar results. But it will be obvious from the examples with *Oxalis*, which gives a very marked colour change, that the method of electrical conductivity measurement is a much more accurate one for following permeability changes than the one depending on colour changes in the leaves. Moreover, it is independent of the nature of the substances in the external solution, whereas whether the colour change takes place in the leaf or not may depend on the chemical constitution of the substance in solution and have no relation to permeability.

Czapek's method, which consists in testing for the presence or absence of tannin in the leaf by means of caffeine, is very similar to the method based on the colour change in leaves. It has all the disadvantages of that method, and the additional one that it involves the use of a microchemical test in place of a very clear colour change easily observable by the naked eye.

For studying permeability changes in the cell in general, the electrical conductivity method here described is to be recommended in preference to earlier methods on account of its accuracy and its general applicability to all plant tissue and all non-electrolytic external solutions, and because it enables the changes taking place to be investigated in a manner impossible with the older methods. It must, however, be clearly understood that the results obtained by its means have direct reference to the permeability of the cell only as regards electrolytes; the results do not necessarily hold, and probably do not hold, for all substances.

In experiments with distilled water and low concentrations of substances, which are continued over a considerable time, a complication is introduced by secondary reactions, such as the effect of substances produced by bacterial action in the medium. We have therefore in these cases also employed another method in which the liquid is constantly renewed, whereby such secondary reactions are eliminated. But during the first twenty-four hours the two methods give practically identical results with the material we have employed. We may therefore postpone the description of this method until a later occasion.

Experimental Results.

In all experiments recorded in this section twenty discs of potato, weighing altogether about 9 grm., were kept in 50 c.c. of solution at 20° C., at which temperature also the conductivity measurements were made. The experiments were generally continued for 24 hours, but in some cases, of which mention will be made, the time of experimentation was considerably longer. The conductivity measurements were all corrected for depression in their value caused by the presence of non-electrolytes in the external solution in the manner described in the previous section of this paper. The conductivities are stated in arbitrary units. To obtain the actual specific conductivities the values given have to be multiplied by a constant.

When exosmosis is complete and equilibrium established between the electrolytes in the external solution and inside the plant cells, the corrected conductivity measurement is about 550, though, as already mentioned, this value is liable to variation corresponding with the weight of potato used.

As we have shown in a previous paper, electrolytes diffuse out from cells even when they are surrounded by distilled water. After 48 hours' immersion in conductivity water of twenty discs of potato, the conductivity of the liquid has increased to 144·8, only about a quarter of the value for complete exosmosis. The curve for distilled water is given in Figs. 4 and 6.

The substances employed in this investigation were the following:

Methyl alcohol	Hopkin and Williams, free from acetone.
Ethyl alcohol	'Absolute alcohol.'
Normal propyl alcohol	Kahlbaum.
Isobutyl alcohol	Kahlbaum.
Isoamyl alcohol	Kahlbaum.
Secondary I. Octyl alcohol, $CH_3(CH_2)_5CHOH{\cdot}CH_3$	Kahlbaum.
Acetone	British Drug Houses, Analytical Reagent.
Chloroform	Hopkin and Williams, pure.
Ether	Tyrer, S. G. 0·717.
Chloral hydrate	Kahlbaum.

Urethane	Kahlbaum.
Aniline	Hopkin and Williams, pure.
Pyridine	Hopkin and Williams, pure.

I. *The Monohydric Alcohols.*

The effect of methyl alcohol on exosmosis of electrolytes was examined in a large number of concentrations varying from $\frac{m}{100}$ to 10 m. The exosmosis during the first 24 hours is exhibited in Fig. 5. It will

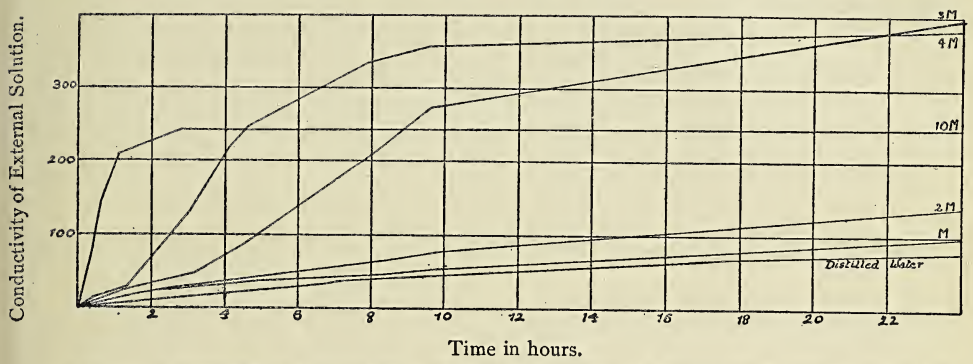

FIG. 4. Exosmosis of electrolytes from potato immersed in solutions of methyl alcohol of various concentrations. The conductivity values are those actually measured uncorrected for the depression in their value resulting from the presence of the methyl alcohol.

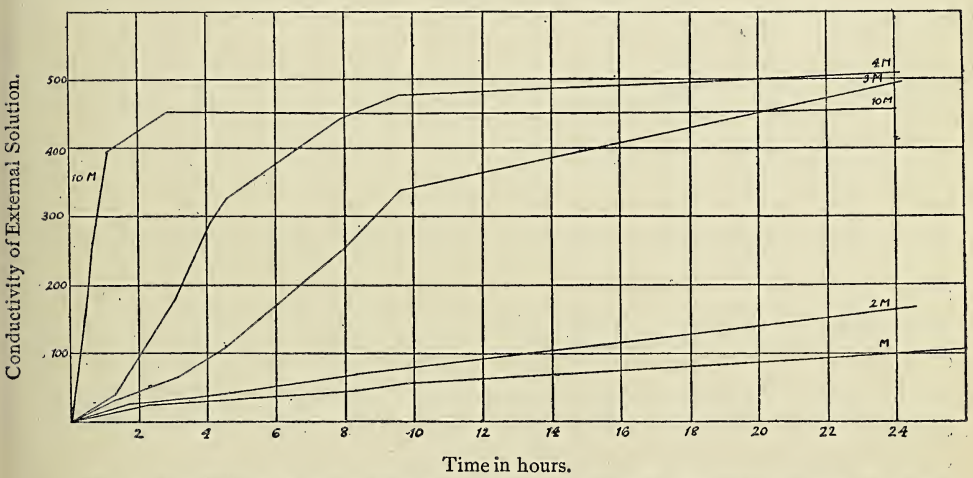

FIG. 5. Exosmosis of electrolytes from potato in solutions of methyl alcohol of various concentrations. The conductivity values in this and succeeding figures are corrected for the presence of non-electrolytes.

be observed that throughout, with increasing concentration, there is an increased rate of exosmosis. From the lowest concentrations up to molecular concentration the permeability is increased very little with increasing concentration; nevertheless, in these lower concentrations increase in

strength of the alcohol does result in increased exosmosis. In a concentration of 2 m. methyl alcohol the permeability increases at a considerably more rapid rate than in the case of a solution of m. concentration, though the difference is only noticeable after three or four hours' immersion in the solutions. A solution of 3 m. methyl alcohol produces more marked exosmosis from the first, but after three hours the rate of exosmosis gradually increases for some hours until this rate is reduced owing to reduction in the concentration of electrolytes inside the cell, the maximum exosmosis not being reached till about 16 to 20 hours.

With 4 m. methyl alcohol the same state of affairs is observable, the permeability increasing more than in the case of 3 m. from the beginning. The marked increase in the rate of exosmosis begins sooner, after about an hour and an half, and the total exosmosis approximates to the maximum

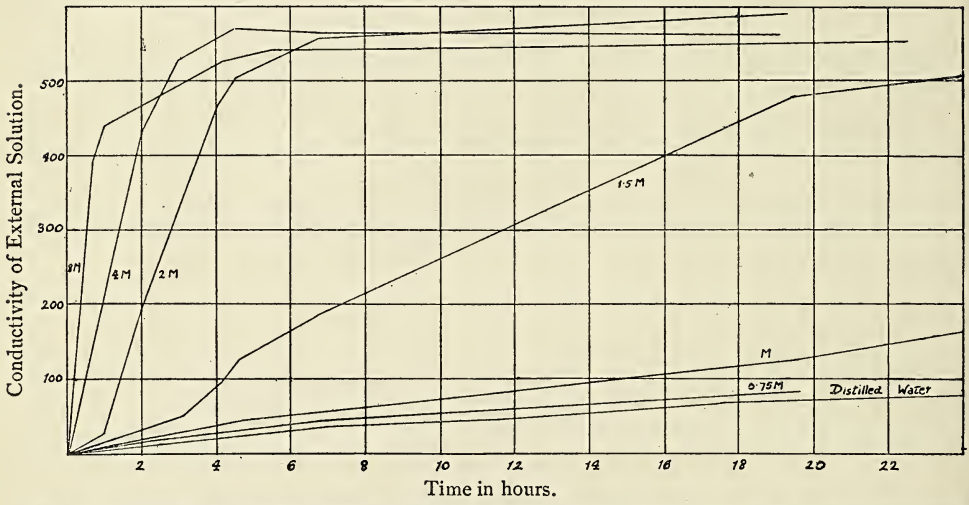

FIG. 6. Exosmosis of electrolytes from potato in solutions of ethyl alcohol of various concentrations.

after 9 or 10 hours' immersion. Higher strengths of alcohol show an increased rate of exosmosis during the early stages, the preliminary period during which the permeability increases comparatively slowly being reduced in duration with increasing concentration of alcohol. In 10 m. methyl alcohol, as the accompanying curves show, the preliminary stage must occupy considerably less time than 20 minutes.

The most numerous series of concentrations was used in the case of ethyl alcohol. The following were the solutions employed: 0·0, 0·75 m., 1·0 m., 1·5 m., 2 m., 4 m., 6 m., 8 m. The results are shown in Fig. 6. They are exactly similar to those obtained with methyl alcohol, except that a smaller molecular concentration is required to produce the same effect on the permeability of the cell. Thus a 1·5 m. solution of ethyl alcohol produces changes in permeability of the cell similar to those produced by

a 3 m. solution of methyl alcohol. The curves show how, as a result of the increased permeability produced by increased concentration, the maximum value of the exosmosis is reached sooner with increasing concentration. Although the curves are only given for the first 24 hours, measurements were made over a considerably longer period. The following table shows that eventually complete exosmosis of electrolytes takes place even with considerably weaker solutions than 2 m., which, according to Czapek, is the limiting concentration above which exosmosis takes place and below which it does not :

Concentration of solution.	*Conductivity after* 2 hrs.	4 hrs.	12 hrs.	24 hrs.	48 hrs.
8 m.	466	525			
6 m.	450	516	538		
4 m.	430	552	562		
2 m.	194	464	570	570	
1·5 m.	33	90	307	509	
m.	20	38	82	156	390
0·75 m.	16	29	59	110	370
Distilled water	10	21	50	79	141

The above table shows that it is impossible to speak of a critical concentration required to produce exosmosis of the cell contents. The rate of exosmosis, indeed, depends on the concentration, but diffusion out from the cell takes place in all concentrations. Czapek's value of 11 per cent. by volume (about 2 m.) as the critical concentration required to produce exosmosis is therefore meaningless. A different result is obtained by Czapek's method according to the time one supposes is required to produce equilibrium conditions. Even with distilled water, exosmosis continues slowly until bacterial action sets in and measurements no longer have reference to the simple action between potato tissue and water. So that by assuming equilibrium to be established after any selected time, any desired value can be obtained as a value of the critical concentration required to produce any particular amount of exosmosis.

But one matter exhibited by Czapek is confirmed in this investigation, which is that any member of the homologous series of primary alcohols has a greater effect on exosmosis than a lower member of the series in the same concentration.

Thus experiments made with normal propyl alcohol show that this alcohol in 0·6 m. concentration is equivalent in regard to its action in producing exosmosis to ethyl alcohol of 1·3 m. concentration. The general course of events is exactly similar to that with the two lower alcohols. The accompanying curves (Fig. 7) show the exosmosis during the first 24 hours for concentrations of normal propyl alcohol from 0·4 m. to m. It should be noted, however, that in the case of the 0·4 m. solution, the maximum exosmosis was approached after 48 hours, although from an inspection of the curves for the first 24 hours this differed little from that

for distilled water. Distilled water after 48 hours only shows a conductivity of about a quarter of this value.

The experiments with higher alcohols, isobutyl alcohol, isoamyl alcohol, and a secondary octyl alcohol, all gave regular results, which might be ex-

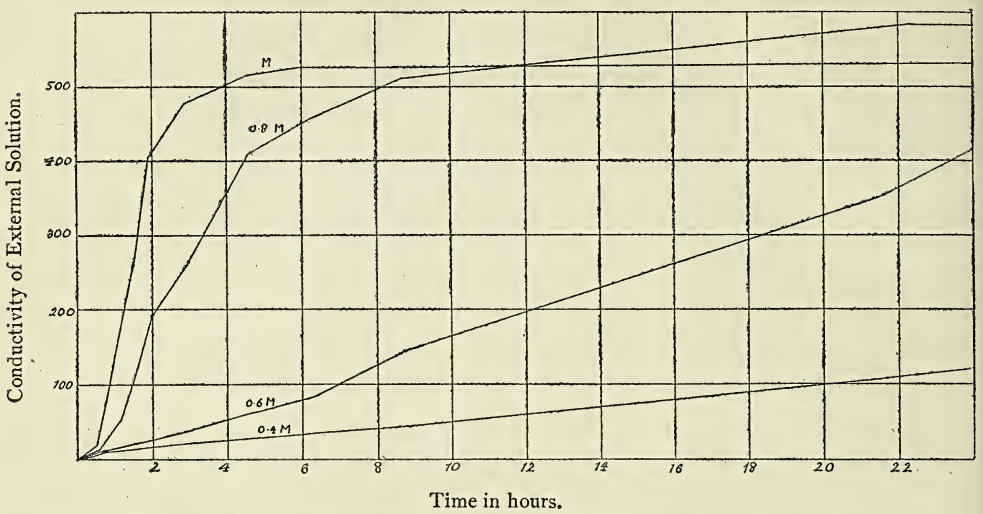

FIG. 7. Exosmosis of electrolytes from potato in solutions of normal propyl alcohol of various concentrations.

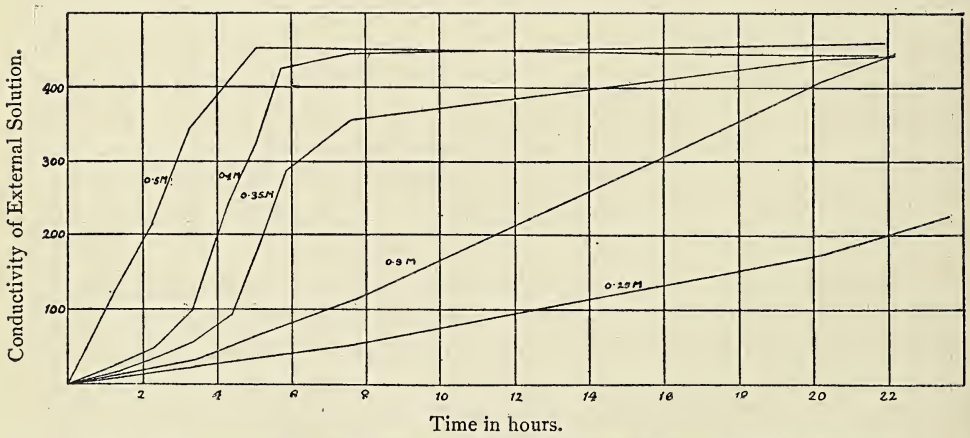

FIG. 8. Exosmosis of electrolytes from potato in solutions of isobutyl alcohol of various concentrations.

pected from those already cited. The results obtained are shown graphically in the accompanying figures. By comparison of the various curves it will be noticed that the action on the permeability of the cell increases with increasing complexity of the molecule. Thus, with the octyl alcohol used, a solution of concentration of only 0·008 m. has a very rapid action in increasing the permeability of the cell.

It might be urged that in the case, for instance, of isobutyl alcohol, concentrations below 0·3 m. produce an exosmosis not differing greatly during the first 24 hours from that produced by distilled water, while solutions above that concentration produce considerably greater exosmosis, and that

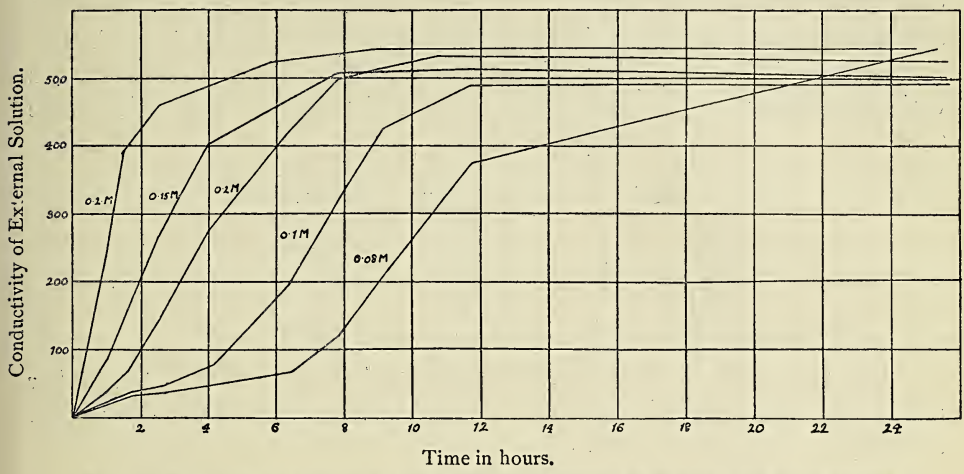

FIG. 9. Exosmosis of electrolytes from potato in solutions of isoamyl alcohol of various concentrations.

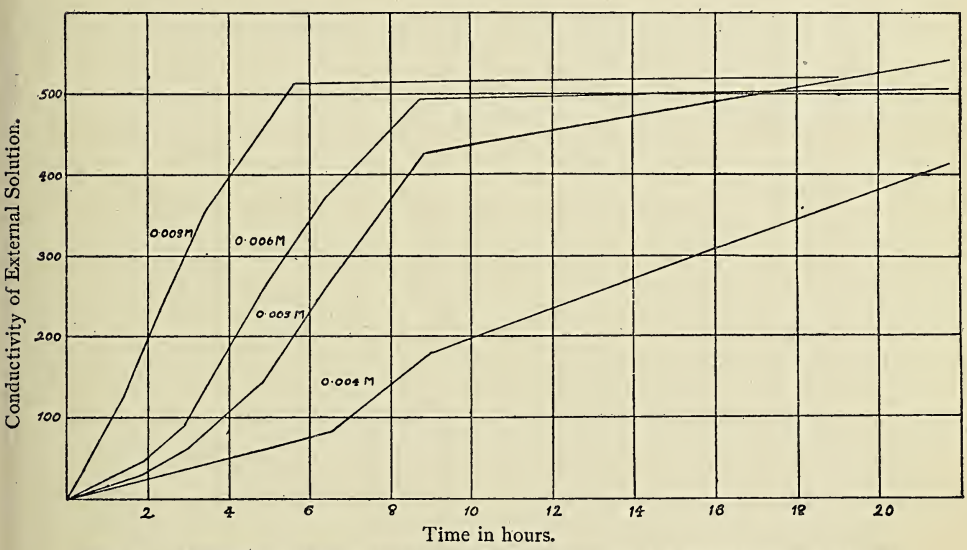

FIG. 10. Exosmosis of electrolytes from potato in solutions of a secondary octyl alcohol of various concentrations.

therefore 0·3 m. is the critical concentration. But there is no reason why the exosmosis during 24 hours should be chosen rather than that during any other time. If 48 hours were chosen, a different critical value would be found; and if a shorter time, as for example 4 hours, were selected, a value

of about 0·4 m. would be obtained; and if 2 hours were chosen it would be 0·5 m.

But even if it were possible to find a critical value for the concentration in this way the values so obtained for different alcohols would not support Czapek's surface-tension theory. Thus, if 0·3 m. is regarded as the critical concentration of isobutyl alcohol required to produce exosmosis of electrolytes, we obtain by comparison with other curves the following values for the various alcohols employed. The values of the surface tension of these solutions are taken from Czapek's paper.

Alcohol.	*Concentration.*	*Surface tension.*
Methyl	2·5 m.	0·79
Ethyl	1·4 m.	0·74
Normal propyl	0·65 m.	0·66
Isobutyl	0·3 m.	0·60
Isoamyl	0·09 m.	0·59
Secondary octyl	0·003 m.	no data

Thus a greater lowering of the surface tension in the case of the higher alcohols as compared with the lower is required to produce the same effect on the permeability of the cell.

II. *Chloroform, Chloral Hydrate, Ether, Urethane.*

Czapek himself admits that chloroform and chloral hydrate do not follow his surface-tension rule, and he therefore attributes to them a specific

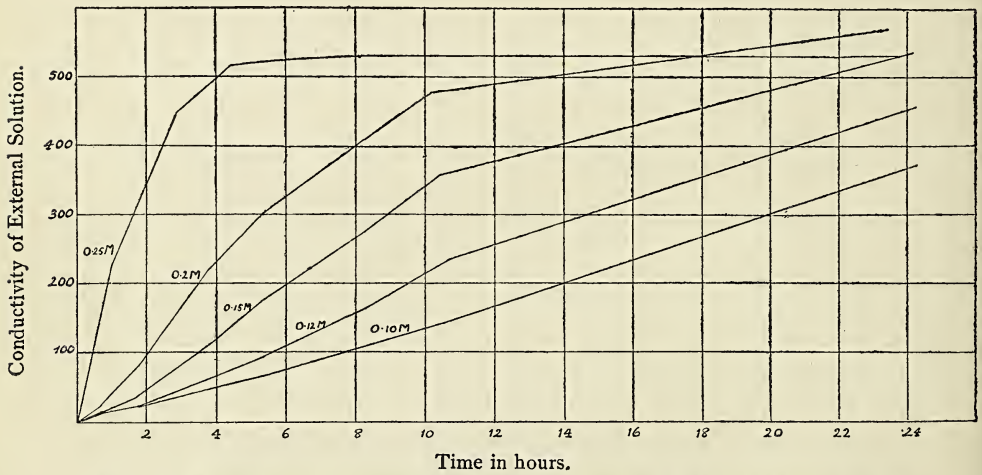

FIG. 11. Exosmosis of electrolytes from potato in solutions of chloral hydrate of various concentrations.

toxic action. Ether, on the other hand, is found to follow the rule. It is difficult to understand what is meant by a specific toxic action, and why, if chloroform and chloral hydrate have a specific toxic action, ether, or for that matter any other substance, should not also have a specific toxic action.

Fig. 11 shows the relation between time and exosmosis for a variety of concentrations of chloral hydrate. It will be observed that the general course of events is similar to that in the case of the alcohols, and that here also it is impossible to find a definite critical concentration above which exosmosis takes place and below which it does not. Chloroform, ether, and urethane give similar results, and it is unnecessary to reproduce the curves here. According to Czapek the surface tension of 0·1 m. chloral hydrate solution is about 0·96. There is no question of the surface-tension rule holding in this case.

As regards Czapek's theory of a specific toxic action in chloral hydrate and chloroform which does not exist in the case of the monohydric alcohols, such a view simply resolves itself into the assumption that in the case of the monohydric alcohols surface tension is the essential factor in producing exosmosis, in the case of chloral hydrate and chloroform it is some other factor. The theory that exosmosis is dependent on one physical factor, and one only, actually breaks down under the test of its author's own observations. We shall discuss this matter more fully in the theoretical part of this paper.

III. *Acetone.*

Experiments with acetone produced results similar to those already recorded for other organic substances. In the case of acetone more than in

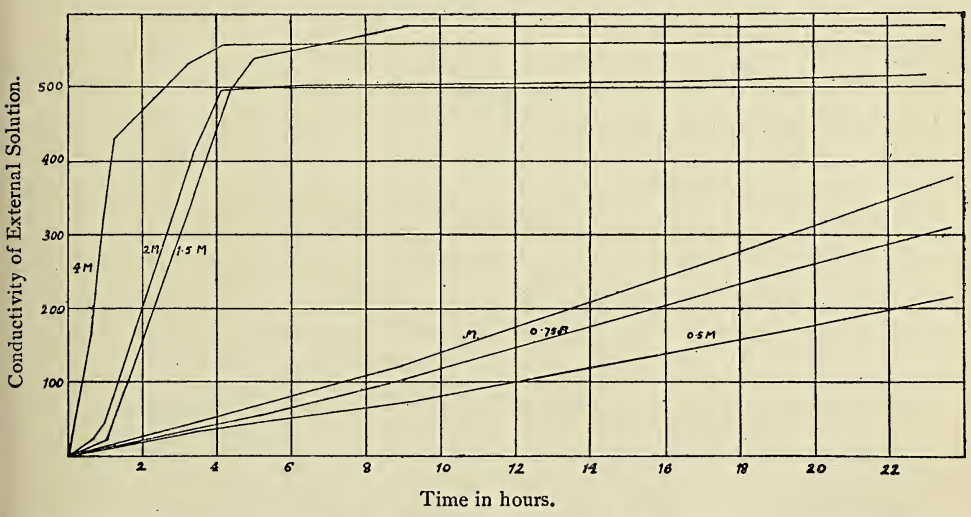

Fig. 12. Exosmosis of electrolytes from potato in solutions of acetone of various concentrations.

any other might it be suggested that there exists a critical concentration with regard to exosmosis between m. and 1·5 m. (cf. Fig. 12). But it will be observed that even here exosmosis continually rises in the lower strengths of solution, and in 0·5 m., the lowest concentration examined, the exosmosis

rises continually to its maximum value. The surface tension of this solution, according to Czapek, is 0·86, so that even if there were such a thing as a critical concentration it would be lower than 0·5 m., and its surface tension higher than 0·86. Specific toxic action might again be invoked to explain this deviation from the 0·68 rule, but, according to Czapek, acetone obeys the law.

IV. *Aniline and pyridine.*

These two aromatic substances are both basic, and have the same surface tension in contact with air. The extent to which their solutions increase

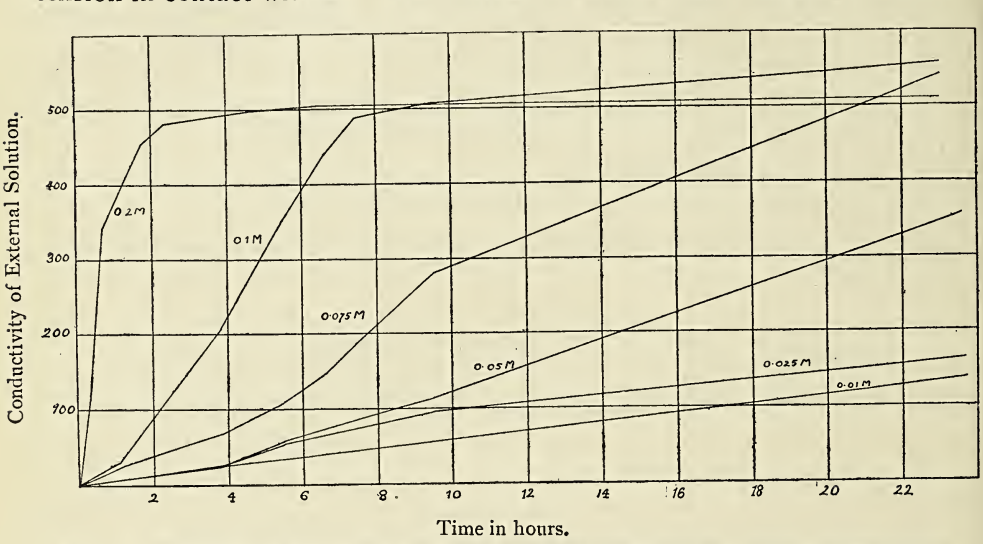

FIG. 13. Exosmosis of electrolytes from potato in solutions of aniline of various concentrations.

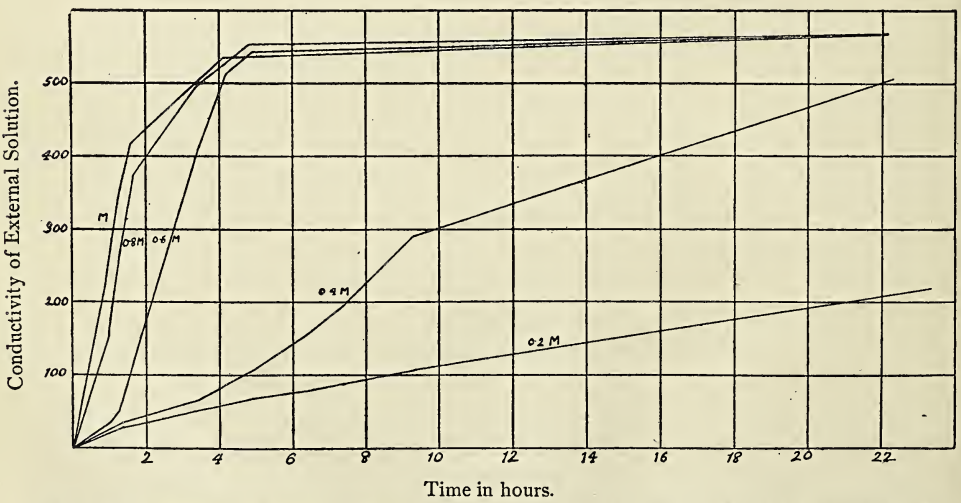

FIG. 14. Exosmosis of electrolytes from potato in solutions of pyridine of various concentrations.

the permeability of potato cells to electrolytes is shown diagrammatically in Figs. 13 and 14. It will be observed that the curves are similar to those obtained with other organic substances examined, but that, in spite of the equality of the surface tensions of the two substances, aniline is considerably more efficient in bringing about exosmosis than pyridine.

No doubt here again Czapek would refer to the specific action of these basic substances, but that of course is only another way of stating that the surface tension is not the deciding factor. It may be mentioned that with both these substances the external liquid becomes coloured a bright yellow colour when exosmosis is complete, as contrasted with the dull pale brown colour produced by the monohydric alcohols.

A General Criticism of Czapek's Theory of the Plasma Membrane.

The criticisms to which Czapek's work has been subjected by earlier writers deal chiefly with his theoretical considerations. In this contribution to the subject we have dealt mainly with the trustworthiness of the experimental evidence on which those conclusions are based. The steps, both experimental and theoretical, which lead up to Czapek's theory of the plasma membrane are these:

1. The measurement of the concentration of a solution of a substance required to bring about exosmosis from plant tissue.

2. The conclusion that there is a critical concentration below which exosmosis from the cell does not take place.

3. The measurement of the surface tension of the solutions used.

4. The conclusion that the surface tension of the solution of critical concentration against air is 0·68, that of pure water being taken as unity.

5. The conclusion that therefore the surface tension of the outer layer of the plasma against air is also 0·68.

6. The measurement of the surface tension of emulsions of neutral fats shows that their surface tension cannot be lowered below 0·68.

7. The conclusion from this that the substances in the outer layer of the plasma important in permeability phenomena are the neutral fats.

We propose now to consider each one of these steps in detail.

1. We have shown in the part of this paper dealing with methods that Czapek's method is crude and cannot be used for following changes quantitatively. By its use only very approximate results are to be expected. Sections of leaves are left in the solutions until an equilibrium condition has been reached. There is no definite time allowed for equilibrium to be obtained, the only criterion for equilibrium being apparently that the desired result is reached. Thus, in the case of tertiary butyl alcohol Czapek required a solution of 3 per cent. by volume to produce exosmosis of tannin

from *Echeveria* leaves. After one day, however, this had not happened, so the experiment was left for three days, when it had.

On the other hand, certain anthocyan-containing objects were not used because they produced exosmosis in less than twenty-four hours from diluter solutions than they should have done according to Czapek's theory. The long time required to produce the desired result in the case of *Echeveria* leaves in tertiary butyl alcohol Czapek explains as due to the slow rate of diffusion of the alcohol ; the rapid exosmosis produced from various tissues containing anthocyan is attributed by Czapek to secondary injury.

It is obvious that with such methods of inquiry any desired result can be obtained, and even for these reasons alone it seems to us that Czapek's results are open to grave suspicion.

2. The results of our own experiments described in this paper indicate that there is no critical concentration below which no exosmosis of cell contents takes place. In our chief experiments we have measured the exosmosis of electrolytes, which can be done with much more accuracy than that of anthocyan or tannin. It is clear that after any time exosmosis above a certain value will have resulted in some solutions and not in others, and it might be supposed that a solution which just produced a certain quantity of exosmosis in a certain time had the critical concentration for exosmosis. This concentration would, however, vary with time chosen.

It may be noted here that Koltzoff (6) has already criticized Czapek for neglecting the time of reaction in his experiments. To this Czapek has replied (4) that he deals only with equilibrium conditions and has therefore eliminated the factor. Our experiments show, however, that Czapek could only have had equilibrium conditions in those solutions in which exosmosis had occurred, and that exosmosis took place, if slowly, in those where it was not measurable by Czapek's imperfect method. The time factor is there; it cannot be eliminated merely by neglecting it. From these considerations we do not accept Czapek's supposition that there is a definite critical concentration required to produce exosmosis.

3. Koltzoff (6) and Vernon (11) have already called attention to the fact that Czapek measures the surface tension of his solutions against air, whereas what he is really concerned with in his experiments is the surface tension between the solutions and the plasma. Czapek assumes that the lowering of the surface tension of a liquid in contact with air, due to dissolved substances, runs parallel to the lowering of the tension when in contact with another liquid. Vernon quotes Clerk Maxwell, who states that by no known means can the surface tension between two immiscible liquids be determined from their surface tensions in contact with air. Thus, the relation between Czapek's surface-tension measurements and the surface tensions actually existing in his experiments is unknown, and hence it is

impossible to draw general conclusions from such measurements. To this criticism Czapek has made no satisfactory reply.

4. Czapek shows that seven substances out of twenty-nine examined by him have a surface tension far greater than 0·68. This has also been pointed out by Vernon. To this criticism Czapek (4) replies that he has already explained the deviation of these substances from the 0·68 rule as being due to their 'specific toxic action', although this is absolutely nothing more than stating the same fact in different words. Vernon suggests that in the cases of these exceptions, the substances probably dissolved the lipoid substances in the membrane, and that it is to be explained on the Meyer-Overton law of partition coefficients. Vernon points out that the same order of concentrations is obtained for tannin exosmosis as for narcosis, in which the results are explicable as due to lipoid substances.

But Schryver (8) suggests that there is no need to assume the results for narcotics as due to lipoids. This author shows that if substances are arranged in the order of their power of disintegrating a cholate gel, that order is exactly the same as found by Czapek in regard to tannin exosmosis, and as Schryver says, the cholate gel 'cannot, by any extension of the meaning of the term, be described as a lipoid', and in any case Czapek's 'purely mechanical conception of cytolysis is clearly no longer tenable'.[1]

In any case Czapek's assumption of specific toxic action is really most destructive of his whole case, for it is an admission that factors other than surface tension may in some cases be the predominating ones in regard to exosmosis.

Again, it cannot even be conceded that solutions which produce equal exosmotic effects have the same surface tension in contact with air. The following table is compiled from the results recorded in this paper. The values are only approximate, but they are at any rate considerably more trustworthy than those of Czapek.

Substance.	*Strength required to produce same exosmotic effect.*	*Surface tension against air (from the data of Czapek and Traube).*
Methyl alcohol	3·2 m.	0·76
Ethyl ,,	1·6 m.	0·70
Normal propyl alcohol	0·7 m.	0·64
Isobutyl ,,	0·33 m.	0·58
Isoamyl ,,	0·095 m.	0·58
Secondary octyl ,,	0·0048 m.	no data
Chloral hydrate	0·17 m.	0·94
Urethane	0·5 m.	0·77
Acetone	1·3 m.	0·69
Aniline	0·08 m.	no data
Pyridine	0·45 m.	,,

[1] On the other hand, we do not regard Schryver's experiments as having any direct bearing on cell problems. The comparison of the cholate gel with the cell rests on the similarity of the power of certain substances to disintegrate the gel, and their power of producing exosmosis from the cell.

It is obvious from these numbers that the exosmosis of electrolytes from the cell, and consequently the permeability changes produced by them, cannot be referred to surface tension alone.

The rate at which the external liquid displaces gases in the intercellular space system may be of importance in this connexion. It is a matter of common knowledge that alcohols displace the air in the intercellular spaces very rapidly, while aqueous solutions do so slowly. Hence cytologists in fixing plant material with aqueous fixatives pump out the air from the tissue in order that the fixing fluid may be brought in contact with the cell membranes bordering on the intercellular spaces. In Czapek's experiments and our own the same considerations hold. The lower the surface tension against air, the easier is the air displaced from the intercellular spaces (cf. Czapek's method of measuring surface tension). Hence in the lower strengths of solution used the rate of exosmosis may be lower than it would be if the air were displaced rapidly as in the higher concentrations of solution. However, with thin sections of tissue the difference is not likely to be very great.

5. The assumption is made that if solutions whose surface tension in contact with air is 0·68 are just strong enough to produce exosmosis from the cell, therefore the surface tension of the outer layer of the protoplasm towards air must also be 0·68. As far as we are aware there is not a shred of evidence in support of this assumption. In his later paper (4) Czapek explains this by supposing that at the interface the substance which most lowers the surface tension will displace those which lower it less. Even if this statement is true, it appears to have no bearing whatever on the problem in hand. For methyl alcohol, for instance, lowers surface tension less than ethyl alcohol, and according to Czapek's assumption ethyl alcohol, if it were present in the plasma membrane, would not be replaced by methyl alcohol in whatever concentration, while if ethyl alcohol were outside and methyl alcohol inside the plasma this would be replaced by ethyl alcohol even if it were below the critical concentration. In short, it would be impossible to accept this step in Czapek's theory of the plasma membrane, even if the previous steps in the argument could be accepted.

6. It is difficult to understand what Czapek means when he asserts that emulsions of neutral fats in water will not lower the surface tension of water below 0·68, as he himself quotes several examples of fats, emulsions of which with water lower the surface tension very considerably below this value.

7. Finally, even if we could accept the evidence that the surface tension of the plasma membrane in contact with air is 0·68, and if it were a fact that fat emulsions cannot lower the surface tension of water in contact with air below 0·68, this is no reason for concluding that the essential substances in the protoplasm as regards its surface tension are neutral fats. For as Czapek

himself admits, substances which could lower the surface tension more than this might be present in the plasma membrane in diluter solution. Thus, for example, he says: 'Meine Beobachtungen widerstreiten nun nicht direkt der Annahme, dass verdünnte Emulsionen von Lezithin und Cholesterin das eigentümliche Verhalten der Plasmahaut bedingen, doch bringen sie auch keinen weiteren Grund dafür bei, dass die Annahme Overtons die allein richtige sein könne.' Similarly, this writer also admits that proteins may be present in the plasma membrane.

From this review of the details of Czapek's work on the plasma membrane, it is clear that neither the experimental evidence nor any part of the theory based upon it can be accepted.

The Application of the Laws of Mass Action to the Question of Exosmosis from Plant Tissue in the Case of Irreversible Changes of Permeability.

When plant tissue is immersed in a solution of a substance we may conclude that after a certain time, owing to diffusion through the intercellular spaces of the tissue and through the cell-walls, a large part of the outer layer of the protoplasm of the cells will be in contact with the solution. If the solute acts either physically or chemically with one or more substances in the protoplasm, the rate at which it does this will depend upon the concentration of the solute at the limiting surface, and also upon the concentration at the limiting surface of the substance with which it reacts. Other factors, such as temperature for instance, may also influence the rate of reaction. The concentration of the reacting substances at the interface between the two phases will depend upon the extent to which the surface tension is lowered according to the principle of Willard Gibbs, but this is only one of a number of factors which may affect the concentration at the interface. Schryver (7) has pointed out that in an action resembling somewhat the one under consideration, namely that of the disaggregating action of salt solutions on globulins, the rate of diffusion of the substances in solution must also influence the rate of reaction. For if diffusion is slow the accumulation of solute in the surface layer may fall below that given by the Willard Gibbs rule, owing to the removal of the substance in the action at the interface at a quicker rate than it can be replaced by diffusion of solute from other parts of the solution. As the rate of diffusion will depend on the viscosity, Schryver concludes that 'the disaggregating action of salt solutions on the globulins is a function of two physical constants of the solutions, viz. the viscosities and the surface tensions'. But of course it is understood that the salt solution has an action on the globulin. The surface tensions and viscosities influence the rate of the reaction; they do not decide whether an action takes place or not. Other factors may come into

play also; thus the effect of electric charges at the surface in influencing the concentration there of the reacting substances cannot be neglected.

While we may conclude that the rate of the action increasing the permeability of the cell is dependent on the concentration of the active substance at the surface of the cell protoplasm, we cannot say what function of the concentration the rate of reaction is. If the action were a purely chemical one we could say from the law of mass action that the rate of reaction is directly proportional to the active mass of the substance in the limiting layer. But besides chemical actions others are possible which might alter the state of aggregation of substances in the protoplasm—by solution, for instance, or by precipitation of a substance from a colloidal solution. In such cases the disaggregation effect is not simply proportional to the concentration of the solution. A reference to the solution or precipitation of certain proteins such as gliadin by alcohol is sufficient to show this, and we may mention Schryver's work (9) on the cholate gel in the same connexion, in which he shows a most curious relation existing between concentration of solutions and their rate of disaggregation of the gel.

Moreover, as far as we know, any substances present in the protoplasm are also present in the limiting layer, and it is quite possible that different substances in the plasma may be affected in different ways by the same solute.

As it is doubtful what relation the concentration of the solute bears to its concentration at the interface, and as it seems likely that this latter quantity will vary at different parts of the tissue owing to variation in rates of diffusion connected with differences in position of cells in different parts of the tissue, it is difficult to draw conclusions as to what function of the concentration of the solution is the change of permeability produced by it.

If, however, we consider a case in which the concentration is so high that the quantity of solute used up is negligible, and if we make the assumption that the concentration at the surface of the plasma membrane remains constant throughout the reaction time, we can from the ordinary laws of mass action obtain an equation connecting the exosmosis with time, and the curve representing this equation is of the same form as those obtained in the experiments described in this paper.

For we may suppose that the increase in permeability of the cell is due to a change in a substance present in the outer layer of the protoplast, whose active mass before the experiment is M. Let us suppose that at any time t of the total amount M, a part m has been destroyed.

The rate at which further destruction of the substances takes place will depend on the quantity left, $M - m$, and on the concentration of the external solution.

Hence $$\frac{dm}{dt} = \alpha f(M-m)\,\phi(C),$$

where $f(M-m)$ is a function of the mass of substance left in the membrane unchanged and $\phi(C)$ is a function of the concentration of the external solution, α being a constant.

If this concentration is so high that only a small quantity is used up throughout the experiment, $\phi(C)$ is a constant for any particular concentration. Also it may be assumed that the rate of change of the substance in the membrane is directly proportional to the amount of it left, when the equation therefore becomes

$$\frac{dm}{dt} = A(M-m),\ A \text{ being a constant,}$$

$$\text{or} \quad \frac{dm}{M-m} = A\,dt;$$

whence, integrating between limits,

$$\log\frac{M-m}{M} = -At$$

$$\text{or} \quad \frac{M-m}{M} = e^{-At}$$

$$\text{and} \quad m = M(1-e^{-At}).$$

Now the rate of exosmosis will be proportional to the amount of the membrane substance destroyed, and also to the difference in concentration of electrolytes inside and outside the tissue.

If at the time t the concentration of the electrolytes in the outer solution is s, and inside the cell s', the rate of exosmosis is

$$\frac{ds}{dt} = \beta m(s'-s),$$

where β is a constant.

If S is the concentration of electrolytes inside the cell at the beginning of the experiment, and if u and v are the respective volumes of the tissue and external solution

$$u(S-s') = vs$$

$$\text{or} \quad s' = S-\frac{vs}{u};$$

whence

$$\frac{ds}{dt} = \beta M(1-e^{-At})\left(S-s\frac{u+v}{u}\right)$$

and

$$\int\frac{ds}{S-s\dfrac{u+v}{u}} = \int\beta M(1-e^{-At})\,dt,$$

from which, integrating between limits, we have

$$-\frac{u}{u+v}\left\{\log\frac{S-s\frac{u+v}{u}}{S}\right\}=\beta M\{t+\tfrac{1}{A}(e^{-At}-1)\},$$

which may be written in the form

$$-B\log(1-ks)=D\left(\tfrac{1}{A}e^{-At}+t-\tfrac{1}{A}\right),$$

where s is the concentration of the external solution at any time t and A, B, D and k are constants depending on the concentration of the external solution, the quantity of tissue used, and the volume of solution.

The curves given by this equation are of the same form as those obtained by us for the exosmosis of electrolytes from plant tissue. In the accompanying figure we give the curves obtained in which the constants B,

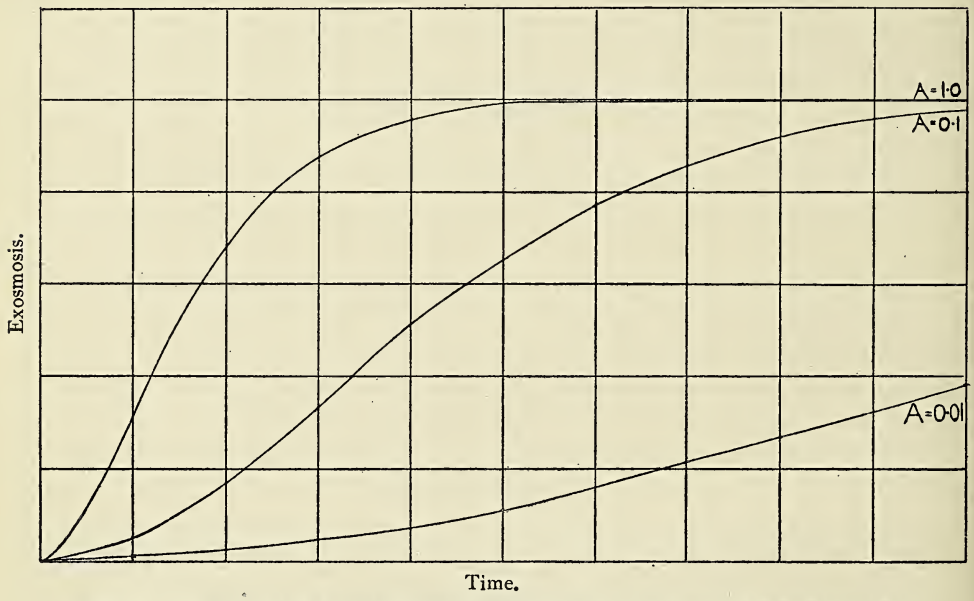

Fig. 15. Curves obtained from theoretical considerations of the relation between time and quantity of exosmosis from plant tissue.

D, and k are put equal to unity, and A, which depends on the concentration of the external solution, is given different values. A comparison of these curves with those for the higher concentrations of each substance experimented with will show how similar they are.[1]

It will be observed that the curve consists of three regions: first a region in which the rate of exosmosis is gradually increasing, then

[1] The system is certainly more complex than we have assumed here. For instance, we have neglected the rate of diffusion of substances in the system. In thick discs and cylinders of tissues this becomes a very important factor, as some of our unpublished researches show. The equation given can only be considered a very rough approximation.

a region during which the rate remains more or less constant, and a final region in which the rate gradually gets less and less. The first part of the curve corresponds to the period during which the impermeable substance in the plasma is rapidly breaking down. The rate at which this takes place gradually gets less until the changed substance remains practically constant in amount; the difference in concentration of electrolytes inside and outside is then the chief factor in determining the rate of exosmosis. This period corresponds to the last part of the curve, in front of which we have the approximately straight transitional part.

With very high concentrations the first part of the curve is extremely short, and in practice readings have to be taken very frequently to show it, and it may be too brief to show graphically on the curves at all.

In all these considerations we have assumed that the concentration of the external solution is so high that it remains practically unchanged throughout the experiments. But if this should become appreciably less as a result of the action, the rate at which the membrane is changed will be much lessened as time goes on. Consequently the rate of exosmosis will be considerably less than it would be if the concentration of the external solution remained constant. The curve we should expect under such conditions is precisely that obtained by us for the lower concentrations of the external solutions we have employed.

Summary.

1. The action of various organic substances on the permeability of plant cells has been investigated by estimating the exosmosis of electrolytes from plant tissue immersed in aqueous solutions of the substances. The exosmosis is estimated by measuring the rise in electrical conductivity of the solution in which the tissue is contained, due regard being had to the depression of conductivity resulting from the presence of the non-electrolytic experimental solutions.

2. The method is compared with various methods employed by former workers, and evidence is brought forward to show that it is more exact and of more general application than those earlier methods.

3. With each organic substance the rate of exosmosis depends on the concentration of the substance employed; the higher the concentration the more rapid the exosmosis.

4. Equimolecular solutions of different substances do not bring about the same exosmosis. Thus, in the homologous series of the monohydric alcohols, the more complex the molecule the greater the exosmosis produced by solutions of equimolecular strength.

5. The rate of exosmosis produced by a solution is not a function of its surface tension alone.

6. It is impossible to find a critical concentration of a solution below which exosmosis of electrolytes will not take place.

7. Each of the assumptions leading up to Czapek's theory of the plasma membrane is discussed, and it is shown that none of them can be accepted.

8. An attempt has been made to deduce a mathematical expression connecting time and exosmosis, and it is shown that the curve representing the equation so obtained from theoretical considerations is of an exactly similar form to those obtained in actual experiments.

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September, 1916.

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On Stigeosporium Marattiacearum and the Mycorrhiza of the Marattiaceae.

BY

CYRIL WEST, B.Sc., F.L.S.

With Plate III and nine Figures in the Text.

NOTWITHSTANDING the attention which has for many years been bestowed upon the Marattiaceae and the frequent references to the presence of a mycorrhiza, our present knowledge of the endophytic fungi which are commonly associated with the roots of this important group of Ferns is very incomplete, and, apart from the observations of Kühn and of Gallaud, practically nothing is known of their morphological and biological characters or of their systematic position.

In his thesis on the Marattiaceae, Kühn (28) reserved a section for a short account of the fungi which he observed in roots of *Angiopteris evecta*, Hoffm., *Kaulfussia aesculifolia*, Bl., and *Marattia alata*, Sm., and, in the case of the last-named genus, claimed to have succeeded in isolating an endophyte which produced spores when grown on artificial nutrient media. But, for reasons mentioned below (p. 90), it seems certain that this botanist was dealing not with the endophytic but with the epiphytic fungal flora of the marattiacean roots. Gallaud (22, p. 40) very briefly described the endophyte which he found in the roots of *Angiopteris Durvilleana*.

The present writer, who was fortunate in having abundant material at his disposal, has therefore attempted to give a more detailed account of these fungi.

Material and Methods.

Material of *Angiopteris evecta*, Hoffm., *Danaea alata*, Sm., *Danaea nodosa*, Sm., *Kaulfussia aesculifolia*, Bl., and *Marattia Cooperi*, Mre., was examined, together with a few pieces of root from a herbarium specimen of *Archangiopteris Henryi*, Chr. et Gies., which was soaked out in 80 per cent. NH_3OH [1] with most successful results.

The greater part of the material was fixed in 75 per cent. alcohol + glycerine: for ordinary histological work this mixture gave very good results, but rendered a careful cytological investigation impossible.

[1] According to the method described by M. Raciborski in Flora, Bd. lxxxi, 1895, p. 153.

[Annals of Botany, Vol. XXXI. No. CXXI. January, 1917.]

Both hand- and microtomed sections were cut; the former were found useful for demonstrating the grosser features, whilst the latter were necessary for determining the finer details.

A considerable number of stains and reagents were used, as it was found necessary to employ a variety of staining methods in order to successfully differentiate the younger parts (especially haustoria) of the fungal mycelium from the tissues of the host-plant.

Fairly good results were obtained by the use of Durand's[1] haematoxylin-eosin combination for differential staining, but the cotton blue + lactic acid method recommended by Jones, Giddings, and Lutman (27, p. 30, foot-note) did not give satisfactory results.

The following stains were also employed either singly or in combination: Kleinenberg's haematoxylin, safranin, azo-blue, picro-nigrosin, methylene blue, and Congo red.

For determining the chemical nature of the cell-walls, &c., various reagents, including chlor-zinc-iodide, H_2SO_4, iodine, phloroglucin + HCl, osmic acid, alkannin, Sudan III, Congo red, and ruthenium red were found useful.

The sections were mounted in Canada balsam or in glycerine jelly.

A. On the Endophyte of the Roots of Angiopteris evecta, Hoffm., Archangiopteris Henryi, Chr. et Gies., Kaulfussia aesculifolia, Bl., and Marattia Cooperi, Mre.

The roots of the first order of all genera and species of Marattiaceae are relatively robust and frequently branch in a monopodial manner at some distance from the caudex. The roots of the second and higher orders are usually long and fibrous, and are provided with multicellular root-hairs (*r.h.*, Text-fig. 9; Pl. III, Fig. 3), which are rather abundant on the fine threadlike terminal rootlets.

In all the above-named genera the roots are characterized by the complete absence of a cortical zone of stereome (cf. *Danaea*), although isolated 'stone-cells' are not infrequently met with near the periphery of the larger roots. The ground-tissue of the cortex consists of typical large parenchymatous cells, many of which are packed with starch.

The endophyte, a brief description of which the present writer (44) has already published, is of general occurrence in the primary and earlier adventitious roots, but its presence appears to be less regular in the later roots of each genus.

It seldom occurs in the aerial portion of the main roots, and is invariably absent from the terminal (i. e. meristematic) region of all roots.

[1] Durand, E. J.: The Differential Staining of Intercellular Mycelium. Phytopathology, i, 1911, p. 129.

A few plants were examined in the roots of which no trace of the fungus could be detected; this condition also obtains in a small proportion of the roots of all plants which have passed their juvenile stage. This may explain Kühn's (28, p. 494; 29, p. 149) failure to find infected roots in his specimens of *Marattia fraxinea*, Sm., and of *Danaea alata*, Sm., and the statements of Campbell (14, p. 215) and of Charles (15, p. 84) concerning the distribution of the endophyte in older roots.

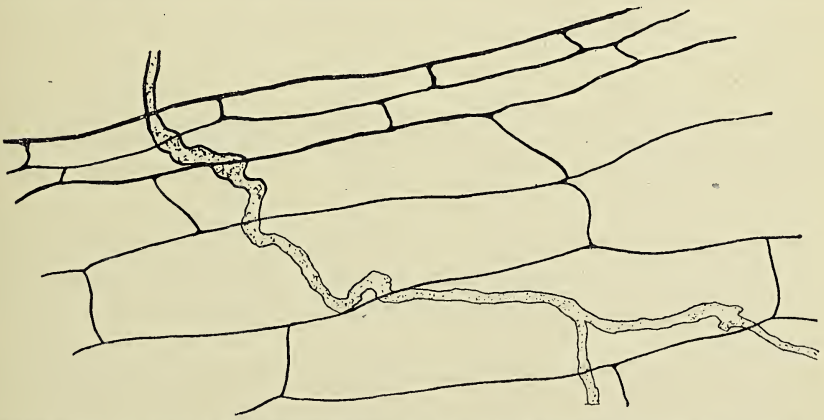
The presence of the mycorrhizal fungus, which can be detected only by an examination of sections of the host-root, does not influence in the slightest degree the external appearance of the host, infected and uninfected plants appearing to be equally healthy and vigorous. Neither does the nature of the soil bear any relation to the presence or absence of the endophyte, which is found in the roots of plants growing in soils ranging from light rich humus to red clay from disintegrated gneissic rocks.[1]

Thus it would appear that in the above-named genera of Marattiaceae symbiosis is not obligate, but facultative.

1. *Distribution, Organization, and probable Life-history of the Fungus in the Tissues of the Host.*

i. *Mycelium.*

Microscopical examination of transverse or longitudinal sections of infected roots shows that fungal hyphae from the surrounding soil may enter the root through any of the epidermal cells in spite of the cuticularization of



TEXT-FIG. 1. Part of a longitudinal section of a root of *Archangiopteris Henryi*, Chr. et Gies., showing infecting hypha of *Stigeosporium Marattiacearum*. × 270.

the outer wall of these cells (Text-fig. 1; cf. Noël Bernard, 10, p. 243). The mycelium is both inter- and intracellular within the tissues of the host, exhibits very few septa, and is copiously branched (Text-fig. 2, *a* and *b*).

[1] The author is indebted to Professor J. B. Farmer, F.R.S., for information regarding the habitat of a number of plants of *Angiopteris evecta*, Hoffm., gathered by him in Ceylon.

When young, the hyphae are hyaline and possess extremely thin delicate walls with a very irregular contour (Text-fig. 2, *a*). At this stage, their contents consist of a finely granular cytoplasm in which many relatively large nuclei are irregularly distributed ; in other words, the mycelium of this fungus exhibits the multinucleate condition typical of the Oomycetes (Text-fig. 2, *a*, *b*).

The walls of the hyphae thicken up with age, assume a light-brown coloration, and become highly refractive. Meanwhile the cytoplasm becomes

TEXT-FIG. 2. Mycelium of *Stigeosporium Marattiacearum*. *a*, *b*, and *c* are drawn to the same magnification in order to show remarkable variation in size and form of the hyphae. From a root of *Angiopteris evecta*, Hoffm. *n*. = nucleus.
The contents of the host-cell are not shown. × 750.

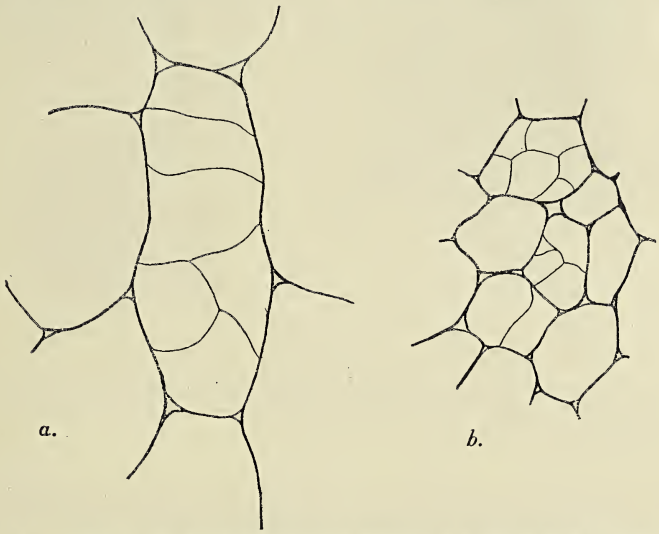
vacuolate and tends to disappear, whilst the oldest hyphae are invariably empty (Text-fig. 2, *c*). In diameter the hyphae are extraordinarily variable, ranging from 1 μ in the younger to 12 μ in the older regions (Text-fig. 2, *a*, *b*, *c*).

As stated above, the mycelium within the tissues of the host is both inter- and intracellular. In the outer layers of the cortex the hyphae are usually intracellular, apparently experiencing no difficulty in penetrating the cell-walls of the host (Text-fig. 1). No obvious constriction of the hyphae is noticeable at the point where they penetrate the cell-wall, nor does the latter develop a tubular sheath around the invading hypha. It is probable that the secretion of enzymes, which results in the solution of the cell-wall, is confined to the apical region of the hyphae, for, even in very old roots, the presence of the fungus never leads to isolation and disorganization of the

host-cells. But a curious effect is produced upon many of the large parenchymatous cells of the inner cortex, which are stimulated to further division, as is shown in Text-fig. 3, *a*, *b*.

However, on reaching the inner layers of the cortex, the hyphae for the most part become intercellular (Pl. III, Fig. 5 A). Several hyphae (eight or more) may frequently be found traversing a single intercellular space (Text-fig. 4, *a*).

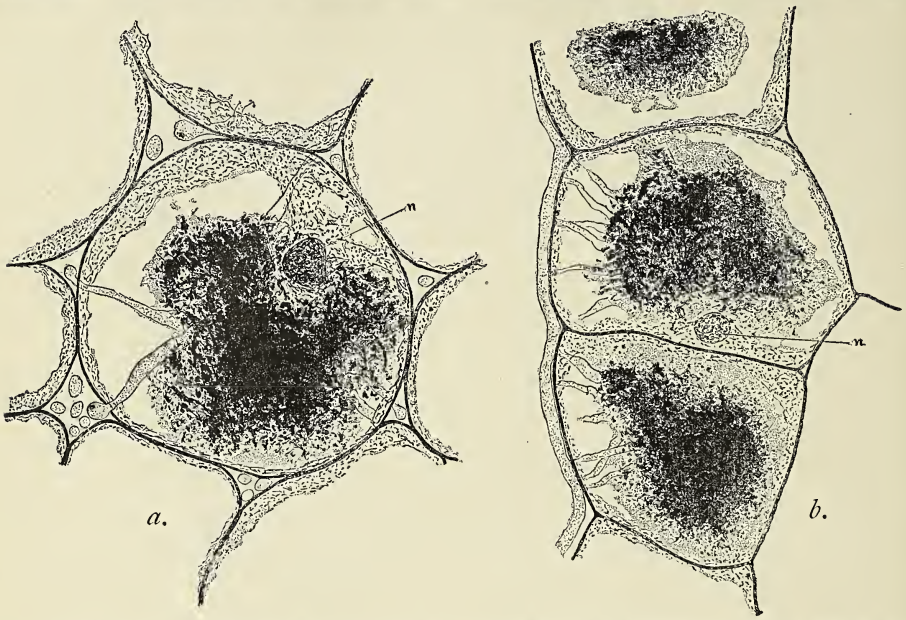
With few exceptions the principal intercellular hyphae pursue a course more or less parallel with the long axis of the host-root (Text-fig. 5; Pl. III, Fig. 5 A), but cross-connexions between two or more of these main



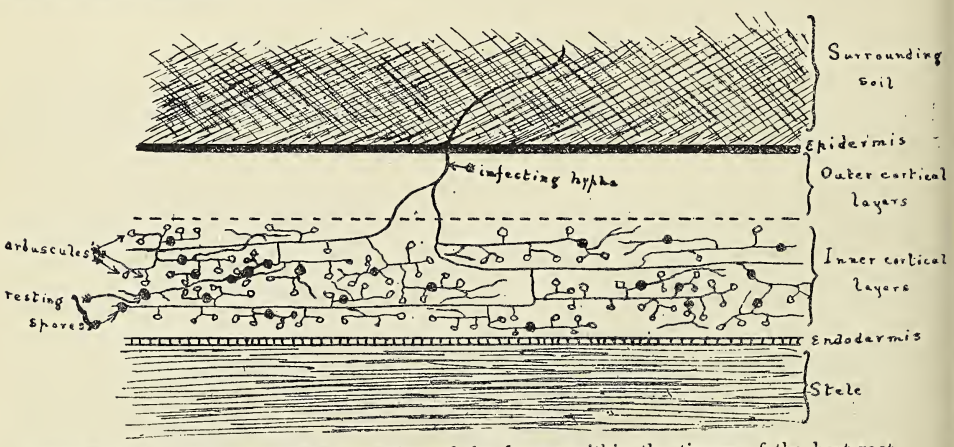
TEXT-FIG. 3. Cortical cells undergoing further division as a result of the presence of the endophyte. *a*. From a transverse section of *Marattia Cooperi*, Mre. *b*. From a longitudinal section of *Archangiopteris Henryi*, Chr. et Gies. The cell-contents are not shown. × 200.

hyphae are not infrequent. From the intercellular hyphae numerous branches of limited growth penetrate the walls of the surrounding cortical cells. These short hyphal branches ramify repeatedly within the invaded cell, where they form peculiar tufts of exceedingly fine mycelial threads. Structures of this nature, for which Gallaud (l. c., p. 223 et seq.) has proposed the term 'arbuscules', appear to be characteristic of most endotrophic fungi, and are undoubtedly homologous with the haustoria of the Peronosporaceae. The ultimate ramifications of these intracellular branches are so fine, and possess such an exceedingly delicate membrane, that even under a very high magnification their true form can be distinguished only after careful treatment. Judging from the infrequency with which it is found, this stage is quickly passed over; the fine threads of the ultimate branches soon lose their individuality, and finally become transformed into structureless, granular conglomerations ('sporangioles' of Gallaud, l. c.,

p. 231 et seq.), which stain a dirty yellow after treatment with iodine and completely resist the action of concentrated sulphuric acid or potassium hydrate. The 'stalk' portion of the 'arbuscules' is more resistant to the



TEXT-FIG. 4. Disorganizing 'arbuscules' of *Stigeosporium Marattiacearum*, as seen in (*a*) a transverse section of a root and in (*b*) a longitudinal section of a root of *Marattia Cooperi*, Mre. *n.* = nucleus of host-cell. × 700.



TEXT-FIG. 5. Diagram showing position of the fungus within the tissues of the host-root. Note: Relative proportions are greatly exaggerated.

enzymes, which are presumably secreted by the cytoplasm of the mycorrhizal cell, and persist for a considerable time after the complete disorganization of the ramifications (Text-fig. 4, *a*, *b*).[1]

[1] The cytological features connected with the digestion of fungal endophytes by their hosts have been carefully studied by Magnus (32), Shibata (40), and Kusano (30).

All stages in the degeneration of the 'arbuscules' are irregularly distributed throughout the inner cortical cells of the host-root. In sections of infected roots, the cells which contain these masses of partially digested fungal mycelium can easily be distinguished, even with the naked eye, as pale yellowish strongly refractive spots, which stand out in sharp relief against the dull brown background of the other cells of the cortex. These clumps or masses of disorganized mycelium persist throughout the life of the host-root, yet the invaded cells show no obvious signs of injury, unless the complete disappearance of starch-grains from these cells can be interpreted as such. On the other hand, the host-cells not only keep the fungus in check, but presumably utilize as food part at least of the products of disorganization of the fungal hyphae. Moreover, the mycorrhizal cells exhibit no signs of approaching death until the root as a whole decays, their nuclei retaining a healthy appearance to the last (Text-fig. 4, *a*, *b*).

It is interesting to find that the mycelium is invariably absent from the tannin-cells of the host even when hyphae are abundant in adjacent cortical cells, and again, the fungal zone of the larger roots of *Marattia Cooperi*, Mre., is always situated *outside* the ring of mucilage-canals (Pl. III, Fig. 7).

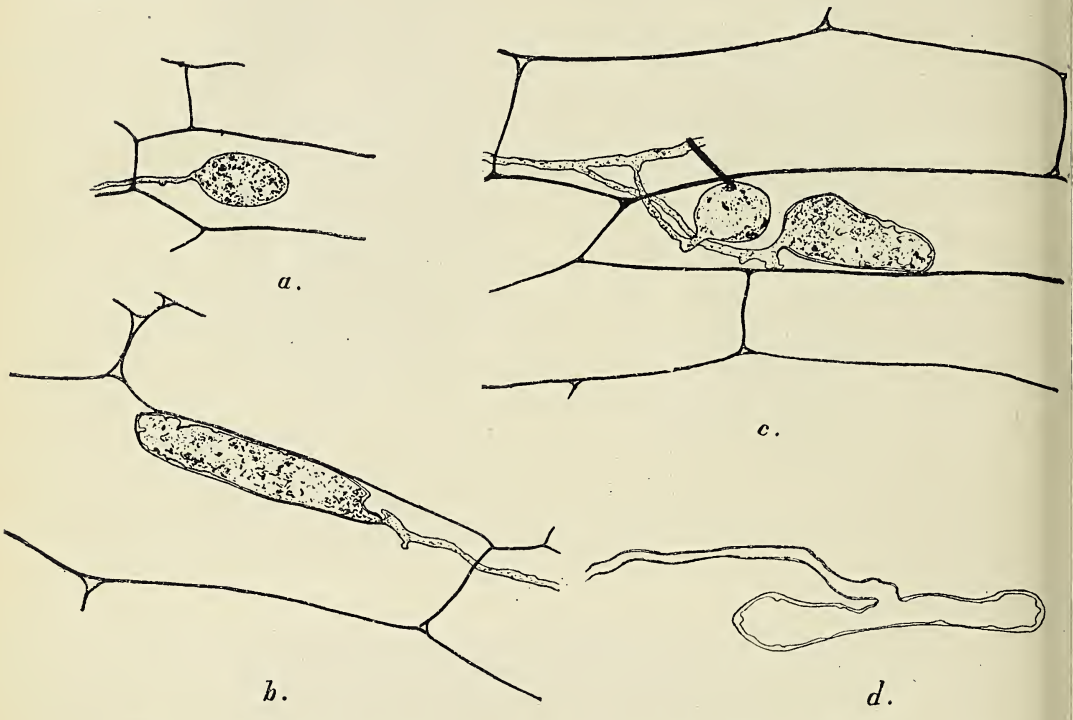
Cook (18) has shown that tannin exerts a deleterious effect upon certain fungi, whilst Gallaud (l. c., p. 317) remarks of endotrophic fungi in general that they are repelled by secretory cells or by chlorenchyma. On the other hand, Peklo (35) found that the fungal symbiont of the mycorrhiza of *Carpinus* and of *Betula* appears to show a preference for this substance.

ii. *Vesicles.*

The terminal portion of many of the hyphae swell up and give rise to structures which are generally known as vesicles (= 'vésicules' of Janse (25), N. Bernard (10), and others). These swellings, which are locally abundant and assume various shapes and dimensions, occupy either an inter- or an intracellular position amongst the cortical cells of the host-root (Text-fig. 6, *a*, *b*, *c*). They are never delimited by a septum from the supporting hyphae, from which they differ only in shape and size. When young they possess a thin hyaline membrane and contain a granular vacuolate cytoplasm in which numerous minute deeply staining bodies of irregular form can be distinguished (Text-fig. 6, *a*, *c*).

As the vesicles increase in size, their wall irregularly thickens up and assumes a yellowish coloration exactly similar to that of the older hyphae, and, as in the latter, the contents of old vesicles tend to disappear. Numerous empty vesicles were observed in some of the roots (Text-fig. 6, *d*). Now the fact that the vesicles rapidly lose their contents militates against the view that they are reproductive bodies of a conidial nature (cf. Campbell (13); also Noël Bernard (10), Fig. 9, p. 249). Neither is there any reason for supposing that they are secretory products of the fungus, and they

obviously are not homologous with the structures designated vesicles by Kusano (l. c., p. 32 et seq.). Gallaud (l. c.) claimed for these structures, which are almost universally distributed amongst mycorrhizal fungi, a function as temporary reserve organs, and, in the opinion of the present writer, this interpretation is the most satisfactory which has yet been given.[1]



TEXT-FIG. 6. Vesicles of *Stigeosporium Marattiacearum* in cortical cells of (*a*, *b*) roots of *Angiopteris evecta*, Hoffm., and of (*c*, *d*) roots of *Archangiopteris Henryi*, Chr. et Gies. Contents of host-cells not shown. × 300.

iii. *Resting spores.*

Special interest attaches to this mycorrhizal fungus, inasmuch as it produces *under natural conditions distinct reproductive bodies* (other than ' vesicles ') *within the tissues of the host-root.*

Distributed among the inner cortical cells of infected roots of every age and size, characteristic thick-walled spores were frequently noticed (Text-figs. 7 and 8; Pl. III, Figs. 1, 2, 5, and 6).[2] These spores, which are usually spherical (32–45 μ), but occasionally ovoid, pyriform, &c., occur

[1] For a full discussion of this question the reader is referred to the works of Gallaud (22, p. 130 et seq.) and of Kusano (30, pp. 36, 37).

[2] The isolated spores observed by Kühn (28, p. 493, Taf. XX, Fig. 39) in *Kaulfussia aesculifolia*, Bl., were probably identical with these.

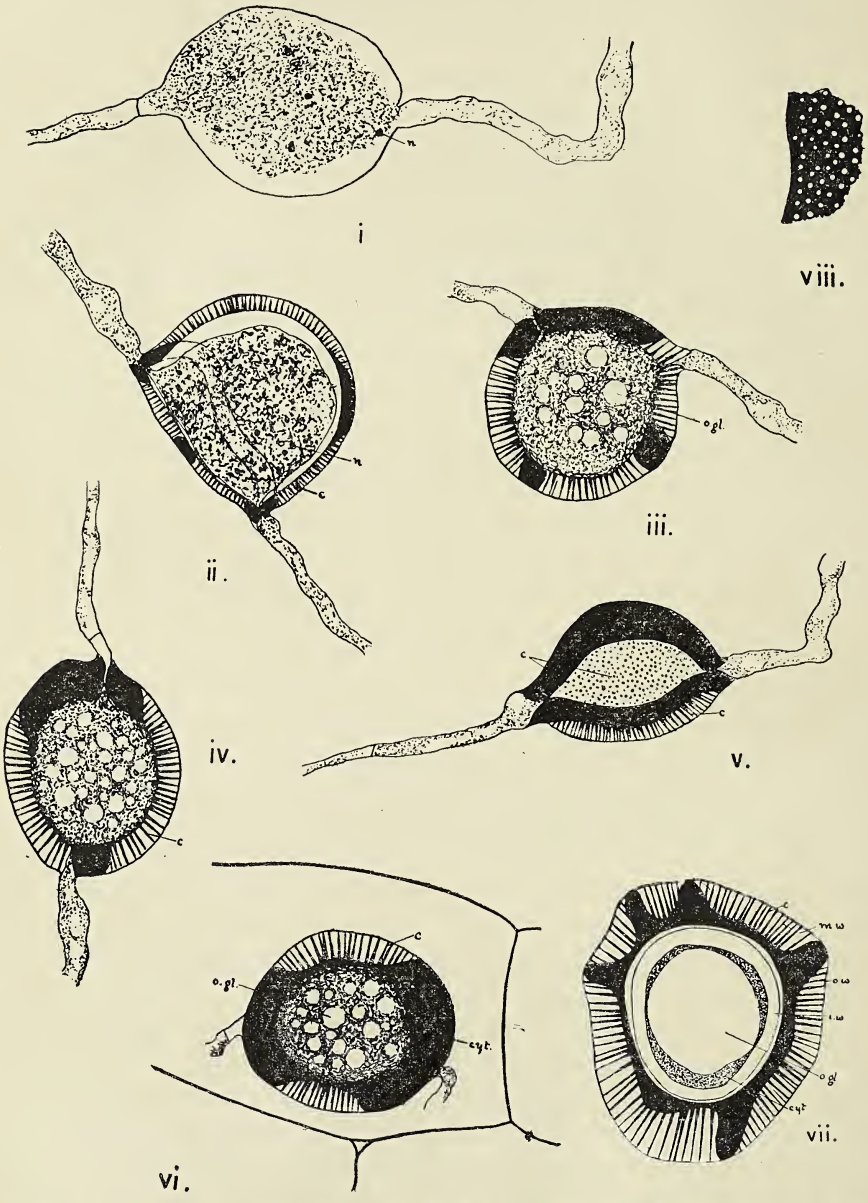
either in a terminal (Text-fig. 7), or more often in an intercalary (Text-fig. 8) position on the hyphae, and were at first mistaken for ripe oospores, to which they bear a remarkably close resemblance, especially during the earlier stages of their development. However, the complete absence both of an oogonial wall and of antheridia is sufficient proof of their non-sexual nature.

a. Development of the resting spores.

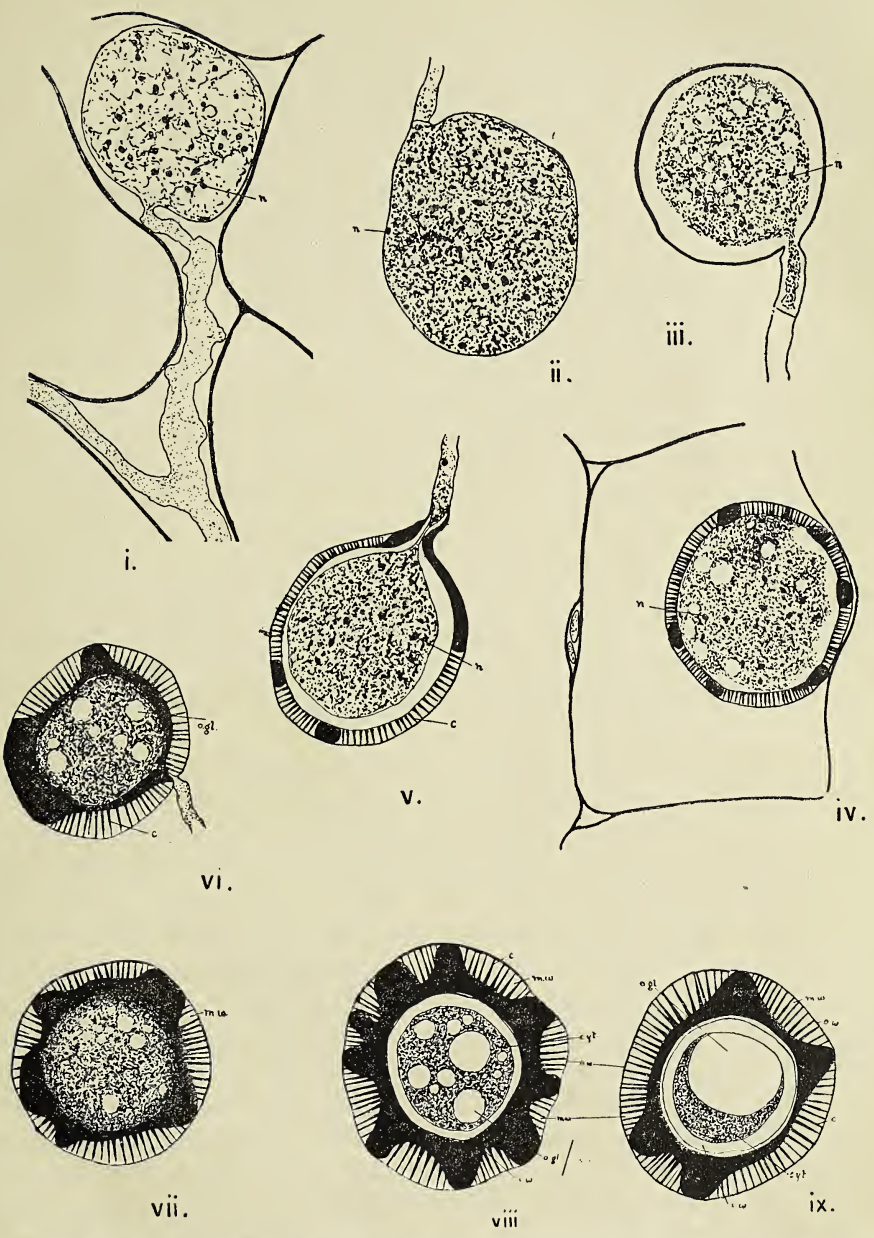
A terminal resting spore originates as a more or less spherical enlargement of the terminal portion of an ordinary young hypha, from which it is usually delimited by a septum (Text-fig. 8, i, ii, iii). The dense granular contents of this enlargement are multinucleate. At this stage the immature resting spore closely resembles a young spherical vesicle, from which, however, it can readily be distinguished by its denser contents and by the uniform thickness of its wall (cf. Text-fig. 8, i, ii, with Text-fig. 6, *a*). The intercalary resting spores (= chlamydospores) are formed in the following manner: Swelling takes place in the course of a young hypha, either equally all round, in which case globular or ovoid enlargements are produced, or in one direction only, in which case the swollen portion has a sub-globose form. In either case the enlargements may or may not be delimited by septa from the supporting hyphae (Text-fig. 7, i). The wall of these swellings, whether terminal or intercalary, is at first similar in thickness to that of the hyphae from which they arise, but differs slightly in its staining capacity. For example, the wall of the young spore stains readily with Kleinenberg's haematoxylin, whereas the hyphal walls take up this stain with difficulty. Very early in the development of the resting spore the middle wall originates as a thin highly refractive layer, in which irregular deep yellow areas can easily be distinguished (Text-figs. 7, ii, and 8, iv, v). At a slightly later stage the first indications of canals or pores can be identified in this wall (Text-figs. 7, ii, and 8, iv, v). The thickening of the middle wall proceeds apace: meanwhile the contents of the spore become more densely granular, and oil globules,[1] which are so conspicuous in the contents of the ripe spore, appear in the cytoplasm and completely mask the subsequent behaviour of the nuclei (Text-figs. 7, iii, iv, and 8, vi, vii).

So far the only indication of the innermost wall is the closer aggregation of the granular contents in the peripheral zone of the cytoplasm (Text-figs. 7, iii, iv, vi, and 8, vi, vii). A number of spores which were artificially crushed at this stage showed no signs of an inner wall; hence it must be concluded that the innermost wall is laid down very late in the development of the spore. Thickening of the middle wall continues until an average thickness of 6 μ is attained, but shortly before this result is arrived at, the inner wall begins to develop.

[1] It may be noted in passing that the presence of oil as a reserve material is characteristic of 'resting' bodies.



TEXT-FIG. 7. i–vii. Intercalary resting spores of *Stigeosporium Marattiacearum* in various stages of development. From roots of *Angiopteris evecta*, Hoffm. × 750. viii. Part of the wall of a ripe resting spore in surface view showing canals. × 1700. *c.* = canal; *cyt.* = cytoplasm; *i.w.* = inner wall; *m.w.* = middle wall; *o.w.* = outer wall; *n.* = nucleus; *o.gl.* = oil globules.



TEXT-FIG. 8. i–ix. Terminal resting spores of *Stigeosporium Marattiacearum* in various stages of development. From roots of *Angiopteris evecta*, Hoffm., and *Archangiopteris Henryi*, Chr. et Gies. *c.* = canal; *cyt.* = cytoplasm; *i.w.* = inner wall; *m.w.* = middle wall; *o.w.* = outer wall; *n.* = nucleus; *o.gl.* = oil globules. × 750.

Whilst the above-described changes have been taking place in the spore wall, the cytoplasm, which at first consisted of dense granular protoplasm completely filling the interior of the spore, gradually decreases in bulk until in the ripe spore a condition is arrived at where the cytoplasm (proteid) occupies only a fraction of the interior of the spore, the remaining space being filled with oil (cf. Text-figs. 7, ii, and 8, ii, with Text-figs. 7, vii, and 8, ix).

Thus, it would appear that the innermost wall is deposited at the expense of part of the original spore contents, whilst the greater part of the remainder of these cell contents is converted into oil. The innermost wall rapidly increases in thickness until in the mature spore an average thickness of 2·5 μ is attained (Text-figs. 7, vii, and 8, ix).

β. Structure of the mature resting spores.

The ripe resting spores possess three distinct walls, namely—

1. An extremely thin smooth outer wall. This wall is colourless and is closely applied to the middle wall, from which it is indistinguishable in unstained preparations, but can readily be identified in stained preparations of crushed spores.

2. A thick (6 μ) middle wall. This wall consists of a transparent straw-coloured matrix which is traversed by a large number of canals or pores (average diameter 0·8 μ), which radiate from the interior of the spore (Text-figs. 7, vii, and 8, ix; Pl. III, Fig. 1, *c*, *d*, *e*, *f*, *g*). Each canal is not of uniform diameter throughout its length, but tapers towards the periphery of the spore (Text-figs. 7, ii–viii, and 8, iv–ix).

The chemical nature of this substance could not be determined with certainty. It is very resistant to every reagent to the action of which the ripe spores were subjected. These included KOH, chlor-zinc iodide, eau-de-Javelle, concentrated HCl, &c., &c., but Kleinenberg's haematoxylin stained it with difficulty (cf. Dastur (19), pp. 207–8).

Irregular bands and markings of a deeper yellow colour ramify through this matrix and are clearly visible both in surface view and in sections of the spores (Text-figs. 7, ii–vii, and 8, iv–ix; Pl. III, Figs. 1, *a–g*, 2, and 5B). The substance of which these bands are composed is equally resistant to the above-mentioned reagents, and all attempts to stain it failed.

3. A moderately thick (2·5 μ) inner wall. This wall develops very late, is generally not closely applied to the middle wall, and presents many of the reactions of cellulose. It is exceedingly tough, and even when the thick middle wall of the spore is crushed artificially this inner wall remains unbroken.

On reaching maturity the spores become detached from their supporting hyphae, and are then found lying free within or between the cells of the host-root, which are sometimes forced apart by the growth of the spores, as is shown in Text-fig. 8, *i*.

On the other hand, it is probable that the pressure of the cell-walls of the host upon the developing spores is responsible for the unusual shape of some of the mature spores (Text-fig. 7, vii).

iv. *The probable life-history of the endophyte.*

In no case, even in very old decaying roots, has a resting spore been observed to germinate *in situ*; it therefore seems probable that germination never takes place until, on the complete decay of the host-root, the spore is eventually deposited in the soil.

Dastur (l. c., p. 199) states, with reference to *Phytophthora parasitica*, that the 'resting' conidia, if kept moist, retain their vitality for over nine months, when they germinate by one or more germ-tubes, but do not necessarily require a period of rest.

It is not unlikely that a similar statement holds good for the resting spores of our fungus; these spores when finally set free in the soil, where they would be preserved under moist conditions, probably germinate direct after a varying period of rest, giving rise to one or more germ-hyphae, which, either at once or after a while, meet with a suitable host-root, the epidermis of which they penetrate in the manner described above.

The thickness of the wall and the absence of a definite papilla indicate that the resting spores are not sporangia, but specialized conidial growths. It is possible that the suppression of vegetative vigour and of sporangia formation on the part of the fungus, due to lack of oxygen or to some other cause (or causes) at present unknown, are determining factors in their production.

According to Magnus (32, p. 216) certain portions of thick-walled hyphae of the endophyte of *Neottia Nidus-avis* persist throughout the winter after the decay of the host-root and germinate ('zu neuem Leben zu erwachen') in the following spring and thus infect fresh host-plants. He refers to these structures as 'sclerotia' or 'cysts'. This fact is interesting, inasmuch as it points to the possible evolution of highly specialized resting spores by the local modification of ordinary vegetative hyphae for a reproductive function. On the other hand, the complex nature of the wall and the striking resemblance which the spores under discussion bear to oospores, especially during the later stages in their development, suggests their origin from parthenogenetic oogonia (=parthenospores), with complete suppression of the male organs.

2. *Cultural Experiments.*

Many attempts to grow this fungus on various artificial media, both in Petri-dishes and in hanging-drops, met with no success. No growth of the fungus resulted when pieces of root of *Marattia* or of *Angiopteris*, the surface of which had *previously been sterilized*, were placed in or upon various artificial media (cf. Rayner, l. c., p. 119). The surface of the root

was sterilized by immersion for varying short periods in very dilute solutions of formalin, mercuric chloride, or hydrogen peroxide, followed by thorough washing in running water. Failing this preliminary superficial sterilization of the host-root, a number of forms appeared in the cultures. The numerous genera represented in such cultures obviously constitute the epiphytic mycoflora of the roots in question and have no necessary connexion with the mycorrhizal fungus.

Bearing these results in mind, it is only reasonable to suppose that the fungal growths which Kühn (28, p. 493, Taf. XX, Fig. 40) succeeded in isolating from roots of *Marattia alata*, Sm., were of a similar nature, for they were obtained from roots which apparently had not been previously sterilized.

In this connexion it is interesting to find that Gallaud (l. c.) invariably failed to obtain cultures of the numerous mycorrhizal fungi which were investigated by him. This observer suggests that the mycelium of the endophyte undergoes some vital change during its sojourn within the tissues of the host-plant. The present writer is of the opinion that Gallaud's explanation is correct.

Noël Bernard (1, 3, 4, 5, 6, 7, 8, 9), however, in the course of a series of cultural experiments extending over a number of years, succeeded in isolating a number of mycorrhizal fungi from the roots of various genera of Orchidaceae and brought to light the remarkable degree of specialization which obtains in these plants. This botanist proved that the germination of the seeds of certain Orchids and the subsequent development of the root-system of the seedlings depended upon their association with their particular fungal symbiont, and in this way solved the problem which in the past had puzzled Orchid growers, namely, the apparent impossibility of raising certain genera of Orchidaceae from seed.

An ingenious theory that tuberization in a number of higher plants depended upon their infection with a fungal endophyte, which brought about an increase in the concentration of the cell-sap, was advanced by this observer (2, 9, 10). Many of these experiments were afterwards repeated and confirmed by Burgeff (11).

Rayner (l. c., p. 120) succeeded in isolating an endotrophic fungus from seeds and from pieces of ovary tissue of *Calluna vulgaris* and in effecting the synthesis of sterile seedlings of the higher plant with this fungus, thereby inducing development of the root-system and vigorous growth under aseptic conditions in closed tubes.

There is good reason to believe that the only parts of our fungus capable of an independent existence outside the tissues of the host are the resting spores and germ-hyphae; hence it was unfortunate that in the only living material available for investigation the vegetative structures of the endophyte were alone represented. On this account no experimental work

on the germination of the resting spores and the subsequent infection of the host-plant could be attempted.

3. *Biological Relations between Host and Endophyte.*

A. *Historical.*

Leaving out of consideration the research upon various ectotrophic mycorrhizas, an abundant literature dealing with the many views put forward to explain the biological significance of the association of endotrophic fungi with the root, or other absorbing organ, of a number of Phanerogams and Vascular Cryptogams, has accumulated during recent years.

But full summaries and discussions of the more important results obtained in this field of investigation, including those of Frank (21), Noël Bernard (2, 7, 8, 9, 10), and Burgeff (11), are to be found in the recent works of Kusano (30) and of Rayner (38). Detailed reference to the literature is therefore unnecessary.

The theories put forward to explain the biological significance of endotrophic mycorrhizas is very briefly summarized below:

1. The mycorrhiza is a real symbiosis with reciprocal advantages; in other words, there is a mutual interchange of material (or other advantages) between host and endophyte (Groom (23), Macdougal (31), Shibata (40), &c., &c.).
   - A. The higher plant provides the fungal symbiont with one or more of the following:
     - i. Habitation.
     - ii. Carbohydrate (especially starch).[1]
     - iii. Shelter from excess of oxygen.[2]
   - B. The fungus benefits the higher plant in one or more of the following ways:
     - i. The fungus takes up humus products from the soil by means of its external mycelium. These humus products are conducted to the internal hyphae, where they are converted into proteids which are in part utilized by the fungus in its own metabolism and in part yielded to the tissues of the host-plant.[3]
     - ii. The fungus is capable of fixing nitrogen directly from the air, with which it builds up organic nitrogenous substances (i. e. proteids) which it yields to the host-plant in exchange for carbohydrate material.[4]
     - iii. The fungus absorbs mineral salts from the surrounding soil.[5]

[1] *Corallorrhiza arizonica*, Macdougal (l. c.); *Thismia Aseroë*, Groom (l. c.).
[2] Janse (l. c.).
[3] Macdougal (l. c.), and others.
[4] Janse (l. c.); *Podocarpus*, Nobbe and Hiltner (34), Ternetz (42).
[5] Stahl (41).

2. The host-plant (Phanerogam) derives the entire, or by far the greater, advantage from the association.[1]
3. The advantages of the association are entirely, or almost entirely, on the side of the fungal partner.[2]

B. *The present investigation.*

The biological relations between the marattiacean roots and the endophyte which inhabits them will now be briefly considered in the light of previous work on mycorrhizal fungi.

Hyphae are never found leaving the host-root. This is shown by the fact that few hyphae are found in the outer cortical layers, and these are obviously only the infecting hyphae, since they are relatively older than the mycelium found in the inner cortical layers of the host-root. This fact may be urged against the hypothesis that the fungus absorbs humus products or mineral salts from the surrounding soil and liberates them, with or without previous modification, in the tissues of the host. This also militates against the view that the mycelium of the endophyte takes the place of root-hairs in the higher plant. Moreover, the distribution of the latter bears no relation whatever to the presence or absence of the fungus. Again, root-hairs are sometimes relatively abundant on young infected roots.

To the question whether the fungus is a facultative anaerobe capable of fixing free nitrogen from the air, no definite answer can be given in the absence of experimental evidence.

But whilst admitting that the fungus is a true parasite, it cannot be said to cause any obvious injury to the plant attacked (cf. the fungus found in the seed and vegetative organs of *Lolium temulentum*, Hanausek (24), Nestler (33)). Kusano (l. c.) has proved conclusively that the facultative parasite *Armillaria mellea* may behave towards the Orchid *Gastrodia elata* either as a true parasite or as an endotrophic symbiont. There is no reason for doubting that the host-plant utilizes part at least of the products of disorganization of the 'arbuscules', but there is no intimate relation between the endophyte and the nucleus of the mycorrhizal cell, such as Groom (l. c.) claims for the mycorrhiza of *Thismia Aseroë*.

Now, apart from its rôle as habitat (and possible shelter from excess of oxygen) for the endophyte, the host-plant provides the latter with all, or at least the greater part, of the food material it requires, because the mycelium in the outer cortical layers of the root, which provides the only connexion between the living mycelium in the inner cortical layers of the root and the free hyphae in the soil, is usually dead and without contents, and therefore cannot function as a channel for the passage of food-material. Moreover, starch invariably disappears from the invaded cells; all stages in the solution of the starch granules, which do not reappear even after the complete collapse of the 'arbuscules' (cf. Groom, l. c., p. 348, and Kusano, l. c.,

[1] *Gastrodia elata*, Kusano (l. c.). [2] Gallaud (l. c.).

p. 36), can be seen in these cells. But this circumstance, whilst indicating that the presence of the endophyte is in some way responsible for the absence of starch from the host-cell, does not necessarily prove that the carbohydrate in question is utilized by the fungus as a food-material.

Since no visible injury is done to the cells of infected roots, it would appear that this fungus shares with the great majority of mycorrhiza-forming fungi investigated by Gallaud (l. c.) the capacity for obtaining all the food it requires from the non-living cell-contents (e. g. starch, sugars, elaborated sap, &c.) of its host.

And the resting spores, with their oily contents, are formed at the expense of the higher plant; hence the advantage of the association is almost entirely on the side of the fungus, the host-plant thriving *in spite of* the presence of the endophyte.

4. *Systematic Position of the Fungus.*

The systematic position of the fungus is of special interest, inasmuch as our knowledge of the phylogeny and relationships of the various mycorrhiza-forming fungi has hitherto been exceedingly meagre.

From the mycorrhiza of the Orchidaceae Noël Bernard (8) obtained cultures of a number of fungal endophytes which he placed provisionally in the genus *Rhizoctonia*. In the same year (1909), Burgeff (11), working on the mycorrhizal fungi of Orchid roots, came to the conclusion that they formed a distinct morphological group, for which he proposed the descriptive name *Orcheomyces*.

Gallaud (l. c., p. 433) observes that, except in the Orchids, the reproductive bodies of the fungal partners of all mycorrhizas remain to be discovered. Dangeard (18 A, 18 B), however, described and figured (l. c., Pl. XV, Fig. 15) not only the mycelium, but also the spores, of a fungus which occurs in the rhizome of *Tmesipteris*, and provisionally referred the endophyte to the genus *Cladochytrium*. Peklo (l. c.), however, claims to have isolated a species of *Penicillium* from the mycorrhiza of *Carpinus* and of *Betula*, whilst Rayner (l. c.) has recently isolated from *Calluna vulgaris* an endophyte for which the new sub-genus *Phyllophoma* has been established.

Judging from its mycelial characters alone, our fungus undoubtedly belongs to the Oomycetes, and exhibits many of the essential features of the sub-family Peronosporae of the Peronosporaceae, where for the present it may conveniently be placed.

According to Clements (16, p. 17), six genera are included in this sub-family; of these, the genus *Phytophthora* most closely resembles the fungus under consideration, sharing with it several important characters, the most noticeable of which are mentioned below.[1]

[1] Treub (43) considered the fungal endophyte of *Lycopodium Phlegmaria* prothallia to belong to the Peronosporaceae, while Noël Bernard (10, p. 252) suggests that the fungal endophyte which occurs in the roots of *Solanum Dulcamara* has affinities with this family; Nobbe and Hiltner (34) claim a similar position for the endotrophic fungus of *Podocarpus* nodules.

Perhaps the most striking character which these genera share in common is the production of thick-walled 'resting' spores. About one-half of the species of *Phytophthora* hitherto described form thick-walled 'resting' spores. Spores of this nature have been described for the following species of *Phytophthora*, viz.:

Species:	Mentioned by:
P. Faberi, Maublanc	Dastur (19), Petch (36)
P. infestans, de Bary	Jones, Giddings, and Lutman (27), Dastur (19 A)
P. parasitica, Dastur	Dastur (19)
P. Colocasiae, Raciborski	Butler and Kulkarni (12)
P. Nicotianae, de Haan	Pethybridge (37)
P. Jatrophae, Jensen	Pethybridge (37)
Cacao Phytophthora (*P. omnivora ?*)	Rorer (39).

And it is not improbable that the parthenogenetic oospores, described by Coleman (17) for *Phytophthora Theobromae*, Coleman, are of a similar nature.

The description and figures of the structure and development of the resting spores of *Phytophthora infestans* published by Jones, Giddings, and Lutman (l. c.), except for a few minor differences, could be applied equally well to the resting spores of the endophyte of these marattiacean roots. And the discovery of ramified haustoria in *Phytophthora infestans* by Delacroix (20, p. 361, Fig. 2) and by Dastur (19 A, p. 5) is also of interest.

The chief points of difference between the two genera may be attributed to the peculiar habitat of our fungus, which no doubt has become associated with special adaptations, amongst which we find an almost complete suppression of certain reproductive bodies generally characteristic of the Peronosporae, namely, the sporangia, oogonia, and antheridia, the modification of the conidia, and a remarkable specialization of the vegetative parts.

Since, however, we unfortunately have no available evidence regarding sexual reproductive bodies in this fungus, nothing precise can be stated as to its affinities, but the marked difference in habit between this fungus and *Phytophthora* is sufficient to make its inclusion in the latter genus unjustifiable.

I therefore propose to establish a new genus, *Stigeosporium*,[1] for its reception, and, since it is undoubtedly normally associated with roots of the Marattiaceae and, so far as is known, is confined to that habitat, I suggest for it the specific name *Stigeosporium Marattiacearum*.

5. *Geographical Distribution of the Fungus.*

This fungus has a remarkably extensive geographical distribution, the same species being found in the marattiacean Ferns occurring in such widely

[1] Or *Stygeosporium*.

separated localities as Eastern Asia (Java, South-West China), Australasia, and Ceylon.

DIAGNOSIS: [1]

Stigeosporium, gen. nov.

Mycelium ramosum ex hyphis inter- et intracellularibus continuis rarissime septatis constans; haustoriis numerosis, extremitatibus in ramulis radiatis valde dissectis; sporis perdurantibus solitariis plerumque globosis raro subglobosis et cet., membrana crassissima irregulariter intenseque colorata.

Differt a *Phytophthora*, cui arcte affine, habitu symbiotico, qua de causa nulla conidia normalia producuntur.

Sp. unica.

Stigeosporium Marattiacearum, West, sp. nov.

Hyphae primum hyalinae demum flavo-brunneae vacuatae 1–12 μ crassae; sporis vel intercalaribus vel terminalibus plerumque globosis 32–45 μ diametro, raro subglobosis vel ovoideis vel pyriformibus; exosporio tenuissimo hyalino levi, mesosporio crassissimo 6 μ crassitudine minute punctulato flavo irregulariter intenseque colorato, endosporio tenue 2·5 μ crassitudine.

Hab. In radicibus subterraneis quae ad quasdam marattiacearum generum orientalium omnium adhuc cognitorum species pertinent.

Distrib. Asia orientalis, Australasia, Zeylania.

B. On the Endophyte of the Roots of Danaea alata, Sm., and of Danaea nodosa, Sm.

The principal roots of both species of *Danaea* examined by the present writer are characterized by the presence of a zone of fibrous cells which is separated from the epidermis by a few layers (2–6) of large parenchymatous cells which constitute the outer cortex (Text-fig. 9; Pl. III, Figs. 3 and 4): in the smaller roots, however, no fibrous zone is found.

In other respects the roots of *Danaea* very closely resemble those of the other genera of Marattiaceae.

An endotrophic fungus was found in nearly every root examined, irrespective of age and size; it is invariably present in the primary and first few adventitious roots of the young sporophyte, but a few of the older roots were found to be free from fungal mycelium.[2]

The mycelium is irregularly distributed throughout the cortex of the smaller roots which do not possess a cortical zone of stereome, but in the larger roots the mycelium is always confined to the few layers of parenchyma *outside* the fibrous zone of the cortex (Text-fig. 9; Pl. III, Figs. 3 and 4).

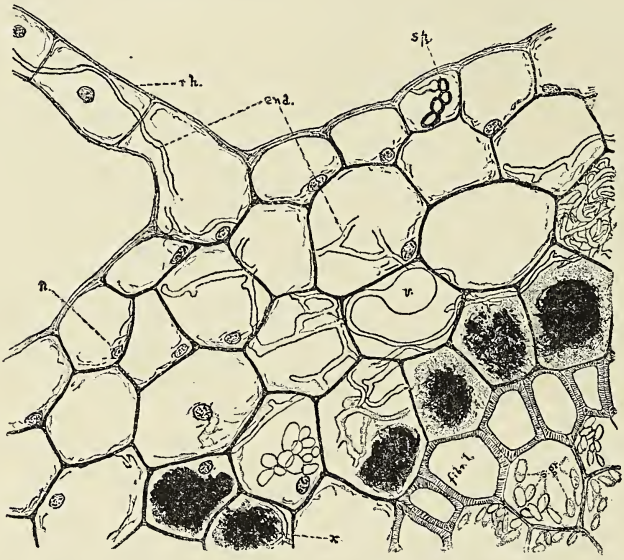
[1] The above diagnosis is an extended form of that which the author published as a note in Annals of Botany, vol. xxx, 1916.

[2] An endophyte exhibiting similar characters was observed in the cells of the prothallia.

In *Danaea* also, the presence of the endophyte does not in any way alter the external appearance of the roots.

Infecting hyphae enter the root either through ordinary epidermal cells or through the root-hairs (Text-fig. 9).

The hyaline non-septate copiously branched mycelium consists for the most part of fine intracellular hyphae which are fairly uniform in diameter (2–4 μ). These hyphae have a delicate membrane and finely granular contents in which no nuclei can be identified with certainty (Text-fig. 9; Pl. III, Fig. 4, *myc.*; and cf. *Stigeosporium*).



TEXT-FIG. 9. Part of a transverse section of a moderately large root of *Danaea alata*, Sm., showing endophyte (*end.*) in outer cortical cells. *fibr. l.* = cortical zone of stereome; *n.* = nucleus of host-cell; *r.h.* = root-hair; *s.gr.* = starch grains; *sp.* = foreign spore-like bodies; *v.* = vesicle; *x.* = disorganized 'arbuscules'. × 300.

From the relatively few intercellular hyphae 'arbuscules' essentially similar to those already described for *Stigeosporium Marattiacearum* are produced. These rapidly collapse, their disorganized remains forming irregular structureless granular masses *within* the host-cell (Text-fig. 9, *x*; Pl. III, Figs. 3 and 4). Vesicles are occasionally found: they are generally globular in form and are never delimited by a septum from the supporting hypha, upon which they occupy a terminal position (Text-fig. 9, *v*; Pl. III, Fig. 4, *v*). Intercalary vesicles were never observed in this endophyte. The hyphae often form peculiar coiled bunches within the mycorrhizal cells (Text-fig. 9): the real significance of this phenomenon, which is not uncommon among endotrophic mycorrhizal fungi, is not yet understood.

So far as it is possible to judge from an examination of sections of infected roots, the hyphae of this endophyte exert no deleterious effect upon

the invaded cells, the only obvious result of their presence being the complete disappearance of starch from such cells.

Unfortunately, no reproductive bodies were observed, consequently the systematic position and relationships of this endophyte could not, even approximately, be determined. No living material of either species of *Danaea* was available for investigation; it was therefore impossible to carry out cultural experiments with this fungus.

That the endophyte which is associated with the roots of *Danaea* spp. is quite distinct from *Stigeosporium* is proved by the following characters, which belong only to the former:

1. *Mycelial characters.*
   - A. Uniform diameter of the hyphae;
   - B. Comparative rarity of intercellular hyphae;
   - C. The hyphal wall does not materially thicken with age;
   - D. Absence of *distinct* nuclei from contents of the mycelium.
2. *Apparent absence of definite reproductive bodies.*
3. *Absence of vesicles of irregular form.*

Summary.

1. A new fungus, *Stigeosporium Marattiacearum*, which forms an endotrophic mycorrhiza with roots of certain genera (*Angiopteris*, *Archangiopteris*, *Kaulfussia*, *Marattia*) of Marattiaceae, is described, and its biology, probable life-history, and systematic position discussed.

2. An unnamed mycorrhizal fungus, which enters into association with roots of the genus *Danaea*, is briefly described.

I take this opportunity of expressing my sincere thanks to Professor J. B. Farmer, F.R.S., for many helpful suggestions, and for allowing me to carry out the present investigation in his laboratory.

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EXPLANATION OF FIGURES ON PLATE III.

Illustrating Mr. C. West's paper on *Stigeosporium Marattiacearum* and the Mycorrhiza of the Marattiaceae.

Fig. 1. *a–g.* Resting spores of *Stigeosporium Marattiacearum*, showing different stages in development. *a*, *b*, *c*, *d* in optical section; *e*, *f*, *g* in surface view. From roots of *Angiopteris evecta*, Hoffm. × 500.

Fig. 2. Mature resting spores of *Stigeosporium Marattiacearum* in a root of *Archangiopteris Henryi*, Chr. et Gies. × 350.

Fig. 3. Transverse section of a small root of *Danaea alata*, Sm., showing endophyte (*end.*) in the cortex. *e.* = endodermis; *fibr. l.* = fibrous layer of the cortex; *rt. h.* = root-hair. × 60.

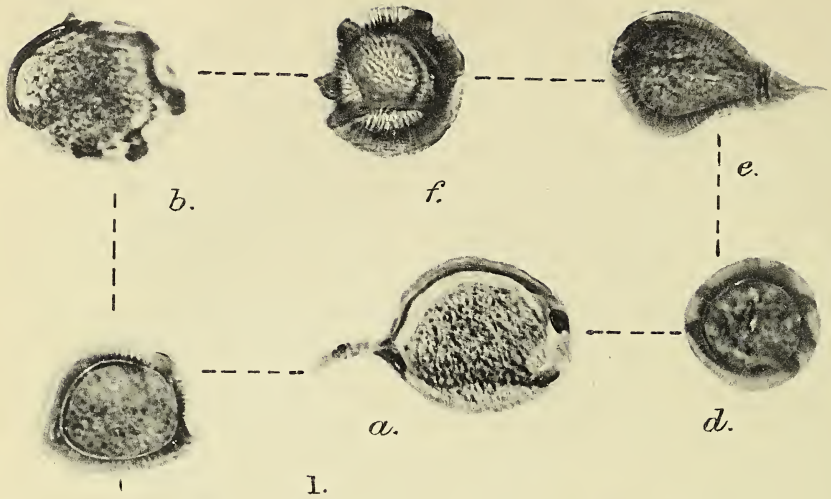
Fig. 4. Part of a transverse section of a small root of *Danaea nodosa*, Sm., showing endophyte (*end.*) in the outer layers of the cortex. *fibr. l.* = fibrous layer; *myc.* = intracellular mycelium; *t.c.* = tannin cell; *v.* = vesicle. × 120.

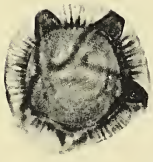
Fig. 5 A. Resting spores (*sp.*) of *Stigeosporium Marattiacearum* as they appear in a longitudinal section of a root of *Angiopteris evecta*, Hoffm. At *x* the intercellular mycelium of the fungus is clearly shown. × 30.

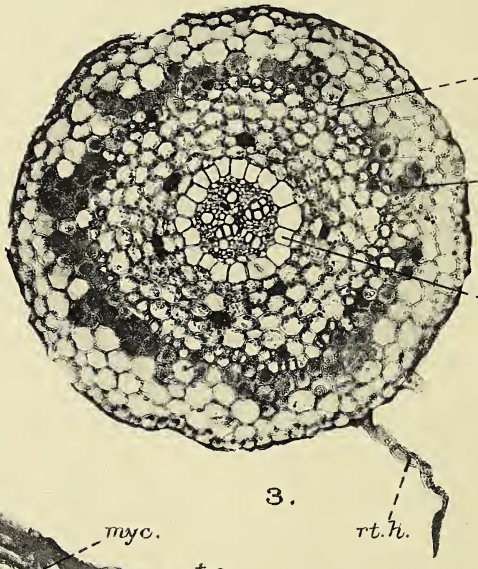
Fig. 5 B. Part of the same more highly magnified to show wall-markings. × 120.

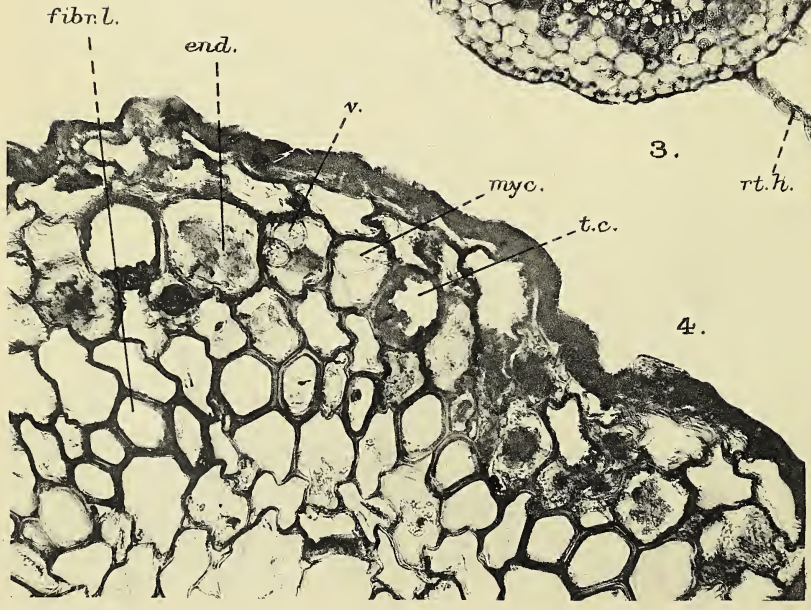
Fig. 6. Part of a transverse section of a moderately large root of *Angiopteris evecta*, Hoffm., showing endophyte (*end.*) in the cortex. *e.* = endodermis; *sp.* = resting spore; *t.c.* = tannin cell. × 35.

Fig. 7. Transverse section of a root of *Marattia Cooperi*, Mre., showing endophyte (*end.*) in the cortex. *e.* = endodermis; *m.c.* = mucilage canal; *t.c.* = tannin cell; *v.* = vesicle. × 30.









C. West photo.

WEST—MYCORRHIZA OF MARATTIACEÆ.

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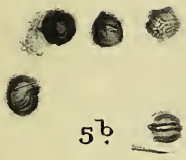

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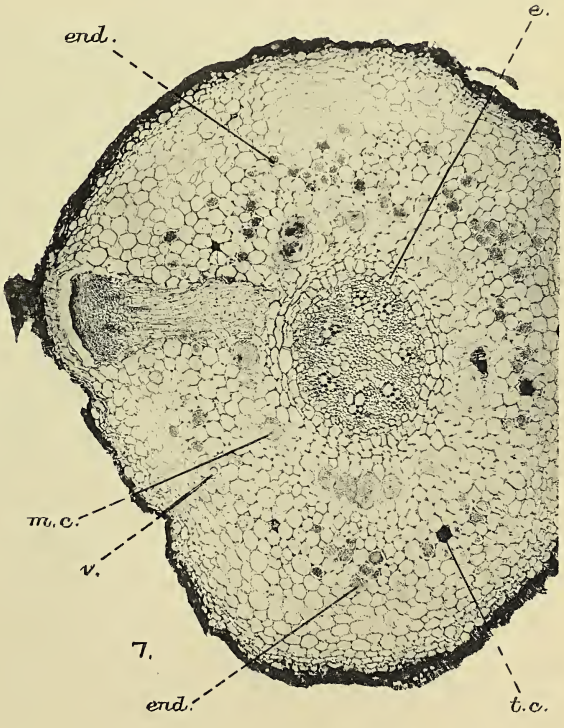

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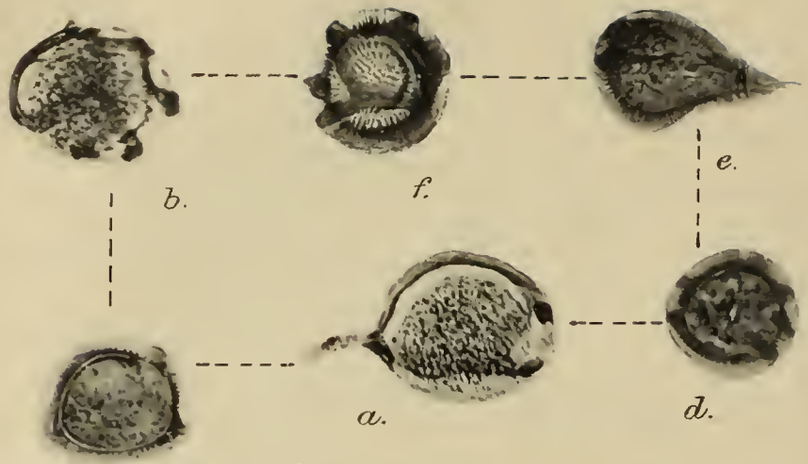
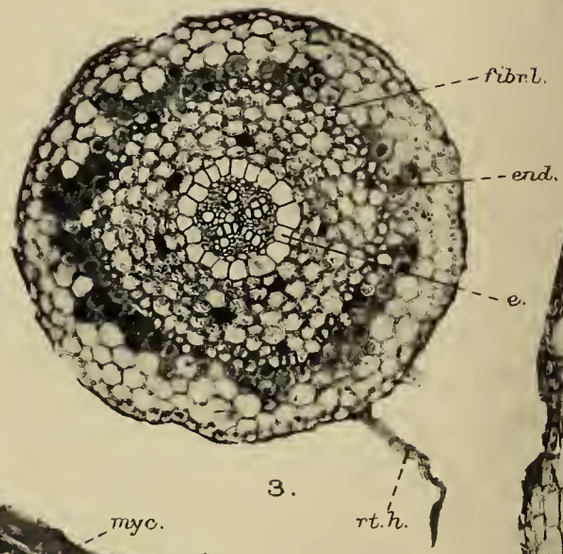
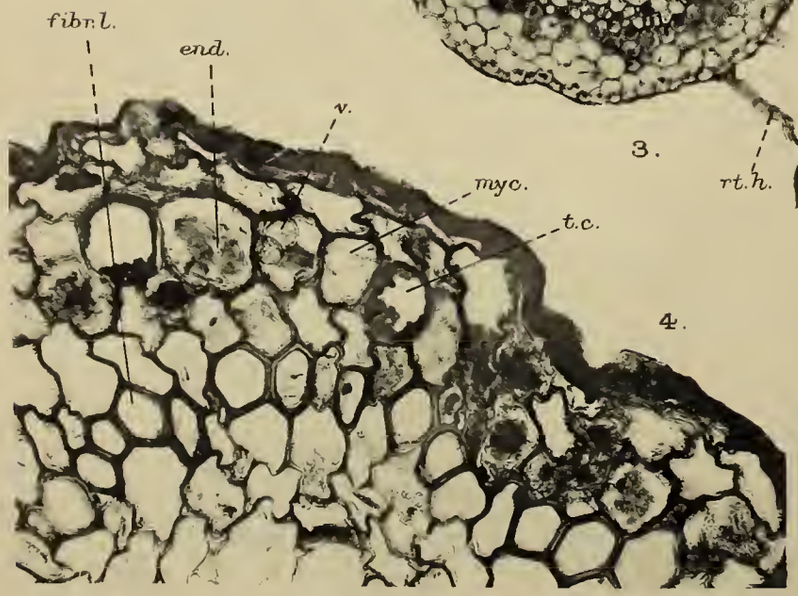
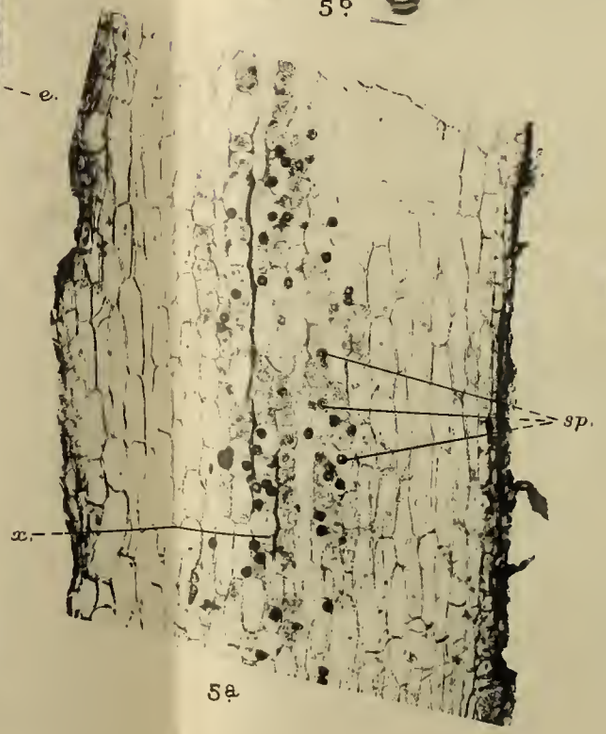
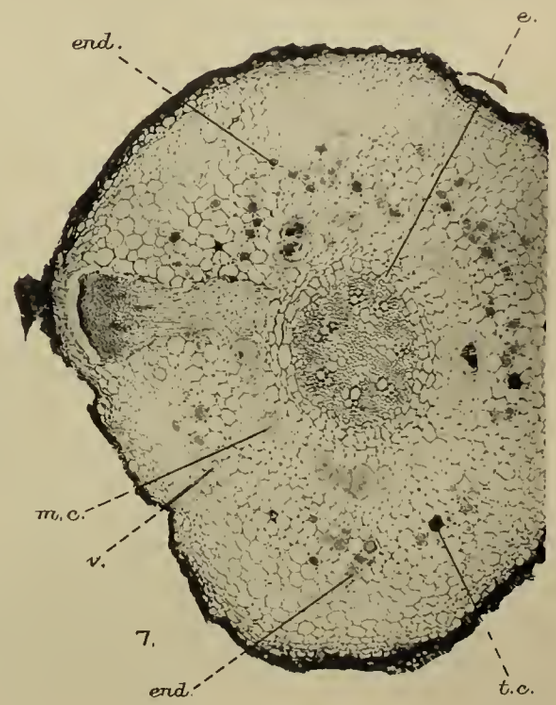










C. West photo.

Huth coll.

WEST—MYCORRHIZA OF MARATTIACEÆ.

A Fossil Wood of Sequoia from the Tertiary of Japan.[1]

BY

KONO YASUI,

Assistant Professor of Botany in the Tokio Normal College.

With Plate IV.

THE Aichi-Gifu coal-field, situated in the middle region of Hondo (the main island of Japan), belongs to the Tertiary, and the lignitic coal from this field contains large quantities of wood belonging for the most part to the *Cupressinoxylon* type. The identification of woods and even genera of living Cupressineous types, as was long ago pointed out by Goeppert, is extremely difficult, and in most cases quite impossible in view of the simplicity of organization of *Cupressinoxyla*. The genus, which can be most definitely diagnosed, is one which is characterized by a unique reaction to wounding. The material which I am about to describe fortunately shows modifications of structure, such as, in living material, are formed as a response to injury, and this evidence, together with other and normal structural features, supplies a sufficiently exact diagnostic description.

Sequoia hondoensis, sp. nov.

Gross Features.

The wood is dark reddish brown in colour, and the annual rings are very narrow but quite distinct, particularly with the lens. The narrowness of the annual increments, as will be shown later, is due to a considerable extent to the action of fossilization on the width of the ring. Making allowance for the exiguity of the zones of woody growth resulting from the conditions accompanying lignitic transformation, it must still be conceded that in life the annual rings were narrow, as would be the case in the later development of an old tree. From the somewhat frequent occurrence of alternate pitting in the tracheides of our specimen, it seems not unlikely that it was derived from the root system of the prehistoric tree. Our specimen was too small and too much distorted to permit of any accurate inference in regard to the diameter of the trunk from which it originated. Traumatic resin canals reveal their presence by thin white lines running across the specimen tangentially in distant annual rings.

[1] Contribution from the Laboratory of Plant Morphology of Harvard University.

[Annals of Botany, Vol. XXXI. No. CXXI. January, 1917.]

Microscopic Organization.

Pl. IV, Fig. 1 shows a transverse section of the wood in the region of a line of traumatic resin canals. The spring tracheides by reason of the thinness of their walls have usually almost entirely collapsed as a result of the maceration and crushing accompanying fossilization. The summer fibres by contrast, on account of the greater resistance to obliteration offered by their much thicker walls, are still largely intact. As indicated above, the growth rings are rather thin, and the transition from spring to summer wood somewhat abrupt. By attentive examination it may be seen that the tracheides of the summer wood are provided with tangential pits. The 'resin cells' are very prominent, and are scattered through the spring and summer wood. The rays are less clearly seen, and as a sequel of compression appear as meandering lines always uniseriate. Occasionally dark resinous contents may be distinguished in the cells of the rays, similar to those occurring in the parenchymatous elements of the wood. In length the ray cells correspond to from two to four tracheides. About one-third of the distance from the top of the figure appears a tangential row of traumatic resin canals, resembling the similar structures in the two living species of *Sequoia*, namely *S. gigantea* and *S. sempervirens*. As my specimen is merely a fragment from a large trunk, it does not show the region of actual injury, but the sporadic and abnormal appearance of the secretory canals under discussion appears to leave no doubt as to their traumatic origin.

Pl. IV, Fig. 2 shows the traumatic resin canals and the surrounding cellular elements, somewhat more highly magnified. The resiniferous cavities are, as in the case of the species of the living genus, surrounded by secretory cells, which are to a very limited extent characterized by the dark brown contents, which mark the so-called 'resin cells' of the wood. The resiniferous elements, in fact, in the case of traumatic resin canals in both living and extinct species of *Sequoia* are comparable rather with the resin-secreting cells surrounding the resin ducts of the wood of the Abietineae, than with the longitudinal parenchymatous strands of Cupressineous woods. Certain of the short cells in proximity to the resin canals are neither resiniferous elements nor 'resin cells', but are short tracheides of the type to which Penhallow gives the name 'parenchyma tracheides'. They in fact correspond very accurately to the structures shown in Text-fig. 40 in his 'North American Gymnosperms'.[1] Attentive examination of this figure shows the bordered pits in the transverse end walls of cells of this type.

Pl. IV, Fig. 3 illustrates the radial view of the wood under discussion. In the centre the abundant parenchyma (only a small part of which consists

[1] Penhallow, D. P.: North American Gymnosperms, Boston, 1908, Text-fig. 40, p. 126.

of true 'resin cells') surrounding the traumatic resin canal can be readily distinguished. A close study reveals septate tracheides ('parenchyma tracheides' of Penhallow) in a more lateral position. The resiniferous space is obviously discontinuous and fistular in the longitudinal direction, as is commonly found to be the case with traumatic resin canals in the living representatives of the Coniferales. Pl. IV, Fig. 4 reproduces the upper region of the foregoing, more highly magnified. A row of short tracheary elements can be discerned a little to the left of the secretory space. Farther to the left lies a row of 'resin cells'. In Pl. IV, Fig. 5 is exhibited a radial view of the uncollapsed spring wood, and at the same time a tangential section of the summer tracheides. This unconformable arrangement is the result of the distortion accompanying fossilization. In our species the radial pits of the walls of the tracheides are usually in a single row in the summer elements, and are frequently biseriate and opposite in the broader tracheides of the spring growth. These pores are round or oval in shape. The lateral pits of the rays are oval with a distinct border, a feature considered by Penhallow to be of diagnostic value for the genus *Sequoia*. Pl. IV, Fig. 6 shows the 'bars of Sanio' in a spring tracheide, much magnified. They present an unusual condition, for Haidenhain's haematoxylin stains them a deep blue as in recent material of *Sequoia*, showing that the pectic cellulose has here for some reason maintained its position and not been macerated away, leaving an empty space, as is more usually the case in fossil coniferous woods, both Mesozoic and Tertiary. It is difficult to imagine why such delicate features have in this case been preserved. Possibly the large amount of tannin present in the cell-wall has acted inhibitively on the common pectic dissolution. The distinct presence of 'bars of Sanio' clearly fixes the general systematic position of our fossil. It has been pointed out by Jeffrey [1] and Holden [2] that traumatic resin canals may occur in the Araucarian series as well as among those Conifers with immediate Abietineous affinities. A satisfactory criterion for separating such woods in those instances where they manifest traumatic resin canals is the presence or absence of the 'bars of Sanio'. Clearly on this evidence our *Cupressinoxylon* belongs to the general Abietineous series, and since only *Sequoia* here possesses traumatic resiniferous spaces, a reference to that genus is unquestionably indicated. Penhallow was the first to call attention to the presence of resin canals in *Sequoia*.[3] He considered them, however, to be normal features of wood structure, and only noted their presence in *S. sempervirens*. Jeffrey has demonstrated that rows of resin

[1] Jeffrey, E. C.: The History, Comparative Anatomy, and Evolution of the Araucarioxylon Type. Proceedings of the American Academy of Arts and Sciences, vol. xlviii, No. 13, Nov. 1912.

[2] Holden, Ruth: Contributions to the Anatomy of Mesozoic Conifers, No. 1. Annals of Botany, vol. xxvii, No. 107, July, 1913.

[3] Cf. North American Gymnosperms and literature there cited.

canals occur in both the living species of *Sequoia* and that they are due to injury.[1]

The structure of our wood as described above, particularly the pitting of the ray cells, the distribution of the so-called 'resin cells', the presence of 'bars of Sanio', and above all the phenomenon of traumatic resin canals, points strongly to an affinity with the living *S. sempervirens*. Many species have been described as ancestral to or closely related to *S. sempervirens* (the Redwood of the Californian coast range). In 1899 Knowlton gave an account of *S. magnifica* as a fossil form from the Yellowstone National Park, and expressed the opinion that it was the ancestor of *S. sempervirens*, but saw no traumatic resin canals in his material.[2] *S. Langsdorfii* has been elucidated from its vegetative habit for both the European and American Tertiary, and it is recognized as the prototype of the living *S. sempervirens*. In 1908 Penhallow described *S. albertensis* as a lignitoid specimen form of the Edmonton series of Canada, and called attention to its close resemblance to the existing *S. sempervirens*. It is of interest that although he had previously recognized the occurrence of vertical resin canals in the wood of the living Redwood, and had even noted horizontal canals in another fossil species, he failed to observe anything of the kind in *S. albertensis*.[3] With the exception of the three species mentioned, we have no really conclusive diagnosis of woods of extinct species of *Sequoia*. In the three species regarded as probably correctly assigned to the genus on the basis of ligneous diagnosis, *S. magnifica* and *S. albertensis* have not yet been shown to produce traumatic resin canals, and by this defect of diagnosis are still somewhat in a dubious position systematically. It accordingly appears that *S. Langsdorfii* (as defined by Penhallow) is on the whole the best authenticated Mesozoic *Sequoia*, although the original diagnosis supplies us with no information as to the important feature of the lateral pitting of the ray cells.

A comparison with *S. sempervirens* and *S. Langsdorfii* indicates that our fossil has a somewhat closer relationship with *S. sempervirens* than the other species. Because it is a fossil and its geographical occurrence is remote from that of the living Redwood (*S. sempervirens*), it seems advisable to assign a new specific name after its place of origin.

The diagnosis of *Sequoia hondoensis*, sp. nov., is as follows:

Transverse. Growth rings clearly defined; transition from spring to summer wood somewhat abrupt; summer wood very prominent, and of from two to ten tracheides in breadth. Spring wood very open and with thin-walled tracheides. Resin cells prominent and scattered throughout

[1] Jeffrey, E. C.: The Comparative Anatomy and Phylogeny of the Coniferales. Part I. The Genus *Sequoia*. Memoirs of the Boston Society of Natural History, vol. v, No. 10, Nov. 1903.

[2] Knowlton, F. H.: Description of Known Fossil Plants from the Laramie of the Yellowstone National Park. Mon. U. S. Geol. Surv., No. xxxii, Part II, p. 761, Pl. CXL, 1899.

[3] Penhallow, D. P.: Report on a Collection of Fossil Woods from the Cretaceous of Alberta. The Ottawa Naturalist, vol. xxii, No. 4, July, 1908.

the wood. Medullary rays somewhat prominent and uniseriate, distant by two to ten or more rows of tracheides.

Radial. Ray cells rather straight, somewhat resinous, equal to two to four tracheides; horizontal walls thick and without pits, terminal walls thin and unpitted, lateral walls with large oval narrowly bordered pits, two to six in number per tracheide and generally in two rows; radial pits of the tracheides round or oval in one or two rows and then opposite; 'bars of Sanio' prominent and staining strongly in haematoxylin.

Tangential. Rays somewhat resinous and from two to eleven cells high. Bordered pits on the tangential walls of the summer tracheides small and in one or two rows. Traumatic resin canals absent in the horizontal plane, and as a consequence seen only in the radial and transverse sections.

The traumatic resin canals occur in tangential series in remote annual rings. They are surrounded by strongly pitted parenchyma cells, with a few so-called 'resin cells', and more externally by septate tracheides (the 'parenchyma tracheides' of Penhallow).

The occurrence of the wood of a fossil *Sequoia* in the Tertiary of Japan completes in an interesting way the evidence for the existence of that genus in Cenozoic time throughout temperate regions of the whole Northern hemisphere. The ligneous structure of *S. hondoensis* also adds somewhat to our knowledge of the phylogeny of the Coniferales. The question of the presence of the genus *Sequoia* in the Mesozoic period need not be raised in this connexion, although it has been pointed out by Professor Jeffrey and his students that it is extremely doubtful if our modern genus antedated the Tertiary, since the types referred to it in the Cretaceous are clearly not anatomically in agreement with the living genus, and do not exhibit even the organization of the *Cupressinoxylon* type.

At the present time there are two opposed views in regard to the phylogenetic position of the *Cupressinoxylon* type of wood. One school recognizes this type as young among the Coniferales and as ancestral to the *Pityoxylon* type characteristic of the Abietineae. A more recent view, for which Professor Jeffrey is sponsor, is that the simpler type of wood found in *Sequoia* and its allies has originated as the result of simplification and specialization from the ligneous type characteristic of *Pinus* and related genera. The genus *Sequoia* stands always at the critical point in these two opposed hypotheses. The study of the living *Sequoia* appears to have made it clear that the view which regards it as more primitive than the Abietineae is due to the error of mistaking the resin canals which occur in the wood of *Sequoia* as a normal feature of structure instead of as the result of traumatic reaction. The discovery in the Mesozoic of Japan of a member of the genus manifesting the same interesting abnormality of the presence of ligneous resin canals appears to furnish another good proof of the derivation

of *Sequoia* from types characterized by the normal occurrence of resin canals in their woods—that is, from the *Pityoxylon* type.

Summary.

1. A wood occurring in the lignitic Tertiary coals of the Aichi-Gifu coal-fields of Hondo is clearly closely related to *Sequoia*, both on the grounds of its traumatic and normal characters.

2. The presence of this type of lignitic remains is further evidence for the widespread distribution of the genus *Sequoia* in the Tertiary of the Northern hemisphere.

3. It supplies confirmation for the hypothesis that the *Sequoias* have come from pine-like ancestors.

DESCRIPTION OF PLATE IV.

Illustrating Professor Kono Yasui's paper on a Fossil Wood of *Sequoia* from the Tertiary of Japan.

Fig. 1. Transverse section of wood of *Sequoia hondoensis*. × 40.

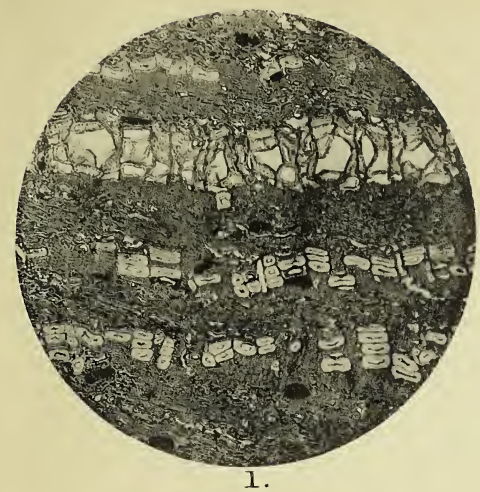
Fig. 2. Transverse section of wood of *Sequoia hondoensis*, showing traumatic resin canals more highly magnified. × 125.

Fig. 3. Longitudinal view of same. × 60.

Fig. 4. Longitudinal view of part of the foregoing more highly magnified. × 125.

Fig. 5. Longitudinal, approximately radial, view of the same. × 60.

Fig. 6. Longitudinal section of tracheid of the same, showing 'bars of Sanio'. × 500.



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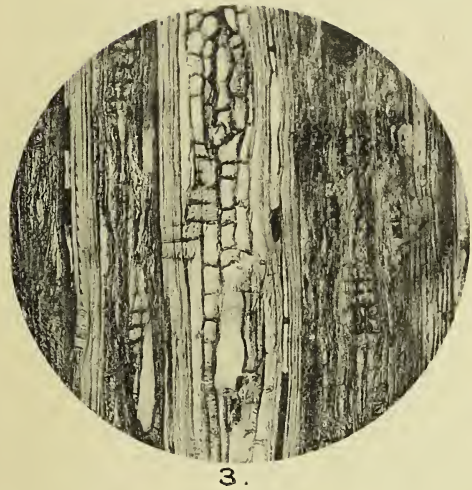

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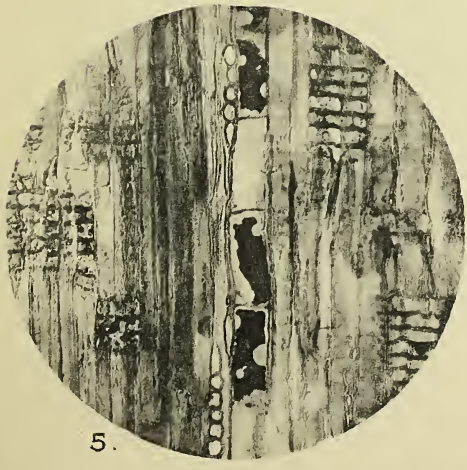

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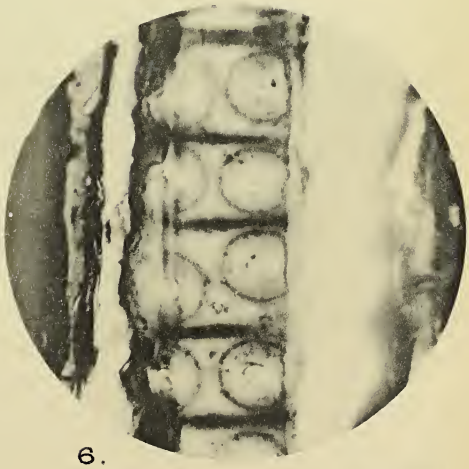

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YASUI — A TERTIARY SEQUOIA.

On the Life-history and Cytology of Chlorochytrium grande, sp. nov.

BY

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With Plates V and VI and two Figures in the Text.

IN the year 1881 Klebs[1] published a paper dealing with the life-histories of a number of new algae belonging to the Protococcales, which he described as being nearly related to *Chlorochytrium*, Cohn. These new forms he assigned to three different genera which he named *Endosphaera*, *Scotinosphaera* and *Phyllobium*. Two years later, Borzi[2] described still another new genus, *Centrosphaera*, belonging to the same group. Since 1883 very little work has been done upon these algae, possibly because of the difficulty of obtaining any of them in sufficient quantity to carry out adequate investigations, but more probably because the distinctions between the genera, as laid down by Klebs and Borzi, are in most cases so insignificant as to render their identification extremely difficult.

The possession of a considerable quantity of living material, which was identified as most probably a species of *Centrosphaera*, Borzi, therefore led Professor G. S. West to suggest in October, 1915, that a careful study of the life-history and cytology of this alga might possibly help to eradicate the confusion existing among these genera.

The alga was originally taken from a dyke in West Yorkshire along with a number of other water plants. Owing to the necessity of following its complete life-history before a definite conclusion as to its identity could be established, it was placed in a small wide-mouthed glass bottle covered with a watch-glass to await an opportunity for further examination. Water was kept in the bottle to a depth of about an inch, and as evaporation took place tap-water was added.

[1] Georg Klebs: Beiträge zur Kenntniss niederer Algenformen. Bot. Zeit., vol. xxxix, 1881, pp. 329–336.

[2] A. Borzi in Studi Algologici, Messina, 1883, pp. 87–97.

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Under these conditions the alga lived for several years, and increased very considerably in quantity. It is entirely submerged, resting upon the substratum, and consists of large unicells growing either singly or in loose globular clusters.

Borzi described two species of *Centrosphaera*: *C. Facciolae*, which occurs normally within bright green tubercles formed on the thalloid colonies of *Lyngbya Phormidium*, and *C. minor*, which is similarly situated among the filaments of *Oscillatoria tenuis*. He found that these forms were also capable of an entirely independent existence, floating freely upon the substratum, and he attributed their presence within the colonies of *Lyngbya* and *Oscillatoria* to the fact that the filaments of those algae, growing and expanding upon the substratum, surround and completely envelop the unicells, which have therefore acquired the power of living endophytically. Hence it is quite possible that the free floating condition of the alga under discussion is not the only one in which it exists, but that it may also grow endophytically.

Experimental Cultures.

From the material provided a number of cultures were prepared, with a view to finding out the effects of different nutrient solutions upon the alga. The vessels used for the cultures were small conical flasks and small wide-mouthed bottles, plugs of cotton-wool being used as stoppers. The whole apparatus was carefully sterilized three times at a temperature of 130° C. before using, and sterilized culture media were used; the material was transferred to the cultures by means of a sterilized pipette.

The following culture media were used:

1. Rain-water.
2. Mineral salt-solution consisting of

Potassium dihydrogen phosphate (KH_2PO_4)	1·0 grm.
Sodium nitrate ($NaNO_3$)	1·0 grm.
Magnesium sulphate ($MgSO_4$)	0·3 grm.
Calcium chloride ($CaCl_2$)	0·1 grm.
Sodium chloride (NaCl)	0·1 grm.
Ferric chloride ($FeCl_3$)	0·01 grm.
Distilled water	1000 c.c.

3. Mineral salt solution of half the above strength.
4. Distilled water.

1. *In rain-water* rapid multiplication by aplanospores took place. The cells were relatively small owing to frequency of multiplication, and the cell-walls were thin.

2. *In the stronger mineral salt-solution* the alga multiplied to a certain extent by means of aplanospores, but not nearly so frequently as in

rain-water. The cells gradually increased in size, the cell-wall thickened, and zoogonidangia were formed which entered into a resting condition.

3. *In the weaker mineral salt-solution* the effects were almost identical with those in the stronger solution, but the cell-walls were less thick.

4. *Cultures in distilled water* were prepared from the original material and also from material which had been in rain-water or in the strong mineral salt-solution for some weeks. In every case the most noticeable feature of the cells after they had been in distilled water for a few weeks was the enormously thickened cell-wall (Text-fig. 1, E). Multiplication by aplanospores did not take place at all, and on one occasion only was reproduction by zoogonidia observed. In this case a quantity of material which had been transferred from the strong mineral salt-solution to distilled water was placed upon a slide for examination. It was kept on the slide under observation for a fortnight, being kept moist with distilled water, and at the end of that time the zoogonidia were produced.

The cells do not seem able to withstand drought. Owing to constant evaporation of the water in which the material was kept, a certain amount of the alga was left stranded on the sides of the bottle in the form of a green scum. The cells under these conditions retained their green colour, and except for a slight shrinkage of the cytoplasm presented the same appearance as normal cells. Three cultures were prepared from this material, in rain-water and the two mineral salt-solutions respectively, in order to find out whether the cells had retained their vitality. No multiplication took place in either of the three cultures, and the cells soon lost their green colour and became disintegrated.

The Vegetative Cell.

Vegetative cells of this alga are spherical or subspherical in shape, but may be ellipsoid (Pl. V, Figs. 1–3); they vary considerably in size. Average-sized spherical and subspherical cells measure from 65–70 μ in diameter, while ellipsoid cells may be 65–75 μ long and 55–65 μ broad. Occasionally very much larger individuals can be found measuring even as much as 104 μ long and 97 μ broad, but this is exceptional.

The Cell-Wall.

The wall of the vegetative cell is usually thin, and in spherical cells is of uniform thickness throughout, while in ellipsoid cells a slight thickening is sometimes noticeable at the narrow ends. Faint concentric striations in the cell-wall indicate that its composition is not homogeneous. When treated with iodine the wall acquires a deep reddish-brown colour; on the addition of concentrated sulphuric acid to the preparation, the outer layers of the wall remain unchanged, while the inner layers gradually darken until

they have assumed an almost black colour. The action of a solution of chlor-zinc-iodine is to turn the inner layers of the wall an intense violet colour, while the outer layers remain unchanged. These two reactions show that the inner layers of the wall are composed of cellulose. To determine the nature of the outer layers a solution of cuprammonia was used. This reagent causes the inner cellulose layers to swell up, and finally to dissolve to form a mucilaginous mass completely filling the spaces in the cell produced by the shrinking of the cytoplasm. If the material so treated is now washed with water and then with a 2 per cent. acetic acid solution, the addition of a solution of ammonium oxalate causes the slow dissolution of the outer layers of the wall, showing it to be composed of pectic substances.[1] This conclusion is confirmed by staining sections of the cells with fuchsin, for the outer pectic layers acquire a deep pink colour while the inner cellulose layers remain colourless; the fuchsin readily dissolves out of the pectic layers in alcohol. The cellulose layer varies in thickness from 1 to 4 μ, while the pectic layer is rarely more than 1 μ thick in the vegetative cell.

Cell-Contents.

The cell-contents are granular and of a dense chlorophyll green, and, owing to the size of the cells and their intense colour, it is impossible to come to any conclusion as to their internal construction from an examination of the cell as a whole. It is possible, in a few ellipsoid cells, to differentiate between a central clear space and an intensely green peripheral portion (Fig. 1), but in spherical cells the chlorophyll entirely masks this, except in very early stages. The external surface of the cytoplasm appears in the living cell to be raised into very numerous, usually rounded lobes (Fig. 2), which give the cell-contents a somewhat mulberry-like appearance. From the centre of the cell a number of radiating lines can be seen on focusing to proceed towards the periphery of the cell, reaching the surface at the base of the depressions between the lobes. This appearance of the living cell led Borzi to conclude that the cell contains numerous rod-shaped chloroplasts, each with its base, either circular or angular by compression, closely applied to the inner surface of the cell-wall, and with a long cylindrical projection, which may be either straight or wavy, winding towards the centre of the cell, where there is a circular colourless area. The accuracy of this description of the chloroplast has been doubted on account of the difficulty of making correct observations in a cell of so large a size and so dense a colour, and the present work has done little to substantiate Borzi's conclusions, for on one occasion only and in a single

[1] The action of the acetic acid in this case is to neutralize any ammonia which may remain, and to convert the pectic substances present into pectic acid, which is soluble in ammonium oxalate solution.

individual has a chloroplast of this description been observed (Fig. 3), though several thousands of individuals must have been examined. The appearance in this case was probably due to vacuolation of the cytoplasm, since the material in which it was found was attacked by a fungus and many of the other cells were abnormal in various ways.

An attempt has therefore been made to determine the exact nature of the chloroplast by means of stained microtome sections of the alga of thicknesses varying from 4 to 8 μ. Different fixing reagents were used, including

i. Corrosive sublimate. 3 per cent. solution in 50 per cent. alcohol, containing 3 per cent. of glacial acetic acid.

ii. Absolute alcohol 3 vols. with glacial acetic acid 1 vol.

iii. Bouin's solution containing

Concentrated aqueous picric acid . .	15 parts
30 per cent. solution of formalin . .	5 parts
Glacial acetic acid	1 part.

The stains used were Delafield's haematoxylin, acid fuchsin-iodine-green, and Heidenhain's iron-alum-haematoxylin, the best results being obtained by fixing with Bouin's solution for twenty minutes and staining with Heidenhain's haematoxylin.

Sections prepared in this way show the presence of a large nucleus in a more or less central position, and a peripheral cytoplasm containing a deeply staining, coarse, granular reticulum, especially thickened at the angles of the network. The spaces within the reticulum are filled by a small-meshed, faintly staining network containing a number of small granules. The pattern of the coarse reticulum varies according to the part of the cell through which the section passes. In a median section (Figs. 4–6) the reticulum is seen to take the form of more or less regular rays which branch towards the periphery and are connected up in various planes with other similar rays. The deeply staining reticulations enlarge slightly at the outside and seem to be connected together by a thin, deeply staining layer surrounding the whole of the cytoplasm. In a few cases only in the stained preparations was there any trace of the apparent lobed structure of the surface of the cytoplasm so characteristic of the living cells.

In a more tangential section (Fig. 7) the reticulum forms a network enclosing more or less polygonal spaces of varying sizes and shapes. These are transverse or somewhat oblique sections of the rays seen in the median sections, and their irregularity is due not only to the varying sizes of the rays, but also to the fusion of adjacent rays. Sections taken through the extreme periphery of the cell show a very characteristic appearance (Fig. 8). The deeply staining reticulations are very much

wider, and the enclosed spaces are more or less circular, and are very much smaller than those towards the centre of the cell.

In none of the stained preparations, however, was it found possible to distinguish any part of the cytoplasm which could be identified as the chloroplast as distinct from the rest of the cytoplasm. The position of the chlorophyll was therefore determined by cutting sections of the living material by means of a freezing microtome. The material was embedded in an aqueous solution of gum arabic made as stiff as possible, this substance being used to ensure the absence of plasmolysis. The sections were transferred at once to a slide, moistened with a drop of water and covered with a cover-slip. By these means the preparations were preserved for three weeks, because the freezing sterilized the material used, and the drying of the gum prevented the ingress of bacteria from the air. When a slide was required for experiment the gum was dissolved by adding a little water, and the cover-slip could then easily be removed.

Sections prepared in this way show that the whole of the protoplast, with the exception of the nucleus and a thin peripheral film of colourless cytoplasm, is saturated with chlorophyll. The surface of the chlorophyll-bearing part of the cytoplasm is seen to be raised into numerous small rounded lobes (Fig. 9), and it is these lobes that give the characteristic mulberry-like appearance to the contents of the living cell. The spaces between the lobes are filled with a colourless granular cytoplasm which is continued over the whole surface in the form of a thin layer which attaches the protoplast to the cell-wall. It is the staining of this surface layer of cytoplasm in the permanent preparations which obscures the characteristic lobing of the chloroplast and renders its identification uncertain. The intensity of staining of the peripheral cytoplasm is identical with that of the internal reticulum, hence it may be concluded that the reticulum is also composed of strands of colourless cytoplasm containing numerous deeply staining granules.

The internal structure of the cell may thus be described as consisting of a wide-meshed cytoplasmic reticulum with a large central nucleus and containing a single massive chloroplast which occupies practically the whole cell except the nucleus and which is raised into numerous small rounded lobes at its surface.

Sections cut with the freezing microtome show the presence in the cytoplasm of a considerable quantity of a bright yellow oil. With osmic acid this oil very slowly assumes a deep brown colour, and is then seen to exist in the form of globules of very varying sizes (Fig. 10); the oil is dissolved out of the cell with alcohol.

The action of iodine upon sections of the living material demonstrates the presence of innumerable minute granules of starch scattered evenly throughout the cytoplasm. They are so numerous that a section

only 6–7 μ in thickness rapidly assumes a uniform indigo colour when treated with iodine.

Within the cytoplasm there are also a variable number of pyrenoids; these are brought out best in sections stained with Heidenhain's haematoxylin. In some cells there appear to be no pyrenoids at all, while in others as many as six large ones, or a considerable number of small ones, may be seen in a single section. In the larger pyrenoids the pyreno-crystal is generally either polyhedral or spherical in shape, and there is a definite but rather narrow surrounding starch-sheath; smaller pyrenoids have usually spherical pyreno-crystals. The pyrenoids appear to multiply by fragmentation, the starch sheath gradually disappears, and the pyreno-crystal splits up into a number of small rounded bodies, each of which becomes the central mass of a new pyrenoid (Fig. 11).

The resting nucleus, as seen in the vegetative cell, is relatively very large (Pl. VI, Figs. 21–3). It has a definite nuclear membrane and is filled with a network differing very considerably from that of the surrounding cytoplasm. There is generally one very large, deeply staining, granular nucleolus, and a number of granules are present in the nuclear network, particularly at its angles. All evidence obtained from stained sections, especially from those stained with Heidenhain's haematoxylin, shows that these granules are of an achromatic nature, and are probably similar in composition to the granules in the outer cytoplasmic network. The chromatin of the nucleus is entirely absent from the nuclear network, and is collected together to form the large nucleolus, which is therefore of the nature of a karyosome. The nucleolus is not homogeneous in structure, but contains frequently one or more less deeply staining areas. In a few cases peculiar conditions have been observed in which the nucleolus appeared to be filled with a number of vacuoles surrounded by intensely staining chromatic substance (Figs. 22 and 23). These may merely have been indications of the incipient decomposition of an otherwise apparently healthy cell; on the other hand, they may possibly have been preliminary stages in the breaking up of the nucleolus to form a chromatic thread preparatory to cell division. In one nucleus three karyosomes were observed, but this was the only cell found containing more than one nucleolus in the nucleus (Fig. 24).

Multiplication by Means of Aplanospores.

When the vegetative cells have reached a diameter of about 65–70 μ, changes begin to take place in the cell-contents preparatory to the formation of aplanospores. Numerous vacuoles appear in the cytoplasm, and after a time the whole of the cell-contents divide simultaneously to form a number of aplanospores (Fig. 31). These are naked granular protoplasts, each consisting of a nucleus and cytoplasm containing uniformly distributed chlorophyll. They are usually spherical and from 5·5 to 6·5 μ in diameter,

and as many as 256 may be formed from a single cell. The inner cellulose layer of the wall of the mother-cell quickly becomes disintegrated to form a mucilaginous mass within which the aplanospores lie for some time (Fig. 32). The outer pectic layer of the wall retains its identity for a considerable time after this, only gradually undergoing dissolution.

Preparatory to the formation of aplanospores the nucleus of the mother-cell, by successive divisions, forms a number of daughter-nuclei without the concurrent formation of daughter-cells; the symmetry of the cytoplasmic network is disturbed (Fig. 25), and vacuoles appear. The apparent vacuolation of the living cell at this stage is probably due not only to the formation of true vacuoles in the cytoplasm, but also to the numerous dividing nuclei (Figs. 29 and 30).

Figures of most of the stages through which the dividing nucleus passes have been obtained, showing that a fairly typical karyokinesis takes place. In the first stages the nucleolus completely disappears, breaking up to form a large number of chromatin granules which become dispersed throughout the reticulum of the nucleus (Fig. 25). A little later these granules become rearranged to form a coiled spireme (Figs. 26 and 27), and at a still later stage each of the component granules is seen to be cleft longitudinally, so that the spireme is double (Fig. 28). The contraction of the spireme and its division into chromosomes have not been observed.

In Fig. 29 are seen the late prophases and the metaphases of division. Details of spindle-formation could not be followed, and the evidence available is not sufficient to determine with certainty the number of chromosomes, but there appear to be about seven. These arrange themselves upon an equatorial plate (Fig. 29, A) and each splits into two; the halves separate and pass towards opposite poles of the spindle (Figs. 29, B and C; 30, D and E). There the chromosomes become closely crowded together (Fig. 30, F), and the reconstruction of the daughter-nuclei begins.

Each of the daughter-nuclei formed within the mother-cell becomes the centre of a little mass of protoplasm saturated with chlorophyll, and the formation of aplanospores takes place when each of these little masses rounds itself off from the rest with the simultaneous formation of as many daughter-cells as there are daughter-nuclei in the mother-cell.

The young aplanospores begin their development within the covering provided by the wall of the mother-cell, and before long each acquires a cell-wall of its own, consisting of a thin layer of cellulose and a still thinner pectic layer (Fig. 32). As the wall of the mother-cell becomes completely disintegrated the developing cells are set free in the water, and they continue to hang together in a loose globular mass for a considerable time (Fig. 33).

Each aplanospore develops directly into a vegetative cell like the mother-cell which produced it, and there is no intermediate period of

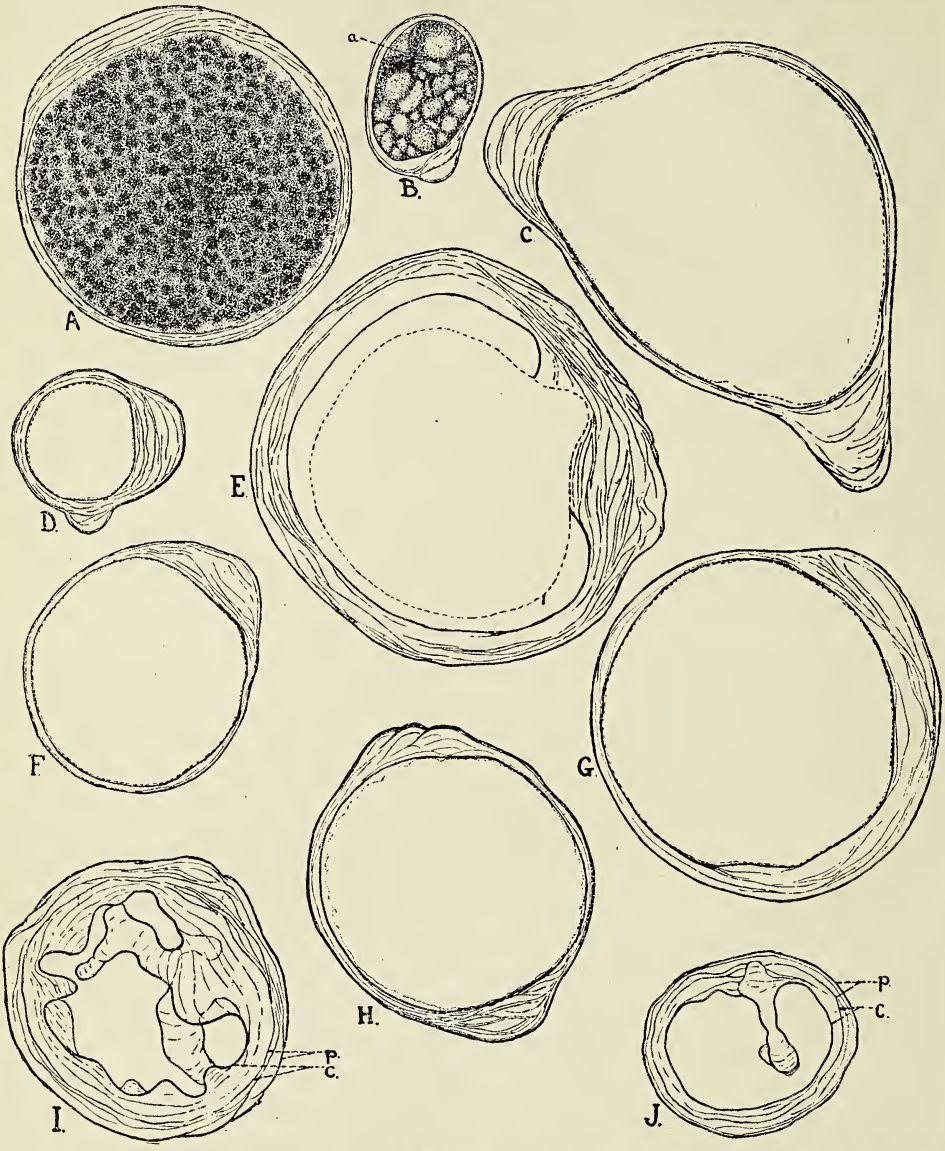
rest. Stained sections of young vegetative cells, of diameter about 18 μ, show an exceedingly simple structure (Fig. 34). There is a more or less central nucleus and a simple chloroplast almost filling the cell, with its outer surface raised into a few rounded lobes; the colourless cytoplasm filling the spaces between the lobes is sometimes very evident, and there may be one or two pyrenoids present. As the cells increase in size the cytoplasmic reticulum becomes much more marked, and the lobes of the chloroplast increase in number until the cytoplasm assumes the structure shown in Fig. 6. The aplanospores produced by a single mother-cell do not all necessarily develop at the same rate; clusters of young cells are often seen in the condition shown in Fig. 33, where one of the cells has attained a diameter of 56 μ, and several others of about 22 μ, while a number of the aplanospores have scarcely begun to develop.

When the cell has developed to its full size a repetition of the foregoing processes takes place, several successive generations of vegetative cells being produced by means of aplanospores before any further changes take place.

Reproduction by Zoogonidia.

After an indefinite number of generations of aplanospore-formation the vegetative cell undergoes important changes, being gradually transformed into a zoogonidangium. This may occur at any stage of growth of the vegetative cell, but usually takes place after the cell has attained its full size; and it is to be noted that propagation by aplanospores has been observed after transformation into a zoogonidangium has begun. The cell-wall becomes enormously thickened in both layers and shows numerous stratifications. The cellulose layer may become 4–8 μ or rarely as much as 11 μ thick, while the pectic layer varies from 2 to 7 μ in thickness; the whole thickness of the wall varies usually from 6 to 15 μ, but specimens have been found with their walls both above and below these limits. The thickening is not distributed evenly over the whole surface of the wall, but in both cellulose and pectic layers it may be concentrated to form one or more projections. Unequal thickenings of the cellulose layer give rise to internal projections of very varied sizes and shapes, which show all the characteristic reactions of cellulose. They may be in the form of small conical papillae showing fine transverse striations, as observed by Borzi, and where they are of this form there are usually several in one cell. Frequently, however, they assume a very much larger size, projecting more than half-way across the cavity of the cell. These long projections may be simple with an enlarged globose or irregular head (Text-fig. 1, J), or they may be branched and twisted, ramifying through the substance of the cytoplasm (Pl. V, Fig. 15). As a rule only one such projection is formed within a single cell, but two have been observed (Fig. 13), and it sometimes

happens that a cell provided with one long projection may also possess one or more small conical papillae. In a few cases, one of which is shown in



Text-fig. 1. Zoogonidangia with variations in thickening of the wall. × 330. A, surface view of specimen showing lobes of chloroplast; B, do., showing depression in cytoplasm at *a* due to internal cellulose projection from the cell-wall; C–H, various cases showing distribution of sculpturing; I and J, sections showing internal thickenings of cell-wall. *p.* pectic layer; *c.* cellulose layer.

Text-fig. 1, I, the whole internal surface is raised into projections of varying sizes.

The unequal thickenings of the pectic layer of the wall produce

external projections very different in shape from the internal ones. They are composed of pectic substances and are solid, striated, and frequently very broad, and their surface is rounded. They vary considerably in position and shape (Text-fig. 1, A–H), and there are usually not more than two on any cell, frequently only one being present. In a few cases (Text-fig. 1, G) the projection is seen to take the form of an elongated ridge in the median plane, sometimes extending as much as half-way round the circumference of the cell. It frequently though not invariably happens that the pectic projection is opposite to the cellulose projection, and where this is the case there is sometimes a small air-space between the two layers, probably formed as a result of the strain produced in the wall by the unequal thickening.

At the same time as the cell-wall is undergoing the above changes, the cell increases greatly in size, and, as a result of the unequal thickening of the wall, it sometimes assumes a somewhat irregular shape (Text-fig. 1, C). The size to which the zoogonidangium may attain varies in different individuals. A fully grown zoogonidangium probably measures on an average about 130 μ in diameter, but specimens have been observed measuring as much as 203 μ long by 127 μ broad, and 162 μ long by 140 μ broad respectively, and as little as 65 μ long by 54 μ broad, including the thickness of the wall, while the production of zoogonidia was observed on a zoogonidangium only 82 μ in diameter.

The formation of the internal cellulose projections from the cell-wall produces corresponding depressions in the cytoplasm of the cell. That these depressions are of secondary development, caused by the invagination of the surface of the protoplast, is shown by the fact that the arrangement of the cytoplasm bordering on the depressions is identical with that of the peripheral cytoplasm of the cell; the lobes of the chloroplast are, however, obliterated by mutual pressure and the colourless film of cytoplasm, which elsewhere lines the cell-wall, appears to be absent from the depressions near their base. Funnel-shaped depressions can be seen in the cytoplasm of the living cell at the surface (Text-fig. 1, B), but owing to the dense colour of the cell it is impossible to trace either the course or the extent of the tubular passages to which they lead except by means of sections.

In consequence of the invagination of the surface, the more or less regular arrangement of the cytoplasmic reticulum characteristic of the vegetative cell becomes disturbed, and in an old zoogonidangium the mesh of the reticulum is usually much smaller than in a young one. Where the internal projections are long an actual splitting of the cytoplasm must eventually take place, because the nucleus, which in vegetative cells is central in position, is frequently seen to lie close to the side of the depression (Fig. 12).

The zoogonidangium contains large quantities of yellow oil, and very

numerous minute starch-grains. Pyrenoids are usually present and are frequently much larger than those in the vegetative cell.

When the zoogonidangium has attained its full size it enters upon a period of rest which may last for some months. Borzi states that zoogonidangia formed in the spring remained in a resting condition until the following autumn, while in the present work most of the zoogonidangia which were found in the material in the middle of October were still inactive at the middle of the following April. Finally, however, the lobes of the chloroplast disappear, and the whole protoplast rounds itself off from the cell-wall and assumes a uniformly granular appearance. Multiplication takes place by the successive bipartition of the whole protoplast (Figs. 17–20) into a very large number of small green zoogonidia, the number varying according to the size of the cell; from some of the larger zoogonidangia as many as a thousand may be produced. The rate at which the successive partitions take place varies apparently with the external conditions; in one case, during the changes which accompanied one partition more than an hour elapsed, while in another the complete change from an 8- to a 32-celled stage was accomplished in forty minutes. No cell-walls are formed during these rapid changes; the cytoplasm divides by simple constriction, and the products of division are intensely granular and are completely saturated with chlorophyll. They round themselves off from one another, and usually dispose themselves towards the periphery of the cell-cavity, but as the divisions become more numerous the whole cavity becomes filled, and the daughter-cells lose their spherical shape, becoming angular by compression. The final products of division are the zoogonidia, and when their formation has nearly been completed a large vesicle is formed on some part of the cell-wall (Fig. 19). This vesicle appears to be produced as a result of changes in the composition of the cellulose layer of the wall, so that it swells up and inflates the outer pectic layer, which, however, never loses its identity. The inner layer of the wall at this point gradually decomposes to form a mucilaginous mass, which fills the vesicle and eventually causes a splitting of the pectic layer, so that the cavity of the zoogonidangium is put into communication with the outside water. At this stage the zoogonidia near the opening round themselves off from the mass within the mother-cell and by means of rotatory movements make their way through the vesicle into the water. This gives an opportunity for those farther within the cell to become free, and in a very short time, often as little as ten minutes, the whole mass of zoogonidia escape one by one from the mother-cell; a few individuals become entangled in the mucilage of the vesicle and are unable to escape (Fig. 20).

The zoogonidia are little oval or pear-shaped masses of naked protoplasm, varying in length from 3·5 to 5 μ, and not more than 2·4 μ in breadth. Their chlorophyll appears to be distributed evenly except at the centre,

where there is a clear spot, probably the nucleus. Exact details of the cytology of the zoogonidia could not be obtained on account of the rarity of their production, which prevented the use of staining methods. It was also found impossible, on the same account, to follow the nuclear changes which take place during the formation of zoogonidia.

The zoogonidia swim about in the water for nearly an hour by means of their cilia ; it sometimes happens that two zoogonidia become entangled by their cilia, but fusion does not take place, and they finally come to rest, round themselves off and grow rapidly, developing directly into vegetative cells. For a short time the young developing zoogonidium has no cell-wall, but after a few days a thin cellulose membrane is formed. The characteristic lobed chloroplast is soon differentiated and development into a vegetative cell continues in exactly the same way as that of an aplanospore. The production of a red pigment has not been observed at any stage of the life-history of this alga.

Affinities and Systematic Position.

A comparison of the life-history of this form with that of *Centrosphaera*, Borzi, shows that undoubtedly the two algae belong to the same genus. In both cases the vegetative cell is globose or ellipsoid with a firm thin cell-wall. Upon reaching a certain size the contents of the cell divide simultaneously into a number of aplanospores which develop directly into vegetative cells. After an indefinite number of generations has been produced in this way the vegetative cell becomes converted into a hibernating zoogonidangium with a characteristically thickened cell-wall. From the zoogonidangium a large number of motile biciliate zoogonidia are produced. These come to rest and grow at once into vegetative cells, which, in their turn, multiply by means of aplanospores.

There are, however, a number of differences which are worth noting. In the alga described above the average diameter of the vegetative cell before aplanospore-formation takes place is 65–70 μ, and of the zoogonidangium about 130 μ. The shape and thickening of the zoogonidangium-wall is very variable ; the internal projections may be short and conical, or long, twisted and even branched within the cytoplasm, and more than one external pectic projection may be present on a cell. The zoogonidia are produced by the successive bipartition of the contents of the mother-cell.

In *Centrosphaera Facciolae*, Borzi, on the other hand, aplanospore-formation takes place when the vegetative cell has reached a diameter of only 30–40 μ, and the zoogonidangia are rarely more than 80 μ in breadth. The zoogonidangium-wall is much less variable ; it bears 1–3 small conical internal projections, and a single large external projection often curved into the form of a spur. Borzi describes the formation of zoogonidia as being due

to the simultaneous division of the contents of the zoogonidangium into the required number of parts.

These differences seem to justify the separation of the alga as a new species of *Centrosphaera* to be named *C. grande*, if the genus *Centrosphaera*, Borzi, can be regarded as an independent genus.

As was mentioned at the beginning of this paper, the identification of algae belonging to this group is exceedingly difficult because of the great similarity of the genera. A careful examination of the original papers and figures by Klebs [1] and Borzi [1] does not reveal a single fundamental character by means of which the genera *Chlorochytrium*, Cohn, *Endosphaera*, Klebs, *Scotinosphaera*, Klebs, and *Centrosphaera*, Borzi, may be distinguished from one another in either the vegetative or the zoogonidangial states. In their habit all are more or less endophytic in water plants ; the first three genera live within the tissues of such plants as *Potamogeton*, *Hypnum*, and *Sphagnum*, while *Centrosphaera* is frequently found growing among the filaments of colonies of blue-green algae, though it may be free floating on the substratum. Since the habit, the vegetative cells and the zoogonidangia are almost identical in all the genera, generic distinctions have been based by Klebs and Borzi upon details in the life-history. Under present circumstances it is therefore necessary to follow the complete life-history of one of these algae, a task which may require any time from several months up to two years for its completion, in order to decide with any degree of certainty to which genus it should be assigned. Even then, the generic distinctions are in some cases so trivial that they might very easily be overlooked.

For example, *Chlorochytrium*, Cohn (1874), and *Endosphaera*, Klebs (1881), both propagate themselves by means of spherical zoogonidia produced as the result of successive bipartition of the contents of the zoogonidangium. In both, the zoogonidia produced from a single cell fuse in pairs near the opening of the zoogonidangium to form zygotes. The distinction between the two genera is based upon the fact that in *Chlorochytrium* the zoogonidia are produced as the final products of an uninterrupted bipartition, while in *Endosphaera* the products of the first five or six divisions provide themselves with a thin wall before they continue to divide, and about eight zoogonidia are produced from each of these secondary cells within the zoogonidangium. But no resting period appears to occur during this interruption in division. The only other distinction between the genera is a detail in the development of the zygote. In both genera the zygote rests upon the surface of a leaf and puts out a tube which penetrates between the cells of the epidermis, and down which the contents of the zygote pass to form at the end of the tube the endophytic cell typical of the alga. In *Chlorochytrium* the part of the zygote which lies on the surface of the leaf

[1] loc. cit.

develops into a permanent spherical stopper of cellulose; in *Endosphaera* it dies away, and very soon no trace of it can be detected.

These two distinctions would seem to be far too unimportant to constitute generic characters, though they might possibly be used to differentiate between two species of the same genus.

Again, Borzi admits that the zoogonidangia and zoogonidia of *Centrosphaera* (1883) are identical with those of *Scotinosphaera*, Klebs (1881), but he separates the two genera because in *Centrosphaera* propagation takes place both by aplanospores and by zoogonidia, while in *Scotinosphaera* only propagation by means of zoogonidia has been observed. If the difference of habitat of these two genera be considered, the difference in their life-history may be explained as an adaptation to environment. *Centrosphaera*, Borzi, is able to multiply by means of aplanospores within the thalloid colonies of *Lyngbya Phormidium*, since the developing cells are able to make room for themselves by separating the *Lyngbya* filaments which are not united to form a compact tissue; it is only comparatively rarely that zoogonidia are formed and escape to establish further centres of growth in other parts of the thallus. In *Scotinosphaera*, Klebs, however, which grows within the leaves of *Hypnum* and of *Lemna trisulca*, aplanospore-formation would be disadvantageous on account of the difficulty which would be experienced by the mass of cells growing together within the relatively compact and inseparable tissue of the host. Zoogonidia, on the contrary, are able to distribute themselves and to develop independently of one another. It would therefore seem better to consider that *Scotinosphaera*, Klebs, was originally identical with *Centrosphaera*, Borzi, but that, having acquired a truly endophytic habit, it has lost the power of aplanospore-formation. The absence of aplanospores in *Chlorochytrium*, Cohn, and *Endosphaera*, Klebs, may perhaps be accounted for in the same way.

A very intimate connexion between *Scotinosphaera*, Klebs, and *Chlorochytrium*, Cohn, can be established through certain species of *Chlorochytrium*, notably through *C. Knyanum*, Cohn, and *C. pallidum*, Klebs, in which propagation takes place only by means of asexual zoogonidia. In these two species the life-history is identical with that of *Scotinosphaera*, and there seems to be no real reason why two generic names should be used for algae so very much alike. The conjugation of the zoogonidia in *Chlorochytrium Lemnae* is considered to be a case of gamogenesis and not a true sexual fusion, hence its occurrence has not been considered sufficient grounds for separating this species from those in which no conjugation takes place.

This discussion indicates that a simplification of these genera would not only be more convenient for purposes of identification, but would also be more correct. The genus *Endosphaera*, Klebs (1881), should certainly be considered as synonymous with *Chlorochytrium*, Cohn (1874), while the striking resemblances between *Scotinosphaera*, Klebs (1881), and *Centro-*

sphaera, Borzi (1883), render it most improbable that they should be regarded as independent genera. Again, the resemblances between *Scotinosphaera* and *Chlorochytrium* are of so much more fundamental a character than the differences between them that it seems advisable to include all of these so-called 'genera' in a single genus *Chlorochytrium*. In this case the new species described in this paper will have to be named *Chlorochytrium grande*, not *Centrosphaera grande*.

Diagnosis.

Chlorochytrium grande, sp. nov. Cellulae vegetativae sphaericae, subsphaericae, v. ellipsoideae, 65–75 μ diam., membrana sat aequali, chlorophora singula magna superficie in lobulos plurimos producta, granulis amylaceis oleo pyrenoidibus numero ludentibus donatae.

Propagatio fit divisione simultanea contentus cellularum in aplanosporas, vel interdum bipartitione repetita in zoogonidia agamica. Zoogonidangia 130 μ diam., membrana 6–15 μ crass., paxillis instructa externis 1–2 e substantia pectica constantibus atque internis uno v. pluribus saepissime maximis et subinde intra plasma ramosis e cellulosa constantibus. Zoogonidia biciliata ovalia v. pyriformia, 3·5–5 μ long., 2 μ lat., cum vesicula evacuata.

Hab. in limo fossae permagnae prope Doncaster, W. Yorks.

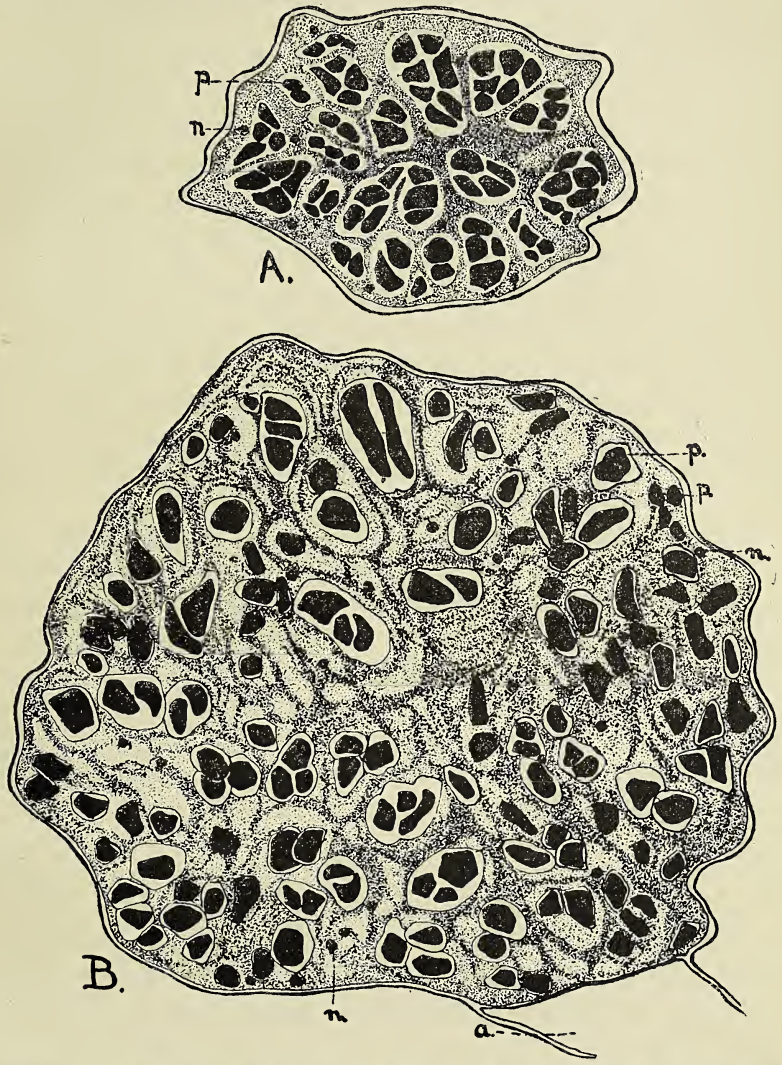
A Note on the Genus *Phyllobium*, Klebs.

The only other genus belonging to this group of algae is *Phyllobium*, Klebs (1881). This undoubtedly stands alone as an independent genus, not only on account of its life-history but also from its external appearance and its cytology. This genus is epiphytic, and the resting cells, which bear considerable external resemblance to those of *Chlorochytrium*, are attached at the base to a vegetative thallus, which in *P. sphagnicola*[1] consists of branching and anastomosing threads, which penetrate through the air-pores into the hyaline cells of the host.

An examination has been made of the cytology of *P. sphagnicola* from material preserved in formalin and very kindly provided by Prof. G. S. West. The material was first washed for two or three days with successive relays of clean water in order to remove all traces of formalin. Sections were then cut and stained with Delafield's haematoxylin, Heidenhain's iron-alum-haematoxylin, and with acid fuchsin-iodine-green. Such sections show a granular cytoplasmic network in the meshes of which are embedded an extraordinarily large number of pyrenoids, each with a starch sheath (Text-fig. 2). On treatment with a dilute solution of iodine the starch sheaths assume a light blue colour, while the pyreno-crystal becomes deep brown. No large nucleus is present in the cell, but under a magnification of 1,435 diameters a number of very small nuclei can be seen in the cyto-

[1] G. S. West: Some Critical Green Algae. Journ. Linn. Soc., Bot., vol. xxxviii, Jan. 1908.

plasmic network. Definite nuclear structures cannot be distinguished, and the nuclei seem to be little more than granules of chromatin, which, with iron-haematoxylin, stain an intense black colour in contrast to the blue colour of the pyreno-crystals.



TEXT-FIG. 2. *Phyllobium sphagnicola.* × 1,435. A, section of small cell showing radial arrangement of cytoplasm with groups of pyrenoids between the rays, (stained with Delafield's haematoxylin); B, section of larger cell stained with Heidenhain's haematoxylin to show the pyrenoids; the cytoplasmic reticulum is irregular. *a.* attachment of branching thallus; *n.* nucleus; *p.* pyrenoid.

In a few cases a somewhat radial arrangement of the cytoplasmic reticulum has been observed (Text-fig. 2, A), but this is most frequently obscured, probably by the distortion caused by the growth of the pyrenoids.

No differentiation of a chloroplast is indicated in these stained sections, and it seems highly probable, both from this and from the external appearance of the cell, that the chlorophyll is diffuse throughout the cytoplasm.

The cells of the colourless branching tubes which form the vegetative thallus appear to be quite empty, no trace being evident in the stained preparations of either cytoplasm or nuclei.

Summary.

In rain-water rapid multiplication of *Chlorochytrium grande* takes place by means of aplanospores, and the cells are thin-walled. In mineral salt-solutions aplanospores are formed more rarely, and the cells become converted into large zoogonidangia with very much thickened walls. In distilled water an enormous thickening of the walls takes place.

The vegetative cells are spherical, subspherical or ellipsoid, 65–75 μ in diameter, with a wall of fairly uniform thickness consisting of an inner cellulose and an outer pectic layer. They contain a wide-meshed cytoplasmic reticulum with a large central nucleus and a single massive chloroplast which is raised into numerous rounded lobes at its surface and occupies practically the whole cell except the nucleus. The cells contain oil, numerous granules of starch, and a variable number of pyrenoids.

Propagation takes place by simultaneous division of the contents of a cell into aplanospores, preceded by numerous successive mitotic divisions of the nucleus of the cell. The chromatin of the resting nucleus is in the form of a karyosome.

The zoogonidangia are very large, averaging 130 μ in diameter. The wall bears one to two rounded external pectic projections, and one to several internal cellulose projections which are frequently large and may be branched within the cytoplasm, which is correspondingly distorted. Starch, oil, and pyrenoids are all present.

Zoogonidia-formation takes place by the successive bipartition of the contents of the mother-cell into numerous biciliate oval or pear-shaped bodies, which escape through a vesicle in the zoogonidangium wall. They develop directly into vegetative cells.

The alga is established as an independent species on account of the very large size of the zoogonidangium and the great thickness and irregularity of its wall.

The generic names *Endosphaera*, Klebs (1881), *Scotinosphaera*, Klebs (1881), and *Centrosphaera*, Borzi (1883), are unnecessary, since the algae thus named can quite satisfactorily be included within the single genus *Chlorochytrium*, Cohn (1874), and the generic distinctions put forward by Klebs and Borzi are inadequate for their retention as independent genera. The new species described has therefore been named *Chlorochytrium grande* rather than *Centrosphaera grande*.

Phyllobium sphagnicola is a coenocyte containing a reticulate mass of cytoplasm in which are embedded numerous small granules of chromatin, and in the meshes of which there are a very large number of pyrenoids. The chlorophyll is probably diffuse throughout the cytoplasm. The cells of the branching thallus appear to have no contents.

In conclusion I wish to express my thanks to Prof. G. S. West for providing the material for this work, and for much help and criticism during the course of the investigation.

The Botanical Laboratory,
University of Birmingham.

DESCRIPTION OF PLATES V AND VI.

Illustrating Miss Bristol's paper on *Chlorochytrium grande.*

PLATE V.

Chlorochytrium grande. All figures × 535, except Fig. 11.

Figs. 1 and 2. Surface view of vegetative cells, showing lobed appearance of chloroplast.

Fig. 3. Cell with apparent parietal chloroplasts, each with an internal projection (probably due to vacuolation of the cytoplasm).

Figs. 4–6. Median sections of vegetative cells, showing rays in the cytoplasm; Figs. 4 and 5 through the nucleus and Fig. 6 through a central cluster of pyrenoids.

Fig. 7. Non-median section, showing cytoplasmic rays cut transversely and obliquely, and numerous small pyrenoids.

Fig. 8. Section through periphery of cell, showing cut ends of rays with deeply-staining substance between.

Fig. 9. Section of living cell, cut with a freezing microtome, showing distribution of chlorophyll (dark), with a colourless nucleus and peripheral layer of cytoplasm.

Fig. 10. Section cut through a living cell, treated with osmic acid to show oil-drops.

Fig. 11. Pyrenoids with starch-sheaths, and fragmenting pyrenoids. × 825.

Fig. 12. Section of zoogonidangium, showing internal cellulose projection from the wall and corresponding depression in the cytoplasm, with the nucleus lying close to its side.

Fig. 13. Section of small zoogonidangium with two cellulose projections from the wall.

Fig. 14. Section of zoogonidangium with wall enormously thickened on one side but with no internal projection.

Figs. 15 and 16. Sections of zoogonidangia with cytoplasm displaced by branched or twisting projections from the wall.

Figs. 17–20. Multiplication by zoogonidia.

Fig. 17. Small zoogonidangium in 16-celled stages of division.

Fig. 18. The same cell after 3 hours.

Fig. 19. The same cell after 17 hours. *v.* vesicle.

Fig. 20. Zoogonidangium with escaping zoogonidia and a very long internal projection from the wall.

PLATE VI.

Figs. 21–30. Sections showing nuclei and nuclear division preparatory to the formation of aplanospores. × 1,435.

Figs. 21–23. Resting nuclei with surrounding cytoplasm, showing variations in karyosome and nuclear network.

Fig. 24. Resting nucleus with three karyosomes.

Fig. 25. Cell in which the cytoplasm has become vacuolated and the karyosome of the nucleus has broken up into numerous granules.

Fig. 26. Beginning of spireme formation; granules collecting together to form a continuous thread.

Fig. 27. Spireme stage of nuclear division.

Fig. 28. Nucleus with spireme cleft longitudinally.

Fig. 29. Cell containing probably thirty-two dividing nuclei. *a*, chromosomes upon an equatorial plate; *b* and *c*, successive stages in passing of chromosomes to opposite poles of the spindle.

Fig. 30. Cell containing probably 128 dividing nuclei. *d* and *e*, further stages in passing of chromosomes to poles of spindle; *f*, crowding together of chromosomes preparatory to reconstruction of daughter-nuclei. *p*. pyrenoid; *f.p*. fragmenting pyrenoid. Cell-wall omitted.

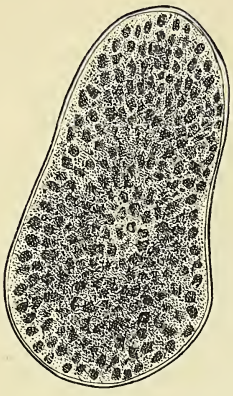
Figs. 31–34. Formation and development of aplanospores. × 535.

Fig. 31. Cell in which simultaneous division of the contents into a number of aplanospores has just taken place.

Fig. 32. Aplanospores developing inside the old wall of the mother-cell, the inner layer of which has disintegrated to form a mucilaginous mass. Each aplanospore has acquired a cell-wall.

Fig. 33. Cluster of cells at different stages of development, set free by the complete dissolution of the mother-cell wall.

Fig. 34. Cytology of the developing aplanospore shown by means of stained sections. *a* and *b*, median sections of very young cells with simple lobed chloroplast surrounding the nucleus; *c*, non median section of a rather older cell with two pyrenoids.



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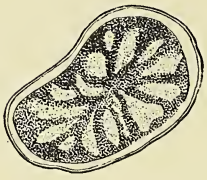

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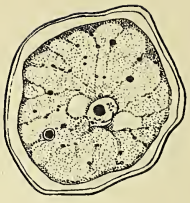

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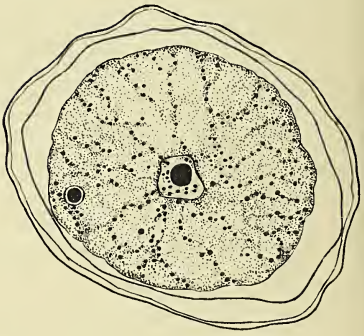

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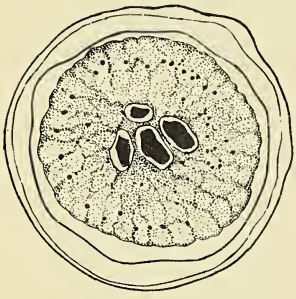

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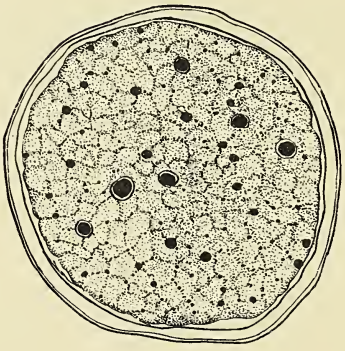

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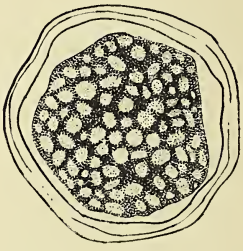

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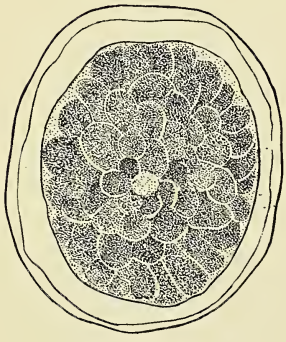

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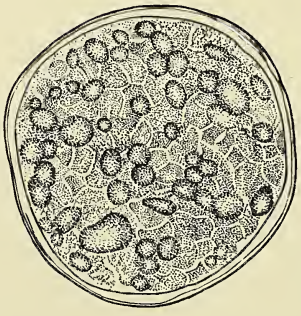

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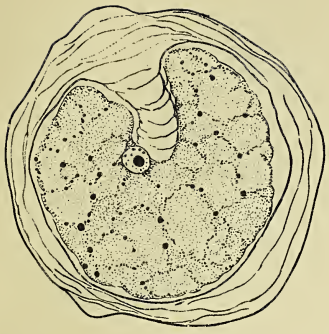
M. BRISTOL — CHLOROCHYTRIUM.



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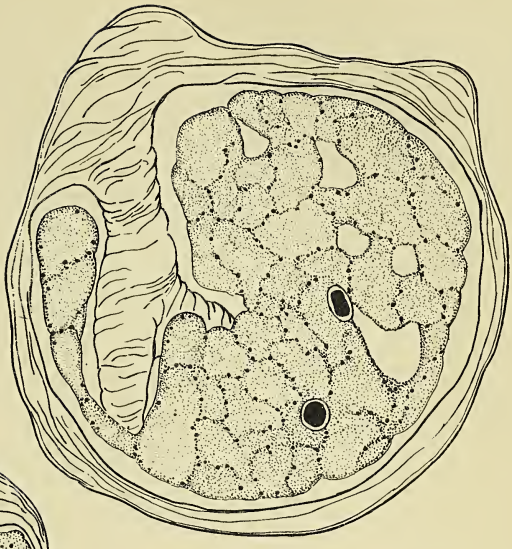

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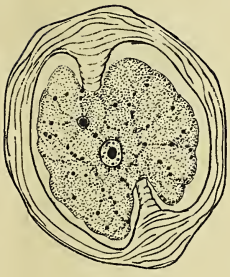

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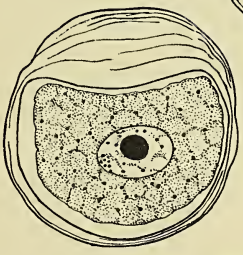

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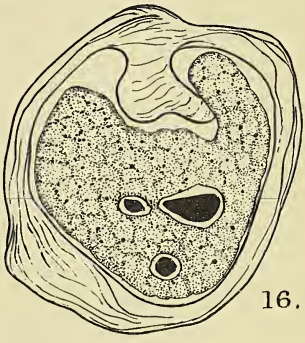

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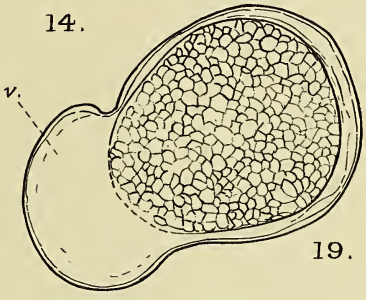

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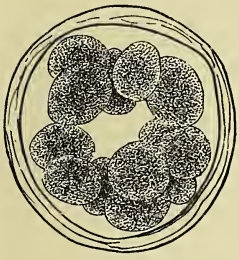

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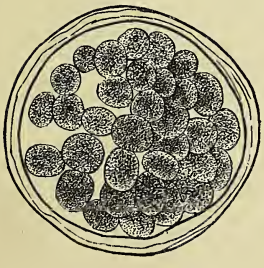

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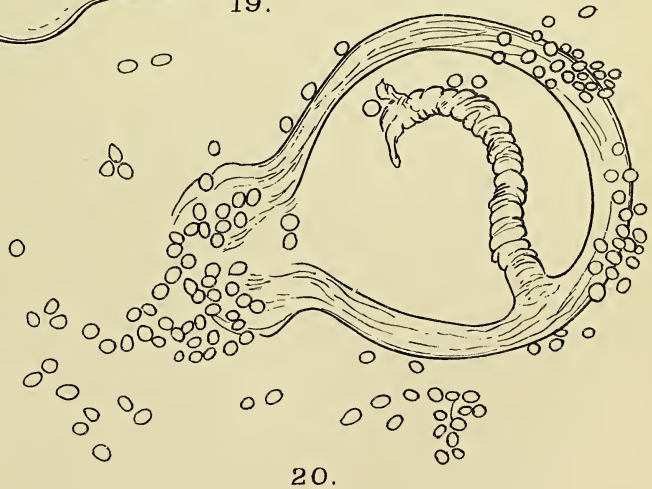

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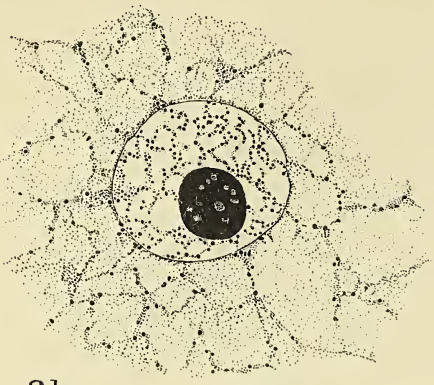

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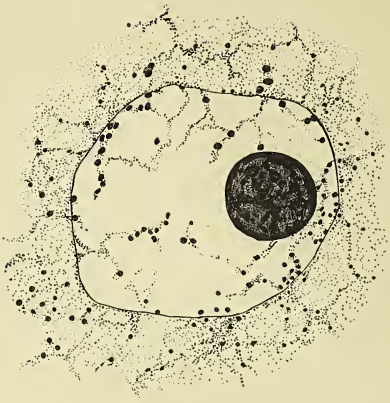

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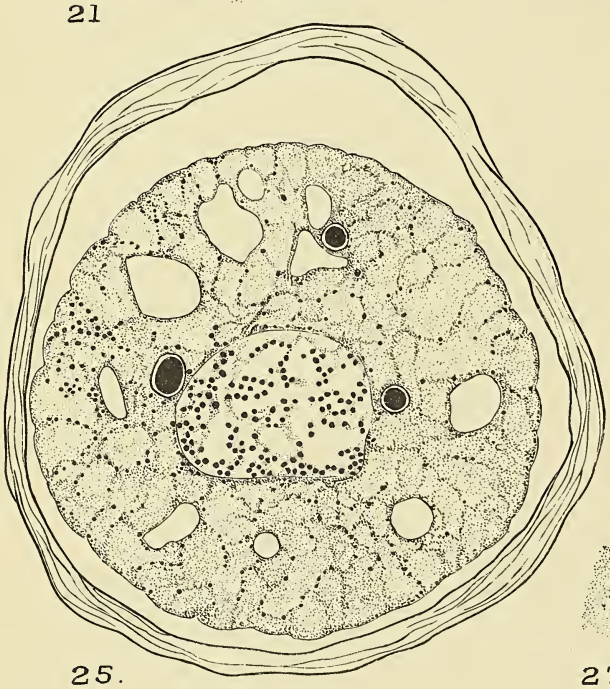

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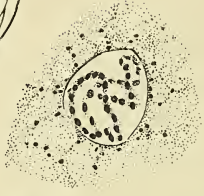

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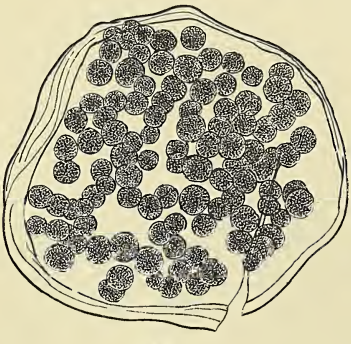

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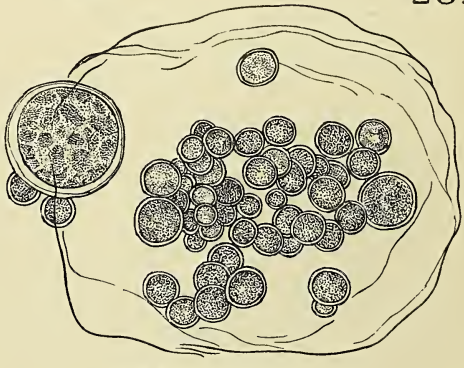
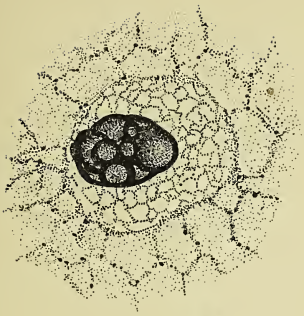


32.

M. BRISTOL — CHLOROCHYTRIUM.



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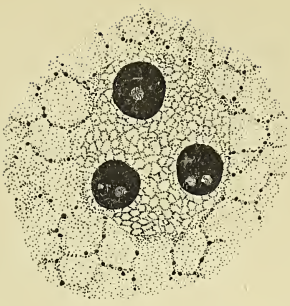

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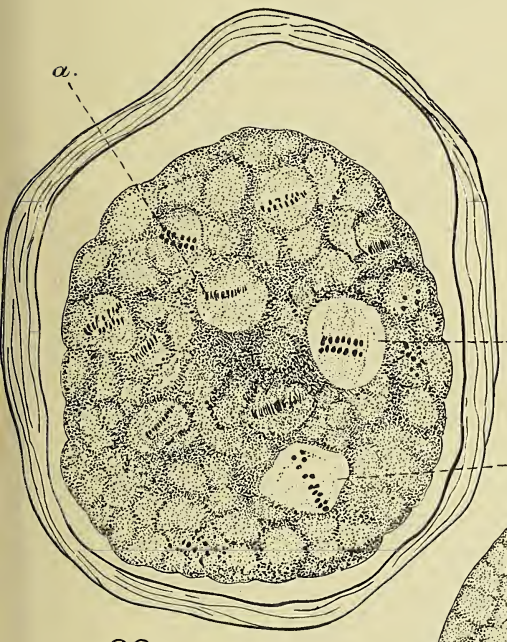

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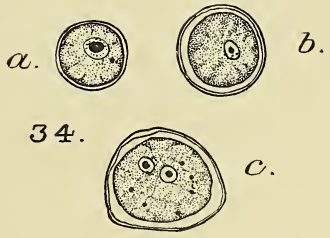

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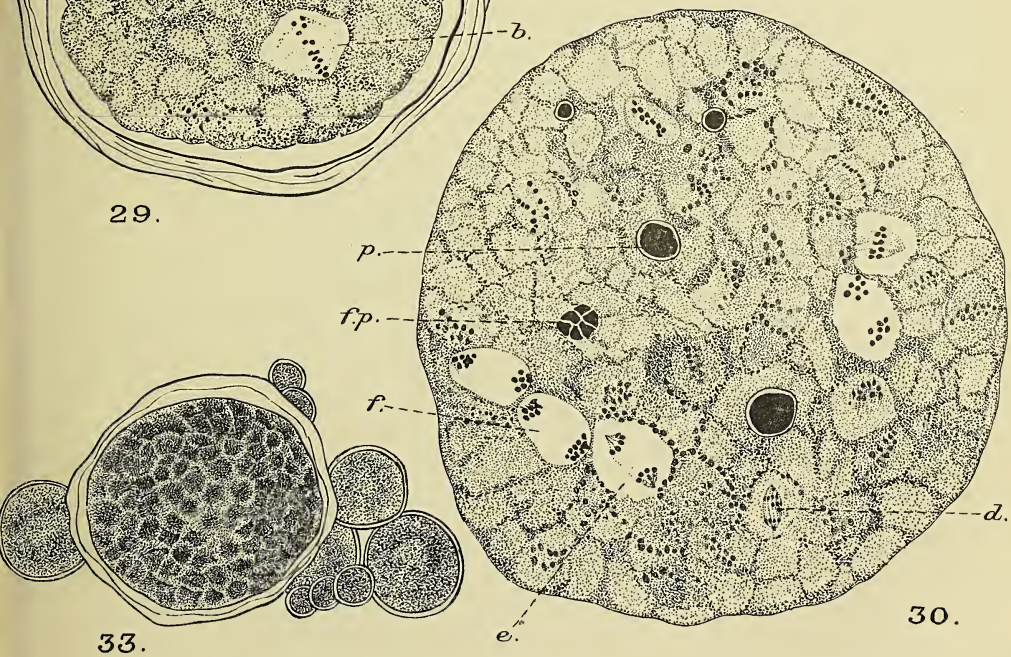

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Spore Germination in Onygena equina, Willd.

BY

WILLIAM B. BRIERLEY

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ONYGENA EQUINA, Willd., is a somewhat rare ascomycetous fungus occurring on decomposing horns and hoofs of cattle, sheep, horses, &c. The fructifications are globular and stalked, pseudo-parenchymatous in texture, and dehisce irregularly. The asci are very evanescent and the spores when ripe fill the peridium as a loose powdery brown mass mixed with 'capillitium fibres'. During the growth of the sporocarp abundant chlamydospores are developed, which form a peripheral crust enclosing the dome-shaped mass of the young fructification.

Prior to Marshall Ward's investigations [1] the conditions of ascospore germination were unknown, although of the related species, *O. corvina*, de Bary [2] had made the significant observation that the spores refused to grow in water or nutrient solutions, or in gastric juice, at any temperature, 'but there was a fine formation of compound sporophores of *Onygena* on the hairs cast up by a white owl . . . which had received the spores of the fungus with a mouse which it had eaten. The fungus developed on the hair from the mouse on which the spores had been strewed and from no other.' Ward also found that ascospores placed directly in water or various nutrient media refused to germinate, but, following the clue given by de Bary's observation, caused their germination by subjecting them to a preliminary digestion in artificial gastric juice. De Bary had tried this method using extract of pig's stomach, and his failure must be attributed to some accessory circumstance.

Marshall Ward found that the chlamydospores also germinated readily after digestion with gastric juice, but later discovered that, in certain nutrient media, their germination was possible in the absence of such treatment, although apparently considerably promoted by it.

On January 2, 1914, a large ram's horn bearing sporocarps of *Onygena*

[1] H. Marshall Ward: *Onygena equina*, Willd., a Horn-destroying Fungus. Phil. Trans. Roy. Soc., B, vol. cxci, pp. 269–91, 1899.

[2] A. de Bary: Comp. Morph. and Biol. of the Fungi, etc., p. 351, 1887.

[Annals of Botany, Vol. XXXI. No. CXXI. January, 1917.]

equina, Willd., was found on the heather moorlands of the Peak District. This was left exposed on the roof of the botanical laboratory at Manchester University, and during the following two years continually produced fructifications of the fungus.[1]

Germination of ascospores. By carefully breaking open a mature ascocarp, pure sowings of the ripe spores may easily be obtained. These were placed in hanging drops of various strengths of nutrient media and kept at different temperatures, but the negative results only confirmed the experience of previous investigators. A preliminary treatment of the spores with artificial gastric juice,[2] or an admixture of this with the nutrient media, readily produced abundant germination after about five or six days at room temperature (16°–18° C.) or three to five days in an incubator at 23° C. The nutrient media were those commonly employed in the laboratory, including various plant extracts, peptone, beer wort, &c. Germination occurred in all; but good subsequent growth was only obtained in a few of those of animal origin, such as peptone and blood fibrin and the more special media described below.

By accident, however, it was discovered that, given a sufficiently long resting period, direct germination may occur in the complete absence of any digestive treatment. Towards the end of January, the exact date being unknown, a number of mature fructifications were picked off the horn and placed in a tin box. This was lost sight of and only rediscovered when the room was turned out for its summer vacation cleaning. The sporocarps were again left lying in the box on a shelf, and it was only on September 13 that the idea occurred to me that possibly the direct germination of the spores might be conditioned by a resting period. Hanging drops of water,[3] gelatine,[4] and coarse glue[5] were immediately prepared, undigested spores from the dried and shrivelled fructifications sown, and the cells left at room temperature. On September 17 abundant germination was noted in the glue and gelatine and a vigorous growth resulted. On September 20 a few abnormal germ tubes, often dilated (Keimblasen), were noted from the spores

[1] The original intention was to make a detailed investigation into the development and physiological relations of *Onygena*, but as it now appears very improbable that opportunity will permit of this, the present fragment has been thought perhaps worthy of separate publication.

[2] Made according to the formula given by Marshall Ward, and consisting of 100 c.c. of 0·4 % HCl (1·3 c.c. of commercial HCl in 100 c.c. H_2O) added to 0·2 grm. pepsine dissolved in 100 c.c. H_2O.

[3] Sterilized tap-water.

[4] 100 grm. of gelatine in 1,000 c.c. of water. Filtered and sterilized in Koch steamer on three successive days.

[5] Fine or pale-coloured glues are usually treated with antiseptics. The coarse glue used was a very dark coloured impure substance in lumps broken out from a large solidified mass. It possessed a strong unpleasant smell and rapidly decomposed, and was obtained for me by a dealer, who described it as 'first boiling'. Twenty-five grammes of this glue were added to one litre of water and heated until it dissolved. It was then filtered and sterilized in a Koch steamer on three successive days.

in water, but here no further growth was obtained. Hanging drops of cow-dung extract,[1] glue, gelatine, and water were then prepared, fresh mature spores were sown, and the cells were left at room temperature and in the incubator at 23° C. These cells were carefully preserved and when necessary the medium was renewed. From September 18 until February 2 no change was observed, but on the latter date the contents of many of the spores in the dung extract in the incubator appeared to become slightly clearer. On February 8 germination was noted, and two days later this was fairly abundant, about 40 per cent. of the spores putting out germ tubes. In the glue germination occurred on February 11, in the gelatine on February 19, whilst no germination occurred in the hanging drops of water. The spores which had been kept at room temperature germinated about the same time, but more irregularly. In this case also no germination occurred in water. The minimum resting period under these conditions was therefore 143 days.

On February 2, 1915, sowings were again made of spores from the original specimens in the tin box, which had thus been in a state of desiccation for twelve months. On February 14 germination occurred in cow-dung extract, on February 16 in glue, and the following day in hanging drops of gelatine. No germination occurred in water. In the nutrient media only about 20 to 25 per cent. of the spores germinated.

On August 15, 1915, hanging drops containing spores from the original material, after eighteen months' desiccation, were again prepared, but no germination could be obtained.

The ripe ascospores are brown in colour with a slightly thickened wall, and if their formation be carefully traced it will be noted that this thickening and coloration occur subsequent to the attainment of their full dimensions. In the unripe condition they are clear and hyaline, and usually lying free within the peridium. By therefore carefully splitting away the peripheral chlamydospore crust of a nearly mature fructification and breaking open the latter, almost pure sowings of the full-grown but unripe spores may be obtained. On March 5, 1914, such spores were placed in hanging drops of cow-dung extract, glue, gelatine, and water, and the cells left at room temperature, 23° C. and 37° C. No germination occurred at the latter temperature, but on March 8 vigorous growths began from about 70 per cent. of the spores at 23° C. and on March 9 from those at room temperature. In water many of the germ tubes were abnormal, and growth ceased after about the third day. These spores had received no preliminary treatment of any kind.

The observations so far described would appear therefore to show :—

(*a*) That the resistance which the mature ascospores offer to direct and immediate germination is correlated with changes in the thickening and coloration of the spore wall.

[1] 50 grm. of air-dried fresh cow-dung well shaken in 1,000 c.c. tap-water, filtered and heated in Koch steamer on three successive days.

(*b*) That the ripe ascospores will germinate after a resting period in the absence of digestive treatment.

(*c*) That digestive treatment with artificial gastric juice eliminates or greatly curtails this resting period.

Influence of low temperature on ascospore germination. Following the successful lead given by Eriksson,[1] with spores of the Uredineae, Marshall Ward tried to bring about the germination of the ascospores of *Onygena* by first subjecting them to a temperature below zero. In only one case was any germination observed, and Marshall Ward[2] himself terms this case 'doubtful', and suggests 'that the successful germination without gastric juice was really that of the chlamydospores'. In my own observations this method was given a very thorough trial. Freezing mixtures, melting ice, and exposure out of doors to frost were used, and with the spores *in situ* in the fructifications, freshly removed as a loose powder, suspended in water, and air-dried from water suspensions upon glass slides. Not one case of germination was observed, and it would seem that the method of forcing the germination of spores by subjecting them to low temperatures is inapplicable to the spores of *Onygena equina*.

Germination of the chlamydospores. The media in which Marshall Ward obtained the germination of chlamydospores in the absence of preliminary digestive treatment were hydrolized horn, and cow-dung and gelatine plus hydrolysed horn; these being maintained at a temperature of 22° to 23° C. In the first case the spores were fresh and germinated well. In the second the spores had been soaked in water for two hours at 35° C. before sowing and the germination was very poor.

In the present investigation hanging drops containing spores were prepared on March 17. Germination occurred on March 23, about 60 per cent. of the spores in hydrolysed horn[3] putting out germ tubes and about 50 per cent. of those in dung extract and gelatine plus hydrolysed horn (equal quantities of dung extract and hydrolysed horn plus 10 per cent. gelatine). In addition, hanging drops of Witte's peptone,[4] glue, water, gelatine, and cow-dung extract had been prepared, and, with the exception of the spores in water, abundant germination (50 to 70 per cent.) occurred after five or six days. In water only a few germ tubes were protruded, and many of these were abnormal and gave no further growth. Chlamydospores treated with gastric juice before sowing possessed no advantage over those lacking

[1] Eriksson, J.: Über die Förderung der Pilzsporenkeimung durch Kälte. Centralb. f. Bakt., Abt. 2, Bd. i, No. 15/16, pp. 557-65, 1895.

[2] Marshall Ward, loc. cit., pp. 277, 279.

[3] 10 grm. of shavings from fresh ram's horn were dissolved in 100 c.c. of boiling 20 % pure concentrated sulphuric acid. This was neutralized with barium carbonate, filtered, and sterilized in the autoclave.

[4] 100 c.c. distilled water, 1 grm. peptone, 1 grm. sodium chloride, heated and then filtered. Sterilized in Koch steamer on three successive days.

such treatment. It would appear, therefore, that Marshall Ward's partial failure in obtaining germination must be set down to some accessory factor.

Some general conditions affecting spore germination. It may be of interest to record a few observations on the more general factors conditioning the germination of spores of *Onygena equina.*

It is well known that treatment with certain acids markedly stimulates the germination of particular fungus spores,[1] and it seemed therefore of interest to ascertain whether the immediate germination of ascospores of *Onygena* after treatment with artificial gastric juice is to be attributed to the digestive action of the latter, or whether it is merely due to the stimulative effect of the hydrochloric acid.

Accordingly observations were made on parallel series of experiments with spores treated in several ways. In the first series the spores were immersed in various strengths of pepsine solution ;[2] in the second in various strengths of hydrochloric acid ;[3] and in the third in artificial gastric juice. As a check three other series were arranged, in one of which the spores were immersed in water, and in the remaining two in various strengths of acetic acid and of lactic acid.[4] After varying periods of time[5] spores from each series were transferred to Van Tieghem cells containing hanging drops of glue, and placed in a dark cupboard at room temperature. The results were very striking in that only those ascospores which had been treated with gastric juice germinated ; and there can be no doubt therefore that the digestive action of this fluid is directly responsible for the stimulation of the spores to immediate development.

Full-grown unripe ascospores when immersed in gastric juice at a temperature of 23° C. for three hours germinated normally. Six hours' digestion reduced their germinative capacity by about 15 per cent. and produced many abnormal germ tubes, whilst twelve hours' immersion almost completely inhibited germination. Ripe ascospores were rendered incapable of germination by digestion for twenty-four hours at 23° C., and their germinative capacity greatly reduced by fifteen hours' immersion, these results being approximately true for the chlamydospores also.

The effect of a low temperature is well marked. *In situ* in the closed fructification the spores are able to withstand repeated freezings, and lost no power of germination when frozen in a solid block of ice on three successive nights. If, however, the spores are in a state of suspension in water or air-dried from such a suspension on a glass slide, they are markedly less resistant, and after fifteen hours' exposure to a temperature below zero or remaining for that period of time frozen in ice, all power of germination is lost. The chlamydospores are slightly more resistant than the ascospores,

[1] See B. M. Duggar : Physiological Studies with reference to the Germination of certain Fungus Spores. Bot. Gaz., vol. xxxi, pp. 38–66, Jan. 1901.

[2] 0·1 %, 0·2 %, 0·4 %, 0·8 %, 1·0 %, 3·0 %.

[3] 0·2 %, 0·4 %, 0·6 %, 0·8 %, 1·0 %, 3·0 %.

[4] Series as HCl.

[5] 1 hr., 3 hrs., 6 hrs., 9 hrs , 15 hrs., 24 hrs.

whilst the full-grown but unripe ascospores are appreciably less resistant than the mature spores. It is worthy of note that ripe ascospores and chlamydospores which have been exposed to a freezing temperature for five hours, and then treated with gastric juice at 23° C. for a similar length of time, retain almost undiminished their germinative capacity. If the reverse treatment be adopted and the spores are first digested and then frozen they become incapable of germination.

Ascospores are highly resistant to desiccation, and, as already noted, will germinate after twelve months' drying *in situ* at room temperature. If air-dried from a water suspension on cover slips and kept at 23° C., they are still viable after a period of seven weeks; at 37° C., after three weeks, but not after four weeks; and at 54° C., not after two hours. Spores air-dried upon human hair or cotton-wool from a suspension in water, and maintained at 23° C., had lost no power of germination after nine weeks; and at 37° C. still showed abundant germination at the end of a similar period. Spores which had germinated and were then dried out on the cover-slip at any temperature proved incapable of further growth.

No appreciable difference is to be noted in the germination of spores occurring in darkness or the diffuse light of the laboratory.

Summary.

The ripe ascospores of *Onygena equina* will germinate directly after a prolonged resting period, which may be curtailed or eliminated by a preliminary treatment of the spores with artificial gastric juice, but not by subjection to low temperatures.

The full-grown unripe ascospores and the chlamydospores will germinate immediately in the absence of digestive treatment.

The Anatomy of the Six Epiphytic Species of the New Zealand Orchidaceae.

BY

K. M. CURTIS, M.A.

With Plates VII—XII.

THE following is an enumeration of the six epiphytic species of New Zealand Orchids dealt with in the present paper, with notes on their geographical distribution.[1]

Earina, Lindl. Besides the two species found in New Zealand, which are endemic, there are four others from the Pacific Islands.

E. mucronata: not uncommon in lowland districts throughout North and South Islands, Stewart Island, and the Chatham Islands; ranges from sea-level to 2,000 ft.

E. suaveolens: North and South Islands and Stewart Island; sea-level to 2,000 ft.

Dendrobium, Swartz. A large genus of about 300 species, most abundant in the Malay Archipelago, but extending as far north as Japan, and southward through Australia and Polynesia to New Zealand. The single species found in New Zealand is endemic, but is closely allied to the Polynesian *D. biflorum,* Swartz.

D. Cunninghamii: North and South Islands, and Stewart Island; sea-level to 2,000 ft.

Bulbophyllum, Thouars. A genus of nearly 100 species with its chief centre of distribution in tropical Asia, but also found in tropical Africa, Australia, New Zealand, and sparingly in South America.

B. pygmaeum: North and South Islands, in the South chiefly on the western side; sea-level to 1,500 ft.

B. tuberculatum: rather restricted in range. Has been seen in the Auckland, Hawke's Bay, and Wellington districts in the North Island, and in the Nelson district in the South Island.

Sarcochilus, R. Br. A genus of about thirty species, most of them from India, the Malay Archipelago, and Australia; a few from the Pacific Islands, and one from New Zealand.

[1] Distribution and morphological descriptions, excerpts from Cheeseman (3).

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S. adversus: occurs in the lowland districts of North and South Islands, Stewart Island, and the Chatham Islands, but is not common.

Earina mucronata, Lindl. Bot. Reg. sub t. 1699.

Stem creeping. Branches numerous, 1–3 ft. long, slender, simple, pendulous or rarely erect, smooth, compressed and two-edged, spotted. Roots long, thick or slender, with smaller branch roots at intervals. Leaves 3–6 in. long, $\frac{1}{6}$–$\frac{1}{5}$ in. broad, narrow linear, acuminate, flat, smooth, thin but coriaceous, very finely striate. Panicle terminal, many-flowered. Flowers rather distant, $\frac{1}{4}$ in. diameter. Sepals and petals pale yellow, lip darker with brownish orange spot at the base.

Roots and rootlets are a dull white colour except near the tips where the velamen has not yet become filled with air and the green of the cortex is still visible. The central cylinder contains from eleven to thirteen alternating groups of xylem and phloem, and is surrounded by a thin-walled cortex occupying the greatest portion of the root (Pl. VII, Fig. 1). Outside the cortex lie the exodermis and a velamen of several rows. Root-hairs are frequently present, especially in flattened specimens where the substratum adjoins. When the velamen consists of four rows, the three outer are somewhat similar to one another. In transverse section their cells are practically isometric; the thickening strands are reticulately arranged, but have a general tangential direction. In longitudinal section the cells are elongated and the strands run more or less parallel to the long axis of the root. The innermost layer of the velamen consists of large cells tangentially elongated in transverse section; the strands on all their walls are radial. Cutinization of walls and strands occurs throughout the velamen and is especially heavy in the cells near the exodermis. The latter consists of a single row of cutinized elements whose continuity is broken by the occurrence, at intervals of usually four cells, of smaller passage cells with cellulose walls. The thickened cells are somewhat compressed tangentially, but are much elongated in the longitudinal direction. Thickening and cutinization occur on their radial and external tangential walls only. The former are finely striated. The two walls forming the angle on the cortical side are of cellulose. In longitudinal section passage cells alternate with cutinized cells. The denser protoplasmic contents and large nuclei of the former present a contrast to the thin protoplasmic lining and small nuclei of the latter.

The cells of the cortex vary in depth from eight in the smaller branches to twenty or more in the principal roots. The unusual thickness of some roots is due chiefly to a greater depth of cortex, but a few additional rows in the velamen may occur at the same time. Two small cells always bound the inner walls of the passage cells. The largest cells lie in the central portion of the cortex, while they diminish in size towards exodermis and

endodermis. Near the outer and inner limits of the cortex the cells are longitudinally elongated, and the walls heavily pitted with round, oval or slit-like pits; in the row adjoining the endodermis the walls are usually reticulately marked. A few of the large central cells are covered with fine spiral thickening strands. Small thin-walled cells containing raphides occur in the two outermost layers of the cortex. The endodermal cells, which are large and elongated, have thick, striated, cutinized walls with numerous pits. When the lumina are small the nuclei are filiform. The passage cells, which lie opposite the protoxylem groups, are a little smaller than the other members of the endodermis and contain denser protoplasm. In a large root they may be slightly cutinized. The cells of the pericycle are elongated in longitudinal section, and the walls, except of those adjoining passage cells, are thickened and cutinized. There are usually twelve, thirteen, or eleven groups of xylem and phloem present. The numerous small elements of the former consist of diversely pitted tracheides and of spiral vessels; the few larger elements are usually spiral vessels. The sieve-tubes are small, but at least one in each group considerably exceeds the others. Separating xylem and phloem are a few sclerenchymatous fibres. Elongated, cutinized cells lie within the vascular region, while those at the extreme centre of the cylinder have walls of cellulose. Chlorophyll occurs in the cortex chiefly in the three rows adjoining the exodermis, and in two or three adjoining the endodermis. The larger cells contain little, if any. In the outer region the two small cells which lie next to the passage cells of the exodermis contain distinctly the most. In addition a few granules may be present in the cellulose cells at the centre of the cylinder. Fungal hyphae branch in numbers through the cells of the velamen. Eventually they pass into a passage cell, where they take several turns round the nucleus, and then proceed into the cortex. Here they seldom penetrate deeper than the fifth cell, where they are reduced to globular masses of disorganizing hyphae connected together through the cell-walls by mere fibrils. If the cortical cell is large it may contain a smaller secondary, in addition to the nuclear, mass of fungus. The nuclei of the cells are always larger when the fungus is present.

The stem has a narrow cortex surrounding an irregular ring of sclerenchymatous fibres, within which lie the numerous large vascular bundles. The cuticle is heavy—at times wider than the epidermal cells. These are cutinized and somewhat elongated in longitudinal section. Their inner walls are thicker than their outer, but the former are the more heavily pitted. In the elongated, pointed elements comprising the cortex the intensity of cutinization diminishes from without inwards, while the quantity of starch contained increases. The cortex gives place abruptly to the sclerenchymatous fibres of the strengthening cylinder. These pass gradually into the oval cells comprising the ground tissue. The latter are filled with dense masses of starch tetrads. The vascular strands which occur

in the ring of sclerenchyma are smaller than those lying in the conjunctive tissue. The phloem of all the strands is protected by fibres whose walls bear several striations. Fibrous elements may be absent from the periphery of the xylem. If present, they usually have larger lumina and contain starch. Small and large spiral vessels are to be seen in the xylem, and tracheides, especially those with equidistant pits, are of frequent occurrence.

The branch is of one order only, and bears about six leaves at an inch or an inch and a half apart. The leaf is continued below in an unbroken sheath to its node where the next lower blade bends out from its sheath. The sheaths are dull in colour, with regular lines or irregular groups of darker brown, due to the complete or partial discoloration of the many sclerenchymatous strands running parallel to the surface (Pl. VIII, Fig. 18). Only the largest of these strands contain vascular bundles, and many of the fibres are devoid of contents. The cells of the general tissue of the sheath are strewn with so many large pits of irregular shape that a reticulate appearance is lent to the walls. This is most noticeable in the spindle-like cells which adjoin or cross the large canals lying between the strands of sclerenchyma. Each of these cells contains a spherical crystal aggregate. Only two or three elements are present in the phloem and xylem of the vascular bundles.

The branch is oval in transverse section. Its cortex is narrow, but within it is a wide band of sclerenchyma in which are embedded most of the vascular bundles. Only a few strands occur in the ground tissue. A heavy cuticle covers the free surface of the small epidermal cells. The outer cells of the cortex are cutinized, but the inner are of cellulose. All are pitted and elongated in longitudinal section and most contain starch. The vascular bundles in the sclerenchymatous ring are seldom large. Usually about six elements are present in xylem and phloem. Those of the central portion of the branch are well developed and may have complete sheaths of sclerenchyma. More often this is absent on the side of the xylem, but two or three cells deep on that of the phloem. The cells of the ground tissue, which are oval in transverse and elongated in longitudinal section, are filled with dense masses of starch.

The leaf is strengthened by the presence of much sclerenchyma (Pl. VIII, Fig. 24). All the bundles are surrounded by large fibrous sheaths; numerous sclerenchymatous strands run immediately within the two epidermes, especially the upper; and a broad strand is present at the edge of the leaf. The isolated strands near the epidermes consist of from one to six fibres. Their contents disappear early, and few pits or striations occur in their walls. There are usually nineteen vascular bundles in the leaf. The elements of their sheaths vary in size; near the xylem they are large and often without striations; near the phloem they are smaller, striated, and pitted, and retain their protoplasmic contents. Most of the smaller

tracheides have their pits arranged in regular order a little distance apart; in the larger the pits are more elongated, but closer together. Cuticle occurs on both surfaces, but is thicker on the upper. The cells adjoining the upper epidermis are somewhat elongated in transverse section, and in tangential section they resemble palisade structure to a certain extent. Below this row they contain numerous chlorophyll granules. In the assimilatory tissue of the lower portion of the leaf the cells are connected together by short tubular extensions, across each of which is a pitted diaphragm. Large intercellular spaces occur in this region. Isolated elements, elongated in tangential section and containing raphides, are to be found between the vascular bundles lying towards the edge of the leaf. Stomata occur on the lower surface. Their walls are extremely thick, and the outer are covered by the cuticle. Above the midrib the cells are large and thin-walled; their protoplasm is vacuolated, and they contain few chlorophyll granules.

Earina suaveolens, Lindl. Bot. Reg. (1843) Misc. 61.

Stems stout, erect or pendulous, slightly compressed, 6–18 in. high. Branches numerous, close-set. Leaves 2–4 in. long, $\frac{1}{3}$–$\frac{1}{2}$ in. broad, narrow linear, acute, rigid, coriaceous, striate, midrib evident. Panicle terminal, 2–4 in. long, many-flowered. Flowers sessile, much closer together than in *E. mucronata*, $\frac{1}{4}$–$\frac{1}{3}$ in. in diameter, waxy-white with a yellow centre; very fragrant.

The root does not usually branch. Its velamen consists of from three to five rows of cells, of which only the inner have cutinized walls (Pl. VIII, Fig. 28). The thickening strands on all the walls are more or less radial. The innermost cells are elongated in both tangential and longitudinal directions; fewer strands are present on their walls than on those of the outer rows. In the exodermis only the outer and the radial walls are cutinized. Three or four thickened cells occur between any two passage cells. Adjoining the latter on the inner side are the usual two small cortical cells containing numerous chlorophyll granules. Reticulately pitted cells are present in the outer and in the innermost rows of cortex, especially in the latter. The larger cells in the middle region have spiral bands or pits of various sizes in their walls. Chlorophyll occurs throughout, but it is denser near the inner and outer limits. Cells containing raphides lie near the exodermis. The walls of the thickened cells of the endodermis are cutinized and striated, while those of the passage and of the one or two adjoining pericycle cells are of cellulose. Twelve groups of xylem and phloem are usually present. As in *E. mucronata*, the former consist of small tracheides and spiral vessels, and the latter of sieve-tubes and companion cells. Sclerenchymatous fibres lie between the groups, but near the centre of the cylinder they give place to large cellulose-walled elements containing a little chlorophyll.

A depth of about twenty cells in the cortex gives a parenchymatous appearance to the stem. There is no complete sclerenchymatous ring, but the outer vascular bundles have extended sheaths which may almost, if not actually, touch. The minute epidermis is bounded by a thick cuticle. The size of the cortical cells is greatest in the central region and diminishes towards either edge. Small intercellular spaces occur between the largest cells, which are oval in shape, and again between the small round cells of the innermost rows. Little marking is visible in any of the walls. What does occur is due chiefly to the occurrence of small pits. Raphides are present in the outer cortex. The phloem sheath of the central vascular strands is frequently four cells deep, and the walls of those of its fibres which adjoin the ground tissue are heavily pitted. The usual elements occur in the somewhat large strands of xylem and phloem. As a rule the vascular bundles are separated from one another by two rows of the large oval cells of the ground tissue. These have thick, heavily pitted, cellulose walls and contain great quantities of starch.

The leaf sheath resembles that of *E. mucronata*, but is firmer in every way (Pl. IX, Fig. 39). Nearly all the large sclerenchyma strands enclose vascular bundles. The cuticle is thick, especially on the outer epidermis. Pits are wide and numerous in the general tissue, particularly in the reticulately marked cells near the canals. The vascular sheaths contain many small fibres, but at the junction of xylem and phloem their elements are larger and have thinner walls.

In the branch a great number of vascular bundles occur throughout the cutinized ground tissue. A heavy cuticle is present. The cells of the four- to five-rowed cortex are elongated in longitudinal section and may contain a little chlorophyll, while the ground tissue is filled with tetrahedrally arranged starch granules. The vascular elements resemble those of the stem. At the centre of the branch the sheaths do not extend round the xylem.

In the leaf the two halves are practically in the same plane, but the edge may be slightly recurved. There are usually twenty-three vascular bundles, and of these five are large. Small sclerenchymatous strands run parallel to the leaf surface immediately within the two epidermes, but they are not as large, nor proportionately as numerous, as in the leaf of *E. mucronata*. The group of fibres at the edge of the leaf lies close to the lower epidermis. There is a distinct furrow above the midrib and a slight one above each of the other large vascular bundles. The cells of three or four rows near the upper epidermis are arranged in palisade formation. The walls are thin and pitted, the protoplasmic contents vacuolated, and fewer chlorophyll granules are present here than elsewhere. This resemblance to water-storing tissue is more noticeable in the cells lying over the midrib. The elements of the epidermis are regular in shape. Their walls

are somewhat thickened and the outer are slightly mucilaginous. Stomata are confined to the lower surface of the leaf, and are not numerous. Spiral vessels occur occasionally in the xylem, but tracheides are the more usual elements.

Dendrobium Cunninghamii, Lindl. Bot. Reg. sub t. 1756.

Stems usually much branched, slender, rigid, wiry, terete and polished, 1–4 ft. long ; usually pendulous, but small specimens growing on rocks or in exposed places are often erect. Leaves numerous, distichous, alternate, $\frac{3}{4}$–2 in. long, $\frac{1}{6}$–$\frac{1}{5}$ in. broad, linear-lanceolate, acute, rigid, and coriaceous, striate and more or less conspicuously 3-nerved ; sheaths truncate, grooved, and transversely corrugated. Flowers $\frac{3}{4}$ in. in diameter, white and pink.

The root as a whole, and all the cells individually, are large (Pl. IX, Fig. 47). Of the usual four rows in the velamen, the three outer are more or less alike, while the inner is elongated radially and has its outer walls inclined to one another. Root-hairs are often present. In transverse section the elements of the velamen immediately over a passage cell look like two or three triangles of different altitudes, standing on a common base, one within the other, and having their apices connected by a zigzag line to the centre of the base. In longitudinal section the walls of these cells resemble portions of concentric circles. Except in these walls the fine thickening strands of the velamen are radial. The exodermal walls may have several striations. They are all cutinized, even the inner tangential. The elements of the cortex are thin-walled and regular in shape. In the central region and near the endodermis they are usually round. Chlorophyll is present, but not in great quantities. Small round pits occur frequently, but spiral bands are seldom to be seen. As in the preceding genus the cortical cells adjoining the endodermis are reticulately pitted. The endodermis is well marked. Its cells are large, radially elongated, and heavily cutinized. The passage cells, which are small, occur about every sixth cell. They are distinctive in being elongated in longitudinal section. The walls of pericycle and conjunctive tissue are cutinized, but the lumina are large. Ten or more strong alternating vascular groups are present. A number of small, and one or two much larger, elements occur in the xylem. The tracheides usually have reticulate or regular equidistant pits. A few fibres may be present in the phloem.

Stem and branch resemble each other closely. A thick cuticle surrounds a peripheral sheath of sclerenchymatous fibres which extends to a depth of about one-fifth of the radius. The cortex is narrow, and consists of thick-walled cells containing a little chlorophyll. Here and there the sheaths of the outer vascular strands join one another. Chlorophyll may also occur in the somewhat thin-walled elements of the ground tissue.

The leaf has a central and two smaller lateral depressions. Near the edge the lower surface slopes gradually towards the upper. No isolated strands of sclerenchyma are present. The rigidity of the leaf is due to the presence of strikingly large vascular sheaths whose outline in transverse section resembles roughly that of the figure 8. The largest strand lies in the centre of each leaf-half. The fibres are small and have many striations except where phloem and xylem meet. Surrounding each sheath is a single ring of small hexagonal thin-walled cells containing numerous chlorophyll granules. Between the vascular strands the cells of the assimilatory tissue are much elongated in transverse section, while above and below this region they are more nearly oval. Numerous intercellular spaces are present in the lower half of the leaf. Club-shaped cells, attached to small basal cells, occur sparingly in the upper epidermis and infrequently in the lower. The epidermal cells adjoining the basal cell are smaller than usual, and their walls are distinguished by the presence of large elongated pits. The inner and outer walls of the two epidermes undergo pectic mucilaginization. The cells of the upper are unusually large. Minute round pits stud the walls, especially those separating two epidermal elements. Above the midrib the cells, which are elongated in the direction perpendicular to the leaf surface, resemble the epidermis in the mucilaginous nature of their walls, in their vacuolated protoplasm, and in the size and shape of their pits.

Bulbophyllum pygmaeum, Lindl. Gen. et Sp. Orch. 58.

Minute, forming densely matted carpets on the trunks of trees or on rocks. Pseudo-bulbs $\frac{1}{2}$–$\frac{1}{6}$ in. in diameter, globose, glabrous. Leaves solitary on the pseudo-bulbs, springing from a minute circular sheath $\frac{1}{4}$–$\frac{1}{3}$ in. long, linear-oblong, obtuse, very thick and coriaceous, grooved down the middle and minutely echinulate above, longitudinally nerved beneath. Peduncles solitary from the base of the pseudo-bulbs, $\frac{1}{3}$–$\frac{1}{6}$ in. long, 1-flowered. Flowers very minute, whitish.

The root is very small (Pl. X, Fig. 57). The velamen consists of a single row of regular, comparatively large cells lacking thickening strands. The inner tangential walls are thick and cutinized; the radial are of cellulose and diminish in thickness towards the surface of the root, where the outer tangential walls are very thin. The thickened exodermal cells have cutinized striated walls and the passage cells are often of equal size. The cells of the cortex have no spiral thickening strands. They are four to six deep, are small at the inner and outer limits, but in the centre are large and oval with distinct intercellular spaces. The longitudinal walls of the row adjoining the endodermis are reticulately pitted. Raphides are present in certain cells of the second layer from the exodermis. Chlorophyll occurs throughout the cortex, but is more abundant in the small cells adjoining the exodermal

passage cells. In transverse section the endodermis has an open appearance due to the presence of two passage cells together and to the large lumina of the cutinized cells. Five groups of xylem and phloem are usual. Their elements are small and seldom more than six in number. The centre of the cylinder is filled with fibres.

In transverse section the stem is sinuous in outline (Pl. X, Fig. 58). The epidermal cells, which are slightly elongated in the tangential direction, have mucilaginous walls. The outer portion of the cortex consists of large semi-collapsed cells with scanty contents. The cells of the inner portion have firm walls with numerous pits which in longitudinal section are frequently arranged in a reticulate manner. Chlorophyll is present in this part of the cortex and in the ground tissue of the centre of the stem. The vascular bundles may have complete sheaths of sclerenchyma, especially if they lie towards the periphery. Their elements are numerous but not large. The xylem consists only of various kinds of tracheides.

The leaf is very thick and has rounded edges. Both epidermes are wide, the upper exceptionally so. Below it are several rows of cells whose shape bears a resemblance to palisade tissue. The general assimilatory tissue consists of somewhat large round cells connected together by tubular processes, in the diaphragms of which two to five circular pits are to be seen. Of the fifteen vascular bundles the midrib and four others exceed the remainder in size. The sheaths are strong, especially on the phloem side, where their elements are unusually large. Xylem and phloem groups are somewhat small. Spiral and pitted tracheides alone are to be found in the former. Single cells containing raphides occur midway between the vascular bundles lying towards the leaf edge. Occasionally a club-shaped sunken cell with elongated pits in its walls occurs in the upper epidermis. Beneath it the 'palisade' cells fit more closely than usual. On the upper surface each cell of the epidermis is produced into a stout papilla over which the thick cuticle is continued. The appearance of the upper half of the leaf is suggestive of water storage. Large vacuoles are present in the protoplasm of the epidermis; pits occur in the walls, especially in those separating two adjacent epidermal cells. In the 'palisade' region vacuoles are numerous, though smaller; pits are of frequent occurrence; the number of chlorophyll granules is small in comparison with that in the lower portion of the leaf. Mucilaginization of the walls occurs throughout; it is most marked in the epidermis, weaker in the 'palisade' region, and least in the general cells of the lower part of the leaf.

In the pseudo-bulb the vascular strands follow one of two courses. Those originating in the upper portion of the stem usually run to the apex with but slight deviation; those, however, which come from the side of the stem bend outward and follow the contour of the pseudo-bulb at a little distance from its edge. Just below the apical cavity they unite to form the

five bundles present in the lowest portion of the leaf. The vascular strands are weak, but small tracheide-like cells serve as an extension of the system. The cuticle is continued a short distance over the rim at the apex of the pseudo-bulb into the cavity. The epidermal cells have thick heavily pitted walls. Raphides occur near the surface. The general mass of tissue consists of somewhat large thin-walled cells containing numerous chlorophyll granules, and scattered profusely among these, but in no definite order, still larger water-storing elements. These lie with their greatest axis in the horizontal plane. Their walls are thin, but are densely covered with fine spiral bands. Pectic mucilaginization takes place in the walls of many of the assimilatory cells. At some of the angles where several join the walls separate along the swollen middle lamella and form intercellular spaces with a mucilaginous lining. In the central portion of the pseudo-bulb storage cells are of less frequent occurrence, while the assimilatory cells, though smaller in this region, contain more chlorophyll. A continuation of the lower end of the leaf blade is distinguishable in the tissue of the apical region of the pseudo-bulb. The leaf in this stage is folded about the midrib with the two halves of the upper surface in contact. Its cutinized upper epidermis may be detected a short distance beneath the cavity. Cutinization of the lower epidermis and separation from the tissue of the pseudo-bulb continue symmetrically from the contiguous leaf-edges, round the two halves of the lower surface, to the point opposite the midrib. The five vascular bundles present at this stage have strong sclerenchymatous sheaths and a rather large development of phloem.

Bulbophyllum tuberculatum, Col. in Trans. N. Z. Inst. xvi (1884), 336 and xxii (1890), 488.

Forms matted patches on the trunks or branches of trees. Pseudo-bulbs $\frac{1}{4}$–$\frac{1}{3}$ in. long. Leaves solitary on the pseudo-bulbs, $\frac{1}{2}$–1 in. long, linear-oblong, acute at both ends, thick and fleshy, slightly concave above, midrib prominent beneath. Peduncles almost filiform, $\frac{1}{2}$–$\frac{3}{4}$ in. long, 2–4 flowered. Flowers $\frac{1}{6}$ in. long, white with a bright reddish-orange lip.

The root is larger than that of *B. pygmaeum*, but its general features are similar (Pl. XI, Fig. 72). The cells of the single-rowed velamen are very large. Their radial walls do not perceptibly diminish in thickness towards the outer surface as do those of the preceding species. No thickening strands occur on any of the walls. In transverse section the exodermal cells are pointed in the radial direction. The cells of the outermost row of the cortex, and sometimes of the second row also, contain abundant chlorophyll granules. Several rows of large cells may be present in its central region. Intercellular spaces are of frequent occurrence, especially near the endodermis. The uniformity of the latter is somewhat broken by irregularity in the size of the cutinized cells and by the presence of two passage cells

together. There are often six groups of xylem and phloem, the former being proportionately the better developed and consisting almost wholly of spiral tracheides. In the centre of the cylinder the cells have thin, slightly cutinized walls.

In the stem the epidermis and cortex are thin-walled. The latter, which is not usually more than eight cells deep, consists of large elements containing raphides, separated by small cells with protoplasmic contents. Spirally marked water-storage cells occur infrequently. The vascular bundles are numerous and the fibres of their sheaths large. Of the latter there are usually two or more rows near the phloem and one near the xylem. Spiral tracheides are of general occurrence, but reticulate and regularly pitted tracheides are also to be seen.

The pseudo-bulb is taller, more pointed at the apex, and contains more vascular bundles than that of *B. pygmaeum*. The regular, slightly elongated cells of the epidermis are protected by a heavy cuticle. The general tissue consists of large spirally marked water-storage cells and small assimilatory cells with numerous chlorophyll granules. Near the periphery of the pseudo-bulb one green cell lies at the angle of juncture of several storage cells, but near the vascular bundles and in the central region several green cells may occur together, and the large elements be segregated in groups of two or three. In longitudinal section assimilatory cells usually surround each storage cell. In the apical portion of the pseudo-bulb the small cells near the surface contain instead of chlorophyll a purple pigment in solution in the cell sap. The vascular strands are weak. A few slightly thickened elements occasionally adjoin the phloem.

The leaf is broader, but proportionately thinner than that of *B. pygmaeum*. Five large and ten smaller bundles traverse its length. The cells of the upper epidermis are extremely large. No papillae are present, but small sunken cells occur at somewhat rare intervals on the upper surface. Below the epidermis are two or three rows of cells with slight contents. Between the vascular strands the assimilatory cells are much elongated in the plane parallel to the leaf surface. Below these again lie more or less round cells connected together by tubular extensions. Large air chambers occur between the small stomata and the assimilatory tissue, and cells containing raphides are present between the outer vascular bundles. One epidermal cell at the extreme edge of the leaf is extended into a short projection, with the result that the leaf margin is sharp.

Sarcochilus adversus, Hook. f. Fl. Nov. Zel. i. 241.

Roots numerous, long, wiry, terete. Stems short, 1–3 in. long, concealed by the imbricated sheathing bases of the leaves. Leaves few, distichous, spreading, 1–2½ in. long, ⅓–¾ in. broad, linear-oblong to elliptic-oblong, obtuse or sub-acute, jointed above the sheathing base, thick and

coriaceous, dark green, often spotted with purple. Peduncles 1–4 from the axils of the lower leaves, slender, 1–2½ in. long, 5–15 flowered. Flowers ⅛–⅙ in. in diameter, green spotted with purple.

The velamen consists of two or three rows of cells, the outer of which are large, thin-walled and covered with fine parallel radial strands (Pl. XII, Fig. 89). The cells within this row are irregular in size, devoid of thickening strands, and cutinized in the walls which adjoin the exodermis. The protective cells of the latter layer are much larger than the passage cells. Spirally thickened water-storage cells occur in numbers from exodermis to endodermis, and raphides are not uncommon throughout the same region. The cortex as a whole contains an unusual amount of chlorophyll. The walls of the endodermis are so heavily thickened that only narrow radially elongated lumina remain. Near the passage cells, which occur singly, the walls of from one to three of the pericycle cells are of cellulose. From ten to thirteen vascular strands are present. In the conjunctive tissue the degree of cutinization diminishes towards the centre of the cylinder, where a little chlorophyll may occur.

A thick cuticle covers the stem. The cortex is nearly one-third of the radius in depth, and is composed of small assimilatory cells and numbers of irregularly scattered large raphides cells. The vascular bundles are well developed, and the sheaths, especially of those near the edge of the vascular region, contain many sclerenchymatous elements on the side of the phloem. Chlorophyll is present in the pitted cells comprising the ground tissue.

The strength of the thick leaf is dependent upon its succulent nature. No sclerenchyma is present except that in connexion with the vascular strands. The sheaths are seldom complete, and only those of their elements which adjoin phloem have heavily thickened walls. Large raphides cells abound. Chlorophyll granules occur from upper to lower epidermis, although in fewer numbers in the upper cells. Xylem and phloem contain numerous elements of regular size.

Summary.

1. The velamen consists of one row of cells in *Bulbophyllum pygmaeum* and *B. tuberculatum* and of two or three rows in *Sarcochilus adversus*; the number in the two species of *Earina* and in *Dendrobium Cunninghamii* is more variable, ranging from three to five, or more.

2. The greatest amount of chlorophyll, in proportion to the size of the root, occurs in *Sarcochilus adversus*, and next in the two species of *Bulbophyllum*. Less is present in the Earinas and in *Dendrobium Cunninghamii*.

3. A great number of spirally thickened water-storage cells are present in the cortex of the root of *Sarcochilus adversus*; they are also to be seen, though less frequently, in the two species of *Earina*.

4. Fungal hyphae are present in the roots of all; they penetrate to the

endodermis in *Bulbophyllum pygmaeum* and *B. tuberculatum*; almost every cell in the outer portion of the cortex of *Dendrobium Cunninghamii* contains one or more large disorganizing masses.

5. *a.* An internal cylinder of sclerenchyma and starch in cortex and ground tissue occur in stem and branch of *Earina mucronata*; there is no complete sheath of sclerenchyma in *E. suaveolens*.

b. A peripheral cylinder of sclerenchyma and chlorophyll in cortex and ground tissue are present in *Dendrobium Cunninghamii*.

c. In *Bulbophyllum pygmaeum* the inner half of the cortex and the ground tissue contain chlorophyll.

d. In *B. tuberculatum* and *Sarcochilus adversus* raphides cells occur throughout the assimilatory tissue.

6. *a.* Isolated sclerenchyma strands are present in the leaves of the two species of *Earina*; a 'palisade' arrangement of thin-walled cells containing little chlorophyll occurs beneath the upper epidermis of *E. suaveolens*.

b. Very large vascular sheaths but no isolated strands are present in *Dendrobium Cunninghamii*; its wide epidermis is mucilaginous.

c. The leaves of the species of *Bulbophyllum* are succulent; mucilaginization takes place in the walls, especially in those of the epidermis; pseudo-bulbs are present.

d. The leaf of *Sarcochilus adversus* is very succulent; great numbers of raphides cells occur throughout.

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Imperial College of Science and Technology.

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EXPLANATION OF THE FIGURES IN PLATES VII–XII.

Illustrating Miss Curtis's paper on the Anatomy of the Six Epiphytic Species of the New Zealand Orchidaceae.

T. S. = transverse section; L. S. = longitudinal section.

PLATE VII.

Earina mucronata.

Fig. 1. Root, T. S.: *cel.*, centre of cylinder where the cell-walls are of cellulose; *cy.*, central cylinder; *ph.*, phloem; *x.*, xylem; *end.*, endodermis; *c.*, cortex; *exo.*, exodermis; *v.*, velamen; *rt.h.*, root-hair. Diagrammatic.

Fig. 2. Root, outer portion, L. S.: *v.*, velamen; *exo.*, exodermis; *p.c.*, passage cell; *t.c.*, reticulately pitted cross-wall; *chl.*, chlorophyll. × 360.

Fig. 3. Raphides cell, L. S., from outer portion of cortex: *r.*, raphides; *p.*, pit; *n.*, nucleus. × 112.

Fig. 4. Cortical cells, root, L. S.: *chl.*, chlorophyll; *p.*, pit; *pr.*, protoplasm; *n.*, nucleus. × 112.

Fig. 5. T. S. two cutinized elements from outer region of cortex of root: lettering as before. × 112.

Fig. 6. L. S. cutinized element; cf. Fig. 5. × 112.

Fig. 7. Root, outer portion, T. S.: *v.*, velamen; *s.*, thickening strand; *b.s.*, section of thickening strand; *p.c.*, passage cell; *f.*, fungus; *f.c.*, coil of fungus; *r.*, raphides; *exo.*, exodermis; K, where cutinization of the wall ceases. × 620.

Fig. 8. L. S. passage cell of root; *i.v.*, cell of innermost row of velamen; *c*, accumulation of fungal hyphae outside passage cell; *c.n.*, coils of fungus with cell nucleus; *p.c.*, passage cell; *h.*, hypha passing to cortex. × 620.

Fig. 9. Stem, T. S., outer portion: *cut.*, cuticle; *epi.*, epidermis; *cu.c.*, cutinized cells; *cell c.*, cells with walls of cellulose; *st.*, starch; *r.scl.*, portion of sclerenchyma cylinder; *ph.*, phloem; *x.*, xylem; *x.scl.*, xylem sclerenchyma. × 360.

Fig. 10. Root, portion of central cylinder, T. S.: *p.c.*, passage cell; *p.x.*, protoxylem; *end.*, endodermis; *peric.*, pericycle; *c.c.*, companion cell; *s.t.*, sieve-tube; *x.*, xylem; *cut.w.*, cutinized wall; *cell.w.*, cellulose wall of elements of centre of cylinder. × 360.

Figs. 11, 12. Small pitted tracheides of root: *p.*, pit; *t.*, unpitted portion of wall. × 620.

Fig. 13. Tracheide of root. × 620.

Fig. 14. Reticulate tracheide of root. × 620.

Fig. 15. L. S. endodermis of branch root: *i.c.*, innermost row of cortex; *end.*, endodermis; *n. end.*, nucleus of endodermal cell; *p.*, pit; *peric.*, pericycle. × 360.

Fig. 16. T. S. large vascular bundle of stem: *ph. scler.*, phloem sclerenchyma; *s.t.*, sieve-tube; *g.t.*, ground tissue. × 620.

PLATE VIII.

Earina mucronata (*continued*).

Fig. 17. L. S. stem, ground tissue and tracheides: *tr.*, tracheides; *g.t.*, ground tissue. × 620.

Fig. 18. Branch and sheath, T. S.: *scler.*, sclerenchyma; *can.*, canal; *gen.tis.*, general tissue; *cor.*, cortex. Diagrammatic.

Fig. 19. Leaf sheath, T. S.: *cut.*, cuticle; *epi.*, epidermis; *can.*, canal. × 360.

Fig. 20. Branch, outer portion, T. S.: *cor.*, cortex; *grd.tis.*, ground tissue; *st.*, starch. × 360.

Fig. 21. T. S.: two fibres from sclerenchyma cylinder of branch. × 620.

Fig. 22. T. S.: leaf, about half-way between edge and midrib; *assim.tis.*, assimilatory tissue; *r.c.*, raphides cell. × 360.

Fig. 23. T. S.: cell from lower portion of assimilatory portion of leaf, showing extensions at junction of two cells. × 620.

Fig. 24. Leaf, T. S.: *assim.tis.*, assimilatory tissue; *m.r.*, midrib; *r.c.*, cell containing raphides. Diagrammatic.

Fig. 25. T. S. stoma of leaf. × 360.

Fig. 26. T. S. leaf edge: *r.c.*, raphides cell; *inter.sp.*, intercellular space; *scler.*, sclerenchyma group. × 360.

Fig. 27. T. S. midrib of leaf: *w.t.*, cells bearing a slight resemblance to water tissue. × 360.

Earina suaveolens.

Fig. 28. Root, T. S. outer portion: *v.*, velamen; *p.c.*, passage cell; *exo.*, exodermis; *r.*, raphides; *fung.*, fungus; K, one of the usual two small cells adjoining a passage cell; *t.c.*, cross-wall of a reticulately pitted cell. × 360.

Fig. 29. T. S. reticulately pitted cell from inner portion of cortex of root. × 620.

Fig. 30. L. S. spirally marked cells, middle of cortex, root: *sp.b.*, spiral band. × 112.

Fig. 31. Raphides cell, L. S., root. × 360.

Fig. 32. T. S. cortical cell adjoining endodermis, root. × 620.

Fig. 33 L. S. cortical cell adjoining endodermis, root. × 360.

Figs. 34, 35. Usual forms of tracheides, root. × 620.

Fig. 36. T. S. central cylinder, root: *end.*, endodermis; *p.c.*, passage cell; *cell.peri.*, cellulose-walled element of pericycle adjoining a passage cell; *cut.w.*, cells with cutinized walls. × 360.

PLATE IX.

Earina suaveolens (*continued*).

Fig. 37. Stem, outer portion, T. S.: *epi.*, epidermis; *f.v.b.sh.*, sheath of a vascular bundle. × 112.

Fig. 38. T. S.: one of larger vascular bundles of stem: *st.*, starch; *gr.tis.*, ground tissue. × 360.

Fig. 39. Leaf sheath, T. S.: *can.*, canal; *r.m.*, reticulately pitted cells of the general tissue. × 360.

Fig. 40. Leaf sheath, L. S.: to show increase in number and size of pits from epidermis inwards. × 360.

Fig. 41. Leaf, T. S.: *m.r.*, midrib; *assim.tis.*, assimilatory tissue. Diagrammatic.

Fig. 42. Leaf, midrib, T. S.: *w.st.*, water-storage cells. × 360.

Fig. 43. T. S. cells from lower portion of assimilatory region of leaf: to show linking of the cells; no chlorophyll indicated. × 620.

Fig. 44. Leaf, T. S., half-way towards edge: *pal.*, palisade-like layer; *r.c.*, ring of somewhat regular assimilatory cells surrounding the vascular bundle. × 360.

Dendrobium Cunninghamii.

Fig. 45. Root, T. S., central cylinder: *end.*, endodermis; *p.c.*, passage cell; *peri.*, pericycle; *cut.tis.*, cutinized tissue; *ph.f.*, phloem fibre; *com.c.*, companion cell. × 620.

Fig. 46. L. S. cortex, root. × 112.

Fig. 47. Root, outer portion, T. S.: *rt.h.*, root-hair; *fung.*, fungus; *rad.m.*, radial strands; *pyr.c.*, pyramidal cells over passage cell; *b.st.*, section of thickening strand. × 360.

Fig. 48. L. S. endodermis, root: *i.c.*, cell of cortex; *p.c.*, slightly elongated passage cell; *cell.p.*, cellulose-walled pericyclic element; *ord.peri.*, cutinized pericyclic element. × 620.

Fig. 49. Root, T. S., centre of cylinder: *cut.tis.*, cutinized tissue; *cell.w.*, central cell with cellulose walls. × 620.

Fig. 50. Stem, T. S.: *scl.*, peripheral cylinder of sclerenchyma; *cor.*, cortex; *gr.tis.*, ground tissue. Diagrammatic.

Fig. 51. T. S. cortical cell of stem: *int.sp.*, intercellular space. × 620.

PLATE X.

Dendrobium Cunninghamii (*continued*).

Fig. 52. Leaf, T.S.: *m.r.*, midrib; *r.c.*, raphides cell. Diagrammatic.

Fig. 53. Leaf, T. S., portion between two vascular strands; *t.c.*, cylinder of small hexagonal cells immediately surrounding a vascular strand; *l.cut.*, cuticle of lower surface. × 360.

Fig. 54. T. S. vascular bundle of leaf. × 620.

Fig. 55. T. S. tissue over midrib of leaf: contents much vacuolated. × 620.

Fig. 56. T. S. upper epidermis of leaf: *cl.c.*, club-shaped cell; *b.c.*, basal cell; *p.*, small round pits common throughout epidermis; *pp.*, elongated pit; *c.*, compact tissue in neighbourhood of basal cell. × 620.

Bulbophyllum pygmaeum.

Fig. 57. Root, T. S.: *p.c.*, passage cell; *v.*, velamen; *exo.*, exodermis; *r.w.*, radial wall of velamen tapering in thickness towards the free surface. × 360.

Fig. 58. Stem, T. S.: *o.c.*, outer portion of cortex; *i.c.*, inner portion of cortex; *f.v.b.*, vascular bundle. Diagrammatic.

Fig. 59. Stem, T. S., outer portion: *e.*, epidermis; *o.c.*, outer portion of cortex; *t.c.*, reticulately pitted cell; *i.c.*, inner portion of cortex. × 360.

Fig. 60. Vascular bundle of stem, T. S.: *gr.tis.*, ground tissue. × 620.

Fig. 61. Pseudo-bulb, L. S.: *cav.*, cavity at apex; *tis.*, general tissue; *ps.b.*, pseudo-bulb; *f.v.b.s.*, vascular strands of stem; *f.v.b.rt.*, vascular strands of root. Diagrammatic.

Fig. 62. Surface view, epidermal cell of pseudo-bulb. × 360.

Fig. 63. Surface view, spiral cell of pseudo-bulb. × 112.

Fig. 64. Pseudo-bulb, T. S. through apical region: *ps.b.*, pseudo-bulb; *cut.*, cuticle of surface of pseudo-bulb cavity and of lower surface of leaf; *u.s.*, upper surface of leaf; *l.s.*, lower surface of leaf; *scl.*, sclerenchyma; *ph.*, phloem. Diagrammatic.

Fig. 65. T. S. vascular bundle of pseudo-bulb; *t.c*, tracheide-like cell. × 360.

Fig. 66. General tissue of pseudo-bulb, L. S.: *sp.*, water-storage cell. × 360.

Fig. 67. Leaf, T. S.: *p.t.*, region of palisade arrangement; *epi.*, epidermis. Diagrammatic.

PLATE XI.

Bulbophyllum pygmaeum (*continued*).

Fig. 68. Leaf, T. S.: *pap.*, papilla; *pr.*, protoplasm; *pal.tis.*, palisade arrangement of cells; on the lower right-hand side three cells are figured without chlorophyll to show connexions. × 360.

Bulbophyllum tuberculatum.

Fig. 69. Stem, outer portion, T. S.: *l.c.*, large vacuolated cell; *rh.c.*, raphides cell; *par.tis.*, parenchymatous tissue. × 112.

Fig. 70. T. S. vascular bundle of stem. × 620.

Fig. 71. L. S. raphides cell of leaf.

Fig. 72. T. S. outer portion of root: *f.w.*, thin outer wall of velamen; *t.w.*, thick radial wall; *c.*, cortical cell. × 360.

Fig. 73. Root, central cylinder, T. S.: *p.c.*, two passage cells together; *cell.peri.*, cellulose element of pericycle. × 620.

Fig. 74. T. S. margin of leaf: *e.c.*, projecting cell of leaf edge. × 112.

Fig. 75. T. S. pseudo-bulb: *m.c.*, water-storage cell. × 360.

Fig. 76. Leaf, T. S.: *m.r.*, midrib; *u.epi.*, upper epidermis. Diagrammatic.

Fig. 77. T. S. lower portion of leaf: *el.c.*, elongated cells between the vascular bundles; *scler.*, part of sclerenchyma sheath; *t.a.*, slight tubular connexion; *pass.*, stomatal cavity. × 360.

Fig. 78. T. S. midrib of leaf: *t.w.*, thickened wall of epidermis. × 360.

Fig. 79. L. S. pseudo-bulb: *m.c.*, water-storage cell; *inter.sp.*, intercellular space. × 112.

Fig. 80. T. S. vascular bundle of pseudo-bulb: *pr.e.*, protective element. × 360.

Fig. 81. L. S. pseudo-bulb, near apex: *pur.c.*, cell containing purple sap; *m.c.*, water-storage cell. × 112.

Sarcochilus adversus.

Fig. 82. Stem, outer portion, T. S.: *p.c.*, parenchyma cell; *r.c.*, raphides cell. × 112.

Fig. 83. T.S. cells of central ground tissue of stem, to show pits. × 360.

Fig. 84. Stem, T.S.: *gr.tis.*, ground tissue. Diagrammatic.

PLATE XII.

Sarcochilus adversus (*continued*).

Fig. 85. T. S. vascular bundle of stem : *com.c.*, companion cell ; *cut.w.*, cutinized cells. × 360.

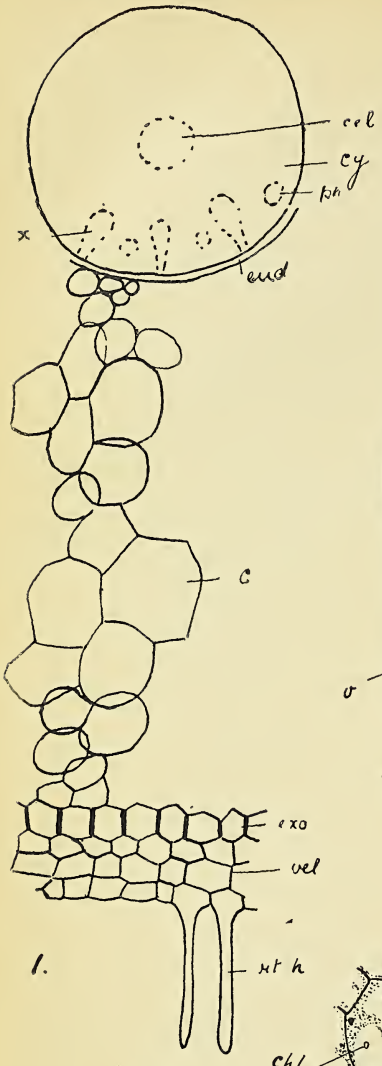
Fig. 86. Leaf, upper portion, T. S. : *r.*, raphides. × 112.

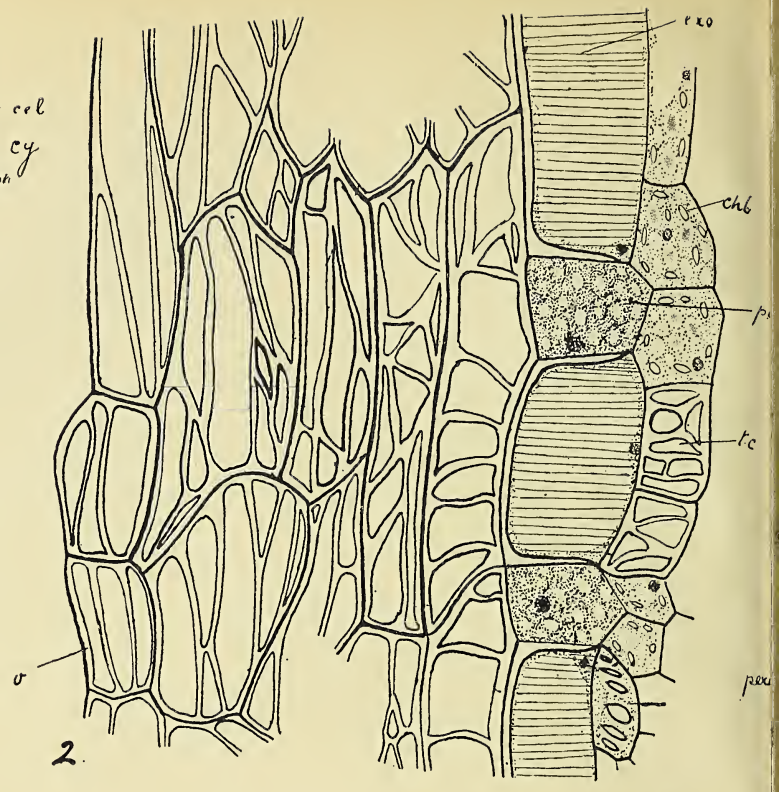
Fig. 87. Leaf, T. S. Diagrammatic.

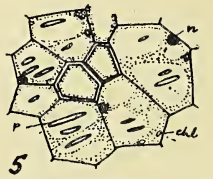
Fig. 88. T. S. large vascular bundle of leaf : *x.*, strong development of xylem. × 360.

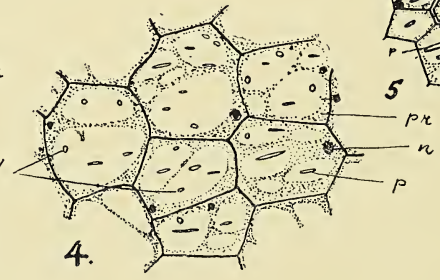
Fig. 89. T. S. outer portion of root : *m.*, radial strands present in walls of outermost cells only ; *l c.c.*, somewhat large cortical cell adjoining a passage cell ; *sp.c.*, spirally thickened cell ; *r.*, raphides. × 620.

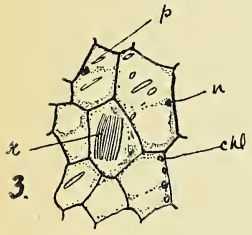
Fig. 90. T. S. central cylinder, root : *cell.peri.*, cellulose element of pericycle ; *cut.c.*, cutinized cell. × 360.

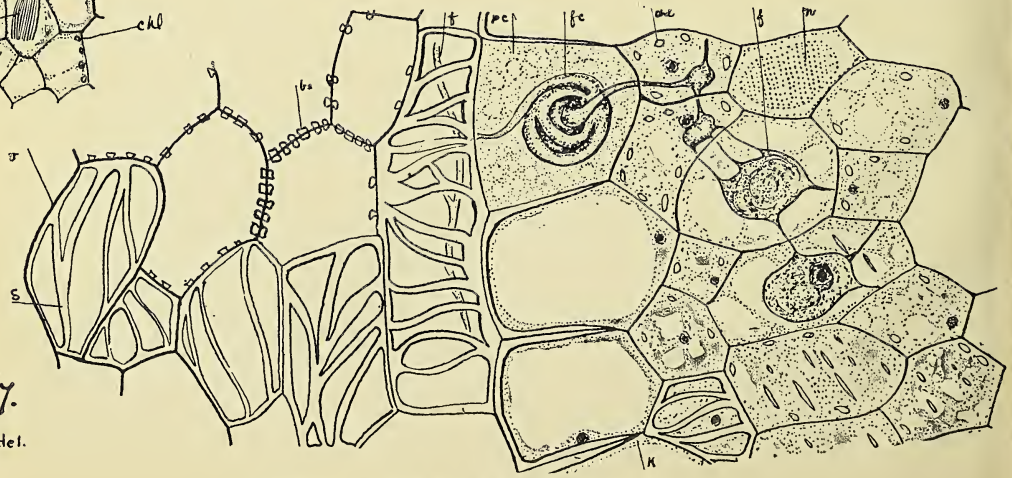












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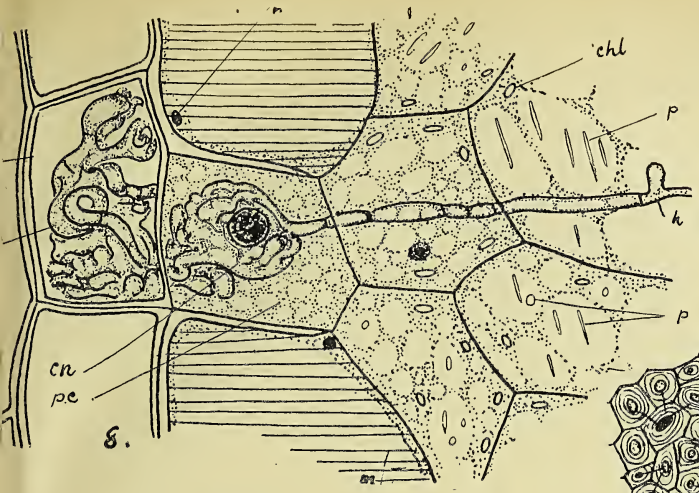

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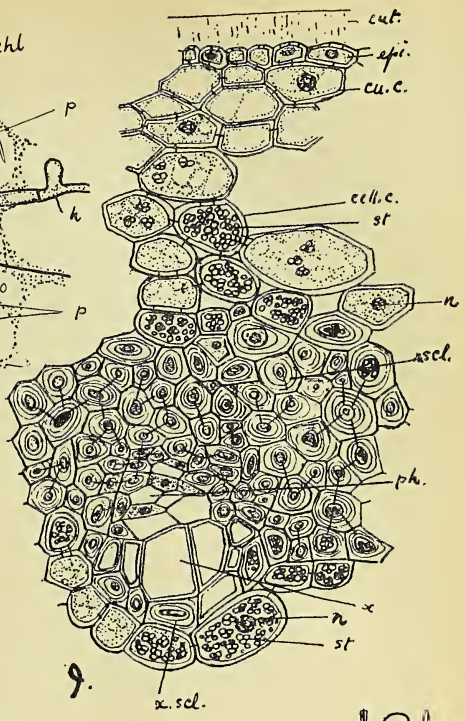

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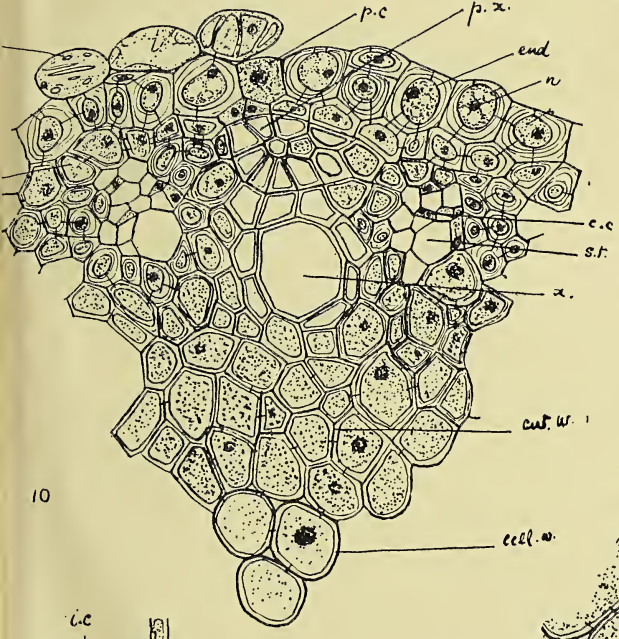

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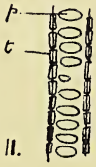

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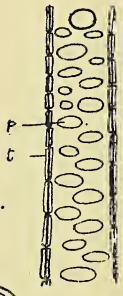

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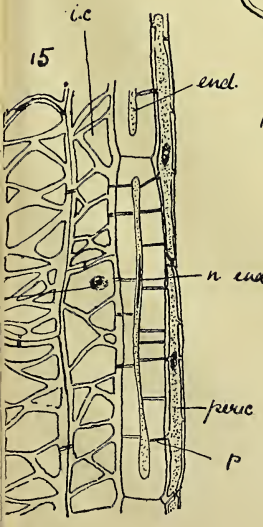

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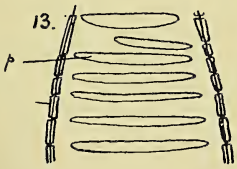

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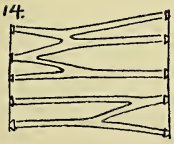

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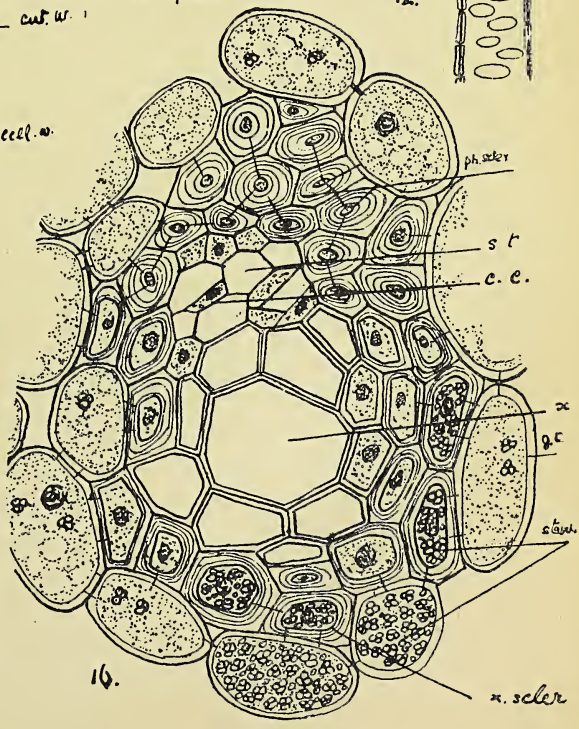

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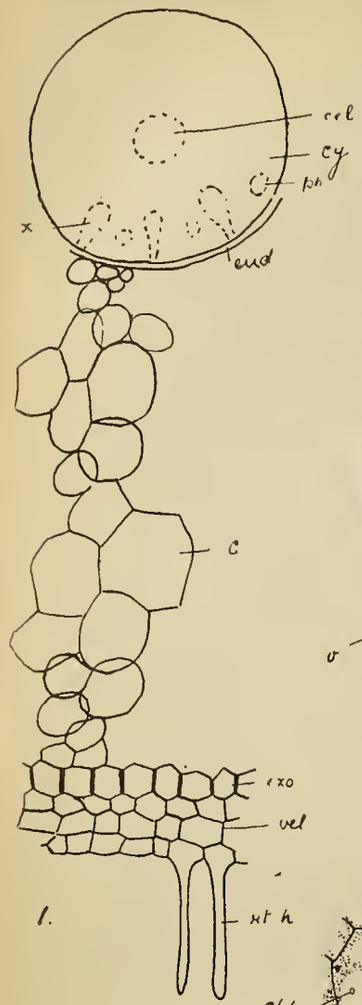

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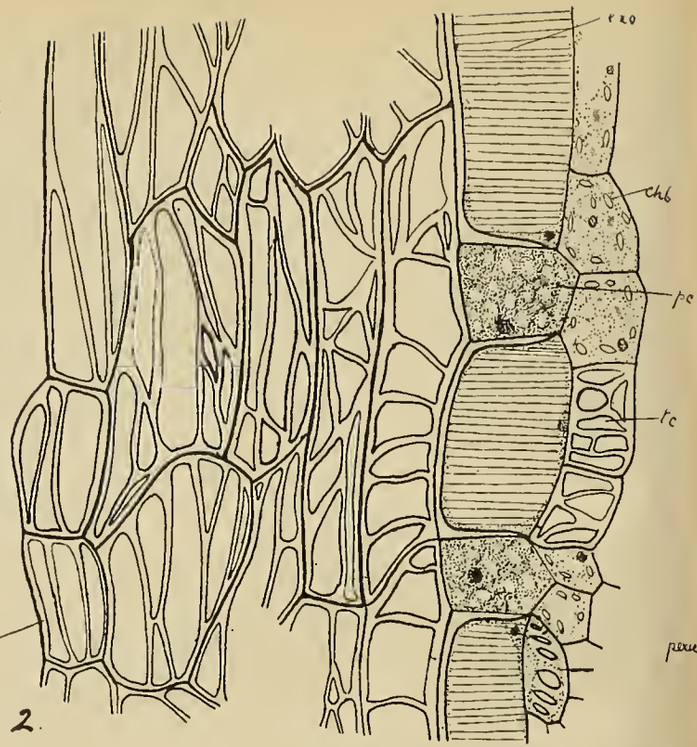

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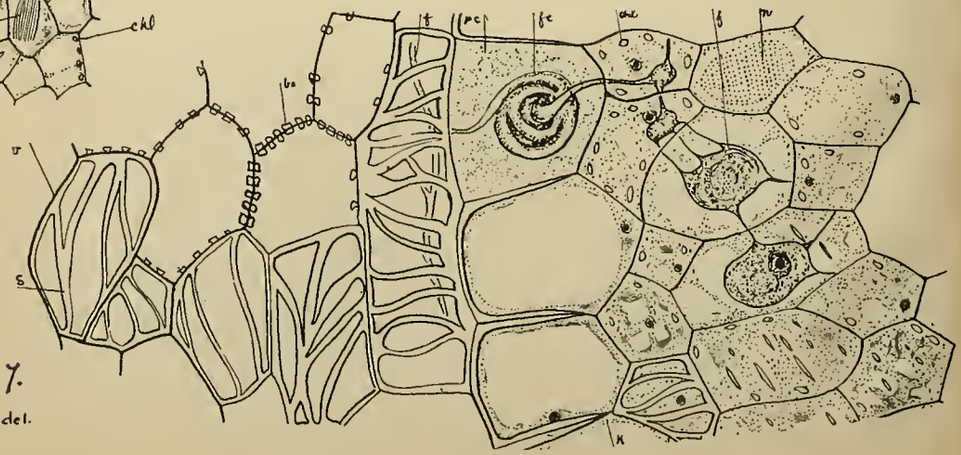

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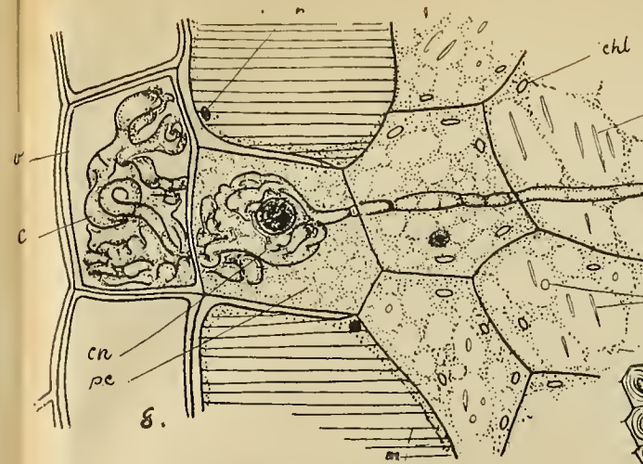

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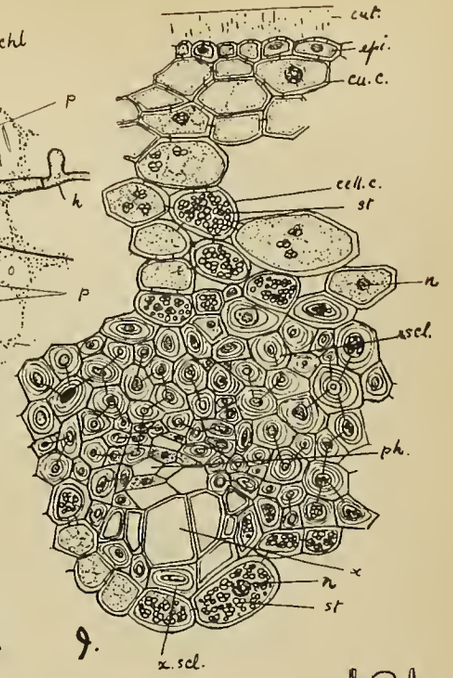

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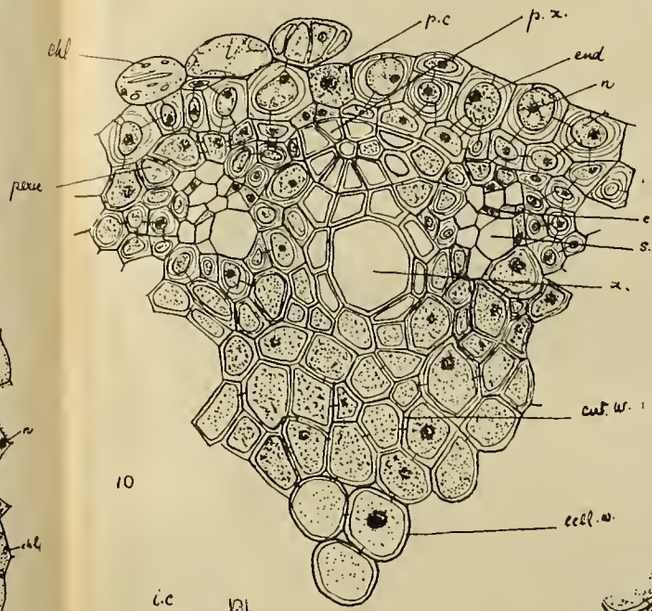

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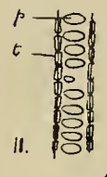

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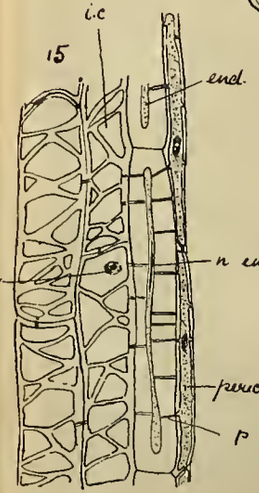

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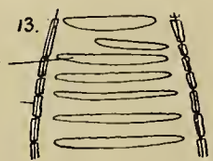

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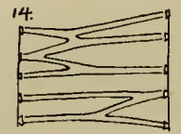

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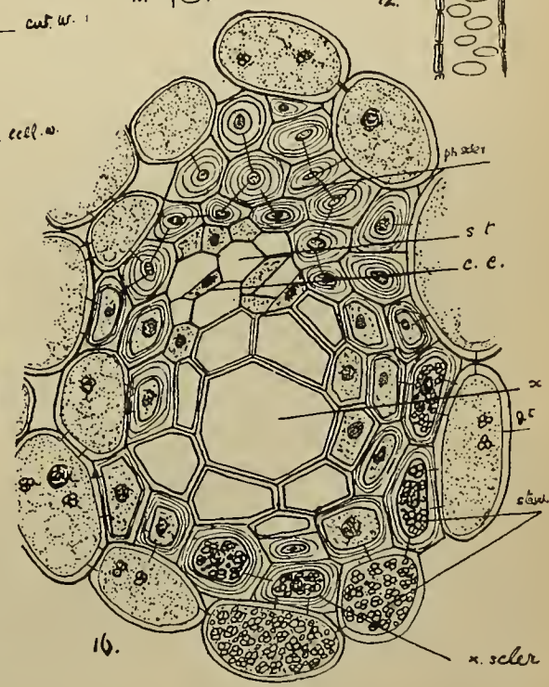

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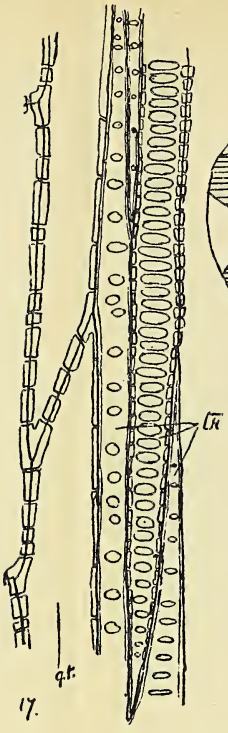

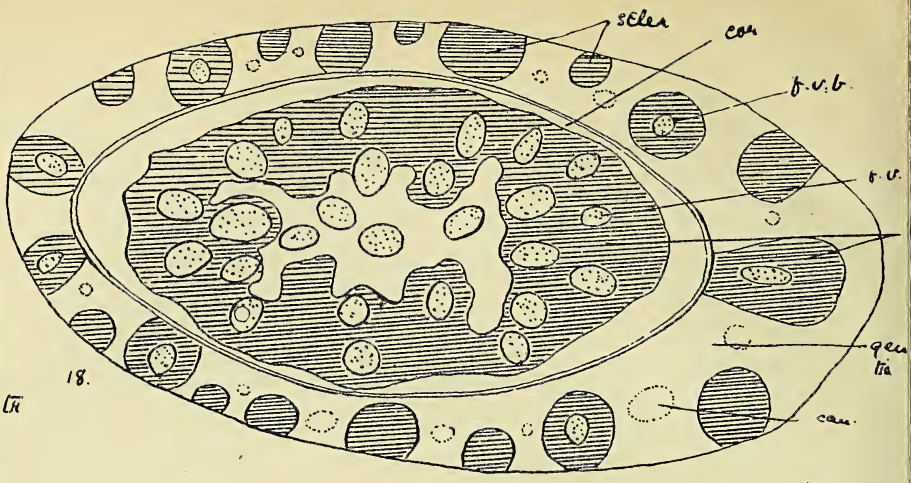

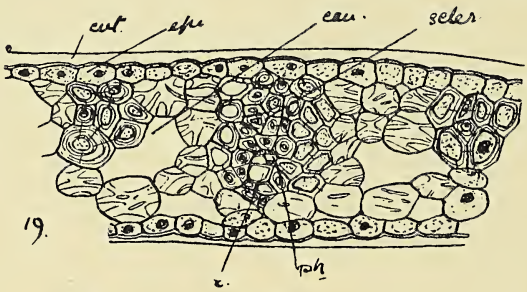

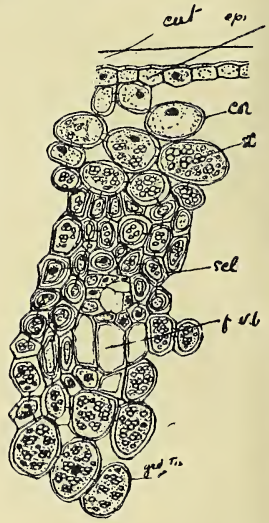

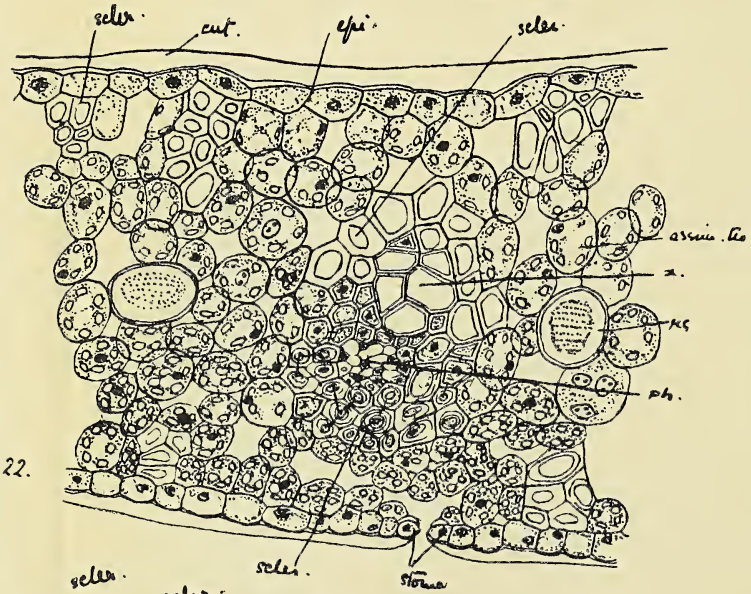

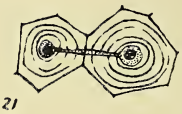

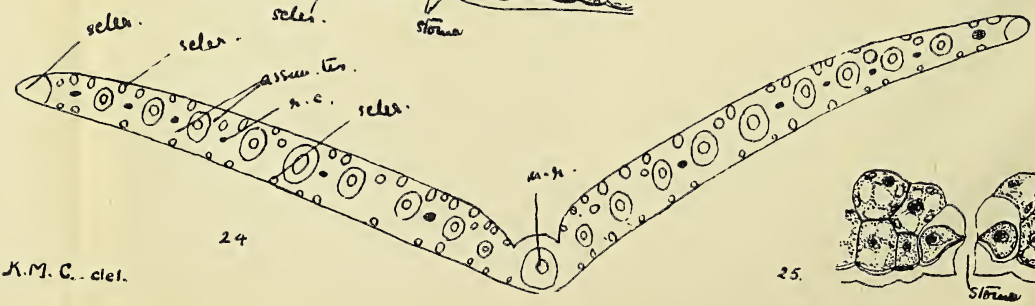

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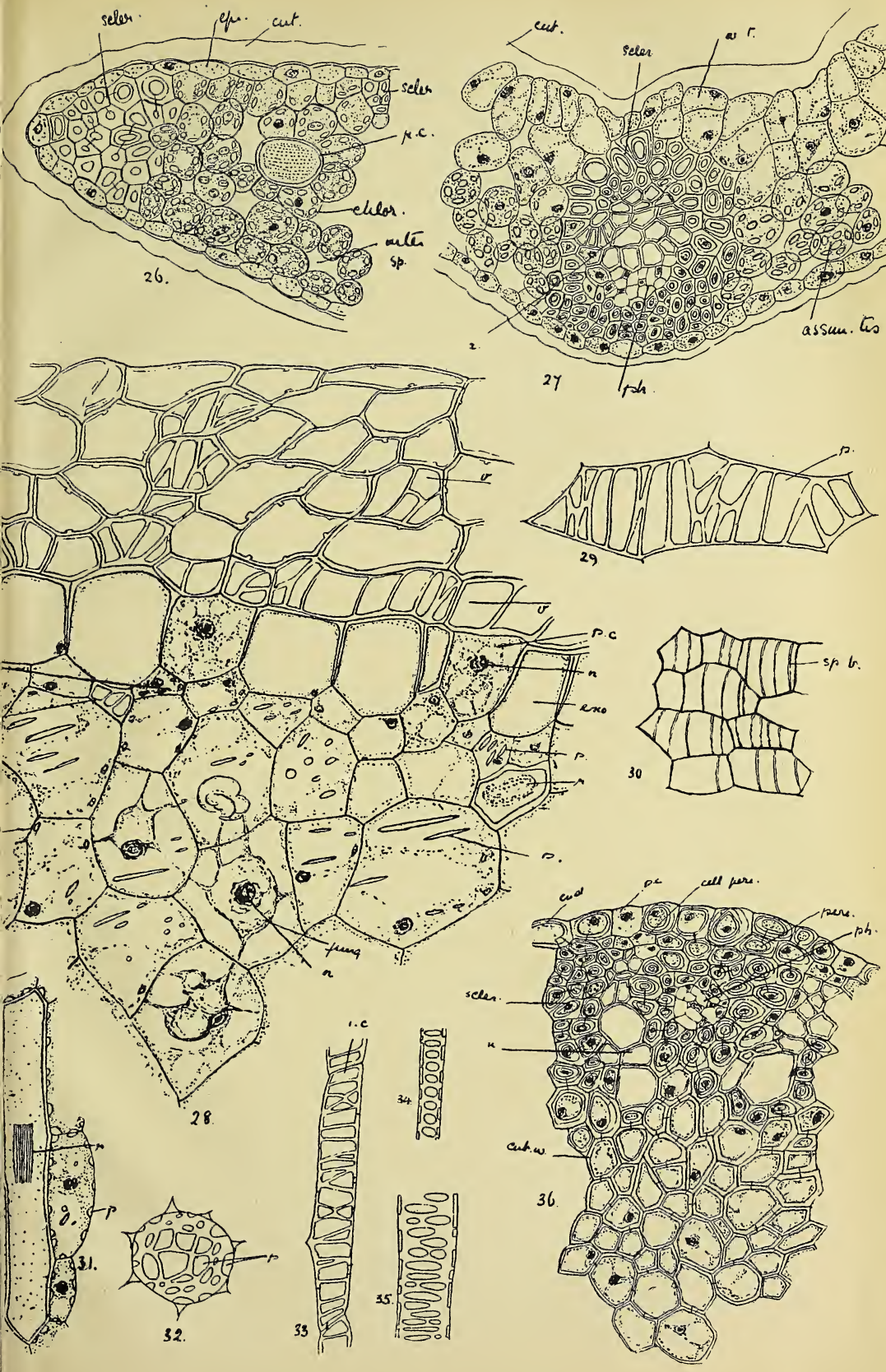
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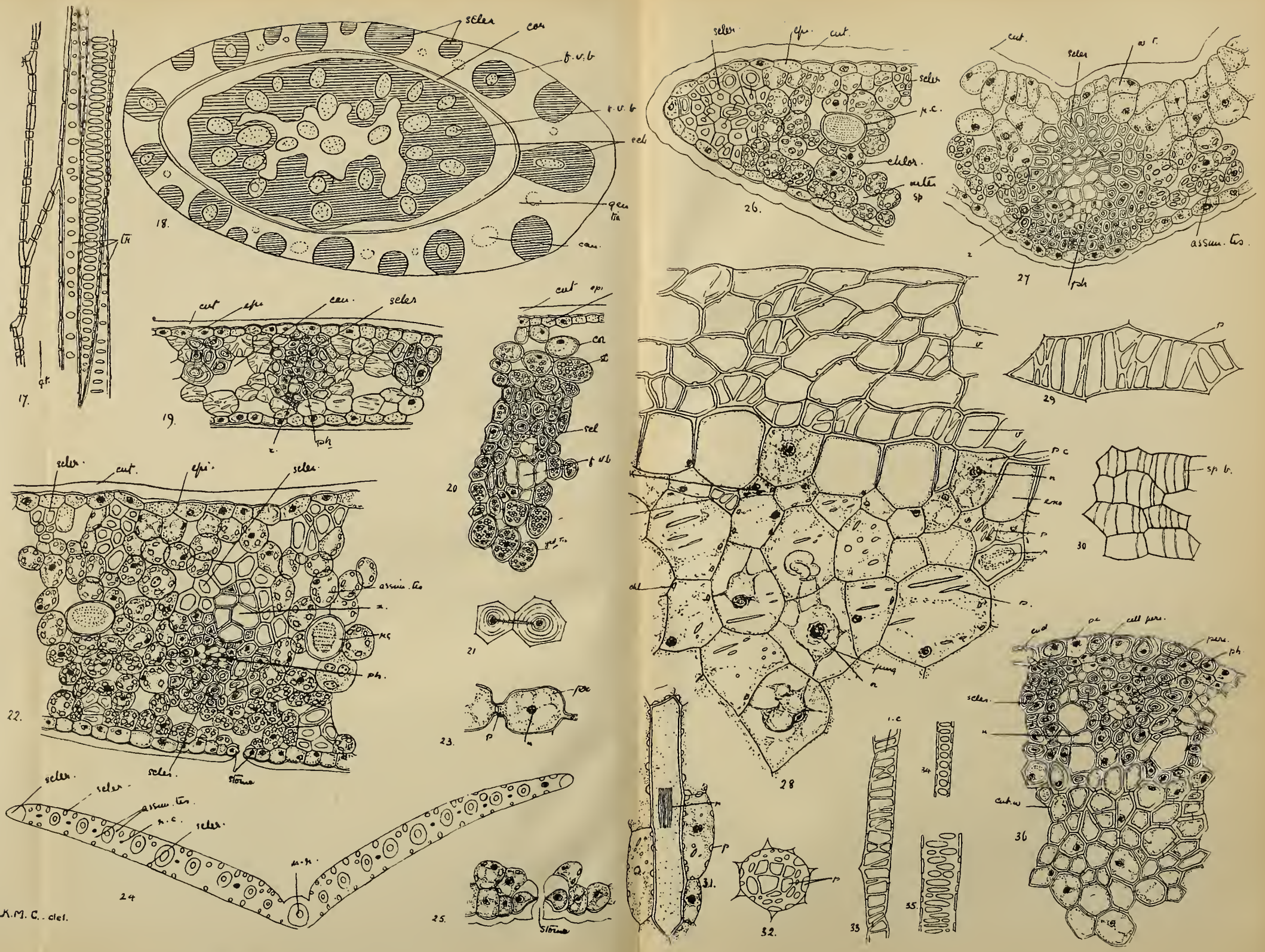

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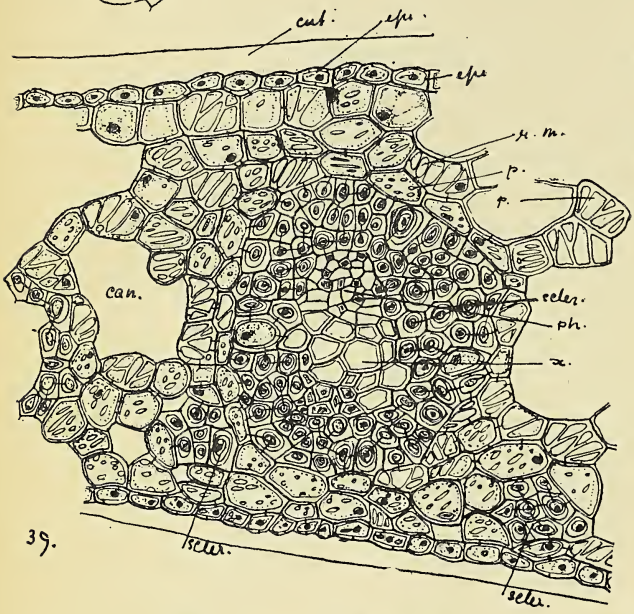

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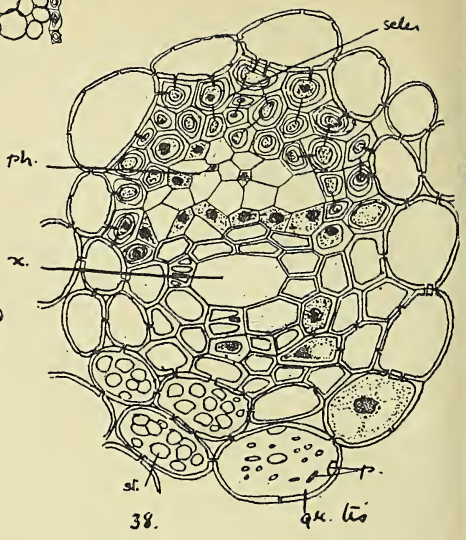

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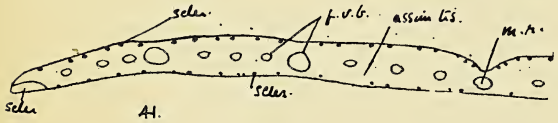

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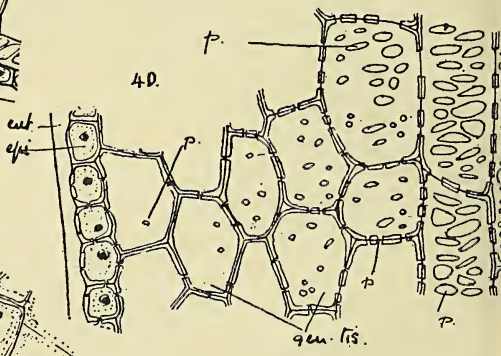

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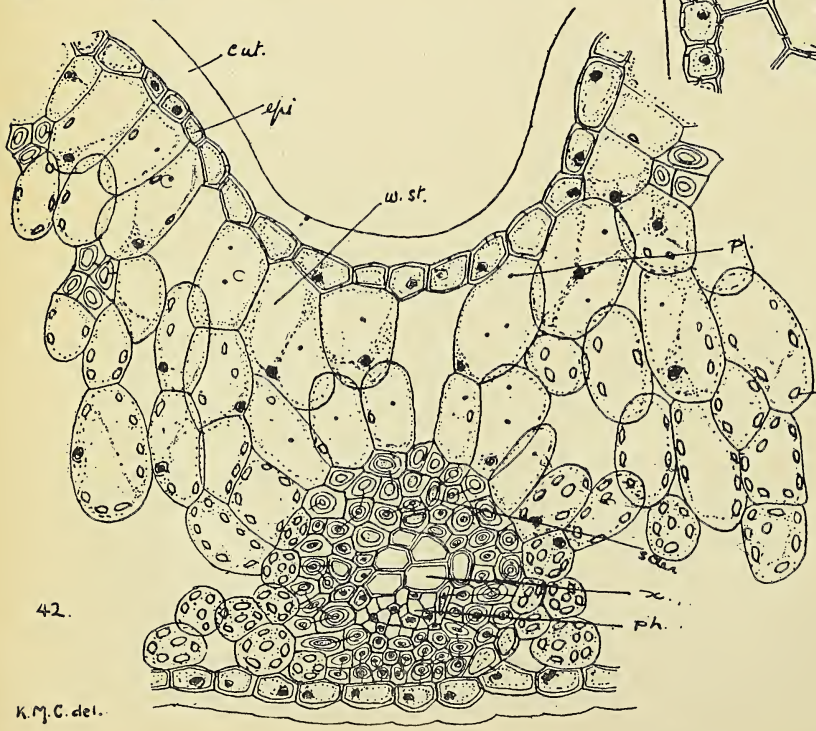

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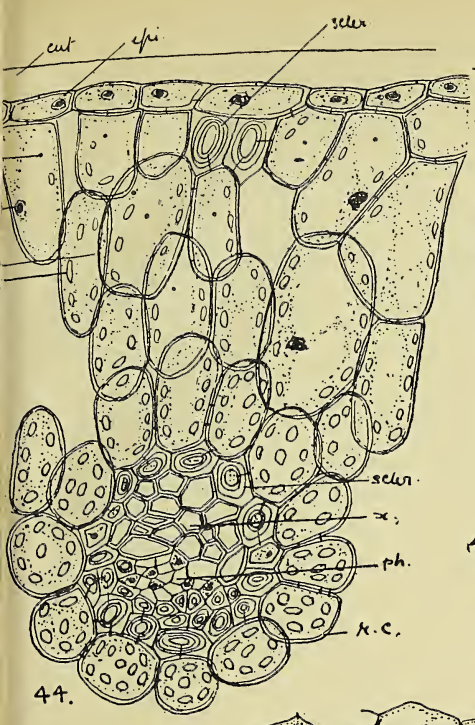

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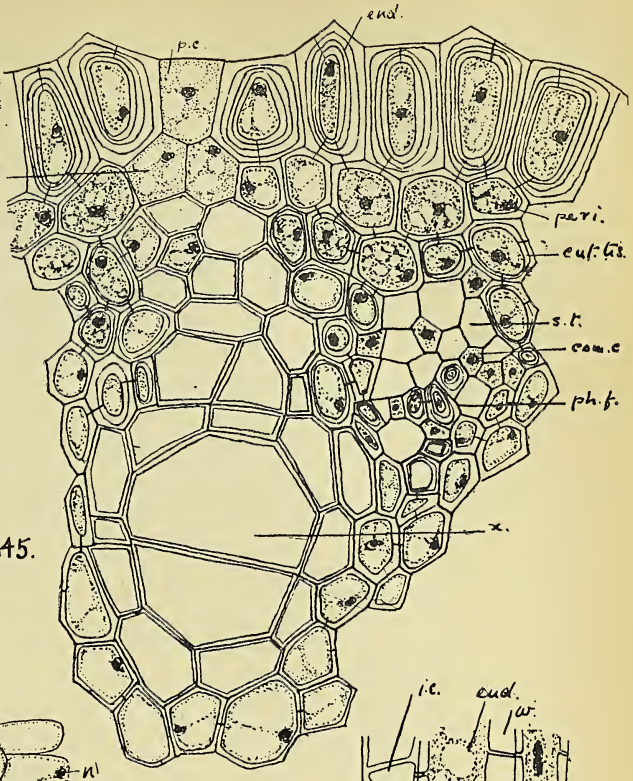

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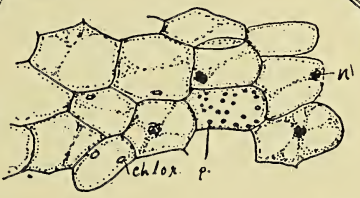

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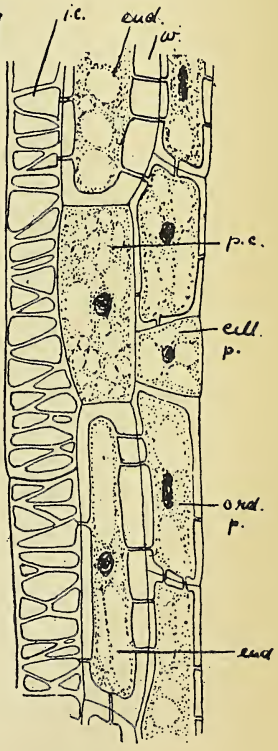

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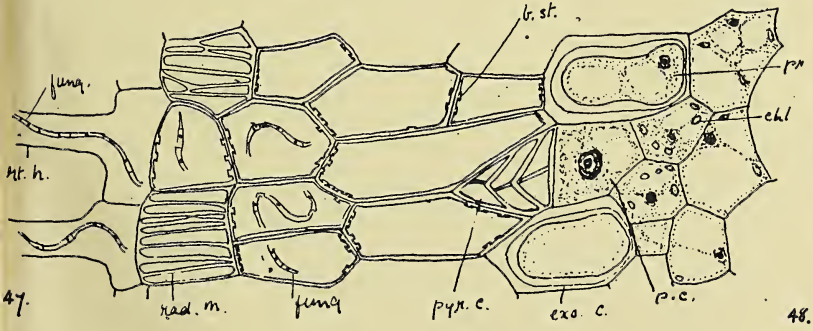

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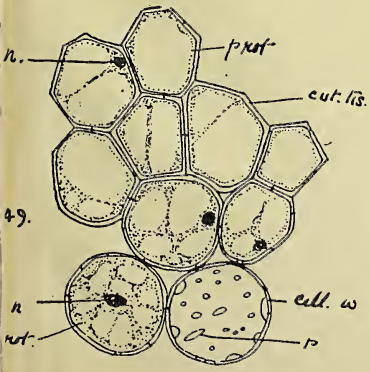

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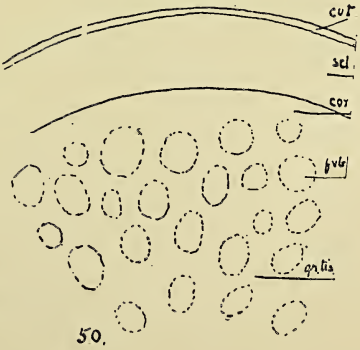

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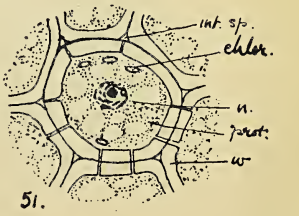

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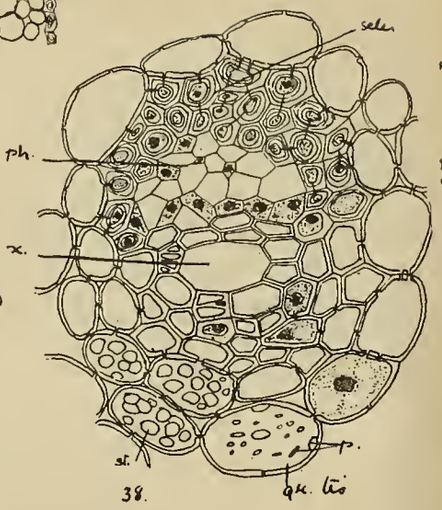
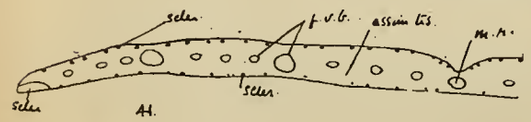
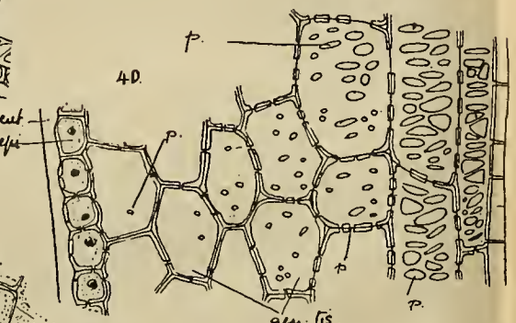
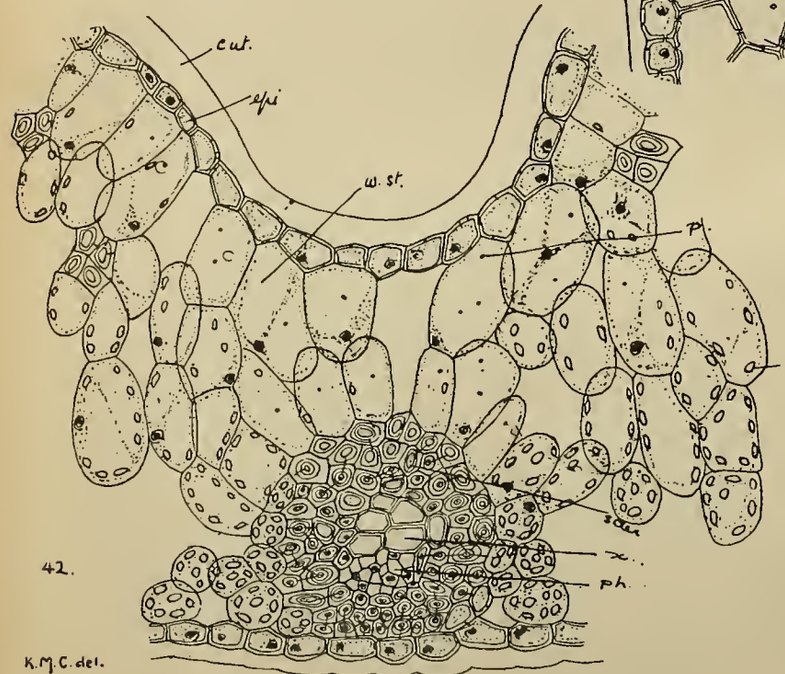
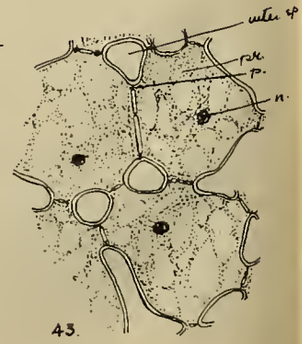
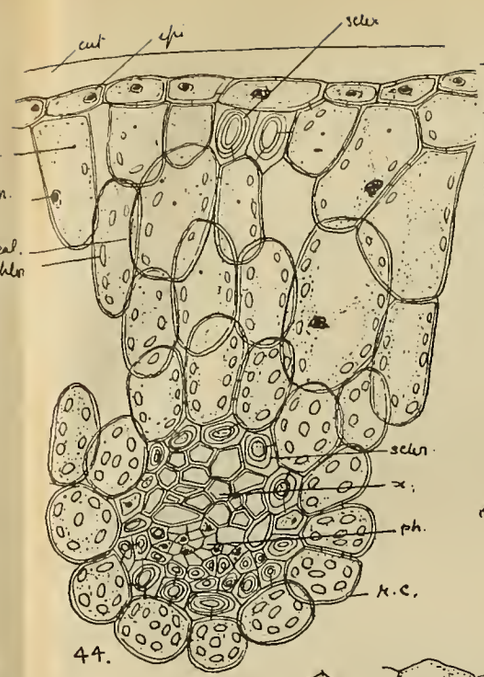


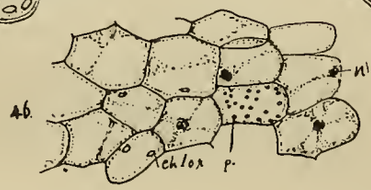


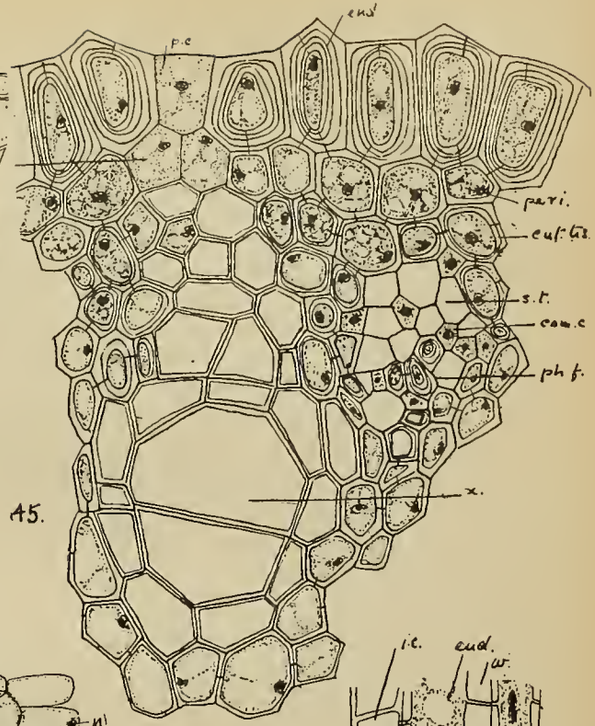


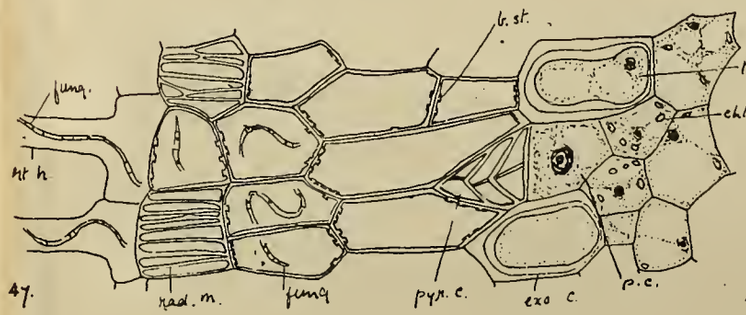


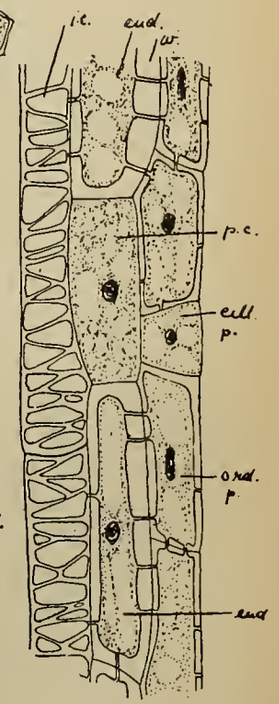


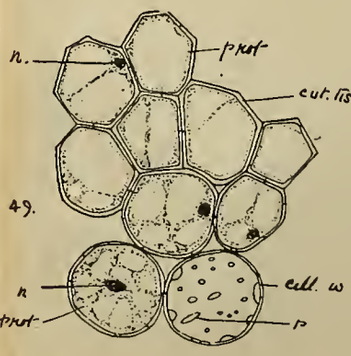


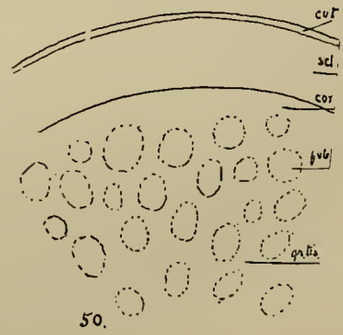


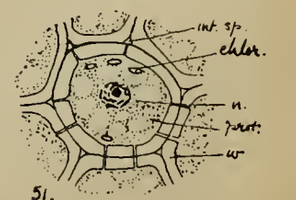




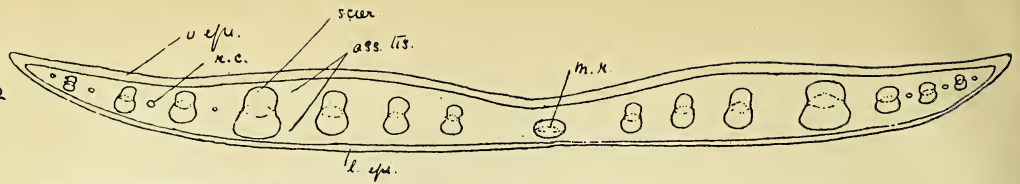








CURTIS—NEW ZEALAND ORCHIDS.



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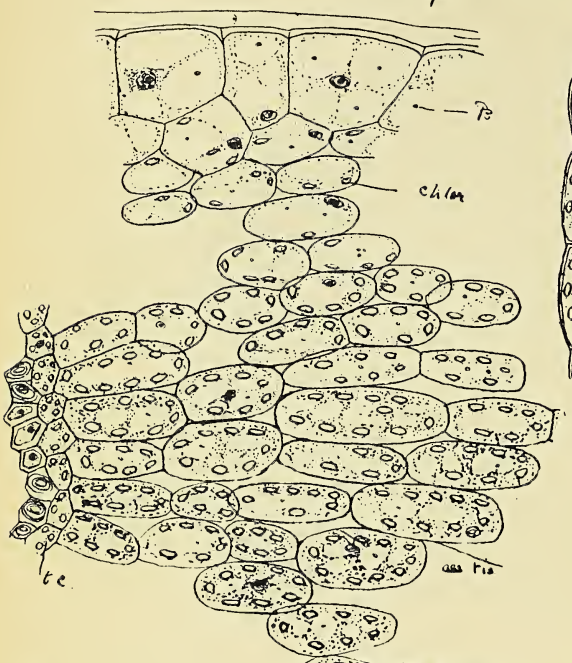

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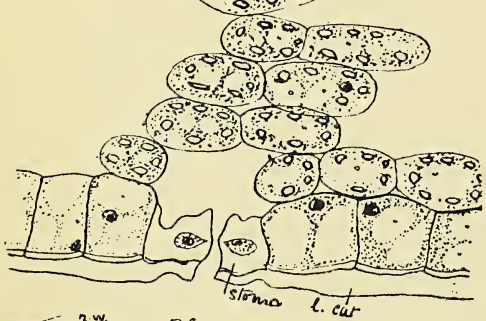

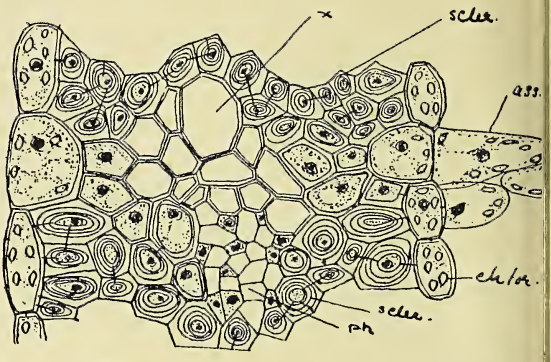

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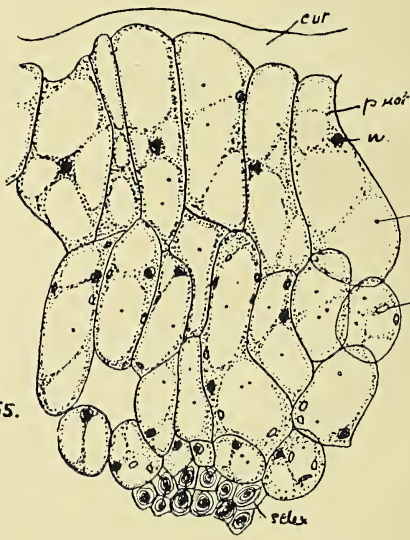

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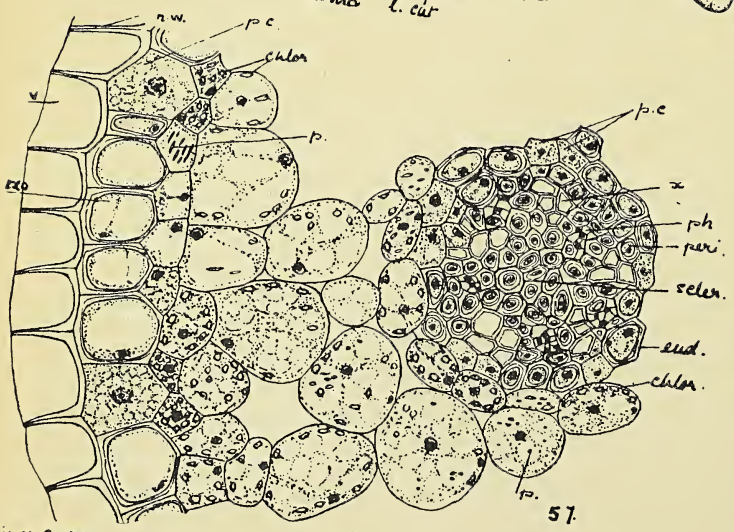

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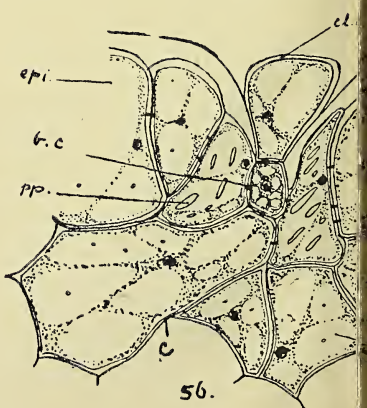

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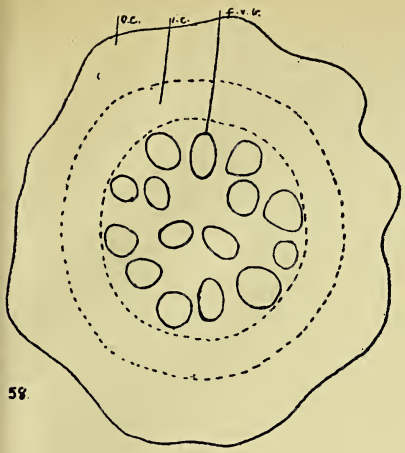

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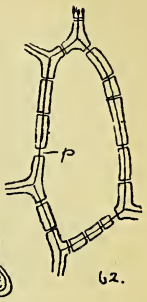

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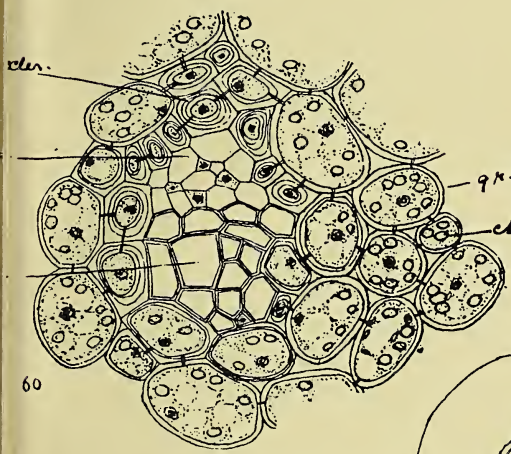

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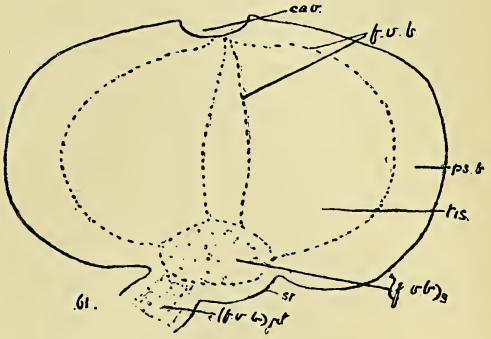

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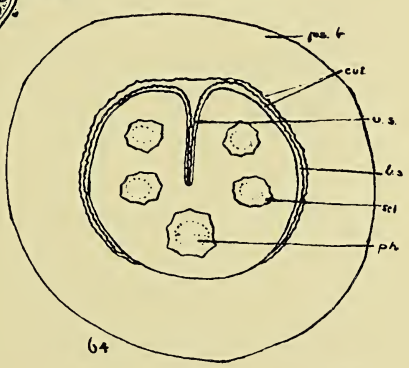

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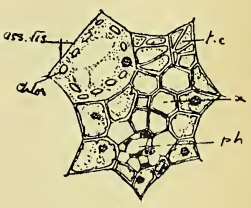

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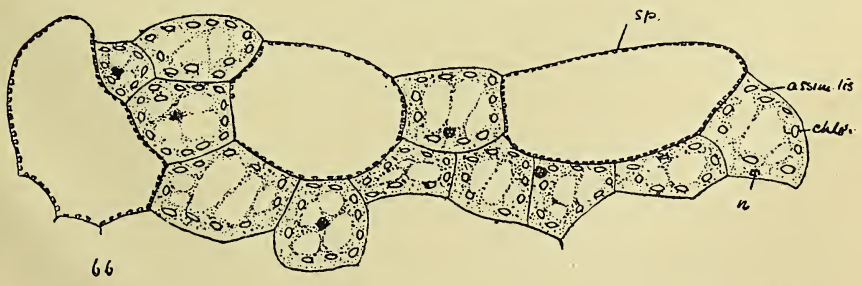

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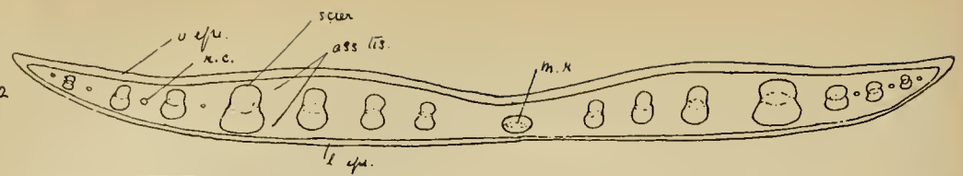

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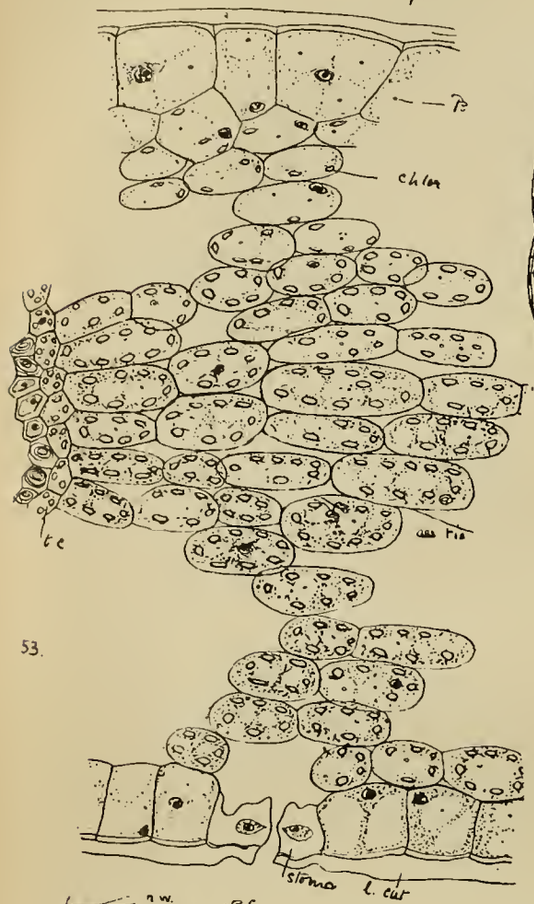

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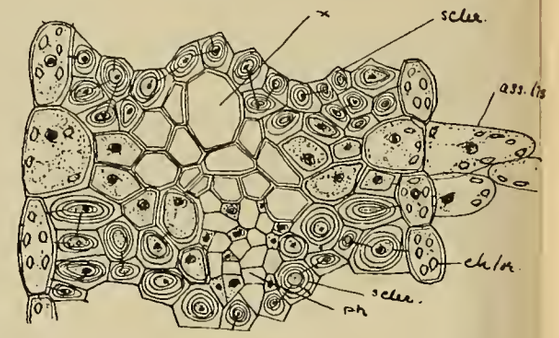

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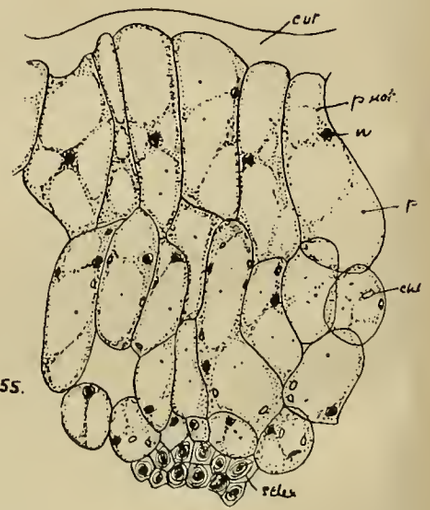

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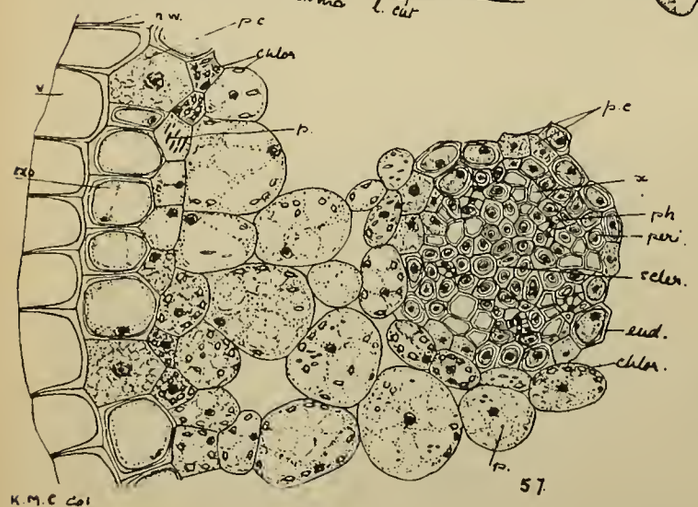

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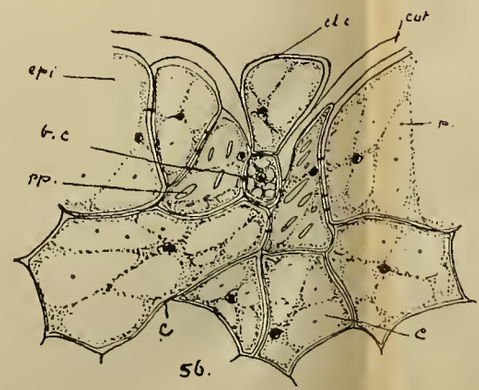

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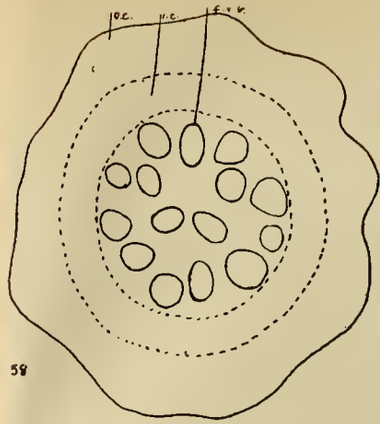

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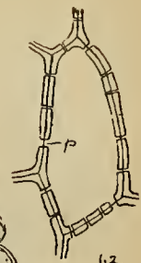

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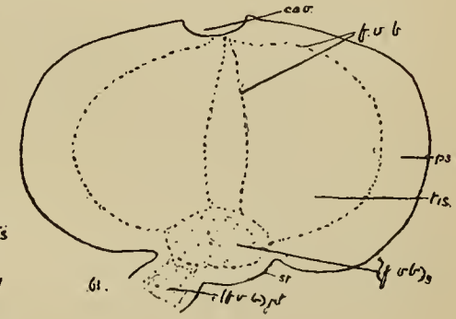

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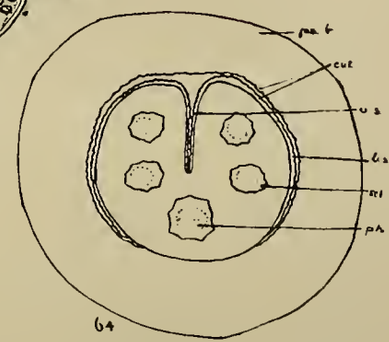

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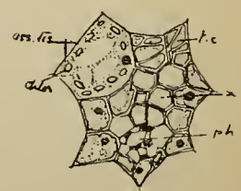

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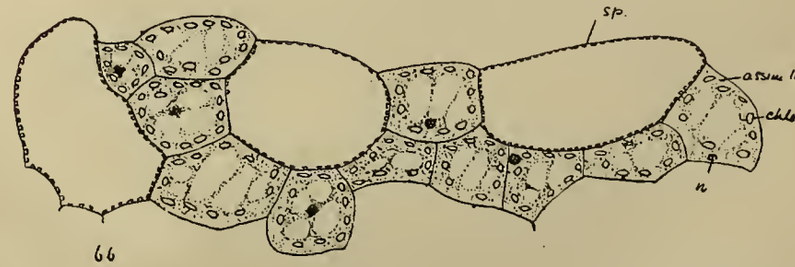

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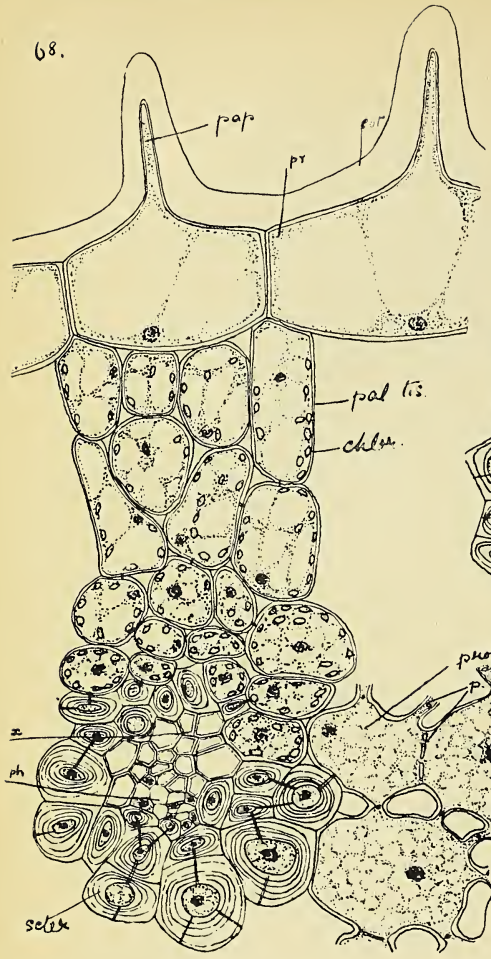
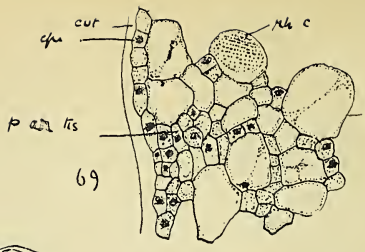
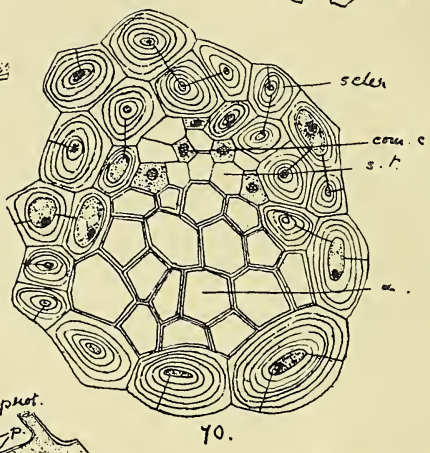
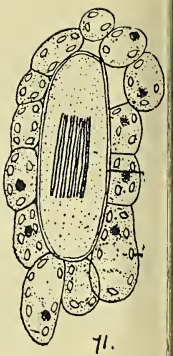
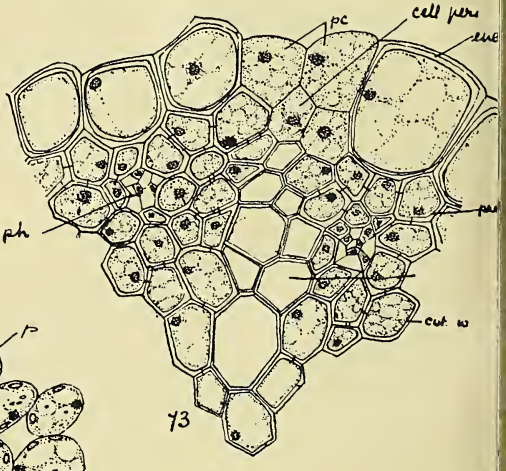
CURTIS—NEW ZEALAND ORCHIDS.

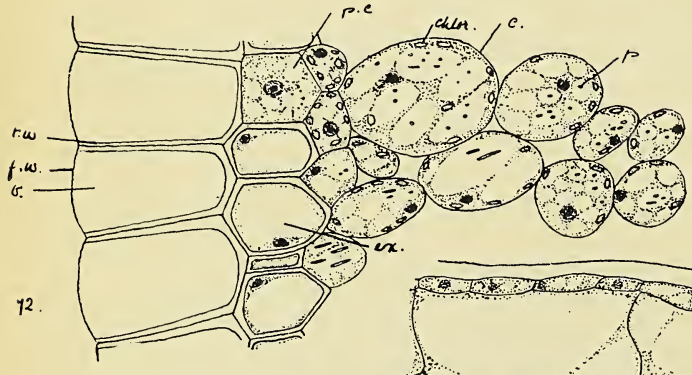


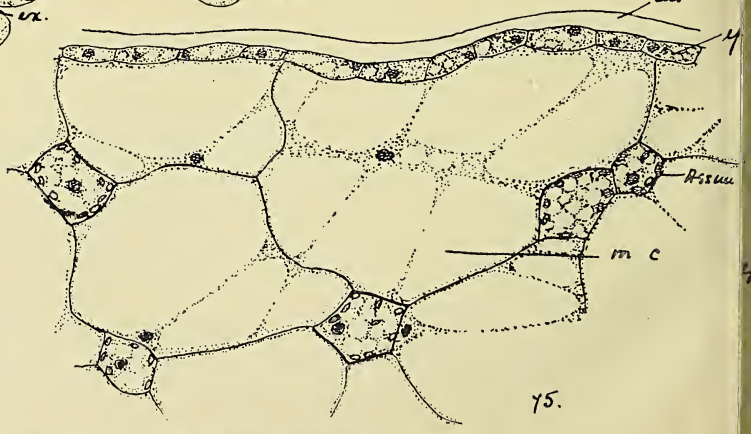


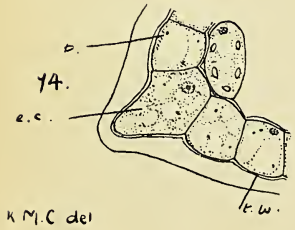












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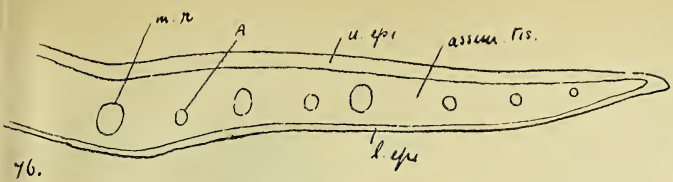
CURTIS—NEW ZEALAND ORCHIDS.



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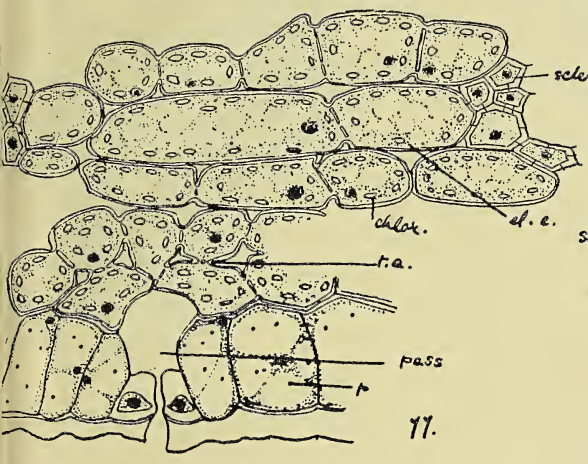

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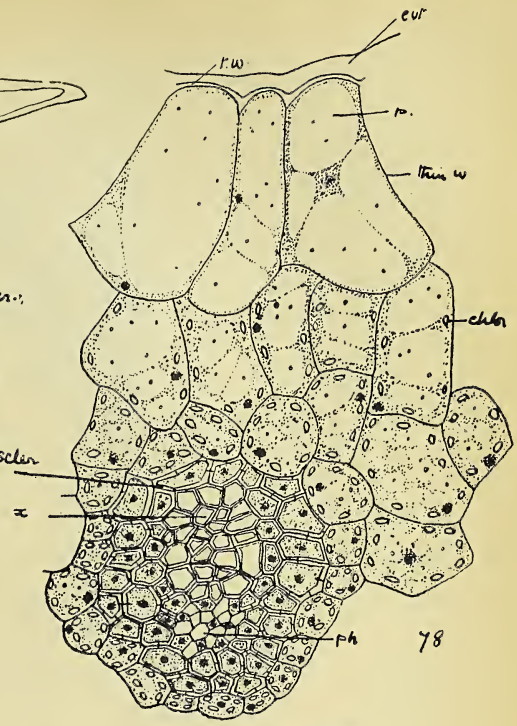

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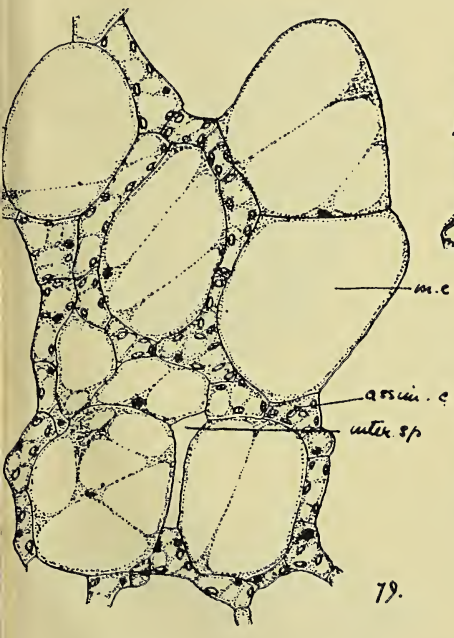

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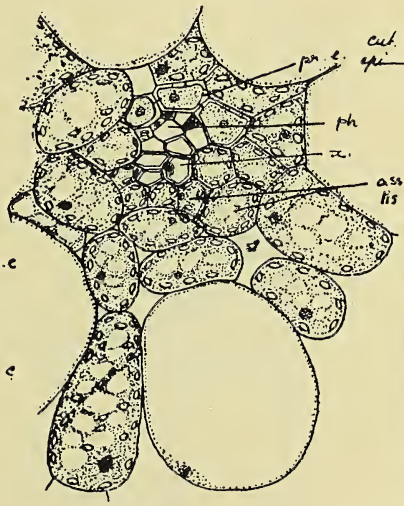

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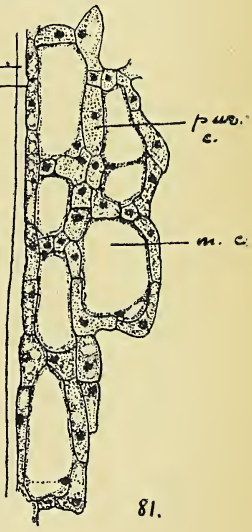

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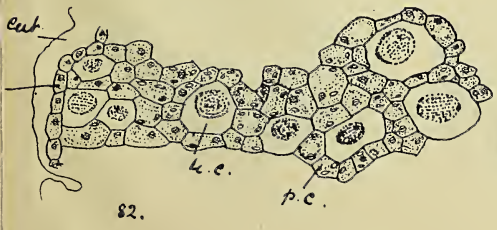

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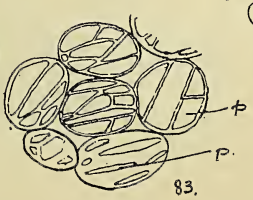

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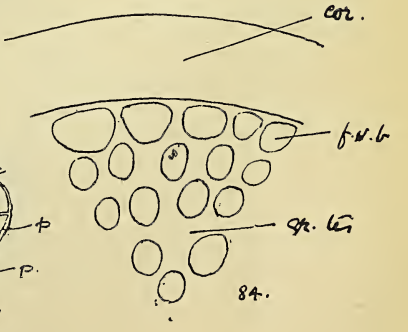

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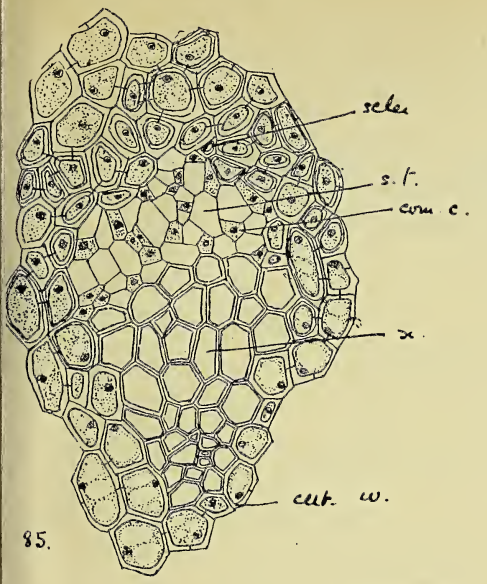

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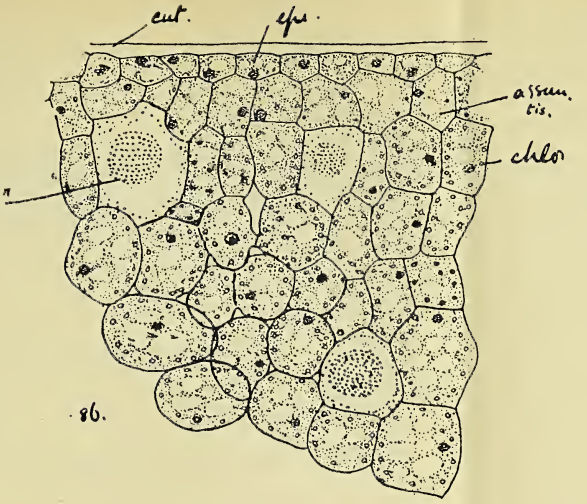

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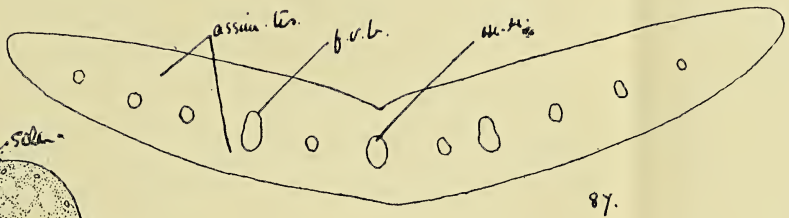

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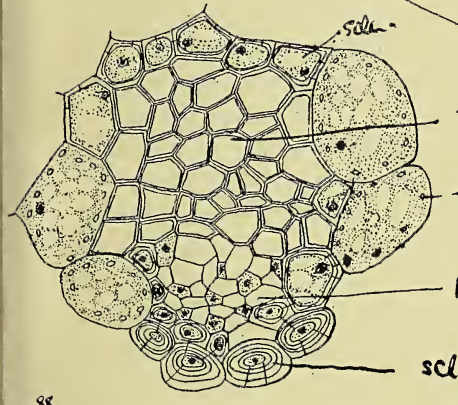

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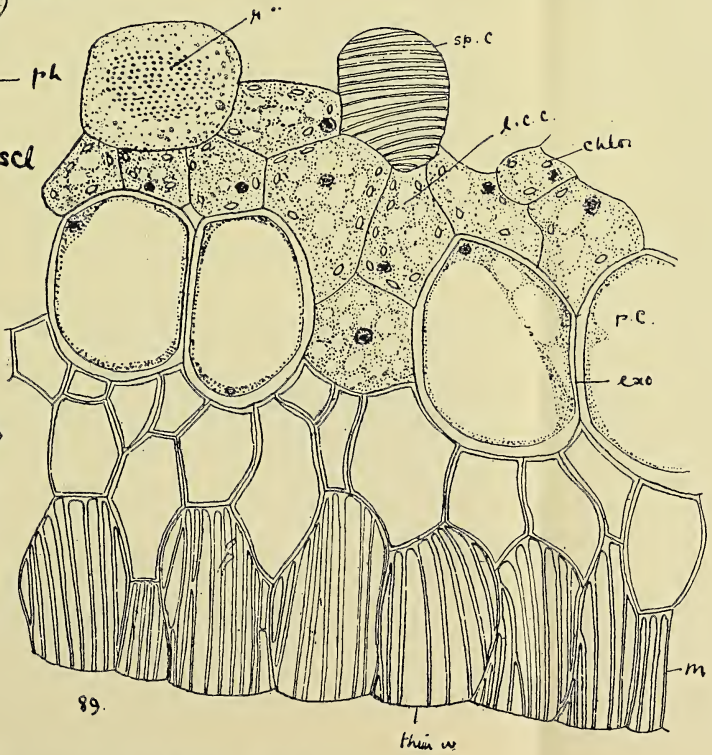

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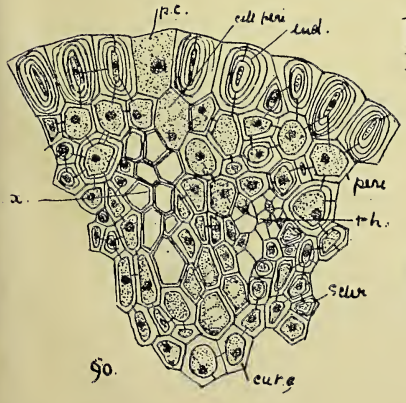

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CURTIS—NEW ZEALAND ORCHIDS.

On the Vascular Anatomy of the Young Epicotyl in some Ranalean Forms.

BY

KATHLEEN B. BLACKBURN,

Lecturer in Botany, Southlands Training College.

With Plate XIII and nineteen Figures in the Text.

THE present paper is an account of some work which has been carried out at Bedford College under the direction of Dr. Thomas. It forms part of a series of investigations into the anatomy of the axis in seedling Dicotyledons, and in particular into that of the epicotyl.

In Ranalean forms the anatomical features of the mature axis have been studied from various points of view by numerous workers, notably by Marié,[1] by members of the Liège School,[2] and by Worsdell,[3] but the beginnings of vascular development in the seedling epicotyl have received far less attention.

The 'Contributions à l'anatomie des Renonculacées', published by the Liège School, comprise monographs on certain Ranunculaceous genera (*Clematis*, *Delphinium*, *Thalictrum*, and *Ranunculus arvensis*), in which detailed accounts of the plumular anatomy are included.

In his work on the 'Morphology of the Central Cylinder in Angiosperms' Professor Jeffrey[4] describes and figures early stages of the vascular structure in young stems of members of the families Nymphaeaceae and Ranunculaceae (*Ranunculus* and *Anemone* spp.).

Professor Gwynne-Vaughan[5] has described early stages of members of

[1] Marié, P.: Recherches sur la Structure des Renonculacées. Ann. Sci. Nat., Bot., tome xx, 1884.

[2] Contributions à l'anatomie des Renonculacées:

Nihoul, E.: *Ranunculus arvensis*. Mém. pub. par l'Acad. Roy. de Belgique, Brux., tome lii, 1892.

Lenfant, C.: Le Genre *Delphinium*. Mém. Soc. Roy. des Sciences de Liège, sér. 2, t. xix, 1897.

Mansion, A.: Le Genre *Thalictrum*. Mém. Soc. Roy. des Sciences de Liège, sér. 2, t. xx, 1898.

Sterckx, R.: La Tribu des Clématidées. Mém. Soc. Roy. des Sciences de Liège, sér. 2, t. xx, 1898.

[3] Worsdell: A Study of the Vascular System in certain Orders of the Ranales. Ann. of Bot., vol. xxii, October, 1908.

[4] Jeffrey: The Morphology of the Central Cylinder in Angiosperms. Trans. Canad. Inst., vol. vi, Toronto, 1889.

[5] Gwynne-Vaughan, D. T.: On some Points in Morphology and Anatomy of Nymphaeaceae. Trans. Linn. Soc., vol. v, pt. 7, 1897.

[Annals of Botany, Vol. XXXI. No. CXXI. January, 1917.]

the Nymphaeaceae in his investigation of the development of polystely in this family.

In view of the great interest attaching to the Ranales on general grounds,[1] and to the Ranunculaceae in particular through the possession of the tubular type of stele to which Professor Jeffrey[2] attaches so much importance, it was suggested that a comparative investigation of Ranalean plumular anatomy in its early stages could hardly fail to yield results of some interest. A nucleus of material was handed over to the writer by Dr. Thomas, and has been supplemented by seedlings raised in the Botany garden of Bedford College, and in the case of many of the British species by those obtained in the field.

Owing to difficulties in obtaining adequate material for a full study of the other families, Ranunculaceae has received the most attention.

The axes of seedlings bearing from one to five expanded foliage leaves, in addition to the cotyledons, were microtomed from the stem apex to the upper part of the hypocotyl.

The seedling morphology shows a number of general features, one or more of which may be departed from in individual cases, though on the whole they are very constant throughout the group.

The first few internodes are relatively short, and the phyllotaxis is two-fifths. The cotyledons are epigeal and, where the first internode is specially short, the first two foliage leaves tend to be opposite and at right angles to the cotyledons. A cotyledonary tube is frequently found.

With the exception of the very specialized Nymphaeaceae[3] the divergence from the type just described is in very limited and definite directions:

(*a*) In *Calycanthus* and in some species of *Clematis* the early foliage leaves are opposite and decussate, and in *Anona* the *phyllotaxis* is one-half.

(*b*) *Hypogeal* germination is found in certain large-seeded forms. It occurs throughout Lauraceae and Anonaceae and in certain species of Ranunculaceae, notably *Anemone nemorosa* and some species of *Paeonia* and *Clematis* (see Text-figs. 3 and 9).

(*c*) The *cotyledonary tube* varies in extent. It is practically absent in *Thalictrum*, but it is well marked in *Anemone fulgens*, *Podophyllum emodi*, and *Eranthus hiemalis* (see Text-fig. 4). In *Anemone apennina* and *Ranunculus Ficaria* there is only one lobed cotyledonary member.

As has been pointed out by Miss Sargant,[4] a *tuberous* swelling of the *hypocotyl* and accompanying suppression of the first few plumular internodes is frequently correlated with a long cotyledonary tube. Accordingly this

[1] See Arber, E. A. Newell, and Parkin, J.: On the Origin of Angiosperms. Journ. Linn. Soc., vol. xxxviii, 1907.

[2] See Jeffrey, loc. cit. [3] See Gwynne-Vaughan, loc. cit.

[4] Sargant, E.: A Theory of the Origin of Monocotyledons, &c. Ann. of Bot., vol. xvii, 1903, p. 77.

feature is found in all the species mentioned above with the exception of *Anemone fulgens*, and also in *A. nemorosa* (see Text-fig. 3).

There is a corresponding general type of anatomy to which the majority of the seedlings described conform, though there are more variants from it than from the common type of morphology.

Each foliage leaf contributes three traces to the axial vascular cylinder, which consists of a circle of separate collateral bundles anastomosing at the nodes. Early cambial activity connects the strands by secondary tissue. The number of primary bundles in subsequent internodes varies with the species, but in the lowest plumular internode there are usually six. These arrange themselves in groups of three on either side of the plane passing through the centre of the two cotyledons, which will be referred to as the cotyledonary plane.

In general the cotyledons each contribute a double bundle, and thus a ring of strands is again completed (see Text-fig. 6). The three plumular and two half cotyledonary strands on each side then fuse, to give what often appears to be a single pair of collateral bundles, on the flanks of the diarch plate. This is usually formed quite high up in the hypocotyl, as described by Dr. Thomas.[1]

Some of the forms described possess independent lateral strands at the base of the cotyledons, and in the case, for instance, of *Ranunculus arvensis* the insertion of their traces is so remarkably like the insertion of the plumular traces in the same species, both as regards the number of strands from each cotyledon and their mode of entry, that one is tempted to suggest the possibility that this is the earlier condition for the cotyledons. The insertion of three strands from each cotyledon is in some cases connected with a tetrarch root, but in the plant referred to the lateral strands die out in the upper part of the hypocotyl. The anatomical similarity between leaf and cotyledon is here borne out by the existence of a series of leaf forms intermediate between the compound leaf of the adult plant and the simple ovate cotyledon.

Just as there were forms whose morphology differed from the general ground plan, so the same or other forms may be aberrant in respect to anatomy.

(*a*) Those forms of *Clematis* seedling whose foliage leaves are opposite show a reduction in the number of strands in the internode, four only being present. The insertion of the leaf-trace strands is also exceptional.

(*b*) In the species presenting both hypogeal and epigeal germination, as in *Clematis*, it is found that this feature has no anatomical consequences.

(*c*) The tuberous habit causes anatomical modifications in several

[1] Thomas, E. N.: Seedling Anatomy of Ranales, Rhoeadales, and Rosales. Ann. of Bot., vol. xxviii, October, 1914.

different directions in the various species showing that peculiarity. An interesting form is to be found in *Anemone apennina* and *Eranthis hiemalis*.

Near the periphery of the tuber is a ring of bundles of plumular origin, in the latter case connected by a well-marked cambium. The cotyledonary traces and some plumular tissue (in *A. apennina*) form a quite separate medullary system. These two groups of vascular tissue join at the base of the tuber.

The other anatomical variations are not so directly correlated with the morphology.

(*d*) The number of strands contributed by each leaf, though prevailingly three, is not always so. The first foliage leaf of *Paeonia arborea* has five traces at its base, whereas quite frequently, in *Anemone sylvestris* and many other seedlings, the strands of the first and sometimes more leaves are reduced to one at the point of insertion. This may be compared with the one double bundle found in the cotyledons, and is probably directly correlated with a reduction in the size of the leaf.

(*e*) The age at which cambial growth first appears is another feature which varies greatly. In *Nigella hispanica*, an annual form, it is first seen at a very late date and does not show great activity, whereas in *Aconitum Wilsonii* the very small amount of primary xylem in the bundles is scarcely differentiated before a complete ring of cambium is present (see Text-fig. 7). It seems possible that the date of appearance of the cambium may be directly correlated with the amount of primary tissue in the bundles.

(*f*) The most conspicuous exception to the general type of seedling anatomy is that found in the genera *Ranunculus*, *Trollius*, and *Caltha*. This is described by Professor Jeffrey[1] in the genus *Ranunculus* and in the young rhizomes of *Anemone pennsylvanica*. It consists of a tubular stele interrupted by leaf-gaps and bounded both internally and externally by a more or less well-marked endodermis, which may, however, be quite absent. The evidence brought forward in this paper suggests that the tubular nature of the stele may be a secondary phenomenon. The significance of the internal endodermis will be discussed later.

Thus, with a few exceptions, the structure of the seedling, both as regards morphology and anatomy, is constant throughout the group, whereas there is considerable diversity in the adult, and this diversity may be found in forms whose seedlings show great similarity.

A consideration of the three genera *Aquilegia*, *Thalictrum*, and *Anemone*, serves to illustrate this point. With the exception of the two tuberous species of *Anemone*, all the seedlings of these genera that have been examined show a remarkable anatomical resemblance to one another, even in histological details, while there are marked differences in the adult anatomy.

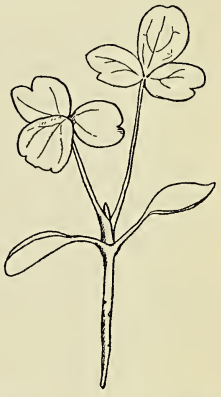
[1] Jeffrey: loc. cit., pp. 615–20.

In *Aquilegia* there is a single ring of bundles with fascicular cambium only, whereas in *Thalictrum* they are arranged in two or three concentric circles. *Anemone* shows various forms of anatomy in the different species. There is commonly a somewhat irregular ring of bundles, but medullary bundles and separate endodermal sheaths are among the anomalies found.

The descriptions of the adult anatomy of the forms described are taken, for the most part, from the published works of M. Marié and Mr. Worsdell.

Ranunculaceae.

Aquilegia. Marié states that there is a ring of bundles with a well-marked fascicular cambium in the adult stem of *Aquilegia.* Small intercalary bundles are also present. The seedlings are small and somewhat wiry in texture. The plumular internodes of the seedling are very short and hidden by the sheathing bases of the foliage leaves, which have long petioles and consist of three practically sessile leaflets. The phyllotaxis is two-fifths. The cotyledons are ovate and stalked and are joined below to form a short cotyledonary tube (see Text-fig. 1). Three species, *A. vulgaris, A. alpina,* and *A. canadense,* have been examined, of which the first is somewhat larger, but otherwise they show almost identical features both morphologically and anatomically. The vascular cylinder consists of a ring of about twelve strands. Secondary thickening begins at an early age (see Pl. XIII, Fig. 1). The sheathing bases of the foliage leaves overlap and so completely encircle everything within them. Each plumular leaf supplies three bundles to the central cylinder, and these enter the ring at an angle of about 120° from one another, so that the lateral strands of adjacent leaves cross. The plumular bundles are reduced to six above the cotyledonary node, and these strands arrange themselves in groups of three on either side of the cotyledonary plane (see Pl. XIII, Fig. 2). The double bundles from the cotyledons are inserted between these two groups, which then rapidly become reduced to single bundles. The phloem groups from the cotyledonary strands fuse with the plumular phloems and a diarch arrangement is attained. The pith and plumular tissue rapidly decrease as the lower levels of the hypocotyl are reached and typical root structure appears.



Text-fig. 1. *Aquilegia vulgaris.* × 1½.

Thalictrum. This genus has been very fully studied from an anatomical point of view by M. Mansion.[1] A very large number of bundles are arranged in the aerial parts of the adult stem in such a way as to resemble

[1] Loc. cit.

strongly that of a monocotyledon. The seedlings of the genus have been described by M. Mansion in the paper referred to above. The young plants differ from those of *Aquilegia* in having petioles to the plumular leaflets. The anatomical features of the four species examined, namely, *T. javanicum*, *T. flavum*, *T. glaucum*, and *T. adiantifolium*, are very similar in the seedling stage. The vascular cylinder consists of a ring of bundles, and at each node three separate leaf-trace strands are inserted. At the cotyledonary node are the usual six plumular bundles, besides the entering cotyledonary traces, which, though single higher up, show their double nature at this level. The anatomy thus closely resembles that of the genus *Aquilegia*, and the general type described for the order.

Anemone. The adult anatomy in this genus has been studied in detail by M. Janczewski [1] and less fully by M. Marié. The latter, in summing up for the genus, says that the cauline bundles are in one circle though of different sizes.[2]

These strands may be surrounded by individual endodermal sheaths. Medullary bundles are found in some species in addition to the normal ring.

I understand M. Janczewski to state that the habit of the stem has definite anatomical consequences influencing the presence or absence of endodermis, cambium, and mechanical tissue. Mr. Sinnott speaks of the genus as being characterized by a multilacunar leaf-trace insertion.[3]

In the young hypogeal stem of *A. pennsylvanica*, Professor Jeffrey has discovered a stelar tube with internal and external phloeoterma communicating through the leaf-gaps.

In all but two of the species examined the seedling anatomy corresponds very closely to that of *Aquilegia*, though the morphological features show a large range of variation. Most species of *Anemone* have a cotyledonary tube, and associated with this, in *A. apennina*, there is a tuberous hypocotyl. In another species, *A. nemorosa*, the tuberous habit is found in connexion with the hypogeal germination.

Anemone montana. This is a small seedling in general habit resembling *Aquilegia*, but with trifid foliage leaves. The internal structure is correspondingly similar to that of *Aquilegia*. Cambial growth is not present in the strands at the leaf base, but is clearly marked and quite diagrammatic in the axis at an early stage.

The lateral strands of the trifascicular trace are nearer the median one at the node than in the last two genera, so that those of subsequent leaves do not so frequently overlap.

[1] Janczewski: Étude morphologique sur le genre *Anemone*. Rev. Gén. de Bot., vol. x 1898.

[2] Loc. cit., p. 167.

[3] Sinnott, E. W.: The Anatomy of the Node as an Aid in the Classification of Angiosperms. Am. Journ. of Bot., vol. i, July, 1914, p. 312.

The lateral strands usually join the vascular axis at a slightly higher level than does the central strand. This is particularly noticeable in connexion with the first foliage leaf, whose median strand is inserted at the level of the cotyledons, whereas the laterals have entered the ring considerably above.

A. vernalis possesses a somewhat smaller seedling, and the foliage leaves are trilobed at the tip. It differs from the last species in several particulars. There is an earlier inception of cambial growth giving a compact ring of merismatic secondary tissue. The pith is smaller. The first internode is relatively long, and in it the plumular strands collect up to form a single pair of rather wide bundles in the intercotyledonary plane. Root structure is rapidly attained.

In *A. vitifolia* the early leaves are more cut up. The structure is intermediate between the two forms already described. In one of the seedlings examined the first foliage leaf contributes only one strand to the vascular cylinder (see Text-fig. 2, strand *a*).

TEXT-FIG. 2. *Anemone vitifolia.* First plumular node.

Anemone virginiana appeared to be similar, though the seedlings were very young. Secondary thickening is instituted comparatively late, as it had not yet appeared in any of the seedlings examined.

A. pulsatilla resembles *A. montana* in external appearance, whereas its anatomy is nearer to that of *A. vernalis.*

A. fulgens. The cotyledonary tube is about half an inch long at the time the plumule breaks through it at the base. The foliage leaves are larger and have longer petioles than in the previous species. Anatomically the species is peculiar in an extreme telescoping of the young axis. The median strands of the first two foliage leaves and the double bundles from the cotyledons all converge at the same level, giving a cruciform appearance at the cotyledonary node. The two laterals of the second foliage leaf and a single one from the first foliage leaf are inserted at two levels higher up in the axis.

A. rivularis is a larger seedling, but its anatomy corresponds closely with that of *A. vernalis.*

A. sylvestris is a smaller seedling, and the small oval laminae of the cotyledons are attached directly to the cotyledonary tube. The lateral

strands of the first few foliage leaves are absent, but otherwise the seedling agrees with the other species in its anatomical features.

The two other species examined form tubers at a very early age.

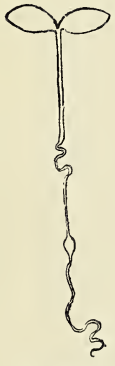
A. nemorosa possesses two sessile hypogeal cotyledons (see Text-fig. 3). The epicotyledonary axis does not elongate, and but a single foliage leaf is formed at the apex of the tuber during the first season. The plumular anatomy shows a ring of small collateral bundles widely separated owing to the tuberous character of the axis. The individual strands frequently have a single radial row of secondary wood elements, and in consequence the wings to the diarch plate of the hypocotyl are very long and narrow.

TEXT-FIG. 3. *Anemone nemorosa*. Seed-coat removed. × 1⅓.

In connexion with this species Marié [1] describes individual endodermal sheaths round the separate bundles, both in the rhizome and aerial stem.

A young rhizome showed a quite normal ring of bundles, but no trace of endodermis was observed.

A. apennina seedlings are noticeable in having a single two-lobed cotyledon. Root structure is arrived at in the cotyledonary petiole as described by Dr. Thomas.[2] The behaviour of the plumular strands in the tuber is anomalous and has not yet been fully worked out. In addition to the strands connecting directly with the central diarch plate there are also five peripheral collateral bundles which join the root stele only at the base of the tuber.



TEXT-FIG. 4. *Eranthis hiemalis*. ⅔ nat. size.

Eranthis. The adult anatomy is unusual as regards its aerial stem in that, as in *Anemone nemorosa*, the individual bundles are surrounded by endodermis.[3] The seedling figured (Text-fig. 4) was too young to make reliable observations on the plumular strands. A year-old seedling bearing a single (? first) foliage leaf showed endodermal sheaths to its leaf-trace strands, but no endodermis was distinguished in the tuber. The structure of the tuber will be further investigated. It shows a ring of irregular bundles connected by cambium and, within the ring, medullary vascular tissues. The xylem in all cases was of isodiametric tracheides.

Delphinium. According to Marié, both annual and perennial species of this genus have, in their adult stems, a vascular cylinder of separate

[1] Marié: loc. cit., p. 58.

[2] Thomas, E. N.: Seedling Anatomy of Ranales, &c. Ann. of Bot., October, 1914, p. 704.

[3] Marié: loc. cit., p. 92.

collateral strands of varying sizes. There would appear to be no interfascicular cambium.

The young stem, as found in the epicotyl of seedlings, has a much more regular ring of bundles connected, particularly in the perennial species, by a strongly developed ring of cambium. The number of strands supplied to the stele from each leaf is usually three. Those of the first two leaves may be two or one, and, according to M. Lenfant,[1] in the adult plant of annual species the number of strands is augmented by others appearing between the median strands and the laterals.

D. formosum. This is a perennial species. The seedling has a five-fid leaf and the cotyledons show a tendency to bifurcate. At a very early age the hypocotyl shows a tuberous swelling. A short cotyledonary tube is present. The base of each foliage leaf also completely enfolds all younger structures. Near the point of insertion of the petiole the leaf-trace strands are three in number and cambial division has taken place within them. The three strands enter the ring of bundles at different points and, at the same level, some of the strands within the vascular cylinder fuse so that, at the age examined, the number of strands in the internode remains about five. Exactly which strands unite varies in different individuals and at different nodes. The inception of a complete cambial ring is at about the level of the node of the youngest fully expanded foliage leaf. The cotyledons each contain a double bundle, but no laterals are present in the petiolar region (see Thomas, loc. cit.). The double bundles join the ring at the same level as the median strand from the first foliage leaf, the laterals of which were inserted at a higher level. In the upper part of this 'double node' are present three strands derived from the second and subsequent leaves. These arrange themselves two on one side and one on the other of the plane passing through the cotyledons. The lateral strands from the first leaf fuse with the single strand mentioned above before the central portion enters the ring, so that the appearance of three strands on either side of the cotyledonary plane is lost because, though present, they unite at different levels.

The strands on the other side fuse below the cotyledonary plane, and the two plumular bundles thus formed pass down the hypocotyl, decreasing in bulk as the lower levels are reached. The cotyledonary strands have become arranged in the form of a diarch root so that these plumular bundles form the customary wings to the diarch plate.

D. occidentale. The first two foliage leaves, though of different ages, are practically opposite and at right angles to the cotyledons. This feature is of fairly frequent occurrence among the seedlings of Ranunculaceae, but in this case it is correlated with a similar anatomical arrangement. The first two foliage leaves have a single strand, instead of the usual three, and

[1] Loc. cit., p. 44.

the two strands are inserted in the vascular ring at the same level. Cambial growth begins at a somewhat earlier age in this species (see Pl. XIII, Fig. 3).

D. luzulinum differs from *D. occidentale* only in that the first foliage leaf has one lateral strand.

D. Ajacis is an annual and differs in several important points from the species described above. Its foliage leaves are produced much more rapidly, so that a seedling of the same age would have many more foliage leaves than that of another species. The leaf bases are not sheathing.

The bundles in the vascular ring are more numerous than in the other species. This is probably correlated with the greater production of leaves. Secondary thickening arises much later and is probably less in extent. There is a relatively large pith. This feature seems to be correlated with the larger number of bundles in the vascular cylinder. In one seedling the first foliage leaf supplies only one lateral to the ring, the other fusing with the median bundle. The central and, if present, the lateral strands of the first foliage leaf join the vascular ring at the same level as the cotyledonary traces. At the cotyledonary node there are a large number of plumular bundles. Stem structure persists for some way down the hypocotyl, differing only from that of the epicotyl in the presence of the two 'mesarch' protoxylem groups derived from the cotyledons. The bundles gradually close up and the number is reduced till the usual six strands, three on either side of the cotyledonary plane, are reached. By reductions of the pith and closing up of the plumular tissue, root structure is established as in the other species, but at a much lower level in the hypocotyl.

Helleborus. H. foetidus is described by Marié as having ordinary dicotyledonous structure in its stem. That is to say, it has a ring of bundles connected later by cambial growth.

The genus is noted for having a sinuous endodermis dipping down between the bundles.

The seedlings and young plants examined showed a noticeable similarity in all the nodes except the cotyledonary node.

Each leaf supplies three strands to a somewhat slender vascular cylinder, but sometimes one or both laterals are missing in the early leaves. The cotyledonary node is quite normal and possesses a pith which persists a short way down the hypocotyl.

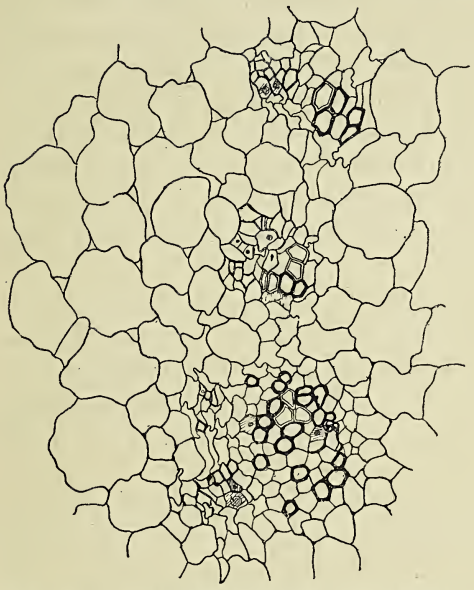
Nigella. The vascular system of the adult stem consists of a ring of strands varying in number and size. Cambial growth may be found at the base of the plant in some species. The seedling structure is similar to that of *Delphinium Ajacis*, but differs in a peculiar distinctness of the individual bundles.

N. hispanica. A meristematic ring does not appear till a very late date, and then it is only in the region of the cotyledonary node. The

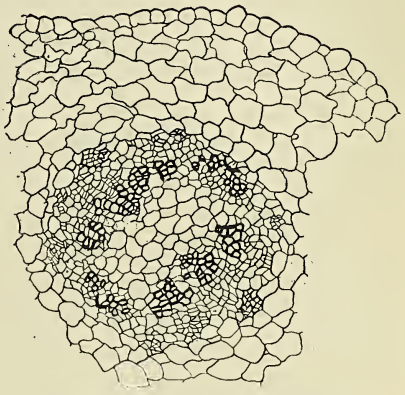
bundles in the internode are very numerous and well marked off from the ground tissue (see Text-fig. 5).

The nodes and internodes are all perfectly distinct, though the first two or three leaves may not have their full complement of strands at the point of insertion. The cotyledonary node is quite typical (see Text-fig. 6).

N. aristata shows similar features.



TEXT-FIG. 5. *Nigella hispanica.* Part of vascular ring in first internode.



TEXT-FIG. 6. *Nigella hispanica.* Cotyledonary node.

Adonis. Perennial species have an extensive cambial development in the adult plant, whereas *A. aestivalis* retains a ring of separate bundles (Marié).

The seedling of the latter species has two large lanceolate cotyledons and a well-marked cotyledonary tube. The first two leaves are much cut up and in an opposite pair at right angles to the cotyledons. Later leaves show the usual two-fifths phyllotaxis. The anatomy of the seedling strongly resembles that of *Delphinium Ajacis*, except that it is the first two foliage leaves which are inserted at a single node. These frequently have both laterals, whereas there are none in the cotyledons. Pericyclic arcs composed of transfusion-like elements are seen at an early age outside the larger bundles.

Myosurus. The whole plant of *M. minimus* is very minute. The part above ground consists of a very short stem, bearing a number of needle-like leaves at the ground level, continued into a long peduncle terminating in a single flower.

In well-developed specimens axillary buds are present in the axils of

the upper and larger leaves. In the peduncle there are five or more distinct collateral bundles surrounded by a lignified pericycle several cells thick.

At the region of the insertion of the leaves the bundles have been connected up by secondary thickening so as to form a continuous cylinder only broken by the entry of leaf traces, three from each leaf.

The seedling of this plant is exceedingly small, with two minute sessile linear cotyledons. The first few leaves are similar and scarcely larger. The anatomy of very young seedlings conforms to the general type for the order. It does so in spite of its minute size owing to the very small elements of which the tissues are composed. The foliage leaves have each three strands, and, owing to the extreme telescoping of the axis, the traces of several leaves are present in the axis at the same time, and the strands of adjacent leaves may fuse. Secondary thickening begins at a very early age, and, considering the ephemeral nature of the whole plant, is surprisingly well developed.

Paeonia. The anatomical peculiarities of this genus are largely in connexion with the leaf trace. According to Marié, the vascular cylinder is of the typical dicotyledonous type with separate bundles connected at an early age by secondary thickening. Mr. Worsdell describes concentric lateral leaf-trace bundles which pass some way down the stem before fusing with the central cylinder, thus forming cortical bundles.[1]

The seedlings are considerably more robust than those of other members of the family, and their germination is hypogeal from a large seed.

P. herbacea. The first foliage leaf is compound in this seedling. The cotyledonary node and hypocotyl swell in a tuberous manner at an early age, but this is chiefly secondary, being largely due to rapid growth of the normal cambium.

The vascular cylinder, though bulkier, is of the usual type found in seedlings of perennials.

The leaf-trace bundle is very large and fan-shaped and contains much secondary tissue. Large buds are present in the axils of the cotyledons, and the cotyledonary node is rather drawn out. The pith is persistent and flanked by plumular bundles, which are augmented by the half-bundles from the cotyledons.

P. arborea is similar in general structure, though it lacks the tuberous swelling at the age examined. The first foliage leaf supplies five strands to the stele in place of the usual three. A slight doubleness in the median strand of the first foliage leaf is to be observed.

P. anomala and *P. officinalis* are similar. In the former, secondary thickening appears to be much later, but this may be not so much a question of age as of rate of production of leaves, for age is here estimated by a number of leaves.

[1] Loc. cit., p. 664.

Aconitum. Marié has described the adult anatomy in a number of species. The vascular system of the stem consists of separate collateral strands arranged either on a single circle somewhat unevenly, as in *Delphinium*, or on two or three circles, thus resembling *Thalictrum*. The tuberous rhizome may exhibit a ring of bundles united by a cambium, or it may show anomalous structures such as the ring of concentric bundles found in *Aconitum Anthora*.

In external appearance the seedlings of this genus resemble those of *Ranunculus*. There is a tendency to the early formation of tubers.

TEXT-FIG. 7. *Aconitum Wilsonii.* First plumular node. M¹, L¹, trace of first leaf; M², median strand of second leaf.

Anatomically, the seedlings are noteworthy in possessing small leaf-trace strands, so that the primary xylem is slight in extent. To compensate for this a cambial ring is instituted very early, but the amount of xylem formed is not great and there are large principal medullary rays which define the bundles even after the formation of secondary thickening.

A. Wilsonii. The seedlings of this genus are glabrous and have a fairly large, long-stalked, reniform foliage leaf. At an early stage there is a tuberous swelling of the hypocotyl and lower part of the epicotyl.

A rather young seedling, with a single expanded foliage leaf, showed an almost complete cambial ring at the first plumular node (see Text-fig. 7).

There were only two lignified elements in connexion with the traces of the second foliage leaf, so that at a slightly older stage the woody tissue is almost entirely secondary. Except for these features the young seedling resembles *Aquilegia*, even to the angular divergence of the three bundles from each leaf.

A. pyrenaicum was examined at an age intermediate between the two stages of *A. Wilsonii*. It appeared to be essentially similar. The foliage leaves are somewhat smaller than in the last species, and covered with short hairs. Tuber formation had just begun and was accompanied by large stores of starch in the cells. The primary wood of the vascular bundles consisted at most of two to three elements, and the rest was all secondary; but, owing to the formation of definite medullary rays, the individual bundles are still clearly delimited.

At the cotyledonary node the plumular strands are collected up into two fan-shaped bundles, the triple nature of which can only be distinguished higher up.

A. Napellus is similar to *A. pyrenaicum*.

Clematis. M. Sterckx [1] has described this genus in great detail. He takes *C. Vitalba* as the type, and describes all stages in the anatomy of the axis, treating the other species comparatively.

In the adult plant the leaves are opposite and decussate. There are six large bundles in the stem, and a varying number of smaller ones alternating with them. The larger ones alone are directly continuous with the leaf traces. Each of the large bundles bifurcates just above the node, and the resulting halves fuse with the adjacent entering strands, of which there are six, three from each leaf. Thus the strands of one node are in position between those of the nodes above and below (cf. *Equisetum*). The smaller bundles are connected above and below with the half-bundles just mentioned.

While the leaves in the adult plant are always opposite, in the seedlings of three species, *C. Vitalba*, *C. Davidiana*, and *C. alpina*, the phyllotaxis is two-fifths.

Germination may be epigeal or hypogeal, and in some cases both conditions may be found in one species, e.g. *C. orientalis*.

The seedling anatomy of the spiral forms strongly resembles that in *Aquilegia*, but that of the opposite-leaved forms is specialized in connexion with this habit.

C. Vitalba. The cotyledons are slightly stalked and epigeal. The first few foliage leaves are ovate and long-stalked with irregularly serrate margins. The phyllotaxis is two-fifths and the internodes are very short (see Text-fig. 8).

According to Sterckx it is not till about the fifteenth foliage leaf that the arrangement becomes opposite and decussate.

[1] Loc. cit.

The vascular axis consists of a ring of strands smaller in number than in the adult plant. The foliage leaves each supply three strands, and their insertion is trilacunar except in the case of the first leaf, whose strands unite at the point of entry. Secondary thickening is present at and near the cotyledonary node in a seedling with three expanded foliage leaves. This is a slightly later appearance than in *Aquilegia*, but otherwise the structure is very similar.

C. Davidiana is very similar to *C. Vitalba* in all respects. This species was not described by M. Sterckx.

C. alpina was only examined in a young stage, but it seemed to correspond with the last two species.

The opposite-leaved habit in the seedling stage, found in the remaining species of this genus, is unique within the family Ranunculaceae. There is considerable variety in the behaviour of this type, even within the limits of one species.

C. recta. The seed is large and flat and germination hypogeal. M. Sterckx has described epigeal specimens.[1] The cotyledons have somewhat long petioles. The plumule emerges from within a short cotyledonary tube. The first internode is usually short, the later ones vary in different specimens; but most commonly the third and subsequent ones are elongated. The first few pairs of leaves are reduced to mere cup-like growths of concurrent scales. The later leaves are ovate with entire margins and are smaller than those of *C. Vitalba*. Axillary buds in the foliage and cotyledonary leaf axils are a feature of this type of seedling.

TEXT-FIG. 8. *Clematis Vitalba.* ½ nat. size.

The anatomical features are very variable, and in the early stages the strands are not always differentiated in all parts of their course. The internodes contain four separate collateral bundles. These are continuous with one another from node to node instead of alternating as in the adult. The leaf traces are three from each leaf, and the laterals of adjacent leaves join one another and are inserted in the middle of one opposite pair of the four strands which open out to receive them, whereas the median strands are inserted in the middle of the other pair. Sterckx figures a complete approximation of the alternate half-strands at the nodes which I have not succeeded in observing, but it seems probable that this may be effected at a later age. Just above the cotyledonary node two of the strands bifurcate, and thus the usual nodal arrangement of three groups on either side of the cotyledonary plane is obtained and root structure is arrived at in the usual manner.

C. orientalis. Germination may be epigeal or hypogeal. The former

[1] Loc. cit., p. 48.

seems correlated with an earlier development of foliage leaves. The first few pairs of leaves in the hypogeal form are frequently reduced to minute concrescent scales (see Text-fig. 9). The scale leaves frequently supply only one strand to the vascular cylinder, and the internodal strands may be reduced to three.

TEXT-FIG. 9. *Clematis orientalis.* Hypogeal form, × ⅔.

C. Flammula. The cotyledons are epigeal (see Text-fig. 10). There is a tendency to increase the number of vascular bundles to five or six in some of the internodes, otherwise it is similar to *C. recta.*

C. viticella is of the hypogeal type and is remarkable in having three strands to its cotyledons. The lateral strands are inserted in the gaps between the three plumular strands on each side, and for a short distance a tetrarch arrangement is present. The intercotyledonary poles soon die out and a diarch root is formed (see Thomas, loc. cit., p. 706).

C. Hendersonii and *C. integrifolia* resemble *C. viticella.*

As a result of his careful study of the genus *Clematis*, Sterckx was led to the conclusion that the opposite-leaved form has been derived from a form with leaves arranged on a spiral phyllotaxy of two-fifths. An investigation of the seedlings of the genus led the writer independently to the same conclusion. The considerations on which this hypothesis is based are as follows:

TEXT-FIG. 10. *Clematis Flammula.* × ½.

(*a*) The phyllotaxis of *C. Vitalba* in the seedling is two-fifths, and only after the formation of about fifteen foliage leaves is the opposite and decussate arrangement arrived at. This seems suggestive on the basis of the principle that ontogeny repeats phylogeny.

(*b*) The opposite leaves and long internodes, possibly correlated with the climbing habit, are not found elsewhere in Ranunculaceae, whereas the two-fifths phyllotaxy and relatively short internodes found in the seedlings of *C. Vitalba*, *C. Davidiana*, and *C. alpina* are characteristic of the seedlings of this order, and this leaf arrangement is usually maintained in the adult plants.

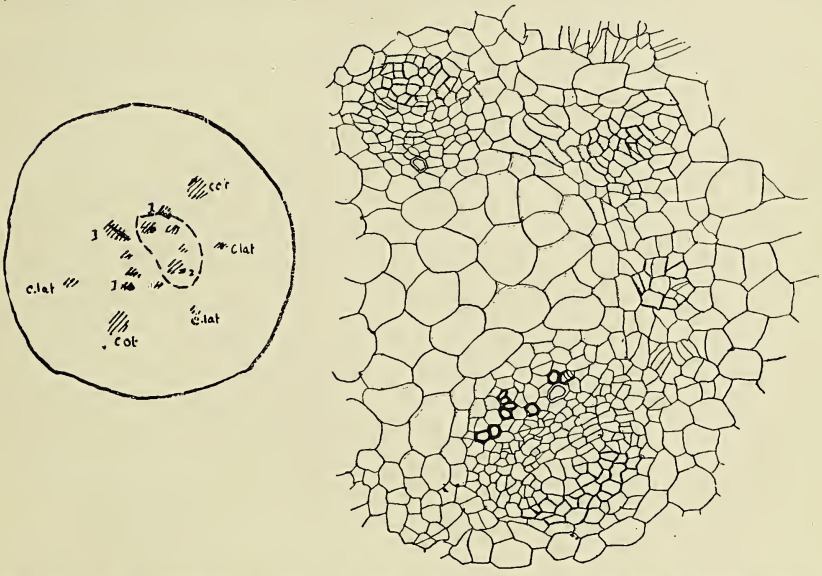
(*c*) In the flower, a notably conservative part of the plant, although the sepals are opposite, the stamens and carpels are spirally arranged.

The consideration of these points, in default of evidence to the contrary, would seem to justify the conclusion that the genus *Clematis* is derived from some form similar to that found in other members of the order, and

that the special characteristics are possibly correlated with its climbing habit.

Ranunculus. Marié considers this genus to be the type from which all the others have been derived, and Professor Jeffrey found in this genus a siphonostelic condition with internal and external endodermal sheaths.

The anatomy varies in the different species. The aerial axis contains a cylinder of separate strands, either with a single endodermis, which may be sinuous, or with a separate endodermal sheath to each bundle. Of the species here described, *R. acris*, *R. sceleratus*, *R. arvensis*, *R. parviflorus*, and probably *R. repens* and *R. bulbosus* show the former condition, while



TEXT-FIG. 11. *Ranunculus arvensis.* First plumular node, showing part of ring of bundles. Inset shows relation to other parts of section.

R. hederaceus, *R. aquatilis*, *R. auricomus*, and *R. Ficaria* present the latter, which is also found in the rhizome of *R. acris* and *R. repens*. An endodermis is found round the petiolar strands as in most plants of the genera of this family.

R. Ficaria stands out owing to its single cotyledon and tuberous habit. The seedling of *R. arvensis* is relatively large.

The seedlings fall naturally into four groups when considered anatomically.

1. *R. arvensis* has been fully investigated by M. Nihoul.[1] This species shows a good series of leaf form, from the well-developed compound leaf to the simple ovate cotyledon. It is chiefly remarkable in showing

[1] Nihoul: loc. cit.

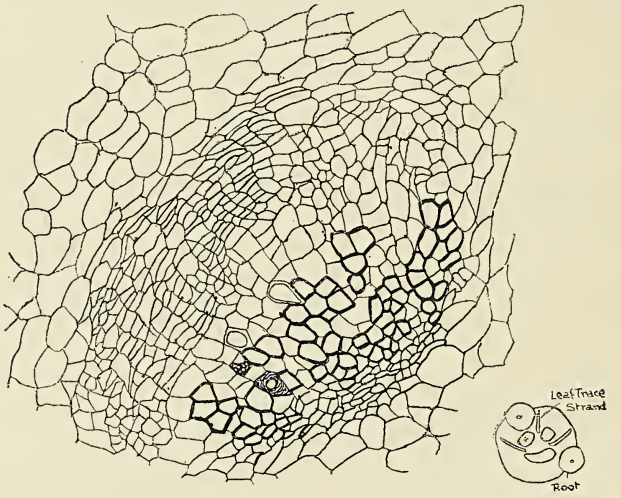
none of the peculiarities of anatomy exhibited by the other species, and conforms to the general type described for the family.

The vascular cylinder consists of a ring of collateral bundles (see Text-fig. 11).

Each foliage leaf supplies three leaf-trace bundles and the insertion is trilacunar. The plumular strands reduce to six above the cotyledonary node. The cotyledons also usually have three strands at their base, though one or both of the laterals may be missing (see Text-fig. 11, inset).

At the cotyledonary node the central strands behave as usual, and the laterals insert themselves in the gaps of the plumular tissue (cf. *Clematis viticella*). The lateral strands fail to form intercotyledonary poles, and a diarch root is obtained.

Secondary thickening begins at an early age, and differs from that



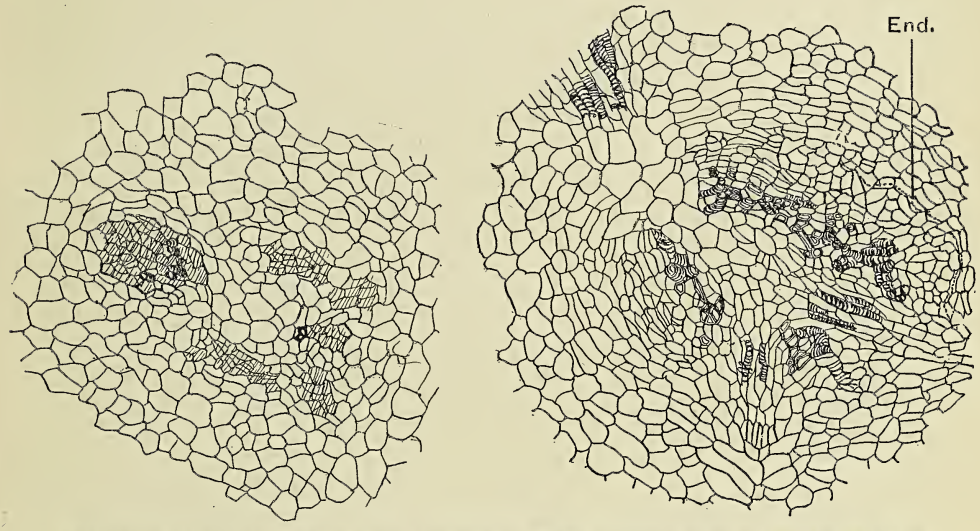
TEXT-FIG. 12. *Ranunculus arvensis.* First plumular node, primary structure disguised by secondary thickening. Inset shows relation of part shown (×) to other parts of the section.

described above for other genera of the order in the absence of principal medullary rays and consequent production of a continuous vascular cylinder. The condition thus produced is suggestive, in appearance, of a siphonostelic ring, but is clearly secondary in origin (see Text-fig. 12). An endodermis could not be detected in young seedlings, but in older plants indications were sometimes seen.

2. *R. acris* may be taken as a type of the second group of forms found in this genus. The seedlings of this species show considerable variation. One young seedling bearing only a single foliage leaf appears very similar to *R. arvensis* at the same age, but it is very much smaller in size and the entering leaf traces have a horizontal course. The usual six plumular strands at the cotyledonary node are not distinguishable. In another seedling of apparently the same age, the primary vascular bundles

are connected up by what appear to be secondary elements arranged in radial rows. An endodermis is distinguishable in connexion with the strands in the leaf and round the axial cylinder. In this particular seedling an internal layer is not distinguishable, but older seedlings show an internal layer also, in communication with the outer through the leaf gaps.

This endodermis is never diagrammatic and is often very difficult to demonstrate. It can sometimes be detected in an unstained section by its highly refractive walls. Gentian violet and Bismarck brown, the double stain used for most of the work described in this paper, will only show up the endodermis in very exceptional cases, and cannot be relied upon. Phloroglucinol as used by Professor Jeffrey was most satisfactory, but as



TEXT-FIGS. 13 and 14. *Ranunculus gramineus.* First plumular node at two stages under the same magnification. *End.*, Endodermis.

this cannot be used for permanent preparation, an attempt was made to find a suitable stain for the purpose. A saturated spirit solution of malachite green, counterstained with borax carmine or eosin in clove oil, was found the most satisfactory relatively permanent stain, although it fades rather quickly.

In quickly growing seedlings a rapid increase in girth causes a tangential stretching of the elements, particularly of the phloem (see Pl. XIII, Fig. 4). In extreme cases this may disguise the radial arrangement of the elements.

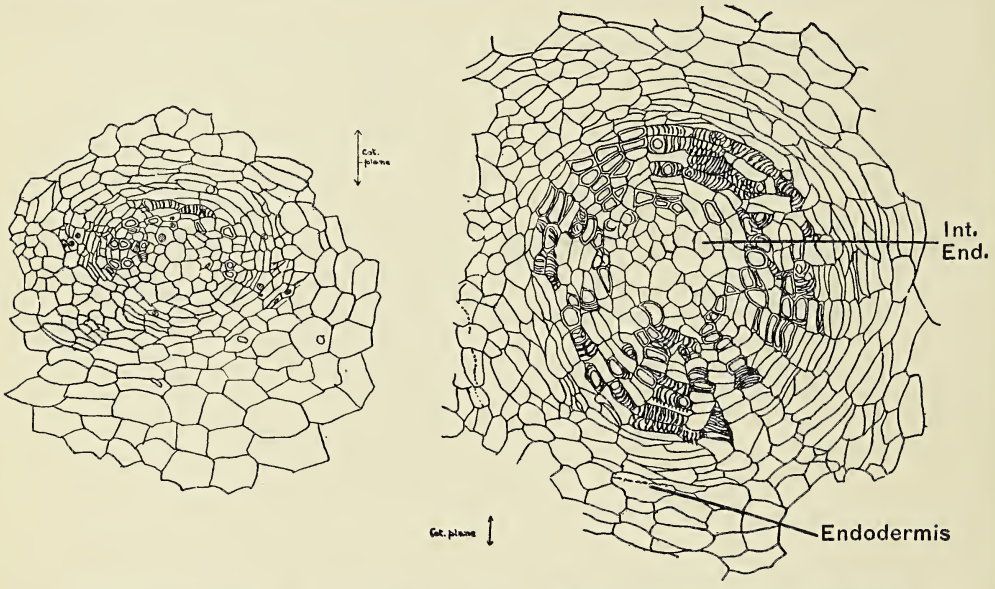
A slow growing autumn seedling cut in the spring showed an entire lack of this feature.

Lateral roots frequently occur at the cotyledonary node.

R. parviflorus shows cambial growth almost before the primary wood

elements are differentiated. There is marked tangential extension of the phloem elements at a very early age.

R. gramineus. The cambium does not appear quite so early in this species, and thus some of the primary elements may be distinguished (see Text-fig. 14). Both internal and external endodermis can be demonstrated in the older seedlings (see Text-fig. 16). The tangential extension does not take place to any great extent as there is little increase in girth of the stem (cf. Text-figs. 13 and 15 with 14 and 16 on the same scale). The axis in this and other species is very telescoped, and in consequence the wood elements are short and the strands sinuous in their course. There are no distinct principal rays between the bundles, so that their identity is almost lost.



TEXT-FIGS. 15 and 16. *Ranunculus gramineus.* First plumular internode at two stages. The same seedlings and magnification as in Text-figs. 13 and 14.

R. bulbosus shows separate strands at an early stage, and in general resembles *R. gramineus.*

R. auricomus is similar to *R. gramineus.* The individuality of the primary strands is more obvious in this species.

R. repens shows extreme tangential stretching of the vascular elements, even in the very young stages, so that the long dimensions of the tracheides appear in transverse section. This seems to be due to the fact that the vascular cylinder is wider than it is long.

3. The aquatic species possess elongated internodes (except the first) in which there are distinct and separate vascular bundles.

R. sceleratus. There are six definite strands in the internode. There is a tendency for these to be lost at the node, but at the cotyledonary node

the six phloem groups are quite distinct. The elements are much less compactly arranged than in the other species.

R. hederaceus. The internodal strands may be two, three, or six in number. At the node there is a ring-like joining-up showing secondary thickening, but the individual strands can still be distinguished although a distinct internal and external endodermis is present.

R. aquatilis and *R. Flammula* are similar. The internal endodermis is the more marked, though both are present at the node.

4. *R. Ficaria.* The apparently single-lobed cotyledon seems to be directly continued into the main root. The tuber is really the first lateral root, and arises later. The first foliage leaf appears to arise at its apex and forces its way through the base of the cotyledonary petiole. A second lateral root is formed at almost the same level, but does not store food (see Text-fig. 17). The anatomy is somewhat special and needs further investigation. The composition of the plumular strand is difficult to elucidate. It is brought into contact with the cotyledonary system at the node, but immediately below the lateral appears again as an independent unit continuous with the vascular axis of the tuberous root, whereas the cotyledonary strand forms the diarch root stele.

TEXT-FIG. 17. *Ranunculus Ficaria.* Nat. size.

Professor Jeffrey[1] examined a number of species belonging to the type which I have called group 2, of which *R. acris* has also been fully described by him. His material was apparently somewhat older than the majority of the seedlings examined in this investigation.

He interpreted the structure found as a siphonostele with leaf gaps through which the internal and external phloeotermal layers are connected, and from this tubular stele he considers that the ring of bundles has been derived by overlapping and lengthening of the leaf gaps and loss of endodermis.

Professor Jeffrey, in company with other anatomists, seems to have used terms, such as siphonostele, describing vascular structure as applying to primary tissues only. Whatever may be the significance of the endodermis, it has been shown that the tubular appearance of the vascular system in the genus *Ranunculus* is due to secondary tissue, and hence the term siphonostele, as now used, cannot be correctly applied to the structure.

The differences between the vascular structure of *Ranunculus* seedlings and more typical forms, irrespective of the question of endodermis, are as follows:

1. The secondary wood elements are irregularly intermingled with

[1] Loc. cit., p. 617.

parenchyma cells, and there are no true medullary rays. This feature is found in the majority of the species, at the nodes in the aquatic species and in the secondary wood of *R. arvensis*, which is otherwise quite comparable in its seedling features with such a form as *Aquilegia*, as it shows a quite definite ring of bundles in the early stages of the seedling.

2. The leaf-trace strands have little primary xylem in their axial portions and its place is taken by very early formation of secondary tissue. This feature is found also in *Aconitum*, but there is no siphonostelic appearance in that genus owing to the formation of wide principal medullary rays.

3. The secondary xylem consists largely of isodiametric tracheides and the elements are frequently tangentially elongated. These features seem to be due to the telescoping of the axis combined with its rapid radial expansion in many of the species.

TEXT-FIG. 18. *Trollius asiaticus.* Part of first plumular node. M^2 is median trace of second leaf.

Trollius. There is a ring of bundles connected by a poorly developed cambium in the rhizome. The aerial stem possesses a ring of bundles surrounded by a sinuous endodermis and with no interfascicular cambium.

The seedlings are not unlike those of *Ranunculus* in external appearance. *T. pumile* showed a tendency to fusion of the cotyledons by one margin. Anatomically they also show resemblances to that genus.

T. asiaticus. The plumular anatomy resembles that of *R. gramineus*, but a greater development of primary tissue distinguishes it, which is observable even after the formation of a quantity of secondary tissue of the '*Ranunculus*' type. See Text-fig. 18, which is part of the ring at the first node and shows distinctly the median trace of the second plumular leaf. No endodermis has been demonstrated, but the evidence is insufficient to state that it is absent. At the cotyledonary node are one or more lateral roots whose vascular system seems largely supplied by plumular tissue.

T. pumile. Only one seedling has been examined. The fusion of the

cotyledonary petioles on one side and the fusion of their strands at the base results in an arrangement at the cotyledonary node precisely similar to that found in *Ranunculus Ficaria*. The plumular structure resembles that found in *T. asiaticus*.

Caltha. The stem contains a ring of bundles, each surrounded by an endodermal sheath.

The leaves of the seedling are completely sheathing and have but a single strand at the base of the petiole. The epicotyledonary structure is difficult to elucidate owing to the production of a large number of roots from the plumule. It seems to be similar to that of *Ranunculus gramineus*, except that a protostelic appearance is produced by the almost complete absence of pith.

Berberidaceae.

Podophyllum. In the subterranean axis of this genus the bundles possess each a separate endodermal sheath and a fascicular cambium is present. They are in a single ring, while those in the aerial parts are scattered, being arranged in three or four irregular circles. The phyllotaxis is one-half.[1]

P. Emodi has a long cotyledonary tube bearing two laminae. The first leaf breaks through at the base of the tube and itself completely encloses the structures within. A lateral root frequently appears at the cotyledonary node.[2]

The anatomy of the seedling in this species differs little from that found in *Aquilegia* except in the phyllotaxy. The lateral root mentioned above appears in the intercotyledonary plane.

Berberis. This is a shrubby form and shows a large amount of lignified tissue in the form of secondary wood. Broad principal medullary rays are found which split up the wood into segments.[3]

In habit the seedlings of the genus resemble those of Ranunculaceae in general features, but a cotyledonary tube is almost absent and the cotyledons sessile. A similar condition was found in some of the species of *Anemone*, for example *A. montana*.

The anatomy of the epicotyl is similar to the generalized type described for Ranunculaceae. It resembles that of *Anemone* in particular in the angular divergence of the strands of the trifascicular trace at their point of insertion. The features of the cotyledonary node and hypocotyl are interesting in view of the appearance of tetrarchy in the roots of some species as described by Dr. Thomas.[4] *B. aquiflorum* is mentioned by Mr. Sinnott[5] as having as many as eleven leaf-trace bundles in the adult

[1] Holm, Th.: *Podophyllum peltatum*; a Morphological Study. Bot. Gaz., vol. xxvii, 1899.
[2] Dickson: Germination of *Podophyllum Emodi*. Trans. Bot. Soc. Edinburgh, vol. xvi, 1885–6.
[3] Solereder's Systematic Anatomy, p. 45.
[4] Loc. cit., p. 708.
[5] Loc. cit., p. 312.

plant. The seedling shows only the usual three. The epicotyledonary anatomy is almost identical with that of *Anemone*. Just above the cotyledonary node the plumular bundles are as usual six in number. At the node the plumular tissue divides in the intercotyledonary plane, but leaves in the middle the protoxylem elements of the median strands. The double bundles of the cotyledons orientate themselves in the usual manner to give a diarch arrangement, and the plumular protoxylem groups thus lie on the same radius as the two phloem strands. Both pith and plumular strands die out at a somewhat lower level, and thus root structure is arrived at.

B. heteropoda is similar to *B. aquiflorum*.

B. lyceum and *B. aristata* show similar epicotyledonary features. In the hypocotyl the four cotyledonary phloem groups persist in the diagonal planes, and the protoxylem groups from the median strands of the first two plumular leaves penetrate farther into the hypocotyl than in the other forms.

Lardizabalaceae.

Only *Decaisnea Fargesii* has been obtained of this order. This and the forms to be described in the following orders are trees, and the seedlings show certain differences from the herbaceous forms of Ranunculaceae. The seedlings are larger and the internodes are elongated. The leaf-trace insertion is as usual trilacunar, but there is a larger number of strands in the vascular ring.

In *Decaisnea* the seedling is large with long-stalked, irregularly oval cotyledons. The first foliage leaf is compound, with three leaflets, and the second has five. The bases of the foliage leaves are not sheathing, as they are in Ranunculaceae.

The plumular internodes are long, while the phyllotaxis is the usual two-fifths. Axillary buds are present both in connexion with the cotyledons and foliage leaves.

The vascular cylinder of the axis consists of a ring of about twenty bundles which are connected at an early age by cambium. The leaf trace has the usual trilacunar insertion, but the median strand is made up of three at its point of entry into the ring. While still in the cortex the leaf-trace strands divide up, the lateral ones each into two and the central one forming a ring of about seven bundles. These latter separate into two adaxial and five abaxial strands, and rearrange themselves so that in the base of the petiole there is a complete cylinder of bundles. At the cotyledonary node the plumular strands are reduced to two flattened arcs of five bundles on either side of the cotyledonary plane. The two separate bundles of the cotyledons, derived by fusion of four at a slightly higher level, insert themselves at the ends of these arcs. The ten plumular bundles reduce to six, and the arcs close up to form a ring which persists far down the hypocotyl and is similar to that found in the epicotyledonary region. Diarch root

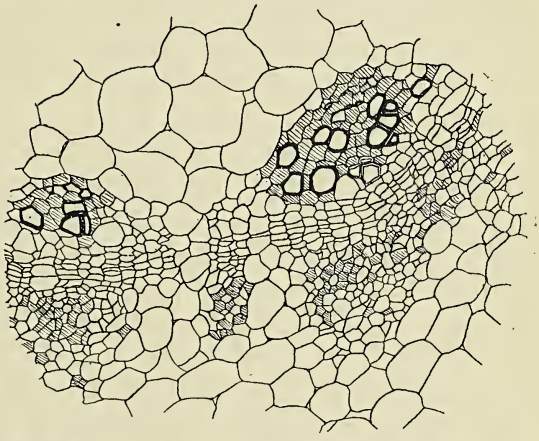
structure is slowly formed by approximation of phloem and xylem groups alternately as described by Dr. Thomas.[1]

Magnoliaceae.

The vascular cylinder in the adult plant consists of a ring of collateral bundles connected by cambium and a large amount of secondary thickening. In *Magnolia* and *Liriodendron* there is a complete cylinder of bundles in the base of the petiole,[2] and the leaf-trace insertion is multilacunar.[3]

There is a ring of bundles in the seedling petiole, but they are reduced to three in the base of the petiole, whereas in the adult plant, according to Worsdell,[4] rearrangements take place largely in the cortex of the stem. This condition may be compared with what was found in the seedling of *Decaisnea*.

Magnolia. Two species of this genus have been examined. The seedlings are large, with practically sessile cotyledons, elongated internodes, and a phyllotaxis of two-fifths. The foliage leaves are large and ovate.



Text-fig. 19. *Magnolia acuminata*. Part of vascular ring, first plumular internode.

M. acuminata. The petiole contains a ring of six bundles which reduce to three in a similar manner to that found in *Decaisnea*, but above the point of insertion of the leaf base. The second foliage leaf has completely enfolding stipules, whose two strands fuse on the side of the axis opposite the leaf insertion and join the ring there, making a minute fourth member to the trace. The stipules of the first leaf are not sheathing and contain no vascular strand. The ring of bundles in the axis is well marked and similar to that of *Decaisnea*. Secondary thickening begins at an early age (see Text-fig. 19).

The cotyledons each contain a double bundle and two laterals. The adjacent laterals from opposite cotyledons fuse and the four bundles so formed enter four gaps in the plumular vascular cylinder. Thus a cylinder,

[1] Loc. cit., p. 709.

[2] Matsuda, S.: On the Anatomy of Magnoliaceae. Jour. Coll. Sci. Imp. Univ. Japan, vol. vi, 1893–4.

[3] Sinnott: loc. cit., p. 312.

[4] Loc. cit., p. 666.

similar to that in the plumule, is formed in the hypocotyl as in *Decaisnea*, but in *Magnolia* passes into a tetrarch root (see Thomas, loc. cit., p. 710).

M. Soulangeana is similar to *M. acuminata.*

Liriodendron Tulipifera. The seedling is smaller and the foliage leaves, which are nearly orbicular, show the apical depression characteristic of the species. The petioles have a ring of four strands, of which the adaxial one bifurcates and the halves rotate to join the central strand within the petiole. The leaf-trace strands are thus reduced to three. The stipules are not completely sheathing and show no vascular strand. Otherwise the anatomy is similar to that found in *Magnolia*, except that the intercotyledonary poles die out in the hypocotyl as described by Dr. Thomas.[1]

Calycanthaceae.

Calycanthus praecox. The cylinder of this plant seems to be quite normal. In the cortex are the well-known four inverted bundles formed by the lateral strands from the leaves. Only the central strand of the five leaf-trace strands, described by Mr. Worsdell, is inserted on the stem cylinder.

As described by Mr. Worsdell,[2] the arrangement of the cortical bundles is similar in the seedling ; they finally insert themselves on the cotyledonary strands at the node. In the seedlings examined the number of leaf traces was three instead of the five described for the adult plant. The features of the main cylinder, cotyledonary node, and hypocotyl are similar to those of *Decaisnea* except for the formation of a tetrarch root.

Although the leaves even in the seedling are opposite, the foliar organs on the peduncle, as pointed out by Worsdell,[3] are spirally arranged. This, he points out, is suggestive in the light of the discovery by Baillon [4] of an exceptional plant of *Chimonanthus* in which the leaves are arranged on a two-fifths phyllotaxy.

Anonaceae.

Anona. The adult vascular cylinder appears to be quite normal. Sinnott states [5] that the leaf-trace insertion is unilacunar though it consists of three strands. The phyllotaxy is one-half. Seedlings of two species have been examined. Germination is hypogeal, and the plumular internodes are long.

A. reticulata. The three strands from the plumular leaves have trilacunar insertion. The lateral strands are inserted near to the median strand, but not in the same gap. The structure of the epicotyl differs from

[1] Loc. cit., p. 710. [2] Loc. cit., p. 669. [3] Loc. cit., p. 669.
[4] Baillon : Sur un *Chimonanthus* à feuilles alternes. Adansonia, vol. ix, 1868–70.
[5] Loc. cit., p. 312.

that of *Aquilegia* in the phyllotaxis and elongated nodes, but the features of the cotyledonary node and hypocotyl are similar to those found in that genus.

Lauraceae.

This order also seems to be noted as having unilacunar leaf-trace insertion in the adult plant.

The seedlings of *Laurus Sassafras* and *L. nobilis* have been examined, but in a rather old condition.

Germination is hypogeal. The first few foliage leaves are mere scales, and the phyllotaxis is two-fifths. The leaf-trace insertion in the seedling is also unilacunar, and the triple nature of the trace is only to be observed above the point of insertion. Secondary thickening in the vascular cylinder had progressed so far that it was impossible to identify the primary bundles with any certainty.

Although unfortunately so little material of Ranalean plants, other than those belonging to the order Ranunculaceae, has been obtained, it was thought worth while to include descriptions of such forms as have been examined. One reason for their inclusion was that they add a little to Mr. Sinnott's information with regard to the leaf-trace insertion, showing as they do that, even in forms with a multilacunar insertion in the adult, as in *Magnolia*, or unilacunar, as in *Anona*, the seedling may show the trilacunar form. A further reason was that although the habit in these tree forms is so different from that of the herbaceous Ranunculaceae, still the seedlings show an essential similarity.

Summary and Conclusions.

The investigation of the structure of the epicotyl in Ranalean seedlings has shown a somewhat remarkable uniformity. There are certain features characteristic of all the seedlings examined, but those of the Ranunculaceae and Berberidaceae show a much closer resemblance to one another.

The general ground plan for the group shows the following features:

(1) a two-fifths phyllotaxy;

(2) a trilacunar leaf-trace insertion;

(3) a ring of bundles connected at an early age by a cambium which forms secondary tissue.

Although no single feature is uniformly present in its typical form, it is uncommon to find deviations, and where they occur they are usually confined to one of the characters. In addition to the general features just mentioned there are certain others characteristic of the herbaceous and shrubby forms of Ranunculaceae and Berberidaceae, as distinct from the plants with tree habit. The axis is very shortened and clothed with the sheathing bases of the petioles; the cotyledons are frequently fused into a tube at their base; the leaf trace in the base of the leaf consists of three

strands, whereas in the tree forms, although reduced to three at the point of insertion, there is often a larger number arranged in a ring at the base of the petiole. The plumular strands become reduced to six at the cotyledonary node. Each cotyledon supplies one leaf trace, which may be double, and a diarch root structure is formed quite high in the hypocotyl, whereas in the arborescent forms the hypocotyledonary vascular arrangement is frequently like that of the stem.

Apart from minor deviations from type, there are three main groups of exceptions to the general structural ground plan for the seedlings of the cohort.

1. *Phyllotaxy.* In *Clematis* the leaves are opposite and decussate in the adult, but it has been shown that the two-fifths spiral in the seedlings of *C. Vitalba* and other species changes ontogenetically to the opposite arrangement. This, taken in conjunction with the strong likeness between seedlings of this species and those of other plants of the family, is strong evidence in favour of the primitive nature of the spiral arrangement.

Calycanthus also has opposite leaves, but Mr. Worsdell has pointed out that this is probably derived from a two-fifths phyllotaxy, and the likeness between the two cases is strong support for either contention. It would seem probable that further data might provide evidence for the derivation of the one-half divergence of *Anona* and *Podophyllum* from the two-fifths condition.

2. *Leaf Trace.* The most notable exception to the general arrangement is found in *Laurus*, in which, though the material was inadequate, the insertion appeared to be unilacunar in the seedling, as in the adult. *Paeonia* is a somewhat curious case in which the first foliage leaf contributes, in some species, five strands to the vascular cylinder, while the later ones have the usual trilacunar insertion. In some cases the first one or two leaves of species belonging to Ranunculaceae contribute a reduced number of strands (one or two) either by the approximation of the strands at the point of entry, or by loss of one or both laterals. This seems clearly correlated with marked reduction in size in the plumular leaves concerned, and may throw some light on the very frequent absence of laterals at the base of the cotyledonary leaves.

3. *The Stem Cylinder.* The main departure from the type is found in the genus *Ranunculus* (also *Trollius*, *Caltha*, and, according to Professor Jeffrey, *Anemone pennsylvanica*), in which there is an internal endodermis and an appearance of a tubular stele. The latter has been shown to be a secondary formation correlated with the slight production of primary xylem found also in *Aconitum*, but the siphonostelic appearance in *Ranunculus* is due to the absence of the principal medullary rays found in that genus.

The presence of the internal endodermis in some species of *Ranunculus* is more difficult to explain. At the age examined, the endodermis is not

usually differentiated in seedlings of the other genera. Its appearance in those of *Ranunculus*, *Trollius*, &c. may be part of the precocity which is observed in the secondary thickening. This seems to be borne out by the fact that endodermis is present round the individual strands in the adult plant, not only in *Ranunculus* where an internal sheath is found in the seedling, but also in other plants, for instance *Podophyllum*, in which the seedling is quite normal. The origin and function of the endodermis has been reconsidered of late years by various writers, for instance by Professor Lang in his recent paper on the Ophioglossaceae,[1] and it seems possible that its phylogenetic importance in the present instance has been over-estimated, especially in view of the specialized geophytic and aquatic habit of the species in which it is found, whereas it is absent in the more normal *R. arvensis*. The order Nymphaeaceae, the other instance of the appearance of an internal endodermis, cited by Professor Jeffrey for the Ranales, is also of aquatic habit, and the presence of an extra endodermis in the stem of water-plants is not an unusual phenomenon (e.g. it is found round the cortical leaf-trace bundles in *Menyanthes*).

To sum up the results of this investigation, there are two centres of special phylogenetic interest:

1. *The Leaf-trace Insertion.* There is a general prevalence of the *trilacunar* trace in the seedlings of Ranales. This strongly supports Mr. Sinnott's conclusions, based on a study of the adult plants of the cohort.

2. *The Type of Vascular Cylinder.* A *single* ring of bundles connected *at a very early age* by cambium is found almost universally throughout the seedlings of the cohort. This single ring of bundles does not appear to be a mere embryonic character, as suggested by Mr. Worsdell,[2] for such forms as do not exhibit specialized morphology due to a geophytic habit show a similar structure throughout life (e.g. *Helleborus foetidus* and *Magnolia*). Interfascicular cambium is usually absent in the adult stems of the herbaceous Ranunculaceae, but it is invariably present at some stage in the seedling.

The siphonostelic appearance described by Professor Jeffrey in *Ranunculus* and *Anemone* is shown to occur also in *Caltha* and *Trollius*. Evidence is brought forward in favour of its wholly secondary origin.

In conclusion I should like to acknowledge with gratitude my deep indebtedness to Dr. Thomas for her constant advice and helpful criticism throughout the progress of this investigation.

Bedford College.

[1] Lang, W. H.: On the Anatomy and Branching of the Rhizome of *Helminthostachys zeylanica*. Ann. of Bot., vol. xxix, 1915, p. 35.

[2] Loc. cit., p. 654.

EXPLANATION OF PLATE XIII.

Illustrating Miss Blackburn's paper on the Vascular Anatomy of the Young Epicotyl in some Ranalean Forms.

Taken from photographs.

$M^1 L^1$, $M^2 L^2$ refer to the median and lateral traces of successive plumular leaves. *cot.* is the cotyledonary trace.

Fig. 1. *Aquilegia alpina.* Transverse section at the first plumular node, showing the beginning of cambial growth. The cotyledonary tube has been removed. × 100.

Fig. 2. *Aquilegia canadensis.* Showing the arrangement of vascular strands at the cotyledonary node. × 80.

Fig. 3. *Delphinium occidentale.* Transverse section of the stem at the first plumular node. × 32.

Fig. 4. *Ranunculus acris.* Transverse section at the second plumular node, showing cambial growth. A few primary xylem elements are differentiated and there is some tangential stretching. × 40.

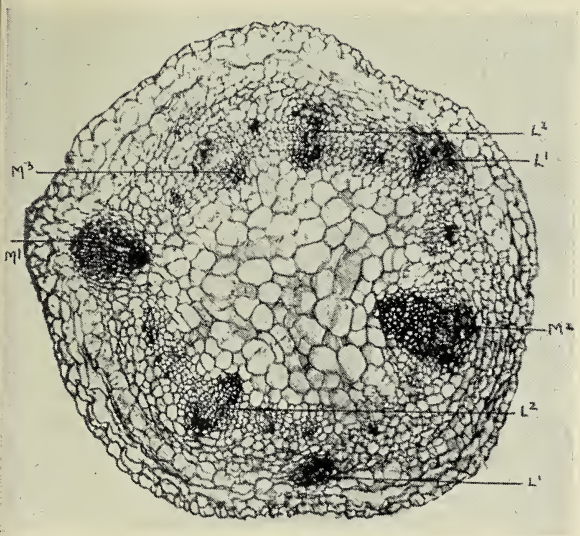


FIG. 1. *Aquilegia alpina.*

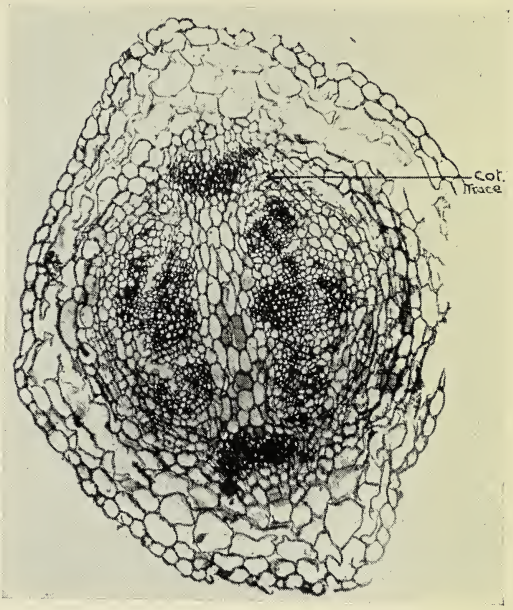


FIG. 2. *Aquilegia canadensis.*

FIG. 3. *Delphinium occidentale.*

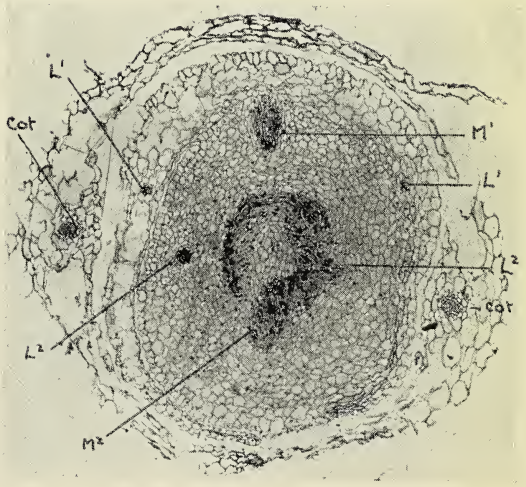


FIG. 4. *Ranunculus acris.*

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BY

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The Effect of one Plant on Another.

BY

SPENCER PICKERING.

With three Figures in the Text.

FIG. 3 shows a pot with two mustard plants growing in earth, on the surface of which rested a perforated tray with five inches of earth in it; through this all the water given to the plants percolated. The tray has been removed so as to be more clearly visible in the photograph. The presence of such a tray makes practically no difference in the behaviour of the plants in the pot below. Figs. 1 and 2 show a like arrangement,[1] but with a crop of mustard growing in the trays: in one case (Fig. 1) the effect has been to reduce the plants in the pot to one-hundredth of their normal size; in the other (Fig. 2) there has been no effect. The only difference in the two cases is that in Fig. 1 the washings from the surface growth were allowed to reach the plants in the pot (though the penetration of any roots through the perforations of the trays was prevented by a layer of very fine metal gauze at the bottoms of the trays), whilst, in the other (Fig. 2), no washings passed, the holes in the trays having been blocked, and such water as the plants required was given to them direct. The conclusion is obvious: the leachings from the plants growing in the trays must contain something which is toxic to other plant-growth.

Such a very simple experiment must definitely settle the question of toxin production, and should have been made long ago,[2] but as a matter of fact it comes only as the (at present) final step in a series which originated in 1895 in observations on the effect of grass on fruit-trees. As so often happens, we start with the more complex problems, and only gradually work down to the simpler ones.

It has now been established with a reasonable amount of certainty that the deleterious effect of one growing plant on another is a general phenomenon. By means chiefly of pot experiments such as those indicated above, the following plants have been found susceptible to such influence: apples,

[1] In Fig. 2 there appears to be only one plant: this is due to a fault in the photograph.

[2] The details of this, and many of the experiments alluded to below, have not yet been published: a description of the other work on the subject will be found in the Third, Thirteenth and Fourteenth Reports of the Woburn Experimental Fruit Farm, 1903, 1911 and 1914.

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pears, plums, cherries, six kinds of forest trees, mustard, tobacco, tomatoes, barley, clover and two varieties of grasses; whilst the plants exercising this baleful influence have been apple seedlings, mustard, tobacco, tomatoes, two varieties of clover and sixteen varieties of grasses. In no case have negative results been obtained. The extent of the effect varies very greatly: in pot

FIG. 1. FIG. 2. FIG. 3.

experiments the maximum reduction in growth of the plants affected has been 97 per cent., the minimum 6 per cent., whilst in field experiments with trees, the effect may vary from a small quantity up to that sufficient to cause the death of the tree. The average effect in pot experiments may be roughly placed at a reduction of one-half to two-thirds of the normal growth of the plant, but no sufficient evidence has yet been obtained to justify the con-

clusion that any particular kinds of plants are more susceptible than others, or that any particular surface crop is more toxic than another; that such differences exist is highly probable, but all the variations observed so far may be explained by the greater or lesser vigour of the plants in the particular experiments in question. Similarly as regards the effect of grass on fruit-trees, though the extent of it varies very greatly, and in many soils is certainly small, we must hesitate to attribute this to any specific properties of the soils in question; for when soils from different localities (including those from places where the grass effect is small) have been examined in pot experiments, they have all given very similar results; and this applies equally to cases where pure sand, with the addition of artificial nutrients, has been taken as the medium of growth.

In searching for an explanation of the effect of grass on trees, various possibilities suggested themselves, and these were excluded, one by one, till the only possibility left was that of the formation of some deleterious substance by the growing grass. It would be impossible in this short communication to give any account of all the suggestions which were negatived, but these included the robbing of the tree of necessary moisture and food by the grass, alterations in the temperature, alkalinity or physical condition of the soil, and alterations in its carbon dioxide and bacterial contents, the exclusion of all which suggestions is embraced in experiments such as those mentioned above, where the grass, or other crop, is grown in a separate vessel, merely resting on the surface of the ground, without any possibility of it extracting anything from the soil in which the plant affected is growing.

From the outset the behaviour of trees in grassed land suggested the action of some toxin: not only is the growth arrested, but a peculiar alteration in the colouring of the bark, leaves and fruit occurs, unlike that attending other forms of ill-treatment: indeed, the high colour developed by fruit under grass is, in some cases, so great, that expert fruit-growers have been unable to correctly name the varieties after being affected, and if this action of grass could be limited, and suitably adjusted to every tree, it would prove beneficial from the point of view of the fruit-grower, if not from that of the tree itself, especially as a limited check to the growth of a tree generally results in heavy cropping. The extent of the grass action which brings about these notable colour changes is very small, for they are apparent in cases of trees weighing about two hundredweight when only three to six ounces of their roots extend into grassed ground. Such an effect is in itself strongly suggestive of toxic action.

To some agricultural chemists the mention of a toxin as being formed in the soil by a growing plant is as a red rag to a bull, chiefly, perhaps, because it conjures up the picture of the plant ejecting some virulent poison; but, though the excretion of toxin from the roots is possible, there is no

need for imagining such an occurrence: all plants in growing leave much root-detritus in the soil, and such disjecta may account for toxic properties, just as well as ejecta. That some forms of organic matter may be highly poisonous to plant-life has been established: soil which has been heated to 125° is very toxic, and there is evidence that toxicity may be produced in it by heating to much lower temperatures ('Journal of Agricultural Science,' iii. 277). In the case of heated soils, the chemical changes are complicated by changes in bacterial character; this may or may not be the case with soil which is growing crops; but there is one feature in common between the two, namely that in both cases the toxin is easily oxidized, and that after oxidation, it acts as a plant nutrient, increasing the fertility of the soil. When in pot experiments such as those mentioned, the leachings from the crop in the trays are kept exposed to the air for about twenty-four hours before being given to the plant, their toxic property is found to have entirely disappeared, and in some cases, indeed, they act beneficially: even a two-inch layer of pumice-stone interposed between the tray and the earth in the pot will admit of sufficient oxidation for a reduction in the toxic effect to be discernible.

A reversal of the effect of grass may also be recognized in field experiments, for in a case where apple-trees were planted and kept clear from grass to a distance of 3 feet from the stems, the trees flourished better at first than those without any grass near them (and, of course, much better than those which had been entirely grassed). But as they grew, and their roots approached the grassed ground, the toxin affected them before it had time to become oxidized, and they began to suffer. Though there are other reasons why land under grass gradually becomes more fertile, the accumulation of the oxidized products of the toxin must constitute an important factor in this enrichment. In certain experiments with apple-trees it was found that soil which had been under grass for ten years induced double as much growth as similar soil which had been under tillage, though, when the turf was replaced on the soil, the trees showed all the bad effects of grass.

From the general character of the action of one crop on another, it follows that the tables may be turned on the grass, and, even in pot experiments, it has been proved that grass in the pots will be adversely affected by apple seedlings in the trays. In practice, of course, it is known that grass and other surface crops are adversely affected by trees. This is generally attributed to the shading effect, and to the robbing of the soil of its nourishment. Doubtless the shading produces bad results in many cases, but it may be questioned whether any serious robbing of the soil occurs, for there is good evidence for believing that ground under trees, even when worked regularly for timber, increases in fertility, just as does ground under grass. At any rate, it has been found that a surface crop

may suffer from trees above it, even in cases where there certainly has been an increase in fertility, and where, also, the shading effect is inoperative, so that the damage to the crop can only be attributed to the toxic action of the trees. Thus, a quarter of an acre of land over which some fifteen apple-trees, twenty years of age, were distributed, was planted uniformly with Brussels sprouts: those under the trees suffered to the extent of 48 per cent. in their growth; but there were patches in the ground where trees had been growing until the preceding winter, when they had been cut down, leaving the roots undisturbed in the soil, and in these patches the sprouts did better than elsewhere to the extent of 12 per cent. In other parts of the ground canvas screens had been erected, at a height of 6 feet above the surface, to simulate, and even exaggerate, the shading of the trees, and under these the sprouts gave exactly the same values as on the unshaded ground. Thus, the trees themselves materially injured the crop, though the soil under the trees was more fertile than elsewhere, and though the shading was inoperative.

Though differences in the toxicity and in the susceptibility of different plants may be overshadowed by differences due to other causes, it is highly probable, as has already been mentioned, that such differences do exist. The only case of differences of a positive character noticed at present in our experiments, is that the effect of a plant on plants of its own kind is generally greater than that on plants of another kind. This may be fallacious; but, certainly, a plant affects its own kind just as much as any other kind; and hence we must conclude that the toxin formed by any individual plant will affect that individual itself. This has been proved by growing plants in pots divided into compartments, so that there was no root interference, and comparing these with other plants grown in similar pots not so divided: in the former case each plant will be affected only by the toxin produced by itself, in the latter it is affected partially by its own toxin, and partially by that of its neighbour, but the amount per plant must be the same in both cases, and, as a matter of fact, the plants all gave the same results, except for a slight advantage in favour of those in undivided pots, due to conditions which can be easily specified.

When a stronger and weaker plant, or an older and younger one, are growing side by side, we find that the latter rarely picks up, and generally gets more and more behind its stronger brother. This cannot be due to the stronger one monopolizing the food-supply; for if it exhausted this supply, both plants would suffer at the same time, and, till that supply is exhausted, both would flourish equally. The inadequacy of any such explanation is demonstrated by taking a pot of soil capable of growing, say, six plants, sowing the seed for three of them first, and that for the other three a certain number of days later. In the case of mustard, when the difference of date is only four days, it is found that, at the end of

growth, some two or three months later, the last sown plants are 60 to 70 per cent. smaller than the others. It is evident that three four-day-old seedlings could not have exhausted the nourishment in $7\frac{1}{2}$ kilos of rich soil so far as to leave insufficient food for three other seedlings; nor can a difference in age of four days in a total life of several months account for such a difference in the weights of the plants. But the results become clear if we take into account the toxic effect of one plant on the other, for the later planted individuals have to start growth under toxic conditions which were absent in the case of those first planted, and throughout their existence their inferiority in size will make them suffer more than their stronger brethren, though the actual amount of toxin in the soil is the same for all. Yet it is found that they are not altogether without their revenge, for the toxin formed by them affects to a certain extent the older plants, and this effect may be traced, even when the feebler plants are only about one-tenth the size of stronger ones. The importance in practice of having seed which will germinate uniformly at the same time, or in having plants of uniform growth in a bed, is demonstrated by these experiments; for a difference of only four days in the germination of one-half of the seeds in the case of mustard reduces the total weight of the whole crop eventually obtained by as much as 20 per cent.

The divided pots have been utilized to ascertain what the effect of crowding in a plantation is when there is no root-interference. In appearance the results were remarkable, for with mustard and tobacco plants at distances of 4, 6 and 9 inches apart, the plants were considerably smaller as the distances between them were greater, the deficiency in their height extending up to 30 and even 50 per cent., and they appeared to be in every way inferior. But these appearances were entirely misleading, for it was found that the weights of the plants were the same at whatever distance they were planted, and at whatever age they were lifted; moreover, this equality held good throughout the plantation, even including the outermost rows: thus interference of the above-ground portions of plants does not affect the amount of growth, but only the quality of that growth.

The results are very different, however, when there is root-interference, as when a number of plants are grown together in one pot, or in one plot in the field; and when the crowding attains to a certain magnitude, the limiting factor is the amount of soil available for each plant: the result of which is that the weights of the plants are inversely proportional to the bulk of soil available (which with soil of uniform depth is synonymous with the area), or, in other words, the total plant-growth is the same, whatever be the number of plants. Thus, in pots containing $7\frac{1}{2}$ kilos of rich soil the total crop was the same with from sixty-four down to sixteen mustard plants (the latter number representing plants at a distance of

1·8 inch apart), or with from sixteen down almost to one plant of tobacco; whilst in unmanured ground in the field, the equality holds good within a wider range, even to plantations with more than 6 inches between the plants in the case of mustard. At the same time, also, the outside rows of plants are very much superior to the inner plants.

This latter superiority is one which is continually noticed in field experiments, though the reverse, judging by mere appearances, sometimes obtains. Whether, when the outside plants appear the worse, they are so in reality, may be doubted; at any rate, in all the cases which have been investigated by weighing, it is a superiority which has existed. But appearances would never have led to a correct estimate of the magnitude of this superiority, for these outside plants are often 100 to 200 per cent. greater in weight than the inside ones. The row next to the outside one generally shows some superiority, but the effect of an external position extends to only about 6 inches from the edge of the plot.

The extra vigour of the outside plants is generally attributed to the extra manure which they have to draw upon; but another factor must now be reckoned with, in the extra facility offered for the oxidation of the toxin at the edge of the plot; and this appears to be an important factor. The question is being investigated on three lines: manure beyond a certain limit does not benefit, and even injures a plant; if, therefore, in a plot where the manure has already attained such a limit, we still find that the outside plants show a superiority, this cannot be due to any further surplus of manure. Such a superiority, we find, still exists in ground manured with 100 tons of dung to the acre, and the superiority is little less, if less at all, than in unmanured ground. But these experiments require repetition and extension before definite conclusions can be drawn from them.

The Relative Age of Endemic Species and other Controversial Points.

BY

J. C. WILLIS, M.A., Sc.D.

IN a recent number of this journal there is a paper by Mr. H. N. Ridley (1) in which he expresses great dissatisfaction with the hypothesis of 'age and area' which I have elsewhere (5, 6, 7) brought forward as being, in my opinion, a general rule which has governed the distribution of the Angiosperms (and some animals at any rate) about the globe. Mr. Ridley also sets forth the case from the Natural Selection point of view; for there is no doubt that the adoption of my hypothesis involves a final abandonment of Natural Selection as seriously operative in this direction. Whatever it may, and doubtless does, effect in individual cases, its results are not sufficiently marked to show themselves in my figures, which deal with large numbers and the long run, and are similar to one another for all groups of plants. Mr. Ridley's paper was evidently written before seeing my subsequent paper on the flora of New Zealand (7), or much that it contains might have been omitted, or left for further consideration.

I shall take Mr. Ridley's points, as given in his summary, in order, paying special attention to the supposition often brought forward, that endemics are chiefly the relics of an old flora; and shall also give further evidence for my hypothesis, which if once fully accepted, will make a great difference in the handling of questions of geographical distribution and evolution at least, if not in other lines of work.

In the earlier portion of his paper Mr. Ridley seems to me to imply that my numerical results are accidental. But, as I have already pointed out, the probabilities against such a thing are inconceivably great. In the first place we have to note the very remarkable fact that the same accident would appear to have happened to all, and we have now the further case of New Zealand, where instead of the estimates of the Ceylon flora I have used actual longitudinal range in the islands, and where every family and genus (for even the genera with one or two species follow the grouping as accurately as those with more, which are quoted in the tables) behaves in the same way. Ceylon is quite put into the shade by the way in which the flora of New Zealand follows my hypothesis.

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Mr. Ridley then goes on to suggest that the figures depend upon the number of specimens in the herbarium, which, if it were literally true in detail, and my Ceylon work were not supported by that upon New Zealand, would be a very damaging criticism. I was careful before publishing, however, to verify that Trimen's figures (4) did, as a matter of fact, in most cases, indicate roughly the area occupied. He does not count duplicates from the same place, and goes, to the extent of perhaps 80 per cent., upon the results already obtained by Thwaites (3), who did the lion's share of the investigation of the Ceylon flora, and that chiefly in the days before there was such extensive clearance for planting. Mr. Ridley complains that investigation stopped with Trimen, but if he will refer to my annual report for 1910 he will find an enumeration of the herbarium, showing that while Thwaites added 6,492 sheets, Trimen added 2,890, and I myself 1,603. But with all the additions since Thwaites's time, the range in Ceylon of the various species has been comparatively little extended. The serious additions to localities made in my time are summed up in a paper by Mr. A. M. Smith and myself (19), but these were completely ignored in my papers under discussion, which are based solely on Trimen's flora. Taking 36 consecutive species at random from Thwaites, and giving them marks according to area as in Trimen, I found that they received 115 marks in the former, 100 in the latter case, and this I think fairly enough represents the general difference that Trimen's work made in regard to the geographical distribution of the species of Ceylon; 13 of the 36 are moved one place up by Trimen, and 12 remain the same; 6 go down a place, and the remaining 5 make larger moves.

That Trimen's figures do as a matter of fact very fairly indicate area is shown by the maps given on p. 12 (6); about 10–15 per cent. of the VR, 5–10 of the R, and smaller proportions of the higher classes are in my opinion wrongly placed, and should be moved up, usually one class. But this movement would make no difference in the figures, as may be easily shown. Let us move up 15 per cent. of the VR species, then 10 per cent. of the increased R's (to allow for the few VR's that should go more than a class upwards), then 5 per cent. of the RR's, and 2 per cent. of the RC's. The result is to replace the table on p. 3 (6) by the following:

TABLE I.

	Endemic.	*Ceylon-Penins. India.*	*Wide.*
VC.	19	45	221
C.	93	120	468
RC.	144	105	318
RR.	162	86	216
R.	191	68	163
VR.	198	68	122
Rarity	4·2	3·4	3·0

a change of 0·1 in the rarity of the endemics and the Ceylon-Indians, which makes no difference to their validity for my argument.

Mr. Ridley further goes on to state that future work on the Ceylon flora will so modify the results obtained from Trimen that any deductions based upon the latter will be valueless. I have already shown how little difference Trimen's work made in Thwaites's results. My own made still less in Trimen's, and the differences will decrease as time goes on. As I have already stated, the effect of the completion work, when once a flora, like that of Ceylon or New Zealand, is reasonably well worked out, is to add new species to the class VR, to move a few VR into R, rather fewer R into RR, and so on in decreasing proportions up the scale. But this leaves the ultimate result the same as before.

In any case the Ceylon figures, estimates though they be, are shown to be fairly accurate and reliable by the fact that they come out with such arithmetically regular gradations along the scale in *opposite* directions for endemics and wides; and this is amply confirmed by the fact that the New Zealand figures, which give actual measurements, agree with them.

I much regret that in my Ceylon papers I did not make sufficiently clear the various conditions that might modify the action of my age and area rule. Partly this was because I thought that many were obvious, and partly because I was thinking more of making the law itself clear. In later papers I have instanced various such conditions, and Mr. Ridley's paper is also of great service in this respect. In the New Zealand paper (7), p. 449, I have said that 'the vast flora of introduced weeds may be left out of account, for there is not the least evidence to show that they would have spread had not *foreign conditions*, or disturbance of the native conditions, been *also* introduced', whilst in a paper which I read to the Linnean Society last year I went into this question in detail, analysing the flora of introductions into Ceylon which I have elsewhere published (8). This list contains 387 plants, and I showed that there was no evidence of spread *without alteration of conditions* except in four cases. Here the spread is but small, and in at least two has been indirectly assisted by man.

It is evident that if man arrive in a country and make extensive clearances, allowing the spread of introduced weeds like the lalang (cf. 1, p. 572), the operation of my law of age and area must be interfered with. For this reason, remembering how much of 'dry' northern and eastern Ceylon was once occupied by agriculture, I do not think that the detailed list of its flora as now made up is very complete, though, owing to the configuration of the island, and the direction of the monsoons, I cannot for one moment admit that this country was ever covered with 'wet-zone' forest. Even there, however, there was a good deal of forest left, while the bulk of the Ceylon flora, whether 'wide' or endemic, lies in the south-west, which at the time of Thwaites was comparatively little encroached upon.

Mr. Ridley does not fully realize that my figures, though it is definitely so stated in several places, refer to area occupied, not to commonness on the ground. On p. 552 he quotes the two Hedychiums as instances of VC species which have now died out at Peradeniya, showing a lack of apprehension of the real drift of my argument. To begin with, he quotes them both as VC, but if he will take the trouble to look again at Trimen's Flora, he will see that this is not quite correct. In the second place, it is not difficult to find them near Peradeniya, though not perhaps on the Kandy road, which is now practically a suburban street. The area occupied by these species remains as before, though evidently the operations of man have reduced their commonness within that area. But even if they were non-existent now in the places where man has introduced a change of conditions, that would not in any way affect my argument, which refers to action under constant conditions. The incoming of man may easily produce as great changes as a change of climate or other geological change, and I much regret that in my earlier statements of the law I did not lay more stress upon the many conditions that may modify its action, as for example by the use of such a covering phrase as 'so long as conditions remain unaltered', but somehow this seemed to me so obvious that it was unnecessary. If a powerful magnet be placed near to the line of fall, it is obvious that an iron ball will not fall in exact accord with the law of gravity, but that fact does not alter the validity of the law.

We may therefore dismiss from further consideration here all such cases as are brought up by Mr. Ridley showing the effects of man's action in the spread of species, or in their destruction, and go on to a further objection raised against the species which Trimen labels VC. Mr. Ridley would apparently regard most of these as also introduced by man. This is, I think, somewhat sweeping. In my Catalogue I have marked by an asterisk those which Trimen or I think to have been possibly or probably so introduced, and they number 52 out of 285, showing rarities VC 12, C 25, RC 5, RR 2, R 4, VR 4, giving a mean rarity of 2·4. Subtraction of all of them makes no difference *at all* in my figures. But in order to satisfy Mr. Ridley, let us omit all the 285 VC's, thus reducing the flora to five classes. Instead of 809 endemics with 3,518 marks, we get 790 with 2,709; instead of 492 Ceylon-Indians with 1,714 marks, we get 447 with 1,222, and instead of 1,508 wides with 4,579 marks, we get 1,287 with 3,071. These, calculated out, give rarities of 3·4, 2·7, and 2·3, in figures running from 1 to 5 only. These are just as useful for my deductions as those actually employed, and the omission of the whole 285 VC species makes no important difference.

The same remarks as have been made upon the effects of the action of man apply to the effects of a change of climate, which in my New Zealand paper, p. 456, I mentioned as one of the factors causing modification. It is obvious that if a serious change of climate occurs—usually in consequence

of some geological change—it will make a difference in the spread of a given set of species about a given area. Mr. Ridley demands geological catastrophes, but apparently (p. 557) even the glacial period is not sufficient.

No one who is unaccustomed to the handling of statistics of large numbers can perhaps easily realize how unassailable my figures actually are. If we move up *one half* of every class, we still get rarity figures of 3·8, 3·0, and 2·6. One must not arbitrarily move up endemics without moving up wides, and the figures cannot be equalized unless all are placed in the VC class, which is an obvious impossibility under Natural Selection, and on my hypothesis could not in any case occur unless the appearance of new species (wide or endemic) were stopped, and perhaps 60,000 further years allowed, as indicated in a valuable paper by Mr. Ridley (2).

The figures given will suffice to show the weakness of Mr. Ridley's attack upon my position. To quote exceptions, as he does, makes no difference. He need not have searched the world for them, but might have found them by the dozen in Trimen's Flora. Probably in about a third of the cases the actual figure there given does not represent the real truth, but that does not invalidate the argument, which simply goes to show that the overwhelming factor in distribution is age, though many other factors are continually at work. Pulling this way and that, however, and not acting upon whole groups of allied forms in the same way, their effects do not show in the figures.

Mr. Ridley quotes about seventy cases in various connexions. Many of these, e. g. those on p. 555, are excellent illustrations of what I have said (6) on p. 22, that a very small accident may kill out a species in the class VR. In others he sets out to show that VC species may easily disappear, but only shows that they have disappeared *in particular localities*. But for the sake of argument let us accept all these cases as showing each that one of my wides is *five* classes too high. Then instead of 4,579 marks for the 1,508, we get 4,929 (i. e. by adding 350), and a rarity of 3·2 instead of 3·0. Or, to make the case as strong as possible, let us omit all the VC species, which Mr. Ridley thinks are introduced, and divide the seventy into two, one half moving the wides down as much as possible, the other the endemics up. Even then, a little calculation will show that the rarities come out (in figures 1 to 5) 3·2, 2·7, and 2·4. My arguments could be carried on from these figures just as effectively as from those that were actually used.

Unless Mr. Ridley can produce large numbers of cases which all move the wides down and the endemics up, it is idle waste of time to bring up exceptions. But the case no longer rests upon estimates; these have been replaced by actual measurements in the case of New Zealand, and Mr. Ridley would have to show that 902 endemics, but no wides, were each under-

estimated in range by 360 miles.[1] Even for Ceylon, to equalize the figures (6, p. 4) 687 endemics must go up a class, which means discovery in new localities, and 699 wides must go down a class, which means proof of wrong identification for localities already recorded.

The New Zealand figures confirm those of Ceylon to the necessary degree of safety. The Ceylon figures served their purpose in drawing my attention to the law of age and area which I based upon them. Had it not been that the species were thus conveniently classified into groups, I doubt if I should have thought it worth while to undertake the enormous labour of determining the area occupied by each, though I was determined to find out all that could be found out about the endemics.

To apply my law to individual cases is to invite mistake, but no amount of evidence of individual exceptions will shake it. Exceptions must be brought up *in groups of twenty allied species, behaving alike.* Assuming that lalang were native (see 1, p. 572) and spread without alteration of the previous conditions, no argument can be based upon it unless it were accompanied by nineteen other exceptional Gramineae. Even the other species of *Imperata* is VR, which at once halves the commonness of the lalang.

Exceptions occur by the dozen, but there is no evidence *in the figures* to show that *similar* exceptions form any large percentage. Probably there are a fair number of relic endemics, of cases where adaptation really occurs, of cases where Natural Selection has been beneficially in operation, and the like, but they do not show in the figures, which simply go to show the overwhelming effect of mere age.

Relative Age of Endemics and Wides.

Mr. Ridley further states that the endemics are nearly all the relics of an old flora rapidly disappearing, and thus brings up the line of opposition which so far I have most frequently encountered. With the view of replying to it in advance, I read at the Newcastle meeting of the British Association a paper with the title 'Are endemics the oldest or the youngest species in a country?' and I shall now proceed to quote a considerable portion of this paper, as Mr. Ridley's objection would obviously make them out to be the older species.

'In several papers recently published, I have brought forward a law which I propose, indicating that the geographical distribution of species (taken in groups of twenty or so allied forms) depends chiefly upon their age within the country, Natural Selection, whatever results it may produce in

[1] Rarity of wides 3·5, of endemics 6·5, in figures from 1 to 10. Each unit represents 120 miles.

individual cases, being apparently of no effect when dealing with many allied species. This law, once stated, has evidently to be hedged round with various provisos, indicating the various causes which may modify its action. One, for example, which seemed to me so obvious that I did not mention it specifically, was that the law would only be strictly operative so long as the conditions remained constant—a change of climate or submergence of part of the country would clearly modify its operation.

'So long as the law was only based upon the estimates given for distribution in Ceylon, so long was its foundation somewhat precarious, and as soon as possible I worked out another flora, that of New Zealand, but with actual distances of spread instead of estimates. This gave confirmatory evidence of the most satisfactory kind, the graduation of the endemic species from few of large spread down to many of small, and of the wides in the opposite direction, being very clearly shown, while at the same time the prediction which I made, that if New Zealand were divided into equal zones the endemic species would appear in them in numbers graduated up to a maximum (or sometimes two), was borne out by the facts in the most convincing manner, leaving no room for doubt that Natural Selection could not be the operative factor in causing their distribution.

'That the longer a species has been in a country, the more area it should occupy, does not seem to be an unreasonable nor far-fetched explanation, but it leaves out of account the structural differences between species, and ignores Natural Selection, and thus runs much against the grain to many botanists, who still base their arguments (though often more or less unconsciously) upon it. In particular they have long been accustomed to look upon the endemic species of small areas as being the oldest in a country, instead of the youngest, and as being in some way expressly suited to the local conditions, though when pressed they are not able to suggest any very clear reason for their belief. There is nothing in the structure of most endemic plants to show that they are in any way adapted to local conditions, nor that they are any older than the species of wider distribution that accompany them, though not seldom something to show the contrary.

'The essential facts which have to be explained in the floras of Ceylon and New Zealand (and observation on other floras shows that they are very general[1]) are that the endemic and the widely distributed species in a given country are arranged in graduated series, showing an increase in number in opposite directions, the endemics increasing from those of wide to those of narrow distribution, the wides in the other direction. I have already shown that Natural Selection cannot account for such regular arrangement of distribution, which shows not only on the total, but also family by family.

[1] The case of England, or of other countries which have been completely altered by man, must be dealt with separately. The advent of man may soon become as important as a geological catastrophe or a serious change of climate.

Some mechanical cause must be responsible, and for that cause I have suggested age.

'Several botanists, while admitting that my figures are not to be gainsaid, are of opinion that my results can be equally well explained by reversing my hypothesis, and considering that youth, rather than age, is responsible for the occupation of large area. I shall try to show that this conception leads logically to an untenable position, and shall also give some crucial test cases, which speak in favour of age.

'Age is an obvious reason for occupying a large area, but youth is not, and we shall require stronger evidence to prove the latter. Obviously it must not be pushed to extremes, and a supplementary hypothesis will be needed to account for the fact that the very latest arrivals are not the commonest species.

'My hypothesis is based upon age *within the country*. What the species may have done in the way of spreading in other countries, or where it was evolved, and when, is immaterial; when it arrives in the country with which we are dealing, it commences to spread, if suitable to the climate and soil, and spreads over an area determined, so long as conditions remain constant, and except in so far as barriers of mountains, broad rivers, sudden changes of climate from one region to another close by, and the like, interfere, by the length of time during which it has been in the country.[1]

'If, however, one try to reverse the hypothesis, one has at once to make choice of two cases. Either the supposition must be that area occupied depends upon youth *within the country*, or upon *absolute* youth. The exact reversal of my hypothesis of course gives the first case.

'On either view a great difficulty arises from the fact that the wides and endemics both show a graduated order of rarity, the former from many of large area to few of small, and the latter in the reverse direction. This fact, which in the Ceylon flora depended upon estimates, shows with actual measurements for New Zealand. There is thus nothing for it but to admit that my hypothesis must be reversed in detail, and that the younger a species is, whether absolutely or within the country, the greater area will it occupy. The same thing follows necessarily from the fact that in Ceylon the species common also to Peninsular India are intermediate in rarity between the endemics and the wides. Whatever hypothesis be adopted, one must admit that these species are intermediate in youth as in rarity.

'A great difficulty for the hypothesis of absolute youth is the fact that the range in Ceylon in no way corresponds with the range outside of it, as one might expect upon this supposition. *Sanicula europea*, for example, and many other species have a vast range outside of Ceylon, and on this hypothesis are therefore presumably very young, yet within Ceylon only

[1] It being of course understood that the law does not necessarily apply to individual cases, any more than Mendel's Law, but to groups of allied forms.

occupy each one restricted locality. *Polyalthia Korinti*, *Garcinia spicata*, *Impatiens oppositifolia*, and forty other species common only to Ceylon and Peninsular India (often only a small southern part of it), and whose absolute range is thus small, presumably indicating age, are yet very common (and therefore presumably very young?) in Ceylon. *Eugenia rotundifolia* (endemic) is very common in Ceylon, and thirteen other endemic Eugenias are very rare. Are the latter very old, the former very young?'

'The hypothesis of youth and area, when the youth is to be absolute youth, cannot be established without calling in Natural Selection of a somewhat remarkable kind. But as I have already pointed out (6, pp. 6–16), Natural Selection must explain the very difficult problem, why every family and genus, in New Zealand as well as Ceylon, shows similar arithmetical arrangement of its species according to the area they occupy.

'We are thus driven to accept the youth hypothesis in the form that it is youth within the country, or exactly to reverse my hypothesis of age and area. But if we do so accept it, we are at once brought up short by the question *why*? What conceivable reason can be given to explain why the two things should be connected? The case of the rapid spread of introduced weeds in islands like Ceylon or New Zealand is often quoted as evidence that foreign species recently introduced spread more rapidly than the local, but ignores three important facts at least: (1) that foreign conditions have also been introduced, e. g. by cutting down of forest, or in other ways; (2) that such weeds are also common in continental areas, as for example at Rio de Janeiro, where the local flora of 7,000–8,000 species is one of the very richest in the whole world, and includes a vast number of species covering enormous areas of distribution, so that its members should be well able to hold their own, and (3) that where they have spread, it has been just as much at the expense of the wides already in the country as of the endemics.

'To return to the main argument, why should a species which is very old (and therefore on this hypothesis very rare) in, let us say, South India, at once spread over a large area if it arrive in Ceylon? Or, to take a concrete case, suppose that *Coleus elongatus*, which at present is confined to the summit of Ritigala (9) in Ceylon, and shows no signs of spreading thence, occurred not on Ritigala, but on one of the hills of South India. Would it at once spread if brought into Ceylon, and if not, why not? Has it at one time existed all over Ceylon, and is the soil of that country now permanently *Coleus elongatus*-sick? Or take it the other way: if carried from Ceylon to South India, would it at once spread? If not, why not?'

Mr. Ridley prefers to consider the endemics as the oldest species in a country. But he must in any case admit that the species confined to Ceylon and Peninsular India, which are intermediate, in area occupied, between the endemics and the wides, are intermediate also in age. We are

thus confronted with a very remarkable case when we deal with such a gigantic and universal genus as *Senecio*, which has hundreds of endemic species in all corners of the earth—Ceylon, New Zealand, Peru, Europe, North America, &c., &c., as well as hundreds of species occupying intermediate areas, and a few occupying large ones. Where did the last-named come from, and at what particular size does a species cease to be one of the doomed, and become an extending and conquering species? Take the case of *Cordia* in Ceylon. *C. Myxa*, which occurs all over the eastern tropics, is C (common), but *C. monoica*, which only occurs in Peninsular India, is also C, while *C. Rothii*, which occurs in Peninsular India, Arabia, and Abyssinia, is only R, and *C. subcordata*, found on most eastern tropical coasts, is VR. *C. monoica* is only endemic to a small area and ought to be one of the doomed; why is it common? What, on Mr. Ridley's views, settles the fate of a species in a country?

The hypothesis of youth (within the country) and area can only be accepted if one be prepared to accept with it the numerous absurdities to which it leads. It is very far-fetched, with no facts to rest upon, and involves a most remarkable amount of rising and falling in the scale of commonness (area of distribution) for which we have no warrant. In fact, it seems to me to require direction of evolution from outside, and in a very remarkable manner. A forthcoming paper, dealing with the distribution of the plants of the outlying islands of New Zealand, seems to me to finally decide the question against it.

Mr. Ridley goes on to state that the endemics must be old, because there is nothing in the land from which they could have been evolved. This is a most remarkable statement, when one remembers that most of them have 'wides' in the same genus. He accuses me of omitting from a list of genera, definitely described as containing five or more endemic species, the monotypic endemic genera, which he will find given in detail in Table XVII of my earlier paper (5). He states that most of these are rare, but if he will look at the figures he will find that they are RR, RC, C, RR, C, C, R, RC, VR, R, R, VR, RC, RR, RC, RC, RC, giving a rarity of 3·7, or considerably less than the endemics as a whole, and much below the rarity of the endemic genera with more than one species, which is 4·2 for those with two or three, 4·6 for *Doona* with eleven, and 5·4 for *Stemonoporus* with fifteen. The rarity of the endemic genera goes in the opposite direction to that which one would expect were they being killed out. Why should genera with many species be nearer extinction than those with one, and the nearer the more species they have?

Mr. Ridley also states that I do not mention the fact that there are many genera which contain only endemic species. These he will find given in detail in Table XXV of the same paper (5). Adding these up, he will find that they only total 169, out of the 809 Ceylon

endemics. And they behave in exactly the same way as do the endemics in those genera which contain wides. Very many of them are as a matter of fact included in the tables on p. 8 (6) to which Mr. Ridley objects. *Doona*, *Stemonoporus*, *Semecarpus*, *Acrotrema*, *Dipterocarpus*, *Shorea*, *Lasianthus*, *Palaquium*, *Gymnostachyum*, *Actinodaphne*, *Bulbophyllum*, and *Cirrhopetalum* in these tables are all genera which contain no wides, but there is nothing in the figures to differentiate them from those which do.

With regard to these endemic species in genera that contain no wides, there are several very remarkable facts which place great difficulties in the path of Mr. Ridley. To begin with, their rarity is 4·2, or almost exactly the same as that of the endemic species in genera that contain wides, or, in other words, they are dying out at much the same rate as the latter. But if so, where does the Natural Selection theory, which implies that the competition will be more severe between species of the same genus, come in? And why are the species of endemic genera rarer than they, and that the more the more numerous they are in the genus? And in these endemics of genera with no wides, why (5, p. 331) is the rarity greater if the genus contains many species than if it contains few, exactly as is the case with the actual endemic genera?

The fact that genera occur with endemics and no wides is no doubt a difficult point to explain, but, as I have just shown, Natural Selection will not explain it. Probably the first arrivals from abroad mutated on arrival, finding themselves in somewhat different conditions, but what is now really wanted is a detailed examination of thousands of genera, to determine if possible the general principles on which specific differentiation occurs. I have made a commencement of this with the Ceylon genera, for example, and find that the endemics separate into three chief classes. The commonest may be roughly represented by a small circle within a larger, and goes on till one gets such a diagram as that of *Doona* (6, p. 14). The next is a small circle touching the large, but outside of it, and there are no materials in Ceylon to follow it farther. The third and last is a small circle at a small distance from the large, and this also cannot be followed any farther in Ceylon. As yet I have not had time to follow out these researches any farther, but I can see in them the possibility of obtaining a good deal of information of great value in the study of geographical distribution.

Why, if Natural Selection is of any avail, do the 112 genera which have no competition with any other more widely distributed species nearly related to them, only contain 221 species, or less than 2 species per genus, while Ceylon as a whole has 2,809 species in 1,027 genera (average 2·7), and the remaining endemics, which have to compete with wides in their own genera, show 588 endemic species in 212 genera (average 2·7 also)?

The next question we have to consider is whether the endemics did

or did not once occupy a greater area. The view adopted by Mr. Ridley apparently is that they are the relics of a once extensive flora which is being driven in.

But if 233 of the Ceylon endemics are VR relics of this flora, why are 222 wides also VR? Are they also relics? And why has Ceylon 222 such relics, while New Zealand has only 21 (with one-fifth the flora of wides), of which at least half have no right to be included in the list (7, p. 452)? Why do the endemics choose mountain-tops to such an extent? Ceylon has 108 of them confined to summits or to small areas in the high mountains (12). Why has New Zealand proportionately fewer, and why in New Zealand do they comparatively rarely occupy one summit only, but more often a small range?

The usual theory of the supporters of Natural Selection is that they have gone up the mountains as the last refuges from the invading flora of the low country. But in such small and uniform countries as South-western Ceylon and New Zealand it is hardly possible to suppose that there was a separate *Eugenia* or *Celmisia* at every few miles. And why did they climb right to the summit? It suggests an unnecessary degree of alarm about the coming competition. Further, it would suggest that endemics are not so incapable of adaptation to new conditions that they need fear the competition at all. If they can undergo the great adaptive changes necessary to reach a summit of 3,000 to 10,000 feet, they must have a very fair capacity for modification, and should be able to hold their own.

Why are the wides which are VR in Ceylon, 222 in number, not found confined to mountain-tops? Are they not dying out, and, if not, why not, when they are as rare as the endemics which are supposed to be doing so? It suggests that they did not care to waste time in modification to suit high altitudes, when they were to be killed out in any case.

But the great difficulty of all, perhaps, for the supporter of Natural Selection is to explain why the dying out of the endemics (assuming that they are doing so) is purely mechanical. Why does every family and genus behave in the same way, whether it does or does not contain wides, and whether it be species in an endemic or in a widely distributed genus? Natural Selection could not cause a mechanical dying out unless it meant that the arrival of the first few widely distributed species (i. e. assuming that they are the younger and arrived later) was the signal for the dying out of the whole of the old flora. Why should a genus, as we have just seen, die out sooner than a species? Why should a genus die out in the regularly graduated way shown in the map of *Doona* (6, p. 14), or in the Tables IV, V, and VI of my last paper (7)? Why should the endemics be most numerous where there are most wides (see below, crucial case No. 1)?

We shall now give two crucial cases (already given at Newcastle), which speak strongly for age against youth, and a third, still more con-

clusive, is given in a forthcoming paper on the islands surrounding New Zealand.

1. *Dispersal of Plants in New Zealand.* All the evidence goes to show that at one time the islands of New Zealand were continuous, but that at some time—whether before or after the separation from Australia is immaterial—they became separated by the formation in the centre of what is now termed Cook's Strait. If the endemics be the older, therefore, it necessarily follows that Cook's Strait would less often interfere with their dispersal through New Zealand than with the dispersal of the (younger) widely distributed species. Taking the distribution of the wides and endemics of New Zealand, zone by zone, in the same way as was done in my New Zealand paper (7), we find

Wides	209	210	237	237	235	242	236	227	215	204	112
Endem. to N.Z.	234	280	330	368	386	537	532	527	516	414	130

From the first line it is impossible to tell where Cook's Strait lies, whether after the 5th, 6th, or 7th number, but a glance at the second line shows a great change after the 5th, and this is in actual fact the position of Cook's Strait. Many endemics come up from the south and stick at the strait.

Similarly at Foveaux Strait, which comes between the last two figures, more than half the wides get across, and a much smaller proportion of the endemics. It is difficult to resist the conclusion that the wides, not the endemics, are the older.

Another very difficult problem for the supporter of Natural Selection is to explain why in this table the maximum of the wides *coincides* in position with that of the endemics. One would not expect to find this, if the former are driving in the latter. The same is the case in Ceylon; for more endemics occur in the wet zone, which also has by far the most wides. Even within the wet zone, the maximum number of both endemics and wides occurs in the same region.

Why, again, do both wides and endemics taper off with very fair regularity towards the ends of New Zealand, and why do the numbers taper down much more rapidly in the case of the endemics? While the wides sink from 235 to 209, the endemics sink from 386 to 234. Age and area will explain all this quite simply, but neither youth and area nor Natural Selection will do so.

2. *Distribution of the Tristichaceae and Podostemaceae.* These families afford an excellent test case for the question of age or youth, for owing to their peculiar morphology one can say with reasonable approach to certainty which are the older forms. He would be a bold man who would say that such forms as *Lawia* in the one family, or *Castelnavia* in the other, with their violently dorsiventral structure, shown in the lichen-like vegetative

body and the extraordinarily modified flowers, were older than such forms as *Tristicha* or *Podostemon*, which are almost radially symmetrical, and come near to the ordinary type of submerged water plant. Yet the latter are widespread and almost universal, covering the whole range of distribution of the families, while the violently dorsiventral forms are all endemic to comparatively small areas, *Lawia*, for example, occurring from Ceylon to Bombay, *Castelnavia* in the Araguaya and one other river in Brazil. It is impossible to talk of local adaptation in these plants, as I have elsewhere pointed out (18); there is nothing to be adapted to. The non-dorsiventral forms are just as common as the dorsiventral, whether in slowly or in swiftly moving water. This family is perhaps the most promising in all the flowering plants in which to study evolution or anything connected therewith; the moment that I saw them growing in the river at Peradeniya, I realized that there was an unrivalled group for the study of Adaptation, in which at that time I was a whole-hearted believer.

Mutation.

Later, Mr. Ridley objects to the mutation theory, and quotes against it numerous examples which show that he confuses mutations with varieties, and with the direct effect of changed conditions, and that he does not clearly distinguish between infinitesimal variations and large changes. He says that 'an organism . . . produces . . . varieties, which if more suitable to the surrounding conditions than the parent form are selected . . .' Does an infinitesimal variation at once produce a variety?

Mr. Ridley does not seem to be quite sure whether he will have Natural Selection with infinitesimal variations or with large changes; he evidently has an uneasy feeling that if he adopt the latter he rules Natural Selection out of court (10, 11), for if it cannot act upon a small beginning, nor determine that a large variation in one direction shall be followed by another in the same direction, it cannot be the determining factor in the production of the finished article, nor can it be its explanation.

So long as we keep to infinitesimal variations, in the literal sense in which they were understood until the coming of the mutation theory, it is quite simple to evolve anything, provided (1) that the variations are fully hereditary without regression, which we now know them not to be, (2) that they are differentiating, and not simply linear, (3) that the necessary variations appear, and (4) that Natural Selection can act—that their appearance gives sufficient advantage to the plant to ensure their survival in at least a majority of cases. But, if we once adopt large changes, the whole case is altered. We know no reason why a large change in one direction should be followed by further large changes in the same direction. There is nothing for it but to admit that the whole of a specific or perhaps even generic change may appear, but Natural Selection has nothing to do with its

appearance; it simply kills out any that are really disadvantageous; and that, it now appears clearly from my work on age and area, it does in the very early stages, when a species is represented by a few individuals only.

The supporters of Natural Selection do not clearly distinguish between *post hoc* and *propter hoc*. Does Mr. Ridley imagine that the spines on one of the Metroxylons (p. 562) began by infinitesimal variation ? If so, did the infinitesimal spines make any difference to the survival of the species? Had the pigs more delicate mouths in those days? Or were there any pigs? But if they did not begin in this way, where did Natural Selection come in? The spines are suddenly produced, and being uninjurious, are not weeded out by Natural Selection, but Natural Selection did not produce them, and cannot be their explanation. The other species, with no spines, survives also.

Does Mr. Ridley suggest that the *Vitex* on p. 564, which on being moved to Singapore proved to be *V. trifolia*, was a distinct species? Did it refuse to cross with *V. trifolia*?

For many years I have kept a note-book in which I have noted down, under the various letters of the alphabet, various questions which may be proposed to the supporter of Natural Selection, and will quote, for Mr. Ridley to answer if he can, a few of those under A :

How did the following commence, and what advantage was gained by the rudimentary beginning, sufficient to ensure the completion of the organ in question?

Phyllodes in *Acacia*?
Sensitive leaves in *Aldrovanda*?
Bulbils in many species of *Asplenium*?
Thorny roots in *Acanthorhiza*?
Simple, lobed, and compound leaves in different Acers?
Dehiscent berries in *Akebia*?
Reversed leaves in *Alstroemeria*?
The formation of adventitious embryos?
Hooked bracts in *Arctium*?
Cauliflory in some *Artocarpus*?
Scaly pappus in *Achyrachaena*?
Anisophylly in many plants?
Sympodia in *Ancistrocladus*?
Pollinia in Asclepiadaceae?

If these, or most of them, arose, as one must believe, directly at one operation, where does Natural Selection come in as formative or explanatory? As they are not harmful mutations it allows them to survive, but that is the end of its activities, so far as we can see. Natural Selection cannot be regarded as the formative agent for differences in individual species, as has been done

in the past; its operations are much more of a destructive nature than of a constructive, and are shown especially in the killing out of individual mutations.

With regard to the Castelnavias which I mention in one of my papers (6, p. 15), Mr. Ridley appears to think that what he terms study in the field rather than the library will some day show differences in the conditions of life sufficient to account for the evolution under Natural Selection of seven different species in successive cataracts in the same river. In the case of the Podostemaceae of India and Ceylon my studies in the field and laboratory (15, 16) together amounted to an average of five hours a day for six years, or an average of 500 hours (four months) for each species. To how many of the species he quotes has Mr. Ridley given that amount of time? Since 1902 I have never ceased to observe these plants, and in Brazil I gave considerable time to their study. During the first period I was an enthusiastic supporter of Natural Selection; but the more I studied them the more I became convinced that they lived under identical conditions, and that Natural Selection had nothing to do with their evolution. In this paper I give a very good crucial case drawn from these families. If the Castelnavias are to be evolved by Natural Selection in response to local differences in conditions, then evolution must be very exact to the most minute differences,[1] and how can one have species that range even over a square mile of varied surface? They live on the same rock substratum in a short stretch of the Araguaya river, and have no external competition whatever, and no differences of conditions can be found between any two except in the imagination—a faculty which was somewhat pushed to excess in the studies of Adaptation which were so largely carried on until the last few years of last century.

Mr. Ridley states that it is only Natural Selection that can answer questions, but, as I have already pointed out, it does so, like the hypothesis of Special Creation which preceded it, by invoking incomprehensibility. Mr. Ridley himself gives a good illustration of this on p. 573, where he states: 'The obvious reason why wide range . . . involves greater commonness is that for some reason the plant has advantages which enable it to spread. . . .' He avoids replying to the second half of my question, by the way. I said that the reply of the Natural Selectionists to the inquiry Why are Ceylon-Indian species commoner than endemics? was that it is because they have a wider range. When asked why the 'wides' have a range in Ceylon that is yet larger than that of the Ceylon-Indians, they can only answer that it is because their range abroad is also larger.

The reply of the Natural Selectionist is always, stripped of its verbiage, 'for some reason this is so'—the reply of the Special Creationist. On

[1] Such, for instance, as the different angle of sunshine at one cataract and the next, or the difference in mean temperature between them.

p. 15 of my Ceylon paper (6) are examples of questions of the kind to which the reply is always such, and Mr. Ridley judiciously avoids these. I do not pretend, and have nowhere pretended, that any modern hypothesis gives a proper explanation of adaptation, which at the moment is perhaps the greatest difficulty of all; but there is no doubt that Natural Selection does not do it satisfactorily. The *how* and the *why* of evolution have yet to be worked out, and that work may be prevented from losing itself in one or two blind alleys, at any rate, by the light thrown on geographical distribution by the present and other researches.

The bulk of Mr. Ridley's attack on mutation may be answered by referring him to the published work of de Vries, which one cannot but infer from his paper that he has not read with great care. Throughout his paper he gives instances of large changes as infinitesimal variations, and then proceeds to kill the case in the summary by saying that the mutation theory is not in accordance with the facts.

He quotes the literature of the fertilization of flowers as a case proving that specific differences are useful. I myself was one of the very last botanists to work seriously at floral mechanisms, good-naturedly chaffed by my friends for adhering to a theory (Natural Selection and detailed Adaptation) which was steadily going the way of all flesh. Having been brought up in the strictest Darwinian school, I devoted five years to this subject and to other 'adaptations'. For this I am now most grateful, for it has shown me the Natural Selection position thoroughly from the inside. But as a result I can only say that it is very rarely indeed that a specific character can be shown to have any importance in this connexion. The plant can sometimes make use of a specific character when there, but it did not acquire that character because of its usefulness.

I have now dealt with the chief points of Mr. Ridley's attack, and may go on to point out that he has made no attempt to parry my own, other than by bringing up exceptions, which have no bearing on figures of large numbers of plants, such as I was dealing with. The attack being upon my Ceylon work, I have confined my answer to that, though New Zealand would have supplied a much better one. He makes no effort to explain why the figures are graduated in opposite directions for endemics and wides; why the Ceylon-Indian species are intermediate in rarity; why the various species show a chain-mail pattern of distribution (6, maps on p. 12); why the endemics are 'dying out' in a mechanical way, one family or genus like another, whether they have or have not allied wides beside them; why every family and larger genus (especially in New Zealand) shows the same general plan of distribution; why the area in which occur the maximum number of wides coincides with that in which occur the maximum of endemics; and so on. Nor does he attempt to meet my arithmetical argument against

Natural Selection (6, pp. 6–16), which may be exactly repeated with illustrations drawn from the flora of New Zealand, where the Ceylon estimates are replaced by actual measurements. If these questions cannot be parried, the case for Natural Selection is a very forlorn hope.

A very valuable result of Mr. Ridley's paper is the stress which it lays upon the various causes which may modify the action of my age and area law. Some of these causes probably come into action in almost every single case of any one individual species, though upon large numbers, and in the long run, they cancel out. All might have been covered had I added to my tentative statement of the law the phrase 'so long as conditions remain constant', or words to that effect. We may enumerate some of these factors here, but the list will no doubt be largely added to. The law itself, however, in my opinion, will stand as valid, when it is applied, like Mendel's, only to groups of forms.

Chance (the operation of causes as yet not understood);

Action of man in opening up a country, cutting of forest, exploring, making fires, &c., &c.;

Interposition of barriers, such as mountains, broad rivers, deserts, arms of the sea, sudden changes of climate from one district to the next, and the like;

Geological changes, especially if involving change of climate;

Serious changes of climate;

Natural selection;

Local adaptation (a species may have a peculiarity which is useful in one country and valueless in another);

Dying out of occasional old species;

Arrival of a species at its climatic limit;

Density of vegetation upon the ground at the time of arrival of a species;

Presence or absence of mountain chains in the land over which the species has to travel in arriving;

Relative width of the union between the country of departure and that of arrival (the wider it is the more rapid may be the spread of the species in the new country),

and so on. There are numerous factors which may exert a disturbing influence, but that no more affects the validity of my law than does the resistance of the air, which prevents a thing from falling in exact accordance with the law of gravity, affect the validity of that law. I am far from denying that in individual cases plants may be relic endemics, or may have had their area of distribution greatly extended by the action of Natural Selection, or in other ways altered; but in large numbers and the long run such things do not show in my figures, which indicate that the overwhelming factor in distribution is simply *age*.

Summary.

The paper is chiefly a reply to the criticisms of my Ceylon work by Mr. H. N. Ridley in the October Annals, but contains a few new facts also. Mr. Ridley's criticisms are in reality answered in advance by my work on New Zealand.

It is shown that the Ceylon results cannot be accidental nor determined solely by the numbers of specimens in the herbarium, and that the figures are far too numerous to be disturbed by bringing up exceptions, as Mr. Ridley does.

Man's action, changes of climate, and similar disturbing factors, were not sufficiently emphasized in my Ceylon paper, but have since been dealt with.

Evidence is then given to show that the endemic species are on the whole the youngest, not the oldest, in a country. There may be relics also, but they are not numerous enough to show in the figures. This is supported by two crucial cases: one showing that the wides of New Zealand take no notice of Cook's Strait in their distribution, while the endemics do; the other based on the local distribution of the highly modified Tristichaceae and Podostemaceae and the cosmopolitan distribution of the little modified forms.

Mr. Ridley's objections to the mutation theory are then considered, and it is shown that the supporters of Natural Selection do not clearly distinguish between *post hoc* and *propter hoc*. Natural Selection cannot explain the origin of the peculiarities which distinguish plants, but can only preserve or destroy them when once formed. The reply of the Natural Selectionist to queries invokes incomprehensibility, as did formerly that of the Special Creationist.

Finally a list is given of factors which may modify the action of my law of age and area.

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The 'Age and Area' Hypothesis and the Problem of Endemism.

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IN a series of recent papers Dr. Willis[1, 2, 3] has brought forward an hypothesis as to plant distribution which is called by him that of 'age and area' and which is based chiefly on his studies of the distribution and affinities of the floras of Ceylon and New Zealand. This hypothesis may be summarized briefly as follows: The area occupied by a given species in a given region in which there occur no well-marked barriers depends in the main upon the age of that species in that region—the older the species, the wider its range. This necessarily involves also the hypothesis that the 'dying out' of a species happens very rarely indeed, and then, it is believed, only by 'accident' or as the result of a geological convulsion or other important environmental change.

The importance of this interesting hypothesis, in support of which Dr. Willis has brought forward an abundance of evidence, would obviously be very great if it should be proved correct. It would upset the traditional belief in Natural Selection as the most important factor in determining distribution. It would make it possible to tell at a glance the relative antiquity of the various elements in any flora and thus to reconstruct with ease the phytogeographical history of a region. It would enable us to identify the most widespread species in a given genus or the most widespread genus in a given family as the most ancient type in that particular genus or family, and thus to clear up at once many vexatious problems of phylogeny. Perhaps no other single hypothesis bears directly upon such a multitude of problems, and its verification is consequently a matter well worthy of our attention. The purpose of the present paper is to bring forward certain facts which

[1] Willis, J. C.: The Endemic Flora of Ceylon, with reference to Geographical Distribution and Evolution in general. Phil. Trans., B, vol. ccvi, 1915, p. 307.

[2] Ibid.: The Evolution of Species in Ceylon, with reference to the Dying Out of Species. Ann. of Bot., vol. xxx, 1916, p. 1.

[3] Ibid.: The Distribution of Species in New Zealand. Ann. of Bot., xxx, 1916, p. 437.

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emphasize the complexity of the whole problem and which throw doubt on the universal applicability of some of Dr. Willis's conclusions.

In the first place there seem to be many factors other than age which are important in determining the extent of territory over which a species is dispersed. No region of any considerable area can be said to be without 'well-marked barriers' of many sorts, such as differences in temperature, moisture, and soil composition, and the presence of competing or parasitic types, all of which are recognized as powerful factors in limiting the area occupied by a species. Ecologists find that even plants growing side by side are frequently living under very different conditions, and that a region which is superficially uniform may actually present considerable environmental complexity and may possess many barriers to certain plant types. A species limited on all sides by effective barriers will not be able to extend its limits, no matter how long it may exist; and with types which have thus reached the boundary of their possible ranges, area of dispersal will obviously afford no clue as to antiquity. A highly specialized form, occupying a relatively narrow 'ecological niche', may in reality be much older than one which from its greater adaptability under diverse environments is able to thrive over a wider area.

Very many species, however, have apparently not yet attained by any means the extreme possible limits of their ranges, and are still expanding. It is among such types that the relation between age and area may be looked for; but even here, there are such decided differences between plants in the rapidity with which they are able to extend their boundaries that no hard and fast rule can safely be laid down. A species with means for rapid dispersal will evidently overrun a wider area in a given length of time than will a more slowly moving type.

Another factor of decided importance in determining the area occupied, and one which is perhaps worthy of special emphasis because it has usually been overlooked, is the growth habit of a species. A. de Candolle noticed many years ago that trees have narrow ranges and herbs wide ones. His list of 117 species which are found over at least half of the land area of the globe includes nothing but herbaceous types. This is probably due in part to the fact that most herbs are able to produce seed in a very short time and in very great abundance, and in part to the fact that their short vegetative period enables them to take advantage of temporarily favourable conditions and to thrive in many places where they would not be able to maintain a permanent existence above ground. It is of interest to note the distribution of the three important growth forms—trees, shrubs, and herbs—represented in Trimen's 'Flora of Ceylon', the data from which furnished the basis for the hypothesis under discussion. Willis divides the species of the island into three groups on the basis of the extent of their range: (1) those which are endemic or limited to Ceylon; (2) those which are of somewhat wider

range but are confined to Ceylon and adjacent Peninsular India; and (3) those which occupy a still wider area. The following table shows the composition, as to growth habit, of these three classes :[1]

TABLE I.

	Trees %.	*Shrubs %.*	*Herbs %.*
Endemic	45	33	22
Ceylon-P. I.	25	35	40
Wide	20	26	54

It is evident that among the endemics, trees possess more than twice as many species as herbs; in the Ceylon-Peninsular Indian class only five-eighths as many, and among the 'Wides' less than two-fifths as many. Shrubs, it will be noticed, are in every case intermediate between trees and herbs.

Still more convincing is a study of the relative areas occupied by members of the three growth forms in Ceylon itself. Trimen divides the species into six classes of progressively greater rarity, using 'Common' in the sense of widespread and 'Rare' in the sense of restricted in range. These classes he designates as Very Common, Common, Rather Common, Rather Rare, Rare, and Very Rare. The percentage of trees, shrubs, and herbs in each class is shown in Table II.[1] (Herbs comprise 37 per cent. of the dicotyledonous flora as a whole.)

TABLE II.

	Trees %.	*Shrubs %.*	*Herbs %.*
Very Common	11	29	60
Common	28	28	44
Rather Common	29	27	44
Rather Rare	35	30	35
Rare	31	33	36
Very Rare	30	33	37

Here again it is evident that herbs preponderate among the 'Common' species but form a much smaller portion of the 'Rare' ones, whereas with trees just the reverse is the case. Figures like the ones cited in these two tables could be multiplied almost indefinitely for other floras. Habit of growth is clearly an important factor in determining the area occupied by a species.

A recognition of the fact that there are many effective influences other than age which decide what a plant's range shall be is not the only difficulty which the 'age and area' hypothesis must meet, for a strict application of it leads to conclusions which are not easy to defend. In the floras of Ceylon and New Zealand, for example, the endemic species have, in the great majority of cases, a much narrower range than do the non-endemic species; and this fact necessarily causes Dr. Willis to conclude that the endemic

[1] Dicotyledons alone considered.

element in a flora is its youngest element, consisting of species which have recently been developed, each in a definite locality, and which have as yet not had time to extend their ranges widely. The non-endemic types he looks upon as the oldest element, the first invaders of a region from abroad which have had time to become widely distributed and common.

This idea that endemics are always young species is open to two objections. First, it disregards the evidence that many endemics are not of local origin but are 'relicts', ancient types which were formerly widespread but which now survive only in isolated corners of the world. We are familiar with many species the range of which is widely discontinuous; for example, that interesting group which is to-day confined to Eastern Asia and to a small area in the south-eastern United States, or that group of antarctic species many of which are also found near the Arctic Circle but nowhere between. In such cases we are driven to the conclusion that these plants were once much more widely distributed, and that if extinction should progress a little farther they would survive in only one of their two present homes. They would then constitute a part of its 'endemic' flora, but would obviously not be of recent and local origin there. Many of the species and genera in Ceylon have to-day a discontinuous distribution similar to that which we have mentioned. They find their co-types or their nearest relatives in the Himalayas, perhaps, in the Malay Peninsula, in East Africa, or in Australia. Many New Zealand species, in the same way, are identical with or closely resemble others found in Patagonia, South Africa, Hawaii, or other distant places, and nowhere else. Such types certainly have the appearance of being remnants of species and genera once much more widely spread, in which a little more 'dying out' would result in the production of forms definitely endemic in one of their present areas. The conclusion is hard to escape that certain of the Ceylon and New Zealand endemics have had such a history. To imagine the monotypic endemic genera, at least (of which there are many), as having arisen by a single leap is to tax heavily the imagination of even an ultra-mutationist.

Another objection to the conclusion that endemics are always of recent development is the fact that in the floras under discussion, and other ancient ones, the endemics include a very much higher percentage of trees and shrubs than do the non-endemics. In a former paper by the writer and Professor Bailey[1] it was shown that in Ceylon the non-endemic species (according to Willis the ancient stock of the island from which the endemics have developed) included only 55 per cent. of trees and shrubs; whereas of the endemic species, 77 per cent. belonged to these woody growth forms. In New Zealand, in the same way, only 19 per cent. of the non-endemic species are woody, but 49 per cent. of the endemics. This rarity of trees

[1] Sinnott, E. W., and Bailey, I. W.: The Origin and Dispersal of Herbaceous Angiosperms. Ann. of Bot., vol. xxviii, 1914, p. 547.

and shrubs in the non-endemic element and their much greater frequency in the endemic element was invariably found in the ancient floras examined and is briefly set forth in Table III.[1]

TABLE III.

	Non-endemic Species.		*Endemic Species.*	
	Woody %.	*Herbaceous %.*	*Woody %.*	*Herbaceous %.*
Australia	38	62	73	27
New Zealand	19	81	49	51
Hawaii	24	76	85	15
Fiji	74	26	98	2
Galapagos	20	80	59	41
Juan Fernandez	6	94	82	18
St. Helena	27	73	77	23
Socotra	15	85	76	24
Mauritius	41	59	87	13
Ceylon	55	45	77	23

In all of these regions the non-endemic element, presumably the original stock, contains a comparatively small proportion of woody forms; but the presumably recent endemic element contains a percentage which averages well over twice as high. This necessarily implies that the production of new species since the colonization of the island has been very much more rapid among trees and shrubs than among herbs. The writer has recently brought forward evidence [2] that just the opposite is apparently the case, and that herbs, from the brevity of their life cycles and their consequent ability to accumulate heritable changes more quickly, are producing new species much faster than are woody plants, where the generation or period from seed to seed is very much longer.

Another objection to the 'age and area' hypothesis is that it necessarily implies a greater antiquity for the herbaceous than for the woody vegetation of the earth. The fact above mentioned, that in all 'ancient' floras the non-endemic element is preponderantly herbaceous, must mean that the original plant population of those regions was composed overwhelmingly of herbaceous species. The general rule which we have cited, that herbaceous species have a much greater average range than woody ones, also implies the greater antiquity of the herbaceous type, if we follow Willis. The consensus of opinion among botanists and geologists, however, is diametrically opposed to this view on account of the abundant evidence that the primitive Angiosperms were woody plants, and that the bulk of our modern herbaceous vegetation is of comparatively recent origin.

In connexion with the hypothesis that 'dying out' of species rarely takes place, we have already spoken of evidence that many plants seem on the high road to extinction. Whether in every case, as Dr. Willis believes, this is due to 'accident' or not, it seems to have been a very common

[1] Dicotyledons alone considered.

[2] Sinnott, E. W.: Comparative Rapidity of Evolution in Various Plant Types. American Naturalist, vol. l, 1916, p. 466.

occurrence, for the very many species, genera, and families among the Angiosperms which are isolated in distribution and in relationships can only be explained (unless we are extreme mutationists) by assuming an enormous amount of extinction to have occurred in the past.

But extinction in this sense seems not to be the only way in which species die out. Dr. Willis believes that the present endemic floras of Ceylon and New Zealand have been developed from species of early arrival in those regions from India and Australia, species which now form the non-endemic and most common element in their respective floras. In the case of Ceylon, however, there are no less than sixty-three genera among the Dicotyledons alone, or 8 per cent. of the whole, which, though not endemic in Ceylon, are represented *only* by *endemic* species. *Dipterocarpus*, *Shorea*, *Hopea*, *Xylopia*, *Euonymus*, *Gymnostachyum*, *Actinodaphne*, *Lasianthus*, *Mangifera*, *Semecarpus*, and others are examples. In New Zealand ninety non-endemic genera of Dicotyledons, or 43 per cent. of the whole, are similarly represented only by endemic species, and among these genera are some of the most important in the islands. In these cases, where is the parent species or group of species in each genus which has supposedly given rise to all these endemic forms and which should now be Very Common? If it has not 'died out', what has become of it? The fact that the proportion of such genera (not endemic but containing only endemic species) is lowest in those regions where the arrival of new species has apparently been of frequent occurrence, and highest in regions which are most isolated, suggests that these parent species tend eventually to disappear altogether. As to what happens to them we cannot be sure. Some may simply be exterminated outright and some, by continual crossing with new forms, may ultimately lose their specific identity. We are tempted to believe that the longer a successfully invading species remains in an isolated area like Ceylon or New Zealand (after its first rapid spread) the less common it tends to become until it is actually 'swamped' out of existence—quite the reverse of the 'age and area' idea.

Certain minor objections may be urged against Willis's conclusions, such as, first, that the great majority of endemic types in Ceylon are on the southern end of the island instead of the northern, the point nearest the bridge to India, where they should be according to analogy from his arguments as to New Zealand; and, secondly, that the flora of New Zealand was in all probability derived not only across a northern bridge from Australia but also, in large part, across a southern bridge from Antarctica at the time of the northward migration of the 'antarctic' flora. This fact should result, according to the hypothesis in question, in the concentration of a much larger number of species at the southern end of the South Island.

There is doubtless much truth in Willis's main contention that, other things being equal, the longer a species lives, the wider the range it will cover.

The chief argument on which the hypothesis is based is the fact, which in the face of the data presented cannot well be doubted, that endemic types have comparatively narrow ranges and non-endemic types comparatively wide ones. To a certain extent, particularly in genera which are rich in endemic species and which seem to be developing new forms rapidly, such as *Impatiens*, *Eugenia*, and *Strobilanthes* in the Ceylon flora, this restricted dispersal among the endemics is doubtless due in part to their youth. In many cases it may also be due to the fact that a given species is a tree and therefore slow to spread. The belief is hard to escape, however, that very many endemics owe their limited distribution to the circumstance that they are remnants of comparatively unsuccessful types which have been exterminated elsewhere and which even in these isolated floras are waging a losing fight against more vigorous and adaptable new-comers. In previous publications the writer has stated his conviction that in ancient insular floras and those of the great land masses of the Southern Hemisphere the endemic element is in general more ancient than the non-endemic, and he sees no reason to modify this belief; for endemics are either 'relicts' and thus very ancient, or else they represent types which have been in the region long enough so that their original characters have been lost. The hypothesis which perhaps seems to fit best all the facts at hand regards isolation as a factor which tends not only to develop new species but also to modify and extinguish old ones; and hence looks upon species in Ceylon and New Zealand which still maintain specific identity with their co-types on the mainland as the newest arrivals rather than as the most ancient members of the flora.

The whole problem of endemism is exceedingly complex. We must recognize that there exist two widely different types of endemic forms—'relicts' and 'indigenes'; we must recognize that species differ in their adaptability and competing power and in the rapidity with which they may extend their ranges, and that these factors are very important in determining whether a plant shall be local or widespread; we must recognize that certain types are phylogenetically younger than others and that their distribution is accordingly affected; and we must recognize that plant types differ widely in the rapidity with which they produce new species, and hence in the rapidity with which an endemic element will arise among them. The purpose of the present paper is to point out certain of these complexities and to show that no single hypothesis like that of 'age and area', however valuable it may be in explaining certain facts, can be used as a key to the whole problem.

Summary.

1. Dr. Willis's 'age and area' hypothesis assumes that the area occupied by a species depends primarily upon its age (the older the species, the wider its range); and that 'dying out' of species occurs very rarely.

2. The following objections may be raised against this hypothesis:

(*a*) Many effective factors other than age determine the area occupied by a species, notably physical and climatic barriers, the adaptability of species under different environments, the rapidity with which they may become dispersed, and the growth form to which they belong.

(*b*) An analysis of various floras shows that the hypothesis necessarily implies that trees and shrubs are producing new species very much faster than are herbs, a conclusion against which there is much evidence.

(*c*) The fact that herbaceous species have a much wider average range than woody ones necessarily implies that the herbaceous element in the vegetation of the world is more ancient than the woody element, a conclusion against which there is also much evidence.

(*d*) 'Dying out' of species is apparently taking place in many cases, both by actual extermination, which causes the last survivors to appear as 'relict' endemics; and by the 'swamping' of isolated members of old species by crossing with newly developed forms.

3. The various factors which determine the occurrence of endemism are discussed, and the complexity of the whole problem emphasized.

A Method of Controlling the Rate of Air Movement in Transpiration Experiments.

BY

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AND

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With one Figure in the Text.

THE influence of air movements on the rate of transpiration of plants has long been recognized but attempts to control this factor during experiments on transpiration have been very few. Investigators have usually contented themselves with periodical observations of the speed of the air in the vicinity of the plant, or else have attempted to eliminate from their results the effects of air-currents and other external factors by directing their attention, not to absolute transpiration, but to 'relative transpiration', i.e. to the ratio between the rate of transpiration from the plant and the rate of evaporation from an atmometer. Such a method is, however, not altogether satisfactory since the response of the plant and that of the atmometer to air-currents are not proportional, as has been demonstrated elsewhere.[1]

On the other hand, attempts to obviate the effects of air-currents by investigations of transpiration in 'still air' are equally unsatisfactory, on account of the difficulty of ensuring that the air is really still. Experience has shown that in spite of precautions that may be taken the air of a laboratory is in constant slight motion, and such chance air-currents will influence the 'shells' of water vapour over the evaporating surfaces and consequently the rate of transpiration will continually vary.

In critical work on transpiration it would therefore seem advisable that work should be carried out neither in 'still air', nor in the open where the wind currents may vary with great rapidity, but under conditions of constant air movement which can be regulated at will.

[1] R. C. Knight, in a paper appearing later in Vol. XXXI.

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Giddings[1] controlled the conditions of air movements in some transpiration experiments by exposing his plants to the wind produced by a fan attached to an electric motor, but he observes that the plants farthest from the fan were probably subjected to chance air-currents also; no steps were taken to remedy this.

The present writers in some unpublished experiments on transpiration attempted a more complete control of conditions of air movement than has previously been obtained.

Preliminary experiments were carried out, using a fan in the manner described by Giddings. The plants under observation were placed in the direct line of the air-current from the fan blades, but, as was to be expected, the results were unsatisfactory for the reason mentioned by Giddings. The experiments were carried out in a greenhouse, but the influence of chance air-currents from doors and ventilators was still considerable unless the fan was made to rotate at a speed so high that it tended to produce rapid transpiration and premature wilting.

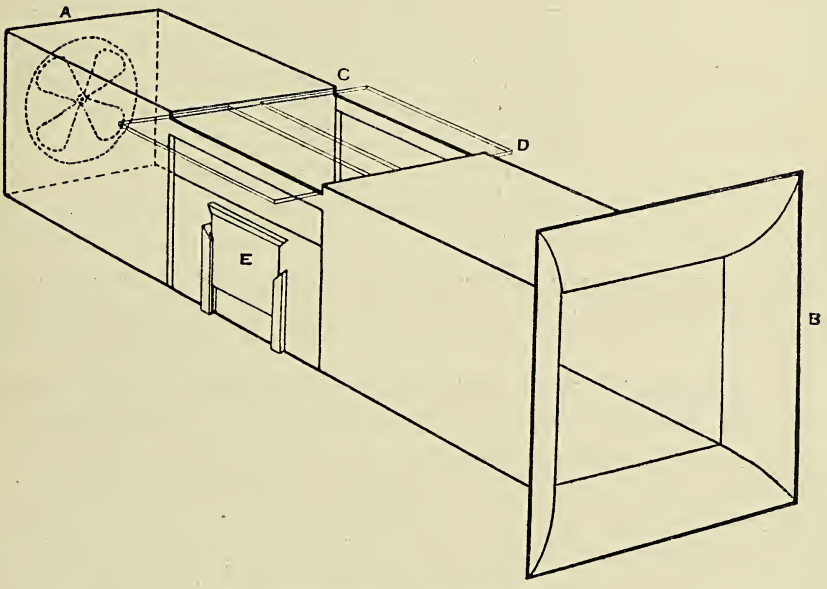
An index to the variations of the rate of air movement was obtained by periodical measurements of the rate of evaporation from a paper or porous-cup atmometer under constant conditions of temperature and humidity.

To obtain the steady movement of air which was desired, a special 'air-flue' was constructed, by means of which the plant could be protected from chance air-currents, and at the same time could be subjected to a current of constant velocity, the velocity being variable at will. The apparatus (see figure) consists of a wooden box 2·25 metres long and 60 cm. in height and breadth. In one end, A, is a circular aperture to accommodate the revolving blades of a fan. The other end, B, of the flue is open, and the four walls are extended by means of bent metal sheets to form a bell mouth, so as to reduce to a minimum the formation of eddy currents at the edges of the opening and the consequent irregularities in the air movement through the flue. Near the centre of the flue a section of the roof and sides is replaced by sheets of plate-glass; it is in this section (C, D), which is 60 cm. long, that the plants under observation are placed. The glass sheet at the top is divided in two, parallel to the long axis of the flue, and the two parts slide in and out in grooves. The front sheet also slides up to facilitate the manipulation of the apparatus or plant inside. This sheet is provided with a small sliding door, E, 18 cm. square, which is convenient for minor operations not requiring the removal of the whole sheet. The woodwork is painted white so that the light may not be unduly reduced. Air is drawn through the flue from B to A by an electrically driven fan working in the aperture at A. Several fans and motors have been tried, but during long-period experiments it was found

[1] Giddings, L. A.: Transpiration of *Silphium laciniatum*. Plant World, xvii, 1914, p. 309.

that the ordinary fan motor is not sufficiently constant in speed but tends to slow down slightly. The most satisfactory motor was found to be one with a governing mechanism attached made by R. W. Paul of New Southgate.[1] With this arrangement the slow air-currents, as low as 5 metres per minute, which have been mostly used, are easily obtainable.

The speed of the air-current is estimated by means of an anemometer except in the case of very low speeds, when the rate of movement of smoke through the flue is timed by a stop-watch.



Semi-diagrammatic view of the air-flue.

The regularity of the air movement in the apparatus was tested by atmometer readings under constant conditions of temperature and humidity. An experiment carried out in a dark room will serve to show the variation which occurred. The dark room is provided with the usual double doors, which are found useful when it is necessary to keep the humidity or temperature of the room above or below that of the laboratory out of which it opens. The table on p. 220 gives an example of the variations in evaporation rate in the dark room as determined by half-hourly weighings of a porous-cup atmometer exposed in the air-flue to a wind velocity of 7 metres per minute. Periodical readings of temperature and humidity were not made,

[1] When the motor reaches a certain speed, centrifugal action causes a weight attached to the shaft to move a lever which breaks the circuit through the coils. The lever works against a spring which, when the speed of the motor decreases, pulls the lever back to complete the circuit again. Thus with a continuous make-and-break mechanism, a regular speed is maintained. This type of motor was designed to drive the clock of a recording drum.

but from the records of a thermograph and hygrograph the variations were :

Temperature, 65° F. to 65·5° F.
Relative humidity, 70 % to 70·5 %.

TABLE.

30-*minute period ending*	*Weight of water evaporated* (*in mg.*).
11.30 a.m.	198
12 noon	197
12.30 p.m.	195
1.0 ,,	198
1.30 ,,	197
2.0 ,,	192
2.30 ,,	195
3.0 ,,	196
3.30 ,,	195
4.0 ,,	193
4.30 ,,	196
5.0 ,,	193

The maximum variation is about 3 per cent., and this includes all errors of weighing in addition to those due to slight variations in atmospheric conditions.

The apparatus has been used and found satisfactory for air movement up to a speed of 25 metres per minute, although the higher speeds are generally not convenient for transpiration experiments.

By means of experiments with atmometers it has been found desirable to use as high a speed as is conveniently possible, since greater regularity is obtained in this way. This is only to be expected, as chance air-currents, which are unavoidable in a laboratory, will have less influence on a rapid air-stream than on a slower one.

The Interrelations of Stomatal Aperture, Leaf Water-content, and Transpiration Rate.

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With four Figures in the Text.

RECENT investigators of transpiration have directed their attention towards the relative importance of the various factors controlling the rate of water loss from a plant. It was formerly held that the function of the regulation of transpiration was efficiently discharged by the stomata, and that, therefore, changes of stomatal aperture and of transpiration rate ran closely parallel; on this assumption Darwin based his horn hygroscope and differential temperature methods of estimating stomatal aperture (7, 8).

Lloyd (1908) was the first to call in question the completeness of stomatal control of transpiration (19); he even went so far as to conclude, from the lack of coincidence of the graphs of transpiration and stomatal aperture, that stomatal regulation of transpiration did not occur except when the stomata were almost, or completely, closed.

Darwin in 1916 (9) concluded that the differences between the graphs of transpiration rate and of stomatal aperture were not sufficiently important to justify the exclusion of stomata as the factor regulating transpiration.

Livingston (17) in 1906 attacked the problem in a different manner. He attempted to eliminate the influence of atmospheric conditions by expressing his results in terms of 'relative transpiration'. He found that the transpiration rate rose during the early morning hours, but the rise was checked before the evaporating power of the air, as measured by the rate of water loss from an atmometer, reached a maximum. In other words, some factor other than external atmospheric changes (which regulate, of course, the water loss of the atmometer) tends to reduce the rate of transpiration after a certain time.

The existence of this inhibiting factor has been demonstrated in various plants by Shreve (21) and Giddings (12) in a similar manner, and also by

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Bakke (1), Trelease and Livingston (22), and Bakke and Livingston (2), using the hygrometric paper method. In addition to this 'early maximum' of transpiring power, a secondary maximum has also been found by Bakke (1), Shreve (21), and by Bakke and Livingston (2) in various plants.

The regulating mechanism concerned in this retardation was not, in Livingston's opinion, stomatal in nature because of its early appearance before any stomatal closure was to be expected. Trelease and Livingston have since demonstrated the correctness of this view by actual porometer experiments, which showed that the maximum stomatal aperture was reached some three hours later than the maximum of transpiring power.

A suggested explanation of the checking of the transpiration rate was forthcoming as the result of an investigation by Livingston and Brown in 1912 (18). They found that the water-content of transpiring leaves was not constant throughout the day, but that it tended to decrease during the morning hours and reached a minimum at about the same time as the evaporating power of the air reached a maximum, i. e. some time after the maximum of transpiring power. The suggestion offered was that as the evaporating power of the air increases a corresponding increase in transpiration results, which reduces the water-content of the leaf. The decrease in water-content, in turn, tends to lower the transpiration rate, by the 'incipient drying' of the walls of the transpiring cells, in a manner similar to that in which a Piche paper atmometer tends to dry out as the result of excessive evaporation.

Shreve (21) has confirmed the work of Livingston and Brown and agrees with their interpretation of the experiments.

Briggs and Shantz (5) in 1916 concluded, as the result of an extensive series of experiments upon the influence of environmental factor on transpiration, that the transpiration coefficient of the plants investigated changes during the day, and they ascribed the change to stomatal control or to lack of water.

Iljin (13) found that the osmotic pressure of the guard cells was much greater than that of the other epidermal cells when the stomata were open, but when the stomata were closed the osmotic pressures of all the epidermal cells were the same. He also found that the graphs of transpiration rate and of the osmotic pressure of the guard cells were parallel, and suggested that excessive transpiration reduced the osmotic pressure of the guard cells thus producing stomatal closure and inhibition of transpiration. He found, however, that the rate at which the stomata closed was not dependent upon the rate of removal of water from the plant. Also he frequently found open stomata in wilted plants.

It may be observed here that two investigators quoted by Lloyd (19, pp. 60, 61) have made observations which supply evidence supporting the theory of the action of incipient drying. Unger (1862) and Comeo

(1878) both found that when transpiration from one surface of a leaf is stopped, the transpiration rate of the other surface is greatly increased.

A possible explanation is that by decreasing the transpiration rate of a portion of the leaf, the water-content was increased and therefore the transpiration rate of the rest of the leaf was increased.

The present investigation is concerned primarily with the relation of stomatal size to transpiration rate. It was undertaken at the suggestion of Professor V. H. Blackman, and I desire to express my gratitude to him for helpful advice and criticism.

Methods.

For the experiments on stomatal aperture the porometer method (Darwin and Pertz, 11) was used and a preliminary investigation was carried out to determine the precautions necessary when using the method. The results of this have already been reported (Knight, 15). In some of the following experiments the self-recording apparatus previously described (Laidlaw and Knight, 16) has been used; in others the method of timing bubbles with a stop-watch (Knight, 14). The usual practice was to attach three leaf chambers to leaves of different ages and to connect all three to the same recording apparatus, thus obtaining an average reading (see Balls, 3, and Knight, 15, p. 75). Sometimes two chambers were employed, and occasionally only one.

Darwin has pointed out (9, p. 415) that transpiration through stomata is most likely to be proportional to the square root of the rate of flow of the air through the porometer, and accordingly he uses the square root of the porometer readings to represent the aperture of the stomata. The same course has been followed in the present work.

The measurements of transpiration were made throughout by means of periodical weighings, the periods generally being of 30 minutes duration.

Concurrently with the records of transpiration rates the evaporating power of the air has also been determined by weighings of an atmometer. Several forms of this instrument have been used, including the Livingston standard earthenware type and a modification of the Piche paper form. The latter was found to respond more quickly to temperature changes than the earthenware types.

By comparing the transpiration and evaporation rates it was possible to obtain figures which were proportional to relative transpiration; but the figures given in the following tables do not actually represent relative transpiration because the rates of transpiration and evaporation were not calculated for unit areas, and also because different atmometers were used in different experiments. For the same reasons the transpiration and evaporation ratios of different experiments are not comparable.

The importance of controlling the conditions of air movement during

transpiration experiments has already been pointed out, and throughout the present work the 'air-flue' elsewhere described (Blackman and Knight, 4) has been employed to produce air-currents of constant velocity. The plants and atmometers under observation were placed close together in the glass section of the flue. At first, weighings were made on a balance standing on the roof of the flue above the plants, the latter being suspended from the balance beam by a wire hook. The fan drawing air through the flue was stopped for a period sufficient to carry out the weighings. It was found more convenient, when more than two plants or atmometers were under observation, to remove each from the flue in order to weigh it, rather than stop the fan for so long a period. When porometer cups were attached to plants to be weighed, it was necessary, of course, to interrupt the record and to disconnect the recording apparatus during weighing.

Various plants were used for the experiments, including *Fuchsia* (garden hybrid), *Antirrhinum*, *Helianthus annuus*, *H. tuberosus*, *Catalpa Kaempferi*, *Ricinus communis*, *Pelargonium zonale* (variety), *Eupatorium adenophorum*, *E. Raffilli*, *Choisya ternata*, *Aucuba japonica* (small and large-leaved forms), and *Veronica speciosa* (variety). Of these, the most useful was found to be *Eupatorium adenophorum*. This plant is easily propagated from cuttings and can be kept healthy throughout the winter in a cool greenhouse. It is suitable for transpiration experiments, because its rate of water loss is sufficiently high to be easily measurable, and is convenient for porometer experiments, owing to the ease with which the leaf chamber can be fixed to the leaf, which is neither hairy nor unduly smooth. The stomata are confined to the under surface of the leaves and are rather small, but are quite active in their response to changes of illumination.

My thanks are due to Mr. W. Hales, Curator of Chelsea Physic Garden, who has, throughout this work, maintained for me an ample stock of the necessary plant material.

Experiments have been carried out, using potted plants and also cut shoots mounted in a potometer. In the former case the pot was enclosed in the usual aluminium sheath and the top covered with rubber sheeting. The whole was placed on a small retort stand and the cups for the potometer were held in clamps. The retort stand was weighed with the plant on a large balance.

In the experiments with cut shoots, the potometer was made from a small burette or graduated pipette which was sealed to one arm of a U-shaped tube. The other arm of this was attached to the cut stem of the plant by a short piece of rubber pressure-tubing, which kept the shoot upright. The curved portion of the U-tube was fastened to a wooden stand, to obviate the need of clamping in an upright position. Porometer cups were held in holes in flat pieces of wood (garden labels answer the purpose well), which were wired to screw-clips gripping the burette or

pipette. These improvised clamps sometimes interfered with the readings of the potometer, but were very convenient to adjust and were relatively light.

Transpiration rates were determined by weighings; readings of the potometer were made simultaneously in order to indicate the amount of water absorbed by the shoot and consequently any changes in the water-content of the plant.

Experiments on the Potometer Method.

Curtis (6) and Livingston (17) have taken exception to methods which involve mutilation of the plant, such as detaching a shoot and using it in a potometer, since such treatment is liable to cause the plant to behave in an irregular manner. Lloyd (19, 20) appears to have found the potometer satisfactory, and as the method is convenient some experiments were carried out to compare the behaviour of plants in pots with that of cut shoots set up in a potometer.

Leaf chambers were fixed to three leaves of each of two shoots on a plant of *Eupatorium adenophorum* and porometer readings taken simultaneously from each shoot by means of the automatic recorders. After a few hours one shoot was severed from the plant, a fresh surface was cut, under water, four inches above the first cut, to exclude the air as far as possible from the water-conducting system, and the cut shoot was mounted in a potometer, the porometer records being continued for some hours with the shoot in the potometer. The graphs of stomatal aperture ran closely parallel until one shoot was severed from the plant; but when this was done the stomata of the severed shoot immediately began to close, the movement lasting about ten minutes. From this point onwards they opened again until, about fifty minutes after the shoot was cut, the two graphs bore the same relation to each other as before cutting. There seems to be no doubt that the temporary tendency to close was due to the handling involved in cutting the shoot and mounting it in the potometer. Other examples of this phenomenon have been described elsewhere (Knight, 15, p. 59).

Records of the stomatal behaviour of shoots in potometers have been continued for as long as three days after setting up, and it has been found that the graphs of stomatal aperture correspond closely for the whole period with those obtained at the same time from potted plants under the same conditions. The differences are no greater than are normally to be found between different potted plants in these circumstances. In the case of the cut shoots, about half an inch of the end of the stem was cut off every day in order to provide a fresh absorbing surface.

Similar experiments have been carried out with regard to the transpira-

tion of severed shoots in a potometer, and the following is a typical example:

A shoot was severed under water at 2.0 p.m. on October 4th, and mounted in the usual manner in a potometer. Porometer and transpiration measurements were made up to 6.0 p.m., and continued from 11.0 a.m. till 3.0 p.m. on October 5th. During that day the stomata responded in the usual manner to the illumination changes and the rate of transpiration also was comparable with that of a rooted plant. Comparison of the potometer readings and weighings showed that the plant was not suffering from lack of water, the quantity absorbed being equal to the quantity transpired. During October 6th transpiration and stomatal movement went on as usual, but it was found at 2.0 p.m. that the rate of absorption of water was slightly below the rate of transpiration and therefore the water-content of the plant was diminishing. There was no apparent wilting, however, until an hour later, when the leaves were distinctly flaccid. At this stage an inch of the end of the stem was removed, to provide a fresh absorbing surface, and the plant immediately began to absorb at a greater rate, so that by 5.0 p.m. the leaves had almost recovered their former turgidity. By 10 a.m. on October 17th the plant, having absorbed during the night more water than it had transpired, was quite turgid, and the observations were continued. By noon, however, wilting had commenced again and the experiment was stopped, although, as the plant was apparently quite healthy, there appeared to be no reason why a freshly cut absorbing surface should not again have enabled it to recover.

It appeared then from these experiments that as far as transpiration and stomata are concerned, a shoot in a potometer is capable in ordinary environment of carrying on its functions normally for some two or three days after its removal from the plant. It was found, as was to be expected, that plants raised out of doors remained normally active for longer periods after mounting in the potometers than similar ones grown in a greenhouse. Since, therefore, most of the experiments in this work lasted less than twenty-four hours, it was considered legitimate to draw conclusions from the behaviour of plants mounted in potometers.

Consideration was given to the possibility that, as the plants were placed in the air-flue during the experiments, the continuous stream of air passing over the leaves might cause some change of stomatal aperture, e.g. the closure due to shaking already referred to. Although the speed of the air-current was never sufficiently high to cause any visible movements of the leaves, nevertheless experiments were carried out to test the possible effects of the wind on the stomatal aperture. Rooted plants and shoots in potometers have been placed in the flue and subjected for short and long periods to air-currents of velocities up to twenty metres per minute. Periods with the air moving have been alternated with periods of fairly still

air, by stopping the fan and closing the ends of the flue with wooden shields. Also control plants have been kept outside the flue. It has not been possible to demonstrate any significant difference between the stomatal records of plants in still air and those subjected to a wind of velocity twenty metres per minute when other conditions are similar.

Another possible source of stimulation of the stomata is the movement of the plants when weighings are made. In the case of *Eupatorium adenophorum*, at least, it was sometimes found that after a weighing had been made the stomata were less widely open than immediately before the recorder was disconnected, and that a few minutes elapsed before they attained their former aperture. This slight movement was, of course, quite distinct from any normal changes due to differences of illumination, &c., and was ascribed to shock. If care is taken in moving the plants, it is a simple matter to prevent any closure at all, and it is very likely that this response to shock is not shown by all plants. It is, however, a matter for precaution.

Experiments on Stomatal Aperture and Transpiration.

This series of some twenty-six experiments was carried out at various times of the year in a greenhouse and most of the species in the list given above were used. In some cases rooted plants were used and in others shoots mounted in potometers. In either case the plant was often made ready for the experiment overnight, in order that in the morning the experiment could be started before the occurrence of maximal values in the functions under observation.

The results, as far as they concern the inter-relationship of stomatal aperture and transpiration, were distinctly discordant. In some experiments the graphs of relative transpiration and stomatal aperture ran closely parallel, a maximum in the case of one coinciding with a maximum in the other. On the other hand, in many experiments there appeared to be no correlation whatever between the two graphs.

Below are given the results of two experiments of the series, representing extreme cases of agreement and discrepancy, respectively, between the graphs of stomatal aperture and relative transpiration.

Experiment 12. The plant used was *Helianthus tuberosus*, a species with large stomata on both surfaces of the leaf and a high transpiration rate. Half-hourly measurements of the evaporating power of the air and of transpiration rate were made, and the ratio of the two gives numbers proportional to 'relative transpiration'. The quantity of water absorbed by the plant during each thirty minutes was determined by potometer readings, and changes of stomatal aperture were recorded by porometer determinations every ten or fifteen minutes. Table I gives the results, which are also expressed graphically in Fig. 1.

In the experiments given in the tables below the weighing of the plant and the reading of the potometer were in each case made one and a half minutes earlier than the weighing of the atmometer, so that the periods represented by the numbers for transpiration and absorption fell one and a half minutes earlier than those represented by the numbers for atmometer loss, although the tables and figures show the respective periods as coincident.

The numbers in the column $\frac{T}{E}$ are proportional to, not equal to, 'relative transpiration', since they are not calculated for unit areas.

The numbers in the last column, 'Stomatal aperture', are obtained from the square roots of the reciprocals of the time readings of the porometer.

TABLE I.

		Plant.		$\frac{T}{E}$	*Stomata.*	
Half-hour periods ending	*Atmometer loss in mg. (E).*	*Transpiration in mg. (T).*	*Absorption in mg.*		*Time.*	*Stomatal aperture.*
					10.25 a.m.	257
					10.33 ,,	257
					10.48 ,,	260
					10.55 ,,	264
11.30 a.m.	400	1030	990	2·58	11.15 ,,	265
					11.25 ,,	269
					11.40 ,,	276
12 noon	425	1270	1110	2·99	11.55 ,,	300
					12.10 p.m.	341
					12.17 ,,	345
					12.25 ,,	298
12.30 p.m.	435	1680	1230	3·86	12.35 ,,	254
					12.45 ,,	245
					12.55 ,,	243
1.0 ,,	430	1340	1100	3·12	1.5 ,,	240
					1.12 ,,	239
					1.25 ,,	239
1.30 ,,	420	930	950	2·21	1.35 ,,	249
					1.45 ,,	243
					1.55 ,,	232
2.0 ,,	420	1050	830	2·50	2.10 ,,	231
					2.15 ,,	234
					2.20 ,,	234
2.30 ,,	490	1020	850	2·08	2.25 ,,	232
					2.40 ,,	227
					2.55 ,,	227
3.0 ,,	490	900	790	1·84	3.10 ,,	226
					3.25 ,,	223
3.30 ,,	455	830	740	1.82	3.45 ,,	222
					3.55 ,,	222
4.0 ,,	415	700	710	1·69	4.10 ,,	223
					4.25 ,,	225
4.30 ,,	360	620	650	1·72		
					4.55 ,,	219
5.0 ,,	300	500	620	1·67		
	Total	11870	10570			

Decrease of water-content of the plant, 1,300 mg.

FIG. 1. Graphs (based on results given in Table I) showing parallelism between stomatal aperture and relative transpiration. The graphs in this and the succeeding figure are plotted on different base lines for the sake of clearness. S = stomatal aperture; E = atmometer loss; $\frac{T}{E}$ = relative transpiration.

The close agreement between the two is clearly shown by the maximum occurring in each between 12 noon and 12.30 p.m., and the small secondary maximum between 1.30 p.m. and 2 p.m.

Experiment 11. A plant of *Helianthus tuberosus* was also used in this experiment, which was carried out in a manner exactly similar to that in Experiment 12. The results are shown in Table II and Fig. 2.

In this case the two graphs appear to be quite independent of each other. Stomatal aperture reached a maximum at 11.45 a.m. and a second maximum at 1.55 p.m., but the relative transpiration graph shows only one maximum, and that between 2.50 p.m. and 3.20 p.m.

From these experiments it is apparent that stomatal size and transpiration rate are not always in close agreement, and this result supports the conclusions reached by Lloyd using similar methods (19, p. 61). Darwin (9), however, claims to have shown a much closer agreement between the graphs of transpiration and stomata than has been found in the present work.

In the above series of experiments, confirmation was sought of the observation of Livingston and others (see p. 221 above) that the maximum of relative transpiration occurs some hours previous to the time of the maximum of the evaporating power of the air. In Fig. 1 above it will be observed that the relative transpiration maximum fell between 12 noon and 12.30 p.m., whilst the evaporating power of the air was greatest between 2 and 3 p.m.

In the other experiment given, however, the positions were reversed and the relative transpiration maximum fell some $2\frac{1}{2}$ hours later than that of evaporation.

A review of the series shows that of twenty-six experiments ten were similar to No. 1 above in showing the 'early maximum' of relative transpiration, whilst of the remainder, six showed the transpiration maximum definitely later than that of evaporation, four showed the two maxima occurring at about the same time, and in six both graphs were falling throughout the experiment.[1]

TABLE II.

		Plant.		$\frac{T}{E}$	*Stomata.*	
Half-hour periods ending	*Atmometer loss in mg. (E).*	*Transpiration in mg. (T).*	*Absorption in mg.*		*Time.*	*Stomatal aperture.*
					10.15 a.m.	2
					10.30 ,,	2
					10.45 ,,	2
11.20 a.m.	410	1270	1280	3·10	11.15 ,,	79
					11.30 ,,	209
					11.37 ,,	266
11.50 ,,	440	1800	1830	4·09	11.45 ,,	283
					11.53 ,,	265
					12.0 noon	224
					12.5 p.m.	203
12.20 p.m.	470	2110	2160	4·49	12.10 ,,	168
					12.15 ,,	156
					12.25 ,,	164
					12.30 ,,	176
12.50 ,,	500	2270	2400	4·54	12.35 ,,	173
					12.45 ,,	156
					12.55 ,,	151
					1.0 ,,	149
					1.15 ,,	169
1.20 ,,	470	2270	2260	4·83	1.25 ,,	181
					1.35 ,,	203
					1.45 ,,	214
1.50 ,,	450	2450	2500	5·44	1.55 ,,	236
					2.5 ,,	208
					2.15 ,,	193
2.20 ,,	450	2500	2490	5·56	2.25 ,,	171
					2.35 ,,	153
					2.45 ,,	129
2.50 ,,	440	2410	2520	5·48	2.55 ,,	117
					3.15 ,,	67
3.20 ,,	330	1860	1920	5·64	3.30 ,,	56
					3.45 ,,	53
3.50 ,,	380	1560	1690	4·11		
					4.15 ,,	40
4.20 ,,	340	1120	1170	3·29		
					4.45 ,,	38
4.50 ,,	315	810	900	2·57		
	Total	22430	23120			

Increase in water-content of plant, 690 mg.

[1] It may be noted here that Fig. 1 above shows the double transpiration maximum observed by some writers, but in the present work this has not appeared with sufficient frequency to warrant any conclusion as to its nature.

FIG. 2. Graphs (based on the results given in Table II) showing the lack of agreement between relative transpiration and stomatal aperture. S = stomatal aperture; E = atmometer loss; $\frac{T}{E}$ = relative transpiration.

The discordant results obtained in this series when considered in conjunction with the water-content of the transpiring plant—as indicated by the difference between the quantity of water transpired and the quantity absorbed—constitute confirmatory evidence of the 'incipient drying' hypothesis of Livingston and Brown (see p. 222 above). In Table I above it will be observed that during the experiment the plant transpired 11·87 grm. of water and absorbed only 10·57 grm., involving a loss to the plant of 1·30 grm. It is also significant that the greatest loss, namely 0·45 grm., was between 12 noon and 12.30 p.m., from which point onwards the graph of relative transpiration falls. From 3.30 p.m. onwards the plant absorbed slightly more water than it transpired and the transpiration rate remained approximately constant. The conclusion is that the excessive transpiration early in the day caused a lowering of the water-content of the transpiring cells and a drying out of the walls, this in its turn exerting a check upon transpiration (and so a decrease in relative transpiration), before the evaporating power of the air began to decrease.

Consideration of Table II above shows the reverse process in action. The total amount of water transpired was 22·43 grm. and the amount absorbed 23·12 grm., a gain of 0·69 grm. Throughout the experiment the rate of absorption never fell appreciably below the rate of transpiration,

only the periods ending at 1.20 p.m. and 2.20 p.m. showing a loss to the plant of 10 mg. in each case.

The plant, therefore, did not suffer from lack of water as in the case of Experiment 12, and consequently there was no incipient drying to check the rate of transpiration. The result was that relative transpiration continued to increase for some time after the maximum evaporating power of the air had been reached.

The whole series of experiments shows the same tendency as that indicated above. When the amount of the water lost by transpiration was markedly less than the amount absorbed, the maximum of relative transpiration regularly fell before that of evaporation. When the plant absorbed more water than it transpired there appeared to be no check upon the rate of transpiration, and relative transpiration showed no maximum value early in the day, and sometimes, as in Experiment 11 above, the maximum was not reached until the stomata were nearly closed.

No case has been found in which actual transpiration has been equal to absorption throughout the experiment, but in several cases transpiration has been greater than absorption during some periods and less than absorption during others. This state of affairs tends to make the graph of relative transpiration rather irregular with no definite single maximum. In these experiments it is not possible to correlate each change of water-content of the plant with changes in relative transpiration. It is possible that this is due in part to the inevitable defects of the method of dividing the experiment into half-hour periods for transpiration determinations, coupled with the possibility of a lag in the influence of the quantity of water absorbed by the stem at one end of the shoot on the rate of transpiration from the leaves some distance away.

It should be noted here that in Experiment 12 quoted above, the actual transpiration rate reached a maximum simultaneously with the relative transpiration rate, although this does not occur regularly when the relative transpiration maximum falls before that of evaporation. The theory of incipient drying is not sufficient by itself to account for the fall of the actual transpiration rate, although it explains the decrease of relative transpiration. If transpiration, absorption, and the water-content of the leaf are related in the manner which Livingston's theory suggests, then, other factors being constant, incipient drying will tend to check an increasing transpiration rate (and, conversely, an increase in the water-content of the leaf will tend to check a decreasing transpiration rate) and to bring it to a constant value in equilibrium with the rate of absorption. But transpiration will show no alternation of maxima and minima without the intervention of some factor other than incipient drying, e.g. stomatal closure or decrease in the evaporating power of the air. Shreve appears to have overlooked this relationship in the explanation offered of the two maxima of transpiration (21, pp. 48

and 49). Shreve's suggestion—that incipient drying checked transpiration, and produced the early maximum, thus allowing the water-supply to catch up with the demand and causing an increase in the transpiration rate up to the second maximum—is clearly inadequate.

There is considerable discrepancy between the results described above and those obtained by other workers. Livingston (17) and Shreve (21), and also Trelease and Livingston (22), found the maximum of relative transpiration to occur *regularly* before that of evaporation. Also Trelease and Livingston report this transpiration maximum as occurring before the maximum of stomatal aperture, whereas from the present work there appears to be no constant relation between the time of occurrence of these three maxima. The explanation may be found in the difference between the prevailing climatic conditions, some of the present experiments having been carried out on very bright days and others on very dull days. Bright days, with a correspondingly high evaporating power of the air, will naturally favour high transpiration rates and incipient drying, whilst on dull days the transpiration rate will be lower and may not exceed the rate of absorption. Both Livingston and Shreve carried out their experiments at the Desert Laboratory at Tucson, where the relative humidity of the atmosphere was very low and the temperature fairly high—conditions favouring the early onset of incipient drying and so producing the early maximum of relative transpiration.

Stomata and Leaf Water-content.

Lloyd (20) found that frequently the stomata continued to open when the water-content of the plant was decreasing, and concluded from this that the stomata were 'ineffectual in maintaining a constant supply of leaf-water'. The results of Trelease and Livingston mentioned above, showing that the stomata continue to open for some three hours after the transpiration maximum is reached, confirm Lloyd's experiments, since the water-content of the leaf must decrease with the onset of incipient drying which occurs before the maximum of relative transpiration is reached. In other words, the stomata continue to open whilst the water-content of the leaf is decreasing.

The same result has been found in the present experiments. When the water-content of the plant is decreasing owing to the excess of transpiration over absorption, the stomata often continue to open, thus tending to increase the rate of transpiration.

Experiment 12 quoted above provides an example of this. Transpiration exceeded absorption (and therefore the water-content of the plant decreased) from the commencement of the experiment at 11.0 a.m. until 3.30 p.m., but the stomata continued to open until 12.15 p.m. A further example is given below.

Experiment 10. The day was bright and hot, the maximum temperature being 91·5° F. A shoot of *Helianthus tuberosus* was set up in the usual way and records of transpiration, absorption, evaporation, stomatal aperture, temperature, and humidity were kept. The results are shown in Table III and Fig. 3.

TABLE III.

The numbers in the 'Water deficit' column represent the excess of total transpiration over total absorption at the end of each period.

	Plant.				$\frac{T}{E}$	*Stomata.*	
Half-hour periods ending	*Atmometer loss in mg. (E).*	*Transpiration in mg. (T).*	*Absorption in mg.*	*Water deficit in mg.*		*Time.*	*Stomatal aperture.*
						9.55 a.m.	171
						10.3 „	239
						10.10 „	265
						10.25 „	299
10.30 a.m.	470	5870	5760	110	12·49	10.40 „	347
						10.55 „	426
11.0 „	580	7100	6730	480	12·24	11.10 „	568
						11.25 „	620
11.30 „	650	7200	6570	1110	11·08	11.40 „	620
						11.55 „	408
12.0 noon	655	5780	5330	1560	8·82	12.15 p.m.	202
		Potometer refilled at 12 noon					
12.35 p.m.	690	4810	4420 (?)	1950	6·97	12.30 „	186
						12.45 „	172
1.5 „	640	3750	3760	1940	5·86	1.0 „	154
						1.15 „	131
1.35 „	600	3270	3330	1880	5·45	1.30 „	121
						1.45 „	113
2.5 „	545	2860	3090	1650	5·23	2.0 „	114

The stomata in this case did not begin to close till 11.40 a.m., by which time the water-content of the plant had decreased by 1·11 grm. owing to the excess of transpiration over absorption. Later in the day, from 12.35 p.m. onwards, the rate of absorption was greater than the rate of transpiration, and the water-content of the plant increased; but in spite of this the stomata continued to close.

It appears from this result that a small decrease in the water-content of the leaf does not produce stomatal closure. The continued opening of the stomata may have been due to increasing illumination, or on the other hand the loss of water may actually have caused the stomata to open as in the case of the preliminary stomatal opening during wilting which was described by Darwin and Pertz (11) and by Laidlaw and Knight (16). Iljin (13) also found open stomata in wilted plants.

Thus the evidence supports Lloyd's contention that stomata do not closely regulate the water-content of the leaf, since there is no stomatal closure in response to incipient drying and consequently no stomatal inhibition of transpiration.

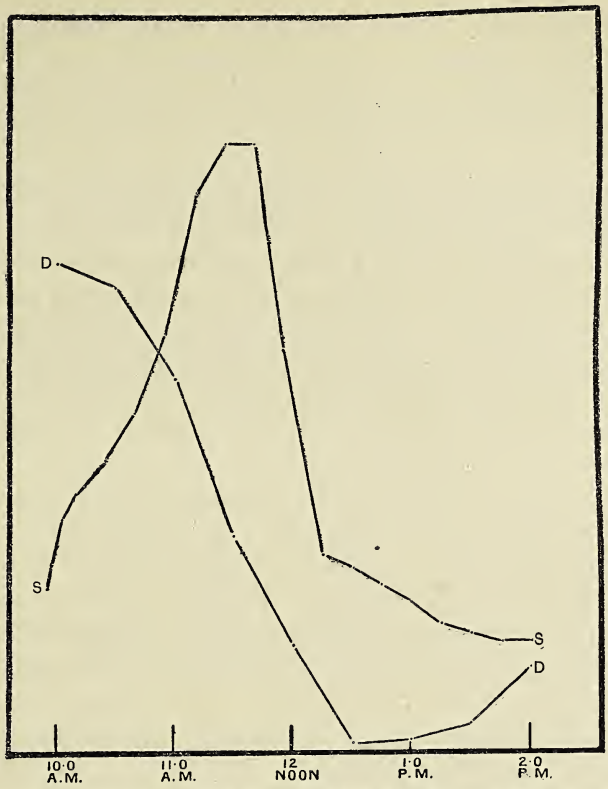

FIG. 3. Graphs (based on the results given in Table III) showing the continued opening of the stomata in spite of the increasing water deficiency. The water deficit numbers are plotted below the zero line so that a downward trend in the graph represents a decreasing water-content, and vice versa. The deficit is assumed to be zero at the beginning of the experiment. S = stomatal aperture; D = water deficit.

EXPERIMENTS IN CONTROLLED ENVIRONMENT.

It appeared likely from the results obtained that, if stomatal changes and the evaporating power of the air were controlled, the rate of actual transpiration might be shown to be dependent upon the water-content of the plant, and an attempt was made to do this.

The experiments were carried out in a dark room where the temperature could be maintained constant to within 0·5° C. and the relative humidity to within 1 per cent. Trials were made with artificial light in order to control the stomata, but it was found that even in the dark the stomata of the plant used—*Eupatorium adenophorum*—were fairly widely open and that the transpiration rate was still fairly high. Accordingly experiments were carried out in the dark, except when weighings were being made, when an electric lamp some distance from the plants was switched on. It was found to have no effect on the stomata. The plants were usually removed to the dark room on the day before the experiment was to be performed in order to ensure that they might reach equilibrium in the changed conditions.

Experiments were carried out in the usual manner, records being made of stomatal aperture, transpiration, absorption, and the evaporating power of the air. The last was merely a check upon the constancy of the atmospheric conditions, and experiments in which it was irregular were discarded.

The results showed the same tendency as before, an excess of absorption over transpiration producing an increase in the latter, and vice versa. When the transpiration rate of a plant had been reduced by the lack of water it was found possible, by decreasing the evaporating power of the air, to reduce the transpiration rate below the rate of absorption and allow some of the water deficit to be made up. Then, on increasing the evaporating power of the air to its original value, it was found that the transpiration rate had increased with the increased water-content. An example of this is given below.

Experiment 46. A shoot of *Eupatorium adenophorum* was cut from a plant which had been in the dark room over night and was mounted in the usual way. The records showed that the rate of transpiration was greater than the rate of absorption and therefore that the water-content of the plant was decreasing, the transpiration rate falling at the same time. The evaporating power of the air was then reduced in the ratio of about 1·6 to 1·0 by stopping the fan in the air-flue. This reduced the transpiration rate still further, and it was now well below the rate of absorption and the water-content of the plant increased. The fan was started again at its original speed after a half-hour period of 'still air', to determine the rate of transpiration with increased water-content. The results are given in Table IV and Fig. 4.

TABLE IV.

No air movement during period 3.30–4.0 p.m.

		Plant.		*Stomata.*	
Half-hour periods ending	*Atmometer water loss in mg. (E).*	*Transpiration in mg. (T).*	*Absorption in mg.*	*Time.*	*Stomatal aperture.*
				11.45 a.m.	160
12.30 p.m.	222	518	330	12.15 p.m.	173
1.0 ,,	218	440	390	12.45 ,,	187
1.30 ,,	220	395	330	1.15 ,,	186
2.0 ,,	218	405	410	1.45 ,,	182
2.30 ,,	222	400	380	2.15 ,,	189
3.0 ,,	220	402	400	2.45 ,,	188
3.30 ,,	220	416	380	3.15 ,,	183
4.0 ,,	135	337	420	3.45 ,,	188
4.30 ,,	225	465	390	4.15 ,,	190
5.0 ,,	225	447	420	4.45 ,,	192
5.30 ,,	222	438	400	5.15 ,,	196
	Total	4663	4250		

Decrease of water-content of plant, 413 mg.

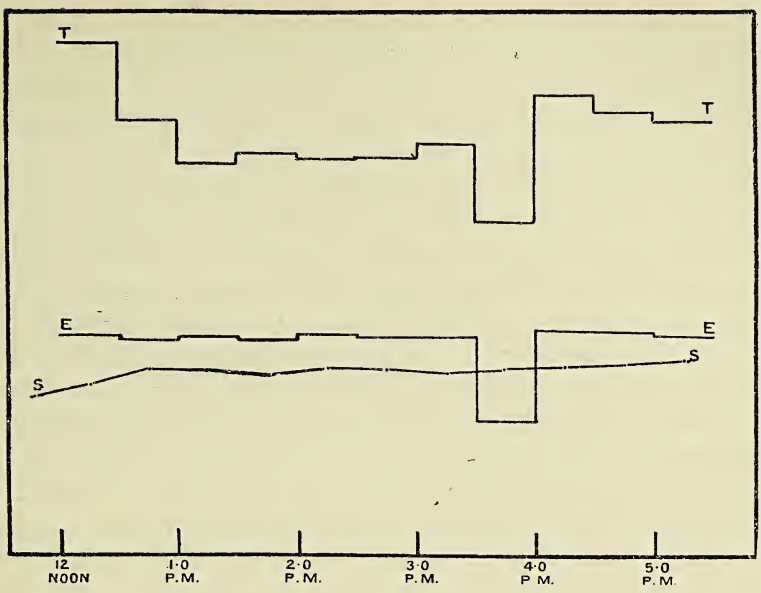

FIG. 4. Graphs (based on results given in Table IV) showing an increase in the transpiration rate as a result of increasing the water-content of the plant by temporarily decreasing the evaporating power of the air. S = stomatal aperture; E = atmometer loss; T = transpiration.

On restarting the fan, transpiration had increased from 400–416 mg. per 30 minutes to 465 mg. per 30 minutes. Owing, however, to the transpiration rate being again greater than that of absorption, the transpiration rate showed a decrease in the subsequent periods.

The stomatal aperture was not quite constant throughout this experiment, but showed a slight tendency to increase as the experiment proceeded. This may have been due to the effect on the stomata of loss of water (see p. 234 above).

CONCLUSION.

It appears therefore from the results of these experiments that in plants under quite ordinary conditions, changes of stomatal aperture and transpiration do not necessarily run parallel. Darwin (9) claims a much closer correlation between these two functions than has been obtained in the present work, but Darwin made no attempt to control his experimental conditions,[1] and most of his transpiration measurements were based upon readings of absorption by the plant from a potometer, uncontrolled by weighing; although, as has been frequently shown, absorption is not by any

[1] It should be noted that Darwin's results were very divergent, as his Tables VII and VIII, pp. 433 and 434, show. He compared the increase of transpiration and the increase in stomatal aperture in response to light, and this ratio, which in accordance with his theory should be unity, varies from 5·5 to less than 0·2.

means necessarily the same as transpiration. The differences between the two can be large enough to influence the transpiration rate without causing any obvious change in the turgor of the leaf, as has been demonstrated by many of the above experiments, so that the fact that a plant is not flaccid is no assurance that its rate of absorption is equal to its rate of transpiration. Darwin (9) is of the opinion that stomata play a primary rôle in the regulation of transpiration, and considers (p. 418) that the water-content does not sensibly affect the rate of transpiration. Lloyd (19, p. 137) concluded that stomatal regulation of transpiration does not occur, except when the apertures are almost or completely closed. In his later work (20, p. 14) he again states that stomata 'are not closely regulatory of the loss of water from the leaf and are ineffectual in maintaining a constant supply of leaf water', basing this on the fact that in many of his experiments the stomata continued to open when the water-content of the plant was decreasing. The experiments in the present paper have confirmed Lloyd's results. Lloyd considers, however, that stomata may limit transpiration 'in a purely passive manner'. This conception is no doubt the same as that expressed on p. 138 of his earlier work (19), namely, that there is no stomatal closure *in anticipation* of wilting, to conserve the water-supply. It is clear, however, that if the difference between the partial pressures of water vapour on the two sides of the epidermis is constant, a decrease in the size of the stomatal apertures is bound to decrease the rate at which moisture diffuses through to the outer air, so that a change in the size of the stomatal apertures must result in a change in the transpiration rate. Limitation of transpiration by stomata in this manner is presumably what Lloyd conceives as the 'passive' regulation. It is unlikely that any one would maintain that stomata are capable of adaptive closure in anticipation of wilting, if by 'wilting' is meant a decrease in the water-content of the leaf. By 'closure in anticipation of wilting', Lloyd doubtless means closure in response to a reduction of water-content, thereby preventing the further loss which would otherwise occur. The response of stomata to loss of water from the leaf has been indicated by the experiments of Darwin and Pertz (11), of Lloyd (20), and of Laidlaw and Knight (16), and also in the present work. A small decrease in the water-content of the leaf does not cause the stomata to close, and as the loss of water proceeds the first noticeable effect of wilting is to cause the stomata to open.[1] Closure finally takes place only at a comparatively late stage of wilting.

Iljin (13) concluded that an excessive transpiration rate caused stomatal closure, but his statements that open stomata are often found in wilted plants, and that the rate of stomatal closure is not dependent upon the rate at which the water-content of the plant is reduced, confirm the work mentioned above.

[1] Lloyd failed to find any evidence of preliminary stomatal opening due to wilting.

Stomatal changes appear to be chiefly influenced by conditions of illumination rather than by small changes in the leaf water-content. Since the water-content may vary within fairly wide limits without causing any stomatal change, the stomata cannot be said to function in maintaining the requisite quantity of water in the leaf by response to changes of water-content, although there is no doubt that stomatal changes can influence the rate of transpiration.

The present work has shown the importance of leaf water-content in influencing transpiration, thus confirming the work of Livingston and Brown (18) in demonstrating that a small decrease in leaf water, producing incipient drying, can reduce the transpiration rate.

The relative importance of incipient drying and stomatal size as the factor controlling transpiration probably depends upon other conditions. From the results obtained in these experiments it appears that in a bright light the stomata are wide open, and that therefore changes in intrinsic transpiring power of the plant are brought about chiefly by changes of leaf water-content. On the other hand, in a dull light it is probable that the lower transpiration rate is the result of the failure of the stomata to open widely, and incipient drying will not occur so long as the supply of water through the stem is maintained.

The direct effect of light on the transpiration of a plant, i.e. the effect of light on the rate of water loss from the evaporating tissues, has not yet been investigated in the present work. Darwin (10) by a new method experimented on this factor and concluded that the direct effect of light might be considerable, the transpiration rate, irrespective of the influence of stomatal change, being as much as 36 per cent. greater in light than in darkness, but the nature of this direct effect is as yet obscure.

Summary.

Experiments have been carried out with various plants under controlled atmospheric conditions to determine the relationship between stomatal aperture and the rate of transpiration.

1. The results have shown that in many cases there is *no* agreement between the two, a decreasing transpiration rate being often accompanied by stomatal opening, and vice versa.

2. The water-content of the leaf was found to be a factor playing a large part in the control of transpiration, a high water-content tending to produce a high transpiration rate, and a lower transpiration rate resulting from decreased water-content.

3. The stomatal aperture is not reduced by slight water deficiency in the leaf; hence the ordinary view that stomata, by their response to incipient drying, are the chief factors in maintaining the water-content of the leaf is not tenable.

4. On the other hand, the stomata are very sensitive to light changes, so that with increasing light intensity the stomata may continue to open whilst the water-content of the leaf is decreasing.

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On the Reduction of Transpiration Observations.

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With one Figure in the Text.

CONTENTS.

1. INTRODUCTORY.

IN the attempt to correlate plant transpiration with the purely physical process of evaporation various methods have been followed; in particular, the transpiration-loss may be compared with that suffered by a water surface (usually circular in shape) of known area, or, as is now more usually the case, with the loss, under similar conditions, of a porous atmometer of the type introduced by Livingston. But it is eminently desirable that the figures obtained by different investigators, under various conditions, should be capable of easy and accurate comparison; and since two atmometers, identical in size and shape, may differ so much in other qualities (e.g. in the porosity of the material of which they are constructed) as to render it impossible to compare the data given by them, some definite method of calibration has become necessary.

The plan adopted in the past and still used by some investigators is to compare the evaporation-loss from the atmometer with that from a circular

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water surface;[1, 2, 3] and, as the areas of the two evaporating surfaces are in general different, to reduce the observations by assuming that the evaporation from a free water surface is proportional to its area. This, except under certain special circumstances, is not even approximately true, and the present research was undertaken with the object of demonstrating the law actually followed, and of showing how the results obtained may be applied to place the figures acquired in transpiration experiments on a more consistent basis.

It has been demonstrated theoretically by Stefan[4] that the rate of evaporation of a circular liquid surface evaporating into an indefinite homogeneous atmosphere is given by the equation

$$V = 4\,ka \log_e \frac{P-p_1}{P-p_0},$$

where V is the volume evaporated in unit time, a the radius of the surface, k the coefficient of diffusion, p_0 and p_1 the pressure of the vapour at the surface and at an infinite distance from it respectively, and P the atmospheric pressure. If p_1 and p_0 be small with respect to P, this reduces to

$$V = 4\,ka \frac{p_0-p_1}{P}.^{5}$$

Renner[6] has made a series of experiments on the rate of evaporation of free water surfaces, and has compared the results obtained experimentally with the values calculated from the formula

$$E = 4\,k\,(\rho_1-\rho_2)\,a,$$

where E is the mass evaporated in unit time, a the radius of the surface, k the coefficient of diffusion, ρ_1 the density of saturated vapour at the given temperature, ρ_2 the vapour density of the surrounding atmosphere at the same temperature. This formula is of a different form from that given by Stefan, and it is to be remembered that, granted the proper conditions, Stefan's formula provides an exact solution. Renner compares the experimental results with those calculated from three formulae, viz. $C4R$, $C4\pi R$, and $C\pi R^2$, where $C \equiv k\,(\rho_1-\rho_2)\,60$.

[1] Cf. Livingston: The Relation of Desert Plants to Soil Moisture and Evaporation. Carnegie Inst. Publications, No. 50, 1906.

[2] Shreve: The Daily March of Transpiration in a Desert Perennial. Carnegie Inst. Publications, No. 194, 1914.

[3] Yapp: On Calibration of Evaporimeters. Appendix to paper On Stratification in the Vegetation of a Marsh, and its Relations to Evaporation and Temperature. Ann. Bot., 1909.

[4] Stefan: Ueber die Verdampfung aus einem kreisförmig oder elliptisch begrenzten Becken. Wied. Ann., xvii, 1882, p. 550.

[5] This equation is misquoted by Brown and Escombe as $4\,ka\,\frac{P-p_1}{P-p_0}$. Phil. Trans., B., vol. cxciii, 1900, p. 251.

[6] Renner: Beiträge zur Physik der Transpiration. Flora, 1900, p. 485 et seq.

An examination of his tabulated results shows that such agreement as exists is purely fortuitous—for some values of a one formula shows fair agreement, for other values the other formulae are invoked. The figures show that the agreement, for example, of the values calculated from $C\pi R^2$ does not necessarily represent any physical fact, but is simply an expression of the mathematical fact that two quite unrelated curves may approximately coincide for a small portion of their length.

The figures obtained from the formula expressing the linear law and those obtained from the formula expressing the area law may be so different that the errors introduced by reduction according to the erroneous formula may be large enough to rob the results of any meaning.

Thus, in the laboratory experiment to determine the relation between the amount of evaporation per unit time from a leaf surface and from a water surface of equal area, Detmer[1] gives the figures for an actual experiment which yields the result

$$\frac{\text{Water lost by 100 sq. cm. of water surface}}{\text{Water lost by 100 sq. cm. of leaf surface}} = \frac{11{\cdot}3}{1{\cdot}99} = 5{\cdot}68.$$

This result was obtained by reducing the experimental figures according to the 'area' law. If we assume Stefan's law for the evaporation from the water surface, and assume further (as Detmer does) that the evaporation from the leaf surface is proportional to its area, the above ratio becomes 2·53—an error of about 120 per cent.! It is not too much to assert that, until reasonable certainty exists as to the true law connecting the evaporation from a water surface with its area, such figures possess no quantitative significance whatever.

Certain deductions from Stefan's law have been subjected to the test of experiment by Winkelmann[2] and by v. Pallich.[3] Apart from the closely analogous experiments on diffusion carried out by Brown and Escombe[4] and more recently by Renner,[5] we are not acquainted with, however, any results which give the laws connecting E and a under various 'everyday' conditions; and the experiments detailed below are offered as a contribution towards the establishment of such laws.

2. Algebraic Statement of Law of Evaporation from Circular Surfaces.

We assume then that the law of evaporation from a circular basin of water or other liquid is given by an equation of the type

$$E = Ka^n \ldots \text{(i)},$$

[1] Detmer: Practical Plant Physiology. (Eng. Translation by Moor, 1909, p. 212.)

[2] Winkelmann: Ueber die Verdampfung von den einzelnen Theilen einer kreisförmigen freien Oberfläche. Wied. Ann., xxxv, 1888, p. 401.

[3] J. v. Pallich: Über Verdunstung aus einem offenen kreisförmigen Becken. Berlin. Akad., Sitz., 106, p. 384, 1897, and Sci. Abs., i, p. 203, 1898.

[4] Loc. cit., p. 223.

[5] Loc. cit.

where E is the evaporation per unit time, and a the radius of the basin—or rather, of the evaporating water surface. K and n are constants, and it is our object to determine the values of K, and especially of n, under various external conditions. Having measured E and a experimentally, we obtain by taking logarithms of equation (i)

$$\log E = \log K + n \log a \ldots \text{(ii)},$$

and on plotting $\log E$ against $\log a$ we should obtain a straight line. The tangent of the angle which this straight line makes with the x-axis at once gives n, whilst $\log K$—and therefore K—is given by the magnitude of the intercept made by the straight line on the y-axis. Thus n and K are easily and accurately obtained from the graph. They may, of course, also be obtained by treating the experimental data by the method of least squares, if the observations admit of such treatment.

If we then find that $n = 1$, this constitutes a verification of the law propounded by Stefan; if $n = 2$, the 'area' law is justified; and in any case, independently of any theoretical assumption, the value of n appropriate to the given external conditions can be obtained without any uncertainty.

This method of procedure appears to us to possess some advantages over those usually employed. In the classic paper of Brown and Escombe, for example, the method of reduction of the results merely shows that the experimental data are better suited by the linear than by the area law. If the logarithms of one set of their data[1] be plotted out in the manner explained above, and a straight line be drawn through the mean position of the points so plotted, it will be seen that the value of n which best fits their data is not 1, but 0·87 (approximately). The points are somewhat irregularly placed, but this is a natural consequence of the extreme difficulty of the experiments, and of the necessary smallness of the apertures employed.

3. Experiments and Results.

In the experiments now to be described, a series of fourteen cylindrical crystallizing dishes, whose radii varied from 2 to 10 centimetres, was employed, each of the dishes being filled to a definite distance (d) from the upper rim; after weighing the dishes, the whole series was set out on a table, each dish being separated from its nearest neighbours by a distance which was several times greater than its own diameter; weighings were then made after the lapse of a definite time and the mean evaporation per hour (E) was thus obtained. Readings of the barometer were taken at the beginning and end of a run, and observations of the relative humidity of the air were made by means of a Regnault hygrometer. A maximum and minimum thermo-

[1] Loc. cit., p. 244.

meter served to show the extreme range of the temperate changes during the progress of an experiment.

Observations were taken in three different localities: (1) in a 'dark' room with blackened walls, having a floor space of about 400 square feet: this room was chosen for its steady temperature properties, as the only portion of its walls which abutted on the external air was that occupied by a moderately large window; (2) in a large room used as a general laboratory, well lighted from above, and with a floor space of about 600 square feet; and (3) in the open air.

It is not necessary to give full details of all the figures obtained in the various experiments. One set of such figures may, however, be of interest as showing both the accuracy obtainable and the magnitude of the various quantities involved. They are the result of an experiment made under 'dark' room conditions with the levels of the evaporating surfaces at the beginning of the experiment 0·7 cm. below the rims of the respective vessels,[1] and are given in Table I below.

TABLE I.

Mean height of barometer = 29″·6 (steady)
Max. temp. = 18°·8 C. Min. temp. = 17°·7 C.
Relative humidity = 63 per cent.
d = 0·7 cm. k = 0·0101. n = 1·69.

a.	*Log a.*	*Time.*		*Loss in weight.*	*Log E (obsd.).*	*E (obsd.).*	*E (cald.).*	*% error.*
cm.		h.	m.	grm.				
10·17	1·0073	43	4	22·10	$\bar{1}$·7103	0·5133	0·5064	−1·34
8·26	0·9170	43	0	15·15	$\bar{1}$·5470	0·3524	0·3579	+1·56
7·03	0·8470	42	58	11·90	$\bar{1}$·4424	0·2770	0·2727	−1·55
5·84	0·7664	42	54	8·60	$\bar{1}$·3021	0·2004	0·1989	−0·75
5·00	0·6990	42	53	6·45	$\bar{1}$·1774	0·1504	0·1530	+1·73
4·38	0·6415	42	54	5·25	$\bar{1}$·0878	0·1224	0·1224	±0·00
3·91	0·5922	42	58	4·373	$\bar{2}$·9977	0·0995	0·1011	+1·61
3·30	0·5185	42	59	3·286	$\bar{2}$·8835	0·0765	0·0758	−0·91
2·84	0·4533	42	59	2·422	$\bar{2}$·7510	0·0564	0·0589	+4·43
2·08	0·3181	42	58	1·484	$\bar{2}$·5384	0·0345	0·0348	+0·87

Average error regardless of sign = 1·47 per cent.

In the above table, the first column gives the radii of the dishes used, the sixth column gives the rate of evaporation from each basin in grammes per hour as deduced from the data given in the preceding columns, whilst the seventh column gives the values of E as calculated from the equation

$$E = 0{\cdot}0101\ a^{1{\cdot}69}.$$

The constants in this equation were obtained, as explained above, by plotting the observed values of log E and log a against each other. It will be seen

[1] At the end of the experiment the values of d, calculated from the known values of the evaporation and the radii, in the above table were increased to 0·77, 0·77, 0·78, 0·78, 0·78, 0·79, 0·79, 0·80, 0·80, 0·81 cm. respectively.

that the observed and calculated values of E are in satisfactory agreement, the percentage error being shown in the last column.

It is not necessary to give full details of any of the other experiments made. A number of the graphs of log E and log a are given in Fig. 1 below, and it will be seen that in all cases the graphs are fairly accurately linear, whilst the variation of n under different circumstances is clearly shown by the varying slope of the different lines.

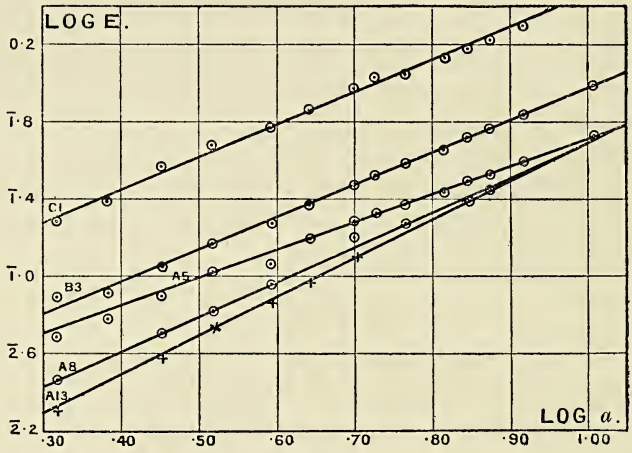

FIG. 1.

The letters appended to the various lines in Fig. 1 are identical with those in the first column of Table II. A reference to this table will therefore show at once the conditions under which the experiment was made.

In Table II below we have a synoptic view of the results of the whole series of experiments. Those indexed A were carried out under 'dark' room conditions; experiments under the index B were made in a room typical of an ordinary laboratory, and those under the index C were made in the open air. The seventh column gives the distance d of the level of the evaporating water surface below the rim of the containing vessel, while the eighth and ninth columns give the values of k and of n in

$$E = ka^n$$

as deduced from the graphs of the logarithms of the observed values of E and of a. (It should be noted that in experiments A 3 and A 4 a maximum and minimum thermometer was not used, and the mean temperatures given are the means of several readings of an ordinary thermometer.)

4. Discussion of Results.

The preceding table brings out several points of primary importance in connexion with the main problem under discussion—the calibration of atmometers. In the first place it is to be noted that under no circumstances

TABLE II.

	Barometer (*Mean*).	*Temperatures in ° C.* *Max.*	*Min.*	*Mean.*	*Rel. Hum.* (*Mean*).	*d.* (*cm.*).	*k.*	*n.*
	inches.							
A 1	30·19	15·1	14·7	14·91	56 %	0·0	0·0307	1·43
A 2	30·14	15·7	15·0	14·35	73	0·3	0·0126	1·60
A 3	30·20	....	....	19·55	73	0·5	0·0187	1·49
A 4	30·78	....	....	19·85	71	0·5	0·0168	1·50
A 5	30·89	22·5	18·7	20·60	69	0·5	0·0158	1·46
A 6	29·94	16·1	15·3	15·70	74	0·5	0·0109	1·62
A 7	29·60	18·8	17·7	18·25	70	0·7	0·0101	1·69
A 8	29·77	18·4	17·8	18·10	73	1·0	0·0079	1·78
A 9	29·60	18·8	17·7	18·25	70	1·2	0·0063	1·86
A 10	29·49	18·8	18·1	18·45	76	1·5	0·0053	1·82
A 11	30·13	15·7	15·0	15·35	73	1·5	0·0048	1·88
A 12	29·95	18·7	17·4	18·05	67	2·0	0·0053	1·99
A 13	29·69	19·8	18·7	19·25	72	2·5	0·0050	1·99
A 14	29·57	20·3	19·2	19·75	71	2·5	0·0056	1·97
A 15	29·83	19·0	18·5	18·75	71	3·0	0·0049	1·97
B 1	30·24	21·7	17·7	19·70	62	0·5	0·0291	1·58
B 2	30·23	21·7	18·1	19·90	60	0·6	0·0268	1·57
B 3	30·21	21·3	18·3	19·80	66	0·7	0·0206	1·65
B 4	29·55	16·8	14·7	15·76	90	2·5	0·0031	2·00
B 5	29·61	17·1	13·9	15·50	84	2·5	0·0047	1·98
C 1	30·21	21·8	18·9	20·35	68	0·5	0·0607	1·67
C 2	29·95	15·7	14·7	15·21	78	2·7	0·0379	1·77
C 3	. . .	2·4	1·4	1·9	66	3·0	0·0097	2·06

does the magnitude of n fall to unity—the value demanded by Stefan's law. The probable reasons for this do not here concern us—these, and several other points of purely physical interest, we hope to be able to publish later through the proper channels. It is sufficient here to note the fact itself.

Again, as the depth of the liquid surface below the rim increases, n also increases, rapidly at first, and then more slowly, until when d is greater than about 3 centimetres, n has become practically equal to 2, and the 'area' law holds good.

Thirdly, if the depth remain constant, and the external circumstances are varied in the direction of a greater disturbance of the atmosphere, n also increases, but only relatively slowly. The experiment C 1, for example, was carried out in the open air, with a gentle breeze blowing sufficiently hard to ruffle continually the liquid surfaces exposed. Even under these conditions the value of n was no greater than 1·67. We found this result somewhat surprising, as we had expected, from *a priori* considerations, that the large disturbance induced in the lines of flow by a steady breeze would tend to make n practically equal to 2. This is, however, by no means the case, as indeed Stefan has indicated.[1]

We may say then that, under all ordinary circumstances, the value of n, for a vessel filled to within 5 millimetres of the brim, varies from 1·5 to 1·7

[1] Loc. cit., p. 411.

(approximately 1·6), and that for a vessel filled to about 3 centimetres from the brim, under similar circumstances, the value of n is practically equal to 2.

Unfortunately the importance of filling the calibration vessel to a definite distance from the rim has not, so far as we are aware, received due recognition. The vessel is usually spoken of as being 'filled' with water; and, where any quantitative details are given, this appears to mean 'filled to within 5 or 6 millimetres of the brim'.[1] Darwin and Acton, indeed, in discussing a very similar problem, recommend the use of a 'shallow vessel';[2] as their results, however, are to be reduced by the area law, it is the very opposite that is required—a *deep* vessel, filled to about 3 centimetres from the brim. Otherwise, the calculated results cannot have any quantitative meaning.

The serious difference that may exist between the true value of n and that usually assigned to it may, as we shall show, introduce errors in the calculated value of the equivalent water surface of an atmometer that may be as great as 30 or 40 per cent. This being so, it is hardly too much to say that any figures obtained from two different atmometers, and any figures—such as those of 'relative transpiration'—based on readings of atmometers calibrated by comparison with water surfaces assuming the area law, cannot by any means be compared one with another. Not only are the numerical magnitudes of the figures involved probably in error, but the degree of the error, depending as it does on the true value of n, is also variable, and is variable by an amount that cannot be estimated so long as the exact conditions of the experiment remain unspecified.

It is evident then, that to remove this unfortunate uncertainty, it is necessary to calibrate an atmometer by means of some quantity that shall possess those qualities which are usually demanded of a standard or unit—that is, it must be constant under constant and easily specified conditions, and must be capable of reproduction at any place or time. The figures cited above seem to show that a free water surface contained in a cylindrical basin fulfils these conditions. The surface is one which can easily be reproduced and the evaporation from such a surface follows a *regular law*. Further, the value of the constants in

$$E = ka^n$$

can readily be calculated. Secondly, after the depth of the free surface of the water below the rim of the containing vessel has reached a certain value, evaporation is proportional to the area, and therefore a water surface of this kind may, without further difficulty, be used for standardizing purposes.

[1] Yapp filled the crystallizing dish used to calibrate his atmometers to 'about 3 mm. below its upper edge'. Loc. cit., p. 311.

[2] Darwin and Acton: Practical Physiology of Plants, 1894, p. 89.

5. Improvements in 'Open-pan' Methods for calibrating Atmometers.

It appears to us that if a water surface be used as standard, two methods of procedure may be followed:

(1) In calibrating the atmometer, three or more basins of varying radii may be used, filled to about 5 millimetres from the rim. The actual value of n may then be determined by plotting log E against log a, as previously explained. Then if E be the evaporation per unit time from the basin of radius a_1, E_2 the evaporation per unit time from the evaporimeter, and a_2 the radius of its equivalent surface

$$\frac{E_1}{E_2} = \left(\frac{a_1}{a_2}\right)^n,$$

or

$$\log a_2 = \frac{n \log a_1 - \log E_1 + \log E_2}{n}$$

giving a_2. The area (A) of the equivalent water surface is then given by

$$A = \pi a_2^2.$$

(2) In the second method, although a single basin is sufficient, two or three basins may preferably be employed to minimize incidental errors. Each basin should be filled to a depth of about 3 centimetres from the rim, when it can safely be assumed that $n = 2$. Then, using the same notation as before, we have

$$\frac{E_1}{E_2} = \frac{\pi a_1^2}{A},$$

or

$$A = \frac{\pi a_1^2 E_2}{E_1}.$$

The equivalent area A should be calculated from each of the basins employed, and the mean taken.

This appears to be the method to be preferred. Such a water surface seems to possess the qualities of constancy and of easy reproduction which are absolutely necessary in every practical standard, and its general use would, we think, remove the great uncertainty which is an undesirable feature of the present methods of calibration. Moreover, as will shortly be shown, a consideration of the relative errors in the different quantities involved will enable us to remove practically every source of uncertainty and to make the calibration as accurate as the circumstances may demand.

6. Example of Method.

Meantime, a practical example will perhaps help to make matters clear:

The transpiration from a small Pelargonium was determined in the usual manner, taking all ordinary precautions. In order to determine the

equivalent area of the transpiring surface of the plant two sets of calibrating vessels, three in each set, were employed. In one set (vessels C, E, and G) the dishes were filled to 5 millimetres from the rim, in the second set (D, F, and I) to 3 centimetres. The following figures were obtained:

TABLE III.

	Radius.	*Loss in weight.*	*Duration of experiment.*
			h. m.
Plant	....	6·45 grammes.	68 12
C.	8·26 cm.	29·55	68 23
E.	7·03	23·38	68 26
G.	5·84	16·55	68 2
D.	7·48	16·80	68 10
F.	6·45	12·15	68 10
I.	5·00	7·51	68 6

The mean rate of evaporation of the plant is therefore 0·0946 gramme per hour. If we assume, as we have shown to be justifiable, the area law for D, F, and I, we obtain, in determining the equivalent water surface (A) of the plant by means of the equation

$$A = \frac{\pi a_1^2 E_2}{E_1},$$

(α) from D, $A = 67{\cdot}45$ sq. cm.,
(β) from F, $A = 69{\cdot}34$ sq. cm.,
(γ) from I, $A = 67{\cdot}48$ sq. cm.,

giving a mean value of 68·09 sq. cm. for A.

In reducing the data obtained from C, E, and G, log E was plotted against log a in the usual way, and the resulting straight line showed that the appropriate value of n was 1·58. Hence using the equation

$$\log a_2 = \frac{n \log a_1 - \log E_1 + \log E_2}{n}$$

we obtain,

(δ) from C, $a_2 = 3{\cdot}168$ cm. or $A = 31{\cdot}53$ sq. cm.,
(ϵ) from E, $a_2 = 3{\cdot}126$ cm. or $A = 30{\cdot}70$ sq. cm.,
(ζ) from G, $a_2 = 3{\cdot}216$ cm. or $A = 32{\cdot}49$ sq. cm.

The mean value of A is therefore 31·57 sq. cm.

Hence we see that the plant loses water at the same rate as a circular water surface 31·6 square centimetres in area filled to a distance of 5 millimetres from the rim of the containing vessel; or at the same rate as a circular water surface 68·1 square centimetres in area filled to a distance of 3 centimetres from the rim. The vital importance of specifying this distance is here shown to be very obvious.

It is equally important that the true value of n should be used in reducing the observations. Suppose, for example, that in the case last discussed we reduce the observations, assuming, as is ordinarily done, the area law. We should then obtain

(η) from C, $A = 46{\cdot}92$ sq. cm.,
(θ) from E, $A = 42{\cdot}98$ sq. cm.,
(ι) from G, $A = 41{\cdot}66$ sq. cm.,

giving as a mean value $A = 43{\cdot}85$ sq. cm. Now, other considerations apart, the steady decrease in the value of A with decrease in the radii of the calibration vessels is sufficient to indicate that something is probably wrong; but, as we have seen, the true value of A is $31{\cdot}6$ sq. cm., and therefore the calculation of A by the usual method introduces an error into the equivalent area of no less than 39 per cent.! This experiment—for, of course, the plant simply serves the purpose of a model atmometer—seems to us to demonstrate beyond reasonable doubt that the method of calibrating an evaporimeter by assuming the area law for a circular basin filled to within a small distance of the rim introduces such errors as to make it quite impossible to compare the results of different instruments; and it seems to be equally true that the figures usually given to show the ratio between the transpiration per square centimetre from a leaf surface and the evaporation per square centimetre from a circular water surface are quite void of any quantitative significance.

As an example of the errors introduced, the figures given by Yapp, who reduces his observations according to the 'area' law, may be taken.[1] His method of procedure, which is given arithmetically, differs slightly from the manner of reduction discussed in this paper, but the underlying principles are identical. Reduced to symbols the argument may be stated thus:

Let E_a be the rate of evaporation from the atmometer, A_a the area of its equivalent water surface. Let E_d be the evaporation from the standardizing dish, A_d its area.

Then it is assumed that

$$\frac{E_a}{E_d} = \frac{A_a}{A_d},$$

or

$$A_a = A_d \times \frac{E_a}{E_d} = 62{\cdot}07 \times \frac{50{\cdot}40}{15{\cdot}51}$$
$$= 62{\cdot}07 \times 3{\cdot}25,$$

using the figures for Yapp's 1908 I atmometer. This atmometer had a superficial area of $141{\cdot}2$ sq. cm., and hence we find that the ratio of the area of the atmometer to that of the water surface which evaporates at an equal rate is

$$\frac{141{\cdot}2}{62{\cdot}07 \times 3{\cdot}25} = \frac{1}{1{\cdot}43},$$

[1] Loc. cit., p. 311 seqq.

or, a square centimetre of atmometer surface evaporates 1·43 times as fast as a square centimetre of free water surface.

Evaporation results are often given by ecologists and meteorologists in linear measure. The method of reduction is illustrated by Yapp in his paper,[1] and the argument may be stated as follows:

Let the rate of evaporation from the atmometer be x c.c. per unit time. Then taking the figures given by Yapp's 1908 I atmometer, the circular water surface from which this would evaporate in the same time must have an area of $62{\cdot}07 \times 3{\cdot}25$ (or $141{\cdot}2 \times 1{\cdot}43$) sq. cm. If d be the depth evaporated, then since the volume of a cylinder $= A \times d$

$$141{\cdot}2 \times 1{\cdot}43 \times d = x,$$

or

$$d = \frac{x}{141{\cdot}2 \times 1{\cdot}43} = \frac{x}{202},$$

which is the figure given by Yapp.

But this result again is based on the assumption that the 'area' law of evaporation is the true one. Let us attempt to find the law of reduction from cubic to linear measure, making no assumptions as to the law of evaporation. If E be the *mass* of water evaporated per unit time from a circular basin of radius r, then

$$E = kr^n.$$

But if d be the depth evaporated

$$E = \pi r^2 d\rho,$$

where ρ is the density of water. Hence

$$d = \frac{k}{\pi\rho}\frac{r^n}{r^2} = K\frac{r^n}{r^2},$$

where K is a constant. If $n = 2$—the usual assumption—then $d = K$, and under these circumstances, *and only under these circumstances*, will the evaporation in linear measure be independent of the radius of the basin employed.

The calibrating dish used by Yapp was filled to about 3 mm. below its upper edge. Under these conditions the appropriate value of n is approximately 1·5. Whence we have

$$d = K\frac{r^{1.5}}{r^2} = \frac{K}{\sqrt{r}},$$

that is, the evaporation in linear measure from a circular water surface is inversely as the square root of its radius. As an illustration, if we had two such dishes, one four times the radius of the other, the depth evaporated in unit time from the larger basin would only be one-half of that evaporated in the same time from the smaller basin. The possibility of the existence of

[1] Loc. cit, p. 315.

discrepancies of this magnitude forces us to the conclusion that very little reliance can be placed on figures for evaporation in linear measure obtained on the assumption of the area law.

7. Effect of Observational Errors and Deductions therefrom.

It remains now to discuss the effect of given errors in the observed quantities on the resulting errors in the computed quantities. The point of most importance so far as we are concerned is the relation between the percentage error introduced into the value of the radius (or area) of the equivalent water surface of an atmometer by a given percentage error in the determination of n.

Let E_1 and E_2 represent the evaporation per unit time from the atmometer and calibrating dish respectively; let r_2 represent the radius of this latter vessel, and r that of the surface equivalent to the atmometer. Then

$$\frac{E_1}{E_2} = \left(\frac{r}{r_2}\right)^n,$$

and, remembering that E_1, E_2, and r_2 are given constants, we obtain at once by differentiating logarithmically

$$\frac{\delta r}{r} = \frac{\delta n}{n} \log_e \frac{r_2}{r}.$$

This result shows, as is otherwise obvious, that, when the radius of the calibrating dish is very nearly equal to the equivalent radius of the atmometer, a large percentage error in n may have very little effect in producing errors in r, and it may be very usefully and easily applied further to reduce any uncertainty that may exist as to the true value of the equivalent radius.

The procedure, then, that appears to us to be safest to follow in calibrating an atmometer is—*first perform the calibration with two or three vessels filled to about 3 centimetres from the brim; reduce the observations, assuming the area law, for each basin separately, and thus obtain a mean value for the equivalent area, in the manner previously demonstrated. This value may be slightly in error, owing to a slight error in the assumed value of* n*; but if we now repeat the experiment, using as a calibrating vessel a dish whose area is as near to this mean value as is conveniently possible, the value of the equivalent area deduced from this last experiment will be accurate enough for all practical purposes.*

8. Concluding Remarks.

During the course of his experiments on atmometry, Livingston first used, then discarded, the method of calibration by comparison with free water surfaces;[1, 2] and in the experiments carried out during the year 1912

[1] Livingston (1906), loc. cit.

[2] Ibid.: Operation of the Porous Cup Atmometer. Plant World, 13, 1910.

and subsequently he has calibrated the instruments by comparison with a standard atmometer.[1, 2] If comparable results are desired by investigators in widely separated laboratories, this entails the possession of secondary standards which have been calibrated by comparison with the standard atmometer—a condition which is evidently inconvenient and far from ideal. Especially when one is aiming at an accuracy of one or one-half of one per cent., it seems preferable to attempt to realize a standard which shall be, as we have already pointed out, invariable under given conditions and capable of easy reproduction at any place or time. The free surface of water contained in a cylindrical vessel and protected by a rim of 3 cm. depth fulfils these conditions. Moreover, the use of a free water surface for purposes of calibration avoids the assumption that the porous properties of the porcelain of the standard atmometers remain unchanged with time.

It *may* be that other factors—such as convection—may still introduce such errors as to preclude the use of the open pan as a standard. But we think that these sources of error have been somewhat too hurriedly invoked to explain observational discrepancies, while the greater sinner—*n*—has been allowed to pass unrebuked.

We do not think that the errors introduced by convection are likely to be as large as is commonly assumed ; were this the case, the logarithmic curves obtained for a series of basins of widely differing radii, tested under all sorts of external conditions, would not be so accurately linear as they actually are. But this point can only be settled by experiment, and it seems to us that it is well worth while to give the perhaps too hastily discarded water surface a further trial.

This is all the more desirable, since the only other alternative—that of standardization by comparison with a standard atmometer—is at best a *pis aller*. The impossibility of standardizing one's instrument oneself is an inconvenience, and the fact that the standard alters as time goes on points to the possibility of the introduction of errors which, small in themselves, may in time accumulate to something very serious.

There are several other points of interest to the physiologist which are, perhaps, outside the scope of this paper ; in particular, the evaporation from small apertures in currents of air of various velocities demands accurate experimental investigation. We have seen that, *ceteris paribus*, the change from a quiet room to a slightly disturbed atmosphere produces a small but definite alteration in the value of *n*, and this change is primarily due to the distortion of the lines of flow of the vapour in the neighbourhood of the evaporating surface caused by the motion of the air. If, therefore, quantitative experiments be made on the evaporation from small apertures in streams of air moving with various velocities, the variation in the values

[1] Livingston : A Rotating Table for standardizing Porous-cup Atmometers. Plant World, 15, 1912. [2] Ibid. : Atmometry and the Atmometer. Plant World, 18, 1915.

obtained for n should give important information as to the magnitude of the air film which is at rest in contact with the surface. Such information could probably be usefully applied to the analogous case of a transpiring leaf surface exposed to a breeze. We hope in the future to communicate the results of experiments dealing with this point.

The greater part of the above work was done in the Physics Department of the University College of North Wales, Bangor, in the summer of 1916, and we desire here to express our appreciation of the assistance given to us by Professor E. Taylor Jones in generously placing at our disposal the resources of his department. Our thanks are also due to Professor V. H. Blackman, who has criticized our manuscript and to whose knowledge of the literature of the subject we owe several of our references.

9. Summary.

Experiments are described which show that the evaporation from a circular water surface is not, as commonly assumed, proportional to its area, unless the depth of the surface below the rim of the containing vessel be greater than two or three centimetres; nor is it proportional to the linear dimensions of the basin, but for basins 'full' of water it is approximately proportional to (radius) $\frac{3}{2}$.

Serious errors—sometimes of the magnitude of 40 per cent.—may therefore arise in determining the water surface equivalent to a given atmometer, and methods of calibration are described by which such errors may be avoided.

The figures usually given to show the relation between the evaporation from a transpiring leaf surface and a circular water surface of the same area are also subject to serious errors, and, simple as the experiment is, stand in need of re-determination.

Roots in Bennettites.

BY

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With Plate XIV.

THE *object of this paper* is to describe and figure fossil rootlets bearing root-hairs petrified with remarkable beauty. These rootlets, in addition to their beauty, have a twofold interest: (*a*) because nothing appears to be known about the roots of *Bennettites*, and the rootlets bearing the root-hairs now described appear to be adventitious rootlets of that interesting genus; (*b*) because, though rootlets of various types are among the commonest of well-petrified plant remains, root-hairs are excessively rare, and there are very few publications in which fossil root-hairs of any genus of any geological age are figured.

The *specimens* to be described are a number of rootlets, all in one slide, in the geological collections of the British Museum (Natural History), No. V. 10158. The area of the plant material cut in this slide is roughly $5 \times 5{\cdot}5$ cm., and the specimen is described in the catalogue as being *Bennettites*, cf. *Saxbyanus*. The section passes obliquely through cortex and leaf-bases, and there is no doubt whatever that it is of *Bennettites*. From the general colour, texture, and other trifles which guide one accustomed to the few specimens of this genus which are known in this country, there is every indication that the slide has been cut from *Saxbyanus*, though there is no record from which block it was cut. It is an old slide, and its early history is lost.

Permeating the *Bennettites* ground-tissue, which is locally broken down, and crossing the mineral gaps, is a large number of stout rootlets, each averaging about 1 mm. in diameter. These run in various directions, and some are traceable for distances of from 1 to more than 3 cm. in oblique lengths. The tracheal and cortical tissues are complete in a number of the rootlets, but their chief feature is their root-hairs. These are excessively numerous, as can be imagined after inspection of Pl. XIV, Figs. 1 and 2. Where there is a break in the tissues of the leaf-bases and the rootlets

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traverse this break, the clear mineral matter filling it is felted with root-hairs. Pl. XIV, Fig. 1, shows only one focal level in a thick slide, so that their quantity may be imagined. Individual hairs may be traced for nearly 8 or 9 mm. across the gaps, and the expanded bases of the hairs, where they are attached to the tissues of the rootlets, are well seen in many places; cf. Pl. XIV, Figs. 1, 2, and 3, *b*.

Description of rootlets. Most of the rootlets are roughly 1 mm. in diameter, but a few branches are present about half this size. Branching is infrequent; though several lengths of rootlets from 1 to over 3 cm. are to be seen, there is only one clear instance of dichotomy, and a couple of apparently lateral branches being given off.

The individual rootlet is composed of a central vascular strand, of which in longitudinal and oblique sections three or four good-sized scalariform tracheides are well preserved. Transverse sections showing the vascular elements clearly are not to be seen. Surrounding the tracheides is a several-layered zone of small, very dark, apparently thickened cells. Outside of the central strand are 3–4 layers of large cortical cells, bounded by similar but smaller cells, vertically elongated, from which the numerous root-hairs spring. All these layers are to be seen in Pl. XIV, Fig. 3.

The rootlets which pass through the unbroken ground-tissue of the *Bennettites* differ in this region only in having *no root-hairs*. Such a hairless rootlet, passing through the mass of parenchyma, can be seen in Pl. XIV, Fig. 4. It is impossible to offer precise proofs, but the impression created in my mind is that the rootlets originate in some way connected with the gum canals of the ground tissue. One must simply await the discovery of further specimens of root-bearing *Bennettites* before the mode of origin of these rootlets can be determined. There remains also the question whether they belong to the plant itself, or are an invasion from the outside. I incline to the view that they are adventitious roots of the *Bennettites* itself. In a few places it appears as though the leaf-base tissues had contributed to the rootlets; and the tracheides of the rootlets are entirely similar to these scalariform elements which form such a characteristic feature of Bennettitalean wood.

Far from the 'histological details of both wood and bast' 'agreeing precisely with the corresponding structures in a recent Cycad', the secondary wood of *Bennettites* spp. has a type of scalariform tracheides which is, so far as I am aware, not found in the secondary wood of any other of the families of higher plants. The perfect agreement of the tracheides of the rootlets with those of the *Bennettites* type seems to me, while not conclusive, contributory evidence favouring the view that they belong to the plant in whose tissues they occur.

No root structures—neither primary nor adventitious—have hitherto been described for *Bennettites* so far as I am aware. Such roots as these,

running in various directions among the breaking tissues of the leaf-bases, can scarcely belong to the main system of the trunk. It is possible that the old leaf-bases of some *Bennettites* gave rise to adventitious buds, and, if so, that these rootlets belonged to them. Indeed, there seems to be a certain amount of evidence accumulating in favour of the view that detachable vegetative buds may have occurred in this fossil group. In the living genus *Cycas*, which is vegetatively so comparable in some ways with *Bennettites*, adventitious buds which ultimately led to 'branches' were described,[1] which had roots running irregularly in and out of the leaf-bases of the trunk. It is true that I did not observe in the living Cycads any rootlets so slender, or any provided with root-hairs like the fossil, but my examination of the living Cycads was not exhaustive, nor are these points of fundamental distinction. Wieland[2] has well illustrated the queer irregular branching of some of the fossil trunks, which is irresistibly reminiscent of those of the Japanese living *Cycas*. It is therefore extremely probable that some adventitious budding took place in the fossil genus.

Though it remains to some extent still a supposition, I conclude that the adventitious rootlets here described belonged to adventitious buds arising from the old leaf-bases of *B. Saxbyanus* itself.

I must register my appreciation of Dr. Smith Woodward's kindness in placing at my disposal the slides and other treasures of the geological collections of the British Museum (Natural History) to facilitate my work on the Bennettitales, of which this paper forms a small part.

[1] Stopes, Marie C., 1910: Adventitious Budding and Branching in *Cycas*. New Phytologist, vol. 9, pp. 235–41, Text-figs. 8–14.

[2] Wieland, G. R., 1906: American Fossil Cycads, pp. 296, Pl. L. Carnegie Inst. Publ., No. 34.

EXPLANATION OF PLATE XIV.

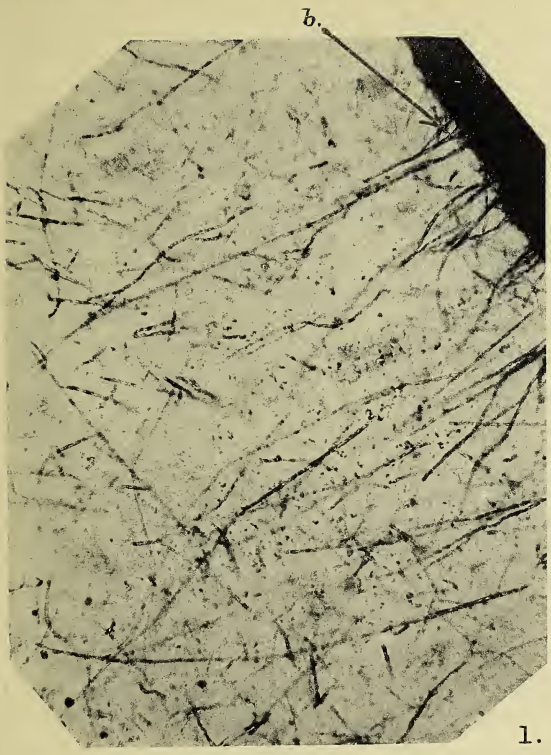
Illustrating Dr. Stopes's paper on Roots in *Bennettites*.

Fig. 1. Weft of root-hairs running in space between the leaf-base tissues. *b*, root-hair base attached to rootlet.

Fig. 2. Free rootlets with root-hairs attached.

Fig. 3. Longitudinal section of rootlet showing cortical and vascular tissues.

Fig. 4. Rootlet running in the tissue cells of the leaf-base: in this position the rootlets are without root-hairs.



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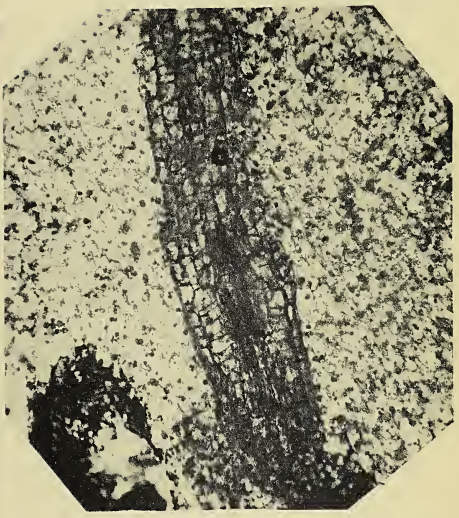

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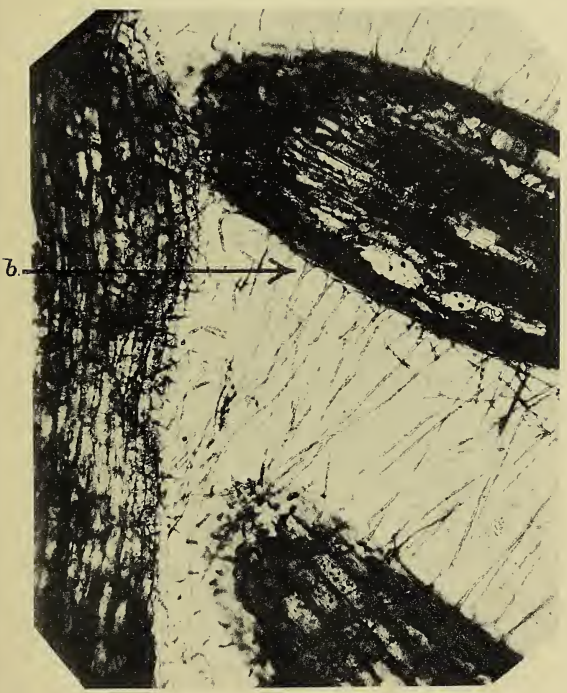
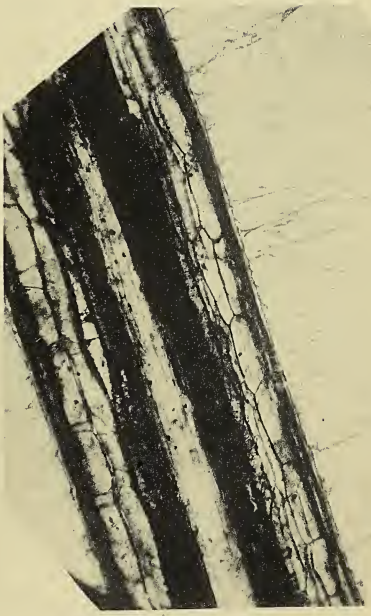


2.



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Huth coll.

STOPES — ROOTLETS IN BENNETTITES.

Irritability of the Pollen-presentation Mechanism in the Compositae.

BY

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IN view of the frequency with which this phenomenon has been found during the past year in the Compositae and its appearance in several common British species, it has been thought advisable to publish this preliminary list of irritable species and varieties. The data form part of a thesis in course of preparation, therefore they will not be discussed fully. The present contribution aims only at recording those species in which irritability has been observed for the first time.

Few previous authors have recorded the exact type of irritability, and none has recorded the degree of irritability observed (1–8 and 11), but in the present list those hitherto neglected features of the phenomenon are recorded thus:

Types of Irritability.

A: pollen presented on touching; no lateral movement of the anther tube.
B: " " " " ; lateral movement, indefinite in direction.
C: " " " " ; lateral movement towards the touch.
C^1: " " " " ; lateral movement towards the centre of the capitulum.
C^e: " " " " with more or less explosive rapidity.

The degree of irritability is noted as slight when it requires careful observation, and notes are added on special cases.

The phenomenon has been well known in the Cynareae since the seventeenth century at least, but very few records are to be found in the literature of the subject of irritable *species*, even in the Cynareae. Juel (4) records thirty-five species in various tribes; Kolreuter (6) records twenty-three species of which thirteen are *Centaureae*, and other authors have recorded only a few more.

The method used in the present investigation was to touch the anther tube gently with a needle and observe the result with a watchmaker's

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eyeglass. The following precautions must be noted for successful observations:

(1) Irritability is shown only under good conditions of heat, light, and dryness. The movement is most active before 3 p.m. On cold or wet days species which show marked irritability under the proper conditions show very little or no movement.

(2) Irritability is frequently shown only after the anther tube has opened and before the style is exserted, but there are many exceptions to this rule, especially in the Cichorieae.

(3) Although several species continue to show irritability after the flowers have been removed from the plant, in most cases movement ceases after this treatment.

(4) The anther tube should be touched *gently*.

(5) The observation of the 'A' type of movement requires careful and sometimes prolonged examination of numerous florets before it can be recorded without doubt or considered absent altogether.

(6) Species which show 'C' under the best conditions may show 'B' or 'A' under less favourable conditions.

(7) Species here recorded as 'A' or 'B' may be found to show 'C' on examination in more favourable circumstances.

The list gives the name of species, place where observed for the first time, and type of irritability, with remarks on the degree of irritability. Many of the observations have been confirmed by colleagues, by students, or by the writer on different occasions. Altogether 233 species and varieties have been examined, and this investigation has yielded 149 records of irritability, mostly new. To facilitate reference an '×' has been placed opposite all British, introduced, or commonly cultivated species. In column 2 of the list K.R.B.G. = Royal Botanic Gardens, Kew; E.R.B.G. = Royal Botanic Garden, Edinburgh; C.U.B.G. = Cambridge University Botanic Garden; C.P.G. = Chelsea Physic Garden; A.C.G. = Armstrong College Garden; F. = in the field. The numbers in brackets in the fourth column refer to the notes at the end of the list.

Genus and Species.	*Place of Observation.*	*Type of Irritability.*	*Remarks.*
Achillea alpina, L.	K.R.B.G.	C	slight
Achillea Clavennae, L.	E.R.B.G.	C	slight
Achillea crustata, Schur.	E.R.B.G.	A	marked
Achillea grandiflora, Bieb.	K.R.B.G.	C	very marked
Achillea holosericea, Sibth. and Sm.	E.R.B.G.	A	slight
Achillea magna, L.	K.R.B.G.	C	distinct
× Achillea millefolium, L.	F.	C	distinct (16)
× Achillea Ptarmica, L.	F.	C	distinct (16)
Amellus annuus, Willd.	K.R.B.G.	C	slight

Genus and Species.	*Place of Observation.*	*Type of Irritability.*	*Remarks.*
Ammobium alatum, R. Br.	K.R.B.G.	A	slight (1)
Anacyclus officinarum, Heyne	K.R.B.G.	C	distinct
Anaphalis margaritacea, Benth. and Hook.	E.R.B.G.	C	distinct
Anthemis canescens, Brot.	E.R.B.G.	A	distinct
Anthemis montana, L., v. grandiflora	K.R.B.G.	C	distinct
× Anthemis nobilis, L.	F.	C	slight
× Anthemis tinctoria, L.	F.	C	distinct
Arnica alpina, Olin and Ladau.	E.R.B.G.	B	distinct
Aster acris, L.	E.R.B.G.	C	distinct
Aster Candollei, Harv.	K.R.B.G.	A	slight
Aster Lipskyi (Hort.)	K.R.B.G.	C^1	marked
Aster longifolius, Lam.	E.R.B.G.	C	variable
× Aster paniculatus, Lam.	E.R.B.G.	A	slight
Aster stellaris, (Hort.)	K.R.B.G.	C	variable
Balduinia multiflora, Nutt.	E.R.B.G.	C	slight but distinct
× Bellis perennis, L.	F.	C	distinct (2)
Buphthalmum salicifolium, L.	K.R.B.G.	B	distinct
Buphthalmum speciosum, Schreb.	E.R.B.G.	C	slow
× Calendula officinalis, L.	K.R.B.G.	A	slight (1)
× Carduus crispus, L.	K.R.B.G.	C	distinct (16)
× Carduus nutans, L.	K.R.B.G.	C	distinct
× Carduus tenuiflorus, Curt.	F.	B	distinct
Cassinia Vauvilliersii, Hook. f.	E.R.B.G.	A	distinct
Catananche caerulea, L.	K.R.B.G.	C	marked (3), Juel records as 'irritable'
Catananche caerulea, L., v. alba.	K.R.B.G.	C	very marked (3)
Celmisia coriacea, Raoul.	E.R.B.G.	C	distinct
× Centaurea aspera, L.	Rouen B.G.	B	distinct
Centaurea atropurpurea, Waldst. and Kit.	E.R.B.G.	C	slight
Centaurea axillaris, Willd.	K.R.B.G.	C^1	distinct
× Centaurea Cyanus, L.	F.	C^1	previously recorded as 'irritable'
Centaurea dealbata, Willd.	K.R.B.G.	C	distinct (12)
Centaurea montana, L.	C.U.B.G.	C	distinct (12)
Centaurea montana, L., v. alba.	C.U.B.G.	C	distinct
Centaurea montana, L., v. lugdunensis, Jord.	K.R.B.G.	C	distinct
× Centaurea nigra, L.	F.	C	distinct but variable
Centaurea praealta, Boiss. and Bal.	E.R.B.G.	C	distinct
× Centaurea Scabiosa, L.	E.R.B.G.	C	distinct (16)
Centaurea sordida, Willd.	E.R.B.G.	C	marked
Centaurea variegata, Lam.	E.R.B.G.	C	distinct
Charieis heterophylla, Cass.	K.R.B.G.	C	marked

Genus and Species.	*Place of Observation.*	*Type of Irritability.*	*Remarks.*
Chrysanthemum carinatum, Schousb.	K.R.B.G.	C	distinct
× Chrysanthemum coccineum, Willd. (= Pyrethrum roseum, Bieb.)	C.P.G.	C	distinct (4)
Chrysanthemum coronarium, L.	K.R.B.G.	C	distinct
Chrysanthemum corymbosum, L.	K.R.B.G.	C	slight
× Chrysanthemum Leucanthemum, L.	F.	C	slight but variable
Chrysanthemum macrophyllum, Waldst. and Kit.	K.R.B.G.	C	slight
Chrysanthemum maximum, Raymond	E.R.B.G.	C	slow but distinct
Chrysanthemum praealtum, Vent.	K.R.B.G.	C	distinct
Chrysocoma Coma-aurea, L.	K.R.B.G.	C	distinct (1)
Chrysopsis villosa, DC.	K.R.B.G.	C	only after presentation has begun
× Cichorium Intybus, L.	F.	C	distinct (16)
× Cineraria stellata, (Hort.)	A.C.G.	A	slight
× Cirsium acaule, Weber.	E.R.B.G.	C	distinct
Cirsium eriophorum, Roth.	E.R.B.G.	B	slight
Cirsium Kerneri, X. (Hort.)	C.U.B.G.	B	distinct (12)
× Cirsium lanceolatum, Scop.	F.	C	distinct
× Cirsium palustre, Scop.	F.	C	marked
Coreopsis grandiflora, Nutt.	K.R.B.G.	A	slight
Coreopsis Grantii, Oliv.	K.R.B.G.	C	'A' distinct, 'C' slight
Coreopsis tinctoria, Nutt.	K.R.B.G.	A	distinct
Crepis blattarioides, Vil.	K.R.B.G.	A	distinct (3)
× Crepis virens, L.	F.	C	distinct (3)
Dimorphotheca aurantiaca, DC.	K.R.B.G.	C	marked
Dimorphotheca Ecklonis, DC.	E.R.B.G.	C	very marked
× Dimorphotheca pluvialis, Moench. (= Calendula pluvialis of gardens)	K.R.B.G.	C	marked
× Doronicum Pardalianches, L.	A.C.G.	C	distinct
× Dugaldia Hoopesii, Rydberg (=Helenium Hoopesii, A. Gr.)	C.P.G.	C	distinct
Erigeron aurantiacus, Regel.	E.R.B.G.	A	distinct
Erigeron Coulteri, Porter and Coulter	C.P.G.	A	distinct
Erigeron glabellus, Nutt.	K.R.B.G.	A	distinct
Erigeron grandiflorus, Hook.	K.R.B.G.	A	distinct
Erigeron macranthus, Nutt.	K.R.B.G.	C	marked
Erigeron multiradiatus, Benth. and Hook.	K.R.B.G.	C	marked (6)
Erigeron Rusbyi, A. Gr.	K.R.B.G.	B	'A' distinct, 'B' slight
Erigeron speciosus, DC.	K.R.B.G.	A	distinct
× Gaillardia aristata, Pursh, v. grandiflora, (Hort.)	K.R.B.G.	C	distinct

Genus and Species.	*Place of Observation.*	*Type of Irritability.*	*Remarks.*
Gerbera hybrida, (Hort.)	K.R.B.G.	C^e	distinct (7)
Gerbera Jamesoni, Bolus.	E.R.B.G.	C^e	marked (7)
Grindelia squarrosa, Dunal.	E.R.B.G.	C	distinct
Helenium autumnale, Lind., v. pumilum, Willd.	E.R.B.G.	C	slight
Heliopsis gratissimus	K.R.B.G.	C	distinct (8)
Heliopsis padula, Wender.	E.R.B.G.	C	slow but distinct
Heliopsis scabra, Dun.	K.R.B.G.	C	distinct (6)
Heliopsis scabra, Dun., v. pitcheriana	E.R.B.G.	C	very slow but distinct
Helipterum Manglesii, Muell.	K.R.B.G.	C	very marked (9)
Helipterum roseum, Benth.	K.R.B.G.	A	distinct (1)
Hieracium grandifolium, Sch. Bip.	K.R.B.G.	C	slight (3)
Hieracium maculatum, Schrank	K.R.B.G.	C	slight (3)
× Hieracium murorum, L.	F.	C	slight (3)
Hieracium pallidum, Biv.	K.R.B.G.	C	slight (3)
× Hypochaeris radicata, L.	F.	C	marked (3)
Inula Conyza, L.	E.R.B.G.	C	slight
Inula glandulosa, Puschk.	K.R.B.G.	C	distinct
Lactuca bracteata, Hook. f.	K.R.B.G.	C	slow (3), 'A' very clear
Lactuca hastata, DC.	K.R.B.G.	C	slow (3)
× Lactuca perennis, L.	K.R.B.G.	C	slight (3)
Lactuca Plumieri, Gren. and Godr.	K.R.B.G.	C	slow (3)
× Lapsana communis, L.	F.	C	marked (13)
Layia gaillardioides, Hook. and Arn.	K.R.B.G.	C	slight
× Leontodon hirtus, L. (=L. nudicaulis, Banks)	K.R.B.G.	C	distinct (3)
Leuzea conifera, DC.	K.R.B.G.	C	marked
× Matricaria Chamomilla, L.	F.	A	distinct
× Matricaria inodora, L.	F.	C	distinct
Moscharia pinnatifida, Ruiz and Pav.	K.R.B.G.	B	'A' distinct, 'B' slight
Odontospermum maritimum, Sch. Bip.	E.R.B.G.	C	marked
× Olearia dentata, Moench.	K.R.B.G.	A	very slight
Othonna carnosa, Less.	E.R.B.G.	C	variable
Othonnopsis cheirifolia, Benth. and Hook.	E.R.B.G.	A	very slight
Perezia multiflora, Less.	C.U.B.G.	C^e	marked (7)
Pluchea Bulleyana, Jeffrey	E.R.B.G.	C	distinct
× Pulicaria vulgaris, Gaertn.	E.R.B.G.	A	distinct
Rudbeckia ampla, Nelson	K.R.B.G.	C	distinct
Rudbeckia triloba, L.	E.R.B.G.	C	slight
× Santolina Chamaecyparissus, L.	E.R.B.G.	C	distinct
Saussurea Yakla, C. B. Clarke	K.R.B.G.	C	distinct

Genus and Species.	*Place of Observation.*	*Type of Irritability.*	*Remarks.*
× Scorzonera hispanica, L.	K.R.B.G.	C	slow but very distinct (3)
Scorzonera purpurea, L.	K.R.B.G.	C	slow but clear (3)
Senecio adonidifolius, Loisel.	K.R.B.G.	C	distinct
× Senecio aquaticus, Hill	F.	B	'A' distinct, 'B' slight
Senecio bellidioides, Hook. f.	E.R.B.G.	C	distinct
Senecio (hortensis)	K.R.B.G.	C	marked (10)
× Senecio Jacobaea, L.	F.	C	'A' distinct, 'C' slight
Senecio Ledebouri, Sch. Bip.	K.R.B.G.	A	slight
Senecio Ligularia, Hook. f.	K.R.B.G.	A	slight
Senecio Ligularia, Hook. f., v. speciosa	K.R.B.G.	C	slight and slow
× Senecio palustris, Hook.	E.R.B.G.	C	distinct
Senecio populifolius, DC.	E.R.B.G.	C	marked (11)
× Senecio squalidus, L.	K.R.B.G.	C	distinct
Senecio tropaeolifolius, MacOwan	E.R.B.G.	C	distinct
× Silybum Marianum, Gaertn.	C.U.B.G.	C	distinct (12)
× Solidago serotina, Ait.	F.	C	distinct
× Solidago Virgaurea, L.	F.	C	slight
× Sonchus asper, L.	F.	C	distinct (3)
× Sonchus oleraceus, L.	F.	C	slight but distinct (3)
× Tanacetum vulgare, L.	E.R.B.G.	A	distinct (16)
× Taraxacum officinale, Weber	F.	C	distinct (3) (17)
Tragopogon orientalis, L.	K.R.B.G.	C	slow (3)
Ursinia cakilefolia, DC.	K.R.B.G.	A	distinct
Ursinia pulchra, N. E. Br.	A.C.G.	C	distinct but variable
Ursinia speciosa, DC.	A.C.G.	B	'A' distinct, 'B' slight

Notes.

(1) *Helipterum roseum* and *Calendula officinalis* are recorded by Juel (4) as not irritable, but it must be borne in mind that these show the 'A' type only, and that species which are irritable in these latitudes may not show that character farther north. *Chrysocoma Coma-aurea* and *Ammobium alatum* are recorded as irritable by Juel but with a ?.

(2) In *Bellis perennis* the movement is sometimes slight, and at other times it has been observed to split the anther tube against the comparatively thick and rigid style. The anther tube is frequently quite loose around the style, and the movement can be seen clearly to be due to the stamens and not to any movement of the style.

(3) The lateral movement in the Cichorieae is slow, and the presenta-

tion of the pollen requires careful observation, as it frequently continues after the style branches have diverged.

(4) Movement is sometimes shown in cut flowers of this species.

(5) This species has orange pollen and dark purple anthers, and the presentation is therefore very easily seen.

(6) In *Erigeron multiradiatus* and *Heliopsis scabra* the movement is greatest when the posterior side of the anther tube is touched, giving a movement towards the centre of the capitulum.

(7) In *Gerbera Jamesoni* and *Perezia multiflora*[1] the outer florets are much more active than the inner, and the greatest lateral movement is towards the centre of the capitulum. In the former half the pollen is expelled at the first touch, and the rest at the second. In the latter all the pollen is expelled at the first touch. In *Gerbera hybrida* the pollen is extruded in small quantity, but more rapidly than in the 'C' type, and the movement is towards the touch.

(8) In *Heliopsis gratissimus* the anthers are forced apart on being touched, and the pollen is presented along the slit, which does not at first extend to the base of the anther tube. In the evening, however, the stamens are more or less free from each other in this species. This breaking of the anther tube has been observed in several other genera, e. g. *Senecio grandifolius*, *Celmisia verbascifolia*, &c., and has been recorded by Moore (10) in *Cratistylis conocephala*.

(9) In *Helipterum Manglesii* the movement towards the touch through an angle of about twenty degrees is immediately followed by a movement in the reverse direction of about half that magnitude.

(10) This seems to be a garden variety of *Senecio populifolius*.

(11) In *Senecio populifolius* the presentation is very distinct, and if the staminal tube is touched at the proper stage the style branches may be seen to protrude and diverge immediately the anthers are retracted.

(12) *Centaurea montana*, *C. dealbata*, *Cirsium Kerneri*, and *Silybum Marianum* were recorded in 1915 (12).

(13) The mechanism of the lateral movement is very clear in *Lapsana communis*; the five filaments can be seen quite well. They are slightly bent, and if one of them be touched with the needle-point it is seen to straighten by contraction while the other filaments become more bent, thus tilting the anther tube in the direction of the stimulated filament.

(14) Irritability of the style has been recorded in the Compositae only in the genus *Arctotis* (4, 9, and 12).

(15) An irritable pollen-presentation mechanism similar to that of Compositae was observed in *Lobelia thapsoides*, Cham., in the Temperate House of the Royal Botanic Gardens, Kew.

(16) Among the British species recorded as 'irritable' by Juel (4) are

[1] First recorded in 1915 (12) under the synonym *Gerbera multiflora*.

Inula Helenium, *Antennaria dioica*, *Tanacetum vulgare*, *Achillea Ptarmica*, *A. millefolium*, *Senecio sarracenicus*, *Centaurea Cyanus*, *C. Jacea*, *C. Scabiosa*, *Cirsium arvense*, *Carduus crispus*, *Lapsana communis*, and *Cichorium Intybus*.

(17) First recorded in August, 1916, v. literature cited (13).

Acknowledgements are due to the Director of the Royal Botanic Gardens, Kew ; the Regius Keeper of the Royal Botanic Garden, Edinburgh ; the Curator of the Cambridge University Botanic Garden ; and to the Curator of the Chelsea Physic Garden, for the facilities which they very kindly granted for the examination of living material.

Summary.

1. Various types of sensitive movements in the Compositae are distinguished.

2. A list of 149 species and varieties showing irritability is given in which the type and degree of irritability observed are recorded.

3. Irritability was found in 64 per cent. of the species and varieties examined, and in all the tribes of the family except the Eupatorieae and the Vernonieae.

4. Notes on special, interesting cases, such as the explosive irritability in the Mutisieae, and the peculiar, slow movement in the Cichorieae, are appended.

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The Spermatogenesis of Polytrichum juniperinum.

BY

CHARLES E. ALLEN.

With Plates XV and XVI.

Introduction.

In a previous paper (Allen, 1912) I have followed the history of the successive generations of cells that occupy the interior of the antheridium of *Polytrichum juniperinum* down to the final division—that, namely, of the androcyte mother-cells. The present study is concerned with the metamorphosis into antherozoids of the androcytes formed by this division. Certain of the observations here to be described have already been briefly reported (Allen, 1908, 1913, 1914).

The methods used in this work were in the main those described in my paper of 1912. For the study of the fully developed antherozoids, the mass oozing from a mature antheridium into a drop of water was exposed for a minute or two to the fumes of osmic acid, or, in most cases, the slide bearing the drop was inverted over a small wide-mouthed bottle containing medium or strong Flemming's solution. After fixation by this means, the slide was allowed to dry thoroughly, drying sometimes being hastened by a brief heating over a flame. Then the slide was immersed in the stain or stains to be used, thoroughly dried again, a drop of clove oil or other clearing agent was placed upon the material, and this was followed by Canada balsam. Dehydration of the stained preparations by alcohol was avoided after a few experiences, since it was found that the alcohol quickly extracts all colour from the cilia. Numerous stains and combinations of stains were used for the antherozoids so prepared; the best results were obtained with acid fuchsin and iodine blue, safranin and gentian violet, and safranin and pyoktanin blue.

Observations.

A description has previously been given (Allen, 1912) of the small darkly staining body present in each young androcyte. This dense granule seems to be identical with that which behaved like a central body in the last preceding mitosis; in the development of the androcyte into an

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antherozoid it is destined, as will be seen, to function as a blepharoplast. When a pair of sister androcytes can be recognized as such (as in Figs. 1 and 2, Pl. XV), the blepharoplast in each is usually to be found in a position corresponding to that of a central body in the division recently completed. The blepharoplast is sometimes (Figs. 1–3) the centre of a system consisting of a few radiations much like those that surrounded the single central body which was found previous to the initiation of mitosis in each androcyte mother-cell. The cytoplasm immediately about the blepharoplast is commonly somewhat more dense than that in other parts of the cell. The cytoplasmic contraction and rounding up, which began in the androcyte mother-cells, have now gone on to a considerable extent. The walls (excepting those laid down in the course of the last division) are much thickened and show evidence of softening. The chromatic substances within the nucleus still exhibit more or less of the forms of the chromosomes; in some cells (Fig. 2) there is often no body as yet recognizable as a nucleole. It was pointed out in the paper just cited that nucleoles make their appearance much later in the androcyte nuclei than they do in the daughter nuclei formed by any of the androgonial divisions. However, in some androcytes of about the same age as those shown in Fig. 2 (e.g. Figs. 1 and 4), bodies appear whose nucleolar nature is evidenced by their usually rounded shape and their affinity for safranin. Later (Figs. 13, 14, &c.), a single fairly large nucleole is always present in the androcyte nucleus. But this nucleole, though sometimes, especially in the earlier stages (Fig. 4), rather irregular in shape, is usually more or less rounded; it is neither so irregular nor so large as the nucleolar masses of the preceding cell generations, which were found to consist of a nucleole about which more or less chromatin was aggregated.

The blepharoplast, originally (Fig. 1) very small, early begins to increase in size. In the androcytes shown in Fig. 2, each blepharoplast is somewhat elongated. The blepharoplasts of the cells shown in Figs. 3 and 4 (which are in the same antheridium as are those shown in Fig. 2) seem to have grown more or less in all dimensions, although that of Fig. 3 is longest in a direction oblique to the plane of the section. Whatever be the differences in this respect at this early period, the growth tendency very soon shows itself exclusively in an elongation (Figs. 5, 6, 7, &c.). Excepting that it lies in the cytoplasm, I cannot find that at this time there is any rule governing the position of the blepharoplast. It may lie in contact with the nucleus or with the plasma membrane, or it may be at any distance, or its different parts may be at varying distances, from the nucleus on the one side and from the plasma membrane on the other. In the early stages of its elongation (Figs. 5, 10, 12), the blepharoplast is likely to be more or less uneven in thickness; sometimes (Fig. 5) one end is noticeably thicker than the other. But as its growth goes on (Figs. 11, 13), it becomes a curved rod of quite

uniform thickness, excepting that it is slenderer toward the ends, one or both of which may be pointed. The blepharoplast is stained deep black in iron-alum-haematoxylin, and deep blue in the triple stain. Some authors—for example, Ikeno (1903) in his study of *Marchantia*, Arens (1907) and the van Leeuwen-Reijnvaans (1908) in their investigations of *Polytrichum*—have described the blepharoplast as growing only into a relatively short body to whose posterior end is attached another thread-like or band-like structure formed by a gradual differentiation within the cytoplasm. Something similar is reported by Walker (1913) for *Polytrichum*. I cannot confirm these descriptions. All the evidence furnished by my preparations indicates that the long, darkly stained rod (shown for example in Figs. 11–14) is a homogeneous body formed by the growth of the originally small blepharoplast. In this respect my conclusions agree with those reached by Yamanouchi (1908) and Miss Allen (1911) in studying the spermatogenesis of Ferns, by Woodburn (1911, 1913) and Miss Black (1913) working with various Hepaticae, and by Woodburn (1915) in his study of *Mnium*.

While the blepharoplast is still comparatively short, another definite cytoplasmic body appears (Figs. 5, 9, 10). This is the large *limosphere*, to use a term suggested by M. Wilson (1911). This body seems to correspond to the one which, in the androcytes of *Marchantia*, was called by Ikeno (1903) the '*chromatoider Nebenkörper*'. It is certain that in *Polytrichum*, at any rate, the body in question is quite dissimilar in behaviour and history to the structure appearing in the spermatids of certain animals to which the name *chromatoider Nebenkörper* was first applied; and for this reason the term introduced by Wilson will be used in the present paper. The limosphere is at first rather faintly stained (commonly taking the orange of the triple stain); it seems to arise by a concentration of a portion of the cytoplasm. Indeed, the bodies shown in Figs. 9 and 10 are so slightly differentiated from the rest of the cytoplasm by their tint and their greater degree of homogeneity that one can hardly be certain either of their exact outline or of their identity with the body which shortly afterwards is so conspicuous. However, a comparison of these figures with Figs. 5 and 12, in which the limosphere is very distinct, although orange in colour, and with Fig. 13, in whose cells also the limosphere is slightly stained, leaves little doubt that it is the same body which is seen in each case. Except for a brief period at the beginning of its existence, the limosphere is deeply stained by iron-alum-haematoxylin and by the safranin of the triple stain. As a rule, the outer portion of the limosphere stains more deeply than most of its internal substance, so that it looks much like a hollow sphere (or, as Figs. 11, 12, &c., show, a ring in cross section). Very commonly there is also some deeply-stained material in the interior (Figs. 11, 12); but sometimes (Fig. 21) the stain is confined entirely to the outer shell. At still other times the limosphere seems to be uniformly stained throughout. This

is especially likely to be true shortly after its first appearance (Figs. 9, 10, 13); it is possible that in such cases there is no differentiation as yet between its outer and inner portions, although sometimes (e.g. Figs. 5 and 12) this differentiation certainly appears at a very early stage. The sections from which Figs. 14, 16, 17, 18, and 20 were drawn were stained first in gentian violet and then in safranin, thus reversing the usual order. Under this treatment the limosphere becomes deep red throughout; probably its apparent homegeneity in these cases is due merely to the unusually deep colour and consequent opacity of its outer shell. This notion is supported by the differentiation shown in the limosphere of Fig. 15, which is drawn from the same antheridium as are Figs. 14 and 16.

While the blepharoplast is growing, the arrangement of nucleus, blepharoplast, and limosphere seems decidedly variable. Cells may be found (compare those represented in Figs. 9–13) which show these three bodies in almost every conceivable spatial relation to one another as well as in almost every possible position within the cell. It does not follow, of course, that there is no relation between the positions of the nucleus, blepharoplast, and limosphere because no constancy in this respect is to be detected. When the androcyte is first formed, it plainly has a polar organization. Later, as we shall see, the different cell organs take up very definite positions with reference to one another, and the cell again displays a marked polarity. It is not necessary to assume that the polarity manifested during the earlier and the later history of the cell is lost in the period now under consideration, simply because, in the changes and shiftings incident to the formation of the limosphere and the elongation of the blepharoplast, these bodies take various positions with reference to the nucleus and to one another.

Fig. 13 shows a condition that is not infrequently found—namely, the presence at the distal end of the antheridium of a small group of androcytes (represented by the two uppermost ones in this figure) that are much larger than any others in the same antheridium and are provided with proportionally large nuclei and blepharoplasts. Similarly placed groups of large androcytes have been found at somewhat later stages than that here shown, but it was impossible to distinguish the details of their structure and they appeared to be in process of breaking down; no unusually large antherozoids have been found; and it is not unlikely that in all cases the giant androcytes cease their development comparatively early and become disorganized. Possibly comparable in a measure with these are the 'double spermatids' of *Mnium* observed by Wilson (1911), which are of about double the normal size, each containing two blepharoplasts, two limospheres, and probably two nuclei. Ikeno (1903) also describes an unusual growth of occasional androcytes in *Marchantia*; however, he thinks these giant cells divide, each thus forming two androcytes of normal size.

By the time the growing blepharoplast has reached a length equal to from two-thirds to three-fourths of the circumference of the cell (Figs. 14–20) its position seems to be more definitely fixed. It now lies close to, and for the most part in contact with, the plasma membrane, with one end near, but apparently not yet touching, the limosphere. This end of the blepharoplast will be referred to hereafter as its anterior end because it is to be concerned in forming the anterior portion of the body of the antherozoid. Arens (1907) finds that in both *Polytrichum* and *Mnium* the anterior end (constituting the whole of what he calls the blepharoplast) lies usually at that side of the androcyte which is towards the apex of the antheridium. I have not found any such regularity in the position of the blepharoplast in my material, nor has Wilson (1911) in his study of the androcytes of *Mnium*. In Figs. 14, 15, 16, and 19, only that part of the blepharoplast is shown which lies on the side of the androcyte turned towards the observer; in Figs. 17 and 18 the blepharoplast is shown throughout most or all of its length. The cell represented in Fig. 20 is unusual in the fact that the blepharoplast is in contact throughout the greater part of its length with the nuclear membrane and so at some distance from the plasma membrane. Apparently it is at about this time that the cilia first appear. At any rate, the youngest cilia-bearing cells that I have seen are those shown in Figs. 16 and 19. In each case the single cilium that is visible seems to be attached to the blepharoplast somewhat behind the anterior end of the latter. It should be noted that the methods used in staining and dehydrating the sections containing these cells were such as to leave little or no colour in structures so slender as the cilia; and also that in sectioning the material the cilia of any particular androcyte are likely to have been in whole or in part cut away. These facts doubtless explain why cilia are often not visible, even in the cases of cells much older than those shown in Figs. 16 and 19; and it is quite possible that the cilia begin their growth at a period earlier than that at which I have first seen them.

In the stages now under discussion, a dark-staining cytoplasmic granule, much smaller than the limosphere, is often conspicuous. Sometimes this granule seems to lie in a vacuole (Figs. 19, 25); sometimes it is elongated (Fig. 16); sometimes there are two similar granules (Fig. 18), of which one may be in a vacuole (Fig. 24, lowermost cell); sometimes (Figs. 14, 15) no such definite body is to be seen. Thus appearances are too variable at this time to justify any general statement concerning these bodies; but it is not improbable that one or more of them may correspond to a similar though larger body which at a somewhat later period seems to be a nearly or quite constant constituent of the androcyte.

The nucleus, which as a rule has remained thus far in the central region of the cell, now moves towards the periphery on that side at which the middle portion of the blepharoplast lies (Fig. 21). The nucleus becomes

pressed against the blepharoplast and the plasma membrane in this region. Fig. 19 represents a cell whose nucleus has assumed the peripheral position fairly early; in other respects, however, this cell seems to be in about the same stage of development as are those shown in Figs. 17 and 18, whose nuclei are still centrally located.

The next conspicuous change to be noted in the developing androcyte is a division of the limosphere. The division is preceded by an elongation of the limosphere in a direction perpendicular to the anterior portion of the blepharoplast, to which the limosphere now seems, at least in most cases, to be attached (Figs. 21, 22). Then follows a constriction (Figs. 23, 24), and finally a division (Figs. 25, 26) into two bodies which usually differ noticeably in size. The smaller of the two, hereafter referred to as the *apical body*, remains in contact with the anterior end of the blepharoplast; the larger one, since it is of approximately the size, and retains essentially the appearance, of the limosphere before its division, will still be spoken of as the *limosphere*. It moves away from the anterior end of the blepharoplast (Fig. 26) and soon comes to lie in contact with the nucleus (Pl. XVI, Fig. 27). The apparently divided limosphere shown in Fig. 20 (Pl. XV) represents a different condition; the androcyte from which this drawing was made is the only one I have found at so early a stage which contained a double (or constricted) limosphere, although less marked irregularities in the outline of this body have sometimes been observed. It is possible, though I think hardly likely, that the condition in Fig. 20 indicates a premature division of the limosphere. Something apparently equally removed from the usual course of events is shown in the lowermost androcyte of Fig. 24, whose limosphere seems to be dividing into four bodies instead of two.

Sometimes, when the apical body and the limosphere are moving away from each other (Fig. 28, Pl. XVI), they seem to be connected by a definite fibre or strand. This has not been observed in the majority of cases, but in many of the androcytes in the antheridium from which Fig. 28 was drawn this connecting strand is plainly visible. If it is generally present at these stages, the staining methods used are not adequate invariably to demonstrate the fact.

At about the time that the limosphere divides, the nucleus begins to elongate in a direction parallel to the length of the blepharoplast. The earliest evidences that I have found of the extension of the nucleus are in the androcytes shown in Figs. 22 and 23, Pl. XV. In each of these cases the nucleus is drawn out into a point that is directed towards the anterior end of the blepharoplast, the posterior part of the nucleus remaining rounded. The elongation of the nucleus seems usually to begin in this way; but there are exceptions to this rule, as in the androcyte shown in Fig. 25, whose nucleus is rounded anteriorly and somewhat drawn out at its

posterior end. It is evident from my preparations that the nucleus does not always begin to elongate at precisely the same stage; for the nuclei of Figs. 26 (Pl. XV) and 27 (Pl. XVI) show no evidence of a change in shape at a time when the apical body has been completely separated from the limosphere.

The further extension of the nucleus is the central feature of the later history of the androcyte. In this process the nucleus stretches out along, and becomes closely applied to, the blepharoplast. Usually the anterior beak of the nucleus increases in length (Fig. 30), the posterior end remaining rounded, sometimes to a relatively late period (Fig. 35). Sooner or later, however, the posterior end also becomes pointed. This may occur quite early (Figs. 28, 31), and probably always takes place before the nucleus has attained a length anywhere nearly equal to the circumference of the cell (compare Figs. 36, 38, and 39). As a rule the anterior portion of the nucleus continues to stretch more rapidly than the posterior portion, consequently the latter remains much thicker down to a very late stage (Figs. 39–41, 43, 44). Fig. 42 represents a condition rarely found, in which the posterior part of the nucleus has developed a slender prolongation nearly as long as that derived from the anterior part; as a result, the nucleus in this instance is thickest in its middle portion. Fig. 47 shows a somewhat similar condition at a later period, in which, however, the posterior prolongation is much shorter than the anterior one. The anterior prolongation of the nucleus is sometimes (Figs. 42, 43) bent into a double curve, as though an external resistance had been encountered in the course of its extension.

Apparently the anterior end of the nucleus never extends quite to the anterior end of the blepharoplast (Figs. 35–38). As the elongation of the nucleus continues and its anterior part becomes more slender until its diameter is little greater than that of the blepharoplast (Figs. 39–44), it becomes increasingly difficult to determine exactly how far forward the nucleus extends. However, even at these later stages, the anterior end of the continuous body that is now being formed by the blepharoplast and nucleus is stained exactly as the blepharoplast was stained before—being, for example, coloured somewhat more deeply by iron-alum-haematoxylin than the chromatic materials of the nucleus, and sometimes retaining the stain when the substances that are certainly nuclear are quite decolorized; it seems highly probable that this, which is to be the anterior extremity of the body of the antherozoid, is formed wholly from the substance of the blepharoplast. This conclusion is supported by the visible persistence of the apical body to a time when the body of the antherozoid is approaching maturity (Figs. 48, 49). The apical body, as we have seen, lies in contact with, and often flattened against, the end of the blepharoplast (Figs. 40, 41, 44, 48, 49). If the prolongation of the nucleus were to extend to the tip

of the blepharoplast, it must push in between the blepharoplast and the apical body; but of such an intrusion there is no indication.

Whether the nucleus, as a result of its elongation, finally extends to or beyond the posterior end of the blepharoplast, I have not been able in most cases to determine. As will appear more fully later, the blepharoplast, except for its short anterior portion, usually becomes indistinguishable while the nucleus is still in the early stages of extension. Rarely (as in Figs. 45 and 46) the blepharoplast is visible as a distinct body until a quite late period. In Fig. 45, representing an androcyte, the posterior portion of whose nucleus is towards the observer, the anterior end of the blepharoplast is seen close to the apical body in the upper end of the figure; it can be traced in the preparation from which the drawing was made, along the anterior half of the nucleus (not shown in the figure), and then along the posterior half of the nucleus, which, with the corresponding part of the blepharoplast, appears in the drawing. The posterior tip of the nucleus, and perhaps also that of the blepharoplast, are hidden from view by the limosphere. Fig. 46 shows a different view of an androcyte found in the same antheridium. In this case also the blepharoplast can be traced nearly the full length of the nucleus. The anterior portion of the blepharoplast, apparently extending beyond the end of the nucleus, is shown, and what seems to be the posterior end of the blepharoplast appears near the upper edge of the figure, in close contact with the nuclear membrane. Here it appears that, just as the blepharoplast extends beyond the nucleus anteriorly, posteriorly the nucleus extends beyond the blepharoplast.

The nucleole usually remains conspicuous until the later stages of nuclear elongation (Figs. 43, 45). Thus far there has been no decrease in the bulk of the nucleus; rather, to judge from appearances, the opposite has happened, for, as the figures show, the nuclei in process of elongation often seem much larger than those of young androcytes (compare, e.g., Figs. 31, 37, and 38, Pl. XVI, with Figs. 14–20, Pl. XV). Some of the apparent increase in size is due to the fact that as the nucleus is elongating it also flattens itself against the plasma membrane, thus, in certain views, giving an exaggerated impression of its bulk. Allowing for this source of error, however, it seems true that the progressive change in shape of the nucleus thus far described is actually accompanied by an increase in its size.

When the nucleus has reached nearly or quite its final length (Figs. 43, 44, 47, Pl. XVI), a portion of it still remains comparatively thick. Now ensues a period (Figs. 45, 46, 48–55) during which the diameter of the nucleus (and consequently its volume) is considerably diminished, not only in the region that has thus far remained thick but in its slenderer portions as well. Until this time the reticulate structure of the nuclear contents has persisted. The diminution in volume which now occurs seems to involve the extrusion of the nuclear sap. As a result, the

chromatic constituents (including perhaps the nucleole, which is no longer recognizable) are pressed more closely together (Figs. 48, 49), and finally (Figs. 50–55) the body of the antherozoid, consisting of the nucleus intimately united with, and indistinguishable from, what remains of the blepharoplast, becomes a slender, coiled chromatic rod, somewhat slenderer or even pointed at its anterior and posterior ends. The exact degree of coiling of this body, and perhaps also its length, vary in different antherozoids (compare, e.g. Figs. 51 and 52); as a rule, the body constitutes about one and one-half coils of a spiral.

While the nucleus is undergoing the changes just described, the body of the blepharoplast, except for the short portion at its anterior end, becomes indistinguishable. This is sometimes the case even in the very early stages of nuclear elongation (Figs. 25, Pl. XV; 27, Pl. XVI). However, in other cells at corresponding stages (Figs. 26, Pl. XV; 30 and 31, Pl. XVI), and even sometimes at a considerably later period (Figs. 32, 33, 35), the blepharoplast is still visible throughout its length. Indeed, in the cells shown in Figs. 32 and 35, the blepharoplast seems to be longer than it was seen to be at any earlier period, as though it had continued to grow in length after the change in shape of the nucleus had begun. Sometimes (Figs. 23, Pl. XV; 29, Pl. XVI) the blepharoplast can be followed for some distance backward from the point at which the anterior end of the nucleus is applied to it, but it disappears before the posterior portion of the nucleus is reached. It seems probable that the difficulty in distinguishing the greater part of the body of the blepharoplast is due, not to an actual disappearance of part of its substance, but to some change which makes it less conspicuous. This might be a change merely in its staining reactions; it might be in part due to a stretching of the blepharoplast after the nucleus has begun to elongate, with a resultant decrease in thickness; or it might be because of an intimate union with, and flattening along, the nuclear membrane. The last explanation, however, would not account for the invisibility of the posterior part of the blepharoplast, with which, at such stages as those of Figs. 23 and 25 (Pl. XV), no part of the nucleus is yet in contact; and, on the whole, a change in the reactions of the blepharoplast to stains seems the most probable explanation of its apparent disappearance. This notion is strengthened by such cases as those shown in Figs. 28 and 29, Pl. XVI. In the androcyte represented in Fig. 28, the part of the blepharoplast that is in contact with the nucleus cannot be traced with any certainty; but that part which extends posteriorly beyond the nucleus is visible, though faintly stained. In other androcytes in the same antheridium, the posterior portion of the blepharoplast is more conspicuous. Fig. 29 represents an androcyte found in another section of the same antheridium. In this cell the blepharoplast is plainly visible from its anterior end to about the middle of that part which touches the

nucleus; at the latter point the blepharoplast seems to end, although it is highly probable that conditions are really substantially alike in the two androcytes of Figs. 28 and 29. Sometimes the blepharoplast can be followed for nearly or quite its full length at a much later period, as in Figs. 45 and 46 already referred to. So it seems necessary to conclude, in spite of the frequent impossibility of tracing the blepharoplast throughout its length, that it actually persists without material decrease in size until a comparatively late stage in the history of the metamorphosis of the androcyte. Eventually, however, the blepharoplast (except for its anterior end) seems completely to disappear; and whether this final disappearance is due to its coalescence with the nuclear membrane or to an actual absorption or destruction of its substance, must be left unsettled.

In the later stages of nuclear elongation (Figs. 38–44), the anterior end of the blepharoplast seems to lie outside the boundary of the remaining cytoplasm—whether or not still covered by the plasma membrane is not clear. Occasionally, as in Fig. 44, the apical body as well as the end of the blepharoplast seems to be partly outside the cytoplast. The projecting tip of the blepharoplast probably corresponds to the cytoplasmic *Höcker* described by Strasburger (1892), and also to the refractive *bouton* of Guignard (1889), although the latter author takes the swelling in question for the anterior end of the nucleus. The appearances in my preparations at this time suggest that the anterior end of the developing antherozoid is pushing out and thus beginning to free itself from the cytoplasm that is not to be used in its formation. Something like this is described by Ikeno (1903) as occurring in the spermatogenesis of *Marchantia*. But I do not find in *Polytrichum* a continuation of this process such as Ikeno's figures suggest for *Marchantia*, namely, a further pushing outward of the anterior end so that the unused cytoplasm is as it were forced to the posterior end of the antherozoid. On the contrary, even after the cytoplasm has greatly diminished in amount (Figs. 51, 52), a portion of what is left remains attached to the anterior end of the body of the antherozoid; and the final disappearance of this anterior mass of cytoplasm is one of the last events in the history of the androcyte.

The same difficulty in the observation of cilia that was noted earlier is met with throughout this history, although portions of one or both cilia are frequently to be seen (Figs. 29, 34, 42, 43, &c.). In sections containing mature or nearly mature antherozoids (Figs. 50–2, 54), considerable portions of the cilia are visible; but they can be seen in their full length and in their relation to the body only in preparations that contain unsectioned antherozoids (Figs. 56–9).

The apical body remains for a long time in contact with the anterior end of the blepharoplast. No apparent material change takes place in its size, form, or density down to the latest period (Figs. 48, 49) at which I have

observed it. It is sometimes approximately spherical (Figs. 25, Pl. XV; 34, 45, Pl. XVI); occasionally (Figs. 26, Pl. XV; 37, Pl. XVI) it is slightly elongated in an axis perpendicular to the blepharoplast; oftener it is more or less flattened against the blepharoplast (Figs. 28, 32, 40, &c.). Sometimes differences appear in the staining reactions of different parts of the apical body (Figs. 26, Pl. XV; 37, 38, 42, Pl. XVI); but there is no uniformity in these appearances, and as a rule the whole body is homogeneously stained. The latest stage at which I have seen the apical body is that shown in Figs. 48 and 49, at which the nucleus has attained approximately its final length. After this time the apical body seems to disappear very quickly and completely. Occasionally a mature antherozoid seems to have a slightly thicker portion just behind its pointed anterior tip (Fig. 59), which at times I have thought might be due to the retention of part or all of the substance of the apical body; although it may also be explained by the presence of the anterior portions of the cilia, lying close to, and indistinguishable from, the body of the antherozoid; at any rate, the part (if any) taken by the apical body in the formation of the mature antherozoid is so small as usually (Figs. 56–8) to produce no perceptible effect.

The limosphere, after the formation of the apical body, places itself, as already noted, in contact with the nucleus (Fig. 27). Sometimes (Fig. 28) the limosphere is flattened in the region of contact; oftener (Figs. 32, 35, 39) the nuclear outline is indented as though it had yielded where the limosphere pressed against it. When the nucleus begins to elongate, the limosphere is usually in contact with that portion of the nucleus which is least stretched out, and whose diameter therefore remains greatest (Figs. 28, 30, 35); and since the elongation of the nucleus is most marked at first in its anterior region, the limosphere thus lies nearer the posterior than the anterior end of the nucleus. Sometimes the limosphere lies at the very posterior end (Figs. 33, 37, 45), or close to this end (Figs. 36, 39, 40), of the nucleus, and therefore farther back than the region of greatest nuclear diameter. However, in the rare cases observed in which there is both a posterior and an anterior elongation of the nucleus (Figs. 42, 47), the limosphere is in contact with the thickest portion of the nucleus, and therefore at some distance from its posterior as well as from its anterior end. At the late stage shown in Figs. 48 and 49 the limosphere is still present, enclosed within the posterior part of the coiled nuclear body (Fig. 48) or at the very tip of the latter (Fig. 49). The same is true at still later stages (Figs. 50–3), and the limosphere is similarly placed in antherozoids seen in sections of nearly or quite mature antheridia (Figs. 54, 55). In sections showing these very last stages, there is no longer a visible differentiation between the outer and inner portions of the limosphere.

During that period in the metamorphosis of the androcyte which is represented by Figs. 30–5, a darkly staining body, distinct from both

the apical body and the limosphere, is generally if not invariably present. This body—hereafter referred to, because of its staining properties, as the *percnosome*—is variously located; thus, in Fig. 33 it is shown near the apical body; in Fig. 35, close to the limosphere and in contact with the posterior part of the nucleus; in Fig. 32, between the limosphere and the posterior part of the blepharoplast; in Fig. 31 (and perhaps in Fig. 30, where the percnosome is partly hidden by the nucleus) it is close to the posterior end of the blepharoplast. In the androcyte shown in Fig. 34 the percnosome was not seen, although it appeared in other cells in the same antheridium. The dark object lying over the apical body in Fig. 27, although comparatively small, is probably also the percnosome. As already noted, at still earlier stages in the history of the androcyte a single small cytoplasmic body is often present, sometimes lying in a vacuole. At later stages, also, there is commonly a single small, darkly stained granule located somewhere in the cytoplasm (Figs. 38, 39, 42), towards the close of the history usually in a vacuole (Fig. 47), and apparently becoming smaller (Figs. 48–50) as the development of the antherozoid proceeds. Occasionally at these, as at earlier stages, there is more than one darkly stained cytoplasmic granule (Figs. 41, 44, 46); but in such a case one of the bodies is usually darker and more definite in outline than the others. If these various appearances are properly to be interpreted as evidencing the persistence of the percnosome during the greater part of the history of the androcyte, it is evident that this body is very small at its first appearance, later grows considerably, and then in turn diminishes in size.

In Fig. 35 still another rounded body appears in the cytoplasm near the posterior end of the blepharoplast, and not far from the apical body, though at a somewhat different depth. In the cell from which this figure was drawn, the body in question was stained orange, as was also the apical body, while the percnosome was dark red, as is usual in triple-stained preparations. The orange-stained body in question was not seen in any other androcyte. Possibly it is to be classed among the disintegration products of those parts of the cytoplasm that are not used in the construction of the antherozoid; indeed, the same suggestion might be made regarding the percnosome but for the fact that its apparent long persistence seems to indicate for it some more special significance.

As the development of the body of the antherozoid proceeds, the volume of the androcyte cytoplasm gradually diminishes. The cell as a whole becomes more and more flattened or lenticular, its short axis being perpendicular to the plane in which the coiled body of the antherozoid approximately lies. This change of form is apparent in androcytes which, like those shown in Figs. 43 and 45, are so placed as to show the coiled nucleus more nearly in edge than in side view. Fig. 50 shows two anthero-

zoids in section at a much later stage, when what remains of the androcyte cytoplasm has been reduced to a very thin layer.

It is not easy to determine in just what order the cytoplasm in different parts of the androcyte disappears. It is possible that the vacuole so commonly present in the older androcytes (Figs. 46, 47), which seems to be somewhat larger in still older cells (Figs. 48–50), and which contains the granule that I have identified tentatively with the percnosome, indicates the beginning of the final disappearance of the unused cytoplasm, and that succeeding stages in the process consist in the progressive growth of this vacuole. At the very late stage shown in Figs. 51 and 52, the diminishing cytoplasmic substance is represented by two masses—one, the larger, is attached to the anterior portion of the antherozoid; a second, irregular in form and including the limosphere, is enclosed within the arc formed by the posterior end of the antherozoid. This latter cytoplasmic mass is best shown in Fig. 53, which represents the posterior part of an antherozoid in the same antheridium as those shown in Figs. 51 and 52. By the time of the maturity of the antherozoid (Figs. 54, 55), the anterior cytoplasmic mass has disappeared, but the posterior mass with the limosphere still remains. This persistent remnant of the unused cytoplasm is the cytoplasmic 'vesicle' of many authors.

The gradual swelling and softening of the walls separating the androcytes, which has been described as beginning very early—even before the division of the androcyte mother-cells—continue until the walls, as definite structures, entirely disappear. The androcytes now lie embedded in a substance, evidently derived from the disintegrated walls, which stains very faintly, often so faintly that it shows no stain at all except by contrast with the adjacent uncoloured portions of the preparation. After the walls are disorganized, each androcyte in an antheridium sometimes seems to be enclosed in an envelope (Figs. 41, 45, 50) composed of a material which is itself faintly stained but is distinct from, and evidently denser than, the substance which otherwise fills the spaces between the androcytes. Sometimes this special envelope is only faintly visible, and often it is quite indistinguishable. Whether or not it is always present but visible only in specially favourable cases must be left an open question. When present, the envelope conforms in general to the shape of the androcyte, being therefore lenticular in the later stages (Fig. 50); but it is turgid, and the space within it is by no means completely filled by the androcyte.

In antheridia containing mature antherozoids (Figs. 54, 55) I have never been able to distinguish this envelope. Antheridia at this stage do, however, differ markedly with reference to the stainability of the substance in which the antherozoids are embedded. In the antheridium from which Fig. 55 was drawn, each coiled antherozoid lies in a clear space

or vesicle, whose form is that of the space enclosed in one of the definite envelopes of a somewhat earlier period (Fig. 50); between these vesicles is an orange-stained substance of variable density. The antheridium from which Fig. 54 was made shows no such distinction between vesicular and intervesicular materials; and the substance in which the antherozoids lie shows no stain, excepting that at places where it abuts upon a perfectly clear space it is seen by contrast to have a faint tinge. These two very different conditions may be seen in antheridia lying side by side in the same section, so that it can hardly be due to differences in the exposure to stains; Figs. 54 and 55 were drawn from different sections (in the same preparation) of antheridia from the same head. It is possible that these differences indicate different stages in the development of the antheridium; but I find nothing to support this notion, and consider it more likely that they represent variations in the conditions within different antheridia.

As the antheridium approaches maturity, there is a considerable shrinkage in the mass of its contents, including the androcytes (or antherozoids) and the substances in which they are embedded. This shrinkage is observable both in fixed and in living antheridia; the contents are contracted, usually towards one side, leaving a large part of the cavity of the antheridium apparently empty. This contraction is doubtless due largely or entirely to the fact that the mature antherozoid is much smaller than the androcyte from which it has been formed, most of the cytoplasm of the latter having disappeared and the antherozoid having taken the form of a flattened coil.

When the opening of a mature antheridium in a drop of water is observed under the microscope, each antherozoid is seen to be enclosed in a sharply outlined drop of clear liquid which is embedded in turn in a denser substance. It is this appearance that has led various observers to describe the antherozoid as enclosed in the wall, or the remains of the wall, of the 'mother-cell'. That there is nothing of the nature of a wall is plain from the history already outlined, as well as from the fact that the substances surrounding the antherozoids gradually dissolve in water. For some time the antherozoids can be observed in rapid movement, each within its own vesicle. As the intervesicular substance is gradually dissolved, the vesicles become separated from one another; but the tenacity of the membrane or layer immediately surrounding each vesicle is such that, even though the mass escaping from an antheridium be left in water for as long as two hours, only an occasional antherozoid escapes from its vesicle. Figs. 56–9 represent antherozoids which were fixed and stained after being set free from the antheridium. Those shown in Figs. 56 and 57 were still enclosed in their vesicles when fixed. It is noteworthy that the outline of the vesicle is quite invisible in such a preparation, and that the intervesicular substance

is represented, if at all, only by a faint tinge, although it was plainly visible before fixation. Doubtless the substances surrounding and separating the antherozoids were largely dissolved in the liquids through which the preparation was passed. The antherozoid shown in Fig. 58 is beginning to free itself, probably having pushed its anterior end beyond the boundary of its enclosing vesicle; and that in Fig. 59 is quite free. The difficulty that the antherozoids of *Polytrichum* apparently find in freeing themselves from the enclosing vesicles has been noted by other observers (most recently by Walker, 1913), in contrast with the readiness with which the antherozoids of other Mosses are set at liberty. It may well be that the conditions in this respect are different in nature from those which obtain in a drop of water under observation in the laboratory.

Each antherozoid in motion shows under a comparatively low power of the microscope a refractive spot; with the oil-immersion objective this spot is seen to be a hollow sphere containing many granules in Brownian movement. On one occasion, when an antherozoid that had become free was under observation, this sphere was seen to burst. There can be no question, I think, that the sphere in question is the limosphere, still embedded in the cytoplasmic mass that adheres to the posterior end of the antherozoid. In the case of fixed and stained antherozoids (Figs. 56–9), the cytoplasmic remnants include various darkly staining bodies, among which it is not usually easy to distinguish the limosphere. Apparently, in the course of the processes concerned in making the preparations, the limosphere is often burst. Attached to the posterior tip of the antherozoid shown in Fig. 57 is a body of somewhat irregular shape whose outer portion is darkly stained; this is probably the limosphere, but the identification is not certain. I have not observed the final discarding of the cytoplasmic remnants by the moving antherozoid; but there can be little doubt that, as in the case of the antherozoids of other bryophytes, this now useless material is thrown off at some time before fertilization occurs.

The cilia are apparently attached to the body of the antherozoid a short distance behind its anterior end. Some of the published figures of Moss antherozoids show the cilia diverging from the extreme tip of the body. It is possible in any individual case that the cilia remain appressed against the body, and therefore indistinguishable from it, for a greater or less distance behind their real point of attachment; but this would hardly happen in every instance, and in a careful study of hundreds of antherozoids I have not found one which gave an indication of an attachment of the cilia otherwise than as shown in Figs. 56–9.

From the description which has now been given of the processes concerned in spermatogenesis, it appears that the material of which the body of the antherozoid is composed has been derived, so far as our present methods allow us to determine, entirely from the blepharoplast and the

nucleus. It is possible that some of the substance of the apical body is retained at the tip of the antherozoid; but of this there is no direct evidence. Except for this possibility, a short portion at the anterior end seems to have been formed by the blepharoplast alone. The remainder of the body consists chiefly of the metamorphosed nucleus; and, if the greater part of the blepharoplast persists at all in the mature antherozoid, it has become so closely appressed against, or united with, the nuclear membrane as to be indistinguishable. A certain difference in reaction to stains is observable between the anterior end and the remainder of the body of the antherozoid. For example, if an antherozoid be stained with safranin and pyoktanin blue, its anterior part shows a greater affinity for the safranin, in which respect it resembles the cilia; the rest of the body takes more of the blue stain. But there is no sharp line of demarcation; rather, the reddish stain at the tip shades gradually into a purple and this in turn into a blue.

The cilia are undoubtedly formed of cytoplasmic substance, probably similar in nature to that of the blepharoplast. The limosphere and percnosome play no recognizable part in the development of the antherozoid, although the latter probably persists until a very late period and the former body is still present in the cytoplasmic remnants that are attached to the mature antherozoid.

The Structures of the Androcyte in various Plants.

Leaving out of consideration the conflicting views concerning the origin and homologies of the blepharoplast, which have been discussed in another place (Allen, 1912), the results of recent work are in substantial accord as to the general features of the history of the blepharoplast and nucleus in the androcytes of those plants whose spermatogenesis has been studied. With reference to the Liverworts, Mosses, and true Ferns, perhaps the most marked difference of opinion that still exists is over the question whether the long cord or band to which the elongating nucleus becomes applied is developed from the blepharoplast alone, or partly from the blepharoplast and partly from a later-formed structure. Upon this point most observers hold to the former view—a view with which the present writer agrees. In *Equisetum* and *Marsilea* (Sharp, 1912, 1914), as in the Cycadales, the blepharoplast, instead of simply growing longer, breaks up into a mass of granules which later unite to form a continuous thread. While the possibility of a somewhat similar occurrence is suggested by the rather knotty appearance of the blepharoplast of *Polytrichum* when it begins to elongate, there is no time when it is visibly resolved into smaller bodies. The spermatogenesis of the Cycadales and *Ginkgo* is distinguished by the fact that the nucleus does not elongate; at most it pushes out a short beak towards the blepharoplast. In these Gymnosperms, too, in contrast with the conditions in Bryophytes and Pteridophytes, all the cytoplasm of the androcyte seems to remain as an

integral part of the antherozoid. As between the Characeae and all the higher groups, an important difference seems to obtain in the fact that in the former, according to the most recent account (Mottier, 1904), the blepharoplast appears first as a slender thread instead of originating as an isodiametric body which later elongates.

With reference to the other structures of the androcyte, the accounts of different observers are extremely confusing. It is highly probable, as already pointed out, that the '*chromatoider Nebenkörper*' described by Ikeno (1903) in the androcyte of *Marchantia* corresponds to the limosphere of *Polytrichum*. As Ikeno points out, Schottländer (1892) had figured a spherical body in the androcyte of *Marchantia* which is probably the '*Nebenkörper*'. Bolleter's (1905) and Humphrey's (1906) descriptions of similar bodies in the androcytes of *Fegatella* and *Fossombronia* are in harmony with that of Ikeno. Humphrey describes the body in question in *Fossombronia* as taking a position between the blepharoplast and the nucleus, where it elongates somewhat—a behaviour that is suggested by one of Ikeno's figures. Finally, Humphrey thinks, the *Nebenkörper* becomes a part (the 'middle piece') of the body of the antherozoid.

Arens (1907) finds, in the androcytes of *Mnium*, a body which he homologizes with the '*Nebenkörper*' of *Marchantia* and *Fossombronia*. The body seen by Arens seems unquestionably to be the same as the limosphere of *Polytrichum*. According to him, it moves to a position between the nucleus and the blepharoplast and is there dissolved, its former position being marked in the later stages of spermatogenesis by a vacuole. If it be remembered that what Arens calls the blepharoplast is but the anterior end of the long body to which the majority of authors have applied that name, it will be seen that the behaviour of the '*Nebenkörper*' in *Mnium* (as well as that of the similarly named body in *Marchantia* and *Fossombronia*) corresponds closely with the behaviour of the limosphere as I have followed it in *Polytrichum* down to the time at which it gives off the apical body.

The van Leeuwen-Reijnvaans (1908) describe a corresponding body, also under the name '*chromatoider Nebenkörper*', in the androcytes of *Polytrichum*. Their figures show that they observed it also taking a position close to the anterior end of the blepharoplast, where they think it disappears before the nucleus begins to elongate.

The bodies appearing in the androcytes of *Polytrichum*, *Atrichum*, and *Pellia* to which Wilson (1911) gives the name *limosphere*, are also in general certainly to be identified with that for which I have used the same name. But Wilson's description is so confused, and so many of the appearances he describes are evidently the result of faulty technique, that it is sometimes difficult to determine whether at different stages he is applying the same name to the same structure.

Walker's (1913) figures of the developing androcytes of *Polytrichum formosum* come much nearer to giving a complete history of the limosphere than do those of any previous writer. In his description, unfortunately, Walker has confused the anterior and posterior ends of the blepharoplast and of the nucleus, and has not altogether succeeded in distinguishing artefact from normality.

Woodburn (1915) shows the limosphere plainly in several of his figures of stages in the spermatogenesis of *Mnium*. He seems, however, to be quite uncertain as to the permanence, or even the identity in different cases, of the body that he figures.

The bean-shaped 'amylaceous mass', described by Guignard (1889) in the androcyte of *Sphagnum*, which persists in the cytoplasmic vesicle attached to the posterior end of the antherozoid, suggests in its behaviour an analogy at least to the limosphere of *Polytrichum*. Campbell (1887) had seen the same body in the vesicle of the antherozoid of *Sphagnum*, and had concluded from its reactions with iodine that it was starch. Still earlier, Roze (1864) had made a similar observation. It remains for present-day cytological methods to determine how closely *Sphagnum* agrees, in this feature of the history of its androcyte, with the Bryales that have been investigated. In this connexion it may be a pertinent fact that several writers have described starch grains as present in some numbers in the cytoplasmic vesicles of the antherozoids of Pteridophytes.

The '*Nebenkern*' found by Yamanouchi (1908) in the androcyte of *Nephrodium* might be thought to correspond either to the limosphere or to the percnosome. Its small size during most of the history and its variable position within the androcyte make its identification with the latter body seem more probable; but its larger size in the later stages of spermatogenesis and its persistence in the cytoplasmic vesicle that is attached to the posterior end of the mature antherozoid suggest a resemblance to the limosphere. It is possible that the very small *Nebenkern* of the early part of this history and the somewhat larger body observed in later stages are not, as Yamanouchi evidently believes, identical. There is even greater uncertainty as to the identity of a body described by Thom (1899) as a 'blepharoplast' or '*Nebenkern*' in the androcytes of *Aspidium* and *Adiantum*. The fact that he finds this body still present and unchanged in form after the elongation of the nucleus has begun seems to indicate that he is dealing, not with the blepharoplast as he supposes, but with another body which probably corresponds to Yamanouchi's '*Nebenkern*'.

The two large, spherical cytoplasmic bodies which Hirasé (1898) first observed in the body cell (androcyte mother-cell) of *Ginkgo* are also possibly homologous with the limosphere. In the division of the mother-cell, one of these bodies passes into each androcyte, where it persists throughout the development of the antherozoid, and is visible in the cytoplasm of the latter

until the moment of fertilization. Another conspicuous body, irregular in form, which appears in the cytoplasm of the mother-cell, passes into one of the two antherozoids and is described also as persisting to the time of fertilization. The latter is, perhaps, the only structure so far reported in plants which may be imagined to be in any way comparable with the sex chromosomes of animals—with the important difference that the resulting cytological differentiation between the male germ cells of *Ginkgo* is in no way connected with chomosome reduction. The case is of special interest because of the strict dioecism of *Ginkgo*.

The van Leeuwen-Reijnvaans (1908) describe a small chromatic body in the androcyte of *Polytrichum*, distinct from the blepharoplast and the limosphere, which at one stage lies in contact with the nucleus and also, to judge from their figure, with the posterior end of the blepharoplast. It is likely that this body is the percnosome. The 'accessory body' described by Wilson (1911) in the androcytes of *Polytrichum*, *Atrichum*, and *Pellia* is also probably in general to be identified with the percnosome.

Walker (1913) shows in several of his figures a small but conspicuous cytoplasmic body (in his Fig. 34 there are two such bodies) in the androcyte of *Polytrichum*. This body, which is without much doubt also the percnosome, is shown in several cases, notably in his Fig. 36, as lying at the posterior end of the blepharoplast. Walker seems to consider the body so located to be the blepharoplast itself—the long cord which is really the elongated blepharoplast being taken by him for a distinct structure.

Nothing shown in Woodburn's (1915) figures of the spermatogenesis of *Mnium* is recognizable with any degree of certainty as the percnosome. In one case (his Fig. 15) he shows a small body lying in a vacuole; in another (his Fig. 14) there is a similar appearance, but in this case he describes the body in question as probably lying upon the surface of the vacuole, and the 'vacuole' itself, which is partly surrounded by a darkly stained layer, is evidently the limosphere. Lewis (1906) and Miss Black (1913) likewise find a single large vacuole, but nothing resembling the percnosome, at certain stages in the history of the androcytes of *Riccia natans* and *R. Frostii*.

Apparently no previous writer has recognized the structure that I have called the *apical body*. However, Walker (1913) figures in certain cases what is probably this body; especially in his Fig. 33, which shows two structures side by side that are quite certainly the limosphere and the newly formed apical body, the latter being in contact with the anterior end of the blepharoplast.

Summary.

Each newly formed androcyte of *Polytrichum juniperinum* contains a small rounded blepharoplast which behaves like a centrosome in the division of the androcyte mother-cell, and which in most cases still lies in the region recently occupied by a spindle pole.

The blepharoplast elongates, places itself in contact with the plasma membrane, and ultimately forms a long, peripherally placed, curved cord. Two long cilia grow out from it; their point of attachment is a short distance behind the anterior end of the blepharoplast.

The nucleus moves into contact with the blepharoplast and stretches out along the latter. The blepharoplast, though visible until a comparatively late stage, ultimately becomes indistinguishable from the nucleus except for its anterior end, which apparently projects a short distance beyond the tip of the elongated nucleus. The nucleus becomes a long, slender, coiled, finally homogeneous body, of about one and one-half turns. The nucleus and the blepharoplast seem to constitute the whole of the body of the mature antherozoid.

At about the time the blepharoplast begins to elongate, a large, spherical body, the limosphere, appears, variously situated in the cytoplasm; soon it comes to lie close to the anterior end of the blepharoplast. In this position it divides unequally; its smaller portion becomes the apical body, its larger retains the appearance of the limosphere before the division and is referred to by the same name.

The apical body remains applied to the anterior end of the blepharoplast until a very late period, but it has not been clearly shown to take any part in the formation of the body of the antherozoid.

The limosphere, after the formation of the apical body, takes a position in contact with the nucleus, nearly always with the posterior portion of the latter. The limosphere persists in the cytoplasm until the time of the maturity of the antherozoid.

During certain stages in the history of the androcyte, another conspicuous cytoplasmic body, the percnosome, seems to be regularly present. It is probably identical with a smaller granule which is generally recognizable both at an earlier and at a later period. This smaller body sometimes—at very late stages usually, if not always—lies in a rather large vacuole.

During this history the androcyte becomes approximately spherical, then, as the bulk of the cytoplasm decreases, lenticular. A portion of the cytoplasm, including the limosphere, remains included within the curve of the posterior end of the mature antherozoid.

The walls which originally separated the androcytes gradually soften and become dissolved. Each antherozoid, when mature, lies in a vesicle which, seen as the contents ooze out of the antheridium, seems to be bounded

by something like a distinct membrane; but in fixed material no trace of such a membrane is to be found at this time, although a similar structure does seem to be present in preparations of some antheridia at an earlier stage. The separate vesicles are embedded in a viscous substance which is probably derived from the material of the broken-down walls.

A part of the work upon which the present paper is based was carried on in the laboratory of Professor Mangin at the Muséum d'histoire naturelle, Paris. It is a pleasure to record here my appreciation of the unfailing courtesy of M. Mangin and his assistants, and of the generosity with which the facilities of the laboratory were placed at my disposal.

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EXPLANATION OF PLATES XV AND XVI.

Illustrating Professor Allen's paper on the Spermatogenesis of *Polytrichum juniperinum*.

The figures were drawn with the aid of a camera lucida, the drawing being at the level of the base of the microscope; some of them with a Zeiss 2 mm. apochromatic objective, 1·40 N.A., and compensation ocular number 18; the others with a Leitz 2 mm. apochromatic objective, 1·32 N.A., and compensation ocular number 18. In each case the magnification is about 3800 ×.

PLATE XV.

Figs. 1, 2. Pairs of young sister androcytes, each showing a blepharoplast in the region occupied by a pole of the spindle in the division recently completed.

Figs. 3, 4. Single androcytes; the blepharoplast in each has grown somewhat.

Fig. 5. An androcyte whose blepharoplast has become a short rod; the limosphere also visible.

Figs. 6–10. Stages in the elongation of the blepharoplast; the limosphere visible in Figs. 9 and 10.

Figs. 11–13. Androcytes with still longer blepharoplasts and distinct limospheres. At the upper end of Fig. 13 two unusually large androcytes.

Figs. 14–19. Different views of androcytes all at about the same stage of development; the blepharoplast has become a curved rod, lying in a peripheral position with one end close to the limosphere.

Fig. 20. An androcyte containing a double (or dividing) limosphere; the blepharoplast is not, at least for a considerable part of its length, peripheral.

Figs. 21–4. Stages in the division of the limosphere. The lowermost androcyte represented in Fig. 24 shows an unusual (perhaps abnormal) condition of the limosphere.

Fig. 25. The apical body is now separated from the limosphere.

Fig. 26. The limosphere is moving away from the region in which the apical body was cut off from it.

PLATE XVI.

Fig. 27. The limosphere is in contact with the nucleus; the dark object lying over the apical body is probably the percnosome.

Fig. 28. Beginning of the elongation of the nucleus; the apical body and limosphere are connected by a cytoplasmic strand.

Fig. 29. Different view of an androcyte at about the same stage as that shown in Fig. 28; part of one cilium is visible.

Figs. 30–6. Stages in the elongation of the nucleus; the limosphere is in contact with the posterior part of the nucleus. Except in Figs. 34 and 36, the percnosome appears as a comparatively large body.

Figs. 37–41. Further stages in the elongation of the nucleus; the conspicuous cytoplasmic granule appearing in Figs. 37–9 is probably the percnosome.

Fig. 42. The nucleus has taken on an unusual form, having pushed out two long beaks, one anteriorly and one posteriorly. A part of one cilium appears.

Figs. 43–7. Still further stages in the extension of the nucleus. A portion of the nucleus, near its posterior end, is still comparatively thick. The androcyte shown in Fig. 45, like that in Fig. 41, lies in a vesicle which is bounded by what looks like a definite membrane.

Figs. 48, 49. The nucleus has reached substantially its final length and, by a diminution in its volume, is becoming slender throughout. A vacuole is present in the cytoplasm, as also in Figs. 46 and 47; in this vacuole lies a granule, perhaps the percnosome.

Fig. 50. Two nearly mature androcytes seen in section; the cilia are conspicuous, and some of the cytoplasm remains, though its volume is much reduced, the cell as a whole being lenticular. Each androcyte lies in a vesicle bounded by a membrane.

Fig. 51, 52. Still older androcytes; the cytoplasm is now in two masses, one attached to the anterior, the other to the posterior, portion of the body of the antherozoid.

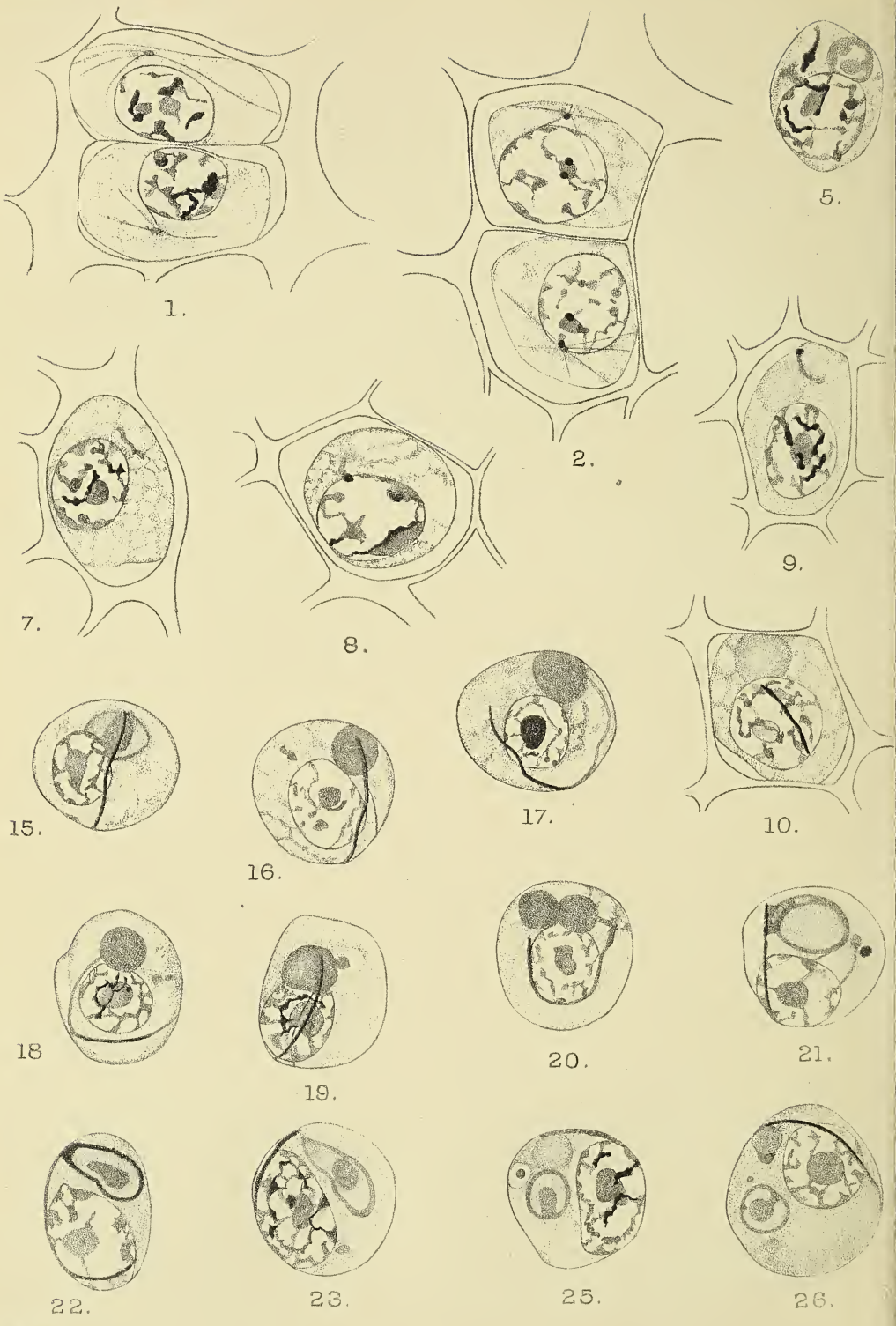
Fig. 53. The posterior end of the body of an antherozoid at the stage of Figs. 51 and 52, showing the posterior cytoplasmic mass, including the limosphere.

Figs. 54, 55. Antherozoids from sections of mature or nearly mature antheridia. In the antheridium from which Fig. 55 was drawn, the intervesicular substance is stained, but no definite vesicular membrane is visible. In the antheridium from which Fig. 54 was made, the intervesicular substance was so faintly stained as to be practically invisible.

Figs. 56, 57. Antherozoids still within their enclosing vesicles, killed after leaving the antheridia. The body shown in Fig. 57, with deeply stained margin and somewhat irregular outline, is probably the limosphere.

Fig. 58. An antherozoid beginning to escape from its vesicle.

Fig. 59. An antherozoid which has become free. Cytoplasmic remnants are still attached to the posterior portion of its body.



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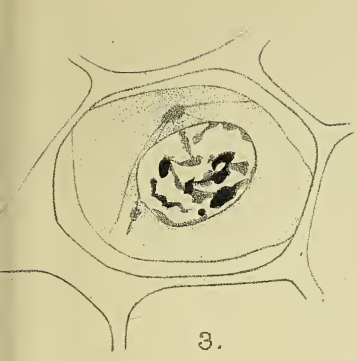
ALLEN — POLYTRICHUM.



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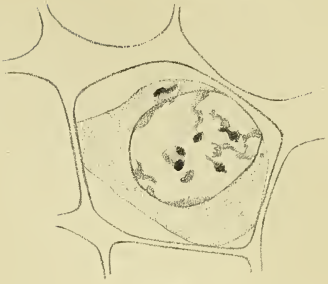

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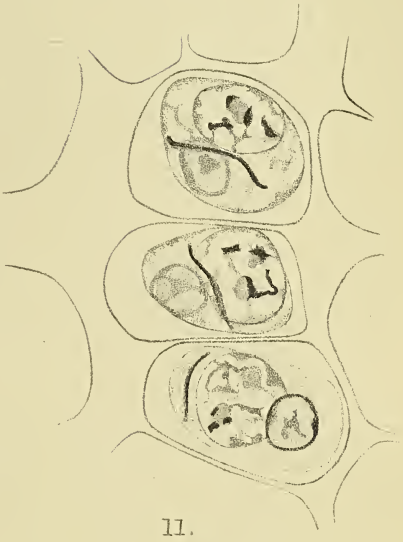

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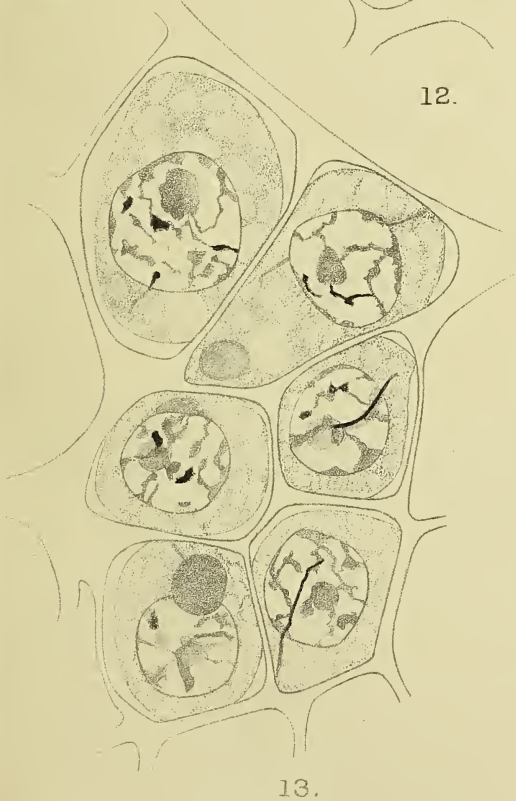

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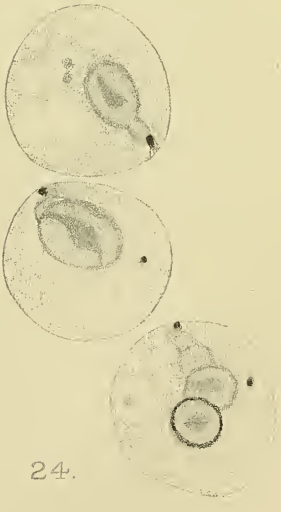

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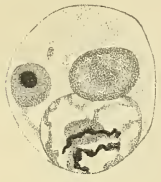

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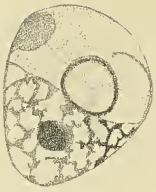

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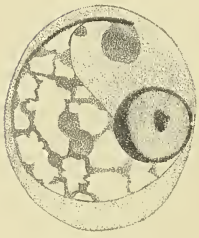

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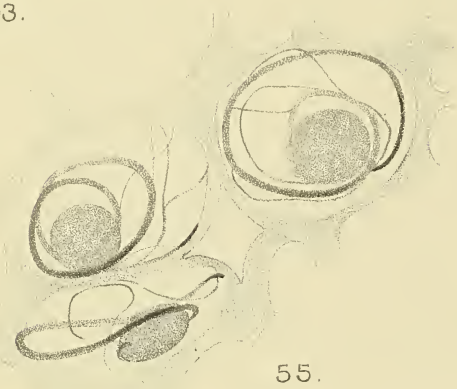

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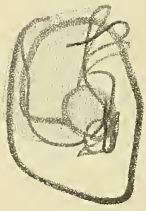

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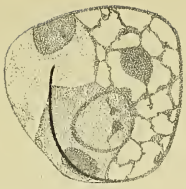

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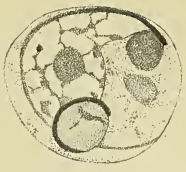

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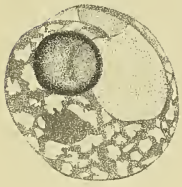

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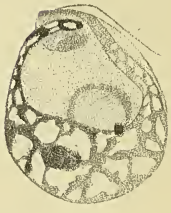

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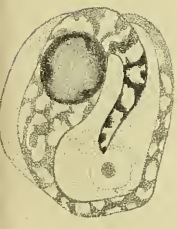

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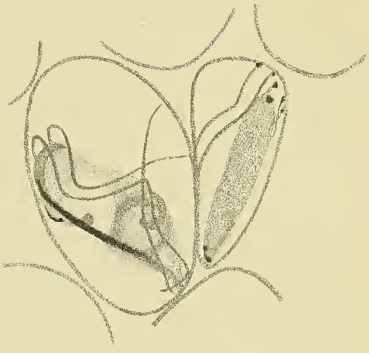

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On the Mechanism of Translocation in Plant Tissues. An Hypothesis, with special reference to Sugar Conduction in Sieve-tubes.

BY

SYDNEY MANGHAM, M.A.,

Lecturer in Botany, Armstrong College, Newcastle-on-Tyne, in the University of Durham.

With two Figures in the Text.

THE transference of products of metabolism from their sources to their destinations, as in the case of sugars formed in leaves and stored or consumed in subterranean organs, may involve journeys over considerable distances, especially in the case of large trees.

While it is highly improbable that any particular molecule, say, of dextrose ever passes immediately after formation rapidly over the whole path from leaf to root tip, yet movement for a certain distance obviously occurs.

Evidence has accumulated [1] showing that, in the case of sugars, sieve-tubes play an important part in the normal process of translocation, and that Czapek's [2] view that these elements provide the chief path for their rapid transference is the correct one.

It is, in practice, scarcely possible to deal with the path taken by assimilates during their passage from the photosynthetic system without reference to the physico-chemical processes involved.

The present paper is the outcome of attempts made to form some mental picture, in terms of physical chemistry, of what actually happens during certain phases of the process under consideration.

It is almost unnecessary to say that any active flow of material through plant tissues must be associated intimately with the properties of living protoplasm, and so with its structure.

Czapek [3] carried out a number of experiments in which he subjected petioles to killing by heat and by chloroform, to plasmolysis, and to

[1] Mangham (1910–11). An account of relevant anatomical and physiological data.
[2] Czapek (1897).
[3] Czapek, l. c., pp. 140–60.

narcosis, and noted the effect of the treatment on the removal of starch from the leaves. He found that killed elements no longer permitted translocation to go on, but that unkilled plasmolysed (5 per cent. KNO_3) cells behaved almost as normal ones. Narcosis prevented translocation, which, however, recommenced on recovery of the cells.

Deleano[1] criticized particular features of this work, but the conclusion drawn by Czapek that translocation is dependent upon protoplasmic activity remains unshaken.

In the paper referred to, Czapek appeared to consider that during translocation sugar is actively taken into the substance of the protoplasm, chemically combined with it, and then excreted again on the other side, where it is taken into the protoplasm of the next cell after diffusing through the intervening cell-wall.[2] The vacuole was regarded as a sort of reservoir of nutriment for the protoplasm.

His views with regard to the rôle of connecting-threads are related to observations depending on a technique which has since been greatly improved upon.

It is interesting to note that Czapek found that translocation occurred in isolated plant portions, provided that a sufficient length (e.g. 12 cm. in one experiment with *Vitis*) of conducting path remained in connexion with the leaf.[3]

Of recent years much work has been done aiming at elucidating the properties and finer structure of protoplasm. In particular, the outer bounding layer, of undefined thickness, generally known as the 'plasmatic membrane', has received attention at the hands of many investigators.[4]

The majority of these researches have been concerned closely with the determination of the permeability of this membrane to various solutes, with the relation of permeability to changes in external factors, with the antagonistic action of some salts upon others, and with the effects of anaesthetics.[5]

Many valuable results have been obtained, and light has been thrown upon the structure of protoplasm and of the membrane in question.

While at the present time the knowledge available is insufficient to permit, for example, of a satisfactory diagrammatic representation of the spatial distribution of the constituent particles of the membrane, yet it seems clear that the protoplasm as a whole forms a heterogeneous system, or colloidal complex, in which water, proteins, and lipoids are important components.

As stated by the Gibbs-Thomson rule, in such a system substances which in any way are able to lower the energy of the surface will, if free to

[1] Deleano (1911). Cf. Schroeder (1911) for abstract and an estimate of the value of Deleano's conclusions.

[2] Czapek, l. c., p. 158. [3] Ibid., pp. 148–9. [4] Blackman (1912).

[5] Atkins (1916) and Bayliss (1915). General accounts and bibliographies are given.

move, tend to accumulate at that surface, while those increasing surface energy will recede from the surface.

It is therefore probable that the protoplasm shows a more or less stratified structure, in accordance with the surface energy relations of the chemical compounds present. Lipoids, for example, owing to their great power of lowering surface tension, would tend to dispose themselves in the outermost layers of the protoplasm.

It is important to bear in mind that in such a complex colloidal system as is here contemplated, since the surface of the components is highly developed, adsorption phenomena will occur to a very large extent.

Substances in solution inside the vacuole may therefore become concentrated at every surface of separation between two different phases, in such a way as to secure the maximum lowering of the energy of the system as a whole.

The protoplasm may then be pictured not merely as a combination of a suspension of particles and an emulsion of droplets in an aqueous medium, with probably also other components in the form of gels, but in addition account must be taken of the fact that in the watery phase various solutes are present, and furthermore that they may exist there in a concentration differing from that obtaining at the actual surface of separation of the water from the other phases.

It is well known that a *reversible equilibrium* exists between the concentration of a solute in its solvent and at the surface of an adsorbing substance introduced into the solution.

To take an example,[1] if equal amounts of charcoal are shaken up with dilute acetic acid in known concentrations, a certain proportion of the acid is removed from each solution owing to local concentration, or adsorption, at the surface of the charcoal particles.

Quantitative estimations have shown that from the weaker solutions a relatively larger proportion is removed than from the stronger ones. This relation can be expressed by a curve of the type shown in Fig. 1.

Put in another way, if to a very fine suspension of charcoal in water acetic acid is added, and the mixture well shaken, the acid will distribute itself throughout the system in a definite manner such that the concentration at the surface of the charcoal bears a specific relation to that of the acid remaining in solution.

If more acid is then added, a new state of equilibrium will be obtained, the concentration of acid becoming increased at the charcoal surface, but relatively more increased in the solution.

Similarly, if more water is added to the original solution, the concentration of the acid at the surface of the charcoal will become decreased, but that of the acid in solution will become relatively more decreased.

[1] Philip (1913), pp. 227–30 and p. 232.

It is clear then, that if changes occur in the concentration of solutes present in a heterogeneous system containing one or more constituents capable of adsorbing them, there will be a succession of readjustments of equilibrium between the concentration of the solute at the surface of the adsorbing phase and that of the solute in the solvent.

Such changes may be brought about in plant cells owing to variations in the supply of both water and of solutes. Thus there would result a state of continuous flux, the solute constantly varying in concentration in the two regions.

It should be realized that the conception here outlined does not imply

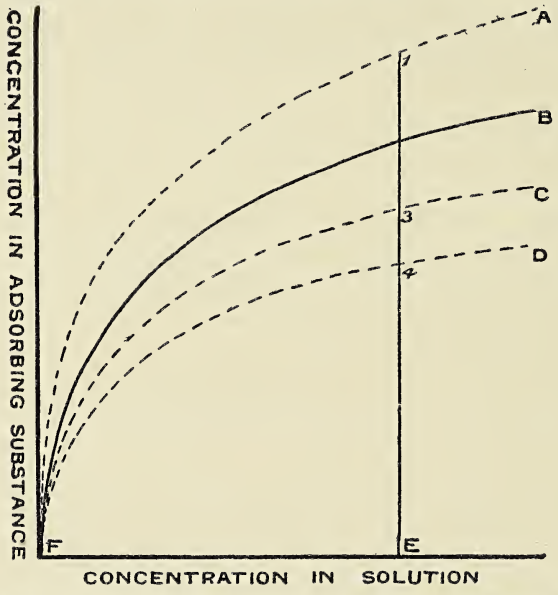

FIG. 1. FB, Type of curve showing reversible adsorption equilibria with a given solute, solvent, and adsorbing phase B. The broken lines represent equilibria with other adsorbing phases A, C, and D, in which the solute is assumed to undergo different degrees of adsorption indicated by E 1, E 3, and E 4.

that particular molecules of sugar are, as it were, fixed on the surface of the adsorbing phase, while others are free to move in the solvent, but rather that molecules are at all times passing from the surface of the adsorbing phase into the body of the solvent, and vice versa, in such a manner that a definite relation between their concentrations in the two regions is maintained while the conditions are constant.

There is therefore the possibility that an individual molecule might remain for a very long period in, say, a bundle-sheath cell, if it managed to confine its peregrinations to the more central parts of the vacuole well out of immediate reach of the protoplasm. Obviously the layer of solution actually bathing the adsorbing substance will be the site of initial re-

adjustments when equilibrium is disturbed; it will take time for the effect produced in this peripheral portion of the vacuole to be transmitted (by diffusion of solute) throughout the whole of the cell sap.

Emphasis must be laid upon the enormous area of the protoplasmic linings of plant cells compared with the volume of the tissue which they compose, for in this way innumerable small reservoirs are provided, and these are readily accessible to the protoplasm. There is therefore available very extensive machinery by means of which translocation can be effected in the manner to be described.

It is now proposed to apply these considerations to the case of sugar transport within the plant.

A normal green plant derives its sugars solely from the cells of the photosynthetic system. That is to say, there is never any question of the sugar having to enter such a plant *from outside*, since it actually arises within the assimilating cells, and presumably within the protoplasm itself.

Now it follows from what has been stated above, that if any of the protoplasmic constituents can adsorb sugar to any extent, there will always exist a tendency to attain the specific relation between the amount adsorbed and the amount remaining in solution.

It should be noted that true adsorption compounds of albumen, lecithin, and glucose are known to occur,[1] so that there is nothing improbable in the supposition that vegetable protoplasm contains elements capable of bringing about local concentration of sugar on their surfaces, possibly in varying degrees. (Cf. Fig. 1.)

When in a given chlorophyll-containing cell a particular plastid has produced sugar, this in course of time will pass into solution in the immediately adjacent liquid phase of the protoplasm, and so into the sap of the vacuole presumably continuous with it; in this the sugar will diffuse in accordance with the ordinary solution laws.

Since, however, particles capable of adsorbing sugar are assumed to be distributed throughout the protoplasm, the sugar will tend to accumulate at their surfaces.

Consequently, the two processes of diffusion through the solvent, and adsorption at the surface of protoplasmic components, would contribute to the transference of the sugar.

If the adsorbing particles were few and relatively distant one from another the ultimate rate of movement of the sugar would be conditioned almost entirely by the rate of diffusion in the continuous liquid phase, that is, it would probably be extremely slow.

If, however, the adsorbing particles were fairly numerous and comparatively close together, the diffusing sugar molecules would not have far to travel before reaching the surface of one of them.

[1] Bayliss, l. c., pp. 57 and 66. Cf. also p. 65.

Finally, if the adsorbing material were present either in very high concentration, so that the particles were only separated from one another by an infinitesimally thin film of solvent, or were actually continuous, forming a meshwork, as is probably the case in a gel, the distance to be traversed before coming into contact with a surface in which concentration could occur would be reduced still more.

The rate of transference would then approximate to that at which condensation on the surface of the adsorbing phase would occur, that is, in all probability it would be extremely rapid.[1]

The specific concentration relations existing between the particular substances concerned at the adsorbing surface and in the layer of solvent immediately in contact with it would, of course, obtain, so that there would be a wave of adjustment to a state of equilibrium, the rate of propagation of which would depend on the rate of spreading of the sugar over the adsorbing materials.

So far, the process has been dealt with only partially, and has been treated as if the sugar formed in the photosynthetic cells passed onwards into protoplasm and solvent containing no sugar. This is simply for purposes of description, and is hardly likely to represent a state of affairs ever occurring in the plant.

Actually the process must be more complex, although conditioned by the principles enunciated above.

It is possible now to pursue the matter somewhat farther.

In the case of a single cell, such as a unicellular green alga, the sugar would diffuse into the vacuole, and the concentration there and at the surface of the protoplasmic constituents would tend to reach a state of equilibrium, which, however, would be continually disturbed by the metabolic changes incidental to growth and reproduction.

In a filament such as *Spirogyra* the conditions would probably be much the same, since the plant is simply a series of equivalent cells all under practically identical conditions.

With such a plant as *Laminaria*, however, the existence of tissue differentiation and of localized meristematic regions adds complications. Here the carbohydrates are produced in those of the outer cell layers into which light penetrates. The inner layers, being shaded, assume other functions.

The soluble assimilates would travel as above described in the cells producing them. But the protoplasm of each cell is continuous with that of its neighbour by means of the connecting-threads in the walls.[2]

The solutes have an unbroken protoplasmatic pathway throughout the

[1] Cf. Bayliss, l. c., pp. 56 and 61.

[2] Sykes (1908). It is here assumed that a real continuity of protoplasm exists, although the exact nature of ' median nodules ' has not been ascertained.

plant, although in most tissues considerable restrictions are placed on speedy transference in quantity from cell to cell owing to the extreme tenuity and comparatively small number of the threads, which oppose rapid motion in much the same way as turnstiles at a barrier limit the rate of passing of a crowd of people from one side to the other.

Similar considerations would apply to the case of a normal green foliage leaf. The sugar formed in the mesophyll would be able to pass from cell to cell by way of the connecting-threads, and so would reach the protoplasm of the bundle-sheath cells near the endings of the fine veins. Its farther path will be dealt with later on.

At all regions in the plant where meristematic tissue occurs, and especially at the cambium, and at the apices of roots and shoots, or where storage is going on, as well as throughout the whole of the living tissues which are continually undergoing respiration, great consumption of soluble carbohydrates occurs. This means that in such cells the concentration of these substances is continually varying.

To form starch reserves, or new cell walls, or to produce fibres or 'stone-cells', sugar must be withdrawn and its concentration become lowered temporarily at the place of withdrawal.

Any such lowering of concentration at one point of the protoplasm will affect the state of equilibrium which should obtain, as described above.

For example, if the sugar concentration is lowered at the surface of a particular protoplasmic particle, then more will pass from the immediately adjacent solution to become concentrated at the surface of this particle in order to re-establish the specific relation.

The solution will accordingly become locally weaker, and this will disturb the equilibrium between it and other particles in the vicinity; the latter will therefore give up a portion of their adsorbed solute, diffusion in the solvent will occur, and its effects be transmitted.

If carbohydrate consumption is vigorous, or long continued, its influence will be far-reaching.

Thus a wave of disturbance and readjustment of equilibrium is propagated, in much the same way as the firing of shells from a battery leads to the depletion of the immediate supply, followed by a replenishing from the reserves, &c., and ultimately from the factory, so that the scheduled relations are maintained between the various stores at intermediate points.

Waves of this type will be sent out from all points where sugar is being transformed, and they will spread in all directions subject to structural limitations.

To a certain extent the cell vacuoles may be compared to a series of small water-tanks from each of which supplies may be drawn off by several pipes, and to which the losses may be made good by small-bored pipes connecting the bases of adjacent tanks. The small tanks must be imagined

to be horizontally disposed and connected ultimately with a main supply pipe capable of delivering considerable quantities of water fairly rapidly.

Thus, if a tap is opened at one of the tanks water will flow out, and this will cause a flow from the adjacent tanks and from the others in turn, and finally water will pass in from the main. When outflow taps are opened at several tanks at once the level of the water in the individual tanks at any moment will depend upon the relations obtaining between rates of loss and supply for each one and the rate of delivery from the main.

In the plant the parenchymatous cells with their vacuoles correspond to the small tanks, the sieve-tubes represent the main supply pipe, while the connecting-threads take the place of the small-bored pipes.

It is desirable to consider the structure of the sieve-tubes in relation to the function here assigned to them.

In the finest veins of the leaves the phloem becomes reduced to a single sieve-tube with companion-cells in connexion with its segments,[1] and finally the sieve-tube mother-cell, by failing to divide, gives rise to 'transition-cells', which are very much like enlarged companion-cells.[2]

The transition-cells are in longitudinal connexion with the sieve-tubes, while laterally they are contiguous with the cells of the bundle-sheath, as also are the companion-cells.

Soluble assimilates from the photosynthetic system must pass into the bundle-sheath. It is highly probable that they then pass into the transition-cells, which, being richly provided with protoplasm, would seem eminently fitted to exert considerable adsorptive suction, so to speak.[3]

In an ordinary leaf vast numbers of bundle-endings occur, so that each need only receive assimilates from a comparatively small number of photosynthetic cells.

Since the transition-cells are in direct continuity with the sieve-tubes, the assimilates could next pass into the latter.

So far, the distance traversed has been very small, equal in fact to the united lengths of a few mesophyll cells. The path over which movement has occurred in the manner described has included only a very few sets of protoplasmic connecting-threads.

Once the assimilates have entered the protoplasm of the sieve-tubes, however, they have a long continuous course practically free from obstacles, as will be seen from the following considerations of structure.

A sieve-tube usually has a very thin [4] wall, and is lined with protoplasm.

As shown more especially by the researches of Hill,[5] the mature sieve-

[1] Strasburger (1891). A number of figures are given.

[2] Haberlandt (1914), p. 367, Figs. 147 A, 147 B. (Reproduced in Mangham (1910–11), Figs. 9, 10.)

[3] Vide infra, p. 305. Cf. Fig. 1.

[4] Comparatively thick walls are to be found in many cases. Cf. Compton (1909).

[5] Hill (1901, 1908).

plates, where investigated, have numerous fine perforations each lined with a protoplasmic tubule through which the fluid contents of adjacent segments are continuous.

Practically the whole area of the plate may be perforated in this way, so that a number of the minute channels occur quite close up against the lateral walls of the tube.

Above the level of the plate the facilities for wave propagation are proportional to the cross-sectional area of that part of the protoplasmic lining containing the sugar-adsorbing constituents. (Cf. Fig. 2.)

By a little consideration of Fig. 2 it readily appears that, owing to the existence of a number of perforations close to the periphery of the plate, the cross-sectional area of the protoplasmic sheath, which is continuous though

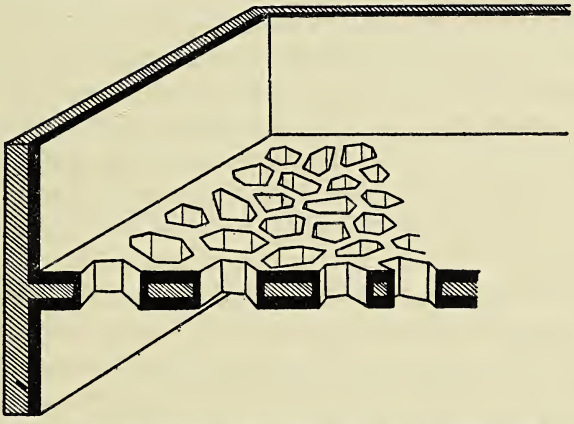

FIG. 2. Very diagrammatic representation of a portion of a sieve-plate and the walls of a sieve-tube to show continuity of protoplasm. Callose is omitted. Cell-wall, shaded. Protoplasm, white (surface), black (in section).

distorted, suffers little, if any, reduction at the level of the plate. At the most it is only as if the tube were provided with a narrow, inwardly-projecting ledge, comparable, save for its irregularity, with an annular thickening of a vessel.[1]

As far then as its effect upon the propagation of a wave of restoration of concentration equilibrium is concerned, the existence of the more central portion of the plate may be almost neglected. It allows the free passage of various albuminous substances, &c., so that the sieve-plate may be regarded as a very satisfactory structural compromise permitting of the two processes referred to, and at the same time retaining sufficient mechanical strength to guard against the collapse of the sieve-tube through pressure exerted by surrounding cells.

Fluctuations in concentration constantly occur throughout the living tissues, especially during periods of vigorous growth and storage.

[1] The sieve-plate should be contrasted with a transverse wall having a single central perforation.

Such disturbances of the equilibrium at any one region will start waves of readjustment, which will be communicated to the sieve-tubes in very much the same way as the turning on of a tap in a dwelling-house causes a flow of water in the main outside.

Owing to their special structure the waves could be propagated much more effectively in the sieve-tubes, which are continuous from leaf to root, than in any other type of plant cell, with the exception of laticiferous elements, to which similar considerations might be applied.

The sieve-tubes are richly provided with connecting-threads on their lateral walls adjoining the companion-cells, and the walls of the latter are freely connected with the phloem parenchyma cells and with the medullary rays.[1]

It is clear then that the views here expressed harmonize well with the histological features of the cells concerned.

The value of connecting-threads for purposes of translocation has hitherto been difficult to estimate. Their rôle becomes very much more comprehensible when the above considerations are borne in mind, and it can be seen that the statements in favour of their use in translocation, which were made by Hill[2] as the result of a study of their distribution and frequency, were more justifiable than their author was led to believe at a subsequent date.[3]

It will be observed that on the hypothesis here formulated the sugar originally coming into existence inside the leaf cells is treated as travelling throughout the plant *without ever passing through the plasmatic membrane, or outer surface of protoplasm in contact with the cell-wall.*

That this layer of the protoplasm in most plants is only with difficulty penetrated by sugars from outside (thus allowing sugar solutions to be used to effect plasmolysis) has been repeatedly shown.[4]

It is interesting to note that Ruhland[5] found the protoplasm of sieve-tubes to be no more permeable to sugars than the protoplasm of other cells.

Another well-known property of protoplasm is its power to prevent the escape of sugar from inside most cells, as in the case of root-hairs, Algae, &c., when placed in water.[6]

It is rather a point in favour of this hypothesis of translocatory mechanism that it does not require alternating passages of the sugar in and out of the protoplasm of adjacent cells through the plasmatic membrane and across the intervening cell-wall.

In this connexion fibres and the 'stone-cells' of fruits are instructive. The walls of these elements often attain considerable thickness, and require

[1] Hill (1908). [2] Ibid. (1901). [3] Ibid. (1908).

[4] Yeast-cells, saprophytic mycelia, &c., are permeable to sugars. [5] Ruhland (1911).

[6] Cf. also sugar retention by blood. It has been suggested that the sugar is held in combination with the proteins of the blood except under pathological conditions, e. g. in diabetes. Cf. Beddard (1905), pp. 708–11.

comparatively large amounts of sugar for their formation. These walls are provided with numerous deep pits, through the closing membrane of which the protoplasm is continuous by means of connecting-threads while the cell is alive. The cells of the date 'stone', which furnish a reserve of carbohydrate for the use of the seedling, are especially favourable for observing similar features.

It is surely much more reasonable to suppose that the sugar needed for the construction of these walls passes in by way of the connecting-threads in the manner above described, than to assume that it passes from cell to cell repeatedly through wall substance of ever-increasing thickness.

In such extreme cases the former supposition seems fairly obvious, but it is here contended that sugar exchange is effected in the same way in cells with walls of ordinary thickness.

It should be borne in mind that the length of connecting-threads is in all cases determined by the thickness of the pit-closing membrane, and that the significance of the thin walls of parenchymatous cells is probably to be found in their extensibility, &c., in connexion with osmotic phenomena and the maintenance of turgor, rather than in any special facilities they may provide for permeation by metabolites.[1]

As mentioned above Czapek stated that plasmolysis of a portion of a petiole did not prevent the removal of assimilates from the leaf.

Deleano[2] considered that the procedure adopted by Czapek gave no guarantee that the more central parts of the petiole became plasmolysed, and he concluded that translocation through plasmolysed cells was not possible. In his own experiments, however, he does not appear to have made certain that the cells were not killed by the plasmolysing solution.

It may be remarked that even when fairly strongly plasmolysed the linings of consecutive sieve-tube segments would be much less liable to become severed than would those of any of the other cells present. In spirit material, for example, the protoplasmic lining is usually contracted about the middle region of each of the segments, but is continuous through the sieve-plates.

If translocation is effected in the manner here suggested, it is hardly likely that plasmolysis (short of killing) would interfere with the efficiency of the sieve-tubes, since the continuity of their protoplasm would probably remain unbroken between the normal portions of petiole above and below the part plasmolysed.

An interesting histological feature which lends support to these views is

[1] In specialized absorptive organs, however, thin walls obviously facilitate the entry of substances from outside, e. g. from the soil solution in the case of root-hairs. Gaseous exchange, too, is similarly aided.

[2] Deleano, l. c. Czapek, however, apparently satisfied himself on this point (l. c., p. 146), and also made sure that the tissues recovered their turgor when the plasmolysing solution was replaced by water.

to be found in the case of the relations of the sieve-tubes of *Cuscuta* to those of the host plant.

Details have been investigated more especially by Peirce[1] and by Thoday (Sykes).[2] The latter found that after the haustorial cells of the parasite had penetrated into the vascular tissue of the host, a very close union was effected by some of them with the sieve-tubes. By absorption of the end walls the protoplasm of these haustorial cells eventually came into actual contact with sieve-fields or sieve-plates.

As the haustorial cells so united themselves take on the structure of sieve-tubes, and are in connexion with the phloem of the parasite, there results a continuity of sieve-tube protoplasm between the two plants.

Here it may be stated that experimental investigation by means of the osazone method for locating sugars in plant tissues[3] has shown that the sieve-tubes of the host, and the haustorial elements united with them, may both contain greater amounts of sugar than occur in the other tissues.

These results, details of which will, it is hoped, be incorporated in a later paper, go some way towards confirming the correctness of the view here taken as to the rôle of sieve-tubes in sugar conduction, and help to emphasize the importance of protoplasmic continuity for effecting translocation.

Albuminous compounds present in the contents of the sieve-tubes may serve to increase the adsorptive capacity of these cells, and thus may add to their power of accumulating sugar. (Cf. Fig. 1.)

The advantage to the plant of close association in a common path of such important metabolites as carbohydrates and nitrogenous organic bodies scarcely calls for comment.

The continuity of protoplasm which occurs in graft hybrids[4] might also be interpreted on lines similar to those taken in the case of *Cuscuta* and its host.

Another point calling for some consideration is that the protoplasm itself can scarcely be exactly the same in constitution and properties throughout the plant.[5]

The very existence of cell differentiation indicates that the protoplasm of a cell of one type differs in some respect from that of another.

The exact nature of these protoplasmic differences can only be surmised, but their results are often easy to observe. In many cases they

[1] Peirce (1893). Cf. Pl. XIII, Fig. 7, and Pl. XIV, Figs. 14, 17, and 18.

[2] Thoday (Sykes) (1911). It is fair to state that in this paper no claim is made to the effect that *absolute continuity of protoplasm* is established (cf. pp. 671, 672). The association is, however, extremely close.

[3] Mangham (1915).

[4] Hume (1913).

[5] Striking examples of differentiation are to be found in the case of the sporangiophore of *Pilobolus*, which actively excretes water, and in the case of nectaries, from which sugary liquids exude.

are expressed by specific chemical activities. Cell-walls may become thickened, either permanently or for the purpose of temporary storage. Production of aromatic compounds may occur in certain types of cells, or a glucoside may be stored for a time in one region, while the enzyme compatible with it is confined to another.

There is also the possibility of change with senescence.

Benedict[1] studied the leaves of various deciduous plants and concluded that the observed facts could best be accounted for by assuming that among other changes a decrease in the permeability of the protoplasm (to water and salts) occurred with advancing age.

Again, the inability of many unicellular organisms to continue purely vegetative reproduction indefinitely has led to the suggestion that sexual fusion in some way rejuvenates the protoplasm.

It would not therefore be surprising to find that certain protoplasts could effect a greater adsorption of sugar than others, so that a higher concentration would be reached in their vacuoles before the protoplasm became adsorptively saturated. (Cf. Fig. 1.)

In such cells sugar would tend to accumulate more than in other cells, and in this manner a directive influence could be exerted upon sugar transference. For example, if the protoplasm lining the sieve-tube segments in the leaf became adsorptively saturated with a lower concentration of sugar than that needed to produce this result in the petiole and stem sieve-tube segments, then sugar would tend to become concentrated more in the latter segments and to be withdrawn from the leaf.

Such gradients in sugar concentration in sieve-tubes have actually been found to occur, in the case of leaves allowed to remain in darkness for a day or two before being examined for sugar distribution by the osazone method.[2]

The views here set forth are in harmony with what was previously known with regard to the flow of sugar in the plant.

Experiments made by Schimper,[3] for example, showed that during darkness sugar disappeared from leaves, and travelled from regions of low to regions of higher concentration, i. e. apparently against the ordinary laws of diffusion.

It is difficult to account for this on lines other than those indicated above. An hypothesis which involves the outward passage of sugar through the plasmatic membrane of one cell followed by the penetration of the membrane of the next protoplast appears to be more arbitrary and less comprehensible than that suggested here.

The present hypothesis enables one to understand how the varying and widespread demands made by the plant upon its sugar supplies are met, viz. by the propagation of waves of readjustment of concentration

[1] Benedict (1915).

[2] Mangham (1910, 1910–11, 1911 (1), 1915).

[3] Schimper (1885).

equilibrium, waves which travel through the protoplasm by way of the connecting-threads, and finally reach the sieve-tubes, which act in part as sugar reservoirs, and in which such readjustments can be made more effectively than in other cells.

The sugar requirements of the cambium cylinders, &c., throughout the plant will result in lateral withdrawal from the sieve-tubes and from such storage tissues as phloem parenchyma and medullary rays.

In this process, as well as in the reverse one of storage, the companion-cells probably play the part of intermediaries.

These cells do not, as a rule, form longitudinally connected series, but are frequently disposed somewhere between the sieve-tubes and the cells of the bundle-sheath in leaves, while in the stem they often occur between the sieve-tubes and the phloem parenchyma or the medullary ray tissue.

It was shown by Gardiner and Hill [1] that the distribution of connecting-threads in the leaf of *Pinus* was such as to suggest that food material could most readily travel to the sieve-tubes by way of the albuminous cells, and Hill [2] demonstrated that the latter, in the stem, were very well connected with the medullary ray parenchyma.

As already mentioned, the companion-cells of the Angiosperms studied in this way were found to have numerous protoplasmic connexions with the sieve-tubes on the one hand, and with the phloem parenchyma on the other.

The albuminous cells of the Gymnosperms are probably physiologically comparable with the companion-cells of the Angiosperms, and it would seem that the rôle in each case is to act as agents between the sieve-tubes and adjacent tissues during the lateral exchange of assimilates. With the present hypothesis the comparatively great development of connecting-threads in the walls of the companion-cells receives a rational interpretation.

Experimental demonstration of this function of the cells under consideration is difficult, for at least two reasons.

In the first place, owing to their small cross-section, and to the absence of longitudinal continuity, the companion-cells do not lend themselves readily to observation, as a little experience shows.

In the second place, it is obvious that translocation might go on effectively by the rapid movement of sugar present in concentrations too low to permit of satisfactory detection by the microchemical methods available.

It is, however, proposed to reserve further consideration of these and kindred points until opportunities permit of describing in some detail results obtained by the use of the osazone method of locating sugars in plant tissues.

The foregoing considerations may be applied to all plants.[3]

For example, the sieve-tubes [4] of the larger Brown Algae, plants which

[1] Gardiner and Hill (1901). [2] Hill (1901).

[3] Saprophytes need further treatment beyond the scope of the present paper.

[4] Oliver (1887) and Sykes (1908).

have localized meristems and grow fairly rapidly, are histologically remarkably similar to those of Angiosperms, and must provide the same facilities for translocation. Some confirmatory experimental evidence in support of this contention has been obtained by the osazone method,[1] but the work is not yet complete.

In such plants as Mosses, which are never very large, and modern Lycopodiales, which are microphyllous with closely arranged leaves, and grow comparatively slowly, demands for vigorous sugar transport are less urgent than in the higher plants; this is correlated with a less advanced development of special conducting cells.

True sieve-tubes have not been demonstrated in the Mosses, but in the larger types there are elements called 'leptoids'[2] which have been assumed to provide conducting channels for assimilates.

Histological examination[3] of the vegetative portions of *Polytrichum* has revealed the fact that the connecting-threads in the leptoids are very numerous indeed, though they show no sign either of being bored out to give slime-strings, or of being enclosed in tubules of callose.

In the terminal walls of the leptoids the connecting-threads are far more numerous than in any other type of cell in the plant. These walls are often very oblique and usually bag-shaped, the end of a cell often giving the effect of being slightly invaginated. In this way the area of the wall is even more increased than it would be if it were simply an obliquely placed plane.

While decisive evidence in support of the view that leptoids conduct assimilates has not been adduced, yet in the light of the considerations above it is clear that their histological features render them very suitable for such a function. The end walls are structurally of a type between the walls of ordinary parenchyma and true sieve-plates, and evidently suffice to meet requirements.

In the modern Lycopodiales the leaf leptome is of a simple type. In most cases numerous sieve-tubes occur in the stem, but they are not provided with companion-cells like those of the Angiosperms.

On the other hand, almost perfectly preserved examples of the fossil representatives of the group have been described as showing no elements which could appropriately be called sieve-tubes,[4] and hence are devoid of secondary phloem, although the xylem underwent considerable secondary increase.

In this connexion the following considerations may be of interest.

[1] Mangham (1911 (2)). [2] Tansley and Chick (1901).

[3] Hume (1913). In correspondence also Miss Hume has kindly supplied certain details of her work on *Polytrichum*.

[4] Seward (1902). In a recent letter Professor Seward has stated that he has no reason for changing the opinions expressed in the paper referred to.

In the palaeozoic Lycopodiales, as in the modern *Lycopodium*, a very characteristic feature is to be found in the numerous closely arranged leaves, the bases of which in many cases practically cover the stem.

Each leaf is a factory for carbohydrates, &c., so that as long as the leaves remain attached and functional, supplies of metabolites are passed into the stem radially at a very great number of places; indeed, practically over the whole surface.

Such an arrangement contrasts very strongly with that to be found, for example, in *Cucurbita*, where a comparatively small number of large leaves are connected with a slender stem having long internodes.

In *Lycopdium* there is usually a fairly well-developed cortex, but the cortical tissue and the contiguous leaf bases of *Lepidodendron* comprise a relatively very much greater amount of parenchyma.

The stele of the modern Lycopods deviates as a rule more or less from the simple protostelic type, and in cross-section exhibits patches, bands, &c., of phloem arranged between areas of xylem, an appearance differing considerably from that presented by transverse sections of *Lepidodendron*, where the xylem has a more or less regular circular outline.

Naturally, no information about the connecting-threads of *Lepidodendron* is available, but there is no reason for supposing that they differed in any important respect from those of living plants.

It is here suggested that in these fossil types there were not present those conditions requiring the production of elements specially equipped to enable rapid transport of metabolites to go on.

If we may judge by the modern Lycopodiales, the ancient types did not grow at all rapidly.

Their numerous leaves must have emptied their stores into the cortical cells near their junction with the stem, so that along the whole length of the leafy stem supplies were constantly being furnished from factories *very close at hand*.

The cortex might be regarded as a large reservoir which kept pace with the needs of the plant by increasing in extent.

It is clear then that in the stem, at any rate while the leaves persisted, very little need for longitudinal translocation existed.

With regard to the root system it may be suggested that the presence of a very large cortical zone continuous with that of the stem probably afforded a sufficient cross-sectional area to permit of adequate translocation through ordinary connecting-threads.

In the modern Lycopods there appears to have been a reduction in cortical tissues, and a more or less complementary development of specialized food-conducting cells.

It is as if, in the course of their evolution, the Lycopodiales, like other plant groups, had hit upon the idea of the sieve-tube, and so had discarded

the cumbersome and uneconomical method of providing for translocation which had existed in *Lepidodendron* and its contemporaries.

On the hypothesis of translocatory mechanism here put forward it can be seen that in *Lepidodendron* there was little, if any, physiological necessity for the development of cells comparable with the sieve-tubes of more recent plants.

Summary.

The paper is the outcome of an attempt to form a mental picture, in terms of physical chemistry, of what goes on in the plant cell during certain phases of sugar translocation.

The work of recent years tends to show that protoplasm contains proteins and lipoids among other substances, and that these form with water a complex colloidal system.

It is probable that one or more constituents are present as gels.

In so far as the component substances are free to move they must be disposed in accordance with their power of lowering the energy of the system; protoplasm may therefore exhibit stratification.

Definite relations have been found to obtain between the concentration of a solute at the surface of adsorbing particles introduced into the solution and the concentration of the solute in the solvent. This state of equilibrium is reversible.

The case of charcoal and acetic acid is cited.

Adsorption compounds of albumen, lecithin, and glucose are known.

It is suggested that in vegetable protoplasm there are present constituents capable of adsorbing sugars from solution.

For any given concentration of sugar present in the liquid phase of the protoplasm, and the cell sap continuous with it, there would be a definite concentration of sugar present at the adsorbing surface.

Any alteration of concentration in either region would lead to a readjustment of concentration equilibrium, which would be propagated as a wave through the system composed of the adsorbing particles and the solution immediately in contact with them.

The rate of propagation of this wave would depend very much upon the degree of approximation of the particles under consideration, and would increase as the distance between them decreased.

Connecting-threads are assumed to provide a continuous protoplasmic pathway, though they impose restrictions varying with their frequency and tenuity.

The structure of the sieve-tube is considered, and it is shown that the sieve-plate would cause little, if any, obstruction to the progress of the wave of readjustment of concentration equilibrium, so that the sieve-tube would

permit the passage of the waves with more effect than would be possible in any other type of cell, except laticiferous elements.

The relations of the haustorial cells of *Cuscuta* to the sieve-tubes of the host plant, and especially the fact that almost complete, if not absolute, continuity of protoplasm is established between the sieve-tubes of the two plants, are interpreted as supporting the views advanced, as also are certain results obtained by the osazone method of locating sugars.

It is suggested that in Angiosperms the 'transition-cells' at the bundle-endings in leaves, and the companion-cells elsewhere, act as intermediaries in the exchange of sugar between the sieve-tubes and adjacent tissues.

In the Gymnosperms the albuminous cells probably have a similar function.

In a normal green plant sugar is synthesized inside the photosynthetic cells, and is not taken in as such from outside.

The hypothesis does not require the passage of sugar outwards through the plasmatic membrane of one cell and inwards through the membrane of the next cell; it thus falls into line with the known small permeability of protoplasm to sugars present outside, and with the power of protoplasm to retain sugars occurring inside the cells.

The structural features of fibres, stone-cells, and the endosperm of *Phoenix* are interpreted as furnishing further evidence in support of the hypothesis.

Differences in the adsorptive properties of the protoplasm in various parts of the plant, together with changes incidental to senescence, may also share in exerting a directive influence upon translocation.

Reference is made to the application of the hypothesis to various plant groups, in particular the larger Brown Algae, the Mosses, and the Lycopodiales.

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NOTE.

GEOTROPISM AND THE WEBER-FECHNER LAW.—Some recent experiments having demonstrated the truth of a statement made by the writer at the Newcastle meeting of Section K of the British Association that the perception of gravity in plants was purely a protoplasmic phenomenon and as such conformed to the Weber-Fechner Law, it has been considered advisable to make a preliminary statement of the work. All details with a description of the apparatus used will be published shortly.

The main points are these:

1. In 1905 Fitting[1] suggested that the Weber-Fechner Law might explain the results he obtained in an investigation with the oblique intermittent klinostat of the minimal angle-differences perceived by the epicotyl of various plants at varying angles to the vertical. He did not, however, develop the suggestion.

2. In 1906 Darwin[2] repeated the suggestion with reference to Fitting's observations on the differences in the presentation times required at varying angles to the vertical, but he did not develop the suggestion.

3. As the Weber-Fechner Law holds good within certain limits for the perception by animals of most stimuli, such as weight, light, sound, &c., and as protoplasm is the only substance common to the various organs involved in the perception, it occurred to the writer that the perception of gravity by plants involved *only* a protoplasmic change. Changes in permeability seemed the most probable mechanism, and such changes involve changes in the electrical resistance of the tissue.

4. Waller[3] has pointed out certain divergences from the logarithmic curve of the Weber-Fechner Law which convert that curve into an $\int$ or sigmoid curve.

5. The writer has examined Fitting's observations on the difference in presentation time required at varying angles and finds that the results give a sigmoid curve.

6. The writer has also examined Fitting's observations on the minimal angle-differences perceived at varying angles to the vertical and finds that, on the hypothesis that 3·6 per cent. is the minimal angle-difference perceived by the plant, forty-eight of the fifty-eight experiments recorded by Fitting give a correct result, and of the ten results not in accordance with this hypothesis two are almost correct and six were repeated by Fitting and the results of the repeated experiments are in accordance. According to Weber the minimal difference in weights which is perceived by man is 3·3 per cent.

7. The writer has investigated, by measuring the changes in electrical resistance of the second millimetre of one side of the root-tip, the changes in permeability

[1] Fitting, H., Jahrbüch. f. wiss. Botanik, Bd. xli, 1905.

[2] Darwin, F., New Phytologist, 1906.

[3] Waller, A. D., Brain, 1895.

produced in the root of *Vicia Faba* on exposure to varying angles, and finds that the permeability increases with the angle to the vertical according to the Weber-Fechner Law, showing the same divergences as found by Waller for the physico-chemical changes in retina, muscle, and nerve in response to various stimuli.

8. The permeability of the cortical cells of both upper and under sides of the root-tip increases, but that of the under side does so to a greater extent. The consequent relatively greater turgidity of the cells of the upper side explains the curvature. The increased permeability, giving decreased turgor, on both sides of the root explains the retardation of elongation of the axis shown by Sachs[1] to take place during curvature.

9. If statoliths or other bodies acted at all their effect would be dependent on four factors:

a. The size of the grains.
b. The number of grains.
c. The angle of the plant.
d. The viscosity of the medium.

Alone, these four factors could never give a resultant which would conform to the Weber-Fechner Law, and if they acted in conjunction with the changes in permeability their effect would be to distort the sigmoid curve considerably. Statoliths are present in considerable numbers in the root-tip of *Vicia Faba*, but the curve is always what would be expected if the excitation due to gravity were a purely protoplasmic phenomenon.

10. The negative geotropism of the shoot is explained by the obvious fact that if the protoplasm has the property of producing 'stem' cells it is different from that which produces 'root' cells, and another of its properties is to react to gravity by changes in permeability which are the reverse of those which take place in the root.

JAMES SMALL.

BEDFORD COLLEGE,
December 28, 1916.

[1] Sachs, J. v., Physiology of Plants (Eng. trans.), 1887.

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BY

A. G. TANSLEY

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On the Anatomy of Two Palaeozoic Stems from India.

BY

THE LATE RUTH HOLDEN.

With Plates XVII–XX.

THE *Glossopteris* flora characteristic of the Permo-Carboniferous of the Southern Hemisphere has received a considerable amount of attention of late years, but there are many problems connected with it which are still to be solved. Up to the present, the vast majority of specimens are known from impressions alone, in marked contrast to the contemporaneous flora of the north. This statement holds true especially for India, where nothing has been described in the way of anatomically preserved material. The Director of the Geological Survey, however, has sent to Professor Seward two specimens which are of exceptional interest from a comparative standpoint.

The first stem is from the Barakar of Deogarh; it consists of several pieces, about 2 cm. in diameter, embedded in a fine-grained grey sandstone. The bark has disappeared completely, and in places the structure of the wood has been obliterated by the vicissitudes of fossilization, but as a whole the state of preservation is excellent.

DADOXYLON INDICUM, sp. nov.

Pith.

As is common in Palaeozoic stems of the Cordaitean type, the pith is large, varying from 5 to 7 mm. in diameter (Fig. 1, Pl. XVII). It differs, however, from *Cordaites* itself, and the nearly related *Mesoxylon*, in the absence of transverse diaphragms, thus agreeing with the only other known Permo-Carboniferous stems from the Southern Hemisphere—*Dadoxylon Pedroi* from Brazil[1] and *D. lafonense* from the Falkland Islands.[2] The tissues of the pith may be differentiated into an inner and an outer portion. The former is composed of irregularly squarish parenchyma cells, and the latter of slightly thicker walled, elongated elements, which are often filled with a dark substance and are probably secretory in nature. These cells are sometimes very long (Fig. 5), but are usually broken up by transverse

[1] Zeiller (1895). [2] Halle (1912).

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or oblique walls (Fig. 4). Both *D. Pedroi* and *D. lafonense* have somewhat similar secretory elements, but they seem more in the nature of canals, the surrounding parenchyma cells being converted into a pseudo-sheath. These in question resemble rather the isolated cells of *Lyginopteris*.

Inserted between the pith proper and the vascular stele, there is a jacket of cells of a different nature. It varies in width, but generally speaking is best developed at the nodes. The smaller diameter of these elements serves to differentiate them in cross-sections (Figs. 2 and 7, Pl. XVIII), while longitudinal sections reveal their characteristically reticulate, tracheoidal markings (Figs. 6 and 17, Pl. XIX). They seem, in fact, to be identical with the transfusion tissue found in the leaves of Conifers,[1] Casuarinales,[2] &c. Instances of their presence in stems are less common, but Rothert [3] reports them in *Cephalotaxus* and Thompson [4] in *Ephedra*. In all these cases, however, they occur as isolated cells, or small groups intermixed with parenchyma, rather than as a solid sheath, while in *Megaloxylon* [5] they are not limited to the periphery, but extend throughout the pith. For strictly comparable structures we must go to *Antarcticoxylon* [6] and to *Mesoxylon Lomaxi* and *M. platypodium*.[7]

Leaf-traces.

As regards leaf-traces, the absence of serial sections through the node renders it impossible to ascertain the conditions with exactness. It seems fairly certain, however, that the internodes were variable in length, a long one being followed by several very short ones. The result must have been that the leaves appeared superficially to be in whorls, but tangential sections prove that each was separated from the next by an appreciable distance. The course of the traces through the wood is almost horizontal until they approach the pith, when they bend steeply downward. The most striking feature is that they are always in pairs. A double trace is characteristic of practically all the Pteridosperms and Cordaitales, with significant variations. The most primitive condition is probably that of *Lyginopteris*, *Heterangium*, *Medullosa*, and *Calamopitys*,[7] where the trace leaves the stele as a single strand and bifurcates either once or repeatedly in the pericycle and cortex. The next stage is represented by *Poroxylon*,[8] where the division takes place some time before the trace leaves the margin of the pith, and the two strands pursue their outward course through the tissues of the secondary wood in close juxtaposition. *Mesoxylon* represents the next stage, for here not only are the two strands distinct at the margin of the pith but, as they penetrate the woody stele and pass out, they diverge more and more. Finally we come to our Indian

[1] Jeffrey (1905). [2] Boodle and Worsdell (1894). [3] Rothert (1899).
[4] Thompson (1912). [5] Seward (1899). [6] Seward (1914).
[7] Scott (1912). [8] Bertrand (1889).

fossil, and here there is no indication that the two traces ever joined. In other words, there is always a distinct intercalary bundle, as in *Ephedra* [1] or any of the living Angiosperms.

Various stages in the exit of the leaf-traces are shown in the photomicrographs. Fig. 7, Pl. XVIII, represents three bundles; the two outer ones are foliar and have commenced their journey towards the exterior, but are still pursuing a longitudinal course; in Fig. 8 they have become bent so that a transverse section of the stem cuts them almost longitudinally; Fig. 9, a tangential section of the stem, shows the traces still farther separated. On one occasion a trace was seen to divide as it passed out through the wood; it is represented in Fig. 12. A somewhat similar case was described by Dr. Scott in the case of *M. platypodium*,[2] where the protoxylem of the strand divided while still in the wood.

Primary Wood.

In describing the structure of the wood, it will be simplest to start with the internode. As is shown in Fig. 2, Pl. XVII, the primary tissue is localized in bundles, of which there are 40 to 50 scattered around the circumference of the pith. All seem approximately the same size, without apparent differentiation into cauline and foliar. In transverse section there is no distinction between primary and secondary xylem, the elements being radially arranged throughout. Longitudinal sections, however, show that the protoxylem is strictly endarch and situated at the apex and along each side of the xylem wedges. Thus tangential sections of the bundle (Fig. 17, Pl. XIX) show the larger spiral and reticulate metaxylem cells bounded on each side by the smaller lumened, often crushed, protoxylem—the whole enclosed on its inner face by the transfusional sheath described above. In radial section the centrifugal development of the bundle is even clearer (Fig. 6, Pl. XVII); at the extreme left is the sheath, its cells becoming longer and narrower towards the wood; next comes the protoxylem, and then the metaxylem, which through a transitional zone of from 15 to 20 spiral and reticulate elements grades into the typical pitted tracheides of the secondary xylem. This arrangement holds for the bundles themselves, but in the interfascicular regions, as in *Mesoxylon*, the secondary wood abuts directly on the pith (Figs. 3 and 4). In the extensive transitional zone between primary and secondary wood proper, this Indian fossil resembles *Cordaites*, *Mesoxylon*, *Poroxylon*, &c., but it differs from them all in the absence of centripetal wood.

We now come to the nodal region, and here conditions are somewhat complicated by the greater development of primary tissue. Instead of being limited to a few elements on the inner margin of the wood, there are distinct groups of primary elements at the apices of the xylem wedges.

[1] Thompson (1912). [2] Scott and Maslen (1910).

Figs. 13 and 14, Pl. XIX, represent two of these groups in transverse section, and Figs. 15 and 16 in longitudinal. The most striking feature about them is the large amount of xylem parenchyma occupying the centre of each group. As far as the writer is aware, such a condition is unique among stems of the Cordaitalean type, though it is characteristic of certain Palaeozoic ferns belonging to the Botryopterideae and allied forms. At the same time, it is worth noting that *Mesoxylon* shows a tendency in this direction (see especially 'Annals of Botany', vol. xxvi, Pl. XC, Fig. 23). It was stated above that during the long internodes there is no distinction between cauline and foliar bundles, but at the nodes a difference may be observed in the relative size of these primary groups, those associated with the leaf-traces being distinctly smaller. This may be made out in Fig. 7, Pl. XVIII.

Secondary Wood.

The secondary wood conforms in general with the cosmopolitan Palaeozoic genus *Dadoxylon*. The tracheides are small and unpitted tangentially, but the radial walls have crowded, one or two serial, bordered pits (Fig. 18, Pl. XIX). These are flattened by mutual contact until often they become roughly hexagonal in outline; the pore is elliptical. The rays are low (2 to 7 cells in vertical series), and throughout they are uniseriate (Fig. 11, Pl. XVIII). This feature is shared by *Cordaites* and *Mesoxylon*, though it is in marked contrast with the broad-rayed *Megaloxylon*, *Poroxylon*, or *Pitys*. Like them too, the horizontal and end walls are thin and unpitted, but the lateral pits are unique. In all previously described woods of this age, the rays communicate with the tracheides by means of several small so-called 'piciform' pits; these are half bordered, and are in groups of 4 to 8 in each cross-field. In our Indian specimen, however, the pits are much larger, and have lost their borders, forming the typical 'Eiporen' of Gothan.[1] They are in groups of 1 to 4, the general tendency being for several small ones to fuse and form a single pit, as has been described by Bailey[2] in various species of *Pinus*. Stages of this process are shown in Fig. 18, Pl. XIX. The importance of the pitting of medullary rays has been emphasized, perhaps unduly, by Gothan (l. c.). Since 'Eiporen' such as are found here are characteristic of the genus *Phyllocladus* and its allies, he proposes to include all woods showing this feature in the form genus *Phyllocladoxylon*. The genus *Xenoxylon* is distinguished by having pits which are even larger, one such embracing the entire cross-field. Some of the pits of our fossil would bespeak its inclusion as *Phyllocladoxylon*, others as *Xenoxylon*. However convenient such a classification may be systematically, its value from a phylogenetic standpoint may be questioned. Among living Conifers, these large 'Eiporen' occur not only in the

[1] Gothan (1905). [2] Bailey (1910).

Podocarpineae, but also in the Taxodineous genus *Sciadopitys* and the Abietineous genus *Pinus*. Among extinct genera, they have been found by the writer in the Araucarian *Paraphyllocladoxylon*.[1] From their presence in this fossil, they must be considered as an interesting case of parallel development, to which no phylogenetic significance should be attached.

Perhaps the most interesting feature of the secondary wood remains to be mentioned—the well-marked growth rings (Figs. 1 and 2, Pl. XVII). Arber was the first to call attention to this peculiarity in Permo-Carboniferous stems from Australia ;[2] since then Seward has called attention to the same phenomenon in *Antarcticoxylon* from the Antarctic,[3] and Halle records rings in two genera from the Falkland Islands.[4] At the same time, David White[5] states that some at least of the South American forms resemble their contemporaries in the Northern Hemisphere in the uniform nature of the spring and summer wood. This fact is of especial interest in view of the well-known mixture of northern and southern floras of Brazil and the Argentine, and may probably best be explained by assuming that the glaciation of this part of Gondwanaland was not as severe or as protracted as elsewhere. That this Indian stem should show distinct annual rings is to be expected from the particularly pure character of the *Glossopteris* flora of that country.

Summary.

To sum up, the chief points of interest in this fossil are:

Pith: large, non-discoid ; differentiated into an inner parenchymatous and an outer, partly secretory, portion ; separated from the vascular stele by a distinct transfusion sheath.

Leaf-traces: paired and invariably separated by an intercalary bundle.

Primary wood: localized into bundles ; strictly endarch ; large amount of protoxylem parenchyma at the nodes ; broad transition zone between primary and secondary wood.

Secondary wood: typically coniferous ; tracheides unpitted tangentially, covered radially by closely crowded, flattened pits with elliptical pores ; rays uniseriate, low, with large fusion pits on radial wall ; annual rings distinct.

Conclusion.

A detailed comparison of this stem with others of the same age has been attempted as the different structures were described, and it is evident that it is closely allied to the other members of the Cordaitales, though it is identical with none. To indicate this relationship it may be called *Dadoxylon indicum*, with the diagnosis as given above. This stem is not without its

[1] Holden (1913). [2] Arber (1905). [3] Seward (1914).
[4] Halle (1912). [5] White (1908).

bearing on the question of the identity of *Cordaites* and *Noeggerathiopsis*. The latter genus was founded by Feistmantel for leaves which are similar to *Cordaites*, but differ in certain details of nervation. It occurs in the chief Gondwana formations—India, S. America, and New South Wales, and has been cited as one of the examples of the difference between northern and southern floras in Permo-Carboniferous times. Recently there has been a tendency to merge this genus with *Cordaites*. A discussion of the pros and cons of the situation is given by Arber[1] and Zalessky,[2] which it is not necessary to repeat. It is sufficient to note here that the anatomy of this Indian stem, which probably bore *Noeggerathiopsis* leaves, indicates that the two genera are very closely allied, but not identical.

Dadoxylon bengalense, sp. nov.

The second stem is from the East Indian Coal Company's colliery at Brahmanbarari in the Jharia coalfield, Bengal. Its source indicates that it is of Barakar (Permo-Carboniferous) age. The cross-section is slightly oval, due probably to the pressure to which the stem was subjected; the greatest diameter is 4 inches, and the length of the smallest of the three pieces, $5\frac{1}{2}$ inches. Their great weight is very noticeable, the specific gravity being 3·6, and in this connexion the following excerpt from a letter by Mr. Fermer of the Indian Geological Survey is of interest:

'The specimen was tested chemically by Mr. G. G. Narke, a student from Nagpur, working in the Geological Survey Laboratory, and found to consist mainly of iron carbonate, with a certain amount of manganese, calcium, and magnesium. The specific gravity of siderite, as given by Dana, is 3·83–3·88. There is no doubt that the substance in which the wood is fossilized is siderite, and the somewhat lower specific gravity of the specimen as compared with true siderite is probably to be explained as due to the cavities that the fossil is seen to contain in places when examined in thin sections under the microscope. It is probable that the substances other than iron, present in the specimen, in part isomorphously replace some of the iron of the chalybite. But from the appearance of one of the sections under reflected light, I should think that a portion at least of the manganese is present in the form of manganese oxide filling interspaces in the fossil. It is to be noticed that the colour of the specimens as examined in hand specimens is black. It has not been determined whether this colour is due to such mechanically included manganese oxide or to mechanically distributed free carbon remaining from the original wood.'

With this description of its external appearance, we may pass on to consider its internal structures. Unfortunately the pith and tissues immediately around it have disappeared, so that it is impossible to obtain any

[1] Arber (1905). [2] Zalessky (1912).

information in regard to the primary bundles, either at the medulla or during their exit as leaf-traces. It may be inferred, however, from the absence of foliar strands in the innermost zone of xylem which has been preserved, that they were ephemeral in their nature, rather than persisting for several years after the fall of the leaf itself, as is the case in certain Mesozoic and living Conifers. In places, traces of the bark may be found, but so indifferent is the state of preservation that no details of its structure could be ascertained. The woody cylinder itself has suffered less from the ravages of time, and a microscopic study reveals all the details of its organization. The most striking feature shown in the transverse section (Fig. 1, Pl. XVII) is the presence of distinct annual rings. That these are true growth rings cannot be questioned ; they surround the entire stem, and it is possible to trace the gradual diminution in size and thickness of wall in passing from spring to summer tracheides, followed by the abrupt transition to the larger lumened, thin-walled elements of the ensuing year. The rings are remarkably broad, extending sometimes from 10 to 12 mm. This character is more striking when compared with that of living Conifers. In measurements made by the writer on a representative collection of woods, the average width of annual ring was found to be from 1 to 3 mm. In many pines 4 mm. is not uncommon, and in the Podocarps and Taxads it is usually as low as 0·5 mm. Penhallow[1] has given figures agreeing in the main with these. The only cases at all comparable with this Indian specimen were *Cupressinoxylon chayennense* (Cretaceous) and *Pityoxylon Aldersoni* (Tertiary), the rings of the former being 10 mm. in width, and of the latter 6–9 mm. To return to the wood in question, their number indicates that it had reached an age of five years, but, as noted above, the tissues adjacent to the pith have disappeared, and it is possible that one or two years' increment may thus have been obliterated. Even so, a much more rapid rate of growth is indicated than that shown by the Conifers of to-day.

The wood itself conforms in a general way to the *Dadoxylon* type so common at that time throughout the world. The tracheides are small, and the pits seem confined to the radial wall. The size and arrangement of these pits are, however, quite distinctive, and serve to differentiate this specimen from others of a similar nature. It will be noticed from Fig. 3 that they practically never stand alone, but are rather in groups of 2–5. Two is the prevailing number, and in this case they are almost invariably opposite. Groups of three are also frequently seen, either all abreast, or one between and above the others. Rarely four may be seen on the same horizontal line, but this is unusual, though groups of four are not uncommon. In no case is there the slightest indication of a 'bar' or 'rim of Sanio' between the pairs of opposite pits. This grouped condition is characteristic

[1] Penhallow (1907).

of the greater part of each tracheide, but towards the ends it is often replaced by the more crowded position which obtains in other Araucarioxyla. In the photograph, Fig. 21, Pl. XX, the small size of the pits is very striking, but it seems probable that the state of preservation somewhat unduly accentuates this appearance. Examination of a number of slides has revealed a few places where the structure of the pit is perfectly retained. From these it is evident that originally they were closely compressed and flattened where in contact; the outside of the border was angular, while the actual opening was an elliptical slit. Further, as in all Palaeozoic woods, there was probably no torus. During the process of fossilization, the aperture was enlarged until it has become circular, the inner margin of the border was darkened so that it now appears as a black rim, while the outer, originally angular, margin is indistinguishable. Gothan[1] figures a specimen where a similar darkening of the inside of the border, coupled with the obliteration of the outside, causes the pits to appear more remote than was actually the case. Tangential sections indicate that this interpretation of the structures in question is the correct one. The beaded appearance of the wall (Fig. 22, Pl. XX) is due to the fact that the entire border has disappeared, leaving a much enlarged aperture to represent the former extent of the whole pit. Nevertheless, by actual measurement, the size of the pits is extraordinarily small. Gothan (l. c. p. 20) gives the dimensions of a number of typical Araucarioxyla varying from 16 μ in diameter in *A. Keuperianus* to 8–9 μ in *A. Tchihatcheffianus*; and it is noteworthy that in this Indian specimen the pits are smaller still, having an average diameter of only 4 μ.

The medullary rays (Figs. 2 and 4, Pl. XVII) present nothing of especial interest. They are always uniseriate, and in height range from 1 to 20 cells, with an average of 6 to 7. The walls are thin and unpitted, except where in contact with tracheides. Here the pits are in groups of 2 to 7 to each crossfield; they are small, half bordered, with an elliptical aperture. It might be suggested that the groups described above as characteristic of the radial walls of the tracheides are really caused by rays which crossed at these points, and that the pits in question are not those from tracheide to tracheide, but from tracheide to ray. It is true that the structure and mode of occurrence are similar, but the fact that the groups on adjoining tracheides are not on the same horizontal line indicates that this explanation is not the correct one. Further, tangential sections (Fig. 4) show conclusively that the grouping on the tracheides bears no relation to the presence or absence of rays, and that the pits are really from tracheide to tracheide.

Having completed the description of this fossil, it is apposite to consider its relation to other similar specimens. The absence of primary tissue

[1] Gothan (1905).

renders any real comparison impossible, but the grouping of the radial tracheary pits recalls certain Devonian stems now referred to the genus *Callixylon*. In 1909 Zalessky [1] described a new stem of the *Pitys* type under the name of *Dadoxylon Trifilievi*; in 1911 [2] he published further observations on this stem and decided that its structure was sufficiently distinctive to warrant the inception of the new genus *Callixylon*, for, in addition to the numerous bundles of primary tissue surrounding the pith, the secondary xylem is distinguished by the peculiar distribution of the radial pits. They are of the normal *Dadoxylon* type,—closely compressed and flattened to a hexagonal outline, but instead of being scattered uniformly over the cell-wall, they are localized in groups of six to thirteen or more. The groups of one tracheide are on the same horizontal line as those of the next, and give a characteristic radially banded appearance to the wood. In this respect Zalessky's stem closely resembles one described by Newberry from the Upper Devonian of the State of Ohio, U.S.A., since named by Penhallow [3] *Cordaites Newberryi*. In 1914 Elkins and Wieland [4] discovered another beautifully preserved specimen of the same general type from the Upper Devonian of Indiana and, on the basis of the pitting, included both stems in the genus *Callixylon*. If we accepted their view that bordered pits are sufficient to define the limits of a genus the Indian fossil should also be referred to *Callixylon*. To facilitate comparison the following table may be of value:

Callixylon (secondary wood).

	C. Newberryi.	*C. Oweni.*	*C. Trifilievi.*	*Indian stem.*
Annual rings	—	+; 6 in 15 mm.	?	+; 10–12 mm. wide
Rays	1–3 seriate	1–2 seriate	1 seriate	1 seriate
	1–20 cells high	1–40 cells high	1–13 cells high	1–20 cells high
	3–6 pits to each cross-field	6–8 pits to each cross-field	1–5 pits to each cross-field	2–7 pits to each cross-field
Tangential pits of tracheides	?	?	+	—
Radial pits of tracheides	Pit circular, 9 μ in diam.; pore diagonal	Pit circular elliptical or irregularly flattened, 10–11 μ in diam.; pore diagonal	Pit flattened or hexagonal, 12–13 μ in diam.; pore diagonal	Pit flattened or hexagonal, 4 μ in diam.; pore diagonal
	2–3 seriate groups of 6–13 groups banded radially	1–4 seriate groups of 3–40 groups banded radially	2–3 seriate groups of 6–13 groups banded radially	1–4 seriate groups of 2–5 groups not banded radially

It seems to the writer, however, that to combine these into one genus is to take an exaggerated view of the importance of radial pitting. While unquestionably constant, its value as a test for affinities is doubtful. Thus

[1] Zalessky (1909).
[2] Zalessky (1911).
[3] Penhallow (1907).
[4] Elkins and Wieland (1914).

in the genus *Pinus* the pits are usually opposite and distant, but Professor Groom [1] has recently described one species from India, *P. Merkusii*, where they are in groups of three or four, comparing this condition with that of *Cordaites Newberryi*. Here the variation is within the genus, but it may occur even within a single species; e. g. some of the Abietineae which have stem pits of the normal *Pinus* type have them grouped in the root as they are in Groom's stems. Were these woods discovered in the fossil state, and pitting used as the criterion for classification, the stem of *Pinus Merkusii* would inevitably be put with the root of *Cedrus*, and so on. Assuming that the pitting of these Palaeozoic Gymnosperms is of the same diagnostic value as that of living Conifers, it seems an unwarranted step to include these four specimens in the genus *Callixylon*, especially when their ages vary from Devonian to Permo-Carboniferous and their sources from America to India.

Accordingly, it does not seem advisable to do more than refer this Indian stem to the genus *Dadoxylon*, with the specific name *bengalense* to denote its source, and the following diagnosis:

Dadoxylon bengalense, sp. nov. Annual rings present. Pits of the radial walls of the tracheides very small (4μ in diameter) and arranged in groups of 2–7.

In conclusion, I wish to thank Professor Seward for an opportunity to study and describe these stems, and for many suggestions in regard to them.

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EXPLANATION OF PLATES XVII—XX.

Illustrating Miss Holden's paper on the Anatomy of Two Palaeozoic Stems from India.

PLATE XVII.

Fig. 1. Transverse section of entire stem, showing large pith, surrounded by vascular tissue with well-marked annual rings.

Fig. 2. Transverse section at internode, showing secretory cells in the pith, small lumened transfusional sheath, primary wood in bundles, succeeded by radially arranged secondary wood.

Fig. 3. Longitudinal section through interfascicular region, showing secondary wood abutting directly on the pith.

Fig. 4. Longitudinal section, showing usual character of secretory cells in pith.

Fig. 5. Longitudinal section, showing unusually elongated secretory cells.

Fig. 6. Longitudinal section through apex of bundle, showing its endarch nature—at extreme left, transfusional elements with tracheoidal markings, becoming more elongated centrifugally; next, protoxylem, metaxylem, and, finally, scalariform elements grading into secondary wood.

PLATE XVIII.

Fig. 7. Transverse section at node, showing two outgoing leaf-traces, separated by a large cauline bundle.

Fig. 8. Transverse section at a slightly higher level, where the two leaf-traces have turned sharply in a horizontal direction.

Fig. 9. Tangential section, showing the two traces widely separated in the secondary wood.

Fig. 10. Tangential section including the pith and innermost part of two bundles.

Fig. 11. Tangential section of leaf-trace embedded in secondary wood, showing low, uniseriate rays.

Fig. 12. Tangential section, showing branching leaf-trace.

PLATE XIX.

Fig. 13. Transverse section of bundle at node, showing group of primary elements, with large amount of protoxylem parenchyma, surrounded by transfusion sheath.

Fig. 14. Transverse section of another similar bundle.

Fig. 15. Longitudinal radial section through apex of bundle, showing especially protoxylem parenchyma, and broad transitional region of scalariform elements before reaching typical secondary wood.

Fig. 16. Longitudinal section through group of primary elements, to show character of protoxylem parenchyma.

Fig. 17. One of the bundles of Fig. 10 more highly magnified, to show how the protoxylem extends down the flanks of the bundle, so that a tangential section includes protoxylem on each side of the metaxylem.

Fig. 18. Radial longitudinal section of secondary wood, showing closely crowded bordered pits of the tracheides and large fusion pits on radial walls of the rays.

PLATE XX.

Fig. 19. *Dadoxylon bengalense*: transverse section, showing annual rings.

Fig. 20. *Dadoxylon bengalense*: radial longitudinal section, showing general coniferous type of wood.

Fig. 21. *Dadoxylon bengalense*: radial section, showing size and arrangement of pits.

Fig. 22. *Dadoxylon bengalense*: tangential section, showing low rays and bead-like characters of tracheide walls, caused by radial pits.

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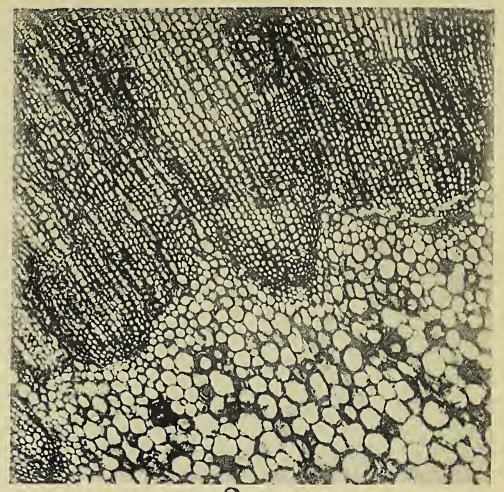

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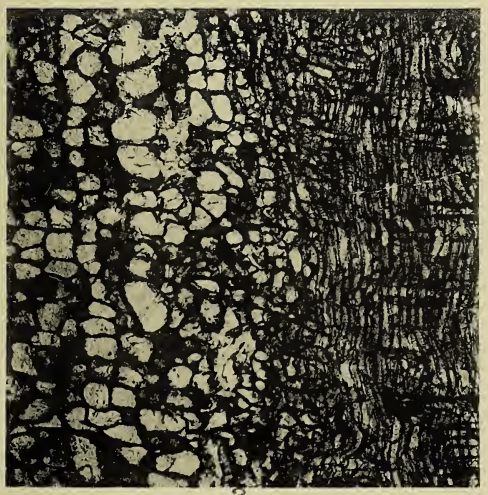

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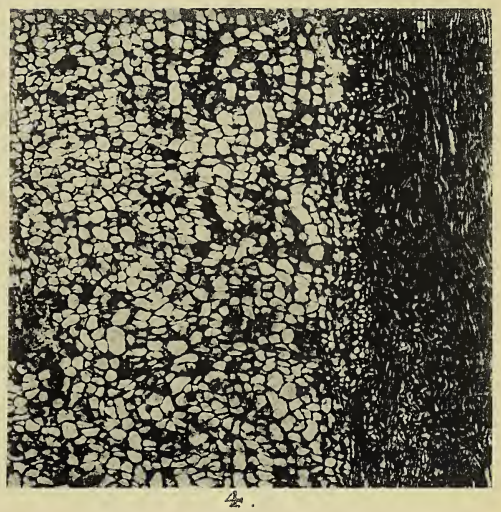

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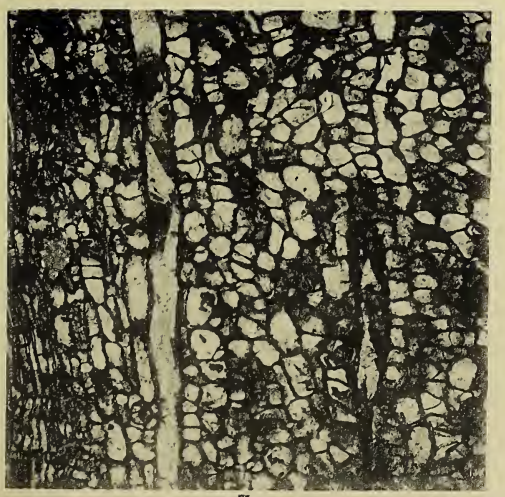

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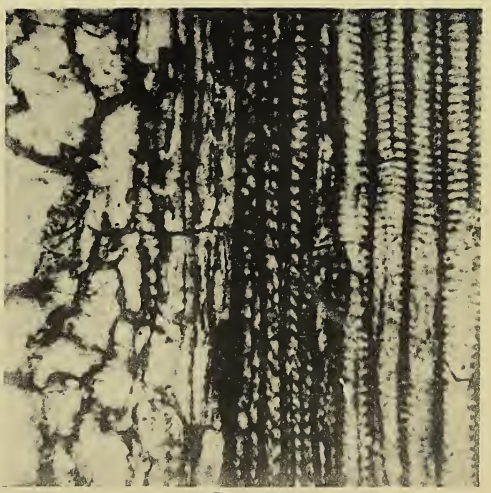

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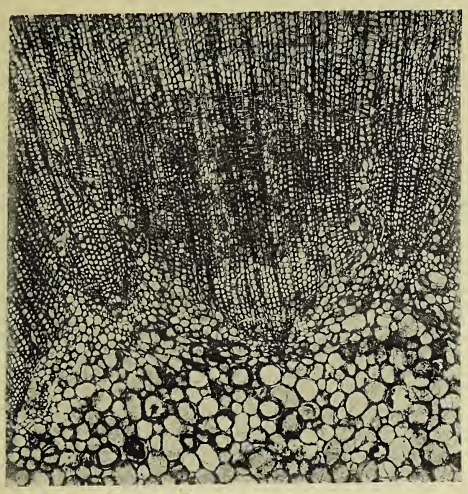
W. Tams photo. Huth coll.

HOLDEN — DADOXYLON INDICUM.



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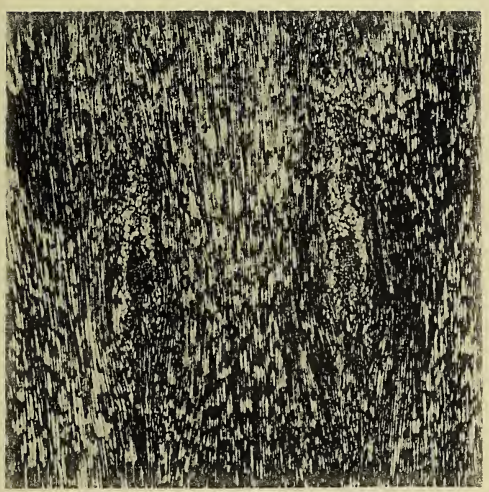

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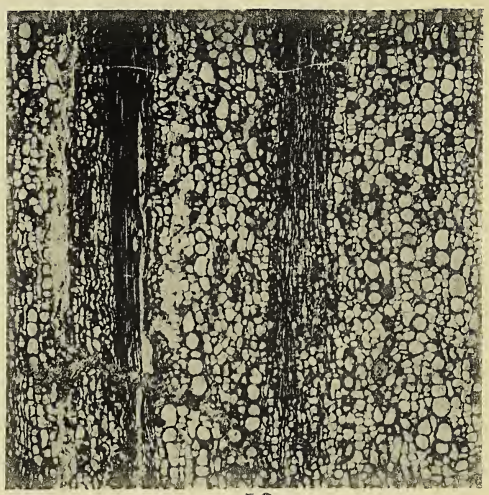

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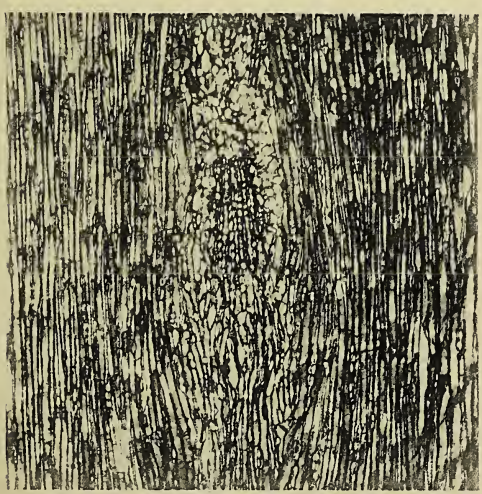

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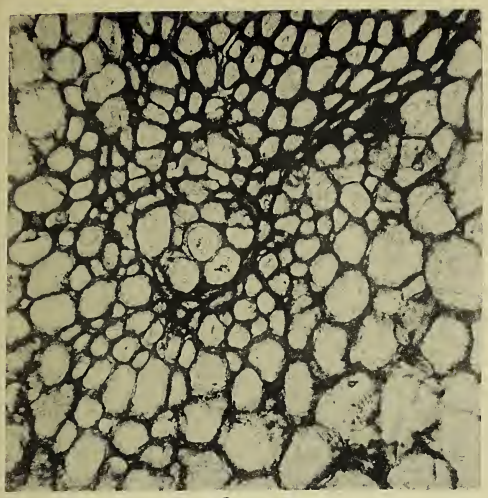

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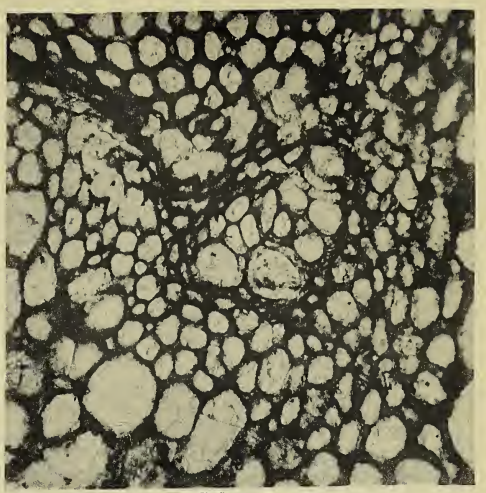

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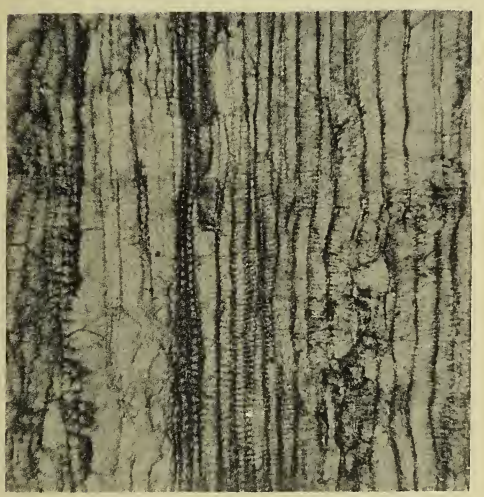

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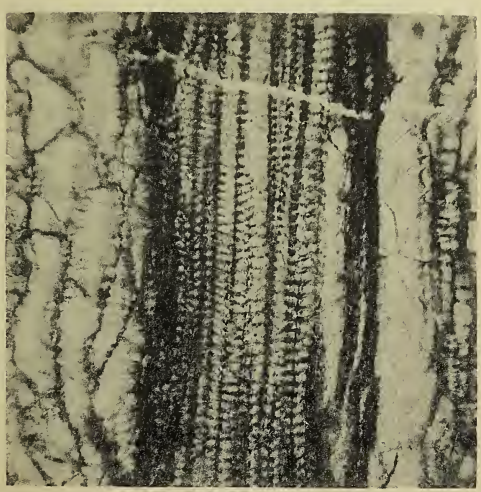

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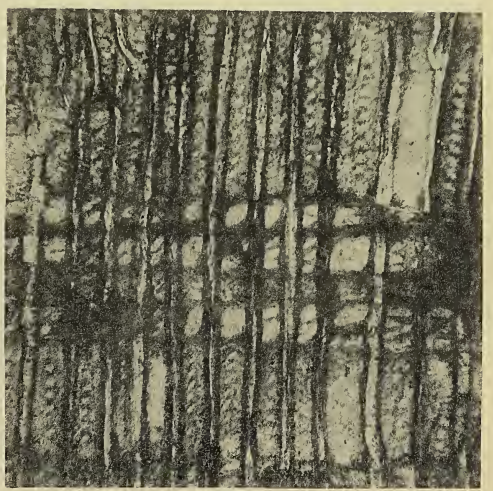

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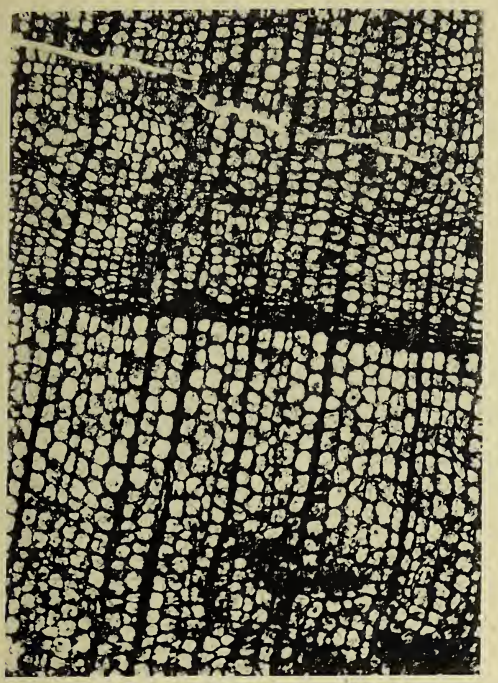

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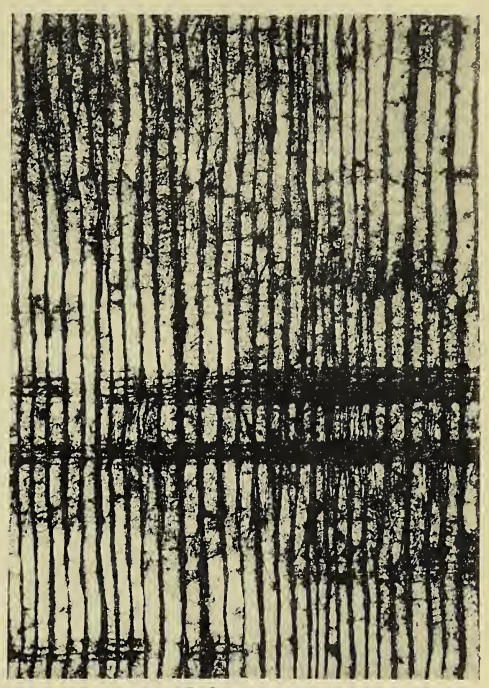

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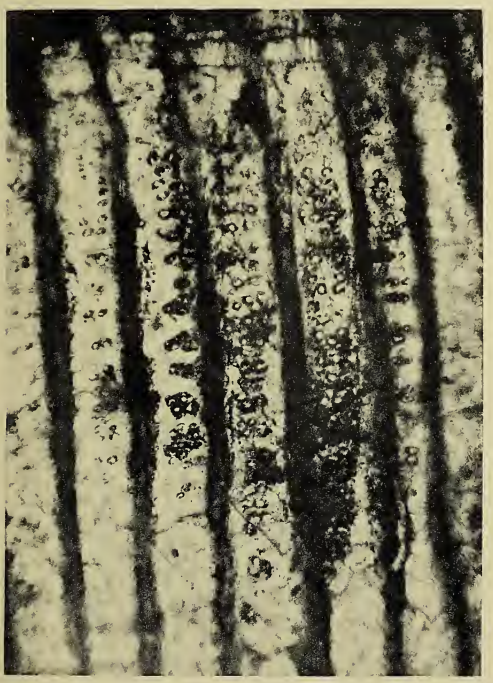

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HOLDEN — DADOXYLON BENGALENSE.

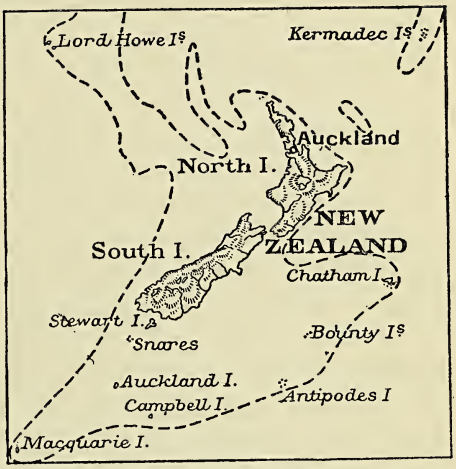
The Distribution of the Plants of the Outlying Islands of New Zealand.

BY

J. C. WILLIS, M.A., Sc.D.

With one Diagram in the Text.

IN a paper[1] upon the distribution of plants in New Zealand, I mentioned that the distribution of the plants in the outlying islands bore out in a very complete manner the hypothesis of age and area which was originally based upon the estimates given in Trimen's Ceylon Flora, and which was confirmed to the point of reasonable certainty by the distribution of the New Zealand flora, where estimates were replaced by actual measurements of longitudinal range in the islands.



New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

On the submarine plateau which soundings (given roughly in the accompanying diagram) show to exist around New Zealand, there are quite a number of islands or groups of islands, viz. to the north the Kermadecs (420 miles away), to the east the Chathams (375 m.), to the south the Snares (60 m.), Aucklands (190 m.), and Campbells (330 m.), to the south-east the Antipodes (490 m.), and to the south-west Macquarie (570 m.). It is fairly certain that at one time or another—it need not have been the same for all—all these were connected directly with New Zealand. East of them the Pacific Ocean descends to enormous depths, only exceeded at one spot upon the globe. It is therefore practically certain that the flora common to these islands and Australia must usually have passed through or near to, or have been evolved in or near to, New Zealand.

[1] Annals of Botany, vol. xxx, 1916, p. 437.

[Annals of Botany, Vol. XXXI. No. CXXIII and CXXIV. July and October, 1917.]

The Kermadec Islands, it will be noticed, are divided from New Zealand by a greater depth than 1,000 fathoms at one part of the intervening sea. The ridge on which they stand leads to the Tongas and Fiji. Now the distribution of their plants in New Zealand does not exactly agree with that of the plants of the other islands, which are not divided from New Zealand by so great a depth of water. It agrees perfectly with my age and area hypothesis, but it shows several very special and interesting features, which tend to indicate that the period when the Kermadecs were united to New Zealand was not quite coincident with that of the union with the other islands. To work out the matter in detail requires geological aid, but my figures come out with such astonishing simplicity and accuracy that it is becoming increasingly clear that evidence based upon 'age and area' cannot be altogether neglected, even in dealing with geological problems.

We shall deal principally with the species common to these islands and New Zealand, or New Zealand and Australia, leaving out those only found elsewhere in New Zealand and South America, which again present a very special problem, indicating that the union with South America was perhaps not exactly synchronous with that with Australia.

No hypothesis as yet put forward, whether Natural Selection or any other, with the exception of 'age and area', will enable us to make any prediction with regard to the distribution *in New Zealand* of the plants of the outlying islands, as to whether they are or are not widespread there; but age and area permits us to do this. The prophecy is obvious, and the fact that it is completely borne out by the actual state of the case has made it worth while to write this little paper.

If age and area be the general rule, then it is evident from the configuration of what we may, for the purposes of this paper, term the New Zealand archipelago, that the earliest arrivals in New Zealand would be the most likely to reach the islands, whilst the later ones would not do so. The three chief groups of islands, which bear enough plants to make argument from their floras by age and area fairly safe, are the Kermadecs, Chathams, and Aucklands. Examination of the little map showing the soundings will show that they lie at more or less the same distances from the narrow strip of less than 1,000 fathoms which runs down from Australia, and which, in the absence of any evidence to the contrary, one must look upon as probably the centre of the line of immigration. It is safe to prophesy, however, that those plants which reach the islands will have been the first to reach New Zealand, and should therefore be more widespread there than those that do not reach them.

Let us now examine the Australian wides of New Zealand which also reach the islands, and classify them according to their range in New Zealand, as was done in Table VIII of the previous paper on that country.

In that table (l. c. p. 449) the total of wides was given as 399. From this we have first to subtract the 98 species which are endemic to New Zealand and the islands only (dealt with below), as is done in Table X (l. c., p. 450), and then further to subtract the wides which reach New Zealand and the islands as well as Australia, 78 in all, and those going only to South America, 10 in number. The remaining 213 species, common to New Zealand and Australia but not reaching the islands, are given in the table.

TABLE I.

Class.	*Range in N. Z.*	*Kerm. Chath. Auckl.*	*Kerm. Chath.*	*Chath. Auckl.*	*Chath. only.*	*Auckl. only.*	*Kerm. only.*	*N. Z. only.*
1	1001–1080 m.	4	10	6	21	—	4	35
2	881–1000	—	4	—	10	—	5	39
3	761–880	—	—	—	2	1	—	26
4	641–760	—	—	—	1	1	1	28
5	521–640	—	—	—	—	—	1	19
6	401–520	—	—	—	—	—	1	17
7	281–400	—	—	—	—	—	3	12
8	161–280	—	—	1	—	—	—	14
9	41–160	—	—	—	1	—	1	7
10	1–40	—	—	—	—	—	—	16
Total		4	14	7	35	2	16	213
Rarity		1·0	1·2	2·0	1·7	3·5	3·6	4·3

An examination of this table discloses at once that the species that reach any of the islands are commoner in New Zealand—usually very much commoner—than those in the last column which do not reach them.[1] Those which reach all three chief groups of islands show the greatest commonness possible; those which reach two groups show less, and those that reach only one group less again. But there is one exception: those reaching the Chathams and Aucklands show greater rarity than those reaching the Chathams only. But the first are only 7 in number, which is rather few to be at all safe for deduction, especially as the single conspicuous exception in class 8 doubles the rarity without any other assistance; were it left out the rarity would be only 1·0.

This leads us to examine the exceptions, which are mostly very conspicuous in the table, and we shall find several points of interest. There are none in the first two columns, but in the third we find one in class 8. This plant, which instead of ranging the whole of New Zealand ranges only over Stewart Island and the south end of the South Island, is given in Cheeseman's Flora as *Carex appressa*, R. Br., and reference to that work shows at once that there is some doubt about the identification, both in New Zealand and in the Chathams. Incidentally it may be noticed, as a very strong argument in favour of my hypothesis, that when a species is found to behave very exceptionally in regard to its distribution—as

[1] Each unit of rarity represents a range of 120 miles, e. g. if 2·0 represents a range of 940 miles, 3·0 represents one of 820.

regarded from the 'age and area' point of view—it is almost always found to be one about whose identification or true nativity there is a doubt. This was well shown in the case of the exceptional number of 21 species found in the last class of the flora in place of the expected 5 or 6 (l. c., p. 452).

In the next column, the plants reaching the Chathams only, there is a conspicuous exception in class 9, *Pomaderris apetala*, which is of such interest that I have devoted to it the last paragraph of this paper. The 16 species in class 10 in the last column have already been dealt with in the preceding paper, as mentioned above. Nos. 2, 3, 4, 9, and 14 of that list range to the islands and are omitted here.

Now if we omit these exceptional cases, the order of rarity comes exactly into line with my hypothesis, the lowest being for the plants of two or three islands and New Zealand, the next for the plants of one group and New Zealand, and the highest for New Zealand only. But in any case, the plants which go to the islands are far commoner (more widespread) in New Zealand than the plants which do not. Now there is no conceivable reason why ranging also to a few little islands should make a species more widespread in New Zealand, unless it be age, which has given them time to spread in New Zealand to the maximum degree. The reverse hypothesis, that dispersal goes with youth, will be rather hardly pressed to explain why youth should ensure that a species should reach more islands. Nor, to go back to yet older views, is there any reason why great dispersal in New Zealand should ensure reaching the islands. If it were so, why were only 45 wides of class 1 selected, and the other 35 left behind, and why 19 of class 2 (instead of any of these 35), leaving 39 behind? It is evident that those which reached the islands were on the whole the first comers to New Zealand. The intermediate position of the species which range only to one group of islands renders the older explanations impossible.

Further consideration of what has been said brings out a very important point which may easily be lost sight of. Twenty species ranged the entire length of New Zealand, and got to two or more island groups; 25 to one island group; and 35 did not reach the islands at all. It is therefore evident that many of these last 60 once ranged to greater or less distances across land which is now submerged. In other words, *submergence may overtake spread*, and greatly reduce, even to extinction, the area occupied by a species. New species, therefore, have probably the best chance of survival and wide dispersal if they arise in the middle of a large continental area, and those that have the best chance of long survival are those which have been fortunate enough to disperse into areas so large that the chance of extermination by submergence or other catastrophe is least.

Other points of interest come out from an examination of Table I. The number of wides which reach two or more islands is 25, reaching one group only is 53, and not reaching any is 213. This indicates age as the

chief factor. Further, in the last column, it will be noticed that though the numbers increase upwards, the highest is not in class 1, which reach Stewart Island, but in class 2, which range only the two main islands. This goes to indicate comparative youth, Stewart having been cut off before many of the plants could reach it.

It is thus clearly evident that the distribution in New Zealand of the Australian wides goes, not with the area covered in the world in general, but with that covered in the New Zealand archipelago, which was entered probably by a comparatively narrow connexion with Australia. This, it seems to me, completely excludes any explanation based on Natural Selection, whilst youth and area can only be made to explain it with the aid of supplementary hypotheses. It excludes the idea of absolute youth, and youth within the country is too far-fetched an idea to be tenable.

We may now go on to the species endemic to New Zealand and the islands, which in the previous paper were treated as wides. They occur nowhere else in the world. They, on my hypothesis, are younger than the wides already dealt with, and should be fewer in proportion to the endemics of New Zealand proper than was the case with those wides. In actual fact they are 98 to 902, against 78 to 213. None of them reach all three of the chief island groups, and only 19 reach two (8 the Kermadecs and Chathams, 11 the Chathams and Aucklands). It is therefore safer to reason from them as a whole, and they give the following table :

TABLE II.

Class.	*Range in N. Z.*	*Species.*
1	1001–1080 m.	41
2	881–1000	21
3	761–880	8
4	641–760	7
5	521–640	5
6	401–520	6
7	281–400	2
8	161–280	3
9	41–160	—
10	1–40	5

This gives an average rarity of 2·9 ; that is to say that, though confined to New Zealand and the islands only, they are far more common in New Zealand than the average of *all the wides* (3·5). The difference of 0·6 represents a range of 72 miles per species more than the mean range of the wides (760 m.). But in actual fact they should rather be compared with the 301 wides that are left after their removal, and these have an average range of 24 miles less, or 736 m. The dispersal of these endemics, however, as is required by my hypothesis, is less widespread than that of the wides which also go to the islands, which, if all be added together, gives a rarity of 1·9, or 120 miles more than the endemics (2·9).

Now, on the hypothesis of Natural Selection, or of youth and area, what conceivable reason can be given to explain why these endemics, which only range to a few small groups of little islands outside New Zealand, are yet more widespread in that country than the great group of 'wides'. Are these endemics younger than the wides as a whole, but older than the wides which reach the islands? If they are older, why did they not reach more islands? And why are they more widespread than the wides which reach to Australia, Asia, or South America, but do not reach to the islands? Nothing but age and area will explain these facts simply and reasonably.

Of the five endemics in class 10, four range far to the south through the Aucklands, only reaching Stewart Island of New Zealand proper, and the fifth, *Lepyrodia Traversii*, is a species as to whose correct identification with the one upon the Chathams I feel some doubt (see Cheeseman's Flora).

Now let us take the case of the species endemic to the islands only, and which do not occur in New Zealand. These, eighty in all, were omitted in my previous paper. None are endemic to the Kermadecs and Chathams, or to the Chathams and Aucklands, but a good many to more than one of the southern groups, which are not so far apart. This would be expected if they are the younger, for the wides would only reach the islands comparatively late. If we take those which are confined to the three principal groups, we obtain the following table:

TABLE III.

Islands.	*Wides.*	*Endemic to these islands only, and N. Z.*	*Endemic to these islands only.*
Chathams	72	50	25
Kermadecs	36	14	13
Aucklands	24	12	9

Twenty species are endemic to the Aucklands with the Campbells or other southern groups, but all but about three or four belong to South American genera. It will be noticed at once that, as in New Zealand itself, the larger the number of wides, the larger that of endemics, a fact very difficult to reconcile with the hypothesis of Natural Selection, or with the dying out of endemics on account of the competition of the wides.

It is thus clear that the floras of the outlying islands of New Zealand, and their distribution, give very conclusive evidence in favour of my hypothesis of age and area. In a later paper I hope to discuss the peculiar features shown by the species common, not to New Zealand and Australia, but to New Zealand and South America.

In conclusion, it may be noted that this work throws a side-light upon the much-discussed problem of the original home of the Maoris. It was mentioned above that *Pomaderris apetala* was a very conspicuous exception in the grouping of the flora common to New Zealand and the Chathams. It

only occurs near to Kawhia, on the west coast of the North Island. Cheeseman remarks that 'the Maoris assert that it sprang from the rollers or skids that were brought in the canoe *Tainui* when they first colonized New Zealand'. It is fairly evident, from the figures, that this legend is quite probably correct, and therefore, as this tree only occurs elsewhere in Australia and the Chatham Islands,[1] that the Maoris came immediately from one or other of these places, perhaps most probably the former, as Kawhia is on the west coast. The origin of the Maoris remains a problem, but their route is perhaps made a little more clear.

Summary.

The distribution in New Zealand of the plants which also reach the outlying islands is here dealt with. Starting from my hypothesis of age and area, the prediction is made that the most widespread plants in New Zealand will be those that reach the islands also, and that those which do not reach them will be less widespread. This is confirmed by the facts in the most striking way, both in the case of wides and endemics.

The endemics which reach the islands are more widespread in New Zealand than the average of all the wides, a fact impossible of explanation by Natural Selection; the hypothesis of youth and area is placed in a difficult position to explain why the younger a species is, the more islands it should reach. The species endemic to the islands only are also grouped in a way very difficult of explanation on any other hypothesis than age and area—the more wides, the more endemics. Other facts are also brought up which render these hypotheses untenable.

It is shown that submergence may overtake spread, even to the extent of killing out a species.

Incidentally, a strong point in favour of my hypothesis is the almost certain way in which it picks out those species where there is doubt about identification or nativity.

[1] Eleven 'wide' species only occur on the islands without occurring in New Zealand, and of these four are Kerguelen species occurring only on the southern islands, and six are tropical forms only found in the Kermadecs. One only, *Leucopogon Richei*, occurs in the Chathams and Australia, and may be classed with *Pomaderris* as a case for which there must be some special explanation.

Further Evidence for Age and Area; its Applicability to the Ferns, &c.

BY

J. C. WILLIS, M.A., Sc.D.

WHEN once the regular graduation of species in a genus or family into series of what we may almost perhaps term 'wheels within wheels' (10, figs. pp. 336–7) has been pointed out, it needs but little investigation to convince oneself that the law of 'age and area' is general in its applicability. So long as it was only based on the estimates made by Trimen for the Ceylon flora, its foundations were by no means secure, but it was soon confirmed to a high degree of certainty by the results for the flora of New Zealand (9), where actual measurements of range were employed. Perhaps the most striking proof of the probable correctness of my deductions is the remarkable result given in Tables III to VI of that paper. The further investigation of the outlying islands of New Zealand (15) showed the operation of the law even more decisively if possible, at the same time giving what appears to me a fairly conclusive proof that the active factor in distribution is age (not youth) within the country.

It would therefore seem that the production of a few more pieces of confirmatory evidence, based like the figures for New Zealand upon actual measurements, should suffice to establish the hypothesis upon a firm basis. In the present paper I give five such pieces of evidence, three of which at the same time go to show that the hypothesis applies not only to the Angiosperms, to which alone I have confined it as yet, but also to the Coniferae and Ferns, and is therefore probably perfectly general in its application.

It is not usually possible to get such clear and convincing evidence as shows in the floras of Ceylon and New Zealand, where the plants can be split into three sections, graduated in order of rarity (Ceylon, Ceylon-Peninsular India, Wide; New Zealand, New Zealand and outlying islands, Wide), though the first and fourth cases given here are such. But once the graduation is seen to hold generally, it will suffice to parallel them in one or more features.

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I. The Orchids of Jamaica.

In order to bring in a country as far removed from Ceylon and New Zealand as possible, I have taken Jamaica, of which a detailed flora is in course of publication by Messrs. Fawcett and Rendle (4), and have used the first volume, which contains the Orchids, a family exceptionally well known in the country.

To go into great detail is unnecessary, and I shall simply describe the method used and the results obtained. Taking a sheet of squared tracing-paper, I placed it over a large map of the island, and with the aid of the large official map identified and marked the localities given, 162 (different) in all. A few of these I was not able to identify, especially such as Ramble, Retreat, and Belvidere, which appear upon the map in dozens of places. I thus noted for each species the squares in which it occurred, and for the unidentified localities I have simply allowed another square. This in actual fact adds almost exactly a square for every other species, but acts equally on all alike. Each of the larger squares of the tracing-paper covered an area approximately $6\frac{3}{4}$ miles square.

To make a fair test of my hypothesis, and to enable comparison with Ceylon and New Zealand, I have divided the Orchids (194 in all) into three groups—those endemic to Jamaica, those found in Jamaica and Cuba only, and those with wider distribution than this (wides). Adding up for every species the number of squares for which it is recorded, we get:

Table I.

No. of squares.	*Wides.*	*Jamaica-Cuba.*	*Endemic.*
1	7	1	30
2	11	2	12
3	13	2	10
4	15	2	4
5	12	3	7
6	10	4	5
7	5	—	3
8	9	—	1
9	6	—	1
10	5	1	1
11	7	—	—
12	1	—	—
13	—	—	—
14	—	—	—
15	1	—	—
16	1	—	1
17	—	—	—
18	—	—	—
19	1	—	—
Total	104	15	75
Average No. of squares occupied	5·7	4·5	3·0

This result, it will be at once seen, is exactly parallel to that obtained for Ceylon and New Zealand. The wides occupy the largest area, the

Jamaica-Cuba species the next, and the endemics the least. Nearly half the endemics are found in one square only. It is of interest to look at the seven wides which only occur in one square, and note that as usual one picks out the most uncertain species in the flora by this method. *Epidendrum ciliare* is a very conspicuous orchid, but is only recorded on the authority of a specimen 'from Jamaica' by Shepherd in Kew Herbarium. *E. patens* is assigned by Swartz to Jamaica, but no specimen has been seen. *Pelexia adnata* was found by Masson in 1781, but has never been seen again. *Pleurothallis Wilsonii* is doubtfully identified with the Porto Rico and Guadeloupe specimens, and is perhaps more likely an endemic. This leaves only three, *Vanilla phaeantha*, *Arpophyllum giganteum*, and *Epidendrum Ottonis*, all collected by Harris, and therefore reliably recorded. Similarly, the one Cuba species that occupied only one square, *Pleurothallis foliata*, was recorded by Wilson (1846–58), and has never been seen again. This and others may quite well have been exterminated in the clearances that have gone on in the last eighty years.

Many may express surprise at the small number of squares from which even the wides are recorded, when it is realized that there are 130 squares in the island; and they may go on to say that further investigation will show that my figures are unreliable. But to this, as in the similar cases of Ceylon and New Zealand, the reply is simple—is there any reason to suppose that collectors deliberately collected wides in preference to Jamaica-Cubas, or to endemics? They are more often recorded simply because they occur more often, and there is no shadow of reason to suppose that the relative results would be affected by any amount of further investigation. And my results depend, not upon absolute figures, but upon comparative.

There is one endemic genus of Orchids in Jamaica (*Homalopetalum*), and as with other endemic genera in Ceylon and New Zealand, it shows less distribution area than the average of the endemics, occupying only two squares against an average of three.

It is thus abundantly clear that my hypothesis of age and area is fully borne out by the facts of the orchid flora of Jamaica.

II. The Flora of the Hawaiian Islands.

As a further piece of evidence in favour of my hypothesis, and from a country which is extraordinarily rich in endemic forms, I have taken the Hawaiian islands, employing as a basis the list of plants given in Hillebrand's Flora (5). There are seven chief islands in the group—Niihau, Kauai, Oahu (on which is the capital, Honolulu), Molokai, Lanai, Maui, and Hawaii. They are separated from each other by stretches of water from ten to seventy miles in width, through which pass currents in a north-

easterly or south-westerly direction, i. e. at right angles to the general direction of the group.

Taking for each member of the flora the number of islands upon which it has been found, one obtains the following result:

TABLE II.

No. of Islands.	*Wides.*	*Endemics.*
All	97 (74)	41
6	—	8
5	1	11
4	5 (4)	55
3	7	80
2	17	113
1	22	273
Total	149 (125)	581
Rarity (in figures from 1 to 7)	2·7 (3·0)	5·6

In great probability, however (see 5, Introduction, p. xvi), some of these wides, viz. twenty-three in those reaching all islands, and one reaching four, were introduced in prehistoric times, and as of the wides only reaching one island or two, most reach Oahu or Hawaii (the largest and most important islands, the former having a great port of call—Honolulu), probably many of the last two classes were recently introduced. This reduces the list of wides to 125, and brings the rarity to 3·0, which, however, makes no difference to the argument.

From this table it is evident that on the whole the wides arrived in the group at an early period, and had already most of them spread over the whole of it before it broke up into the existing islands, which stand upon a submarine plateau. The way in which the wides reached the group does not matter to the present discussion, whether overland, or by other means. Only fifty-one of them arrived too late to spread over the whole of the islands before they became broken up, and of these there is good reason to suppose that many are comparatively very recent arrivals.

A considerable number of endemics were also evolved in time to reach the outer boundaries of the present archipelago before it broke up. It must be remembered that upon my hypothesis all plants, given long enough time and absence of serious barriers, will ultimately cover the whole area (cf. 11, p. 20, Table XIII, columns from G onwards). In the Hawaiian islands the whole area is mountainous, so that plants of all elevations can probably spread with comparatively equal facility, though it would seem probable that as the suitable areas of high elevation are of necessity smaller, in most cases, the plants of high altitudes would most often tend to spread less rapidly than those of low. This, therefore, must be added to the list of causes that may modify the action of age and area, as given in a previous paper (13, p. 206).

The forty-one endemics found in all the islands are thus explained as due to the continual growth of the number by new arrivals from lower in the scale. But when the islands separated, there would still be many that had not yet covered the whole area, and as the endemics are younger, these would be more numerous than in the case of the wides, in proportion at any rate. They would increase in the usual way from those occupying large down to those occupying small areas. Once the islands were well separated, they would necessarily be confined, in the great majority of cases, each to its own island, its two islands, or whatever number it then occupied. The actual facts of the table above bear out these suppositions completely.

If, as there seems just reason to suppose, endemic species may themselves give rise to other endemics, then the numbers *now* found on one island are probably much in excess of those that actually occurred at the moment of separation. The sudden rise from 113 on two islands to 273 on one goes to support this supposition.

The facts of the Hawaiian flora thus fit in easily with my hypothesis of 'age and area', but without considerable use of supplementary hypotheses, based merely on supposition, cannot be made to fit any other.

III. The Distribution of *Callitris* in Australia.

So far I have not attempted to include within the operation of my 'age and area' hypothesis anything but the Angiosperms, but a very little investigation shows that it is in reality more general. Happening to have in my possession a monograph of *Callitris* (1), I investigated its distribution. The genus is confined to Australia. *C. glauca* occupies the whole range of the genus, covering Australia and Tasmania. Two species come into a second class, *C. verrucosa* (New South Wales to West Australia) and *C. tasmanica* (New South Wales to Tasmania), while a third class includes *C. robusta*, *Drummondii*, *calcarata*, *intratropica*, *arenosa*, *rhomboidea*, *Macleayana*, and *propinqua*. Lastly, a fourth class—those of very narrow distribution—includes *C. oblonga*, *gracilis*, *Muelleri*, *tuberculata*, *Roei*, *Morrisoni*, and a *sp. nov.* Considering the small size of the genus (eighteen species), this grouping fits in very well with my hypothesis, and goes to show that it applies also to the Coniferae.

IV. The Ferns of New Zealand.

I shall now go on to show, in two short essays, that the Ferns also follow the law of 'age and area'. In order to reduce the fern floras of New Zealand and Hawaii to a common denominator in a group in which the nomenclature has been so much in a state of flux, I have used the names as settled by Christensen (3), but the result is almost exactly the same as if I had left them untouched.

Leaving age and area entirely out of the consideration, it is generally accepted that the Ferns are on the whole a much older group than the Angiosperms. One may therefore obtain a good crucial test as to whether it is age or youth that goes with area occupied. If the endemics be older species dying out, then it is evident that fern endemics should occupy less area than angiosperm endemics; but if, on the other hand, my hypothesis be accepted, then they should occupy more. The actual facts give us for the Ferns:

TABLE III.

	Number.	*Rarity* (Figs. 1–10).
Wides (including those only reaching the outlying islands)	104	3·2
Endemic to New Zealand proper	24	3·8

The corresponding figures for Angiosperms (9, p. 449) were 3·5 and 6·5. The Ferns therefore, whether wide or endemic, show a wider distribution in New Zealand than the Angiosperms. This is especially the case with the endemic Ferns, which range on an average 724 miles, against a range of 400 for an angiosperm endemic.[1] This agrees with what is required under my hypothesis, but is very difficult to harmonize with the idea that endemics are old species dying out.

Why, whilst the endemics show this great difference in favour of the Ferns, the wides should only show an increased range of thirty-six miles is not altogether clear. The explanation which I would suggest is that as we know that fern spores may be carried to great distances by the wind, and as we have actual examples in which Ferns have arrived in new places over considerable distances with no intermediate halting-places, e. g. the summit of Ritigala in Ceylon (12), or the sterilized island of Krakatoa near Java (6, 8), land connexion is not so necessary as it is in the case of Angiosperms. There is no reason why new Ferns should not occasionally arrive in New Zealand subsequently to the breaking of the land bridge, though the figures already given rather go to show that most of them arrived by that way. If we accept this hypothesis, which is not very far-fetched, there is no difficulty in understanding why there are so many wides with but a small range in New Zealand, though they may be widely dispersed abroad. It does not affect the question of the endemics, because one of these which became established beyond the sea would cease to be an endemic. Nor does this behaviour of the wides give any encouragement to the supporter of Natural Selection, or of the idea of dying out of old species.

We may get a clearer insight into this question by splitting the Ferns of New Zealand into four groups, according as to whether they do or do

[1] Each unit of rarity represents 120 miles of range; 6·5 means 400 miles, 5·5 means 520, and so on.

not reach the outlying islands (Kermadecs, Chathams, Aucklands), or some of them. This gives us the following table:

TABLE IV.

Range in N. Z.	*Wides reaching Islands.*	*Wides not reaching Islands.*	*Endemics reaching Islands.*	*Endemics not reaching Islands.*
1001–1080 miles	27[1]	4	5[3]	5
881–1000 „	12[2] (2 K)	8	1	5
761–880 „	1	3	2	5
641–760 „	1 (K)	8	1	2
521–640 „	1 (K)	9	2	2
401–520 „	—	5	—	—
281–400 „	1 (K)	5	1	1
161–280 „	1 (K)	3	—	1
41–160 „	—	1	—	1
0–40 „	—	2	—	2
Total	44	48	12	24
Rarity	1·7	4·6	2·8	3·8
Angiosperms	2·4	3·9	2·8	6·5

K means a species reaching the Kermadecs only among the islands, though ranging beyond them into the rest of the world.

This is a very interesting table. In the first place, it shows that a sprinkling of wides have come in from the Kermadecs, which have supplied part of its flora to, as well as received part of their flora from, New Zealand. If we subtract those which only reach the Kermadecs among the islands, we get 27 in Class I, 10 in Class II, and 1 in Class III with an average rarity of 1·3, or nearly as widespread in New Zealand as possible. The 10 all range to Foveaux Strait, which evidently was formed very early in the geological history of New Zealand. The one species in Class III ranges from Stewart Island up to 200 miles south of North Cape, and reaches the Chatham, Auckland, Campbell, and Antipodes islands, as well as Australia.

Turning to the second column, the first thing one notices is the irregular graduation of the numbers from top to bottom. This is a very strong point in favour of my suggestion made above, that many of these wides may have entered New Zealand after the breaking of the land bridges. Only 8 of the 48 reach Stewart Island; 10 more reach Foveaux Strait, and 23 reach the North Cape. Their ranges are much more scattered about the islands than those of the species in the first column, which again is a point in support of my hypothesis. It is also favoured by the fact that the rarity of these species is much greater than that of the

[1] Reaching in all 53 of the three chief groups (K., Ch., Au.), and 18 other groups (Campb., Antip., Macquarie, &c.).

[2] Reaching in all 14 of the three chief groups, and three others.

[3] Reaching in all 6 of the three chief groups.

corresponding Angiosperms, which are much less able than the Ferns to pass over long stretches of water. The rarity of the Ferns which had also reached the islands is much less than that of the corresponding Angiosperms, as would be expected by reason of their greater age.

It will be noticed that the fern wides which do not reach any islands are rarer than the endemics which do not do so. On my hypothesis as to the late arrival of some of the Ferns, this is intelligible enough, but it is an awkward difficulty for the Natural Selectionist, or the supporter of the idea of the dying out of endemics.

Passing on to the third column, one notices at once that the endemics, as would be expected from their age, show a marked accumulation at the top, and this is also shown in the last column, though, as would be expected, the latter shows more species in the lower classes. The one endemic in the third column that is below the fifth class has a range that includes the Chathams, Aucklands, and Antipodes, and evidently only touched New Zealand with the outer part of its circle of distribution: its centre of origin was probably somewhere between the Chathams, Aucklands, and Antipodes.

The three chief groups of islands are the Kermadecs (north), Chathams (east), and Aucklands (south), and it is interesting to note the proportionate numbers that reach them:

TABLE V.

Reaching.	*Ferns.*				*Angiosperms.*			
	Wides.		*Endemics.* (*N. Z. and Islands.*)		*Wides.*		*Endemics.* (*N. Z. and Islands.*)	
		Rarity.		*Rarity.*		*Rarity.*		*Rarity.*
K., Ch., Au.	7	1·0	—	—	4	1·0	—	—
K., Ch.	4	1·2	1	1·0	16	1·3	8	2·2
Ch., Au.	10	1·0	1	7·0	11	2·6	9	2·3
	14	1·07	2	4·0	27	1·8	17	2·2
K.	6	4·6	—	—	16	3·6	7	3·8
Ch.	17	1·5	5	1·8	36	1·6	33	1·8
Au.	—	—	4	3·5	10	4·6	34	4·0
	23	2·3	9	2·5	62	2·6	74	3·0
Total	44		11		93		91	
Total of wides in N. Z.	92				301			

The Ferns are distinctly the more widespread group: the first class (K., Ch., Au.) should be especially noted, and it should be observed that all its members, both Ferns and Angiosperms, show the maximum possible commonness in New Zealand, ranging the islands from end to end. The second degree of commonness is found in the second class, the third in the third, with the single exception of the endemic fern reaching the Chathams and Aucklands.

If my hypothesis be accepted, another prediction may be made as

[1] Perhaps a case of accidental transport, but see above on this page.

follows. No other hypothesis, be it noted, allows of any predictions. One will expect the Ferns to show a graduated arrangement by zones similar to that shown by the Angiosperms in Tables III to VI (9), and as they are on the whole a good deal older, one will expect them to have spread farther along the islands of New Zealand, so that the curves should be flatter. At the same time, one will expect to find them taking less notice of the presence of Cook's Strait, as they were probably developed early enough to have crossed that part of New Zealand before the strait was formed. Turning to the actual facts, one gets:

TABLE VI.

		0–100 m.	100–200 m.	200–300 m.	300–400 m.	400–500 m.	500–600 m.	600–700 m.	700–800 m.	800–900 m.	900–1,000 m.	1,000–1,080 m.
Ferns:	*Wides*	75	77	84	84	79	78	69	69	61	58	39
	Endemics	15	18	23	21	21	21	21	19	18	19	8
Angiosperms:	*Wides*	209	210	237	237	235	242	236	227	215	204	112
	Endemics	234	280	330	368	386	537	532	527	516	414	130

It is evident that the prediction is borne out by the facts. The fern curves are flatter, and they, as well as the 'wide' Angiosperms, take no notice of Cook's Strait (between the 5th and 6th figures), which holds up so many endemic Angiosperms. Similarly, they take less notice of Foveaux Strait, between the last two figures. One gathers the impression that the angiosperm wides and the fern endemics are on the whole of somewhere about the same period, very broadly speaking.

Another prediction one may make is that as the Ferns are older, more (proportionately) should have reached the outlying islands. This one finds to be the case, for 44 out of 92 do so, against 95 out of 301 Angiosperms.

More of the Ferns, too, should have reached even farther than this, and thus those that were once endemic would have become wides, leaving a smaller proportion of endemics. The fern endemics are only 24 to 92, against 902 angiosperm endemics to 301 wides.

It is thus clear that the Ferns also obey the law of age and area, and throw a good deal of light upon various problems associated with it.

V. The Ferns of the Hawaiian Islands.

Taking the Ferns from Hillebrand's Flora (5), and naming them afresh from Christensen's Index, one gets the following:

TABLE VII.

Islands.	*Wides.*	*Angiosp.*	*Endemic.*	*Angiosp.*
All	36	74	20	41
6	—	—	—	8
5	2	1	5	11
4	2	4	5	55
3	7	7	10	80
2	8	17	14	113
1	5	22	17	273
Total	60	125	71	581
Rarity (figures 1–7)	2·8	3·0	4·3	5·6

As in New Zealand the endemics are a good deal more common (widespread) than the corresponding Angiosperms, but the wides are not, and again I would suggest that this fact is due to comparatively recent arrival of a good many forms by transport of the spores through the air.

Just as in New Zealand, too, there are fewer endemic Ferns in proportion to the wides; they are 71 to 60 against 581 to 125; and many more in proportion reach all the islands (more than $\frac{1}{4}$ against 1/14).

It is evident that the facts of the Hawaiian fern flora agree with those of New Zealand, and go to support my hypothesis of age and area.

It is thus evident that the law of age and area is very general, if not universal, in its applicability. The Orchids of Jamaica show its operation as clearly as did the floras of Ceylon and New Zealand. The distribution of the flora of the Hawaiian islands agrees with it; that of *Callitris* in Australia shows that it probably applies to Coniferae, and that of the Ferns of New Zealand and Hawaii that the Ferns also obey the same law.

In the Ferns of both countries, there appears a very clear departure from the normal trend of the figures, though it is not in a direction to encourage those who believe in Natural Selection as governing areal distribution, or in the hypothesis that endemics are old species dying out. In both cases the endemics, which must on the whole be older than the endemic Angiosperms, are much more widespread than the latter, as would be expected upon my hypothesis. The wides, however, do not show this; they have only a slightly greater distribution than the angiosperm wides. It is evident that this is probably due to the same cause in both cases, and I have suggested that it is due to the fact that Ferns may be easily carried as spores, and so may continue to arrive up to the most recent times.

But if we accept the law as thus wide and general in its applicability, it is evident that it must have some bearings upon other branches of botanical work, and it may be worth while briefly to call attention to some of these, in so far as they affect lines in which I have myself worked.

In systematic botany, for example, the tendency in recent times, as

exemplified in Engler and Prantl's 'Natürlichen Pflanzenfamilien', has been to split up the older families into smaller, and to a certain extent to do the same with the genera. This splitting is on the whole fully justifiable, and there are yet some cases, it seems to me, where it will have to be done; but, on the other hand, there is no doubt that it may easily be carried too far. From my work on age and area it is now fairly clear that in systematic work the geographical factor should be taken into consideration to a greater extent than has hitherto been the case. In the last twenty years the tendency has undoubtedly been in this direction, and the genera and families are tending more and more to be split into groups which show geographical as well as structural affinities. But it would seem as if more stress yet must be laid upon the former.

The figures given in my various papers on this subject afford no evidence to show that any species are actually dying out. Many people at once jump to the conclusion that by this I mean that *no* species are dying out, but this is by no means the case. What the figures show is that such cases are too few to be seen in them in an unmistakable way—and this fact, by the way, shows much more clearly in those sets of figures which (as in the case of New Zealand) are the result of actual measurement, than in the figures for Ceylon, which were merely estimates. New Zealand may quite well contain 20–30 or more species which are in process of dying out, but their presence is not shown by the figures, and could not be, unless they all belonged to one or two families.

But if this be so, then it is no longer safe to assume a greater discontinuity in distribution than may be accounted for by known geological changes, except in those cases where we may with reasonable probability invoke accidental transport by air or water. And no isolated example of geographical discontinuity can be accepted as of value without other confirmatory cases. Numerous North Temperate Zone species, for example, occur again in Australia or New Zealand, without there being any in the intermediate regions, so far as we know. This we may perhaps put down to alternating glacial periods. Fern spores again, we know, may be carried almost indefinite distances by wind, and may germinate if on arrival they strike a suitable spot. Probably this does not often happen, but it almost certainly does at times occur, so that we may accept as quite possible any distribution that may occur for any group of Ferns, without needing any other supplementary hypotheses.

But in the flowering plants such long-distance transport is very rare, and we have little evidence to show that it occurs for instance between one continent and another. Probably the Orchids and Composites are best suited to such transport. On the summit of Ritigala, for example, which is separated by about 40 miles of 'dry' country from other 'wet' mountain country in Ceylon, I found (12) 49 wind-carried wet-zone plants. Of these

no less than 24 were Ferns or Lycopodiums, and 20 Orchids, while the remaining five were made up of two each from the Compositae and Asclepiadaceae, and one of the Apocynaceae. It is thus evident that even at a range of 40 miles most of the parachute mechanisms begin to break down. In any case the families in which long-distance transport by wind may occur are few. Or, to take the case of dispersal by birds, there is little or no good evidence for any beyond, say, at the most 1,000 miles. Or yet again, where there is a marine current, transport may occur by its means to great distances, provided the climates at the two ends are not hopelessly dissimilar. In the Maldives, for example, Prof. J. Stanley Gardiner and myself (16) showed that several of the water-borne species must probably have come from Malaya or from the Seychelles—a long distance in either case.

But there are quite a number of cases in which none of these causes can be called in as aid, and yet a great discontinuity is accepted in the geographical distribution. To take an extreme instance, the genus *Cryptotaenia*, DC. (Umbelliferae), is described as having one species in Canada, one in Calabria, and one in the Cameroons. If there were, say, twenty to thirty more with the same distribution, then it is obvious that some explanation would have to be found. But with this one case only, it is evident that the identification of these three species as belonging to the same genus must rest upon the convergence of evolution or upon a parallel development or polyphyly (14, p. 446). Such cases are very numerous, though there are not many with quite so marked a discontinuity as that quoted.

Or, as another case, let us take the family Lardizabalaceae, sometimes split off from Berberidaceae, chiefly on the ground of climbing habit, unisexual flowers, and polycarpellary ovary. There are seven genera, of which five occupy the region from the Khasia Mts. (Assam) to Japan, whilst *Boquila* and *Lardizabala* are found in Chile. Now if the first five were found in Australia or New Zealand, this would be normal enough; these countries have many groups in common with temperate S. America, and even species. But from eastern Asia to Chile is too great a gap. The fleshy fruit could not be carried by birds to such a distance in such a direction, and we have no longer any right to invoke the dying out of linking forms in the intermediate region, without direct evidence. It *may* have occurred, but we require evidence to prove it. This being so, it seems to me almost impossible, *on the present evidence*, to regard the Lardizabalaceae as a monophyletic unit. The two isolated groups may have had a common ancestor not very far back, but on the existing evidence it will be safer to reunite them to Berberidaceae (or Menispermaceae, if preferred), as two separate sub-groups. This is a thesis which might easily be developed to any extent, but I have simply chosen these two examples to illustrate my main point, that geographical affinity is required as well as structural in settling relationship.

It appears to me very unsafe to assume that two very similar forms are *necessarily* very nearly related when they live in places as to whose former geological connexion we have no evidence, and between which accidental transport is almost impossible. Other evidence than mere structural similarity is now required to prove close relationship. We must have geographical affinity as well. A number of groups at present accepted as monophyletic will probably prove to be more or less polyphyletic, as is proving to be the case with the old and once apparently well-defined genus *Acrostichum* (2).

Again, it is worth while pointing out that 'age and area' gives strong evidence in favour of mutation. If we accept infinitesimal variation literally, it is, as pointed out in a recent paper (13, p. 202), easy to evolve anything, but four provisos, and they are all very large, must be made. Unfortunately for Natural Selection, none of them can be shown to hold good, and the result has been gradually to force its supporters, as illustrated in Mr. Ridley's recent paper (7), to abandon infinitesimal variation, and postulate for something larger. They have not yet fully realized that by doing this they have really abandoned the essential point of their position. There is no evidence to show that a small—not infinitesimal—variation in one direction can be followed by others in the same direction. To take the examples given on p. 203 (13), can it for a moment be supposed that the formation of bulbils in *Asplenium* and many other plants began in easy stages, not to speak of infinitesimal? Can one conceive of them as beginning as rudimentary bulbils? Or can one expect to see a slightly dehiscent berry, a partially reversed leaf? How can intermediate stages exist between cauliflory and normal flowering, between pollen and pollinia (especially pollinia with translators—an essential family character, the only one really discriminating Asclepiadaceae from Apocynaceae), between sympodia and monopodia? If once really infinitesimal variation is abandoned, there seems nothing for it but to take the plunge and admit that mutations of considerable size may occur. And if so, Natural Selection cannot be causative.

But to return to the bearings of age and area on the question, it seems clear that it shows that endemic species confined to small areas are in reality, in the great majority of cases, new species commencing their career. At the commencement of their life, and for a long period afterwards, Natural Selection comes in, but chiefly as an agent to determine the non-survival of really disadvantageous mutations. How many of these occur we have no means of knowing; those which survive are but few in number—only 902 in New Zealand since very far back in time. These one may look upon as having successfully passed through the Natural Selection sieve, but, as I have already pointed out, a very small accident may easily kill out other commencing species which, if left uninterfered with, might also have passed through. A fire on a mountain-top in Ceylon might easily kill a local

endemic. *Didymocarpus Perdita*, Ridley, perhaps belongs to this class. Mr. Ridley (7, p. 555) collected two specimens, and the species has never been seen again; probably he exterminated it in its earliest stages. *Christisonia albida*, Thw., found (once only) at Hakgala in Ceylon, has never been seen again, though there is a botanic garden there, and the forest has been most carefully ransacked. Thwaites describes it as white, so that perhaps it was weeded out by Natural Selection for lack of the brownish colouring matter of its family, and yet it was so different in other features as well that Hooker accepted it as a Linnean species.

Once this early stage is passed, my work on age and area goes to show that the further distribution of species depends on their mere age far more than on any other factor.

But the species which are thus found commencing, and which have apparently passed through the Natural Selection sieve, are not, in the great majority of cases, distinguished from their congeners of the same country simply by small and unimportant differences, but by well-marked characters, which very often are sufficiently numerous and well defined to rank the species as Linnean.

In the case of *Coleus elongatus*, for example, the species is distinguished from *C. barbatus*, its nearest relative, and from all other species of Asia, by the peculiar raceme-like inflorescence, and by the five equal teeth of the calyx, in place of the one large upper and four small lower teeth of other Colei.

Unless, therefore, evidence can be brought up to prove that such species have arisen by the dying out, or the killing out, of intermediate forms, there seems nothing for it but to admit that considerable mutations may take place. In numerous cases, too—for instance, *Coleus elongatus*—intermediate stages are not possible, so that probability goes against supposition of the dying out of intermediate forms. In any case, 'age and area', taken together with previous work, speaks very strongly in favour of mutation as the immediate means by which new species have arisen.

Summary.

Five further pieces of evidence are given, confirming, and extending the application of, the hypothesis of age and area.

The range of the Orchids of Jamaica is shown to go in the order—endemics least, Jamaica-Cuba species next, wides greatest.

The flora of Hawaii is shown to be easily explicable on the same hypothesis, the wides ranging much more than the endemics, which are graduated down to a maximum (in one island only).

Callitris is taken as an example to show that Coniferae are also included in the operation of the law.

The Ferns of New Zealand and Hawaii are also studied. The endemic species show a much greater range than the endemic Angiosperms, a result to be expected on my hypothesis, but contrary to what one would expect if endemics are dying out. The wides only range a trifle farther than the wide Angiosperms, which I put down to the probability that new species may arrive after the breaking of the land bridges. Cf. especially the numbers and rarities in Table V.

Lastly, it is pointed out that more care must be taken, in view of these results, to consider geographical as well as structural relationship in forming genera and families, and that this work gives strong evidence in favour of mutation.

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'Relative Transpiration' as a Measure of the Intrinsic Transpiring Power of the Plant.

BY

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THE term 'relative transpiration' was first employed by Livingston in 1906 (8) as the expression for the intrinsic transpiring power of a plant. It was defined as the amount of water lost per hour from one square cm. of plant surface, divided by the amount lost per hour from one square cm. of a water surface under similar atmospheric conditions, and this number, of course, represents the area of water surface which would evaporate water at the same rate as unit area of the plant.

Since 1906 the methods of measurement of evaporation and the units employed have been modified, but the conception of 'relative transpiration' remains unaltered and has been used by various investigators, e. g. Shreve (12) and Livingston and Hawkins (10).

The process of transpiration is essentially, of course, the evaporation of water from a wet surface, and consequently changes in atmospheric conditions, such as temperature, humidity and air movement, which cause an increase or decrease in evaporation, also produce similar effects upon transpiration. Thus among the factors influencing transpiration an important part is played by atmospheric conditions, and therefore most measurements of transpiration are markedly affected by these external conditions.

In an experimental investigation of the effect of any single factor on a physiological process such as transpiration it is often desirable that the effect of all other factors should remain constant throughout the experiment, so that any change of behaviour under experimental conditions may be ascribed to its proper source. Thus when experimenting upon the influence of, say, light on transpiration, whilst varying the light conditions the evaporating power of the air should be maintained constant, otherwise changes in the rate of transpiration would not necessarily be the result of changes in illumination.

Under ordinary conditions the evaporating power of the air is constantly changing owing to the day and night fluctuations of temperature and

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humidity and the more or less irregular changes in the rate of air movement; so that under greenhouse or field conditions it is almost impossible to keep the evaporating power of the air constant. To avoid this difficulty Livingston made use in his experiments of the conception of 'relative transpiration'. His method was to measure, in addition to transpiration, the rate of evaporation of water from an atmometer placed near the plants under observation, and to consider only changes in the *relative* rate of these two processes as of physiological importance.

He assumed that by this means the direct effect of atmospheric conditions on transpiration could be neglected in the interpretation of his results. In other words, any changes in the relative rates of transpiration and of evaporation, i. e. changes in the 'relative transpiration' of a plant, could be attributed to changes of internal factors, that is, to factors other than those controlling the evaporating power of the air.

The assumption underlying this principle is that changes in the atmospheric environment affect equally the rate of transpiration of a plant and the rate of evaporation from a water surface. This assumption is, however, hardly warranted by a consideration of the facts. With reference to the standardization of various types of atmometer, Livingston (7, p. 22) has pointed out, and Briggs and Shantz (3, p. 636) have emphasized the fact, that atmometers of different sizes and shapes are not really comparable under changing conditions. For example, a change of environment which doubles the rate of evaporation from one atmometer does not necessarily double the rate of evaporation from another of different size or shape. In exactly the same manner the size and shape of the plant may modify the influence of environmental changes on its rate of transpiration. Renner (11) has indicated the part played by the size and shape of leaves in the response of transpiration to external conditions. He found that while under the influence of a considerable air movement transpiration is proportional to leaf area, as the speed of the air diminishes transpiration becomes more and more nearly proportional to the diameter of the leaf. Therefore in comparatively still air a small leaf transpires relatively more than a large leaf. This is to be attributed to the changes brought about by wind in the nature of the diffusion stream extending from the evaporating cells to the drier external air.

It appears, therefore, that it is not possible to compare either one atmometer with another or one leaf with another under changing environmental conditions, so that it seems hardly justifiable to compare an atmometer with a plant.

A further *a priori* criticism of the 'relative transpiration' method is to be found on consideration of the structure of the leaf. A portion of the path along which the diffusion stream passes from the evaporating mesophyll cells to the outside air, is situated actually within the leaf,

and being protected by the epidermis, is therefore not subject to the direct influence of movements of the outside air. In the case of the atmometer, however, the whole of the path of the diffusion stream is exposed to air movements, and it is theoretically possible, by means of a sufficiently rapid current of air, to reduce the moisture in the air close to the evaporating surface to the same concentration as that in the general atmosphere. In the case of the leaf, it is only at the surface of the epidermis, and not of the evaporating mesophyll, that this minimal concentration can be obtained, so that it is to be expected that changes in the speed of air movement will have less influence on the transpiration rate of the plant than on the rate of water loss from the atmometer. This difference is easily demonstrated by experiment.

Experiment I.

A shoot of *Eupatorium adenophorum* was set up in a potometer and removed to a dark room where conditions of temperature and humidity could be kept constant. In addition, the conditions of air movement were under control, the plant being placed in an air-flue (described elsewhere, 2) fitted with a fan by means of which any desired rate of air movement could be produced. The plant could be kept in comparatively still air by stopping the fan and closing the ends of the flue with pieces of wood. Transpiration was determined at 30-minute intervals by weighing, and the quantity of water absorbed by the plant was measured by readings of the graduated tube of the potometer. The evaporating power of the air was determined by weighings of a Livingston standard atmometer placed close to the shoot, which was subjected to still air and moving air alternately for varying periods. The results are shown in Table I.

TABLE I.

Period no.	*Half-hour period ending:*	*Absorption by shoot in c.c.*	*Transpiration in mg. (T).*	*Atmometer loss in mg. (E).*	$\frac{T}{E}$	*Conditions.*
1	12 noon	0·21	213	108	1·97	Still air.
2	12.30 p.m.	0·31	318	190	1·67	Wind. Speed of 7 metres per min.
3	1.0 ,,	0·32	312	188	1·66	
4	1.30 ,,	0·35	320	187	1·71	
5	2.0 ,,	0·31	322	188	1·71	
6	2.30 ,,	0·26	223	110	2·03	Still air.
7	3.0 ,,	0·25	230	115	2·00	,, ,,
8	3.30 ,,	0·33	335	196	1·71	Wind.
9	4.0 ,,	0·33	334	193	1·73	,,
10	4.30 ,,	0·34	340	194	1·75	,,

The numbers in the column $\frac{T}{E}$, although not reduced to unit area and unit time, are proportional to ' relative transpiration '.

If ' relative transpiration ' is to be taken as a measure of the ' transpir-

ing power' (Bakke, 1; Livingston and Hawkins, 10) or 'transpiration coefficient' (Briggs and Shantz, 3) of the plant, it indicates that in period 2 the power of the plant to evaporate water decreased in the ratio 1·97 to 1·67, in period 6 it increased to 2·03 and decreased again to 1·71 in period 8. Taking the means of the respective series, the transpiring power in still air is to that in moving air (7 metres per minute) as 2·00 is to 1·71, a decrease of 14·5 per cent. There appears to be no reason for this because the maximum variation of $\frac{T}{E}$ in periods 2, 3, 4, 5, 8, 9 and 10, when conditions were practically constant, was 1·66–1·75 (less than 6 per cent.), and in periods 1, 6 and 7, 1·97–2·03 (3 per cent.). The actual amount of transpiration in moving air was about 50 per cent. greater than that in still air, and it might be suggested that such a high rate produced a deficiency of water within the leaf, resulting in incipient drying of the cells (see Livingston and Brown, 9) and a consequent reduction of the transpiring power of the plant. Consideration of the relation of the quantity of water transpired to the volume absorbed in the above experiment, however, disposes of this possibility. During periods 2 and 5 the net loss of water to the plant (transpiration minus absorption) was 8 and 12 mg. respectively, and during periods 3 and 4 the plant gained 8 and 30 mg., i.e. at 2.0 p.m. there were 18 mg. more water in the plant than at 12 noon, so that the transpiring power should be higher in period 5 than in period 1, instead of lower. Also a gain to the plant of 30 mg. was accompanied by the transpiration of 320 mg. (period 4) and a loss of 8 mg. by the transpiration of 322 mg. (period 5) and 318 mg. (period 2), so that it appears that the incipient drying caused by the small changes of water-content indicated above is not sufficient materially to affect the transpiring power of the plant.

There is also the possibility that the movement of the air might have caused the stomata to close more completely than they had already done in response to darkness, thus producing a corresponding reduction of transpiration. No measurements of stomata were made in the experiment quoted above, but a series of porometer experiments has been carried out both in light and darkness, and it has not been possible to demonstrate any significant stomatal change in response to air movements up to a speed of 20 metres per minute.

As there is no apparent reason to account for the reduction of the transpiring power of the plant, there appears to be no escape from the view that the reduction of the rates $\frac{T}{E}$ is due to the fact that an increase in the speed of air movement accelerates the rate of water loss from an atmometer to a greater degree than the rate of transpiration from a plant. Such a result is to be expected from the considerations above.

The results of the experiment described above, which is one of about twenty similar ones, do not agree with the conclusions of Shreve (12). This writer remarks (p. 14): 'It was found that while a marked increase in wind velocity was always accompanied by an increase in the actual transpiration rate, this increase did not appear in the relative transpiration, thus showing that the plant was affected in the same manner as the atmometer.' The experiments in question were carried out with potted plants in the open air, with conditions no doubt varying continuously and irregularly, as the graphs of evaporation show. Unfortunately no comparison was made, in the tables and graphs given, of the transpiration and evaporation with different wind velocities.

Livingston (6), in comparing the relative transpiration method with the direct method in which hygrometric paper is used, states (p. 18): 'That the relative transpiration graphs exhibit marked accelerations and retardations that are only slightly, or not at all, indicated by the graph from the direct method seems to be explained by the supposition that the atmometers are much more sensitive to changes in the surrounding conditions than are the plants.' The experiment was carried out in the open air and it seems not unlikely that some of the irregularities in the relative transpiration graph are to be explained by the different response of plant and atmometer to air movement. The graphs from the direct method would fail to exhibit these irregularities owing to the fact that the portion of the leaf under observation is protected from air movement by the hygrometric paper used.

In their capacity to influence the rate of water loss from a leaf and an atmometer, relative humidity and temperature stand in contrast to air movement, for there is no *a priori* reason why the first two factors should not affect equally the rate of transpiration and the rate of evaporation from an atmometer. Air movements affect the diffusion gradient, on which the rate of water loss depends, by altering the distance between the evaporating surface and the point of minimum water concentration in the manner which has been indicated above, and this alteration is not the same in plant and atmometer. On the other hand, changes of relative humidity and temperature affect the diffusion gradient by altering the (water) concentration difference or diffusion potential, which will influence plant and atmometer alike, irrespective of structure. Thus, subject to differences of conductivity of heat and of specific heats, the response of the plant to temperature and relative humidity changes should be the same as the response of the atmometer.

Experiments have been carried out to ascertain the influence of changes of temperature and relative humidity upon plant and atmometer.

Experiment II.

The method was similar to that used in Exp. I. The plant, *Eupatorium adenophorum*, and the atmometers[1] were placed in the air-flue (2) in a dark room, and a stream of air was drawn through at a speed of 7 metres per minute. Weighings of the plant and the atmometers were made at 30-minute intervals, for which purpose each was removed from the flue for two minutes. When the rates of transpiration and evaporation had become practically constant, the temperature of the room was raised by means of an electric radiator, and the rates of transpiration and evaporation were again determined at intervals (90-minute periods in the experiment given). The temperature continued to rise slowly during the rest of the experiment, and simultaneously the relative humidity tended to decrease. An attempt was made to keep the latter constant by watering the floor of the dark room, but with the changing temperature the humidity was found somewhat difficult to regulate with any degree of accuracy.

Readings were made of the absorption of water by the plant from the potometer as in Exp. I, in order to determine whether there was any tendency towards wilting.

The results appear in Table II.

TABLE II.

Time.	*Temp.* ° *C.*	*Relative Humidity per cent.*	*Plant*		*Loss from Atmometers.*	
			Absorption.	*Transpiration.*	*I*	*II*
			mg.	*mg.*	*mg.*	*mg.*
11.30 a.m.	15·9	83·5	420	415	177	153
12 noon	15·9	83·5	390	407	176	149
12.30 p.m.	16·0	83	420	411	180	158
1.0 p.m.	16·0	83				
	16·7	82				
	17·2	82	1400	1402	604	525
2.30 p.m.	17·6	81				
	17·8	80				
	18·0	80·5	1540	1536	671	578
4.0 p.m.	18·2	80·5				

Dividing the experiment into three 90-minute periods, representing three different average temperatures, we find that the change of rate of

[1] The atmometers used in this experiment and in Exp. III, quoted later, were paper ones of a modified Piche type (see Livingston, 7). Atmometers of porous earthenware have also been used in the present experiment and give similar results, but there is a distinct lag in the response to temperature changes, presumably owing to the slowness with which the relatively large mass of water and earthenware assumes the changed temperature of the surrounding air.

water loss from the atmometers is proportional to the change of transpiration rate:—

Period.	*Plant.*	*Atmometers.*	
		I	*II*
(1) 11.30 a.m.–1.0 p.m.	1233	533	460
(2) 1.0 p.m.–2.30 p.m.	1402	604	525
Ratio $\frac{\text{Period (2)}}{\text{Period (1)}}$	1·14	1·13	1·14
(3) 2.30 p.m.–4.0 p.m.	1536	671	578
Ratio $\frac{\text{Period (3)}}{\text{Period (1)}}$	1·25	1·26	1·26

The possibility of change of stomatal aperture was considered, and porometer experiments have been carried out to ascertain the behaviour of stomata when subjected to changes of temperature of the same order as those in Exp. II. A porometer leaf-chamber was attached to a leaf of a potted plant in the greenhouse, and stomatal changes were recorded by means of the automatic recorder described elsewhere (5).[1] After a time the plant was removed to the dark room, when the stomata closed rapidly. When the aperture had become constant, the temperature of the dark room was raised by means of the radiator used in Exp. II, and the record of stomatal aperture was continued for several hours—in one case eighteen. The results showed no significant change of stomatal aperture on raising the temperature. The relative size of the apertures, assuming them to be proportional to the square root of the reciprocals of the porometer readings (see Darwin 4, p. 423), were as follows in one typical experiment:

Before raising temperature—max. aperture		92
	min. „	89
After raising temperature—max.	„	94
	min. „	89

To test the influence of humidity changes upon transpiration and evaporation rates, experiments have been carried out on lines similar to those of Exp. II above. The plant and various atmometers were exposed in the air-flue in the dark room to a stream of air moving at a speed of 7 metres per minute, and determinations of water loss were made at intervals. When the rate of loss had been shown to be constant, the relative humidity of the atmosphere was increased by spraying the walls and floor of the room with a syringe, the determinations of water loss from plant and atmometers being continued. With the rise of humidity the temperature tended to decrease and this was checked by the use of the radiator.

[1] Since the publication of the paper referred to, the apparatus has been modified so as to dispense with the constant temperature bath; the modified recorder is easily portable.

Experiment III.

A shoot of *Eupatorium adenophorum* and three paper atmometers of different sizes were used. When the rates of water loss had been practically constant for two hours, the humidity was raised, and after allowing about half an hour for the conditions to come into equilibrium the rates of transpiration evaporation were again determined.

The results are given below:

TABLE III.

Time.	*Temp.* ° *C.*	*Relative Humidity per cent.*	*Plant*		*Loss from Atmometers.*		
			Absorption.	*Transpiration.*	*I*	*II*	*III*
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
1.0 p.m.	21·3	76					
1.30 ,,	21·3	76·5	410	395	97	180	321
			410	390	98	175	322
2.0 ,,	21·4	76					
2.30 ,,	21·4	75·5	410	397	97	175	333
3.0 ,,	21·4	75·5	420	403	98	175	330
3.30 ,,	21·5	80					
	21·5	80·5					
	21·5	81	960	920	224	401	748
5.0 ,,	21·5	81					

Comparing the 90-minute periods 1.30–3.0 and 3.30–5.0 we get:—

Period.	*Plant.*	*Atmometers.*		
		I	*II*	*III*
(1) 1.30–3.0	1190	293	525	985
(2) 3.30–5.0	920	224	401	748
Ratio $\frac{\text{Period (1)}}{\text{Period (2)}}$	1·29	1·31	1·31	1·32

As in the experiments with temperature, experiments were carried out to determine the behaviour of the stomata under varying conditions of humidity. The method used was exactly similar to that described for the temperature experiments, and, with a change of relative humidity from 77 per cent. to 84 per cent., the relative stomatal apertures as calculated from porometer readings were:

Humidity 77 per cent.—max. aperture 96
min. ,, 93
Humidity 84 per cent.—max. ,, 95
min. ,, 93

It is to be concluded, therefore, that changes of relative humidity affect the rates of evaporation and transpiration proportionately.

From the foregoing experiments it appears that changes in 'relative transpiration' do not necessarily represent changes in the intrinsic transpiring power of a plant unless conditions of air movement are maintained constant, owing to the unequal response of plant and atmometer to

changes of wind velocity. In other words, the influence of air movements upon transpiration cannot be eliminated by comparing the transpiration rate with the rate of water loss from an atmometer.

This method of comparison can, however, be used to eliminate the influence of changes of temperature and relative humidity on transpiration, since these two factors act equally upon the rate of transpiration from a plant and evaporation from an atmometer. It is clear that *only when wind velocity is constant* does 'relative transpiration' give a satisfactory measure of the intrinsic transpiring power of a plant.

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A Contribution to the Study of the Marattiaceae.

BY

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With Plates XXI and XXII and thirty-three Figures in the Text.

Contents.

Introduction.

JUDGING from the geographical distribution of the modern representatives of the Marattiaceae, it would seem that this group of Ferns occupied a far more prominent position in the flora of the past. The palaeontological record lends support to this hypothesis. Moreover, it has long been recognized that in these Ferns the stelar system has attained to a stage in complexity far in advance of that of any other modern group of Vascular Cryptogams. It is therefore not surprising that these plants have formed the subject of repeated investigations. But many difficulties are encountered whenever an attempt is made to reconcile the statements of previous writers respecting even the more important details of the structure and development of the vascular tissues in this interesting group of Ferns. And it can safely be said that, with the possible exception of *Angiopteris*, there is still not a genus of Marattiaceae of which we possess a complete account.

Annals of Botany, Vol. XXXI. Nos. CXXIII and CXXIV. July and October, 1917.]

For this reason, the opportunity of throwing fresh light on several points which were in dispute, and of adding to our knowledge of the adult sporophyte of the hitherto little-known genus *Danaea*, was welcomed by the present writer, who desires at the outset to express his indebtedness to Dr. S. E. Chandler, F.L.S., for his kindness in supplying him with a large number of plants of *Danaea alata*, Sm., and of *Danaea nodosa*, Sm., which were collected by him during a recent visit to the West Indies.

Material and Methods.

Apart from the complete series of plants of *Danaea alata*, Sm., and of *Danaea nodosa*, Sm., already mentioned, material of *Angiopteris evecta*, Hoffm., *Kaulfussia aesculifolia*, Bl., *Danaea simplicifolia*, Rudge, and of several species of *Marattia*, including a large specimen of *Marattia Cooperi*, Mre., was available for investigation.

In order to facilitate the elucidation of the complicated arrangement of the vascular strands in these ferns, a number of wax models of the vascular tissues were built up. The method described by Farmer and Hill (29, p. 375) was adopted with a few modifications.[1] Although this method is very laborious, it has many advantages, inasmuch as every detail of the complicated branching and anastomosing of the vascular strands, both in the stem and in the leaf-bases of the adult plant, can readily be determined.

For the histological work, a number of stains and reagents, including safranin, Kleinenberg's haematoxylin, eosin, gentian violet, Bismarck brown, iodine, phloroglucin + HCl, chlor-zinc-iodide, KOH, and concentrated H_2SO_4, were employed. Prolonged staining in a saturated alcoholic solution of safranin, followed by rapid staining in Kleinenberg's haematoxylin, was found most satisfactory for demonstrating the presence of an endodermis.

Most of the preparations were mounted in Canada balsam or in glycerine jelly, but a few were mounted in euparal (46, p. 247). Euparal has a low index of refraction ($n = 1{\cdot}483$), and shows up the endodermis, when present, even in the regions where its histological characters are otherwise difficult to observe.

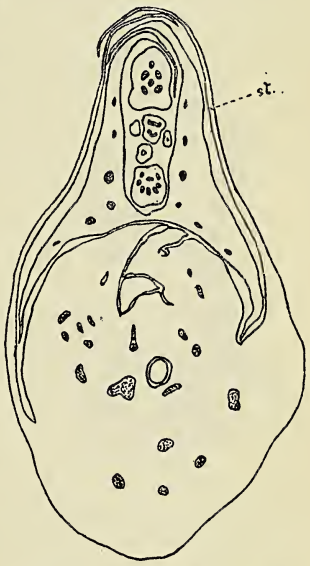
[1] Owing to their large size and extreme complexity, it was found necessary to strengthen these models (1) by introducing into the wax long pieces of copper wire bent to the required shape, and (2) by giving them several coats of enamel, which, on drying, formed a rigid covering to the wax and effectively prevented its gradual subsidence during hot weather. Electro-plating with copper was found impracticable owing to the uneven deposition of the metal, which resulted in the formation of numerous centres of weakness and did not add materially to the strength of the models whilst greatly increasing their weight.

Morphology.

1. *Danaea.*

The very young sporophyte of both species of *Danaea* examined by the present writer exhibits a perfectly radial symmetry (Pl. XXI, Fig. 4), the first few leaves being arranged in a rough spiral on the stem, which is relatively elongated as compared with sporelings of *Angiopteris*, *Macroglossum*, or *Marattia*[1] of similar age.

In the case of *Danaea*, we find within the limits of a single genus two distinct trends of organization. Whereas in *Danaea nodosa*, Sm., the radial symmetry is maintained in the adult plant, in *Danaea alata*, Sm., the shoot-apex very soon bends through an angle of approximately 90° (Pl XXII, Fig. 9, A and B), and continues its growth in the horizontal plane. The stage at which this change in the direction of growth of the shoot takes place varies slightly in different plants, but it always occurs very early. As a result, the adult plant of *Danaea alata* exhibits well-marked dorsiventrality, the leaves, which are simply pinnate, arising from the dorsal and lateral surfaces of the horizontal rhizome, as is shown in Pl. XXI, Fig. 2. Very occasionally, however, a leaf arises from the ventral surface of the rhizome.



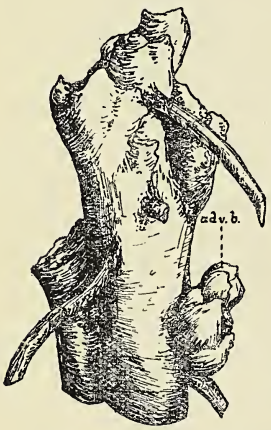
Text-fig. 1. *Danaea alata*, Sm. Transverse section through the apex of an old plant, showing bud-protection. *s.t.* = stipule. × 4.

All the leaves grow more or less vertically upwards, the bend taking place in the region of the basal pulvinus of the petiole. The roots, which branch freely in a monopodial manner,[2] arise in no very definite order from all sides of the rhizome, but there is usually one to each leaf of the adult plant. Prominent stipules with distinct commissures occur at the base of the petioles and provide a most efficient means for protecting the growing apex, the stipule of any leaf completely enveloping the next younger leaf whilst the latter is at an early stage in its development (Pl. XXI, Figs. 5 and 6; Text-fig. 1). Moreover, numerous peltate hairs or scales occur on the petiole. It is worthy of note that those Ferns which bear relatively few leaves at a time generally possess highly developed protective devices (e.g. Ophioglossaceae; and cf. Campbell, 20, pp. 191–2).

[1] The young plants of *Marattia fraxinea*, Sm., investigated by Kühn (42) appear to be exceptional.

[2] It is difficult to reconcile this fact with Kühn's (43, p. 149) statement that in the case of *Danaea alata* 'die Wurzeln scheinen normal unverzweigt zu sein'.

On the other hand, the adult sporophyte of *Danaea nodosa*, Sm., which is a much larger plant than *Danaea alata*, Sm., has a very different habit. The stem, upon which the leaves are inserted in an irregular spiral, is constructed on a radial plan, but in a few of the specimens examined the axis had assumed an oblique position relatively to the ground-level; the change in the direction of growth of the stem apparently has no effect upon the arrangement and position of the leaves, which adapt themselves to the altered conditions by bending in the region of the basal pulvinus, whilst the long robust roots, which are equally numerous on all sides of the stem and which bear no definite numerical relation to the leaves, grow more or less vertically downwards, branching freely in a monopodial manner, often at a considerable distance from the stem. This gives a curious appearance to the larger specimens of *Danaea nodosa*, such as that represented in Pl. XXII, Fig. 8, the caudex of which had attained a diameter of 4 cm. and a length of more than 40 cm.



TEXT-FIG. 2. *Danaea nodosa*, Sm. Part of the caudex of an adult sporophyte, showing a young adventitious bud (*adv. b.*). Nat. size.

In this species also, stipules with distinct commissures occur near the base of the petioles.

The stem was unbranched in every specimen examined; what at first sight appeared to be a lateral branch on one of the stems on closer examination proved to be an adventitious bud, which had arisen from one of the old leaf-bases (Text-fig. 2).

This method of vegetative propagation is not uncommon in *Angiopteris* and *Marattia*, and it is a general practice among fern-growers to propagate these two genera by means of their adventitious buds, which are often produced in large numbers (cf. Buchanan, 16; also Hofmeister, 33, p. 255).

Gwynne-Vaughan (30, p. 266) called attention to masses of meristematic tissue which he observed in the leaf-bases of *Archangiopteris* and of *Kaulfussia*, and suggested that they might represent the rudiments of adventitious buds. The present writer has noticed the occurrence of similar masses of meristematic tissue in the leaf-bases of *Kaulfussia* and of two species of *Danaea* (Text-figs. 10, A, and 8, B), but only in the case of *Danaea nodosa* has their ultimate development into leafy shoots been observed.

Vascular Anatomy. Apart from the scanty observations of the earlier botanists (Brongniart, 13, p. 439, Pl. XXXIII, Figs. 2 and 3; Karsten, 40, p. 198, Pl. IX, Fig. 10; Mettenius, 49, p. 524; Kühn, 43, p. 147), practically nothing was known of the arrangement of the vascular strands in the genus *Danaea* until 1902, when Brebner (12) published a full account of the

development of the vascular system in the young sporophyte of *Danaea simplicifolia*, Rudge.

Jeffrey (36) very briefly described the arrangement of the vascular tissues in young stems of *Danaea*, in which he observed a tubular central cylinder interrupted by foliar gaps, and a single medullary strand which fused with the wall of the stelar tube above the foliar gaps. According to this investigator, the medullary strand later develops into a tube or a series of strands; finally, the stelar system is further complicated by the appearance of another series of strands. This arrangement of the vascular strands was compared with that which obtains in *Matonia*.

In his monograph on the Psaronieae and Marattiaceae, Rudolph (51) described the course of the vascular strands in a small piece of an old stem of an unidentified species of *Danaea* from Brazil. This stem showed a radial organization, but unfortunately the lower portion was missing. A series of outline drawings of transverse sections of this stem, in which the individual bundles were numbered (l. c., Taf. III, Figs. 5–14, 16), illustrated the description; such a method, however, is most unsatisfactory because the extreme complexity of the structures concerned leads to confusion in the mind of the reader.

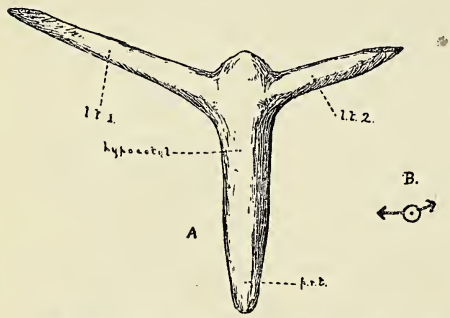
Campbell (20) has recently published a critical study of the development and arrangement of the stelar tissues in three species of *Danaea* (*D. jamaicensis*, *D. Jenmani*, and *D. elliptica*). This botanist puts forward the view that the stele of the very young sporeling consists of a sympodium of leaf-traces which below merge insensibly into the diarch primary root, and suggests that in these young sporelings there is no stem in the strict sense. Only a passing reference is made to the vascular anatomy of the adult plant, which, apart from an increase in the number of leaf-traces and commissural strands, is assumed to be essentially similar to that of the young plant (l. c., p. 175).

Thus, it is evident that no satisfactory account of the stelar anatomy of the adult sporophyte of *Danaea* has yet been published. Since this is the only genus of Marattiaceae at present known which includes both radial and dorsiventral species, it seemed that a comparative study of the development and arrangement of the stelar tissues in *Danaea alata* and *Danaea nodosa* respectively might yield both useful and interesting results. With this end in view, three wax models (Text-figs. 3 and 4, A; Pl. XXI, Fig. 1 A), showing clearly the transition from the simple stelar system of the young sporeling to the highly complex arrangement of vascular strands in the adult plant, were built up.[1] These models, although constructed from three plants, form what is practically a continuous scheme of the stelar system in the rhizome of *Danaea alata*, Sm., and for this reason the following description of the vascular anatomy of the genus *Danaea* is mainly

[1] Unfortunately the actual specimens from which these models were built up were not sketched before being cut up; however, Pl. XXI, Fig. 2, and Pl. XXII, Fig. 9 B, respectively represent plants of about the same age as those from which the two larger models were made.

based upon a study of these models. But, owing to the difficulty of manipulating thin layers of wax of uneven thickness with any degree of accuracy, the curvature of the basal portion of the adult rhizome is not represented in the large model (Pl. XXI, Fig. 1 A). For a similar reason, the slight curvature of the apex of the small rhizome, which was about to assume its horizontal growth, is not shown in the model represented in Text-figs. 4, A, and 5.

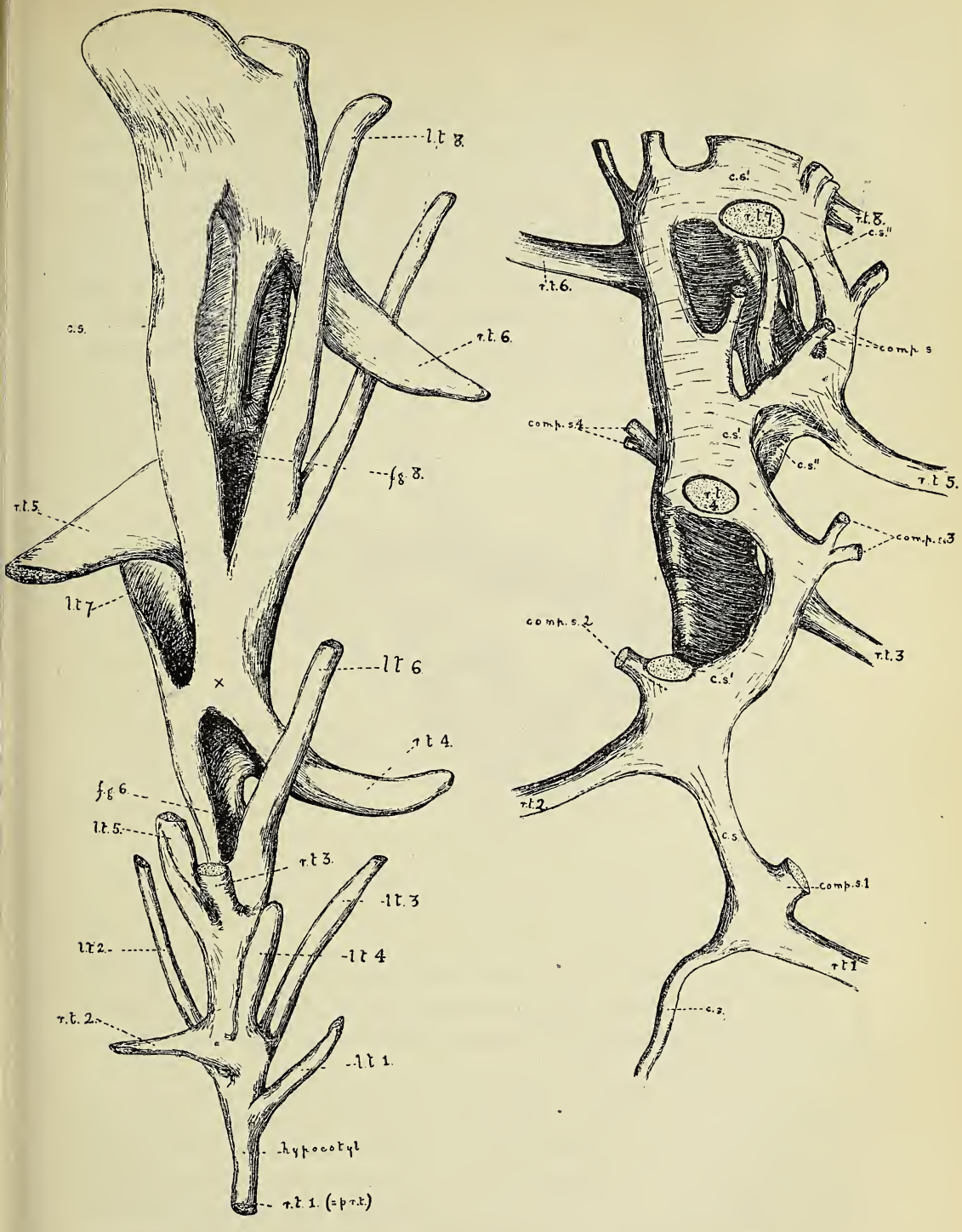
Danaea alata, *Sm.* The present writer's observations on the development of the vascular tissues in a large number of very young sporelings of *Danaea* for the most part confirm the account given by Campbell (20) as regards this genus. The vascular strand of the embryo plant is formed very early, being first differentiated in the cotyledon; tracheides make their first appearance at the junction of cotyledon and primary root, and from thence the development of the tracheides works upwards and downwards until a simple vascular strand, continuous from the tip of the cotyledon to the apex of the primary root, is produced. The trace of the second leaf, which arises nearly opposite the cotyledon (Text-fig. 3, B), unites with the primary vascular strand in the so-called hypocotyledonary region. As Campbell (20) rightly points out, the strands belonging to the first two leaves of the young sporophyte are quite distinct in this region, being separated from one another by a very irregular layer of small parenchymatous cells. Lower down, however, they appear to form a single more or less oval strand (as seen in transverse section) which merges insensibly into the diarch bundle of the primary root. A model of the vascular system of a young plant which had attained to approximately this stage of development is represented in Text-fig. 3, A. It will be noticed that there is as yet no sign of a second root.



TEXT-FIG. 3. A. *Danaea alata*, Sm. Model of the stelar system of a very young sporeling. Side view. *l.t.* 1, *l.t.* 2, leaf-traces. B. Diagram showing divergence of the first two leaves.

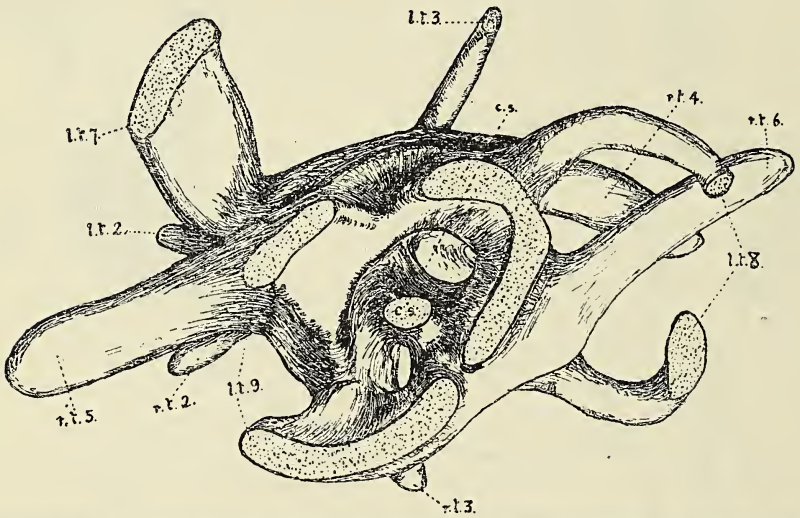
Text-figs. 4, A, and 5 represent a model of the stelar system of a rather older sporophyte which had developed eight leaves. Traced downwards, the simple traces belonging to the third and fourth leaves (Text-fig. 4, A, *l.t.* 3 and *l.t.* 4) gradually approach and finally coalesce, and still farther down join the trace of the second leaf close to its union with the vascular strand of the first leaf or cotyledon. The trace of the first adventitious root (Text-fig. 4, A, *r.t.* 2) joins the stem-stele[1] near the point where the traces of the third and fourth leaves become united. Thus, in the young

[1] The convenient descriptive term 'stem-stele' is here employed for the common vascular tissue produced by the close association or union of the earlier leaf- and root-traces.



TEXT-FIG. 4. A. Model of the stelar system of the rhizome of a young sporophyte of *Danaea alata*, Sm., viewed from below. B. Model of the internal stelar system of the rhizome of an old sporophyte of *Danaea alata*, Sm., viewed from above. *c.s.*, commissural strand; *c.s.'*, internal stelar system arising out of the commissural strand; *c.s.''*, commissural strand of the internal stelar system; *comp.s.*, compensating strand; *f.g.*, foliar gap; *l.t.*, leaf-trace; *p.r.t.*, trace of the primary root; *r.t.*, root-trace.

sporophyte of *Danaea*, the stelar system is not complicated so early by the influence of the adventitious roots as in the case of *Angiopteris*, in which the first adventitious root is given off nearly opposite the first leaf (cf. Farmer and Hill, 29, Pl. XVI, Fig. 1, with Text-fig. 3, A, of the present paper). In a similar way the simple traces of the fifth and sixth leaves (Text-fig. 4, A, *l.t.* 5 and *l.t.* 6) traced downwards appear to approach one another and ultimately enter into close association, giving rise to a single strand, which is more or less crescent-shaped in section. This strand at a still lower level becomes united with the fused traces of leaves 3 and 4. The trace of the third root joins the stem-stele close to the point of fusion of the traces of the fifth and sixth leaves.



TEXT-FIG. 5. *Danaea alata*, Sm. Front view of the model represented in Text-fig. 4, A. *c.s.*, commissural strand; *l.t.*, leaf-trace; *r.t.*, root-trace.

Up to this point, the description of the earlier stages in the development of the vascular system in the genus *Danaea* as given by Campbell (20, p. 174) so closely agrees with the arrangement of the bundles as I have found them in the species studied by me, that I cannot do better than quote his summary word for word:

'The vascular system in the young sporophyte of *Danaea* begins as a single axial strand, which is continuous through the cotyledon and root. At a very early period a second vascular bundle or stele is formed in the second leaf connecting with the primary strand, and this is followed by a similar single strand or stele in each succeeding leaf, up to about the seventh. Up to this time, except for the steles of the secondary roots, the whole vascular system is built up of united leaf-traces and there is no cauline bundle in the strict sense of the word, although we may speak of the bundle, or stele of the stem, as soon as there is a solid central strand formed below

the junction of the earlier leaf-traces. This primary stele never has the form of a true protostele, however, as the xylems belonging to the separate leaf-traces can be recognized and the compound nature of this central bundle is unmistakable.'

It has already been pointed out that the sporeling of *Danaea alata* is radial in structure; correlated with this fact, we find that the sympodium of leaf- and root-traces which together make up the vascular system of the young sporophyte is likewise based on a radial plan, the traces of the first few leaves being arranged in a rough spiral.

It may be noted in passing that the vascular tissue of the embryo plant of other megaphyllous Vascular Crytogams (e.g. Ophioglossaceae, *Isoëtes*) may consist solely of a system of leaf- and root-traces, hence it is not surprising that a similar condition should obtain in the remarkable megaphyllous Marattiacean Ferns.

A distinct leaf-gap is formed by the trace of the sixth leaf, above the point of insertion of which the stem-stele is definitely crescentic in transverse section. Campbell(20) maintains that the stem-stele in this region still consists solely of leaf- and root-traces, but the present writer is of the opinion that part, at least, of the vascular tissue of the 'siphonostele' with large leaf-gaps (cf. Text-fig. 4, A) which marks the next stage in the elaboration of the stelar system of *Danaea*, is made up of elements which have a truly cauline origin and serve to connect up adjacent leaf-traces.

A root-trace (Text-fig. 4, A, *r.t.* 4) joins the stelar cylinder above the gap formed by the trace of the sixth leaf; in this region the siphonostele forms a complete cylinder (= solenostele) uninterrupted by leaf-gaps (the region indicated by a × in Text-fig. 4, A). The first commissural strand arises by proliferation of the vascular tissues on the inner surface of the stelar tube opposite the point where the trace of root 4 joins the stem-stele (above leaf-gap 6 in Text-fig. 4, A); considered from the point of view of water-conduction, this strand forms a direct continuation of the root-trace. Traced upwards, this strand passes across the medullary ground-tissue from the upper end of one leaf-gap to the apex of the next leaf-gap above, which it helps to close, and since, generally speaking, a root-trace joins the outer surface of the stem-stele at these points, the commissural strand at first forms an auxiliary internal conducting system of cauline origin, which continues to connect up the points of insertion of the relatively large and important root-traces. During these earlier stages, the commissural strand obviously fills a relatively subordinate position, as compared with the main stelar cylinder, but, as Brebner (12, p. 536) rightly pointed out, the special advantage of such an arrangement is obvious, since any root probably does not reach the soil until after the related leaf has unfolded.

Now, whereas the trace of the seventh leaf consists of a single strap-shaped strand which splits into two whilst still within the stem-cortex, the

eighth leaf-trace dichotomizes near its base (Text-fig. 4, A, *l.t.* 8), whilst the traces of later leaves depart as two distinct strands, which, instead of arising from the base of the gap, as in the case of the single trace of the earlier leaves, arise from the sides of the gap, usually at different levels (Pl. XXI, Fig. 1 A, *l.t.* 1–4). Further branching and occasional anastomosing of the strands of the leaf-trace occur in the cortex of the stem.

The siphonostelic condition is maintained for a short time only; sooner or later, owing to the crowding of the spirally arranged leaf-traces, the gap above one leaf-trace fails to be repaired till after the exit of the trace of the next leaf; in this way a simple dictyostele is produced (Pl. XXI, Fig. 1 A).

The change in the direction of growth, to which reference has already been made, takes place in the specimen under discussion at the level of the apex of the gap made by the departure of the traces of the third leaf represented in the large model; this region is indicated in Pl. XXI, Fig. 1 A, by the two arrows.

Correlated with the change in the direction of growth there is an important difference in the organization of the vascular tissues, the radial configuration of the stem-stele being replaced by one in which dorsiventrality is well marked. This change is indicated at an early date by the formation of a large gap (*v.g.* in Pl. XXI, Fig. 1 A) in a position where one would expect to find a gap formed by the departure of the traces of leaf 5. However, the traces of this leaf, instead of continuing the spiral in which the earlier leaf-traces are arranged, depart from the stem-stele at a point almost opposite the exit of the preceding leaf-trace. In this connexion it is interesting to find that the commissural strand, after leaving the inner surface of the vascular cylinder above the gap of leaf 4, *takes a sharp bend* (indicated by a × in Pl. XXI, Fig. 1 A) *towards the upper end of this large ventral gap* before continuing its course towards the apex of the next leaf-gap above (i. e. the gap made by the departure of the traces of the fifth leaf), which it helps to close. A root-trace (Pl. XXI, Fig. 1 A, *r.t.* 1) fuses with this commissural strand immediately below the point where the latter joins the inner surface of the stelar cylinder.

The stem, meanwhile, steadily increases in diameter, the dictyostele opening out to a corresponding degree; at about this stage the latter becomes fractionated, perforations, other than leaf-gaps, occurring in the stelar cylinder.

On the dorsal and lateral surfaces of the dictyostele the leaf-traces become more crowded and more complex, frequently leaving the stem-stele as six or more separate strands, among which the two main laterals can readily be recognized by their stronger development (cf. Farmer and Hill, 29, p. 378). The strands of the leaf-trace depart from the basal and lateral margins of the foliar gaps, anastomosing at irregular intervals with the strands which can still be regarded as belonging to the original dictyostele

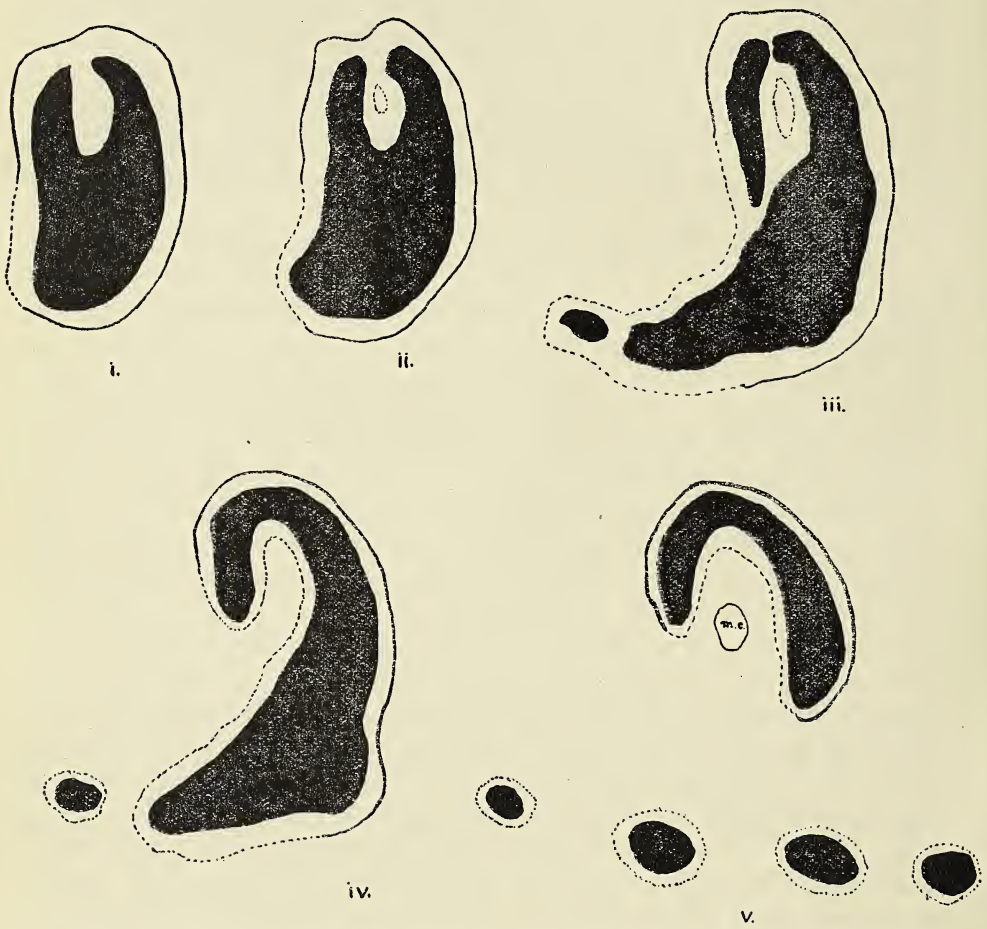
as well as with one another. On the other hand, in accordance with its dorsiventral organization, only a single weakly developed leaf-trace (i. e. *l.t.* 8 in Pl. XXI, Fig. 1 A, and Pl. XXII, Fig. 7) takes its origin from the ventral surface of the stem-stele, although a number of large diamond-shaped gaps, closely resembling leaf-gaps, occur in this part of the stele.

In *Danaea* the *internal* vascular cylinder arises by the gradual elaboration of the original commissural strand. In its development the internal vascular system passes through a series of stages strictly analogous to those of the original vascular cylinder. For comparison, Text-figs. 4, A and 4, B, which represent respectively a model of the stelar system of the rhizome of a young sporophyte of *Danaea alata* and a model of the *internal* stelar system of the rhizome of an adult plant of the same species, are placed side by side.

It has already been stated that the commissural strand (Fig. 1, A, *c.s.*) at first consists of a small solid vascular bundle which pursues a somewhat zigzag course through the medullary ground-tissue, fusing with the main stelar system at the apex of the leaf-gaps, which it helps to close ; in other words, this commissural strand itself functions as a compensating (*Ersatz*) strand. Traced upwards, this strand rapidly gains in importance, and instead of fusing directly with the outer stelar cylinder at the apex of the leaf-gap (i. e. the gap made by the departure of *l.t.* 5 in Pl. XXI, Fig. 1 A), it gives off a branch (Text-fig. 4, B, *comp.s.* 1) which functions as the compensating strand. The commissural strand then crosses over towards the upper end of the next leaf-gap above ; meanwhile, the number of its vascular elements are considerably augmented by the addition of those of a root-trace (Text-fig. 4, B, *r.t.* 2) which passes through this foliar gap and fuses with the commissural strand. In this way a large solid mass of vascular tissue, more or less oval in transverse section, is produced. A short massive compensating strand (Text-fig. 4, B, *comp.s.* 2) leaves this vascular mass and, anastomosing right and left with bundles of the external cylinder, assists in closing the gap formed by the exit of the meshed segment, which at this stage constitutes the leaf-trace. The departure of this compensating strand produces a distinct gap, comparable with a leaf-gap, in the central stelar system, which now opens out to form an incomplete cylinder of vascular tissue. Text-fig. 6, i–v, represents a successive, but not consecutive, series of transverse sections of this transitional region (indicated by a × in Pl. XXI, Fig. 1 A) ; it is seen that a well-marked 'pocket' is produced, at first phloem (Text-fig. 6, i) and then characteristic ground-tissue parenchyma (Text-fig. 6, ii, iii) appearing in the centre of the xylem core.

The subsequent stages in the development of the internal stelar system proceed along lines essentially similar to those of the original solenostele ; in brief, the internal vascular cylinder, thus inaugurated, increases in size *pari passu* with the widening of the outer cylinder and of

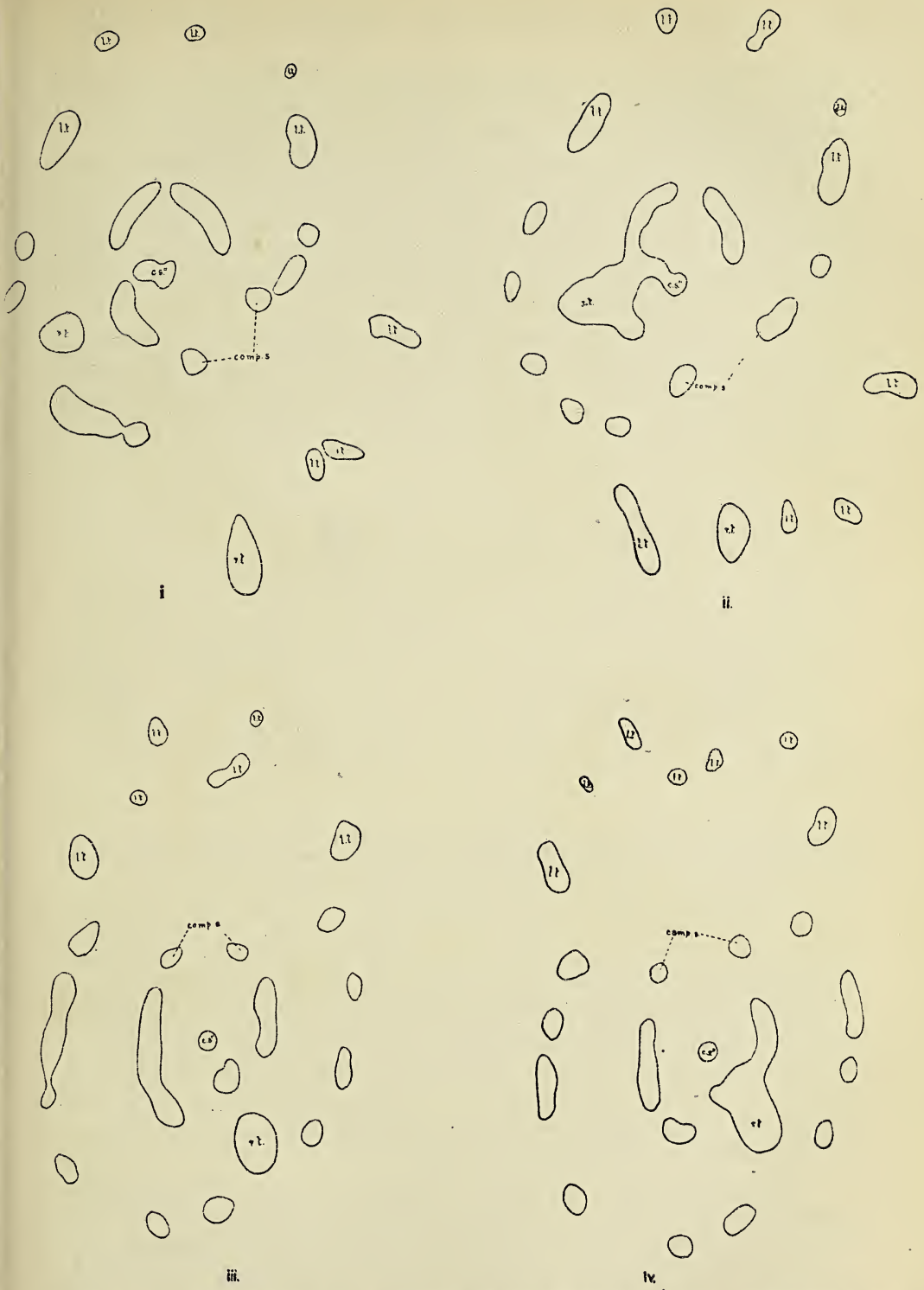
the stem, further complications resulting from the increasing importance and crowding of the compensating strands. Sooner or later, the gap above one compensating strand fails to be repaired until after the exit of the next, with the result that the internal vascular cylinder becomes broken up into a typical dictyostele, or, more strictly speaking, a 'perforated' dictyostele (cf. Tansley, 64, p. 65), since a few relatively small



TEXT-FIG. 6. i–v. *Danaea alata*, Sm. The diagrammatic figures illustrate in successive, but not consecutive, transverse sections the changes that occur in the internal vascular system of the rhizome of an adult plant at the transitional region (marked by a × in Fig. 1 A, Plate XXI). The smooth contours denote the endodermis, while the dotted contours mark the outer limits of the phloem. The shaded areas = xylem. *m.c.*, mucilage canal.

gaps, other than those formed by the departure of the compensating strands, occur in the internal cylinder.

An interesting parallel may also be drawn between the leaf-traces and the compensating strands, which respectively pass through an ontogenetic series of stages starting with a simple single strand, passing through



TEXT-FIG. 7. i–iv. *Danaea alata*, Sm. Diagrams illustrating transverse sections of the rhizome of an old plant at successively higher levels. They are in fairly close sequence, but are not consecutive. The contours mark the outer limits of the phloem. *c.s.''*, commissural strand of the internal stelar system; *comp.s.*, compensating strand; *l.t.*, leaf-trace; *r.t.*, root-trace.

an intermediate condition where the strand forks near its base, and ending with two independent strands which originate, not from the base, but from the lateral margins of their respective gaps (Text-fig. 4, A and B).

The root-traces are inserted directly upon the outer surface of the *internal* vascular cylinder of the stem (Text-fig. 4, B).

Eventually a commissural strand (Text-fig. 4, B, *c.s.''*) is differentiated from the inner surface of the internal stelar system, to which it bears exactly the same relation as the original commissural strand (Text-fig. 4, A, *c.s.*) does to the external stelar system.

In the specimen of *Danaea alata* under discussion, the commissural strand of the internal stelar system leaves the inner surface of the cylinder near the insertion of a root-trace (Text-fig. 4, B, *r.t.* 4), and above the gap formed by the exit of a compensating strand (Text-fig. 4, B, *comp.s.* 2)

TEXT-FIG. 8. A and B. *Danaea nodosa*, Sm. Transverse sections of the stem of a large plant, showing three concentric zones of bundles and a central strand. *adv.b.*, adventitious bud; *c.s.*, commissural strand; *comp.s.*, compensating strand; *l.t.*, leaf-trace; *r.*, root.

passes slowly across the central ground-tissue to the point of insertion of the next root-trace (Text-fig. 4, B, *r.t.* 5) above the gap of the next compensating strand (Text-fig. 4, B, *comp.s.* 3), and thus forms an accessory conducting system, which serves to connect up the points of insertion of the root-traces. Further complications arise later by the branching and anastomosing of the original commissural strand of the internal stelar system and by the appearance of a second strand, whilst weak commissures are differentiated across the central ground-tissue and serve to join up the two main commissural strands.

Even in the largest specimens of *Danaea alata* and of *Danaea nodosa* examined by the present writer, no distinct third vascular *cylinder* was present, the innermost stelar system (if we are entitled to designate it as such) consisting only of a few weakly developed anastomosing and branching commissural strands (Text-fig. 8, A and B).

Danaea nodosa, Sm.

In its development, the stelar system of *Danaea nodosa* passes through a series of stages in elaboration identical with those described above for *Danaea alata*, while the stele of the adult rhizome differs from that of *Danaea alata* only in its larger proportions and perfectly radial symmetry, the leaf- and root-traces being given off equally all round the stem-stele; a detailed description of the structure and development of the vascular system of *Danaea nodosa* is therefore unnecessary.

However, an examination of the vascular anatomy of an ill-nourished adult specimen yielded interesting results. Judging from its slender elongated rhizome, on which definite internodes could be distinguished, this specimen had fallen upon evil days.

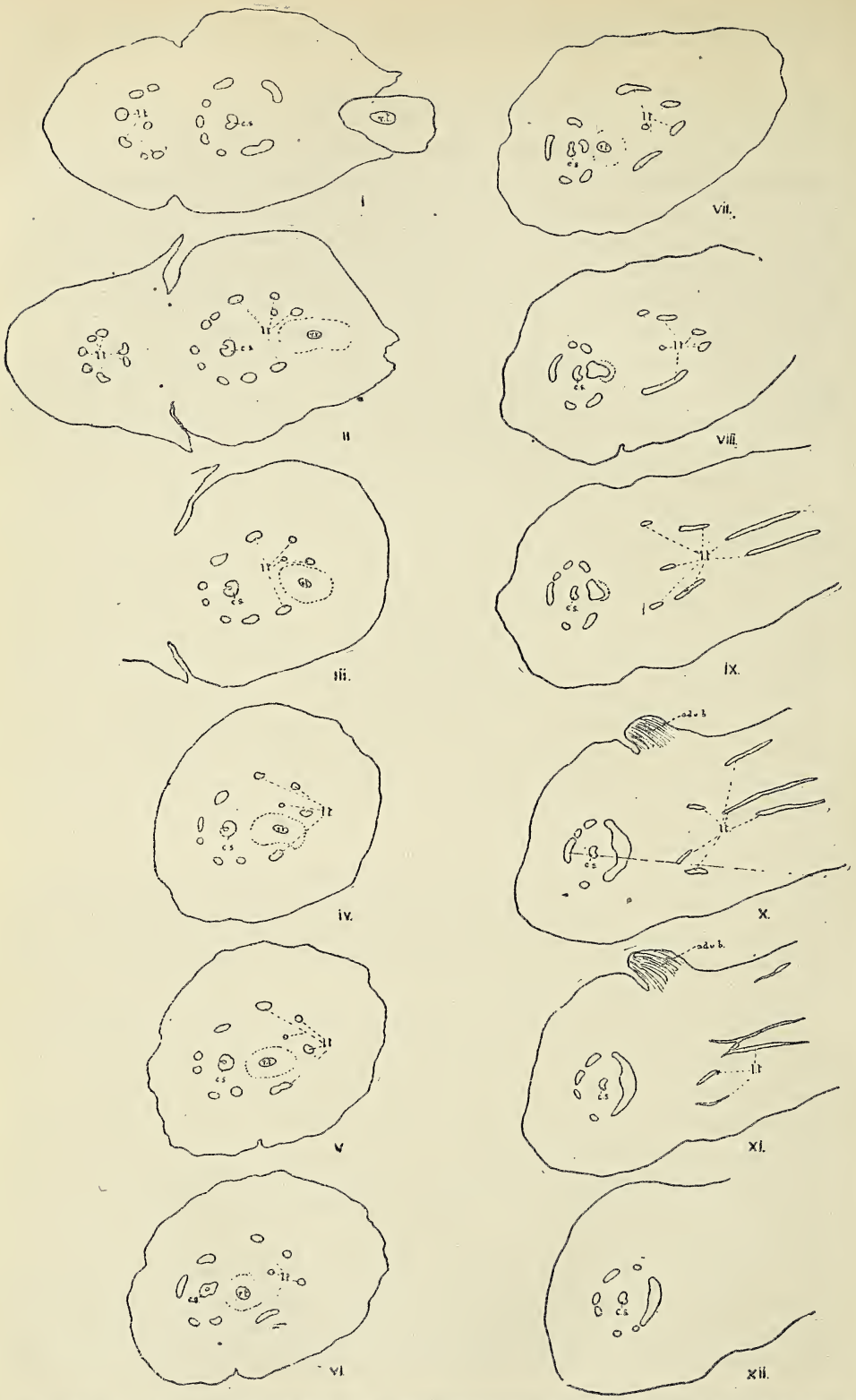
The stele of this rhizome consisted of an outer network of vascular strands enclosing a ground-tissue parenchyma which was traversed by a single strongly developed commissural strand (Text-figs. 9, i, and 14, A). The leaf-traces, which are made up of several (4–6) strands, pass off from this outer network of bundles leaving large foliar gaps. But since the insertions of the spirally arranged leaves are widely separated from one another, the foliar gaps seldom overlap. In the internodes the commissural strand opens out into a solenostele (Text-fig. 9, ii–vi) and below each leaf-gap in turn branches into two almost equal parts, one of which functions as a compensating strand, joining on to the strands bounding the leaf-gap and closing it in front, whilst the other passes slowly across the central parenchyma towards the next leaf-gap above, where the same sequence of events is repeated.

A root-trace usually joins the commissural strand in the internodal region and plays an important part in the formation of the solenostele, but sometimes the root-trace joins the outer lattice-work of bundles at the point where the compensating strand fuses with the main vascular cylinder, as is shown in Text-fig. 9, viii–x.

Thus we find that the stem of this ill-nourished adult sporophyte of *Danaea nodosa* not only approximates in size to that of the sporeling, but it permanently retains a type of stelar organization which marks only a passing phase in the development of the vascular system of the normal sporophyte (cf. Lang, 44, p. 51).

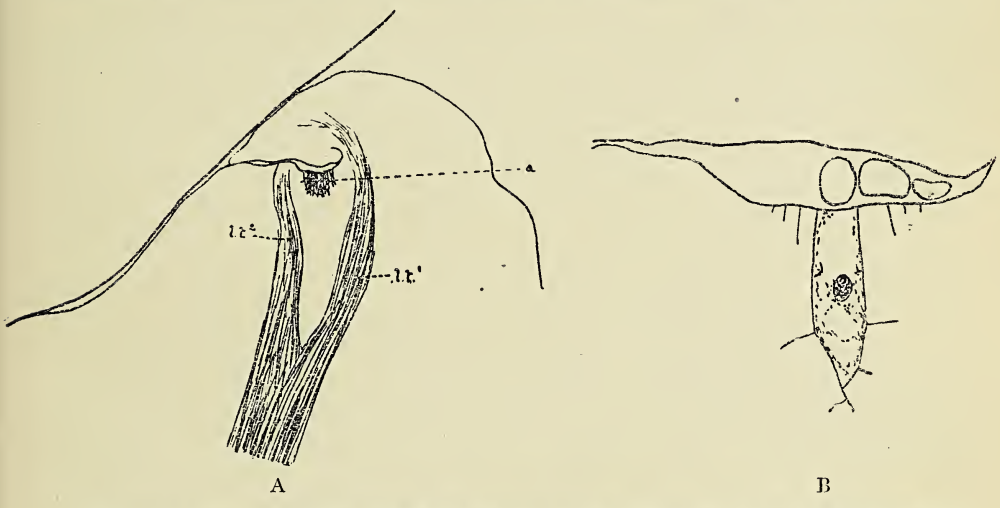
It is possible that the ontogenetic evolution of the stelar system is here arrested at an earlier phase than usual, the requirements of the plant having become simpler.

Adventitious Buds. In *Danaea nodosa*, Sm., adventitious buds arise from a group of meristematic cells situated at the extreme base of, and towards one side of the swollen leaf-base of the parent plant (Text-figs. 8, B, and 10, A). It has already been pointed out by Lang (45, p. 706) that



TEXT-FIG. 9. i–xii. *Danaea nodosa*, Sm. These diagrammatic figures illustrate in successive, but not consecutive, transverse sections the changes and rearrangements that take place in the vascular system of an ill-nourished adult rhizome. For description see text. *adv.b.*, adventitious bud; *c.s.*, commissural strand; *l.t.*, leaf-trace; *r.t.*, root-trace. × 3.

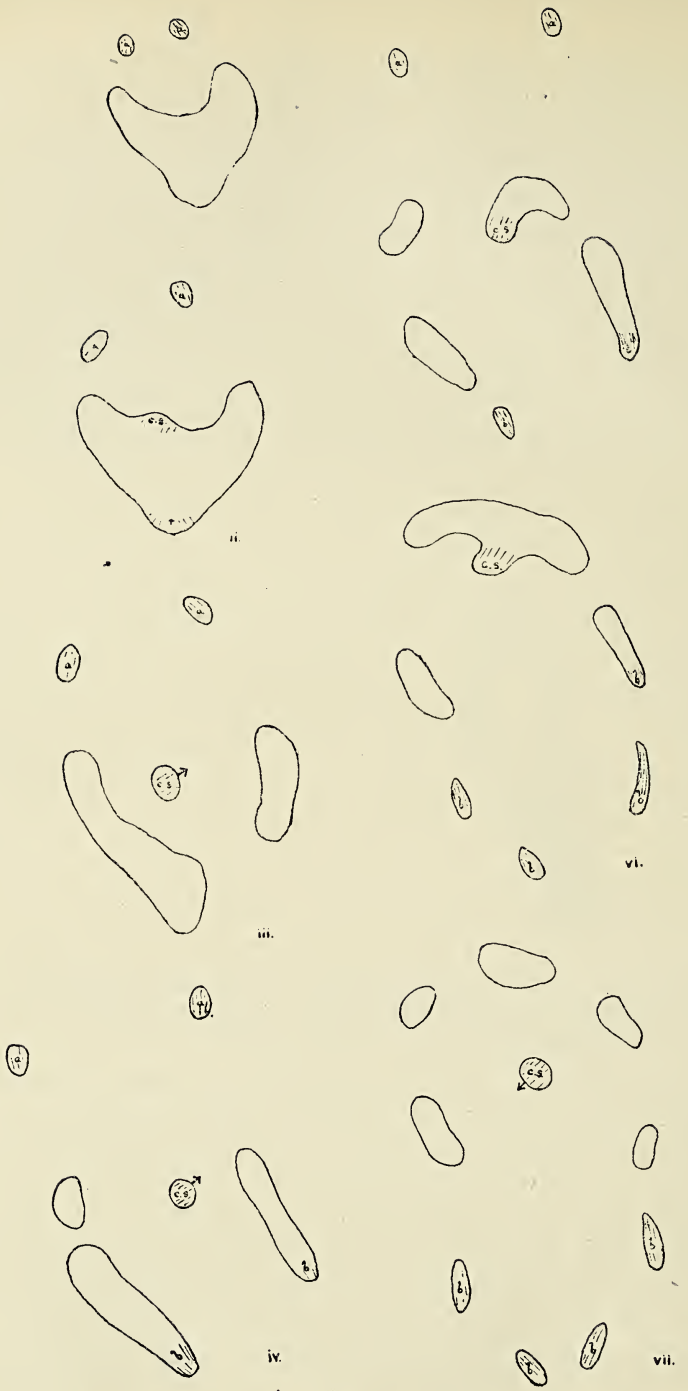
vegetatively produced plants tend in their development to pass through stages in elaboration similar to young plants which take their origin from a zygote (cf. Jones, 37, p. 27). This statement also holds good for the adventitiously produced plants of *Danaea nodosa*. At the apex of the stem of the adventitious bud there is a single elongated initial cell, similar in every respect to that found at the stem-apex of the sporeling (cf. Text-fig. 10, B, with Text-figs. 20, B, and 21, B). Moreover, the second leaf of the adventitious bud is formed almost exactly opposite to the first leaf (cf. Text-fig. 10, A, with Text-fig. 3, B). Not only does the adventitious bud actually arise upon the basal pulvinus of the petiole (although very close to the stem), but its vascular supply is derived directly from one of the foliar traces (Text-fig. 8, B); it cannot therefore be regarded as a *branch* of the parent stem.



A B

TEXT-FIG. 10. A. Median longitudinal section through the apex of an adventitious bud of *Danaea nodosa*, Sm. *a.*, apical cell of the stem; *l.t.*¹, *l.t.*², traces of the first and second leaves respectively. × 8. B. Apical cell of same more highly magnified. × 360.

Vascular Anatomy. In the vegetatively produced plant the vascular tissue differs in its origin from that of the sexually produced plant, inasmuch as it arises as a simple strand from one of the numerous leaf-traces of the parent plant; also, the earlier stages are hurried over or absent altogether, a fact which may be correlated with the relatively greater importance of the first leaf of the adventitious bud and with the early development of the commissural strand. Otherwise the stages in the elaboration of the stelar system are almost identical with those described above for the sporeling. The protostele at once becomes crescentic after the departure of the first leaf-trace; the latter departs from the stem-stele as two strands which branch whilst still within the cortex of the stem. The trace of the first root fuses with the stem-stele opposite the point of departure of the first leaf-trace, whilst immediately above this point the commissural strand (Text-fig. 11, *c.s.*) arises by proliferation of the vascular tissues. A relatively



TEXT-FIG. 11. i–vii. *Danaea nodosa*, Sm. The diagrams illustrate transverse sections of a hand-cut series of an adventitious bud at successively higher levels. They are in fairly close sequence, but are not consecutive. The contours mark the outer limits of the phloem. *a*, meristeles of first leaf-trace; *b*, meristeles of second leaf-trace; *c s.*, commissural strand; *r*, root.

large leaf-gap is formed by the departure of the trace of the second leaf from the convexity of the crescent-shaped stem-stele ; this leaf-trace consists of four strands which pass off at various levels from the margins of the foliar gap (Text-fig. 11, v-vii). The stem-meristeles meanwhile branch and anastomose, and the commissural strand pursues a roughly spiral course through the central ground-tissue, fusing with the stem-meristeles above the foliar gaps. In this way, then, the protostele becomes directly transformed into a typical dictyostele without the intervention of a solenostelic stage. The subsequent stages are essentially similar to those described by Brebner (12) for the sporeling of *Danaea simplicifolia*, Rudge, and by the present writer for the young sporophyte of *Danaea nodosa*, Sm., the radial symmetry being retained throughout.

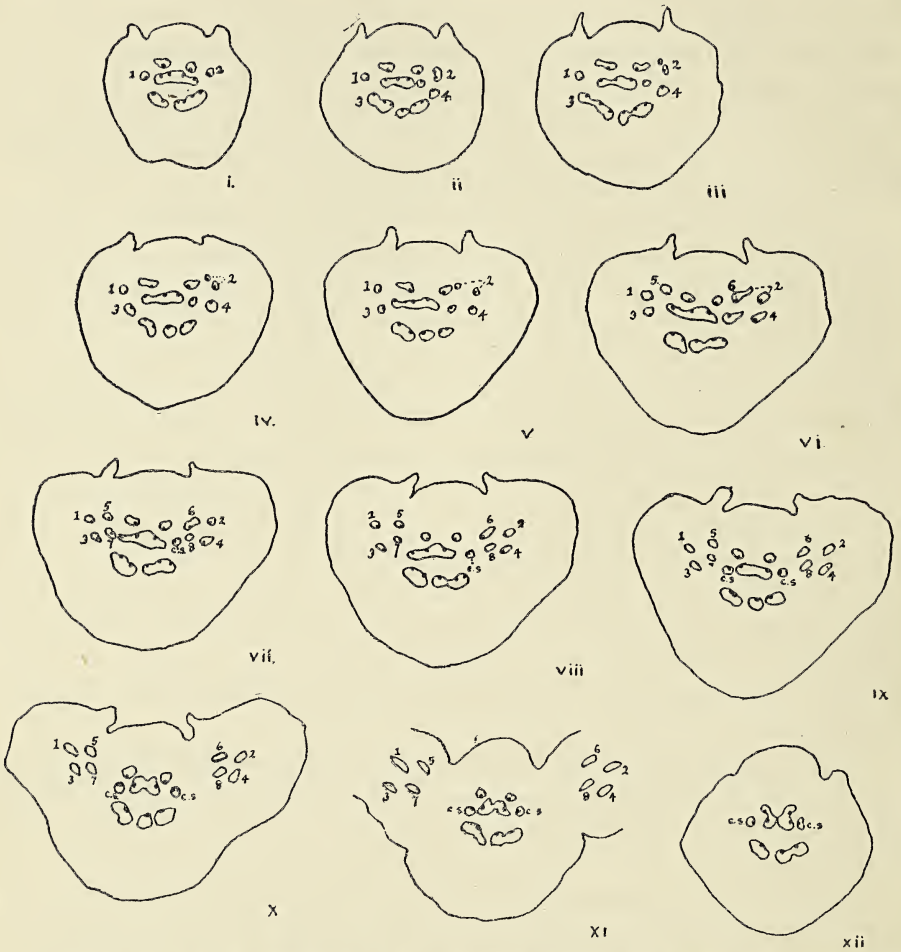
Vascular Anatomy of the Petiole of the Adult Sporophyte. The leaf-trace,[1] both in *Danaea alata* and in *Danaea nodosa*, is made up of an abaxial arc of several strands and of adaxial wings derived from the two strong lateral strands which generally branch two or three times whilst still within the cortex of the stem (Pl. XXI, Fig. 1, A). No strands from the inner cylinder contribute directly to the leaf-trace, but a varying number of internal accessory strands, similar in every respect to those described by Gwynne-Vaughan (30) for *Kaulfussia* and for *Archangiopteris*, arise from the strands of the abaxial arc near the base of the petiole, travel across the ground-tissue, and unite with the terminal strands of the primary ring. The presence of these so-called internal strands of the leaf-trace is a very characteristic feature of this group of Ferns. Above the region of the basal pulvinus the strands of the horseshoe curve anastomose repeatedly ; sometimes the terminal (i. e. adaxial) strands fuse together to form a single large strand with adaxially directed protoxylem groups (Text-fig. 12, i–ix).

The vascular supply of the pinnae is interesting, but since a considerable variation in the number and shape of the vascular strands was found among the specimens examined, two typical cases are described in some detail below.

Case I. The arrangement of the strands represented in Text-fig. 12, i, was found a short distance below the point of insertion of the lowermost pinnae. Each leaflet of the lowermost pair receives four strands ; three of these (numbered 1, 3, 5 and 2, 4, 6 respectively) proceed from the flank of the horseshoe curve, while the fourth (numbered 7 and 8 respectively) arises from the edge of the large strand which is formed by the fusion of the terminal (i. e. adaxial) strands of the petiolar curve with the internal accessory strands of the basal pulvinus. Occasional branchings and anastomosings occur in these pinna-traces (Text-fig. 12, ii–vii). Branches arising

[1] For a brief, but comprehensive, summary of previous work on this subject consult Tansley, A. G. : Evolution of the Filicinean Vascular System. Reprint from New Phytologist, vols. vi and vii, 1907–8.

from the ends of the large terminal strand (Text-fig. 12, vii–xii, *c.s.*) assist in repairing the gaps made in the petiolar curve by the departure from its flanks of strands 1, 3, 5 and 2, 4, 6 respectively, and their function is therefore strictly analogous to that of the compensating (= *Ersatz*) strands in the stem-stele. Finally, the terminal strand divides into two, the



TEXT-FIG. 12. i–xii. *Danaea alata*, Sm. The figures illustrate in successive, but not consecutive, transverse sections the changes and rearrangements that occur in the vascular strands of the rachis, including the region of the lowermost pinnae. The position of the protoxylem groups is denoted by the black areas. *c.s.*, compensating strand. × 6. For description see text.

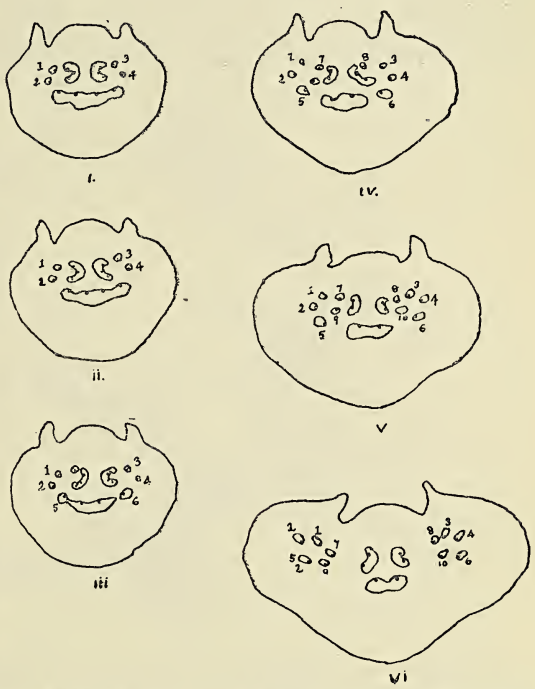
respective halves fusing with the two adaxial strands of the curve, as shown in Text-fig. 12, x–xii. The petiolar curve at a level immediately above the point of insertion of the lowermost pair of pinnae is represented in Text-fig. 12, xii.

Case II. In the second case the arrangement of the petiolar strands at a level immediately below the point of insertion of the lowermost leaf-

lets is essentially similar to that described above, except that in this case the terminal strands of the curve do not fuse to form a single strap-shaped strand (Text-fig. 13, i). Five strands pass to each leaflet, viz. four strands (numbered 1, 2, 5, 7 and 3, 4, 6, 8 respectively in Text-fig. 13) from each flank of the horseshoe curve and a strand (numbered 9 and 10 respectively) from the incurved end of each of the terminal (i. e. adaxial) strands. It will be noticed that in this case no compensating strands are produced; the arrangement of the strands of the petiole at a short distance above the point of insertion of the pinna-traces is shown in Text-fig. 13, vi.

Thus it would appear that the vascular supply of the pinnae is based on the same lines in every genus of Marattiaceae in which this point has been investigated (cf. Gwynne-Vaughan, 30, p. 262 et seq.).

Several pulvinoid swellings or 'nodes' are normally present on the petioles of adult plants of *Danaea alata* and *Danaea nodosa*. These structures have been interpreted as representing the position of abortive pinnae (cf. Brebner, 12, p. 537), but this interpretation has up to the present been based on poor evidence. In the course of the above investigation, however, a single case was noticed where a rudimentary vascular strand left the main petiolar curve on one side only in the region of one of these nodes. This strand soon petered out, ending blindly in the parenchymatous ground-tissue of the petiole.



TEXT-FIG. 13. i–vi. *Danaea alata*, Sm. The figures illustrate in successive, but not consecutive, transverse sections the changes and rearrangements that occur in the vascular strands of the rachis below the point of attachment of the lowermost pinnae. The position of the protoxylem groups is denoted by the black areas. × 6. For description see text.

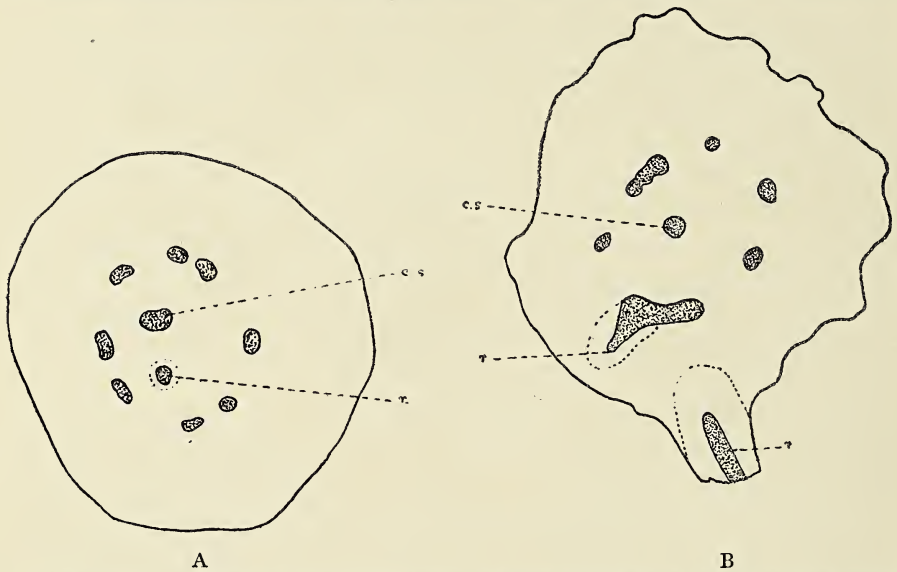
2. *Kaulfussia*, Blume (= *Christensenia*, Maxon).[1]

The genus *Kaulfussia*, of which only one distinct species (i. e. *Kaulfussia aesculifolia*, Bl.) is at present known, possesses a relatively slender prostrate rhizome with a very well-marked dorsiventral configuration. According to Kühn (42, p. 462), lateral branching of the rhizome some-

[1] Carl Christensen, Index Filicum, 1906.

times occurs, but the rhizome was unbranched in every specimen examined by the present writer. The numerous roots which arise from the ventral surface and flanks of the rhizome greatly outnumber the leaves, which form two ranks on the dorsal side of the rhizome. The leaves of *Kaulfussia* are not crowded, consequently distinct internodes can be distinguished (cf. the ill-nourished specimen of *Danaea nodosa* described above [p. 375] and cf. Text-fig. 14, A with 14, B).

The development and arrangement of the vascular strands in the very young sporeling have been carefully studied by Campbell, who, in his monograph on the 'Eusporangiatae', maintains that the vascular system of *Kaulfussia* is at first essentially similar to that of *Danaea*, consisting of a sympodium of the traces of the earlier leaves. He states, however, that



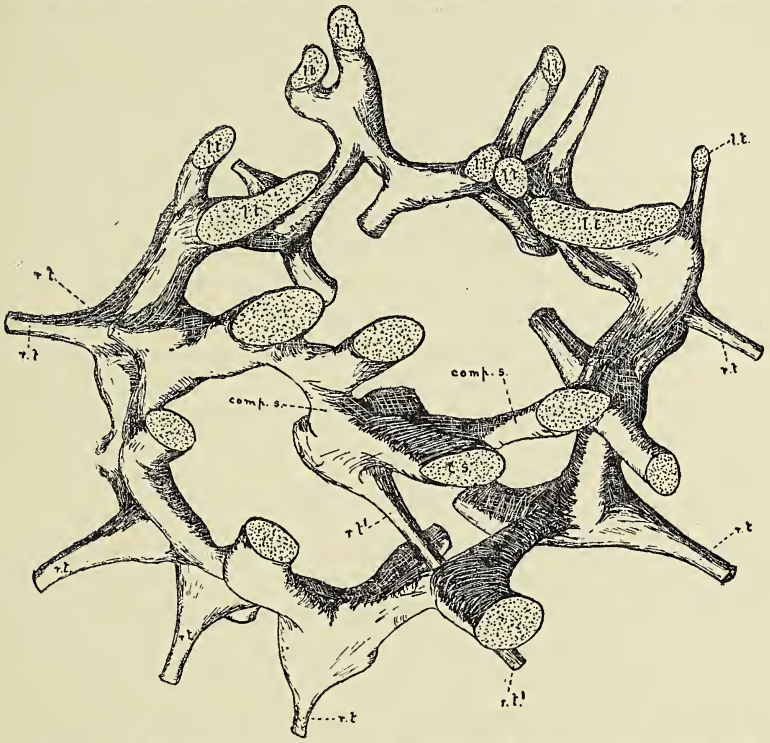
A B

TEXT-FIG. 14. A. *Danaea nodosa*, Sm. Transverse section of the rhizome of an old (ill-nourished) specimen. × 5. B. *Kaulfussia aesculifolia*, Bl. Transverse section of the rhizome of an old specimen. × 4. *c.s.*, commissural strand; *r.*, root.

the marked dorsiventrality of the adult plant has already impressed itself on the morphology and vascular anatomy of the young sporophyte, a distichous arrangement of the leaves being evident from the beginning, while the traces of the first and of all succeeding leaves are inserted upon the same side of the stelar system. Between the solid stele found in the very young stem and the dictyostelic cylinder of the older rhizome, Campbell (20, p. 184, Fig. 168, J, K) observed a transitional siphonostelic condition.

The vascular anatomy of the adult plant of *Kaulfussia* has already been investigated by Kühn (42), Farmer and Hill (29), and Gwynne-Vaughan (30). However, for comparison with that of the dorsiventral species of *Danaea*, the present writer examined the stelar system of the adult rhizome and petiole.

In the internodal regions of the adult rhizome of this fern there is a single ring of branching and anastomosing bundles, from five to twelve in number, surrounding a single commissural strand (Pl. XXI, Fig. 3; Text-figs. 14, B, and 15). The leaf-traces, which consist of several strands, pass off as large meshed segments from the dorsal region of the stelar system, leaving wide foliar gaps (Pl. XXI, Fig. 3; Text-fig. 15). The commissural strand (*c.s.* in Pl. XXI, Fig. 3, and in Text-fig. 15), which in this genus, according to Campbell (20, p. 184), arises late in the development of the vascular system, pursues a somewhat sinuous course through the central



TEXT-FIG. 15. *Kaulfussia aesculifolia*, Bl. Front view of a model[1] of the stelar system of a portion of the rhizome of an old plant. *c.s.*, commissural strand; *comp.s.*, compensating strand; *l.t.*, leaf-trace; *r.t.*, root-trace (cf. Pl. XXI, Fig. 3).

ground-tissue, and below a leaf-gap gives off either a single strand, which immediately dichotomizes, or, more often, two separate strands, which, fusing with bundles of the peripheral ring, help to close the foliar gap (Pl. XXI, Fig. 3, and Text-fig. 15, *comp.s.*).

A root-trace (Pl. XXI, Fig. 3, and Text-fig. 15, *r.t.'*) usually joins the commissural strand immediately before the latter gives off a branch (or two branches) to close the leaf-gap; the great majority of the root-traces, however, are inserted indiscriminately upon any of the stem-meristeles and apparently bear no intimate relation to the number and position of the leaf-traces. In the oldest specimens examined, the stelar anatomy had

[1] This model, now in the Botanical Museum of the Imperial College, was prepared by Mr. T. G. Hill.

not increased in complexity beyond this stage, hence it would appear that in the genus *Kaulfussia* the arrangement of the vascular strands in the adult rhizome is *simpler* than in *Danaea*.

Vascular Anatomy of the Petiole. The arrangement of the vascular strands in the petiole of *Kaulfussia* has already been dealt with at some length by Bertrand and Cornaille (2) and by Gwynne-Vaughan (30); according to the last-named botanist (l. c., p. 263), several (1–5) internal strands leave the abaxial strands of the arc, pass across the central ground-tissue, and unite with the adaxial terminal strands of the horseshoe curve; hence they are not continued up as separate strands beyond the region of the pulvinus. The two terminal strands sink in towards the centre of the petiole and eventually fuse together across the median plane to form a single large strand; this is the arrangement of the vascular strands as seen in a transverse section taken at some distance below the point where the petiole branches (Text-fig. 16, i; and cf. Bertrand and Cornaille, l. c, Fig. 86, p. 169). But in the region immediately below the point where the petiole branches, the large central bundle divides into six strands (Text-fig. 16, iv); of these six strands, two pass into each of the three main branches accompanied by three strands from the peripheral ring of bundles (Text-fig. 16, v–vii); these together form an anastomosing ring of strands in each of the three primary branches of the petiole. Two or more strands leave the ring of bundles to supply the secondary branches of the petiole (Text-fig. 16, viii–x).

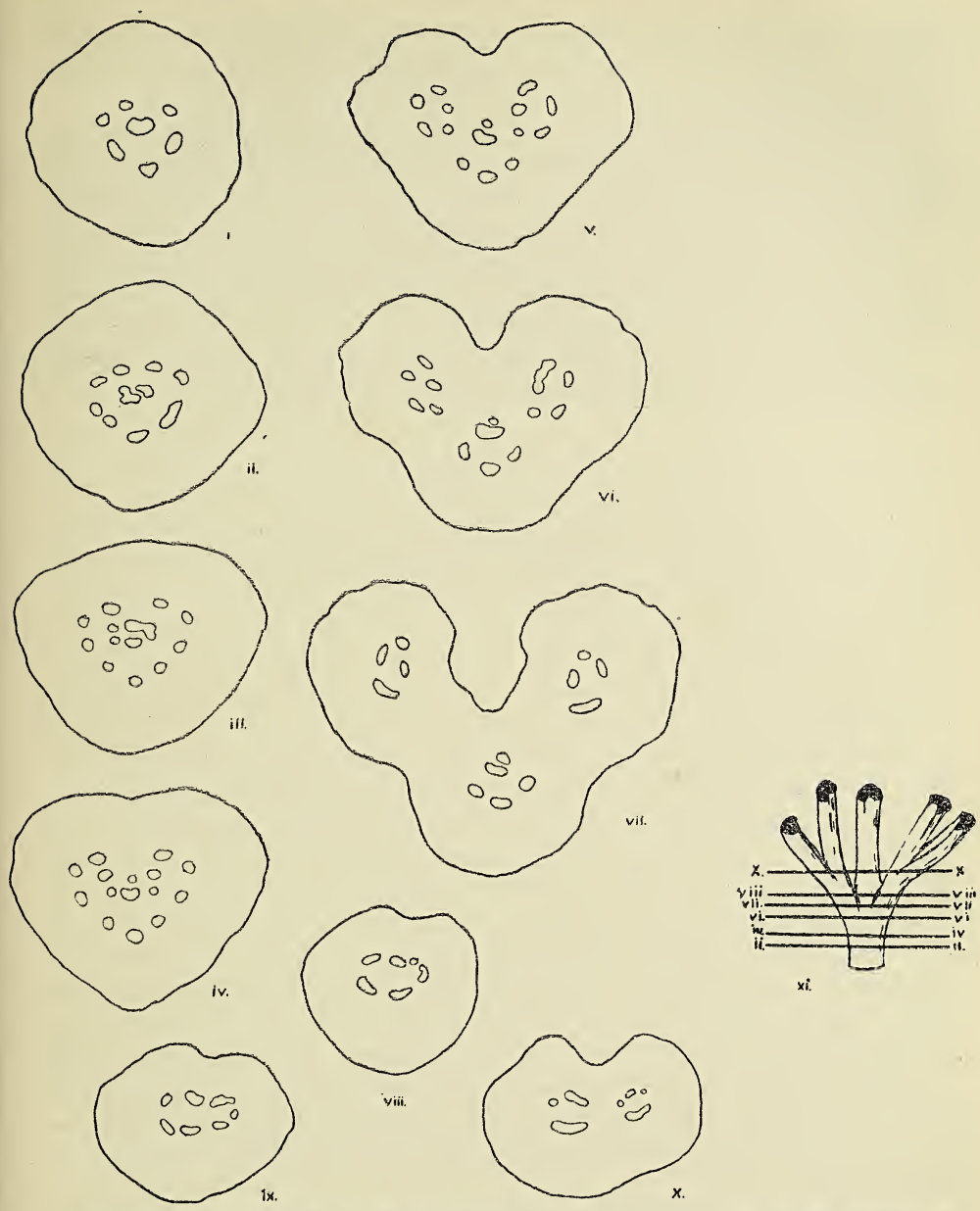
3. *Archangiopteris*.[1]

Our knowledge of the general morphology of this monotypic genus is still far from complete, and it is impossible to decide from the information available whether the stem of the *adult* plant has a radial or a dorsiventral organization. Gwynne-Vaughan (30), who investigated the anatomy of the petiole and of a small fragment of the stock, states that the arrangement both of the leaves and of the vascular strands in the stem indicates a radial symmetry. An examination of herbarium specimens by the present writer lends support to this view.

Archangiopteris Henryi has simply pinnate leaves and closely resembles the larger species of *Danaea*.

According to Gwynne-Vaughan (30, p. 261), the vascular system of the stem consists of a single dictyostelic ring of two to four small strands, which anastomose with each other in a somewhat irregular manner; in addition there is usually a small internal (=commissural) strand which runs free in the central ground-tissue through the greater part of its course, and from time to time approaches the dictyostelic ring, fusing with those

[1] For information regarding the morphology of the frond of *Archangiopteris Henryi*, the reader is referred to Pteridographische Notizen, published by H. Christ and K. Giesenhagen in Flora, Bd. lxxxvi, 1899.



TEXT-FIG. 16. i–x. *Kaulfussia aesculifolia*, Bl. These diagrammatic figures illustrate in successive, but not consecutive, transverse sections the changes and rearrangements that take place in the vascular system of the petiole of an adult plant in the regions indicated on xi. × 6. xi, nat. size.

meristeles that are about to close a foliar gap. This central strand soon separates off again and passes on across the central ground-tissue to the next leaf-gap above, which it helps to close up (cf. the young sporophyte of *Danaea*); root-traces arise from the external surface and sides of the stem-meristeles, a root-trace invariably arising from the point where the

central strand fuses with a meristele of the dictyostelic ring (cf. 30, Plate X, Fig. 6). In this way a direct water-channel is formed between a root and the next leaf above.

Two strands only are given off to supply the petiole, but as they pass outwards through the cortical region of the stem, they divide into several (eight or nine) strands, which, at the base of the petiole, are arranged in a typical horseshoe curve (cf. 30, Pl. X, Fig. 12).

The vascular anatomy of the petiole closely resembles that of *Kaulfussia* and of *Danaea*, internal accessory strands being found in the region of the basal pulvinus of all three genera.

Also, the manner in which the pinnae obtain their vascular supply from the rachis is essentially similar in these three plants.

4. *Marattia.*

The adult sporophyte of *Marattia* possesses an upright, tuberous, more or less conical stem surmounted by a rosette of large pinnate leaves; the remainder of the stem surface is almost entirely covered by the huge leaf-bases. Numerous stout adventitious roots anchor the stem to the soil.

Apart from a passing reference by Holle (34), the vascular anatomy of this genus was first studied by R. Kühn (42), who found in the stem of comparatively young plants of *Marattia fraxinea* a peripheral ring of anastomosing vascular strands surrounding a single axile bundle (l. c., Taf. XVIII and XIX, Fig. 22). In rather older stems of the same species this investigator showed that the stelar system was further complicated by the appearance of a second zone of anastomosing strands (l. c., Taf. XVIII and XIX, Figs. 30–32). His account of the arrangement of the vascular strands is rather difficult to follow, but he seems to show that compensation for the departure of the leaf-traces from the outer zone of bundles is provided for by two strands which leave the inner zone and, passing obliquely outwards, help to close up the foliar gap. The central strand likewise gives off two branches which assist in closing the gap formed in the inner zone. It will be noticed that Kühn describes for the comparatively young plant of *Marattia fraxinea* a type of stelar structure which is fundamentally similar to that of the adult sporophyte of the radial species of *Danaea* (e. g. *Danaea nodosa*, Sm.).

Farmer and Hill (29) gave a brief account of the development of the vascular system in the sporeling of this same species of *Marattia*, and showed (l. c., pp. 378–9) that the protostele found at the base of the stem of the young sporophyte opens out to form a siphonostele with extremely large foliar gaps (l. c., Pl. XVII, Figs. 20 and 21). Sooner or later commissural strands of an attenuated form are developed within the siphonostele. The earlier leaf-traces are single, but those subsequently formed fork once in the stem-cortex; the dichotomy extends farther

back in the successively produced leaves, till it is obvious at their first origin at the base of the foliar gap.

Lotsy (47, p. 681) briefly states, with reference to *Marattia sambucina*, that 'Der Stamm hat eine weniger komplizierte Dictyostele als *Angiopteris*'.

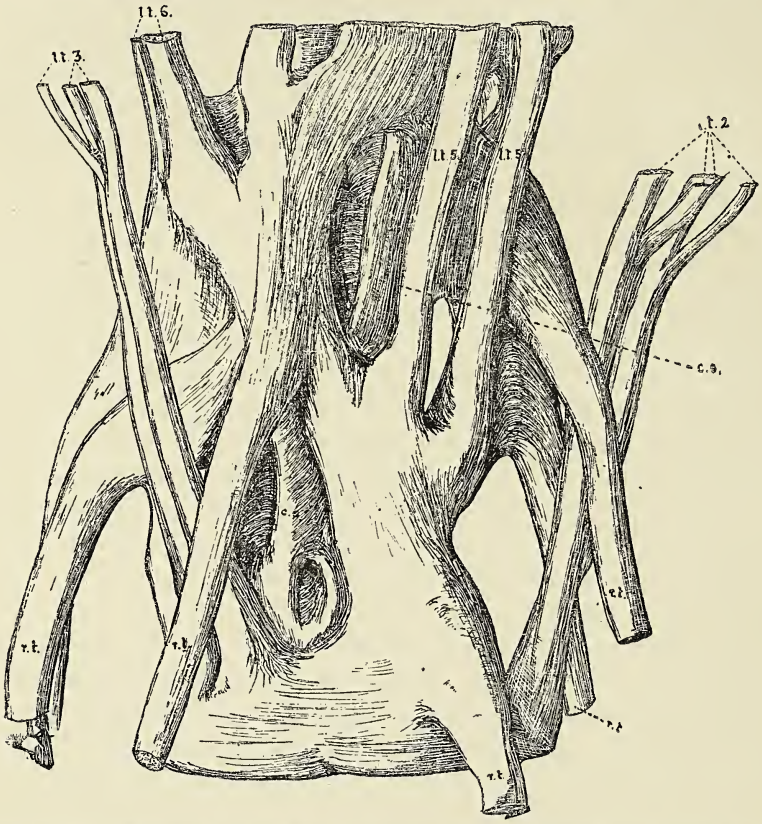
Campbell (20), as a result of his investigations upon the vascular anatomy of young sporophytes of *Marattia Douglasii* and *Marattia sambucina*, brings this genus into line with *Danaea* and *Kaulfussia* by maintaining that the solid strand which occurs at the base of the stem is composed of the united traces of the earlier leaves. This botanist also describes (20, pp. 191–3) the anatomy of the stem of a small adventitiously formed plant of *Marattia alata*; his description of the stelar system of this species agrees essentially with that given for *Marattia fraxinea* by Farmer and Hill (l. c.).

The most recent and complete account of the development of the stele in this genus, however, was published by Charles (23), who had the great advantage of working with abundant material of *Marattia alata*. At the base of the young sporeling this observer finds (l. c., p. 97) a protostele from which the transition to a solenostele takes place suddenly and without the intervention of a distinct medullated monostelic stage. Later, a medullary strand arises, and at first behaves like the commissural strand in the young sporophyte of *Danaea*, but as the leaf-traces become more crowded, the medullary strand divides into a number of branches, which join the stelar cylinder above the leaf-gaps. Eventually, however, a second cylinder is produced by anastomosing and branching of the medullary strands (l. c., p. 92, Fig. 3). The main root-supply joins the external system, the medullary system having only a few small roots.

The model represented in Text-figs. 17 and 18 was built up from a series of transverse sections of the caudex of an adult plant of *Marattia Cooperi*, Mre., the base of which had unfortunately completely decayed away. A transverse section of the existing basal region of this specimen showed a ring of strap-shaped bundles surrounding a single central bundle (Text-fig. 19), whilst a similar section taken just below the apex of the same plant showed two concentric zones of strands (Text-fig. 18). The region of the stelar system at which the transition between these two conditions takes place is represented in this model, from a study of which it is seen that the stem-stele of this plant is made up of a simple dictyostelic cylinder with extremely large foliar gaps (cp. Farmer and Hill, l. c., p. 379) and an internal accessory system which at first consists of a single commissural strand, a short branch from which assists in closing the foliar gap. Subsequently, however, further elaboration of the internal conducting system takes place by branching and anastomosing of the original commissural strand, whereby an internal vascular cylinder is produced (Text-fig. 18). Only a single compensating strand (Text-fig. 18, *comp.s.*) leaves this inner cylinder to assist in closing the gap formed in the outer cylinder by the departure of

the leaf-trace. No gaps other than leaf-gaps occur in the external vascular cylinder.

The double leaf-traces, which are arranged in a fairly regular spiral,[1] leave the outer cylinder from near the base of the leaf-gaps and at first pass out to their respective leaves so gradually, branching meanwhile in the cortical ground-tissue (Text-figs. 17 and 18), that the traces of a number of leaves appear in a transverse section of the stem and present an appear-



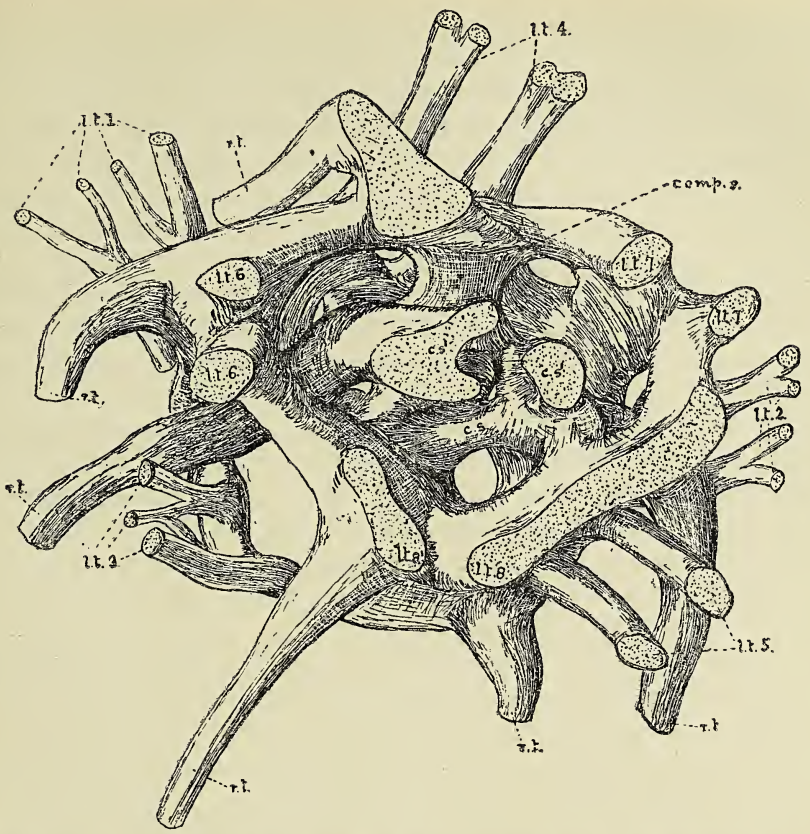
TEXT-FIG. 17. *Marattia Cooperi*, Mre. Model of the stelar system of the caudex of an adult plant, viewed from one side. *c.s.*, commissural strand; *l.t.*, leaf-trace; *r.t.*, root-trace.

ance very similar to that of the outermost ring of bundles of the stem-stele in *Danaea*, with which at first sight they may very easily be confused. However, the model clearly demonstrates their true foliar nature; anastomosings between adjacent leaf-traces never take place.

Usually a root-trace fuses with the main vascular cylinder just below the point of departure of the leaf-trace (Text-fig. 17), and there is generally one root to each leaf, as Holle (34) pointed out long ago. Other root-traces join the central stelar system at irregular intervals (Text-figs. 17 and 18).

No third cylinder was developed in the specimen examined. It

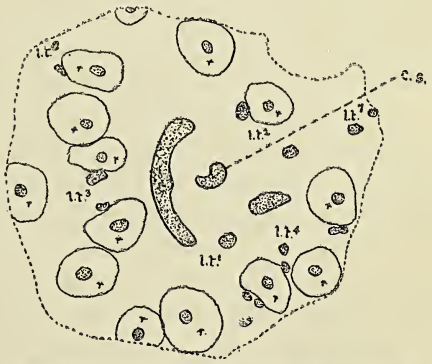
[1] The leaf-divergence = 144° approximately.



TEXT-FIG. 18. *Marattia Cooperi*, Mre. Model of the stelar system of the caudex of an adult plant, viewed from above. *c.s.* = commissural strand; *c.s.'* = central cylinder; *comp.s.* = compensating strand; *l.t.*, leaf-trace; *r.t.*, root-trace (cf. Text-fig. 17).

is probable that further complications arise in the stelar system of the huge tuberous stems of very old plants, but, unfortunately, no very old plant of *Marattia* was available for investigation.

Vascular Anatomy of the Petiole. Gwynne-Vaughan (30, pp. 264–5) found a large number of internal strands within the typical ring of bundles just above the basal pulvinus of a petiole of *Marattia fraxinea*; traced upwards, these internal strands decreased in number by a succession of fusions, which, however, take place so gradually that a number of the



TEXT-FIG. 19. *Marattia Cooperi*, Mre. Transverse section of the basal region of the stem of an old plant, showing numerous roots (*r.*) and leaf-traces (*l.t.*) embedded in the ground-tissue. The leaf-bases had been artificially removed. The leaf-traces are numbered consecutively, *l.t.*[1] being the youngest shown. *c.s.*, commissural strand. × 1·5.

internal strands continue their course from the pulvinus far up into the region of the rachis, where, according to Bertrand and Cornaille (2, pp. 162 and 212), the primary branches of the petiole are supplied direct with strands from the internal system, in addition to those they obtain from the outer ring.

5. *Angiopteris.*

The adult sporophyte of *Angiopteris*, which closely resembles in size and in habit that of most species of *Marattia*, also has a bulky tuberous caudex surmounted by a rosette of huge leaves, which are usually twice-pinnate.

The first account of the vascular anatomy of the genus *Angiopteris* was published in 1853 by Harting (De Vriese and Harting, 27), who found that the vascular system of the stem consisted of a confused network of bundles which traversed the ground-tissue in all directions. This investigator, however, not only failed to distinguish the foliar traces from those of the intracortical roots, but made no attempt to trace out the actual course of individual vascular strands.

In 1864 Mettenius (49) published an important monograph in which the course of the bundles in the stem and leaf-bases of an old plant of *Angiopteris* was described in great detail. He showed that the very numerous vascular strands of the tuberous stem form a number of concentric cylinders of anastomosing bundles each having the general form of an inverted cone. Meshed segments leave the outermost cylinder to supply the leaves; the gaps in this cylinder formed by the departure of the leaf-traces are compensated by corresponding segments from the next inner zone. In a similar manner segments from the third cylinder compensate for the gaps produced in the second, and so with successive inner cylinders. Mettenius (l. c., p. 590) also stated that strands from the second cylinder sometimes contribute directly to the leaf-trace.

De Bary (1) pointed out that the stem-stele of a young plant of *Angiopteris* consisted of a 'Bündelrohr' with foliar gaps, while Leclerc du Sablon (54) gave a brief account of the vascular anatomy of the young plant as it appeared in a series of transverse sections of the stem.

Shove (58) described the vascular anatomy of a large specimen of *Angiopteris* and in most of the important details confirmed the results obtained by Mettenius. This plant, however, exhibited a dorsiventral structure which was especially well marked towards its base, where the lower surface was quite destitute of leaves, but thickly covered with roots. This observer (58, p. 505; Pl. XXVIII, Figs. 5 and 6) found that, correlated with the external dorsiventrality of the plant, the meshes of the vascular network were much longer on the ventral root-bearing side than on the dorsal leaf-bearing side. All the strands of the leaf-trace were derived solely from the outermost

ring of bundles at the time being, no strands from the second cylinder contributing directly to the leaf-trace.

The year 1902 was marked by a great advance in our knowledge of the stelar system in this genus, when Farmer and Hill (29) published a full account of the arrangement and development of the vascular tissues in *Angiopteris evecta*; this paper was illustrated by means of a series of wax models representing the stelar skeleton of this plant at various stages in its development. In this way, a most accurate description of the successive stages in the elaboration of the complicated vascular system of the adult plant was obtained. A gradual transition was traced from the solid axile rod of vascular tissue (= protostele) of the very young sporeling to a hollow cylinder or siphonostele, and with perforations corresponding to foliar gaps enclosing a core of pith, which they regarded as distinct from the now tubular stele. Sooner or later, the gap above one leaf fails to be repaired until after the exit of the traces of the next leaf; in this way a typical dictyostele is produced. Meanwhile commissural strands are differentiated across the central parenchyma and serve to connect the opposite sides of the stelar cylinder. 'Finally (l. c., pp. 377–8) the siphonostele opens out to a considerable width (l. c., Pl. XVI, Fig. 6), whilst the axile commissures assume an ever-growing importance forming a sort of sympodial column. . . . The leaf-traces also become more complex, and anastomoses take place at irregular intervals with the strands which can still be recognized as the relics of the original siphonostele, as well as with one another. Irregularities also begin to become apparent as to the relative height at which the two members of the leaf-traces become freed from the plexus of tissue, and a stage is thus reached at which the vascular skeleton appears to consist of a stout axile strand surrounded by upwardly diverging zones of steles which ultimately pass out above to the leaves. The complexity and obscurity is primarily due to the commissural strands which connect up the margins of siphonostelic foliar gaps, and the whole arrangement is to be correlated with the presence of the bulky parenchyma of the stem.' According to these observers, the roots of *Angiopteris* sometimes unite with the more central strands, though far more commonly with those peripherally situated, but Shove (l. c., p. 506) states that the majority of the roots originate from the inner zones, although a few arise from the outer ones, usually at the points where the strands anastomose.

In his monograph on the 'Eusporangiatae', Campbell (20, p. 200) states that 'in the early stages *Angiopteris* appears to agree closely with the other Marattiaceae in the development of its vascular system, but the single central stele without leaf-gaps is retained much longer than in the other genera, and it also becomes much larger and has a better-developed xylem, and the open dictyostele, formed from the anastomosing of the early single leaf-traces, characteristic of *Danaea* and *Marattia*, is not present'.

Thus it is seen that the anatomy of this genus has already been

carefully elucidated; the present writer's investigations on this genus, although covering most of the ground, merely confirm previous results.

Vascular Anatomy of the Petiole.[1] The vascular anatomy of the petiole of *Angiopteris* appears to differ from that described above for *Marattia* only in the larger number of internal strands which arise from the strands of the abaxial arc; as many as five separate concentric rings of bundles were counted by the author in a transverse section of the base of a petiole of an old plant.

6. *Macroglossum.*

This recently discovered genus resembles *Marattia* and *Angiopteris* both in size and in the form of its upright, nearly globular, bulky stem, upon which the leaves are spirally arranged (cf. Copeland, 26; also cf. Campbell, 21). In the form of its simply pinnate leaves, however, it more closely resembles *Danaea*,[2] whilst its sporangia are very similar to those of *Archangiopteris.*

We are indebted to Campbell (21) for the only account of the anatomy of *Macroglossum* that has yet appeared. According to his account (l. c., p. 661), no true cauline stele is developed in the young sporeling, the vascular system of the axis of which is at first composed only of leaf- and root-traces. A single root is formed for each of the early leaves.

Sections of the basal region of the stem of a rather older specimen showed a type of stelar structure not far removed from that of the young sporophyte. In the centre there were five strands; of these five strands, one was comparatively large and somewhat crescentic in transverse section, whilst the remaining four bundles, which were of smaller size, were arranged in pairs; the latter probably represent the double leaf-traces.

It is to be regretted that the author (l. c., pp. 662–3) only briefly refers to the anatomical features exhibited by the adult plant, which, as indicated above, unites within itself certain characters of four other genera of Marattiaceae.

Apical Meristems.

1. *Stem.*

The structure and arrangement of the generative tissues at the apex of the massive stem and roots of the Marattiacean Ferns has been for many years the subject of much divergence of opinion.

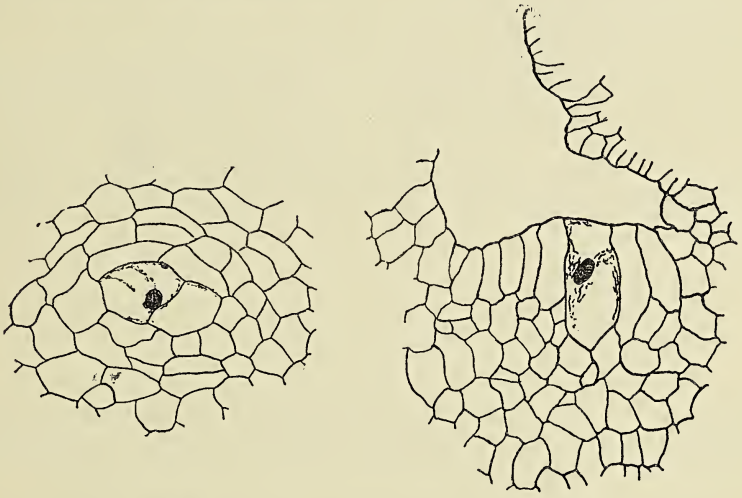
[1] For an exhaustive account of the arrangement of the vascular strands in the petiole of *Angiopteris*, *Marattia*, and *Kaulfussia*, the reader is referred to Bertrand, C. E., et Cornaille, F.: Études sur quelques caractéristiques de la structure des Filicinées actuelles, in Trav. et Mém. de l'Université de Lille, t. x, Mém. 29, 1902.

[2] Moreover the gametophytic characters of *Macroglossum* and *Danaea* agree in (1) the structure of the spermatozoids and in (2) the presence of a large suspensor (cf. Campbell, 21).

As long ago as 1857 Hofmeister (32 and 33) ascribed a three-sided apical cell to the stem of all known Vascular Cryptogams, and referred to *Marattia cicutaefolia* as an example of the Marattiaceae.

Holle (34, p. 218; 35, p. 21) found a four-sided, long-drawn-out initial cell at the apex of the stem of *Marattia cicutaefolia*, and believed that a similar condition obtained in the stem of *Angiopteris evecta*; but he admitted that in the latter genus the origin of the cells at the stem-apex cannot with certainty be traced back to the divisions of a single cell.

Jonkman (38 and 39), on the other hand, described and figured (39, p. 225; Pl. VI, Figs. 13 and 18) a small-celled meristem at the apex of the stem of young sporophytes of *Marattia* and of *Angiopteris*, while Shove (58, p. 522) satisfactorily demonstrated several initial cells in the apical region of an old plant of *Angiopteris*.



A B

TEXT-FIG. 20. *Danaea alata*, Sm. A. Transverse section of stem-apex of a very young sporeling showing the apical cell. B. Longitudinal section of stem-apex of a very young sporeling showing the apical cell. × 350.

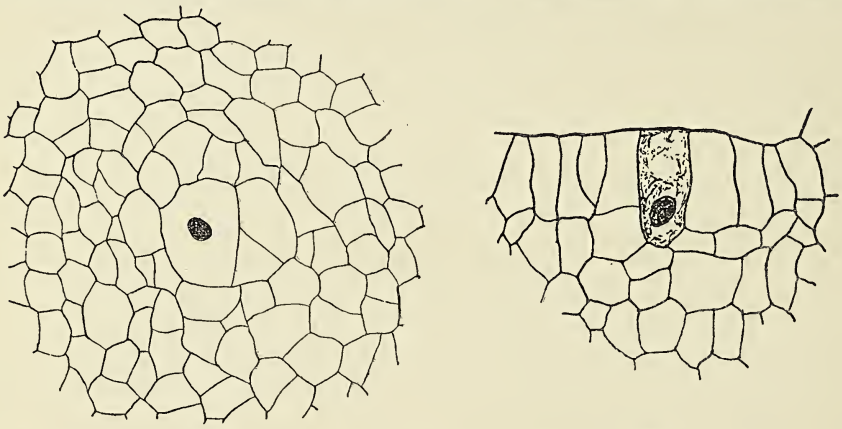
According to Brebner (10), Farmer and Hill (29), and Charles (23), a single initial cell occurs at the apex of the stem of sporelings of *Danaea simplicifolia*, *Angiopteris evecta*, and *Marattia alata* respectively. A similar view is held by Campbell (18, 19, 20, and 21), who also includes in this category the genera *Kaulfussia* and *Macroglossum*.

For our knowledge of the cell-divisions at the apex of the stem of adult sporophytes, we are indebted to the investigations of Bower and of Charles. In an earlier communication, Bower (4, p. 579) stated that his observations on a well-grown plant of *Angiopteris evecta*, var. *pruinosa*, Kuntze, pointed clearly to the existence of a wedge-shaped apical cell, which was represented in Pl. XXXVII, Fig. 9 of that work. This botanist (7, p. 327), however, subsequently arrived at the conclusion that in strongly

grown plants of *Marattia fraxinea* and of *Angiopteris evecta*, the stem-apex is devoid of an apical cell, the meristem being referable, in some cases at least, to a group of four or five initials of exactly similar size and shape, which meet at the intersection of two more or less perpendicular lines.

Charles (23), working on *Marattia alata*, gave a full account of the series of stages of increasing complexity which lead up to the formation of a meristem of several cells at the stem-apex of the older plant.

The present writer's observations on the structure of the stem-apex in a large number of young sporelings of *Danaea alata*, Sm., and of *Danaea nodosa*, Sm., confirm Campbell's (20) account of the apical growth in young plants of this genus, a single very distinct apical cell being found at the apex of the stem of every specimen examined. This apical cell varies consider-

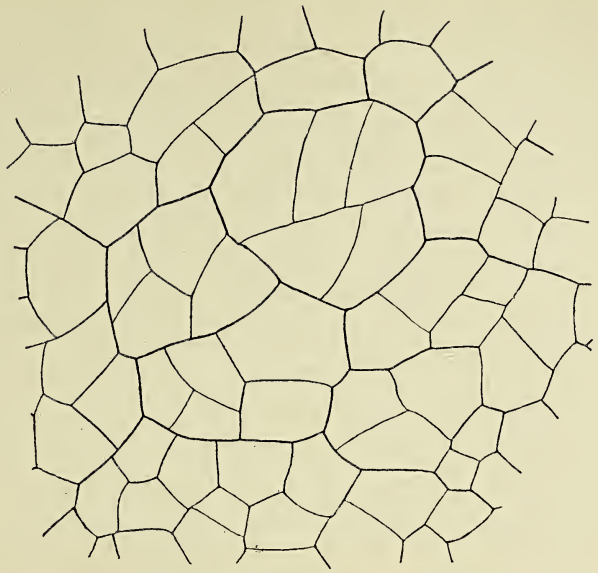


A B

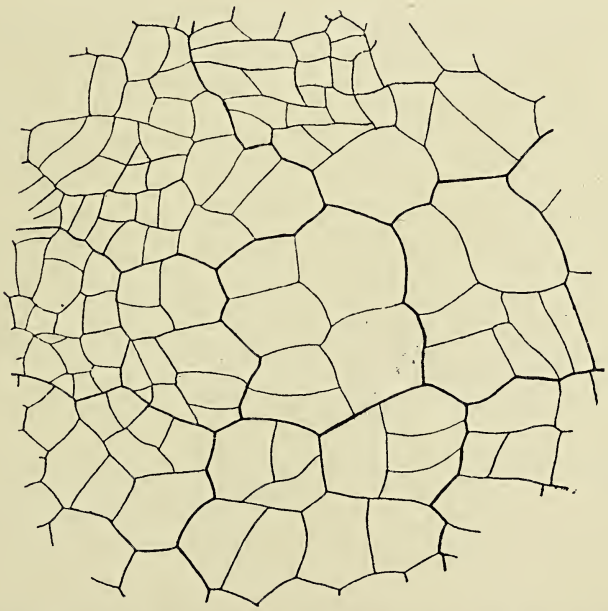
TEXT-FIG. 21. *Danaea nodosa*, Sm. A. Transverse section of stem-apex of a very young sporeling showing the apical cell. B. Longitudinal section of stem-apex of a very young sporeling showing the apical cell. × 350.

ably in shape, but generally has an irregular triangular outline in transverse section (Text-figs. 20, A, and 21, A), whilst in longitudinal sections it appears as an elongated cell which may be either pointed or truncate below (Text-figs. 10, B, 20, B, and 21, B). In slightly more advanced sporelings, the apical cell appears roughly four-sided in transverse section, and is generally more or less truncate below. At a later stage one or more lateral segments of the apical cell do not pass over into permanent tissue, but retain their meristematic condition indefinitely, and thus become the equivalents of, and assume a similar function to, the original apical cell. In other words, each of these cells contributes to the slow growth in length of the stem by dividing periclinally; consequently, at the apex of large well-grown stems, a meristematic region, such as that represented in Text-figs. 22 and 23, is found.

Campbell (20) states that in *Kaulfussia* the apical cell of the young rhizome is roughly triangular in transverse section, and oblong with a broadly truncate base in longitudinal section, but gives no account of the

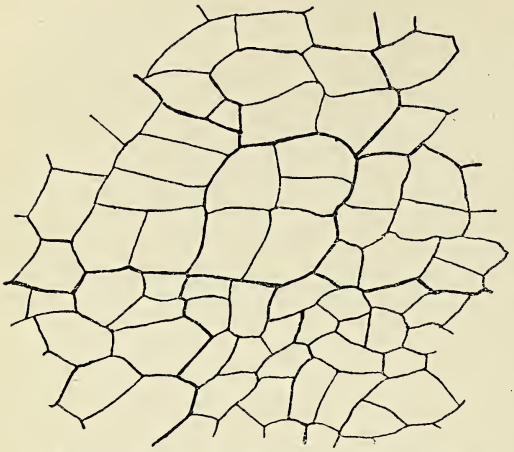


TEXT-FIG. 22. *Danaea alata*, Sm. Transverse section of stem-apex of a large sporophyte showing three meristematic blocks. × 220.



TEXT-FIG. 23. *Danaea nodosa*, Sm. Transverse section of stem-apex of a large plant showing meristematic region. × 220.

structure of the apex of the older rhizome. The present writer has found that a meristematic region, similar in all essentials to that described above for *Danaea*, occurs at the apex of the old rhizome of *Kaulfussia aesculifolia* (Text-fig. 24).



TEXT-FIG. 24. *Kaulfussia aesculifolia*, Bl. Transverse section through the apex of an old rhizome showing initial cells. × 180.

The stem-apex of *Marattia* and of *Angiopteris* was not examined by the writer, since Charles (23) for the former, and Bower (4, 5, 7, 9) for the latter, have already published a full account of the changes which take place at the apex of the stem in these genera, and have shown that whilst the apical growth of the young sporeling may be traced back to the segmentation of a single initial cell, in older plants a group of equivalent initials constitute the apical meristem.[1]

2. *Roots.*

Russow (54, p. 107, Taf. VIII, Fig. 158) expressed the opinion that the apical growth of the roots in the Marattiaceae takes place by means of several (*Marattia* = 7–10; *Angiopteris* = 12–18) relatively large prismatic or pyramidal initial cells, the outermost of which give rise to the cortex and epidermis, whilst the more central form the axile vascular tissue and the tissues of the root-cap.

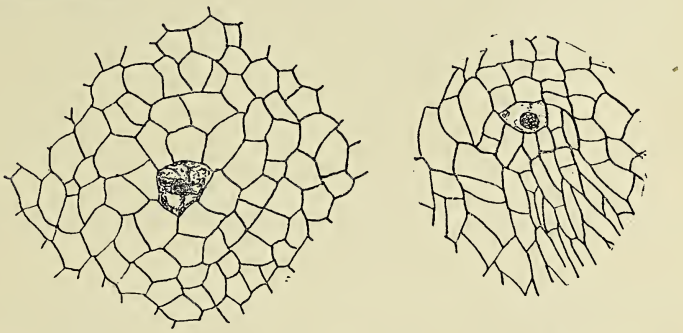
Holle (34, p. 217) gave a brief account of the apical growth of the roots of *Marattia cicutaefolia* and of *Angiopteris evecta*, in which he stated that the number of initials at the root-apex was correlated with the size and strength of the roots, a single four-sided apical cell being found only in the weaker roots. Schwendener (56, Taf. VI, Figs. 3 and 4) described and figured four initial cells at the apex of the roots of *Angiopteris evecta* and of *Marattia Kaulfussii*.

Van Tieghem and Douliot (66) briefly referred to the apical growth of the lateral root in *Angiopteris Durvilleana* and in *Marattia laevis*, and figured (l. c., Pl. XXVI, Fig. 407) a lateral root of *Marattia* with a single large triangular apical cell.

[1] Several initial cells are found at the apex of the stem of other Vascular Cryptogams in which the shoot is relatively bulky; e.g. *Lycopodium* (Russow, 53; Strasburger, 61), *Phylloglossum* (Bower, 6), *Isoëtes* (West and Takeda, 71), and certain species of *Selaginella* (Russow, 53; Bruchmann, 14, 15).

Bower concluded (7, p. 315) that his own observations on roots of *Marattia fraxinea* and of *Angiopteris evecta* bore out Schwendener's conclusions rather than those of Russow. Farmer (28, p. 268) stated with reference to the root of the embryonic plant of *Angiopteris evecta*, that 'the apical cell, which is at no time very clear, is subsequently replaced in most cases by a group of initials', and suggested that some connexion may exist between the robust condition of the root and the structure of its apex.

In a long paper devoted to the subject of apical growth in roots, Koch (41) put forward the view that no *persistent* apical cell is present in roots of *Angiopteris*, the function of the apical cell being temporarily assumed by one of four particularly large cells that are found at the apex of the roots of this genus.



A B

TEXT-FIG. 25. *Danaea alata*, Sm. A and B. Apex of small roots in transverse and longitudinal section respectively, showing the single initial cell. × 200.

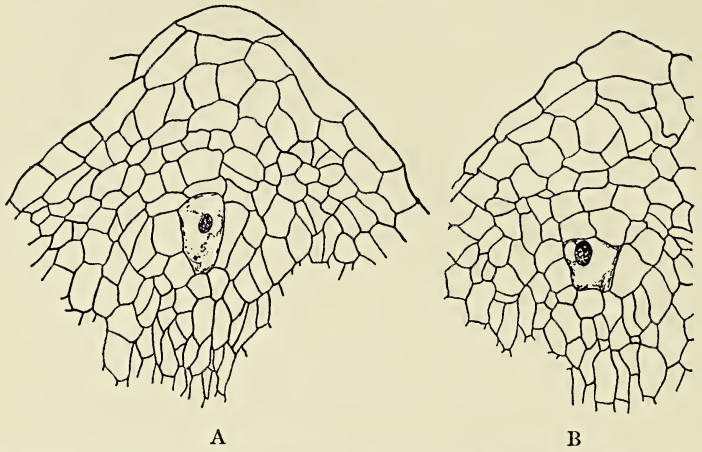
According to Jonkman (39, p. 224), the apical meristem of the primary root of *Marattia* and of *Angiopteris* consists of 'un groupe de quatre cellules environs' (l. c., Pl. VI, Figs. 15 and 16).

Brebner (10, p. 119) concluded that a single initial cell was present at the apex of the primary root of *Danaea simplicifolia*, but that the adventitious roots possessed a group (sometimes four) of equivalent initials.

The most important contribution to this subject is the recent comparative work of Campbell (20 and 21), who finds a single initial cell at the apex of the primary root of *Angiopteris*, *Danaea*, *Kaulfussia*, *Marattia*, and *Macroglossum*; this botanist adds, however, that in the later roots of *Danaea*, *Marattia*, and *Macroglossum*, the single apical cell is replaced by a group of apparently equivalent initial cells which are wedge-shaped in longitudinal section.

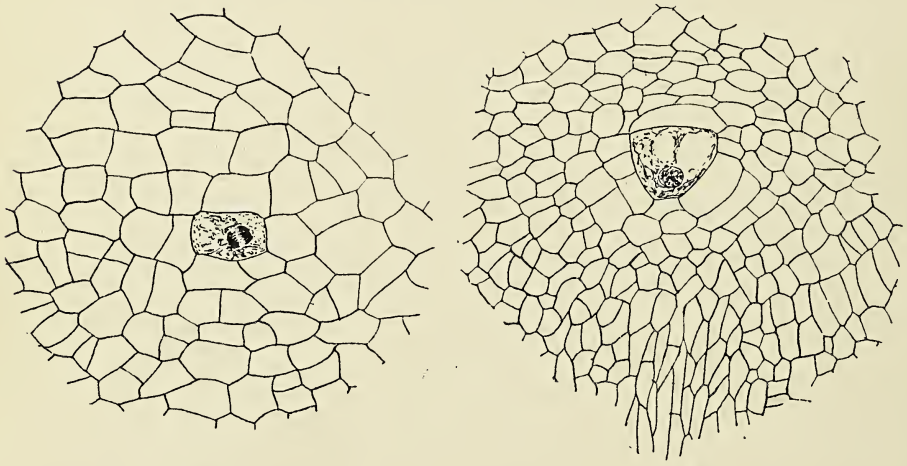
The present writer, working on *Danaea alata*, *Danaea nodosa*, *Angiopteris evecta*, *Kaulfussia aesculifolia*, *and Marattia Cooperi*, has obtained results on the whole very similar to those of Campbell. A single apical cell of moderately large dimensions and of variable shape is found at the apex of the primary and earliest adventitious roots of the above-named genera and

species (Text-figs. 25, A and B, 26, A and B, 27, A and B). At the apex of later roots of moderate size a block of about four equivalent initial cells is found (Text-fig. 28), but the largest and most bulky adventitious roots possess a definite apical meristem (Text-figs. 29, 30, A and B), consisting



A B

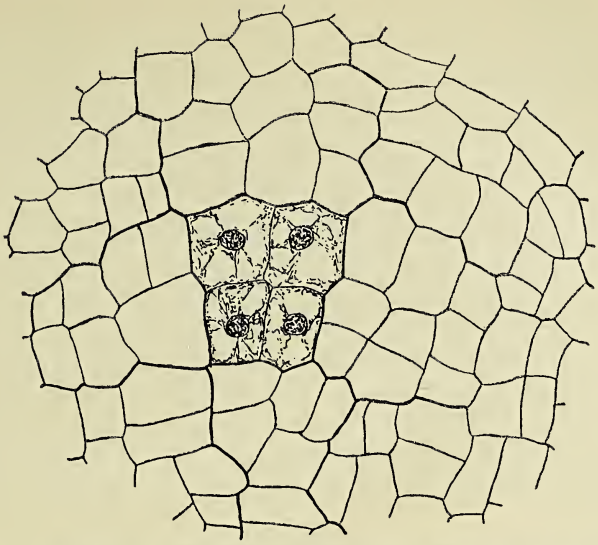
TEXT-FIG. 26. *Danaea nodosa*, Sm. A and B. Longitudinal sections of small roots showing variation in shape of the single initial cell. × 180.



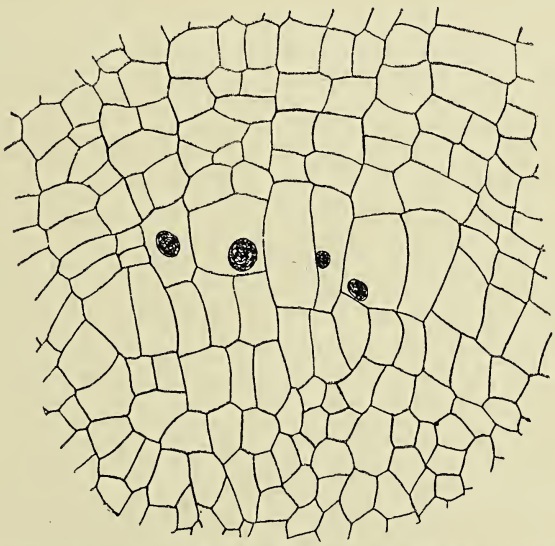
A B

TEXT-FIG. 27. *Marattia Cooperi*, Mre. A. Transverse section of the apex of a small root showing a single initial cell. B. Longitudinal section of the apex of a similar root showing the single initial cell. × 180.

of a number of independent, usually wedge-shaped initials, which in median longitudinal sections of the roots in question appear to be arranged in a fan-shaped manner as described and figured by Campbell (20, p. 177, Fig. 162). Hence it would appear that in the case of the Marattiacean *roots*, the number of initial cells found at the apex depends upon the bulk, and



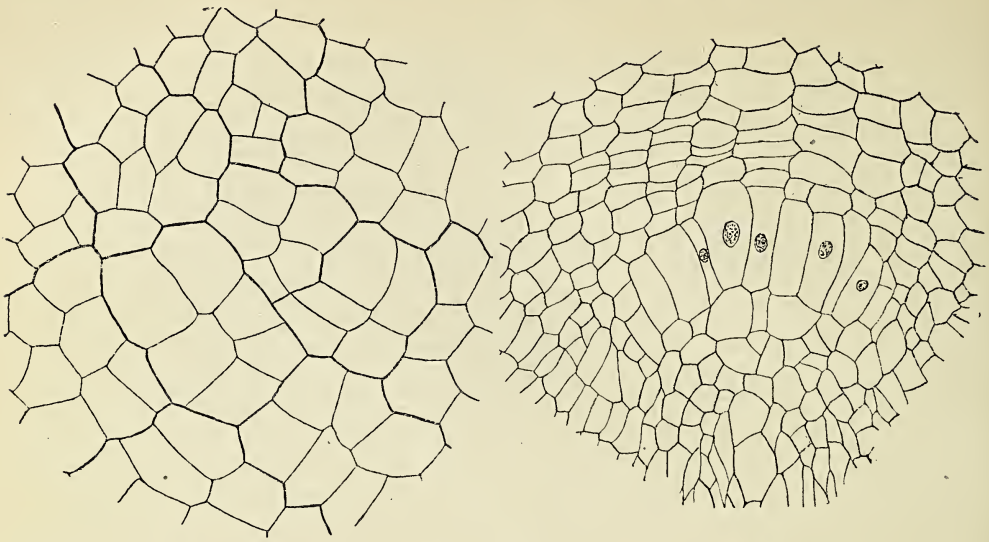
TEXT-FIG. 28. *Danaea nodosa*, Sm. Transverse section of the apex of a large root showing four equivalent initial cells. × 240.



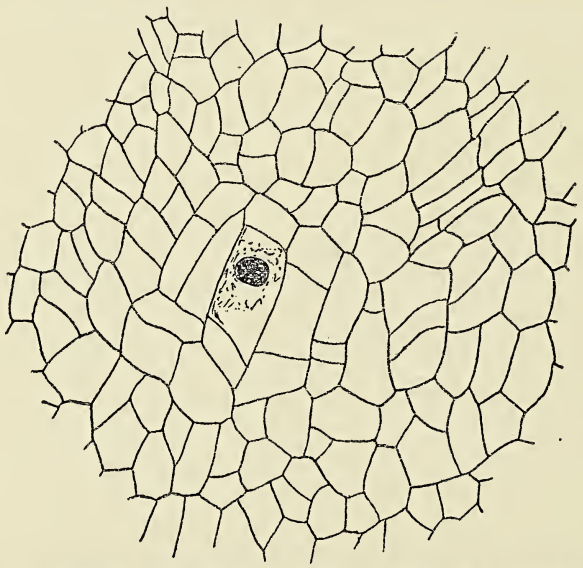
TEXT-FIG. 29. *Danaea alata*, Sm. Longitudinal section of the apex of a large root showing meristematic region. × 220.

not necessarily upon the age of the roots in question; the stronger and more robust the root, the larger the number of equivalent initials which constitute the apical meristem.

The adventitious roots of *Danaea* and of *Kaulfussia* originate from the undifferentiated stelar tissues in the very young region of the stem (also cf.



TEXT-FIG. 30. *Marattia Cooperi*, Mre. A. Transverse section of the apex of a large root showing meristematic region. B. Longitudinal section of the apex of a large root showing meristematic region. × 180.



TEXT-FIG. 31. *Kaulfussia aesculifolia*, Bl. Transverse section of the apical region of the rhizome, showing early origin of a root from the vascular meristem. × 240.

Bower, 5, p. 90 and Pl. IX, Fig. 30; and Farmer and Hill, 29, p. 391, for *Angiopteris*; Charles, 23, p. 96, for *Marattia*). In the first instance there appears to be a single initial cell of elongated prismatic form, from which segments are cut off parallel with its long axis (Text-fig, 31). In the larger

roots certain of these lateral segments assume a function equivalent to that of the mother-cell from which they have arisen (cf. Bower, 5, Pl. IX, Figs. 30, 31, and 32). Segments are also cut off from the distal end of these cells; these apparently go to form the root-cap.

Histological Notes.

The histological details of the structure and development of the vascular tissues in the young sporophyte have already been critically examined by Campbell (20 and 21); recapitulation of the facts by the present writer, whose observations, extending over several hundred serial sections of young sporelings of *Danaea alata*, Sm., and *Danaea nodosa*, Sm., entirely confirm those of Campbell, would therefore be quite superfluous. However, since several points of interest have arisen from the present investigation on the histology of *Danaea*, especially with reference to the older sporophyte, a few notes on the more important details are set forth below.

As in all other known genera of this family, the anomalous position of the protophloem, first pointed out by Shove (58, p. 522, Pl. XXIX, Fig. 28), was very obvious in every species of *Danaea* examined by the present writer.

The protoxylem of the stem-meristeles is generally mesarch, occasionally endarch, whilst in the leaf-trace meristeles it invariably occupies an endarch position.

Even in quite old plants of *Danaea* the cells of the ground-tissue are capable of reassuming meristematic activity; this fact was clearly shown in the case of an adult plant of *Danaea alata* in which the conducting tissues of one of the stem-meristeles had completely decayed away, probably as the result of fungal attack working up from the decayed basal region of the rhizome. The cells immediately surrounding this meristele had become actively meristematic, and had produced around it a continuous layer of periderm-like cells, the walls of which, however, were unsuberized.

1. *Endodermis.*

Whilst it is not proposed to attempt to enter upon a lengthy discussion of the real significance of the endodermis in the Marattiaceae, a few remarks upon the peculiar distribution of this morphological layer in the genus *Danaea* may not be out of place, especially as certain botanists (e.g. Jeffrey) have assigned to it far-reaching importance in questions of stelar anatomy.

For the sake of brevity, the statements which have already been published as to the presence or absence of this layer in the various genera of Marattiaceae are briefly summarized in the following table:

Genus.	*Examined by.*	*Stem-stele.*	*Leaf-trace.*	*Root-stele.*
Danaea	Kühn (43)	+		
	Brebner (11)	+ *	–	+
Kaulfussia	Kühn (42)	–	–	+
	Farmer and Hill (29)	+		
	Campbell (20)	+ *	+	+
Archangiopteris	Gwynne-Vaughan (30)	–	–	+
Marattia	Russow (53)		–	
	Holle (34)	–	–	+
	Thomae (65)		–	
	Kühn (42)	–	–	+
	Farmer and Hill (29)	+		
	Charles (23)	+	+	+
Angiopteris	Sachs (55)	–	–	
	Holle (34)	–	–	+
	de Bary (1)	–	–	+
	Thomae (65)		–	
	Shove (58)	–		+
	Farmer and Hill (29)	+		+

+ denotes presence of an endodermis; – denotes absence of an endodermis; * = not recognizable in older rhizomes.

According to Jeffrey (36, p. 122), the phloeoterma (=endodermis) of the Marattiaceae is characteristically present in the stem of the young plant even when it is absent in the adult. He adds that the primitive medullary strand is generally surrounded by a well-marked phloeoterma.

Leclerc du Sablon (54) remarks, with reference to *Angiopteris*, that 'Le peu de netteté de l'endoderme est le caractère spécial'.

Such wide differences of opinion would suggest that in this group of Ferns the distribution of an endodermal layer is most erratic, and that there is very little constancy in the position of histologically differentiated endodermal cells. The present writer's observations on *Danaea* spp. have shown that this is actually the case. When present, the endodermis is very conspicuous,[1] and can readily be recognized in sections stained with safranin and haematoxylin, especially when they are mounted in euparal. A very distinct endodermis surrounds the stele of the young sporophyte, being easily distinguished in the stem and petiole as well as in the root. But in older plants no general rule can be laid down as to its distribution; for instance, all attempts to demonstrate its presence in the leaf-traces and petiole met with no success; on the other hand, an endodermis is invariably found in roots of all ages.

Occasionally an endodermal layer can be observed completely surrounding a stem-meristele (the central strand, or strands, are included in this category), but more frequently this layer is incomplete, or absent altogether.

No trace of an endodermis could be demonstrated round the stem-meristeles of a number of sections which were carefully examined after treatment with concentrated H_2SO_4.

[1] According to Rumpf (52, p. 24, Taf. II, Fig. 48), the endodermal cells of the Marattiaceae belong to his *primary* type.

2. *Mucilage Canals, Tannin Cells, and Tannin Ducts.*

A comparative account of the structure and development of the various secretory tissues found in the Marattiaceae was published by the present writer (68) in a recent number of this journal, to which the reader is referred. Since the publication of that paper, however, the writer has observed in a moderately large root of *Marattia Cooperi* a mucilage canal traversing the endodermis (Text-fig. 32). When it is remembered that in the roots of this plant the mucilage canals arise very early just behind the growing point, it becomes apparent that, apart from the cells of the ground tissue, others, which would normally become endodermal cells, may be concerned in the formation of the canal. Moreover, stelar tissues in the strict sense also take part in the formation of the canal.

TEXT-FIG. 32. *Marattia Cooperi*, Mre. Transverse section of a root showing a mucilage canal (*m.c.*) traversing the endodermis (*e.*). *c.*, cortex; *r.h.*, root-hair; *xy.*, xylem. × 30.

3. *Secondary Thickening.*

No trace of secondary thickening, such as that described and figured for *Angiopteris* and for *Marattia* by Farmer and Hill (29, p. 388, Pl. XVIII, Figs. 26, 28), was observed in the stem of either species of *Danaea* examined by the present writer; neither was it found in the rhizome of *Kaulfussia aesculifolia*; but regular tangential division of the pericyclic parenchyma was noticed in old roots of *Marattia Cooperi*, Mre. The cells which exhibit this secondary activity appear to be sister cells of the endodermis (cf. Farmer and Hill, 29, p. 388).

4. *Mycorrhiza.*

The Fungi which are normally associated with the roots of this group of Ferns have recently been described by the writer (69 and 70). Further reference to the mycorrhiza of the Marattiaceae is therefore unnecessary.

5. *Cavity Parenchyma* (= *Tyloses*).

Cavity parenchyma was frequently met with in both species of *Danaea* examined. Series of intrusive thin-walled cells of the ground-tissue, similar in appearance to those described by Brebner (12, p. 544, Pl. XXIII, Fig. 22) for *Danaea simplicifolia*, and by McNichol (48, p. 408, Pl. XXV,

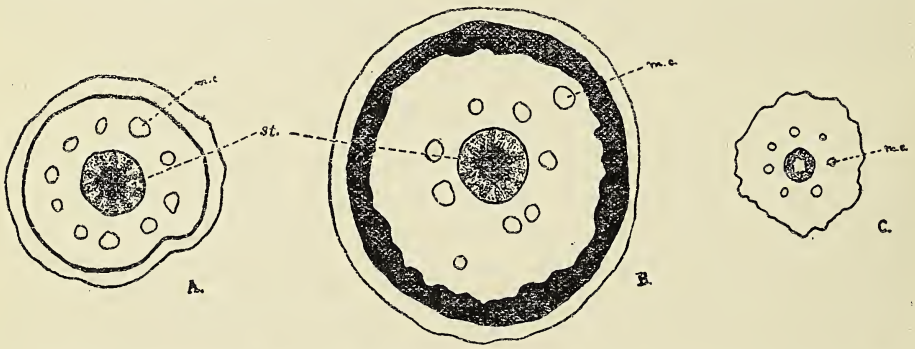
Fig. 13) for *Angiopteris* and *Marattia*, were frequently found completely blocking the lumen of the protoxylem elements of the vascular strands both in the stem and in the petiole.

6. *Leaf Structure.*

The adult leaf of *Danaea nodosa* and of *Danaea alata* has a leathery texture, and exhibits histological characters which are generally associated with a xerophytic habit. In other words, the leaf is provided with a thick cuticle, whilst the ground-tissue of the lamina exhibits differentiation into a palisade layer and spongy mesophyll; large intercellular spaces occur in the latter. Numerous stomata are found on the lower surface of the leaf; as Campbell (20, p. 152) has already pointed out, each fully developed stoma is surrounded by a series of accessory cells, which are more or less spirally arranged.

7. *Root Structure.*

The general anatomical structure of the root, which is identical throughout the family, is already well known and needs no further



TEXT-FIG. 33. Diagrammatic transverse sections of roots of (A) *Danaea alata*, Sm., (B) *Danaea nodosa*, Sm., and (C) *Kaulfussia aesculifolia*, Bl. *m.c.* = mucilage canal; *st.* = stereome. All × 10.

description. However, a comparative study of the distribution of stereome in the roots of the first order in the various genera and species of Marattiaceae is of interest, since it can readily be correlated with the general habit of the plant.

In the principal roots of *Danaea nodosa* and of *Danaea simplicifolia*, two of the radial species, the stereome forms a fairly wide zone near the periphery of the cortex, whilst lignified tissue (excluding the tissues of the xylem) also occurs in an axile position (Text-fig. 33, B). Considered from the point of view of mechanical efficiency, this distribution of the stereome would enable these roots to withstand effectively both crushing and pulling strains. Now, although the basal portion of the massive more or

less upright caudex of these species rests upon the soil (often consisting of loose forest humus), the greater part of its weight is supported by the roots, which apparently function in much the same way as the so-called prop roots of certain mangrove plants, e. g. *Rhizophora*. In this connexion it should be remembered that the *stem* of all known genera of Marattiaceae is characterized by the complete absence of sclerenchyma.[1]

On the other hand, the main roots of *Danaea alata*, which is a much less bulky plant with a horizontal rhizome (Pl. XXI, Fig. 2), differ from those described above in the much feebler development of the peripheral zone of stereome (cf. Text-fig. 33, A, with 33, B). Whereas in the roots of *Danaea alata* this zone is frequently only a single layer of cells in thickness, in the roots of *Danaea nodosa* and of *Danaea simplicifolia* as many as six layers were observed. A corresponding difference was noticed by Campbell (20, p. 179) between the roots of *Danaea jamaicensis* (= dorsiventral species) and of *Danaea elliptica* (= radial species) respectively.

If we except the 'stone'-cells which occur sparingly in the cortex of roots of *Angiopteris*, this peripheral zone of stereome is entirely wanting from the main roots of all the other known genera of Marattiaceae (Text-fig. 33, C), the adult plants of which possess either a strictly dorsiventral rhizome (e. g. *Kaulfussia*) or a massive conical stem (e. g. *Angiopteris*, *Macroglossum*, and *Marattia*). In both cases the entire weight of the plant is borne by the substratum, the stem therefore requiring anchorage only; this need is well supplied by the main roots, which act as stays, their anatomical structure being well fitted for withstanding a pulling strain.

Discussion.

Two main points of theoretical interest emerge from the statements found in the preceding pages. Firstly, the question as to whether the radial or the dorsiventral type of symmetry is primitive in the Marattiaceae, and secondly, the question as to whether the results derived from a comparative study of the morphological, anatomical, and histological characters in this group of Ferns give any clear indication of the probable affinities of this family.

We will now briefly consider the first question. According to Campbell (20), the organization of the embryonic plant (excepting *Kaulfussia*) is from the very beginning of its development strictly radial, the vertical growth of the young sporeling being initiated by the primary segmentation (or basal) wall of the fertilized ovum; this wall in all genera of Marattiaceae is always transverse, with the result that the first leaf, or cotyledon, arising from the half of the embryo which is turned away from the

[1] 'Stone'-cells are sometimes found near the periphery of the cortex of the rhizome of *Danaea alata*.

archegonium, grows straight upwards and eventually pierces the prothallium, emerging on its upper surface (cf. Campbell, 20, p. 138, Fig. 108, A). The root, which in this family is a strictly endogenous structure, as soon as its apex is established, rapidly increases in length, and with the cotyledon, which in the meantime has been growing actively upward, forms almost a straight line, so that the young sporophyte may be described as bipolar (cf. Campbell, 20, p. 145). Thus the evidence derived from a consideration of the Marattiacean type of embryogeny points clearly to a radially organized ancestry for this group of Ferns.

Strong evidence in favour of this view can also be derived from a comparative study of the structure and development of the skeletal framework in this family. In every genus and species, with the exception of *Kaulfussia*, the shoot of the young sporophyte, for a time at least, is radially organized, the earliest leaves forming an irregular spiral around the stem, and in the case of *Marattia*, *Macroglossum*, certain species of *Danaea* (e. g. *D. nodosa*, *D. elliptica*, and *D. simplicifolia*), and probably *Archangiopteris*, this radial organization of the shoot is permanently retained. However, in other species of *Danaea*, as for example *D. alata*, *D. Jenmani*, &c., the apex of the shoot sooner or later bends over and growth proceeds horizontally, the leaves being for the most part confined to the dorsal and lateral surfaces of the rhizome whilst the roots arise from all sides of the stem.

Lastly, we have in *Kaulfussia* a plant in which a dorsiventral structure is indicated at a very early stage, the second leaf arising quite close to the cotyledon and on the same side of the stem-apex (cf. Campbell, 20, p. 156; 19, p. 79). The distichous arrangement of the leaves, thus early initiated, is retained permanently on the slender creeping rhizome of this genus. Campbell (l. c., p. 218) maintains that *Kaulfussia* is probably the most primitive of the living representatives of the Marattiaceae; in the opinion of the present writer, however, this view is untenable, since not only is this genus the most aberrant type externally, but the comparatively simple type of vascular anatomy found in the adult rhizome is to be regarded as a derived or specialized condition correlated with the habit of the plant.

I have omitted *Angiopteris* from the series traced above because there has been considerable divergence of opinion with regard to the symmetry of the axis of this plant. According to the observations of Campbell (20, p. 201), plants of *Angiopteris* growing upon *level* ground are always strictly radial in structure.

Farmer and Hill (29, p. 380) remark that 'both *Marattia* and *Angiopteris* also exhibit a tendency to dorsiventrality, but it is not very marked in young plants', while Shove (58, p. 521) states that the stem of *Angiopteris* examined by her presented definite dorsiventrality. Charles

(23, p. 84) found that the tendency to dorsiventrality shown by older *Angiopteris* stems did not appear in *Marattia*, although the specimens of *Marattia* which she examined were gathered from steep banks (23, p. 83).

An examination of a very considerable number of plants of *Angiopteris evecta* of all ages and sizes was undertaken by the present writer in order to decide this question, with the result that of thirty-six specimens examined twenty were found to possess a strongly dorsiventral stem (cf. Pl. XXII, Fig. 10),[1] six had an obliquely ascending axis, whereas only ten exhibited a strictly radial configuration of the stem. The present writer was therefore led to the opinion that the genus *Angiopteris* does show a marked tendency towards dorsiventrality, especially in older plants. Against the view that the change in direction of growth of the stem depends entirely upon the slope of the ground, it can be urged that several of the large plants of *Danaea nodosa* examined by the author were gathered by Dr. Chandler from a very steep bank, and yet they exhibited a strictly radial symmetry.

The statements of Miss Charles (23, pp. 83–4) with reference to *Marattia alata* also show that the configuration of the shoot is quite independent of the position of the plant. No signs of dorsiventrality were observed in *Marattia*.

In the case of the genera and species with upright radial axes, the skeletal framework is developed more or less uniformly all round, and, if we except the leaf-insertions, it is usually so, also, in the horizontal rhizomes of the adult sporophytes of *Danaea alata* and of *Angiopteris evecta*; however, Shove (58, p. 521) asserts that the specimen of *Angiopteris* which she examined presented definite dorsiventrality, not only in its external morphology, but also in its vascular anatomy, the meshes of the stelar lattice-work on the lower (= ventral) surface being long drawn out and with few anastomoses between the strands.

The present writer agrees with Farmer and Hill (29, p. 380) that the occurrence of large diamond-shaped gaps, similar in many respects to leaf gaps, upon the ventral surface of the skeletal framework of the markedly dorsiventral adult rhizomes of *Danaea alata* and of *Kaulfussia* indicates that the dorsiventrality, which these plants now exhibit, was probably acquired from a radially formed ancestor, the interior anatomical characters corresponding to such a disposition having been to a varying extent retained.

In the following table the degree of dorsiventrality exhibited by young and old plants respectively of all the genera and species of Marattiaceae at present known is summarized in convenient form:

[1] In the specimen of *Angiopteris* figured, which was quite an old plant, the bases of only two weakly developed leaves were found on the ventral surface of the stem; the roots, on the other hand were restricted to the ventral surface.

	Young Sporeling.		*Adult Sporophyte.*	
	Orientation of Shoot.	*Organization of Shoot.*	*Orientation of Shoot.*	*Organization of Shoot.*
Marattia	Vertical	Radial	Radial	Radial
Macroglossum	,,	,,	,,	,,
Archangiopteris	,, (?)	,, (?)	,, (?)	,, (?)
Angiopteris	,,	,,	,, Obliquely ascending, or horizontal	,, Dorsiventral ,,
Danaea simplicifolia	,,	,,	Radial	Radial
,, *nodosa*	,,	,,	,, or Obliquely ascending	,, ,,
,, *alata*	,,	,,	Horizontal	Dorsiventral
Kaulfussia	,,	Dorsiventral	,,	,,

Two main factors can be recognized as having played an important part in the evolution of the wide range in form of the Marattiacean stem, namely (i) the remarkable megaphylly of these plants and (ii) the complete absence of stereome (other than isolated 'stone'-cells) in their stem-tissues. In every genus the adult fronds are relatively large and require a large surface for their attachment, although it should be remembered that in many species comparatively few leaves are unfolded at once, and that these leaves are confined to the apical region of the stem. In this respect the Marattiaceae and Ophioglossaceae show close agreement.

In order to support, and also to supply sufficient surface for the attachment of the large fronds, it would be necessary for an upright stem to become greatly elongated, in which case a development of stereome (such, for example, as we find so well exemplified by the V-shaped bands of sclerenchyma that occur in the stem of certain tree-ferns) would be essential. It is probably for this reason that none of the existing Marattiacean Ferns has adopted the 'tree' habit. The nearest approach to this type of stem is found in *Danaea nodosa* and *Danaea elliptica*, where the comparatively massive shoot assumes either a vertical or an obliquely ascending growth, and largely depends upon the stout adventitious roots for its mechanical support (Pl. XXII, Fig. 8).

While still retaining a strictly radial organization of the shoot, three genera of Marattiaceae (e. g. *Angiopteris*, *Marattia*, and *Macroglossum*) have adopted the squat, massive type of stem, which not only offers an extensive superficial surface for the attachment of the leaves and roots, but also relegates to the substratum the task of bearing the greater part of its weight. In these genera the roots act as stays and assist in keeping the plant anchored to the soil.

The most economical solution of this problem, however, is found in the relatively slender horizontal rhizome; here again the soil to a great extent relieves the stem of the necessity for supporting the weight of the leaves. This type of stem occurs in most species of *Danaea* (e. g. *D. alata*, *D. Jenmani*, &c.), in *Kaulfussia*, and sometimes in *Angiopteris*. With

reference to the last-named genus, it is interesting to note that the majority of the stems which exhibit pronounced dorsiventrality are generally more elongated and less bulky than the radial ones.

The general conclusions which may be drawn from a comparative study of the morphological, anatomical, and histological characters of the Marattiaceae may be briefly summarized as follows:

1. The modern representatives of the Marattiaceae, as exemplified by the six genera *Angiopteris*, *Archangiopteris*, *Danaea*, *Kaulfussia*, *Macroglossum*, and *Marattia*, undoubtedly form a very natural and homogeneous group, as is shown by the following characters which they share in common:

i. Arrangement of the vascular strands in the stem.
ii. Structure of the vascular strands in the stem; e. g. anomalous position of the protophloem; structure and irregular distribution of the endoderms; position of the protoxylem; structure of the xylem and phloem elements.
iii. General morphology of the frond.
iv. Arrangement of the vascular strands in the petiole.
v. Absence of sclerenchyma from the stem-tissues.
vi. Bud-protection devices.
vii. Anatomy of the root.
viii. Presence of an endotrophic mycorrhiza (West, 70).
ix. Apical meristems of stem and root.
x. Presence of lysigenous mucilage canals (West, 68).
xi. Presence of tannin cells or tannin ducts (West, 68).

These genera also show a striking similarity with regard to their

xii. Spore-producing members, e. g. the radiate uniseriate type of sorus (cf. Bower, 8).
xiii. Embryogeny (cf. Campbell, 17, 18, 19, 20, 21).
xiv. Gametophytic structures, e. g. prothallia, sexual organs (cf. Campbell, 17, 19, 20, 21).

2. The Marattiacean Ferns occupy an isolated position among modern Vascular Cryptogams.

Considered from the point of view of their morphological, anatomical, and histological characters, as summarized above, the Marattiacean Ferns are very sharply marked off from all other modern groups of Vascular Cryptogams, with the possible exception of the Ophioglossaceae. But whilst admitting with Campbell (20, p. 218) the possibility of a common origin for the Marattiaceae and Ophioglossaceae from the same primitive stock, the present writer is of the opinion that these two families have proceeded along widely divergent lines from this common plexus, with the result that by comparing *individual* surviving genera from these two families

it is impossible to demonstrate any satisfactory evidence of phyletic relationship.

The geographical distribution of the living genera of Marattiaceae suggests that these plants represent the remnants of a much larger group, which, according to the available evidence derived from what is provisionally believed to be their palaeontological record, dates back to the lower coal measures.

A considerable number of fern-like fructifications, e.g. *Ptychocarpus* (Renault, 50), *Cyathotrachus* (Watson, 67), *Scolecopteris* (Strasburger, 63), *Danaeites*, &c., bearing a striking superficial resemblance to the sporangia or synangia of the modern genera of Marattiaceae, have been described from palaeozoic strata. Among these fossil fructifications are found types which closely resemble the spore-producing members of each of the surviving genera; this fact in itself would indicate that the modern genera of Marattiaceae are of equal antiquity, and suggests their possible multiple derivation from some primitive stock or plexus. As Bower (8, p. 73) pertinently remarks, perhaps no feature in the Marattiaceae is more remarkable than the persistence of type from the remote past to the present day.

Unfortunately, however, we cannot at present fix upon any links from strata of more recent geological eras, and, moreover, it is quite possible that all, or most, of these fern-like fossil fructifications which have been referred to the Marattiaceae are in reality the male fructifications of Pteridosperms; this evidence should therefore be accepted with reservation.

The doubts cast upon the hitherto generally accepted view of the Marattiacean affinities of the fossil genus *Psaronius* by Farmer and Hill (39, pp. 382–3) have been materially strengthened by the recent observations of Solms-Laubach (60) on the anatomy of this genus.

Summary.

1. A comparative account of the structure and development of the stelar system in the Marattiaceae, with special reference to the adult sporophyte of *Danaea*, is given, and the question of the symmetry of the sporophyte in this group of Ferns is discussed. A primitive radially symmetrical type of shoot is distinctly suggested.

2. The single apical cell found in the apex of the stem of the young sporeling is later replaced by a group of equivalent initial cells or by a meristematic region.

3. A single large apical cell occurs at the apex of the primary and earliest adventitious roots. At the apex of the later adventitious roots of moderate size a group of about four equivalent initial cells is found, while the more robust roots generally possess a definite meristem consisting of a number of independent initial cells. In brief, the number of initial

cells found at the apex of the Marattiacean roots is clearly related to the bulk, and not necessarily to the age of these roots.

4. The six genera which comprise the Marattiaceae show remarkable uniformity in their morphological, anatomical, and histological characters, and constitute a very homogeneous and natural family, which probably occupies an isolated position amongst modern Vascular Cryptogams.

In conclusion, the author wishes to express his sincere thanks to Professor J. B. Farmer, F.R.S., not only for suggesting that this work should be undertaken, but also for his constant help and valuable advice throughout the course of this investigation; to Dr. S. E. Chandler, F.L.S., who supplied him with much valuable material of *Danaea*; and to Mr. C. H. Wright, A.L.S., for help in the identification of material.

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EXPLANATION OF FIGURES ON PLATES XXI AND XXII.

Illustrating Mr. West's paper on *Marattiaceae*.

ABBREVIATIONS.

c.s., commissural strand; *c.s.'*, central stelar system; *c.s.''*, commissural strand of the central stelar system; *comp.s.*, compensating strand; *l.t.*, leaf-trace; *p.*, prothallium; *p.r.t.*, trace of the primary root; *r.t.*, root-trace; *st.*, stipule; *v.g.*, ventral gap.

PLATE XXI.

Fig. 1 A. *Danaea alata*, Sm. Model of the stelar system of the rhizome of a moderately large specimen, *viewed from below*. In this model the transition from the simpler vascular structure of the young sporophyte to the more complicated vascular structure of the adult sporophyte is clearly shown. The leaf- and root-traces are consecutively numbered in order of priority. An unusual feature of this plant was the absence of adventitious roots from the basal region of the rhizome; it was probably due to this abnormality that the older portion of the rhizome had so far successfully resisted decay; there is normally one root to each leaf of the young sporophyte. (N.B. The natural proportion between the length and breadth is here exaggerated in the ratio 1 : 2.)

Fig. 1 B. Tranverse section of the stele in the plane α, β, of the same model, showing almost perfect solenostelic structure.

Fig. 2. *Danaea alata*, Sm. Rhizome of an adult plant, showing the well-marked dorsiventrality. Nat. size.

Fig. 3. *Kaulfussia æsculifolia*, Bl. Model of the stelar system of a portion of the rhizome of an old plant, *viewed from below* (cf. Text-fig. 15).

Fig. 4. Sporeling of *Danaea alata*, Sm. Nat. size.

Figs. 5 and 6. Bud-protection in *Danaea alata*, Sm. The stipules (*st.*) of the young leaf completely envelop the next younger leaf. Slightly enlarged.

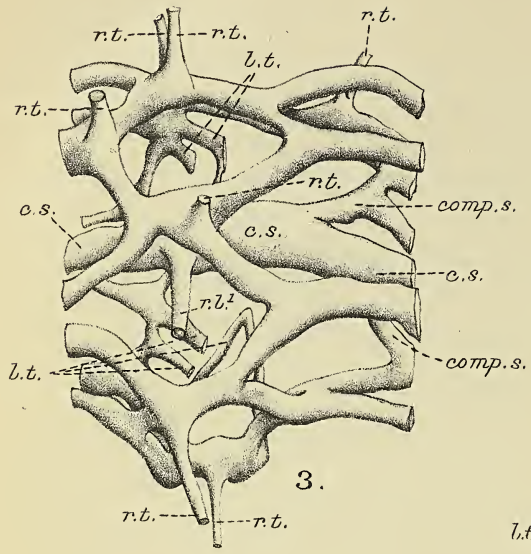
PLATE XXII.

Fig. 7. *Danaea alata*, Sm. Front view of the model represented in Fig. 1 A.

Fig. 8. *Danaea nodosa*, Sm. Large sporophyte, showing habit. The leaves had been artificially removed. × $\frac{1}{5}$.

Fig. 9. A and B. Sporelings of *Danaea alata*, Sm., showing the inception of the dorsiventral habit. × $\frac{1}{2}$.

Fig. 10. *Angiopteris evecta*, Hoffm. Rhizome of a moderately large plant, showing well-marked dorsiventrality. × $\frac{1}{4}$.



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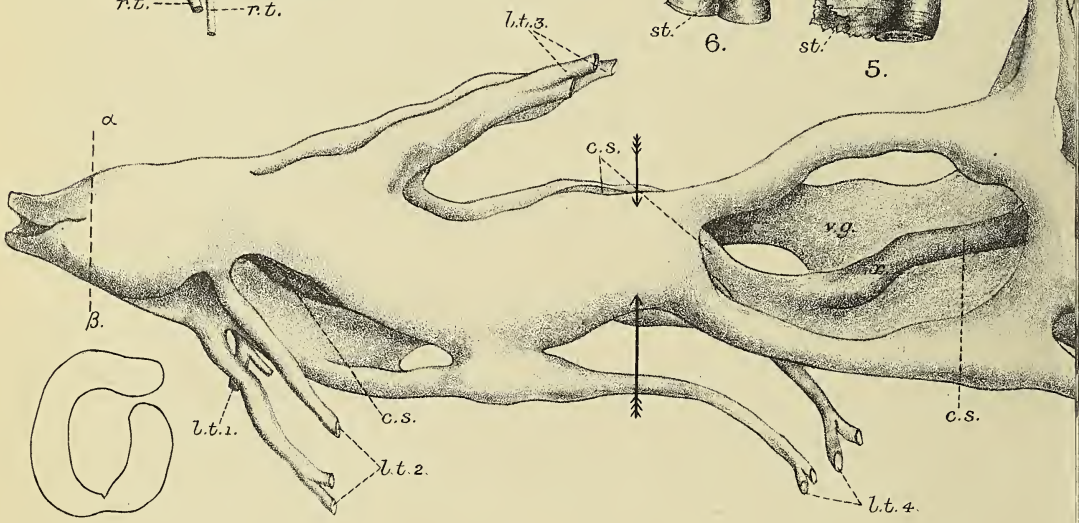

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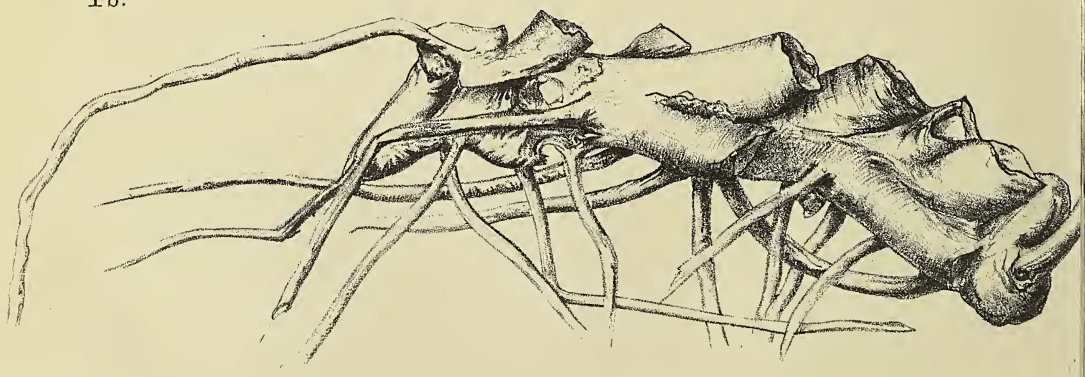

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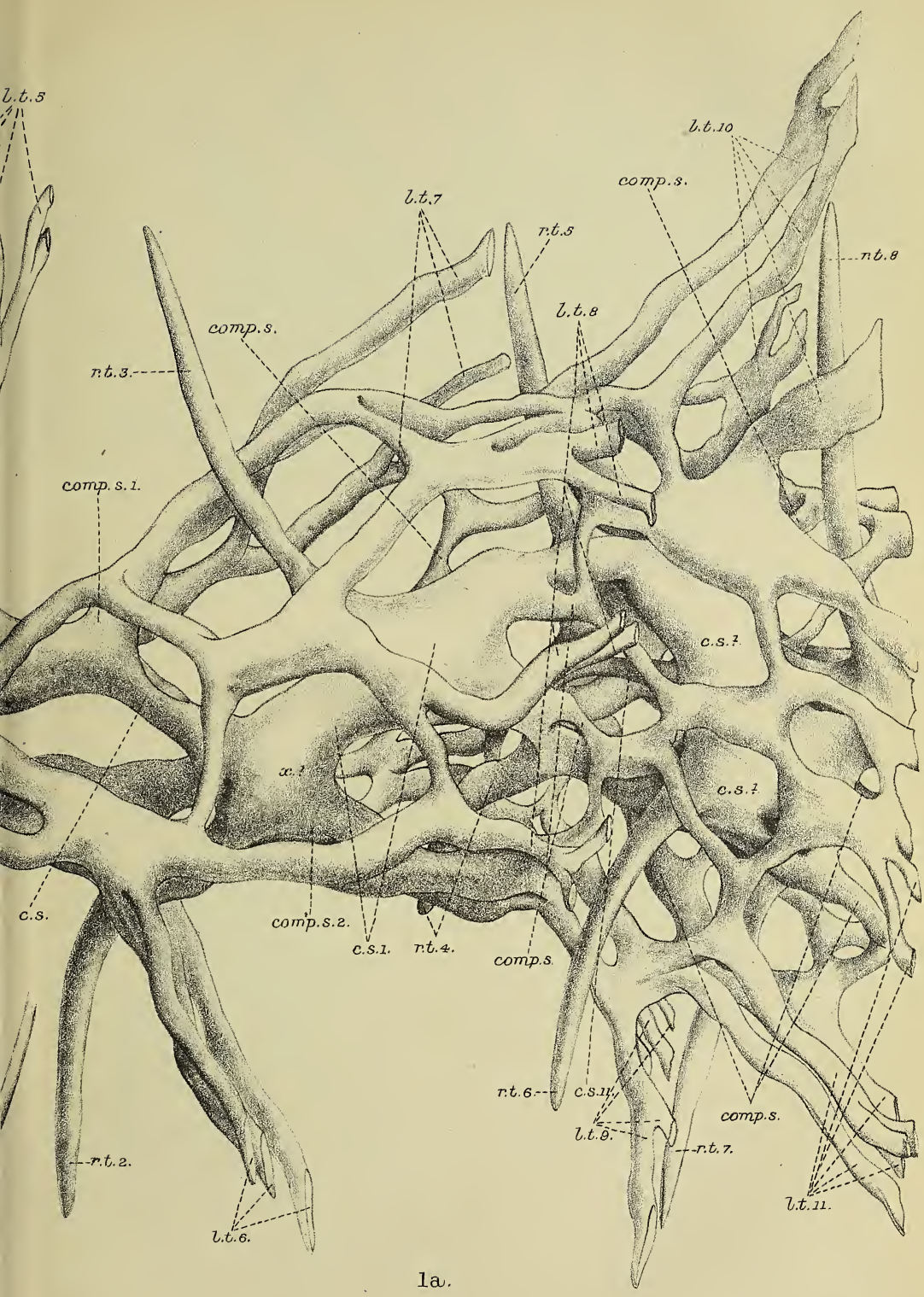

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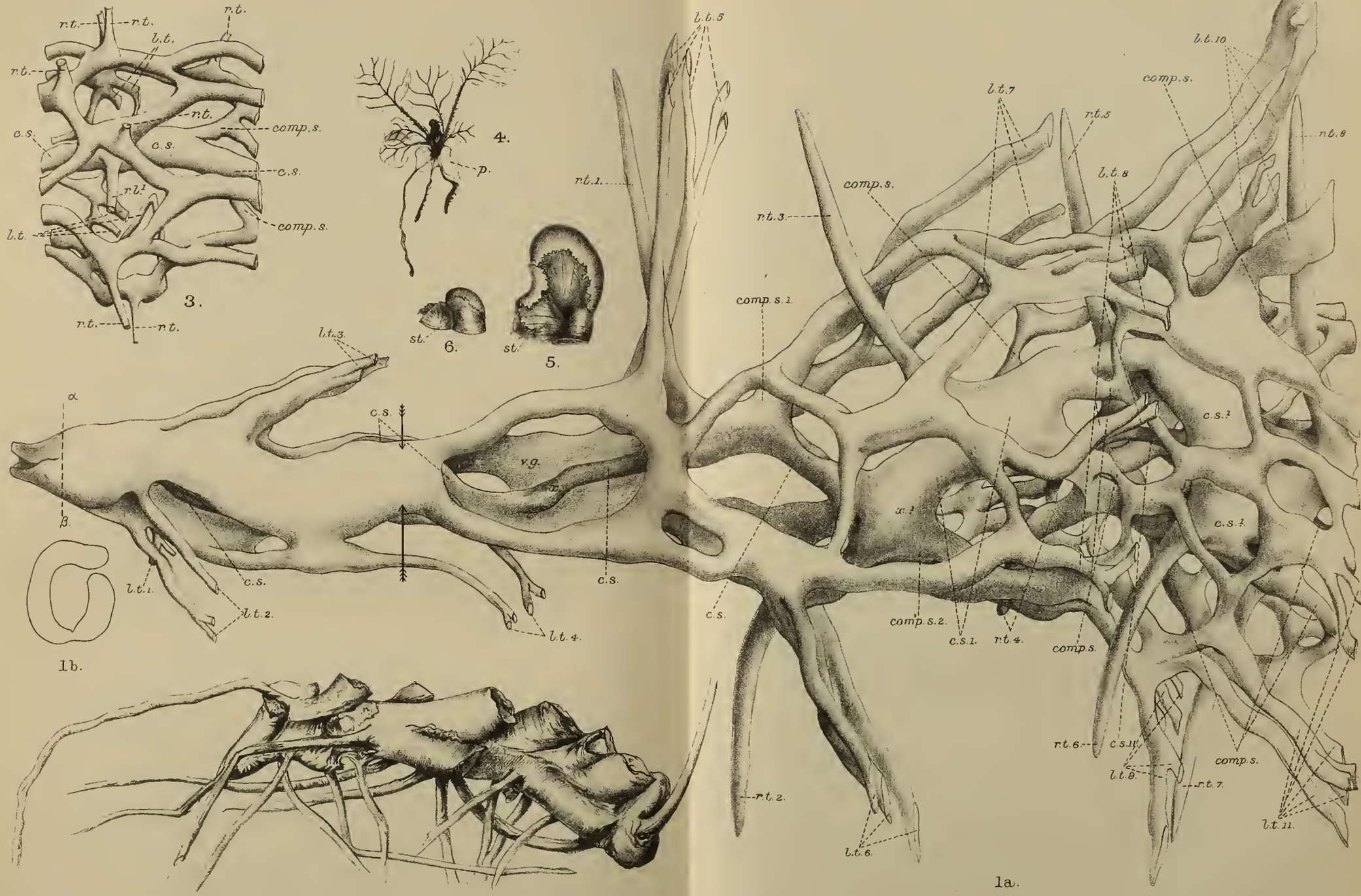

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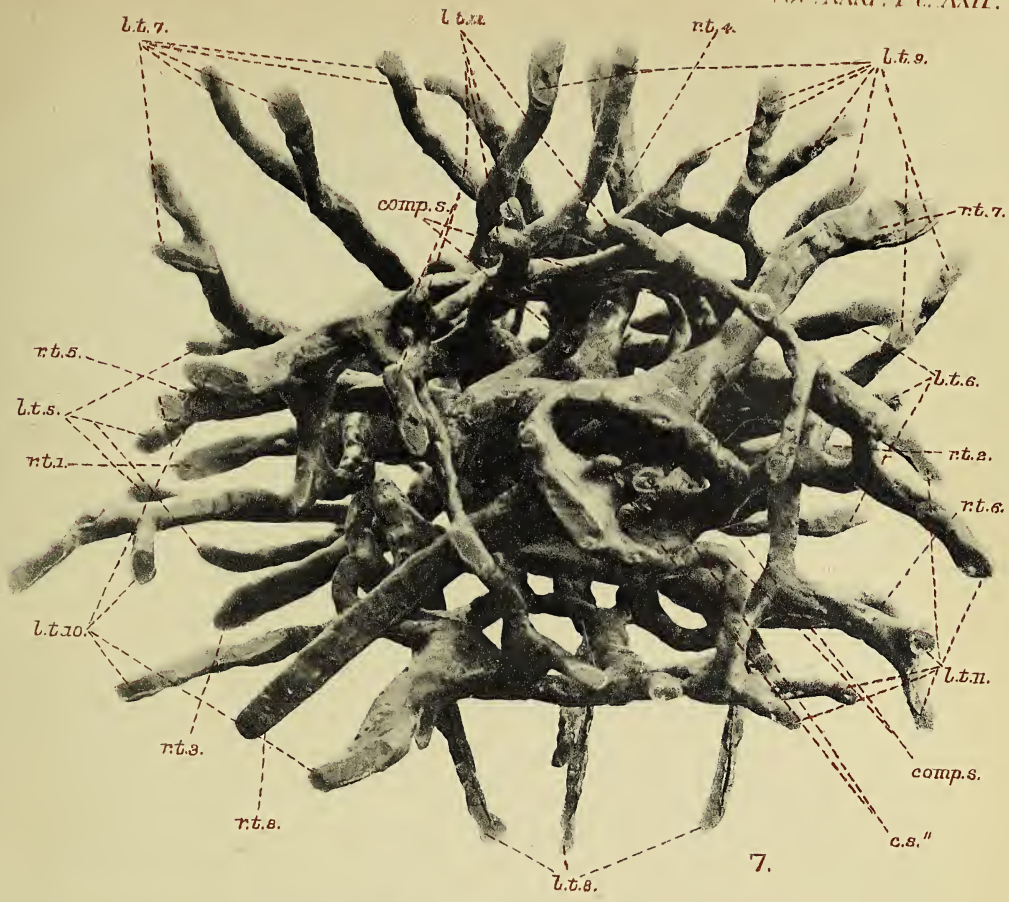
WEST — MARATTIACEAE.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Studies in Permeability.

V. The Swelling of Plant Tissue in Water and its Relation to Temperature and various Dissolved Substances.[1]

BY

WALTER STILES

AND

INGVAR JØRGENSEN.

With ten Tables and nine Figures in the Text.

CONTENTS.

INTRODUCTION.

THE passage of substances between plants and their surroundings, and between cell and cell, is a subject of fundamental importance. Thus among the problems to which such phenomena have application may be mentioned those of carbon assimilation, translocation, transpiration, and root absorption, with their bearing on agriculture; the extraction of substances from living or dead plant tissue; and even such problems as those involved in the preservation of vegetables and fruits. Nevertheless, in spite of the wide importance of the subject, the known facts are few and often not very definite, so that the information so far available does not enable us to attempt an analysis of the processes involved. For although the single term 'permeability' is used in relation to these phenomena, it is to be expected that the processes are numerous and not all of the same nature.

Obviously a detailed analysis of the processes of permeability is

[1] The terms swelling and shrinkage are used in a general sense and refer to the total gain or loss of water by the cell under various conditions. Pfeffer used the term swelling in a restricted sense to indicate the water imbibed by the protoplasm, as distinct from that absorbed by osmosis.

[Annals of Botany, Vol. XXXI. Nos. CXXIII and CXXIV. July and October, 1917.]

required, similar to that which has been attempted in the case of carbon assimilation, in which, even if our knowledge is still very imperfect, we have a considerable amount of information regarding the complexity of the processes involved and the factors influencing them. Without such an analysis the putting forward of theories is clearly of little help to the advancement of the subject, and undoubtedly, as with carbon assimilation, the development of the subject has been hampered by undue speculation.

As to the water relation in permeability, after the extensive pioneer investigations of de Vries and Pfeffer, little work of any value was done, and no advance in the subject was brought about until the recent introduction by A. J. Brown (3, 4, 5) and by F. F. Blackman (6) of fresh methods of attack in which changes are measured quantitatively and are followed from time to time, thus rendering possible an investigation of the kinetics of the changes taking place.

Method of Experiment.

The observations on the passage of water between the cell and surrounding medium recorded in this paper have been made on potato and carrot. The changes with time have been followed quantitatively by weighing the tissue experimented upon at intervals, as was done in the experiments of A. J. Brown (3, 4, 5) on the intake of water by seeds of barley.

The method of experimentation is as follows: Cylinders of tissue having a diameter of 1·8 cm. are cut from the potato or carrot by means of a cork-borer. These cylinders are then cut up by means of a hand microtome into a number of discs having a definite thickness. In all the experiments dealt with here the thickness was 2 mm. After they are cut, the discs are rapidly dried between blotting-paper and then weighed out into lots of 10 or 20 grm. It is not difficult to obtain such lots which contain the same number of discs and differ in weight by not more than 2 per cent. The weight of each such set is taken to the nearest centigramme; more accurate weighing is not justified on account of errors arising from other causes, namely drying of the discs and variability of the samples. Each lot of discs is transferred to a bottle or flask containing a comparatively large volume of liquid (100 c.c.–700 c.c.) which is kept at constant temperature in a thermostat. At intervals the discs are removed from the liquid, their surfaces are dried as rapidly as possible with blotting-paper, and they are weighed. They are immediately replaced in a fresh quantity of the experimental liquid.

Alterations in the weight of the discs are almost entirely due to the passage of water into or out of the tissue. The weight of the small quantity of dissolved substances which may pass into or out of the cells is negligible compared with the weight of water which passes across the tissue boundaries.

From the data so obtained curves are constructed, showing the relation between time and the absorption by, or exosmosis from, the tissues under different conditions.

The Variability of the Tissue used and the Probable Error of Experiment.

Although it might be expected that potato and carrot would yield very uniform tissue, so that individual variations would be largely eliminated in experiments with 20 grm. of tissue (40 discs), yet actual measurements show this is far from being the case. The variability of such tissue in regard to water absorption and excretion is indeed surprisingly large. Probably the previous history of the tissue is in a great measure responsible for this variability, although inherent differences may possibly play a part. In the experiments with potato the same variety was used throughout so that inherited differences should be reduced to a minimum. It is a matter of common knowledge that organs such as potato and carrot gradually lose water during storage, and evidently different degrees of desiccation of different samples will give rise to notable differences in regard to absorption or exudation of water.

In Table I are shown the amounts of water absorbed by nine separate lots of potato discs cut at the same time and subjected to the same experimental conditions. In these experiments, as they were cut, the discs were dried between blotting-paper and weighed out into lots of 20 grm. It will be observed how wide are the variations between individual samples, and that these are indeed due to differences in the tissue itself is indicated by the fact that the differences are maintained throughout the experiment.

Table I.

Water absorbed by 20 grm. of Potato from Distilled Water.

Sample.	*Gain in Weight of 20 grm. of Potato after various times.*				
	0·8 *hr.*	3·8 *hrs.*	18·15 *hrs.*	47·3 *hrs.*	70·8 *hrs.*
	grm.	grm.	grm.	grm.	grm.
1	0·52	1·08	2·20	2·77	3·04
2	0·53	1·37	2·49	3·05	3·22
3	0·59	1·43	2·63	3·31	3·60
4	0·36	1·08	2·31	2·60	3·40
5	0·70	1·49	2·93	3·66	4·12
6	0·61	1·63	2·63	3·28	3·60
7	0·56	1·36	2·33	2·70	3·24
8	0·50	1·39	2·33	3·00	3·67
9	0·57	1·53	2·47	3·17	3·57
Mean	0·55	1·37	2·48	3·05	3·50
P_M	±0·02	±0·04	±0·07	±0·075	±0·07

It is possible to form an idea of the degree of accuracy of the mean values by calculating their probable error. From Table I it will be observed

that the probable errors (P_M) of the mean results there recorded lie between 2 and 4 per cent.

With carrot the variability of the tissue is somewhat greater, as the following table shows:

TABLE II.

Water absorbed by 20 grm. of Carrot from Running Tap-water.

Sample.	*Increase in Weight of 20 grm. of Carrot after various times.*			
	21·03 *hrs.*	44·4 *hrs.*	68·8 *hrs.*	123·5 *hrs.*
	grm.	grm.	grm.	grm.
1	2·53	2·41	2·60	2·48
2	2·52	2·20	2·54	2·57
3	2·24	2·37	2·74	2·69
4	2·14	2·26	2·56	2·46
5	3·09	2·83	3·00	3·00
6	3·21	3·36	3·66	3·66
7	2·79	3·00	3·57	3·61
8	3·10	3·34	3·25	3·22
9	2·66	3·00	2·74	2·87
Mean	2·70	2·75	2·96	2·95
P_M	±0·08	± 0·10	±0·07	±0·10

It is thus possible when working with nine samples to obtain results in which the probable error of the mean result of the nine samples is about 3 per cent. Thus differences of about 9 per cent. have a reasonably certain significance in relation to different experimental conditions. This is not a particularly high order of accuracy having regard to the labour entailed when sets of nine different samples are used. We therefore adopted the following method in order to reduce the sampling error:

All the discs required for a particular experiment in which the results were to be compared were cut, dried with blotting-paper, and then thoroughly mixed before they were weighed out into lots of 10 or 20 grm. This preliminary mixing has the result of reducing the sampling error considerably, as the following table shows. Thus when carrot is used with only four different samples the probable error of the mean does not generally rise much above 2 per cent. With potato it is possible to work with sets of three samples and obtain this same degree of accuracy.

TABLE III.

Water absorbed by 20 grm. of Carrot from Distilled Water.

Sample.	*Increase in Weight of 20 grm. of Carrot after various times.*					
	0·5 *hr.*	1·0 *hr.*	2·0 *hrs.*	5·0 *hrs.*	22·5 *hrs.*	49·5 *hrs.*
	grm.	grm.	grm.	grm.	grm.	grm.
1	1·43	1·95	2·75	3·17	3·48	3·65
2	1·50	2·11	3·10	3·45	3·87	4·00
3	1·47	2·02	2·85	3·10	3·40	3·75
4	1·47	1·94	2·74	2·92	3·19	3·62
Mean	1·47	2·00	2·86	3·16	3·47	3·75
P_M	±0·01	±0·03	±0·06	±0·07	±0·10	±0·06

In the majority of cases the differences recorded are many times larger than any that could be accounted for by sampling error, and they may therefore be regarded as having a real significance.

By this method it is only those experiments conducted at the same time with material from the same general sample of mixed discs that can be directly compared, as otherwise differences due to previous history and inherent causes may become evident. An example of this we shall have an occasion to quote in the section dealing with the influence of temperature.

We may take this opportunity of calling attention to the necessity of determining the error of experiment in plant physiological work. Probably little plant tissue is so uniform as potato and carrot, and it is shown here how necessary it is, even with this tissue, to determine the probable error of experiment before drawing conclusions from observed differences in behaviour in relation to different conditions. In very few plant physiological researches has this very necessary calculation been made, with the result that conclusions are constantly drawn for which there is really no experimental evidence.

Experimental Results.

(*a*) *The Swelling of Potato and Carrot in Water.*

When living tissue of potato or carrot is immersed in distilled water there is a passage of water into the cell. The rate of this process, rapid at first, gradually slows down until a condition of equilibrium is reached.

The curves marked P_D and C_D in Fig. 1 illustrate the general course of this swelling in distilled water at 13° C. It will be observed that, in the case of carrot, 90 per cent. of the total swelling takes place during the first two days. The maximum swelling is reached after about six days; little alteration takes place during the next six days, at the expiration of which time the experiments were discontinued.

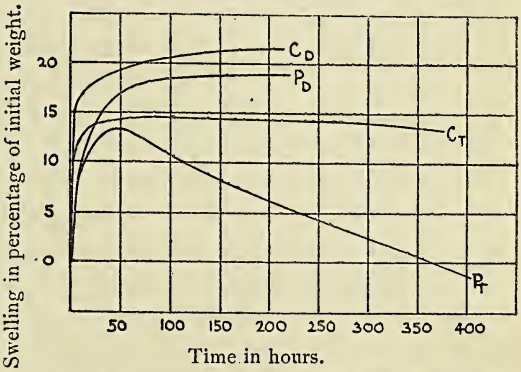

Fig. 1. Swelling of potato and carrot in distilled and in tap water. C_D, carrot in distilled water; C_T, carrot in tap-water; P_D, potato in distilled water; P_T, potato in tap-water.

The course of swelling with the potato is similar, but the initial rate of swelling is less rapid (see Fig. 2). This fact we have noticed throughout our experiments. Equilibrium is, however, reached in about the same time as with carrot, and is maintained for a considerable time just as with that tissue.

The behaviour of the two tissues with tap-water is different. With carrot there is the same rapid initial rate of swelling, but the maximum value of this is less and is reached somewhat sooner, after which there is a slight, though distinct, gradual shrinkage of the tissue for the next eleven days. Having regard to the course of events observed with potato to be immediately described, there is little doubt that this shrinkage would have continued until death of the tissue finally resulted.

The line in Fig. 1 marked P_T illustrates the course of swelling of potato in running tap-water. The tissue swells at first in the same way as in distilled water. As with carrot a lower maximum swelling is reached than with distilled water, and in this case after about 2·5 days, and then immediately a shrinkage of the tissues sets in, which was continued very uniformly until the end of the experiment. Thus, after 17 days in running tap-water, the potato tissue had shrunk to its original weight.

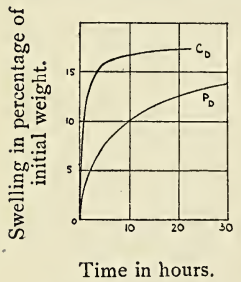

FIG. 2. Swelling of potato and carrot in distilled water. C_D, carrot in distilled water; P_D, potato in distilled water.

De Vries and Pfeffer explained the plasmolysis of plant tissue in more concentrated solutions of various substances on the theory that the limiting layer of the protoplasm of the cell acts as a semi-permeable membrane, allowing the passage of water, but not permitting the passage of the particular solutes. On this view such a swelling as we have recorded for potato and carrot would have to take place, if it is assumed that the various crystalloidal substances of the cell cannot pass through the membrane, or can pass only with great difficulty and slowness. If the cell wall were absolutely elastic the intake of water should continue indefinitely, as equilibrium could only be reached when there was the same osmotic concentration inside and out. On the other hand, if the cell wall were perfectly inelastic and quite incapable of stretching, water would pass in as before on account of the difference in osmotic concentration on the two sides of the cell boundary. This will result in an increase in pressure inside the cell which tends to force water out of the cell. Under these conditions water will pass in by osmosis until the pressure forcing water into the cell by osmosis is equal to the pressure forcing water out of the cell owing to its compression inside. In this condition the cell will be in equilibrium with the external medium in regard to the exchange of water.

In reality the condition of affairs is intermediate between these two extremes. Although the rigidity of the cell wall prevents indefinite increase in volume of the protoplast, yet the wall is capable of a certain amount of stretching so that a certain increase in volume takes place. Hence the actual course of events may be considered as follows: Water passes into the cell in consequence of the difference in osmotic pressure on

the two sides of the plasma membrane. This produces an increase in pressure inside the cell and in consequence the cell wall stretches. As the water passes in, the pressure inside the cell increases, and continued stretching of the cell wall takes place until ultimately the pressure of the water inside is equal to the osmotic pressure forcing water into the cell.

That such high turgor pressures can exist in the cell as are indicated by these considerations has been generally admitted by physiologists, and they are indicated also by the very turgid condition of discs of tissues so swollen in distilled water.

We have considered the swelling of plant tissue in water in terms of the original theory of de Vries and Pfeffer. However, we may perhaps indicate that we use this theory only as a working hypothesis which explains the facts; we do not wish to urge that it is the only hypothesis which will explain the facts or that it is either correct or complete.

The depression of swelling observed when tap-water is used instead of distilled water may be explained on this hypothesis by the supposition that certain salts in the tap-water are absorbed and bring about an increase in permeability of the cell. The subsequent diffusion out of dissolved substances from the cell as a result of this lowers the osmotic pressure of the cell, and so water passes out into the external liquid.

(*b*) *The Influence of Temperature on the Swelling of Potato and Carrot in Water.*

Our earlier experiments on the influence of temperature on the swelling of plant tissue were made on a number of different days. The results obtained showed no correlation between the swelling and the temperature, as the curves exhibited in Fig. 3 indicate.

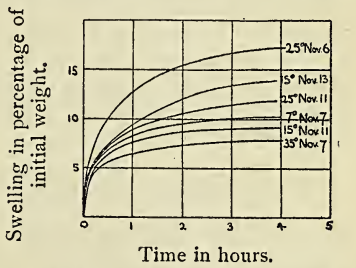

FIG. 3. Swelling of different samples of carrot at different temperatures.

Such a result can only be due to variations in the samples used in the experiments conducted on different days. The sampling error is indeed reduced as regards the comparison of results obtained from experiments started at the same time, by the thorough mixing of the discs as already described; but the error is not eliminated as regards experiments done at different times, and necessarily on different samples of potatoes or carrots.

In order therefore to avoid this difficulty the measurements of swelling at different temperatures were made at the same time on discs all taken from the same mixed sample. The swelling at four temperatures was measured, the vessels containing the discs being kept during the experi-

ment in four different thermostats. The swelling of potato at 10° C., 20° C., 30° C., and 40° C. measured in this way is shown in Table IV.

TABLE IV.

Swelling of Potato in Distilled Water at Different Temperatures.

10° C.		20° C.		30° C.		40° C.	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
		0·65	5·6	0·53	9·1	0·60	19·6
1·23	4·4	1·27	8·7	1·28	12·6	1·30	14·4
1·95	5·8	2·00	10·2	2·00	15·1	2·02	10·3
2·85	7·2	2·87	11·8	2·87	17·7	2·87	5·8
4·98	8·9	5·03	14·5	5·05	20·6	5·08	−5·8
6·57	10·3	6 65	16·2	6·67	22·5	6·08	−8·2
18·80	19·8	19·23	20·6			19·68	−14·6

These results are shown graphically in Fig. 4. They clearly illustrate the fact that the temperature exerts a marked influence on the rate of water absorption by potato tissue—the higher the temperature the more rapid the swelling. With higher temperatures, however, secondary actions occur which transform the swelling into a rapid shrinkage. Measurements were not taken at temperatures higher than 40° C., but the results obtained leave little doubt that the initial period of swelling would be less at higher temperatures, and the shrinkage still more rapid. The results are analogous to those obtained by F. F. Blackman for carbon assimilation, where assimilation falls off with time at higher temperatures owing to secondary actions.

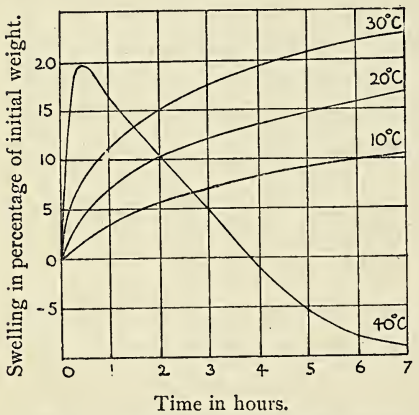

FIG. 4. Curves illustrating the influence of temperature on the swelling of potato in distilled water.

The influence of temperature on the swelling of carrot is similar to its influence on potato, although it is not so marked. The numbers obtained by experiments are given in Table V.

TABLE V.

Swelling of Carrot in Distilled Water at Different Temperatures.

10° C.		20° C.		30° C.		40° C.	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0·63	9·4	0·52	10·8	0·52	12·6	0·58	12·0
2·27	15·2	2·28	16·0	2·28	16·5	2·35	10·9
3·13	16·6	3·15	17·2	3·13	17·3	3·22	8·8
14·90	20·1	15·13	18·8	15·08	16·6	15·23	−1·7

There will again be observed the increase in the rate of swelling with

increased temperature and the subsequent shrinkage at higher temperatures. It would appear that even after half an hour the shrinkage at 40° C. has already commenced. In Fig. 5, where these results are shown graphically, the first part of the curve of swelling at this temperature is assumed.

From the values obtained from fifteen to twenty hours, in both the cases of carrot and potato, it seems reasonable to conclude that temperature exerts no marked influence on the equilibrium value of maximum swelling, apart from the secondary action at higher temperatures to which reference has already been made.[1]

The rate of swelling at any particular moment is given by the tangent of the angle made with the time axis by the tangent drawn to the curve at that particular point.

In order to measure the effect of temperature on swelling it is necessary to compare the rates of swelling at the same stage of swelling at the different temperatures.

The numbers given in Table VI are obtained in this way. The table shows the comparative rates of swelling at different temperatures when the tissue has increased 10 per cent. in weight, i.e. when it has swollen to about half the maximum possible swelling.

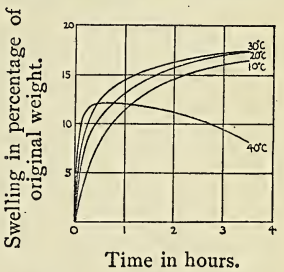

FIG. 5. Curves illustrating the influence of temperature on the swelling of carrot in distilled water.

It does not follow, however, that carrot and potato are in the same absolute stage of swelling, as we cannot conclude that the initial conditions of the two tissues are the same in regard to water-content and turgor pressure. Nevertheless, although the comparison of the temperature coefficients of potato and carrot obtained here must be made with a certain amount of reservation, yet the recorded differences are striking and indicate considerable differences in constitution of the two tissues.

TABLE VI.

Rate of Swelling of Potato and Carrot at Different Temperatures.

Temperature.	*Potato.*	*Carrot.*
10° C.	0·15	1·3
20° C.	0·45	1·7
30° C.	1·2	2·5

These numbers give the following values for the temperature coefficient of swelling:

TABLE VII.

Temperature Coefficients of Swelling of Potato and Carrot.

Temperature.	*Potato.*	*Carrot.*
10°–20° C.	3·0	1·3
20°–30° C.	2·7	1·6

[1] Cf. Pfeffer (14), p. 138: 'Since by a rise of temperature of 15° C. the (osmotic) pressure is only raised from 100 to 105·5, it is evident that temperature can never exercise any marked direct effect turgor in plants.'

It is not advisable to enter into a discussion of the meaning of these temperature coefficients while our analysis of the processes involved is so incomplete. As the rate of swelling is dependent not only on the permeability of the protoplasm to water, but also on the elasticity of the cell wall and probably on other factors also, as for instance permeability to substances dissolved in the cell sap, it is clear that at present we can do no more than record the fact that temperature increases the rate of swelling in the manner indicated. Moreover, it does not follow that tissue in which the inherited factors are different or which has previously been subjected to different external conditions will give the same values for the temperature coefficient of swelling as recorded here.

(*c*) *The Influence of Dissolved Substances on the Swelling of Potato.*

1. *Sodium chloride.*

It was shown by de Vries that above a certain concentration of solute, solutions of various substances brought about a contraction of the protoplasm of the cell away from the wall. This was explained by de Vries and Pfeffer as due to the outer layer of the protoplast acting as a semi-permeable membrane which allowed water to pass through, but which prevented the passage of the dissolved substance. A solution of the same osmotic concentration as the cell sap should produce no effect whatever, but above that concentration it should bring about plasmolysis. Thus, the terms hypotonic, isotonic, and hypertonic were introduced to describe solutions respectively lower, equal, or higher in osmotic concentration than the cell sap.

If in the case of hypertonic solutions the cell wall should shrink to some extent along with the protoplast, water should be exuded and the tissue should lose weight. As in distilled water the cell wall stretches, so it is reasonable to expect it to be capable also of shrinkage, and the experiments of Miss Delf (6), in which the rate of shrinkage in length of tissues in such solutions is measured, show that this is the case.

The following table shows the swelling and shrinkage of potato tissue in various strengths of sodium chloride solution. The values are given in terms of percentages of the original weight of the tissue:

TABLE VIII.

Swelling and Shrinkage of Potato in Sodium Chloride Solutions.

Distilled Water.		$\frac{N}{12}$ *NaCl.*		$\frac{N}{10}$ *NaCl.*		$\frac{N}{8}$ *NaCl.*		$\frac{N}{7}$ *NaCl.*		$\frac{N}{6}$ *NaCl.*	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0·57	6·4	1·15	4·0	0·75	1·8	0·92	0·6	1·72	−1·6	1·62	−4·6
3·12	13·6	3·28	3·6	3·18	1·8	3·25	1·2	2·42	−3·0	2·42	−5·6
6·80	17·2	7·12	2·6	6·92	1·2	5·83	0·4	3·25	−3·0	3·20	−6·8
21·80	22·4	22·03	2·8			7·00	0·2				

These results are shown graphically in Fig. 6. They show clearly the effect of increasing quantities of sodium chloride in the external solution in producing a progressive decrease in swelling until above a certain concentration shrinkage is produced.

It will be observed how, after a certain time, the swelling or shrinkage reaches a position of approximate equilibrium which is maintained for at least sixteen hours. On the de Vriesian view this indicates that the sodium chloride does not enter the tissue in any quantity. For if it did enter the cell it would either alter the permeability or it would not. In the latter case, since the substances dissolved inside the cell cannot pass out, the osmotic pressure inside would be raised, and, when the concentration of sodium chloride inside and outside was the same, the tissue should ulti-

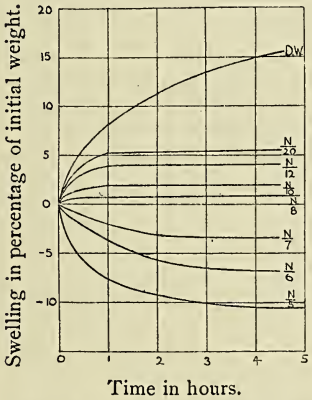

FIG. 6. Curves illustrating the absorption and excretion of water from potato in sodium chloride solutions of various concentrations.

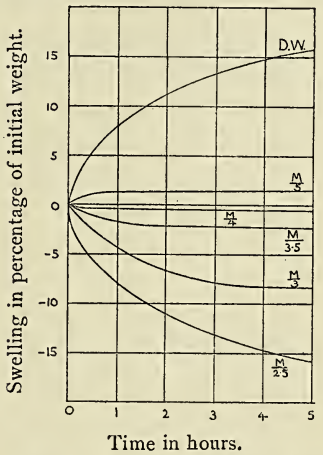

FIG. 7. Curves illustrating the absorption and excretion of water from potato in sucrose solutions of various concentrations.

mately swell to the same extent as in distilled water. Similarly, if the permeability to the substances inside the cell were decreased, there would still be swelling as in distilled water. On the other hand, if the permeability were increased so that dissolved substances inside the cell diffused out, one would expect increased swelling, at first due to the increase in salt concentration inside the tissue, followed by a shrinkage as the permeability increased and the internal solutes diffused out. This is not indicated by the curves obtained with sodium chloride, although such is indeed the course of events observed with some substances which for other reasons we know bring about an increase of permeability.

It will be noted that with these samples of potato a solution of $\frac{N}{8}$ sodium chloride was approximately isotonic on the osmotic hypothesis with the cell sap.

A similar series of experiments with carrots showed, however, that a solution of sodium chloride of a concentration of about $\frac{N}{3}$ was required to reduce the swelling to zero.

2. *Sucrose.*

In Fig. 7 are shown graphically the results obtained for the swelling of potato in solutions of cane sugar of various concentrations. It will be observed that the results are almost identical with those obtained with sodium chloride. A solution of concentration $\frac{M}{4}$ is approximately isotonic, the shrinkage in this solution being almost negligible. Thus, an isotonic solution of sucrose has a concentration of almost double that of an isotonic solution of sodium chloride. This is exactly the result to be expected as sucrose is completely undissociated in solution, while sodium chloride is almost completely dissociated into its constituent ions.

3. *Ethyl alcohol.*

In the solutions of ethyl alcohol employed the general course of swelling is quite different from that obtained with sodium chloride or sucrose.

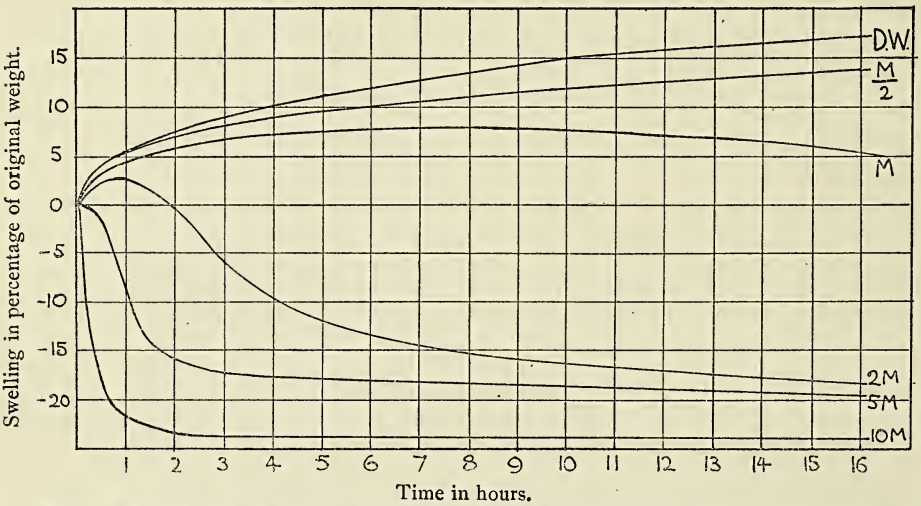

FIG. 8. Absorption and excretion of water by potato in solutions of ethyl alcohol of various concentrations.

If ethyl alcohol behaved similarly to these substances we should expect a concentration of ethyl alcohol of about $\frac{M}{4}$ to be isotonic, and solutions of higher concentration to bring about shrinkage and those of lower concentration swelling. As a matter of fact swelling takes place at first in much higher concentrations than this. Thus, potato swells at first in a solution eight times as strong (2 *M*). In this strength, however, the swelling phase is of short duration and reaches a low maximum after about half an hour.

Shrinkage then commences and continues for at least sixteen hours, after which time the tissue may have lost in weight by about 20 per cent. (Fig. 8). In higher strengths than 2 *M* the preliminary swelling phase is reduced, and in solutions of 5 *M* and 10 *M* shrinkage is considerable after half an hour. It is clear that the higher the concentration of alcohol the more rapid is the rate of shrinkage.

With concentrations less than 2 *M* the preliminary swelling period is more marked. Thus, in a gram-molecular solution maximum swelling is reached after about ten hours and is a higher value than the maximum swelling in the case of a 2 *M* solution. With $\frac{M}{2}$ ethyl alcohol the maximum is not reached even after sixteen hours, nevertheless the swelling is lower than in distilled water.

The explanation of these phenomena on the membrane hypothesis is simple. Ethyl alcohol is supposed to penetrate the membrane, and in so doing to alter its constitution in such a way that it becomes permeable to the dissolved substances inside the cell. In the first stage of the process, therefore, the rise in osmotic pressure inside the cell owing to the addition of the alcohol produces swelling in what would have been hypertonic solutions if the alcohol had not passed through. If the membrane remained unaltered the cell should ultimately swell as much as in distilled water, provided no reactions took place between the alcohol and cell contents. But if the membrane is rendered more permeable to the solutes dissolved in the cell sap, these will diffuse out of the cell, slowly at first, then more rapidly as more of the membrane is changed, and finally more slowly again as the difference in concentration inside and outside of the cell becomes small. The exosmosis of the dissolved substances reduces the osmotic pressure inside the cell, which therefore loses water to the external medium. As the destruction of the membrane will take place more rapidly the higher the concentration of the alcohol, the exosmosis from the cell, and consequently the shrinkage, will be more rapid the higher the concentration. The curves given in Fig. 8 show that this is exactly what actually occurs.

Moreover, that the exosmosis of dissolved substances takes place as this explanation supposes, has already been shown in regard to electrolytes in the preceding paper in this series (16). Reference may be made to the series of curves obtained for ethyl alcohol given in Fig. 6 of that paper, in which the exosmosis of electrolytes from potato tissue immersed in various strengths of ethyl alcohol is shown. It will be observed how exactly the exosmosis curves and swelling curves correspond.

It is thus necessary to distinguish between the shrinkage due to plasmolysis and that due to injury. So Osterhout (11) distinguishes between true and false plasmolysis, the latter being the shrinkage which results from injury.

4. *Octyl alcohol.*

In the preceding paper of this series it was shown how the complexity of the molecule in the series of monohydric alcohols influenced the exosmosis of electrolytes. The higher the alcohol the lower is the concentration which produces equal exosmosis. This relation ought also to appear in regard to the swelling of tissue in solutions of different alcohols; that is, the higher the alcohol the lower should be the concentration required to produce the shrinkage which is correlated with exosmosis.

This is found to be so, and the case of octyl alcohol, the most complex alcohol used, may be cited. It was previously found (17) that the exosmosis produced by a 1·4 *M* solution of ethyl alcohol was produced by a concentration of a secondary octyl alcohol

$$CH_3.(CH_2)_5.CHOH.CH_3$$

of 0·003 *M*. The swelling in three strengths of this alcohol are indicated in the following table. In 0·001 *M* the swelling is approximately the same as in distilled water. In 0·002 *M* the swelling is the same as in 0·001 *M* after one or two hours, but after sixteen hours the swelling is depressed just as in the case of 0·5 *M* ethyl alcohol. In a solution of concentration 0·003 *M* the swelling is the same after 1·3 hours, but by the end of sixteen hours there is a great shrinkage.

TABLE IX.

Swelling of Potato in Solutions of a Secondary Octyl Alcohol.

Time in hours.	*Swelling in percentages of original weight.*			
	0·000	0·001 *M.*	0·002 *M.*	0·003 *M.*
1·33	7·2	7·2	7·3	7·4
16·50	16·9	16·9	13·9	−19·2

The form of the swelling curves given for ethyl alcohol may be regarded therefore as the typical form of such curves when the substance in solution outside the tissue enters the cell and produces changes which render the cell permeable to the solutes inside.

5. *Sulphuric acid.*

That alcohols enter the cell is not easy to show by direct measurement; it is, on the other hand, comparatively easy to show by direct measurement that acids, or at any rate the hydrogen ions of their molecules, are readily absorbed by plant cells. This has been done in the first three papers of this series (15, 16, 8), and it has also been shown (14, 8) that the absorption of the hydrogen ion is accompanied by the exosmosis of electrolytes from the cell. It would be expected then that the swelling of potato in acids would follow a similar course to the swelling in alcohols, that is, there would be a preliminary swelling, the magnitude of which

would depend on the dilution of the acid, followed by a shrinkage which would appear sooner the stronger the solution.

Fig. 9 shows that this is the case. With $\frac{N}{50}$ sulphuric acid the maximum swelling is reached in an hour, and with acid as dilute as $\frac{N}{1,000}$ the tissue begins to shrink after four hours' immersion. Shrinkage was observed in $\frac{N}{2,000}$ acid by the sixth hour of immersion, and even $\frac{N}{5,000}$ acid brought about shrinkage before the elapse of twenty-four hours.

It will be observed that in the lower strengths of sulphuric acid the swelling is at first actually greater than in distilled water. It is possible that this may be referred to the well-known phenomenon of increased swelling of proteins in acid solutions as compared with pure water, a phenomenon on which much stress has been laid by Fischer (7), who successfully shows the similarity between the swelling of such proteins as gelatine and blood-fibrin with certain animal tissues, frog's muscle and sheep's eyes.

The observed increased swelling of potato in dilute acid solutions appears to be a definite increase and not merely due to variations in the samples, as the differences are for the most part considerably greater than the probable error. These are given in Table X.

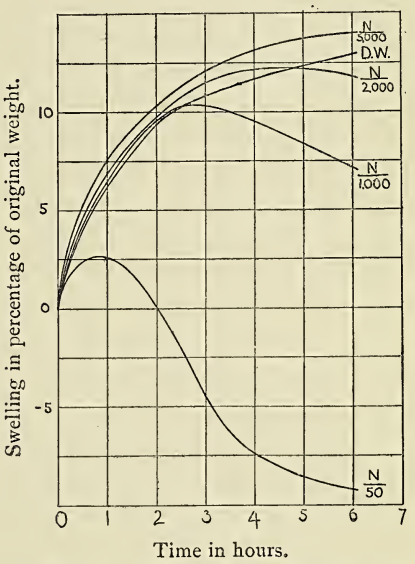

FIG. 9. Curves illustrating influence of different concentrations of sulphuric acid on the swelling of potato.

TABLE X.

Swelling of Potato in Various Concentrations of Sulphuric Acid.

Distilled Water.		$\frac{N}{5,000}$ H_2SO_4		$\frac{N}{2,000}$ H_2SO_4		$\frac{N}{1,000}$ H_2SO_4		$\frac{N}{50}$ H_2SO_4	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0·50	4·0 ± 0·05	0·58	5·7 ± 0·2	0·50	4·5 ± 0·0	0·50	4·3 ± 0·0	0·25	0·37
2·00	9·4 ± 0·3	2·23	10·7 ± 0·25	2·17	9·9 ± 0·2	2·00	9·5 ± 0·25	1·02	0·53
3·60	11·2 ± 0·4	3·67	12·8 ± 0·3	3·63	12·1 ± 0·15	3·58	10·0 ± 0·3	2·52	−0·40
5·85	12·7 ± 0·35	5·88	14·0 ± 0·35	5·87	11·8 ± 0·25	5·88	7·3 ± 0·25	3·32	−1·16
7·00	13·5 ± 0·3	7·05	14·8 ± 0·25	7·03	11·4 ± 0·25	7·00	5·1 ± 0·4	4·37	−1·59
21·90	17·4 ± 0·15	24·18	13·0 ± 0·1	24·78	2·6 ± 0·4	24·20	−7·5 ± 1·3		

However, as we hope to deal on a later occasion both with the question of the action of dilute solutions and with the general behaviour of plant tissue towards acids, we do not propose to enter into a detailed discussion of these results here.

Concluding Remarks.

In the preceding pages we have recorded the results of some preliminary experiments on the exchange of water between the plant cell and the surrounding medium. The chief difference between these experiments and most of the earlier ones on the same subject lies in the fact that the method employed is quantitative and the reaction is followed with time. In work with such complex systems as the plant physiologist has to deal with, it is extremely easy to draw incorrect conclusions and to refer observed effects to wrong causes, and this is especially the case where the course of the reaction with time is not followed. It is only necessary for us to refer to the conclusions drawn by van Rysselberghe (18) from his experiments on our subject, as an example of this. Thus, Miss Delf (6) has recently shown that van Rysselberghe's conclusions are false, and that it would have been impossible for him to have drawn them if he had made measurements in his experiments from time to time. Again, it is only by quantitative work that an analysis of complex processes will ever be made and the laws underlying the processes discovered. The whole history of physics and chemistry exemplifies this.

By far the most important work on the subject here dealt with which has appeared since Pfeffer's fundamental researches, is that of Miss Delf (6) on the effect of temperature on the passage of water out of the cell when slightly plasmolysed, in which the method employed was quantitative and sensitive and in which the change with time was followed. Her conclusion that the rate of passage of water out of the cell is greatly increased by rise of temperature agrees well with the results of the authors' experiments, which show a similar effect of temperature on the rate of intake of water by the cell surrounded by distilled water.

As regards the magnitude of the coefficient, it is impossible to make any definite statement. The actual rate of swelling depends on the previous history (environmental and genetic) of the tissue employed, and it is at least not improbable that the temperature coefficient of swelling may show similar variations with the previous history. For there are at least four different processes concerned, namely, the absorption of water by the cell wall, the absorption of water by the protoplasm, the passage of water into the vacuole, and the stretching of the cell wall, and if these are differently affected by temperature and also by the factors to which they are subjected by previous history, the temperature coefficient of swelling will depend on the previous history.

This complexity of the system, as we have already said, makes it difficult to draw conclusions as to the causes of the observed phenomena. With quantitative data and proper methods, however, mathematical treatment becomes possible and will in the future no doubt be a great aid in the analysis required for an understanding of the processes of plant physiology. However, although mathematical treatment is possible in regard to the subject dealt with in this paper, we have avoided this, as it seems to us considerably more data are required before this can be done profitably in this connexion.

The explanations so far put forward to account for swelling and plasmolysis probably do not take enough account of the complexity of the system. Of the two chief theories, one, that of de Vries and Pfeffer, explains the absorption of water on the basis of semi-permeable membranes and osmosis.[1] The protoplast is supposed to be surrounded by membranes which are permeable to water and impermeable to certain substances.

The argument advanced against this theory, that salts get into the plant, and therefore the plasma membrane cannot be impermeable to salts, is frivolous, for in the first place the plasma membrane may undergo reversible changes in regard to its permeability,[2] and, secondly, plasmolysis would still take place if the membrane were not absolutely semi-permeable (as no membrane is) but let through the salt more or less slowly.

Although we do not think the theory of de Vries is likely to be a complete expression of the facts, yet we may point out that there is not a fact recorded in this paper which cannot be explained on its basis. The swelling of tissue in distilled water, the plasmolysis in sodium chloride and sucrose, the behaviour in solutions of substances with poisonous properties and which are known to enter the cell, such as acids and the primary alcohols, are all phenomena which follow directly from the semi-permeable membrane theory. On the other hand, unpublished work of Stiles and Kidd indicates that sodium chloride passes rapidly into the cells of potato and carrot, while Brooks (2), using de Vries's method of tissue tension, concludes that sucrose as well as sodium chloride passes readily into the scapes of the dandelion.[3]

Such difficulties as this have caused some workers on animal cells, e.g. Moore, Roaf, and Webster (9, 10) to reject the osmotic theory altogether and to explain all permeability phenomena as due to the presence of

[1] Pfeffer was well aware of the capacity of protoplasm to absorb water on account of its colloidal nature, but apparently did not regard it of importance in such observations as are recorded here. Cf. Pfeffer (14), p. 136.

[2] 'Apparently it is by temporary or permanent alterations in the specific nature of the plasmatic membrane that an absorption (or excretion) of a particular substance may be temporarily or permanently permitted or prevented' (Pfeffer, 14, p. 102).

[3] Pfeffer points out (14) that the formation of starch in the plastids, or the accumulation of other carbohydrates, when plants are supplied externally with sugar, indicates that sugar can be absorbed under certain circumstances.

a colloid in contact with a crystalloidal solution. The difficulties of this theory (see e. g. Bayliss, 1) are so great, and it has explained so little, that it has found practically no support. Martin Fischer (7), as a result of an extensive series of experiments with gelatine, blood fibrin, frog's muscle, and sheep's eyes, has also rejected the osmotic theory and attempted to explain swelling of animal and plant tissue and related phenomena as due to the colloidal nature of the tissue. In view of the fact that the causes of the swelling of colloids are not by any means settled, and especially when it is considered that the swelling may be an osmotic phenomenon, this explanation of the swelling of living tissue is not so far very helpful. Probably the most striking result of Fischer is the exact similarity in the effect on acids in increasing swelling of gelatine and blood fibrin on the one hand, and muscle and sheep's eyes on the other. This may very well be the same phenomenon, and the indication of an increased swelling in dilute acid in our experiments may also be due to the colloidal nature of the protoplast, but it scarcely justifies Fischer's conclusion that in all cases the swelling in water is due to the production of acid as a result of the conditions of experiment. In our experiments a very low hydrogen ion concentration is sufficient to kill the cell, which would appear to dispose completely of the view that the total swelling of plant tissue in water is due to the formation of acid in the protoplast.

In such a complex organization as the cell it is unlikely that any one single explanation will explain all the phenomena of permeability. We know that there are at least three structures of which account must be taken: the cell wall, the protoplast, and the vacuole. We also know that at the junction of two immiscible phases the properties of the material may be different from those in the body of either phase, and indeed we find that de Vries postulated two limiting membranes to the protoplast, an outer one surrounding the protoplast (the plasma membrane or ectoplast), and an inner one surrounding the vacuole (the vacuole wall or tonoplast).[1] It would for instance be reasonable to suppose that the protoplast behaved towards external solutions in much the same way as gelatine or blood fibrin, while at the same time it formed a semi-permeable membrane between the vacuole and external solution.[2] Such a theory might be elaborated to explain the permeability of the cell, but we feel, as we have already emphasized in previous papers in this series, that we know so little

[1] The arguments for the existence of plasmatic membranes with diosmotic properties distinct from those of the body of the protoplast have been stated by Pfeffer (13). They cannot be regarded as very convincing.

[2] 'The general principles previously laid down as governing the exchanges of substance taking place between the protoplast and the external world would still hold good, even though no differences existed between the diosmotic properties of the general mass of the protoplast and the limiting membranes which enclose it; if, for example, the entire thickness of the primordial utricle behaved like a single very thick plasmatic membrane' (Pfeffer, 14, p. 107).

of the facts of permeability, that a great amount of experimental work is needed before the presentation of fresh theories is justified.

Summary.

1. A method for investigating the passage of water between the cell and its surroundings is described, which is quantitative and allows the investigation of the kinetics of the changes taking place.

2. The probable error of experiment is determined, and the means of reducing it indicated.

3. The tubers of potato and roots of carrot in distilled water absorb water (swell) for some days before equilibrium is attained. Equilibrium is maintained for a considerable time.

4. The rate of swelling of carrot is much greater than that of potato.

5. The previous history of the tissue influences greatly the magnitude of the swelling.

6. In tap-water, i. e. a weak solution of a number of salts, the magnitude of swelling is less than in distilled water and equilibrium is not maintained for so long (especially in the case of potato), a shrinkage of the tissue supervening. This is presumably due to the absorption of substances which cause an increase in permeability.

7. Increase of temperature increases the rate of swelling. The temperature coefficients (Q_{10}) determined were, for carrot, about 1·5, and for potato, about 3·0. These are probably not definite values characteristic of the tissue, but probably vary with the previous history of the tissue.

8. In solutions of sodium chloride and sucrose the swelling of potato is reduced with increase of concentration of the solution. With the sample of tissue used sodium chloride produced no swelling in a concentration of about $\frac{N}{8}$ and sucrose in a concentration of about $\frac{M}{4}$. No swelling of the carrot used took place in a concentration of about $\frac{N}{3}$ sodium chloride. With concentrations above these values swelling took place; with lower concentrations, shrinkage. The shrinkage obtained in these cases is identical with the shrinkage usually called plasmolysis.

9. In the case of certain substances which enter the cell, such as the primary alcohols, preliminary swelling takes place in solutions of much higher concentration than isotonic solutions as determined by experiments with sodium chloride and sucrose. Subsequently shrinkage or depressed swelling occurs in all concentrations, which is correlated with the alteration of the permeability of the protoplasm and the death of the tissue. This shrinkage is due to toxic action and not plasmolysis.

10. Acids behave similarly to the primary alcohols, and produce the preliminary swelling and the subsequent shrinkage correlated with toxic action.

11. The bearing of the facts described on some theories of permeability is discussed.

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Note on a Method of demonstrating the Heat of Respiration.

BY

M. C. POTTER.

With one Figure in the Text.

THE method of demonstrating the heat of respiration described in most text-books follows that devised by Sachs and, one is tempted to think, is frequently quoted without practical trial. In this method a number of germinating seeds are placed in a funnel supported in a beaker which contains an alkaline solution to absorb the CO_2. The funnel and seeds are covered by a bell-jar to protect them from external variations of temperature, a tubule at the summit of the bell-jar serving for the introduction of a thermometer and for ventilation. A similar apparatus with a thermometer plunged in seeds which have been killed by boiling is used as a control. This contrivance is very inconvenient. A funnel sufficiently large for the purpose of the experiment necessitates a large bell-jar, and thus the apparatus becomes both expensive and cumbersome. For various reasons it cannot always be depended upon to give satisfactory results, and, as the heat of respiration is an important laboratory experiment, it may be of some interest to describe a simple apparatus which I devised and have used for many years with success.

In every experiment certain precautions must be taken to guard against experimental error. In the directions usually given to demonstrate the rise of temperature consequent upon respiration, the calibration of the thermometers and the necessity for guarding against the variations of temperature of the surrounding air are generally noted. But no account is taken of any increase of temperature due to fermentation or putrefaction. This calls for special comment, and neglect of this factor may give rise to serious error. Thus when seeds merely killed by boiling are used as the control (as is commonly recommended) putrefaction generally takes place, and it is often found that the experiment apparently does not succeed as the control registers the higher temperature.

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For the success of this particular experiment under any method, the following conditions must be satisfied:

(*a*) a sufficiently large number of seeds so that the heat produced may not be dissipated;

(*b*) a supply of oxygen;

(*c*) a means of escape for the CO_2;

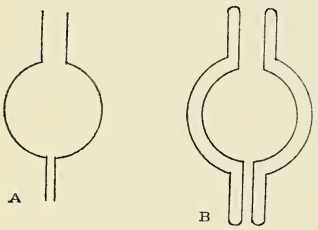
(*d*) screening from the changes in temperature of the surrounding air;

(*e*) efficient control;

(*f*) the prevention of putrefaction.

The form of apparatus which I have found convenient consists of flasks of about 500 c.c. capacity with a short ventilation tube fused at the base for the escape of the CO_2, these being enclosed in a box and packed with cotton-wool (in annexed figure, A). By this means the first four conditions enumerated can be satisfied.

I invariably employ three of these flasks. The first (I) contains living seeds thoroughly soaked before introduction into the flask. The second (II) contains seeds soaked and then boiled to destroy their power of germination. The third (III) contains seeds soaked and boiled in a solution of 0·5 per cent. mercuric chloride, with the object of killing the seeds and at the same time effectually sterilizing them.



To soak the seeds before introduction into the flasks is a necessary precaution, especially if peas are used, otherwise they would swell and break the flasks. The usual procedure is to soak the seeds for II and III and then effect the necessary boiling, and while these are cooling to soak the seeds for I. In this manner it is possible for the experiment to commence with the three flasks at the same initial temperature. Since the germinating seeds in I are damp, it is necessary that the control seeds should be damp also, in order to minimize any error due to the loss of heat from evaporation.

The three flasks thus prepared are placed in a box and packed round with felt or cotton-wool. It may be safely assumed that any variation of temperature due to evaporation or to changes of room temperature will be common to the three flasks.

Flask III is the control. In an ordinary room or laboratory its temperature will fluctuate, but does not rise above that of the room, and any rise above this room temperature in the other flasks will be due to respiration in I or putrefaction in II.

Flask I gradually rises in temperature as the activity of respiration proceeds, and in the course of about forty-eight hours (if peas are employed)

may register 2·0 centigrade above the control III. This may be taken as the rise of temperature due to the respiration.

The behaviour of Flask II is most instructive. Its temperature gradually rises, but more slowly than is the case with I, and after two days or so it will be found to possess a higher temperature than that of I. This rise of temperature is due to the action of various fungi and bacteria which invariably gain a footing on the dead unsterilized seeds.

As the experiment proceeds it is often found, after about a week, that the seeds in I are attacked by moulds and bacteria, and hence the later temperature difference is due partly to the seed-respiration and partly to putrefaction. It is only in the initial stages that the experiment gives a true indication of the heat evolved during respiration, unless the seeds have been sterilized before insertion.

The experiment is usually kept under observation for about fourteen days. By taking readings at frequent intervals, say twice a day, of the differences of temperature between I and II and between II and III, and employing them as ordinates, very instructive graphs can be obtained showing the progress of the heat of respiration and that of the heat of putrefaction.

A very simple method which yet gives excellent results and would be useful in school work may also be mentioned. The seeds are placed in three boxes instead of three flasks: ordinary chalk boxes placed upright answer admirably. A small hole is made at the top to serve for the introduction of the thermometer and suitable perforations at the opposite end for the escape of the CO_2. By this extremely simple means results similar to those given by Sachs's method may readily be obtained.

A more refined experiment may be performed by using a modification of the Dewar flask (Fig. B). Since 1903 I have employed flasks of this description with the addition of a drainage tubule, the vacuum diminishing very considerably the loss of heat from radiation ('Proc. Roy. Soc., B.,' 1908). These flasks are somewhat expensive and hardly suitable for students' use. But they are excellent for research and demonstration purposes, and several sets of the simpler apparatus can easily be provided so that the students may set up and work out the experiments individually.

With this form of apparatus many instructive experiments may be carried out. Thus by closing the end of the drainage tube in I and allowing the CO_2 to accumulate the fall of temperature due to decrease of respiration under these conditions can be noted. Or by filling the flask I with CO_2 or hydrogen or nitrogen one may observe the failure of the seeds to germinate under such conditions and the consequent absence of any rise in temperature.

Ganong ('Plant Physiology') also recommends vacuum flasks, but uses an alkali to absorb the CO_2 in place of the drainage tubule.

Pierce ('Botanical Gazette,' 1908, 1912) has employed the Dewar flask with drainage tubule in his investigations on measuring the number of calories liberated by germinating peas, and has also emphasized the necessity of measuring the heat equivalent. In his experiments he has also avoided the error due to the heat of putrefaction by sterilizing the seeds.

Armstrong College,
Newcastle-upon-Tyne,
March, 1917.

Notes on Equisetum debile, Roxb.

BY

SHIV RAM KASHYAP.

With three Figures in the Text.

1. The Endodermis.

IT has long been known that the form and position of the endodermis of the stem in the genus *Equisetum* is very variable. Three different types may be distinguished: (i) one endodermal ring round each vascular bundle, (ii) two layers of endodermis, one outside and the other inside the ring of vascular bundles as a whole, (iii) one endodermal layer round the ring of vascular bundles as a whole. These different types are not restricted to different species of the genus exclusively, but two may occur in different parts of the same plant. The following, for example, is the condition of the endodermis according to Sadebeck (vide 'Nat. Pflanzenfamilien'). In the internodes of the aerial shoots of *E. Heleocharis* the endodermis is of the first type; in *E. hiemale*, *E. ramosissimum*, *E. trachyodon*, and *E. variegatum* it is of the second type; and in *E. palustre* and *E. scirpoides* it is of the third type. In the internodes of the underground portion of the shoots of *E. hiemale*, *E. ramosissimum*, and *E. trachyodon*, however, the endodermis is of the first type, though in the aerial portion it is of the second type. In the internodes of the underground portion of the shoots of *E. sylvaticum* the endodermis is of the second type, though in the aerial portion it is of the third type. In most investigated species, however, according to Sadebeck, this difference in the aerial and underground portions of the shoot is not present, e.g. in *E. arvense*, *E. maximum*, *E. palustre*, *E. scirpoides*, *E. Heleocharis*, and *E. variegatum*; but in the underground tuberous swellings of *E. palustre*, *E. arvense*, and *E. sylvaticum* Pfitzer found an endodermis of the first type.[1]

If we do not take into consideration the exceptional case of the underground tuberous swellings of the three species mentioned above on account of their great modification, it is clear from the above description that in those species where two different forms of the endodermis occur in different parts of the stem, an endodermis of the first type is associated with the

[1] For further details see Engler and Prantl: Nat. Pflanzenfamilien.

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endodermis of the second type, and an endodermis of the second type is associated with one of the third type. Since underground organs, e. g. the root of vascular plants, retain their ancestral structure for long periods and through many phyla, it appears that an endodermis of the second and the third type has been gradually derived from that of the first type. This view is borne out by the writer's observations on the endodermis of *E. debile*. *E. debile* is the most variable species of the whole genus as regards the form of the endodermis in the stem. In a very general way it may be stated that in the internode of the underground rhizome the endodermis belongs to the first type; in the aerial vegetative shoot it is of the first type near the node but of the second type in the internode; and lastly in the fertile shoot

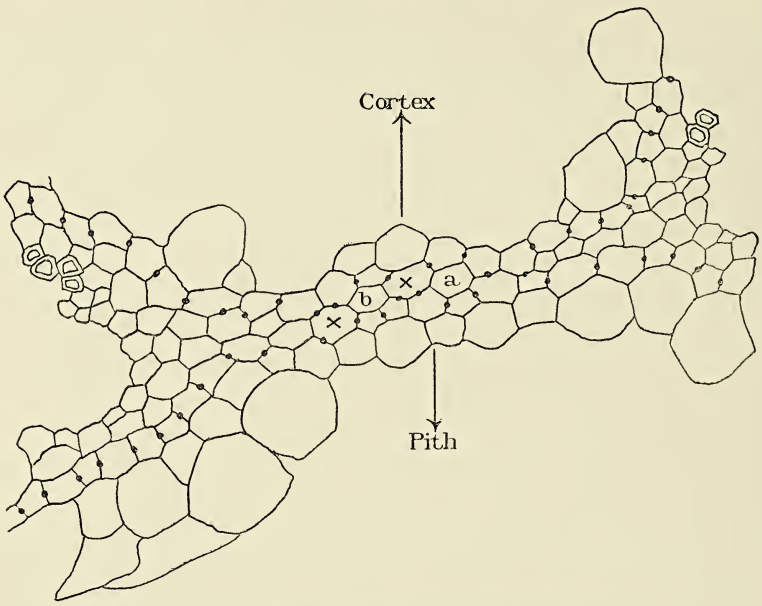

FIG. 1. *Equisetum debile*. (For explanation see the text.)

below the cone as well as in the cone, so far as it can be traced upwards, it is of the first type. At the same time, in the internodes of the underground as well as the aerial portions, the two types, first and second, merge into each other. The transition is met with at all places in the internode irrespective of its nearness to the node. In those species where two layers of the endodermis occur, one external and the other internal to the ring of the vascular bundles, the band of parenchyma lying between the two layers in the interfascicular region is usually very narrow owing to the external endodermis being bent inwards in that region. In *Equisetum debile* these two layers are actually fused in certain places so that a complete closed ring of endodermis is formed, not only round one or more bundles, but isolated rings of endodermis are also pinched off in the interfascicular region, pro-

ducing small islands of parenchymatous tissue of one to six or seven cells. These isolated rings may be close to each other so that one cell is actually common to the two rings (Fig. 1, ×), or the endodermis round the parenchymatous cells may be quite independent (Fig. 2, *a*). In Fig. 1 the two rings *a* and *b* are connected by one cell having suberized bands on four of its walls, two belonging to one ring and two to the other. In other cases two of the bands may actually occur on one wall, the four bands belonging to two rings occurring on three walls as seen in Fig. 3 at *a*.

It is easy to understand the formation of these islands if we suppose that the second type of the endodermis is gradually passing into the first by the fusion of the two layers at various points. But that is hardly likely in view of what has been said and what is to follow. It is more difficult to understand how these islands could be formed if the endodermis of the first

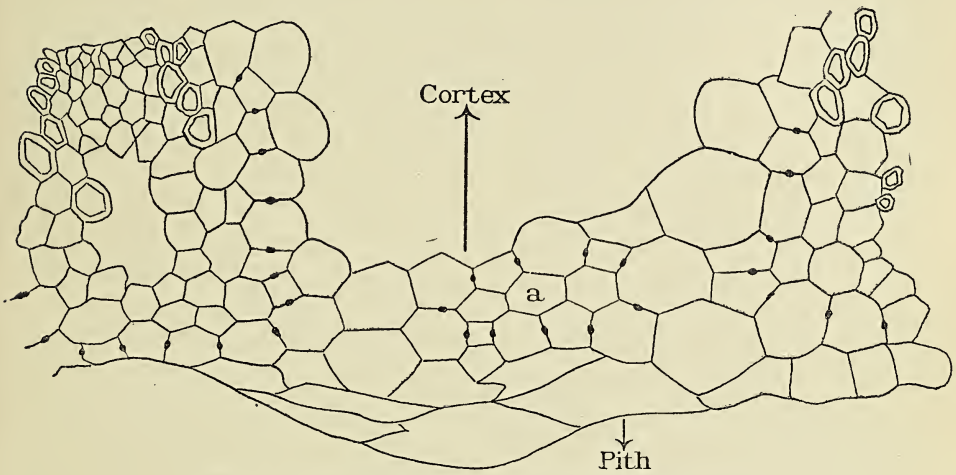

FIG. 2. *Equisetum debile.* (For explanation see the text.)

type at the node is giving rise to the endodermis of the second type in the internode.

In the aerial fertile region below the cone, and in the base of the cone itself, the endodermis is of the first type everywhere. It may be due to the fact that the internodes here are very short. Since the fertile part of the shoot, however, is known to be more conservative and retains its ancestral structure for a longer time than the vegetative shoot, it is likely that this arrangement indicates the ancestral features of the stem, especially when taken along with the structure of the rhizome. In the cone itself, even near its base, the characteristic bands on the radial walls of the endodermis are lost, but the layer can be distinguished on account of the large size and regular arrangement of the cells. As we ascend higher, however, even these

distinctive characters gradually disappear and the endodermal cells can no longer be distinguished from the neighbouring parenchymatous cells.

In the region of the hypocotyl in the young plant the changes in the form of the endodermis are as follows: The root is usually triarch without pith and with an external endodermis having suberized bands on radial walls. Higher up some parenchymatous pith appears in the centre and phloem comes to lie all round the xylem ring, as seen in a transverse section, and has a surrounding endodermis. At this stage a small bundle is given

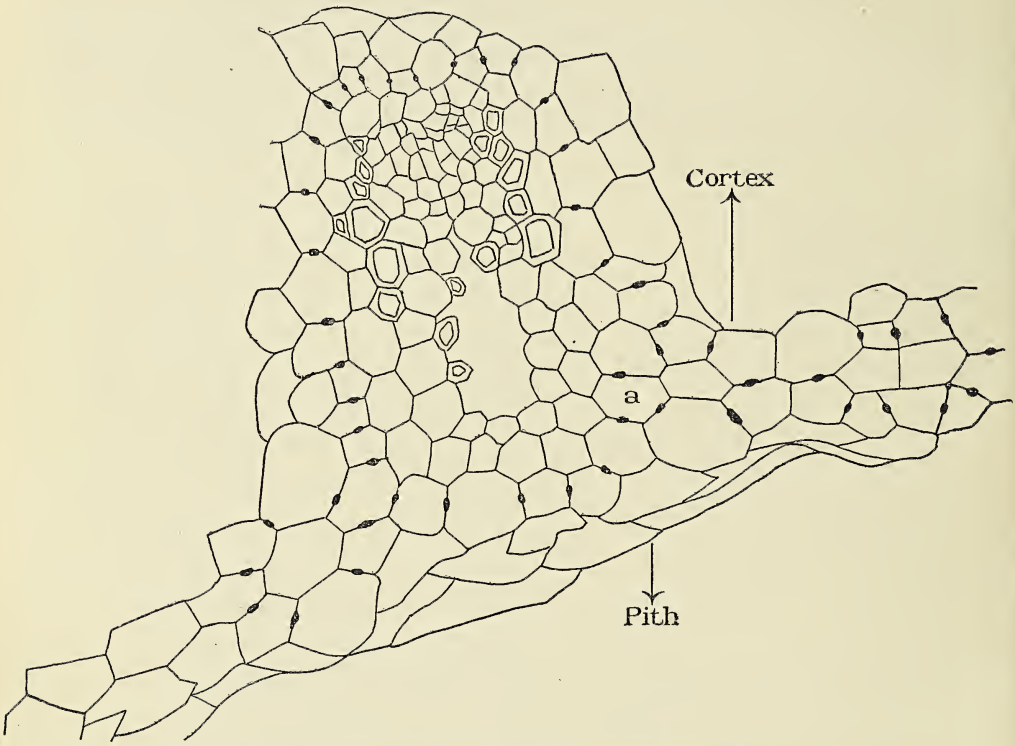

FIG. 3. *Equisetum debile.* (For explanation see the text.)

off for a branch, leaving a gap in the stele. A little higher the gap is closed. Then the three leaf-traces are given off for the first three leaves and the main stele also becomes separated into a few bundles (usually three). External endodermis here cannot be made out, though it becomes distinct a little higher up on the outside of the bundles only, but a little later it becomes continuous. At this place the internal endodermis appears as a small ring surrounding sometimes a single cell, or may be represented by a single cell with all its walls having suberized bands. Gradually the internal ring becomes wider and both layers persist up to the growing point. Thus, in the hypocotyl the internal endodermis forms an inverted cone, gradually

tapering downwards and disappearing first above the origin of the first leaf-traces. In other words, considered from below, it arises immediately after the giving off of the first leaf-traces. Each of these leaf-traces, like the traces higher up in the adult stem, have their own endodermis.

The significance of the islands of parenchyma surrounded by endodermis is not quite clear. Probably they represent vestiges of bundles which have gradually disappeared owing to the habitat of the plant, which is generally swampy soil. It is interesting to compare in this connexion the condition of the normal vascular bundles, which are also greatly reduced and partly replaced by air-spaces.

2. The Prothallium.

The writer published an account of the structure and development of the prothallium of *Equisetum debile* in 1914 ('Annals of Botany', January 1914). The material of that investigation was obtained chiefly from the river bank, where the prothallia grow in large numbers, and only the youngest stages were observed by growing spores in the laboratory. Last year as well as this year spores were germinated and prothallia were allowed to go on growing for several months, and some interesting observations were made. In some experiments spores were made to germinate at some distance from each other (by shaking some spores in water and sprinkling a little of this water on the soil in the pot, which was watered from below), leaving plenty of room for the growing prothallia, and it was observed that all the prothallia grew large, having a circular outline, bore archegonia only at first, and attained a diameter of nearly 2 mm. in forty-five days. They went on growing and some are growing even now (January 1917), having been produced from spores sown in November last. Even the very young stages showed a meristem all along the circular margin, and this meristem is always present along the whole margin in the bigger specimens. In a few cases, however, it was found that the prothallium showed a more vigorous growth on one side and the meristem was localized on a part of the margin. This was probably due to the mode of germination, owing to the spore having formed a short filament first, while in the other case it formed a mass of cells. Even in those cases where the meristem was localized on a part of the margin in the young stage, it would have gradually spread to the rest of the margins also, as shown by some other specimens which were a little older.

In other experiments the spores were grown thickly. In these it was observed that the prothallia grew in thick clusters and remained very small even after two to three months. They showed a distinctly apical growth and the meristem was restricted to one part of the margin. Naturally the prothallia were elongated antero-posteriorly, and the largest were about one

millimetre in length and considerably less in width. They bore archegonia or antheridia. The occurrence of antheridia alone on the prothallia is interesting, as they are never found alone in the prothallia, growing at a distance from each other in the laboratory or in the wild state. In the latter state the prothallia are always scattered, clusters never having been observed. In the scattered prothallia archegonia are always formed first and antheridia arise only on the older prothallia. The prothallia arising in clusters from thickly sown spores are thus extraordinarily similar to those of other species of *Equisetum* figured by Buchtien and others. The lobes, however, in the prothallia of *Equisetum debile* are always erect in whatever condition the prothallia may be growing, thus differing from the lobes of those of other species which are spreading. It is possible that in some cases at least the prothallia of other species of *Equisetum* remain small owing to the spores having been sown thickly. It would be interesting to find out the behaviour of the spores of some other species of *Equisetum* when sown far apart.

It may also be mentioned that though in nature the prothallia do not last more than a few months, dying before the end of April, it is possible to keep them growing for a longer time, and perhaps for some years. The stoppage of growth and ultimate death is apparently due to two causes—the formation of the embryo and the heat of the summer. During 1915–16 some prothallia were kept in a glass-house from September 1915 to July 1916. The embryos as they were developed were cut away and the prothallia were protected from the heat in the glass-house. Thus they had been living for three months more than their ordinary life when unfortunately all the flower-pots containing them were submerged under water during a heavy downpour of rain and the prothallia were all killed. During the last two months of their existence the growth was very slow and parts of the prothallia died, leaving separate lobes growing independently. They would have very likely resumed their active growth in September again if they had lived up to that time.

Summary.

1. *The endodermis.* The endodermis in *Equisetum debile* is very unstable. At the nodes of the underground and aerial sterile shoot and in the fertile region the endodermis surrounds each vascular bundles separately. In the internodes of the underground and aerial sterile shoots there is a transition from the separate endodermis round each bundle to two endodermal layers, one external and the other internal, round the ring of bundles as a whole. This transition is independent of the distance from the node of that part of the internode where it occurs. The two rings of endodermis fuse here and there, leaving islands of parenchymatous tissue in the interfascicular region. At the point of junction of the two layers a

single cell may show radial bands on three or four of its walls or two bands on the same wall.

2. *The prothallium.* If the spores are sown thickly the prothallia remain small and show one growing point only and usually bear only one kind of sex organ. If the spores germinate at a distance from each other, leaving enough space for the prothallia to develop fully, the latter become very large and develop a meristem all round on the circular margin.[1] It is possible to keep the prothallia growing for a few months longer than their natural period of life by removing the embryo and protecting the prothallia from heat, and probably possible to keep them growing for more than one year.

[1] These prothallia bear archegonia at first and develop antheridia later on.

Organic Plant Poisons.

I. Hydrocyanic Acid.

BY

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With three Figures in the Text.

THE study of organic plant poisons presents one fundamental difference from that of inorganic poisons. *Inorganic* compounds containing the toxic elements must of necessity be present in the soil or nutrient medium in which the plant is growing, must be absorbed by the roots, and must be translocated within the plant either in the same form or as some other compound determined by the metabolism of the particular species concerned. In no case can an inorganic substance be developed within the tissues unless the essential basis has been supplied by absorption. The degree of virulence of such toxic elements is frequently determined by the chemical form in which they are presented to the roots, some compounds of an element such as copper being far more toxic than others. *Organic* poisons, on the other hand, are frequently developed within the plant in the ordinary course of metabolism. When this occurs they do not appear to function as poisons, but enter in some way into the economy of the plant. If, however, these substances be presented to the roots for absorption, even in very low concentration, they may be toxic to a high degree. Many of these organic compounds are of considerable commercial or economic interest, so that much research has been done on their presence in various species of plants, giving rise to a great bulk of literature, largely pharmaceutical in character. The action of these same substances when applied to the roots has hitherto been largely overlooked, and little work has been published except for a very few compounds. This is in striking contrast to the vast literature dealing with inorganic poisons, and may be largely due to the fact that our knowledge of the organic constituents of the soil is very incomplete, owing to the great difficulty of identifying and isolating them, whereas the determination of inorganic constituents is less difficult and has been vigorously pursued for the last hundred years at least.

It has long been known that various poisons act as stimulants to the

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animal body if they are utilized in very small quantities, and this knowledge has been made full use of by medical science. In some quarters the idea gradually evolved itself that *all* poisons behave as stimulants under favourable conditions, and it was assumed that this applied to plants as well as animals. In 1896 the position of affairs was thus summed up by Hüppe:[1] 'Jeder Körper, der in bestimmter Concentration Protoplasma tödtet und vernichtet, in geringeren Mengen die Entwickelungsfähigkeit aufhebt, aber in noch geringeren Mengen, jenseits eines Indifferenzpunktes, umgekehrt als Reiz wirkt und die Lebenseigenschaften erhöht.' This theory has been frequently put to the test, especially with regard to metallic compounds, and the results indicate that it is by no means a universal law, but that while toxic compounds reach an indifferent point as the concentration decreases, it does not always happen that stimulation occurs with still greater dilution.[2] There is less experimental evidence with regard to organic compounds, so during the last four years an attempt has been made at Rothamsted to gain further information with regard to some of the more common and economically interesting substances.

Hydrocyanic Acid.

This substance has possibly attracted more attention than any other plant constituent on account of its deadly poisonous nature and its presence in many articles used as food for man and animals. Numerous cases of poisoning by Soy beans (*Phaseolus lunatus*) and Sorghum (*Sorghum vulgare*) have occurred, and the economic importance of this fact has given an impetus to investigations on the subject. Prussic acid is formed by a goodly number of plants, representative of a variety of natural orders, of which Rosaceae furnishes a considerable number of instances. The presence of prussic acid in the oil of bitter almonds had long been known, but in 1830 Robiquet and Boutron Charlard[3] recognized that it did not really exist as such in the fruit, but that water was essential for its formation; they realized that bitter almonds contained a principle—amygdalin—which under certain conditions gives rise to prussic acid. It has since been recognized that hydrocyanic acid as such rarely occurs in plants, but that it is evolved by the interaction of cyanogenetic glucosides (such as amygdalin) and enzymes (such as emulsin) in the presence of water. Usually these two essential principles are localized in the same plant or parts of plants, but in some cases one may be present without the other, when there is no formation of hydrocyanic acid unless the missing constituent is supplied in some way. Amygdalin was the first cyanogenetic

[1] Hüppe, F.: Naturwissenschaftliche Einführung in die Bakteriologie. Wiesbaden, 1896. Quoted by Copeland, Bot. Gaz., 1903, vol. xxxv, p. 83.

[2] See Brenchley, W. E.: Inorganic Plant Poisons and Stimulants. Cambridge University Press, 1914.

[3] Robiquet et Boutron Charlard: Ann. Chim. et Phys., vol. xliv, 1830, pp. 352–82.

glucoside to be recognized (1830), but in 1906 Greshoff[1] tabulated about a dozen cyanogenetic compounds which had been discovered by various observers in the Vegetable Kingdom, several being associated with particular plants or groups of plants.

The function of these cyanogenetic compounds in the metabolism of the plant is very obscure, and investigators express considerable disagreement on the matter. Latham (1886)[2] indicated a possible relationship between certain cyanogenetic compounds and various albuminous bodies that occur in animal tissues, suggesting that possibly the latter may be built up from substances derived from the decomposition of the former. Hébert[3] (1898) stated that A. Gautier had put forward the theory that hydrocyanic acid forms the basis of the synthesis of the vegetable albuminoids. The theory is that absorbed nitrates are dissociated, partly because of their great dilution in the cell sap, and partly because of the natural acidity of sap. The free nitric acid reacts with formic aldehyde in the green cells, giving rise to HCN, CO_2, and H_2O.

$$2\,HNO_3 + 5\,CH_2O = 2\,HCN + 3\,CO_2 + 5\,H_2O.$$

It was suggested, however, that the HCN when formed does not remain as such, but is promptly transformed in most cases.

In 1904 Treub[4] made a very full exposition of the theory that hydrocyanic acid is the starting-point for the production of primary nitrogenous bodies in plants. He suggested that in most plants, directly the HCN is formed, it enters into such stable compounds that its presence cannot be detected. Later on he stated that possibly HCN is the first recognizable product of the assimilation of nitrogen and perhaps even the first nitrogenous compound that is formed. Treub[5] repudiated the suggestion of the protective function of HCN on the grounds that it has neither a nauseous smell nor a taste that is repugnant to animals, and that it has even been found that some lower members of the Animal Kingdom, such as nematodes, arachnids, and others, are definitely encouraged by the presence of hydrocyanic acid in the tissues of the plants on which they feed and live.

Guignard[6] in dealing with the cyanogenetic glucosides found in plants concluded that they functioned as reserve materials, though perhaps of an unusual nature. He suggested that while they appeared to be nutritive, yet possibly their actual rôle varies with their constitution.

In 1906 Dunstan and Henry[7] summed up the march of ideas concerning the function of hydrocyanic acid in plants thus:

[1] Greshoff, M.: Bull. Sc. Pharm., vol. xiii, 1906, pp. 589–602.
[2] Latham, P. W.: Brit. Med. Journ., vol. i, 1886, pp. 629–36.
[3] Hébert, A.: Ann. Agron., vol. xxiv, 1898, pp. 416–40.
[4] Treub, M.: Ann. Jard. Bot. Buit., vol. xix, 1904, pp. 86–145; vol. xxiii, 1909, pp. 85–118.
[5] Ibid., vol. xxi, 1907, pp. 107–14.
[6] Guignard, L.: C. R., vol. cxli, 1905, pp. 1193–201; vol. cxlvii, 1908, pp. 1023–8.
[7] Dunstan, W. and Henry, T. A.: Report Brit. Ass. York, 1906, pp. 145–57.

1. That it is a waste product of no metabolic importance.

2. That it is a means of protection against marauders.

3. That it is an intermediate product in the synthesis of proteids, a view to which Henry[1] himself is favourable.

Though Treub's idea as to the function of hydrocyanic acid in metabolism received support from many other investigators, Guerin raised considerable opposition to it. Guerin[2] held that the relative rarity of hydrocyanic acid in plants constituted a serious objection to the adoption of the hypothesis that it is one of the materials at whose expense nitrogenous matters are elaborated. He felt that this hypothesis, which admits that HCN constitutes the first product of assimilation in green plants, does not meet, in every case, objections from the chemical point of view; that, while admitting that in certain cases HCN serves for the building up of albuminoid matters, one must also admit that its rôle completely escapes one when it is found engaged in those complex molecules which constitute the glucosides. The leaves of elder and passion flowers, at the time of their fall, contain as much cyanogenetic glucoside as during the preceding months, so that it is very difficult to attribute to this body the rôle of a reserve material. Also, it is still less satisfactory to see in these compounds a protective substance against the attacks of animals and insects. Altogether Guerin maintained the incompleteness of our knowledge as to the rôle of hydrocyanic acid in plants, and emphasized the need for further investigation in order to solve the problem, which is one of great importance in vegetable biology.

In view of Treub's suggestion as to the part played by HCN in plant nutrition a few experiments have been made on the action of HCN when supplied to plants from the outside. It is evident that while certain (and perhaps most) plants can elaborate some amount of HCN within their tissues, yet this same substance is most toxic in nature when offered from outside. Townsend[3] tested the effect of hydrocyanic acid gas upon grains and other seeds under different conditions. He found that *dry* seeds are very resistant to the action of the gas—that short exposures do not affect germination at all, and that exposure for several months to gas generated from 1 grm. KCN per cubic foot of air does not altogether destroy the power of germination. *Damp* seeds, however, are far more affected, the length of soaking determining the reaction to the hydrocyanic acid gas. HCN is also capable of holding the germination of soaked seeds in abeyance for some considerable time without destroying their vitality even when other conditions are favourable to growth.

[1] Henry, T. A.: Sci. Prog., vol. i, 1906, pp. 39–50.

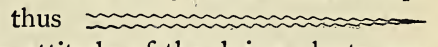
[2] Guerin, P.: Revue Scient., vol. viii, 1907, pp. 65–74, 106–10.

[3] Townsend, C. O.: Bot. Gaz., vol. xxxi, 1901, pp. 241–64.

Molliard[1] grew radishes under aseptic conditions in glass tubes, offering them 1 part in 1,000 of various nitrogenous compounds added to a mineral solution free from nitrogen, with 5 per cent. pure glucose. A control set received the same solution but no nitrogen. After six weeks' growth the order of usefulness of the compounds, as shown by the mean dry weights, was: (1) urate of sodium, (2) aspartic acid, (3) glycine, (4) legumin, (5) sodium cyanide, (6) amygdalin, (7) hydrocyanic acid, (8) leucine. As a matter of fact the plants with the last three substances did not look much better than the controls with no nitrogen. It is difficult to attach much importance to these results since the concentration of HCN used—1/1,000—is most highly toxic to plant life, as the Rothamsted experiments have shown, and also the control plants grown under aseptic conditions without any source of nitrogen would naturally fail to develop, as there is little or no store of nitrogenous reserves in the seed of the radish.

The toxic action of hydrocyanic acid on plants is as well marked with animals. Fungi are as a rule able to resist the action of poisons much better than are the higher plants, but prussic acid is a very violent poison even for them.[2]

Experiments have been made at Rothamsted to see whether the theory of stimulation with weak concentrations holds good with regard to hydrocyanic acid. Numerous experiments were carried out with peas and barley in water cultures in various strengths of nutrient solutions, and no sign of stimulation was found in any case, even with as little poison as 1 part in 1,000,000,000, below which concentration it is hardly conceivable that any effect can be produced. Great care was taken that the prussic acid and other substances used were of the highest possible degree of purity. The prussic acid was made afresh for each set of experiments, and was distilled from pure potassium cyanide and tartaric acid. The distillate was taken in distilled water and the concentration of the solutions was estimated by Liebig's method with silver nitrate.

The initial experiments on peas demonstrated the great toxicity of hydrocyanic acid. The concentrations ranged from 1/1,000 to 1/10,000,000, and the plants were grown in the usual Rothamsted food solution. Within two days the plants with 1/1,000 HCN were killed without making any development, and not only so, but the green colour had entirely disappeared from the plumules. Lower strengths, to 1/10,000, took rather longer to kill the plants. A little shoot growth was made in some cases, which at first appeared quite healthy, but the roots utterly failed to develop. The roots became intensely contracted, thus  and rapidly lost their turgescence. The attitude of the dying plants was most characteristic. The stems shrivelled from the junction with the

[1] Molliard, M.: Bull. Soc. Bot. France, lvii, 1910, pp. 541–7.

[2] Clark, J. F.: Bot. Gaz., vol. xxviii, 1899, pp. 289–327, 378–404.

cotyledons upwards, so that the shoots fell over, giving the plants the appearance of literally 'lying down to die'. This is quite unlike the usual phenomenon when pea plants are killed by poison or otherwise, since as a rule a fairly upright position is maintained to the very end. With concentrations down to 1/100,000 the plants remained apparently unaffected for a day or two, but eventually all were killed and completely withered within two or three weeks. In some cases the contraction of the roots was so intense that they were completely withdrawn from the solutions. In no single instance were any laterals formed, and, as is usual, the roots were always seriously affected earlier than the shoots.

Down to this limit of concentration no ultimate growth had been possible under the conditions of experiment. With 1/400,000 HCN, however, a change occurred. At the beginning the roots showed contraction towards the tips, but there was some attempt to put out laterals, which were very short and were chiefly developed above the surface of the water. For some long time this condition of affairs continued; the laterals continued to form, but refused to enter the solutions for about four weeks. At last they proceeded to elongate, and though the roots remained bunchy in appearance and medium in length for some time longer, yet they finally became more normal in appearance, though the plants remained rather small to the end.

As the concentrations decreased still farther the symptoms of poisoning gradually disappeared and the plants approached the controls more nearly. It is probable that some slight toxic action continued to manifest itself even with 1 part HCN in 4,000,000 or 10,000,000 solution. It must be borne in mind that the exact concentration at which certain phenomena occur cannot be fixed definitely for all experiments. These plants were growing from Oct. 3 to Feb. 4, during the slow-growing period of the winter months. It is more than probable that the time of year, amount of available light and heat, and many other factors interact in determining the exact action of a particular concentration in any experiment, but still, within a certain range, the sequence of phenomena remains constant.

In the experiments with barley, Feb. 16 to April 18, the concentrations of HCN ranged from 1/100,000 to 1/1,000,000,000, and both strong and weak food solutions[1] were tested. In both cases with the strongest solu-

[1]

	Strong nutrients.	*Weak nutrients.*
	grm.	grm.
Potassium nitrate	1·0	0·2
Sodium nitrate	—	0·5
Magnesium sulphate	0·5	0·1
Calcium sulphate	0·5	0·1
Potassium di-hydrogen phosphate	0·5	0·1
Sodium chloride	0·5	0·1
Ferric chloride	0·04	0·04
Distilled water, to make up	1 litre	1 litre

tions of HCN growth was entirely suspended for about a month. At the end of this time most of the plants started into growth, slowly at first, more rapidly later on, so that at the end of the experiment they were healthy and fairly normal in type, but very small in size. With lower concentrations the initial delay in starting was less marked or did not occur at all, but the toxic action of the poison was very evident to the eye down to

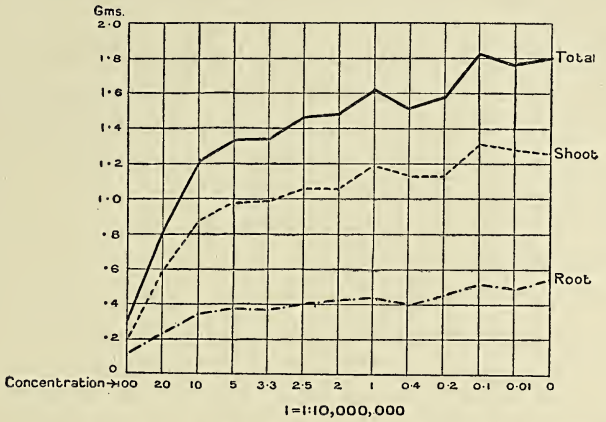


FIG. 1. Average dry weights of 10 series of barley plants grown in strong nutrient solutions in the presence of differing amounts of hydrocyanic acid. Feb. 16–April 18, 1914.

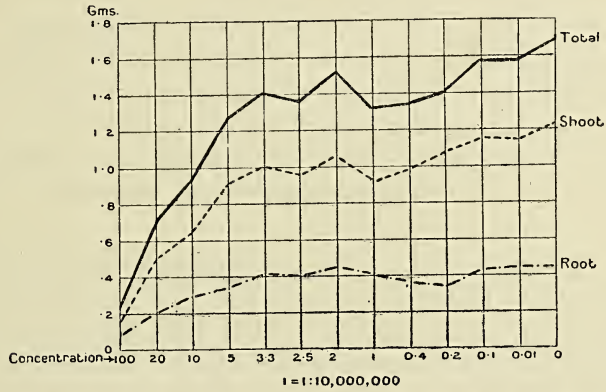


FIG. 2. Average dry weights of 10 series of barley plants grown in weak nutrient solutions in the presence of differing amounts of hydrocyanic acid. Feb. 16–April 18, 1914.

a very considerable dilution. From the outset the plants growing in the weaker food solutions were rather less developed than in the strong solutions, but the same order of development was maintained (Figs. 1 and 2). It was soon evident that the roots were able to withstand more poison in the strong solution than in the weak. With high concentrations, as 1/100,000 and 1/500,000, the roots began to make growth at an earlier date in the strong nutrients, so that the plants pulled ahead and ultimately made

more growth (as shown by the total dry weight) than the plants grown in the weak food solutions with the same concentrations of prussic acid. This difference was evident throughout the life of the plants, though it was less obvious in the later stages.

The experiment was repeated later in the year, April 25 to June 9, with similar results, except that as growth was more vigorous at the later date all the differences were magnified, so that the graph of the dry weights showed a much steeper curve.

The delay in development followed by more or less normal growth may perhaps be due to the behaviour of the poison itself. Prussic acid in the presence of water tends to hydrolyse gradually, giving ammonium formate, which may possibly be assimilated by the plant. Although high concentrations of prussic acid are so toxic, it seems evident that at certain lower strengths the poison simply paralyses the tissues, preventing growth entirely without destroying vitality. As the HCN hydrolyses in the presence of water in the slightly acid food solution the paralysing effect slowly wears off and eventually the plant starts into growth, which is more or less normal in character according to the amount of permanent injury the HCN was able to inflict in addition to acting as a paralyser. It may well be that this paralysing action is simply a manifestation of a toxic action which shows itself by inhibiting growth without actually killing the tissues, so that when the inhibitor is removed the plant is in many cases able to exercise its inherent recuperative power and to make more or less growth according to circumstances. Possibly, too, this temporary inhibition of growth without ultimate loss of vitality is analogous to Townsend's results (quoted earlier in the paper) on the power of hydrocyanic acid gas to suspend the germination of damp seeds for considerable periods without destroying their power to start growing when the inhibitory agent is removed.

In view of the possibility of the formation of ammonium formate in the solution a set of plants was grown with formic acid (H_2CO_2) as the poison in strengths corresponding to those used with prussic acid, 1·704 formic acid being equivalent to 1 part prussic acid. 1·704/50,000 formic acid was very variable in its action, as some plants were killed by the poison while the rest started into growth after an initial check and eventually produced quite good plants, though the total weight remained behind that of the controls. Plants grown with the same strength of HCN were killed outright at the very beginning. With 1·704/100,000 formic acid there was some delay in starting into growth, but the plants soon began to make headway and presented a striking contrast to those which were grown in the corresponding strength of HCN. Below this concentration the formic acid played no part, as plants in 1·704/500,000 were as good or better than the controls, and lower strengths gave no indication

of toxic or stimulant action. This indicates that if the HCN is hydrolysed into formic acid or its derivatives the highly toxic substance is replaced by another which is practically indifferent in its action except when it is present in relatively large quantities. It is evident, though, that the higher concentrations of prussic acid do not merely paralyse growth, but kill the plant so that there is no possibility of recovery if and when the poison is hydrolysed.

In order to see whether the toxic action is due to the cyanogen radicle independent of the combination in which it is presented to the roots, a series of barley was grown with sodium cyanide replacing the HCN in equivalent concentration (1·815 sodium cyanide = 1 HCN). A parallel

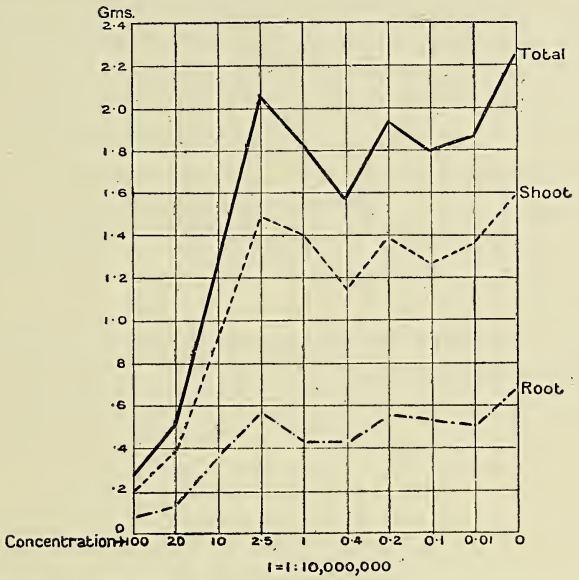


FIG. 3. Average dry weights of 8 series of barley plants grown in strong nutrient solutions in the presence of differing amounts of sodium cyanide. April 23–June 9, 1914.

series with prussic acid was grown alongside, and it was found that the results were similar with both combinations of the cyanogen radicle, though growth was rather more seriously depressed with the 1·815/500,000 sodium cyanide than with the 1/500,000 HCN. This indicates that the toxic agent in prussic acid is the cyanogen radicle, and that it acts in the same poisonous way when presented in other combinations than that of HCN (Fig. 3).

The experiments have shown that prussic acid and sodium cyanide are highly toxic to plants down to a weak concentration, the poisonous action still being evident at 1/4,000,000 strength and even lower. Although the dilution was carried down to 1/1,000,000,000 prussic acid no trace of stimulation was obtained in any case. In this respect HCN behaves in the

same way as the various inorganic poisons, zinc, arsenic, and copper, which were tested in earlier work.

Summary.

1. Prussic acid is very toxic to peas and barley. All strengths up to and including 1/100,000 killed peas outright, either immediately or after a short interval of poor growth. All strong concentrations kill barley, but with 1/100,000 a period elapses during which no growth occurs, after which a little progress is made, though the plants never attain any size.

2. The peas killed by prussic acid shrivel from the cotyledons upwards and the roots contract so intensely that they are often completely withdrawn from the nutrient solution. Barley roots decline to enter strong solutions at all, but often put out laterals which stop short at the surface of the solution and form the characteristic bunchy root so often seen with this plant when in the presence of poison.

3. Formic acid is comparatively harmless to barley, except in very strong concentrations, whereas sodium cyanide is quite as toxic as prussic acid, indicating that the cyanogen radicle is the toxic agent in the cyanogenetic compounds.

4. No trace of stimulation in peas or barley has been obtained with any of the compounds tested.

The Controlling Influence of Carbon Dioxide.

IV. On the Production of Secondary Dormancy in Seeds of Brassica alba following Treatment with Carbon Dioxide, and the Relation of this Phenomenon to the Question of Stimuli in Growth Processes.

BY

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With Plate XXIII and five Figures in the Text.

CONTENTS.

§ 1. INTRODUCTION.

IN previous papers (Kidd, 1 and 2) it has been shown that carbon dioxide in relatively small quantities in the atmosphere inhibits the germination of seeds. The actual percentage of carbon dioxide required to produce inhibition was found to vary with temperature and with oxygen supply. At 3° centigrade 2 per cent. to 4 per cent. carbon dioxide produced inhibition, whilst at 20° centigrade 25 per cent. to 30 per cent. carbon dioxide was required. With 5 per cent. oxygen, 9 per cent. to 12 per cent. carbon dioxide produced inhibition, but with 20 per cent. oxygen 20 to 25 per cent. carbon dioxide was required (temperature 17° C.).

In all seeds tested, except those of *Brassica alba*, germination in the normal way was found to follow the removal of the inhibiting carbon dioxide. In the case of *Brassica alba* seeds, the dormancy induced by carbon dioxide continued, however, after the removal of the inhibiting

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gas-mixtures. This induced dormant condition of White Mustard seeds following as an 'after'-effect of carbon dioxide inhibition is here termed 'secondary dormancy' (cf. Crocker (3), p. 114 et seq.).

Normally, *Brassica alba* seed germinates quickly and uniformly in twenty hours at ordinary temperatures with a high percentage of germination.[1] The behaviour of *Brassica alba* seeds showing secondary dormancy as an 'after'-effect of short CO_2-treatment was in striking contrast to this. The typical behaviour of a sample of *Brassica alba* seed removed from an inhibiting partial pressure of carbon dioxide is shown by the following table:

TABLE I.

*Number of days after removal from the inhibiting gas-mixture.**	*Number of germinations.*
6	17
16	49
44	73
68	103
365	135

Number of seeds used = 153.

* 20 % CO_2; 20 % O_2 for 19 days.

In this experiment, which was extended to twelve months, during the whole of which period the seeds lay on damp sand in a moist atmosphere, it is seen that a few seeds are constantly germinating in a sporadic manner. This slow sporadic germination is typical of *Brassica alba* seeds showing secondary dormancy. After twelve months, in this particular case, eighteen seeds out of an original sample of 153 inhibiting seeds still remained ungerminated. These eighteen seeds finally gave rise to perfect seedlings.

Occasionally it was found that the secondary dormancy produced was not complete. A rush of germinations took place during the first few days after the removal of the seeds from the inhibiting gas-mixtures, but after this period, however, characteristic sporadic germination set in. For example, in ten days 41 per cent. of a sample of eighty inhibited seeds had germinated, whilst only 5 per cent. more germinated in the following twenty days.

The object of the work described in the present paper has been to discover, if possible, the controlling causes of this secondary dormancy which follows as an 'after'-effect of carbon dioxide inhibition in the case of *Brassica alba*.

§ 2. The Production of Secondary Dormancy in Seeds of *Brassica alba*.

In view of the fact that the degree of secondary dormancy had been observed to vary considerably, a complete series of experiments was conducted in order to determine the optimum gas-mixtures with regard

[1] The *Brassica alba* seeds used in this investigation gave germination tests of 98 % to 100 %.

to carbon dioxide and oxygen for the production of secondary dormancy as an 'after'-effect.

In these experiments an arbitrary period of ten days was allowed before determining the number of seeds showing secondary dormancy. This period was chosen as a result both of our general experience and of specific experiments, to one of which we have already referred. In result it was found that a high percentage of seeds showing prolonged secondary dormancy could only be obtained under quite limited conditions.

Oxygen.

The following experiments deal with the question as to how far the degree of secondary dormancy is influenced by the concentration of oxygen used during the primary period of inhibition. Two series were conducted:

Table II.

Effect of various Partial Pressures of Oxygen (25 *per cent. Carbon Dioxide present*) *on the Production of Secondary Dormancy in Seeds of* Brassica alba.

No. of seeds in experiment.	*Approximate percentage of oxygen. 25 % CO_2 in all cases.*	*Days.*	*Germinations whilst in presence of inhibiting gas-mixtures.*	*Number of inhibited seeds transferred to air.*	*Germinations after removal of the seeds to air. Number of days—* 3	4	5	6	7	8	9	10	*Percentage of seeds showing secondary dormancy.*	*Percentage of seeds (other than those which germinated in the presence of CO_2) showing injury to the radicle.*
30	0 %	21	0	30	3	8	13	16	16	16	18	22*	0 %	80 % (approx.)
30	0 %	15	0	30	0	5	13	19	21	24	24	24*	0 %	60 %
30	5 %	21	0	30	8	9	12	12	12	12	13	14	53 %	23 %
50	20 %	17	7†	43	0	1	1	1	2	2	2	2	96 %	5 %
30	30 %	21	15†	15	0	0	0	0	0	0	1	1	93 %	0 %
30	75 %	21	14†	16	0	1	1	2	2	2	3	3	81 %	6 %

Mean temperature of laboratory during inhibition period, 15° C.
* Remainder dead.
† All showing injury to radicle.

with 25 per cent. carbon dioxide present (Table II); and with 35 per cent. carbon dioxide present (Table III). The amount of oxygen present was found to influence the degree of secondary dormancy. Thus in Table II it is seen that with concentrations of oxygen below 20 per cent., the percentage of seeds showing secondary dormancy decreases with the fall of oxygen. With 20 per cent. oxygen present 96 per cent. of the seeds show secondary dormancy, and higher concentrations of oxygen have little effect, the percentage of seeds showing secondary dormancy remaining high.

In Table III the results are of a similar nature. In the absence of oxygen no secondary dormancy is obtained, whereas with 15 per cent. or 30 per cent. oxygen present approximately half the seeds exhibit secondary dormancy. The fact that the amount of secondary dormancy obtained

TABLE III.

Effect of various Partial Pressures of Oxygen (35 *per cent. Carbon Dioxide present*) *on the Production of Secondary Dormancy in Seeds of* Brassica alba.

Number of seeds used.	*Approximate percentage of* O_2 *: 35 %* CO_2 *present in all cases.*	*Days.*	*Germinations whilst in presence of inhibiting gas-mixtures.*	*Number of inhibited seed transferred to air.*	*Germinations after removal of seeds to air.* *Days.* 4	5	6	7	8	10	*Percentage of seeds showing secondary dormancy.*	*Percentage of seeds (other than those which germinated in the presence of* CO_2*) showing injury to the radicle.*
50	0 %	21	0	50	17	23	27	30	32	35*	0 %	78 %
50	15 %	21	5	45	4	13	15	17	22	24	47 %	10 %
50	30 %	21	3	47	4	12	13	13	17	22	53 %	4 %

Mean temperature of laboratory during inhibition period, 13° C.

* The 15 ungerminated seeds were dead.

with 35 per cent. CO_2 does not rise much above 50 per cent. in comparison with the 96 per cent. of secondary dormancy obtained with 25 per cent. CO_2 finds an explanation in the following section dealing specifically with the effect of various concentrations of carbon dioxide on the production of secondary dormancy.

It thus appears that for the production of secondary dormancy the seeds must be supplied with oxygen during the primary period of direct inhibition by carbon dioxide.

In the last column of the above tables the percentage of seeds (other than those germinating in the presence of the inhibiting gas-mixtures) which showed injury to the radicle is given. A marked correlation appears between the injury to the radicle and percentage of seeds showing secondary dormancy: the greater the injury the less the amount of secondary dormancy produced.

Where secondary dormancy is almost complete, the percentage of seeds showing injury is practically nil. In fact it may be said that any seed which after ten days in air shows typical secondary dormancy (in other words, is practically indistinguishable from a newly swollen normal seed) is certainly uninjured with regard to the radicle. The condition with regard to injury or non-injury of the seeds showing secondary dormancy was examined by the removal of the seed-coats, a treatment which, as pointed out in a previous paper (Kidd, 1, p. 416), will cause germination at any time during the period of secondary dormancy.

Carbon Dioxide.

The following experiments deal with the question as to how far the degree of secondary dormancy is influenced by the concentration of carbon dioxide used during the primary period of inhibition.

These experiments were conducted in three series: with 20 per cent. oxygen present, with 10 per cent. oxygen present, and with 0 per cent. oxygen present.

Table IV gives the results of the series of experiments with a range of carbon dioxide concentrations, 20 per cent. oxygen being present in all

Table IV.

Effect of various Partial Pressures of Carbon Dioxide (20 *per cent. Oxygen present*) *on the Production of Secondary Dormancy in Seeds of* Brassica alba.

Number of seeds.	*Approximate percentage of* CO_2*: 20%* O_2 *in all.*	*Days.*	*Germination whilst in presence of inhibiting gas-mixtures.*	*Number of inhibited seeds transferred to air.*	*Germination after removal of the seeds to air. Number of days—* 3	4	5	6	7	8	9	10	*Percentage of seeds showing secondary dormancy.*	*Percentage of seeds (other than those which germinated in the presence of* CO_2*) showing injury to radicle.*
50	25 %	17	7	43	0	1	1	1	2	2	2	2	96 %	5 %
50	50 %	17	4	46	1	6	15	24	30	30	30	34	26 %	65 %
50	80 %	17	0	50	0	16	22	26	29	29	29	32	30 %	66 %

Mean temperature of laboratory during inhibition period, 15° C.

cases. The percentage of carbon dioxide used during the primary period of inhibition has a marked effect upon the degree of secondary dormancy obtained. When high percentages of carbon dioxide are used for the production of primary inhibition, the percentage of seeds showing secondary dormancy as an 'after'-effect is small and at the same time a considerable percentage of seedlings show injury to the radicle. The optimum treatment for the production of secondary dormancy in these experiments, in which the concentration of oxygen present amounts to 20 per cent., is with 25 per cent. carbon dioxide.

The results of the experiments with a range of carbon dioxide concentrations and 10 per cent. oxygen present in all cases (Table V) are of a similar nature. Following the treatment with the higher percentages of carbon dioxide injury occurs and the number of seeds showing secondary dormancy is small. Both the maximum 'after'-effect of secondary dormancy (73 per cent.) and the minimum percentage of injury (2 per cent.) occur in these experiments after treatment with 10 per cent. carbon dioxide.

TABLE V.

Effect of various Partial Pressures of Carbon Dioxide (10 *per cent. Oxygen present*) *on the Production of Secondary Dormancy in Seeds of* Brassica alba.

No. of seeds used.	*Percentage of CO_2 (10 % O_2 in all).*	*Days.*	*Germination in the presence of the gas-mixtures.*	*No. of inhibited seeds transferred to air.*	*Germination after removal of the seeds in air. Number of days.* 1	3	4	5	6	7	10	*Dead.*	*Percentage of seeds showing secondary dormancy.*	*Percentage of seeds (other than those which germinated in the presence of CO_2) showing injury to the radicle.*
50	5 %	21	36	14	1	6	7	9	9	9	9	3	14 %	21 %
50	10 %	21	5*	45	0	5	9	10	10	11	12	0	73 %	2 %
50	15 %	21	5*	45	0	1	3	4	8	10	16	1	62 %	4 %
50	25 %	21	0	50	0	6	10	15	17	19	19	3	56 %	10 %
50	50 %	21	0	50	0	10	13	18	18	21	25	0	50 %	20 %
50	70 %	21	0	50	0	20	24	30	36	37	41	7	4 %	70 %
50	90 %	21	0	50	0	12	19	27	30	32	40	9	2 %	88 %

Temperature during inhibition period, 13° C.

* Show injury to the primary root.

In the series of experiments with no oxygen present (Table VI) no secondary dormancy was obtained. The main fact emerging is that secondary dormancy cannot be produced by the exposure of seeds to carbon dioxide alone in any concentration. This result is in accordance with the experiments described in the previous section.

TABLE VI.

Effect of various Partial Pressures of Carbon Dioxide (*no Oxygen present*) *on the Production of Secondary Dormancy in Seeds of* Brassica alba.

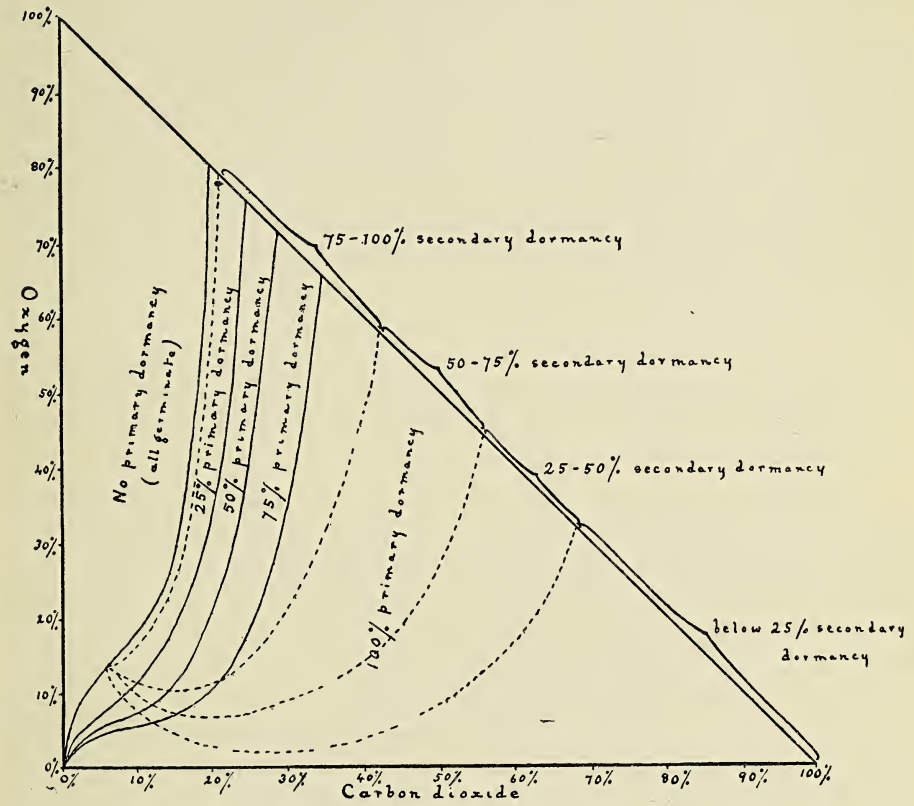
Number of seeds used.	*Approximate percentage of CO_2 (no O_2 present).*	*Days.*	*No. of germinations in presence of CO_2*	*Number of seeds transferred to air.*	*Germination after removal of the seeds to air. Days.* 6	13	15	15	15	15	15	16	*Percentage of seeds showing secondary dormancy.*	*Percentage of seeds (other than those which germinated in the presence of CO_2) showing injury to the radicle.*
30	0 %	21	0	30	6	13	15	15	15	15	15	16	0 %*	70 %
30	25 %	15	0	30	0	5	13	19	21	24	24	24	0 %*	60 %
30	50 %	15	0	30	0	6	24	26	26	26	27	30	0 %	20 %
30	80 %	15	0	30	0	2	4	8	11	13	15	15	0 %*	100 %
30	100 %	21	0	30	0	0	0	0	1	2	3	5	0 %*	100 %

Temperature during inhibition period, 13° C.

* All seeds still ungerminated after 10 days in air were either dead or so badly injured with regard to the radicle that they finally decayed.

Marked injury to the radicle occurs in these experiments in the absence of oxygen, and a correlation between subsequent germination and injury to the radicle thus appears again.

With the intermediate concentrations of carbon dioxide, following which the injury occurring is much less,[1] the correlation between germination and injury is not so great. Thus, following 50 per cent. carbon dioxide in the absence of oxygen, only 20 per cent. of the seeds showed visible injury to the radicle although all germinated and none showed secondary dormancy. Nevertheless, the conditions in the absence of oxygen are undoubtedly injurious, and do, if maintained, result in death. If the



TEXT-FIG. 1.

internal reactions which cause injury in the embryo are also those which are responsible for germination, as the correlation observed in the above experiments indicates, this particular case is of interest in that it shows that these internal changes initiate growth in their early stages before they have proceeded far enough to produce *visible* injury and cell-death.

Summing up the results of these experiments dealing with the conditions necessary for the production of secondary dormancy in *Brassica*

[1] This in itself is remarkable, and is to be attributed to an effect of carbon dioxide in depressing anaerobic processes which give rise to toxic products (cf. Kidd, 4).

alba seeds, it appears (i) that the phenomenon does not occur if oxygen has not been present during the primary period of inhibition or if carbon dioxide has been used in too high a concentration; (ii) that those conditions during the primary period of inhibition which prevent the subsequent occurrence of secondary dormancy, are found at the same time to exercise an injurious effect upon the radicle which is visible when the seeds subsequently germinate, and that a correlation exists therefore between internal changes in the radicle resulting in injury and occurrence of germination.

The relation of secondary dormancy to the conditions of oxygen and carbon dioxide concentration used during the primary inhibition period can be clearly seen from the accompanying diagram (Text-fig. 1). An 'after'-effect amounting to 100 per cent. secondary dormancy is only obtained within quite narrow limits of possible inhibiting mixtures of carbon dioxide and oxygen. It is only in the region of carbon dioxide concentration immediately above the critical concentration needed to produce inhibition, that the maximum degree of secondary dormancy is obtained.

Relation between the Percentage of Secondary Dormancy produced and the Duration of the Primary Period of Inhibition in the Presence of Carbon Dioxide.

In addition to the facts described above with regard to the influence of excess of carbon dioxide or lack of oxygen during the primary period of inhibition upon the degree of secondary dormancy, it has been found further that the percentage of secondary dormancy is dependent upon the length of the primary period of inhibition in the presence of carbon dioxide. Tables VII and VIII give the results obtained in two experiments. It is clear that the full effect of secondary dormancy is not produced at all quickly, but that at ordinary temperatures a period of two to three weeks in the presence of carbon dioxide is required. The significance of this fact will be discussed later.

TABLE VII.

Time Factor in the Production of Secondary Dormancy.

Number of days in the gas-mixture (20 % CO_2 = 16 % O_2).	*Germinations 10 days after removal from the inhibiting gas-mixture.*	*Approximate percentage of secondary dormancy.*
2	100 %	0 %
5	66 %	34 %
10	57 %	43 %
17	17 %	83 %

30 seeds in each experiment. Mean temperature of laboratory, 12·5° C. (in the dark).

TABLE VIII.

Time Factor in the Production of Secondary Dormancy.

Number of days in the gas-mixture (30 % CO_2 = 14 % O_2).	*Germinations* 10 *days after removal from the inhibiting gas-mixture.*	*Approximate percentage of secondary dormancy.*
1	100 %	0 %
3	60 %	40 %
4	40 %	60 %
8	40 %	60 %
12	6 %	94 %

Mean temperature of laboratory, 14° C.

It has been shown that oxygen is necessary for the production of secondary dormancy in seeds of *Brassica alba*, since in the absence of oxygen during the primary period of inhibition injury occurs which prevents the phenomenon. Carbon dioxide is necessary because in the absence of this gas the primary period of inhibition in the presence of oxygen does not occur at normal temperatures. But it is not clear whether secondary dormancy is due to the specific action of carbon dioxide or simply to slow secondary changes occurring in the tissues of the fully swollen seed independently of the action of the carbon dioxide. In order to decide this point, fully-swollen seeds were prevented from germinating for a period of seven days by exposure to a subminimal temperature. On return to normal temperatures a full percentage of germination resulted, thus showing that carbon dioxide exercises a specific action in the production of secondary dormancy.

§ 3. Changes in the Seed-coat or Embryo of *Brassica alba* accompanying the Production of Secondary Dormancy.

It is clear that the causes underlying the persistence of the dormant condition of *Brassica alba* seeds must be looked for in changes produced either in the testa or in the embryo during the primary period of inhibition in the presence of carbon dioxide.

It has already been shown that when such changes, due to the lack of oxygen or to toxic concentrations of carbon dioxide, amount to injury to the radicle, secondary dormancy does not occur.

It is convenient at this point to analyse, on the lines of previous research work on dormancy in seeds, the possible causes controlling the phenomenon of secondary dormancy in uninjured seeds of *Brassica alba.*

A. A change in the seed-coat during the period of primary inhibition in the inhibiting gas-mixtures:

(i) Resulting in decreased permeability of the testa to oxygen sufficient to cause the prolonged secondary dormancy observed.

(ii) Resulting in decreased permeability of the testa to carbon dioxide, owing to which the tension of respiratory carbon dioxide in the tissues of the embryo during the period of secondary dormancy does not fall below

a critical value in relation to oxygen supply and temperature at which germination can take place.

(iii) Resulting in increased mechanical resistance, owing to which either (*a*) the full swelling of the embryo by physical water uptake to the critical point at which germination and further growth become possible is prevented, or (*b*) owing to which the radicle is unable to burst its way through the seed-coats by growth although the water supply is not the limiting factor.

B. Changes in the embryo during the period of primary inhibition in the inhibitory gas-mixtures, owing to which the embryo becomes less sensitive to growth conditions; in other words, there is a rise in the threshold value of the necessary growth stimulus, so that the same value in the case of some critical growth condition (e. g. of oxygen supply, CO_2 tension, moisture, temperature, hydrogen ion concentration, or other internal factor), which in the case of the untreated seeds was sufficient, although near the critical minimum, to cause germination, becomes subminimal after treatment with carbon dioxide.

Changes in the Permeability of the Seed-coats to Gases.

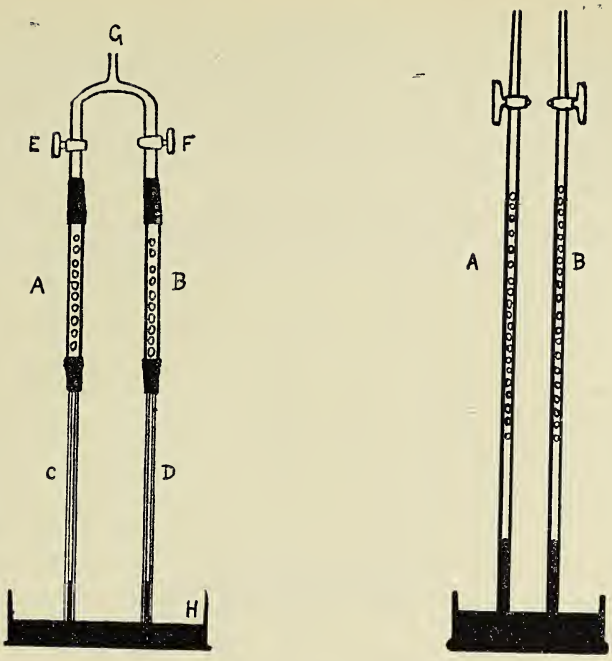
In order to test whether any change in the permeability of the testa to gases takes place during the immersion of the seeds in atmospheres of carbon dioxide, direct experiments were carried out. These were conducted in two series. In Series A the permeability of the testa to carbon dioxide was determined, whilst in Series B the object of the experiments was to ascertain the permeability of the seed-coats to oxygen.

Series A.

The principle of these experiments was as follows:

A number of fully swollen White Mustard seeds, inhibited and non-inhibited respectively, were brought suddenly into an atmosphere of pure CO_2; then the rate of CO_2 uptake was compared in either case. When no further uptake occurred, the atmosphere of CO_2 was quickly replaced by an atmosphere of nitrogen and the rate at which the CO_2 escapes was measured.

Fig. 2, *a*, represents the apparatus employed. The seeds are placed in the wide-bore tubes A and B, which are then connected to the capillary tubes C and D by means of rubber connexions as is shown in the diagram. The stop-cocks E and F are opened and a rapid current of CO_2 is turned on from G. The stop-cocks are then shut and the mercury bath H is raised into position. When absorption of CO_2 is complete, a current of nitrogen is passed through the apparatus in the same way, but after bringing the mercury bath into position an initial negative pressure is created by suction before shutting the stop-cocks. A modified form of the apparatus, in which the rubber connexions are avoided, is also shown in Fig. 2, *b*.



a. *b.*

TEXT-FIG. 2.

TABLE IX.

Exper. 1. *Passage of Carbon Dioxide into the Seed.*

Ten inhibited seeds showing secondary dormancy compared with ten normal seeds fully swollen after one day on wet sand. The outer testas were removed in both cases.

	Mercury rise in millimetres.	
Time.	*Inhibited seeds.*	*Non-inhibited seeds.*
6 min.	33	33
11 ,,	38·5	39
16 ,,	40·5	41
60 ,,	52	54
3 hrs.	59	61
17½ ,,	94	89

TABLE X.

Exper. 2. *Passage of Carbon Dioxide out of the Seed.*

The same seeds as in the above experiment, the carbon dioxide being displaced by a current of nitrogen after seventeen and a half hours.

	Mercury fall in millimetres.	
Time.	*Inhibited seeds.*	*Non-inhibited seeds.*
5 min.	8·5	8·5
10 ,,	12·5	13·5
16 ,,	14·5	16·5
30 ,,	16	21
50 ,,	17	24
80 ,,	20	32

TABLE XI.

Exper. 3. *Passage of Carbon Dioxide into the Seed.*

Eight inhibited seeds showing secondary dormancy compared with eight normal seeds fully swollen after one day on wet sand. Both outer and inner testas were left intact.

	Mercury rise in millimetres.	
Time.	*Inhibited seeds.*	*Non-inhibited seeds.*
5 min.	12	12
10 ,,	27	27
35 ,,	43	43
150 ,,	71	71

Exper. 4. *Passage of Carbon Dioxide into the Seed.*

Twenty inhibited seeds immediately after removal from 30 per cent. carbon dioxide compared with twenty normal seeds fully swollen after one day on wet sand. In this experiment the non-inhibited seeds allowed a quicker uptake of carbon dioxide than the inhibited ones, which was to be expected from the known difference between the initial internal concentrations of carbon dioxide within the seeds, i.e. a difference of 30 per cent. approximately.

TABLE XII.

Exper. 5. *Passage of Carbon Dioxide out of the Seed.*

Same seeds as in the above experiment, the carbon dioxide being displaced by a current of nitrogen after four hours. Weight of twenty seeds = 0·24 gramme. Approximate volume = 0·25 c.c.

	Escape of carbon dioxide.	
Time.	*Inhibited seeds.*	*Non-inhibited seeds.*
85 min.	0·125 c.c.	0·135 c.c.
16 hours	0·175 c.c.	0·195 c.c.

Exper. 6 and 7. *Passage of Carbon Dioxide into and out of the Seed.*

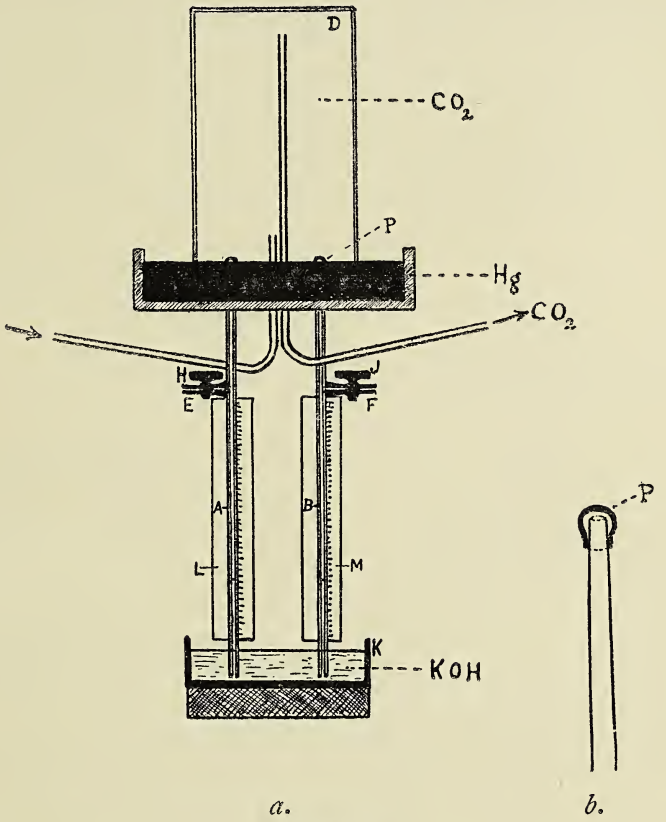
The same seeds as in the above experiment, carbon dioxide being again run into the apparatus. The subsequent rise in four and a half hours amounted to 0·140 c.c. in the case of the inhibited, and 0·160 c.c. in the case of the non-inhibited seeds. The carbon dioxide was then again displaced by nitrogen, and the subsequent escape of CO_2 from the seeds proceeded at an equal rate.

Series B.

In these experiments, free testas of equal size were obtained by cutting off a sector of the seed and carefully removing the embryo. The permeability to oxygen of testas from inhibited and non-inhibited seeds respectively was compared by direct experiment.

Text-fig. 3, *a*, represents the apparatus employed. The testas, P, to be compared were fitted over two narrow glass tubes, A and B, of equal bore,

to which they were securely fastened with silk thread (see Text-fig. 3, *b*). These glass tubes were inserted through corks in the bottom of a wooden trough. The joint between the testa and the glass tubes was sealed by mercury held in the trough. The glass vessel D, through which a current of carbon dioxide was slowly passed, was placed over the trough as shown in the diagram. For a few minutes a rapid current of oxygen was passed through the tubes A and B via the lateral connexions E and F. At a given moment the stop-cocks H and J were turned off, leaving an atmosphere of



Text-fig. 3.

pure oxygen in the tubes A and B, the lower ends of which were allowed to dip into a concentrated solution of potassium hydrate contained in the vessel K. Now, since any carbon dioxide which penetrates the testa-membrane was immediately absorbed by the KOH-solution, the actual rate of the passage of oxygen from the tubes A and B respectively through the seed-coat membranes was shown by the rise of the solution in these tubes, which was readily measured on the scales L and M. In this way the permeability of any two testas was directly compared.

The results thus obtained are tabulated below:

TABLE XIII.

Results of Experiments on the Permeability of the Seed-coats of White Mustard Seeds to Oxygen.

Exper. 1.

Remarks.	*Rise of KOH columns after an interval of:*			
	½ *hr.*	1½ *hrs.*	3½ *hrs.*	4½ *hrs.*
Testa from an inhibited seed.	6 mm.	12 mm.	23 mm.	25 mm.
Testa from a non-inhibited seed.	6 mm.	12 mm.	28 mm.	31 mm.

Both inner and outer testas present. Temperature 15° C.

Exper. 2.

Remarks.	*Rise of KOH columns after an interval of:*		
	1 *hr.*	1⅓ *hrs.*	2 *hrs.*
Testa from an inhibited seed.	9 mm.	11 mm.	15 mm.
Testa from a non-inhibited seed.	7 mm.	9 mm.	12 mm.

Both inner and outer testas present. Temperature 15° C.

Exper. 3.

Remarks.	*Rise of KOH columns after an interval of:*		
	1 *hr.*	1⅓ *hrs.*	3 *hrs.*
Testa from an inhibited seed.	9 mm.	11 mm.	20 mm.
Testa from a non-inhibited seed.	7 mm.	9 mm.	17 mm.

Both inner and outer testas present. Temperature 15° C.

Exper. 4.

Remarks.	*Rise of KOH columns after an interval of:*		
	20 *hrs.*	24 *hrs.*	40 *hrs.*
Testa from an inhibited seed	42 mm.	62 mm.	72 mm.
Testa from a non-inhibited seed.	43 mm.	63 mm.	73 mm.

Both inner and outer testas present. Temperature 15° C.

Exper. 5.

Remarks.	*Rise of KOH columns after an interval of:*					
	20 *min.*	40 *min.*	60 *min.*	80 *min.*	100 *min.*	120 *min.*
Testa from an inhibited seed.	4 mm.	13 mm.	21 mm.	28 mm.	47 mm.	53 mm.
Testa from a non-inhibited seed.	7 mm.	15 mm.	24 mm.	34 mm.	56 mm.	66 mm.

Inner testa only present. Mean temperature of laboratory, 15° C.

NB.—During these experiments the height of the barometer showed no appreciable change.

It is to be noted that the series of experiments dealing with the permeability of the testa to oxygen are less satisfactory inasmuch as single testas only are compared, and, moreover, a considerable amount of handling is unavoidable in setting up the experiments. In the series of experiments dealing with the permeability of the testa to carbon dioxide, the average effect of a number of seeds is measured and the seed-coats are intact.

The outstanding result of the above experiments is that the testa of White Mustard seeds, both in the inhibited and non-inhibited condition, is very permeable to oxygen and carbon dioxide. It cannot be said, however, that the experiments show that the testa of the inhibited seed is less permeable to either oxygen or carbon dioxide than that of the normal seed.

The conclusion indicated is that neither lack of oxygen nor accumulation of carbon dioxide in the embryo controls the phenomenon of secondary dormancy. This conclusion is confirmed by the results of experiments described in a following section, in which it was found that germination of inhibited *Brassica alba* seeds could not be forced either by increasing the oxygen pressure or by lowering the internal carbon dioxide concentration in the seeds by exposure to a vacuum (see Table XXII).

Changes in the Mechanical Resistance of the Seed-coats.

Crocker and Davis (5) have shown in the case of *Alisma Plantago*, a water-plant, the seeds of which normally lie under water, that while the seed-coat is readily permeable to water, the factor responsible for the non-germination of these seeds is the mechanical resistance of the testa to physical swelling of the embryo, whereby the latter remains dormant owing to an insufficient water content for growth and germination. When the testa is weakened by treatment with acids the expanding force of the seed-contents is sufficient to rupture it. Further swelling and uptake of water by the embryo can then take place, and a point is finally reached at which growth begins. The same result is obtained by experimental rupture of the seed-coat.

In the normal germination of White Mustard seeds it is clear that the rupture of the seed-coats by the radicle takes place as the result of growth,[1] and not as the result of physical swelling of the embryo. This may be concluded from the fact that no rupture of the seed-coats occurs in seeds which are prevented from germinating by lack of oxygen.

It is conceivable, however, that the action of carbon dioxide may toughen the testa, with the result that seeds set to germinate under inhibiting conditions of carbon dioxide do not swell to the point at which sufficient water is present for the growth of the embryo after removal from the inhibiting gas mixture. Direct experiment proves, however, that this is not the case. The following table compares the weight of fully swollen normal seeds, just before and just after germination respectively, with that of inhibited seeds. The latter are on the whole slightly heavier.

TABLE XIV.

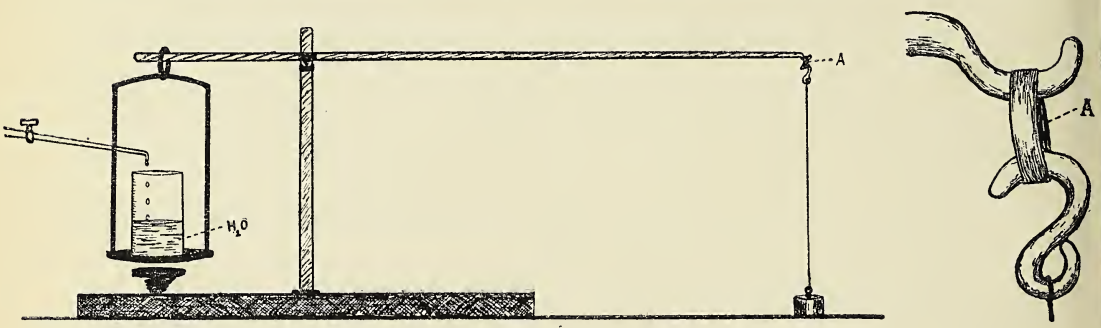
Comparison between the Weight of Inhibited Seeds from Damp Sand and fully swollen (20 per cent. actually sprouting) Non-inhibited Seeds.

Weight of 200 inhibited seeds.	*Weight of 200 fully swollen non-inhibited seeds (soaked for 20–25 hours).*	
2·41 grm.	2·41 grm.	
2·51 grm.	2·41 grm.	
2·40 grm.	2·24 grm.	
Average = 2·42 grm.	Average = 2·35 grm.	
	2·10 grm.	All germinated.

[1] Histological examination of seeds during the period immediately preceding germination showed that the root forces its way through the seed-coats by growth pressure. No enzyme action was indicated.

While it is thus clear that no change under the influence of carbon dioxide has occurred in the mechanical resistance of the testa sufficient to prevent the embryo from swelling to its normal size, and while we must conclude that sufficient water is present for the growth of the radicle to proceed, a further possibility remains. An increase in the breaking strain of the testa during the primary period of inhibition by carbon dioxide, although insufficient to produce any measurable effect on the physical swelling of the embryo, may yet prevent the growth of the radicle and so account for the non-germination of the seed.

Müller (6) draws a conclusion for certain seeds that the breaking strain of the testa is nominally only slightly less than the expansive force of the embryo, both lying in the region of three and a half atmospheres. The conclusions of Crocker (3) with regard to *Amaranthus retroflexus* may also



Text-fig. 4.

be cited in this connexion. The seeds of this plant will not germinate immediately after gathering, but they will do so after a month or more of dry storage. The reason for this is that the colloids of the seed-coats by a slow process of hysteresis in the dry condition take on new characters which give them a much lower elasticity and breaking strain. When the seeds are soaked after this change has occurred, they are able to germinate by rupture of the seed-coats. If, however, germination is now prevented by keeping the wet seeds at subminimal temperatures, the colloids of the seed-coats in their saturated condition recover their former strength by a reverse process of hysteresis. The embryo is then no longer able to rupture the seed-coat when the temperature is raised, and a prolonged period of secondary dormancy ensues.

In view of these results of Crocker and of Müller, the following direct experiments were carried out to compare the breaking strain of the seed-coats of *Brassica alba* in the case of normally germinating seeds and in the case of seeds showing secondary dormancy after CO_2-treatment.

The apparatus used is represented in Text-fig. 4.

Seed-coat rings (A) were obtained from thick median sections of seeds. These seed-coat rings varied in breadth from 2 μ to 20 μ, a micrometer reading being taken in each case at the point of rupture. In each experiment a number of rings from different seeds were taken and the average weight supported (in grammes per 1 μ of breadth) was calculated. This is possible since the relationship between the breadth of the ring and its breaking strain was found to be approximately linear over the range used. The sensitiveness of the apparatus was increased by making the arms of the balance of unequal length.

TABLE XV.

Mechanical Resistance of the Testa-breaking Strain of Seed-coat Rings.

Non-inhibited Seeds.

Time in germinator.	*Number of seed-coats tested.*	*Average weight supported by testa-ring per μ of breadth.*
3½ hrs.	11	3·3 grm.
6 hrs.	9	2·3 grm.
10 hrs. (testas only soaked)	8	1·9 grm.
10 hrs. (whole seeds soaked)	9	1·4 grm.
2 days (3 actually germinating)	7	1·0 grm.
3 days (kept under water)	7	1·2 grm.

Inhibited Seeds.

	Time after removal from the inhibitory gas-mixture.	*Number of seed-coats tested.*	*Average weight supported by testa-ring per μ of breadth.*
1st Sample.	5 days *	13	2·9 grm.
	15 days	14	1·3 grm.
	23 days	11	0·9 grm.
2nd Sample.	Immediately	10	1·2 grm.
	5 days	10	0·9 grm.

* No germinations occurred in this sample of inhibited seeds up to a period of two months.

The results of these direct measurements indicate that the condition of secondary dormancy in White Mustard seeds cannot be attributed to an increase in the breaking strain of the seed-coats by which the growth of the radicle is prevented. When the lowest values obtained for seeds undoubtedly in a condition of secondary dormancy are compared with the values obtained for untreated seeds on the point of germination, the figures are from 1·3 to 0·9 as against from 1·4 to 1·0. The results further indicate that the breaking strain of the seed-coat is subject to considerable variation. In the case of freshly soaked untreated seed it is clear that the breaking strain of the seed-coat continues to fall for some time after they have become water-saturated and pliant. In the case of inhibited seeds showing secondary dormancy, continued exposure on damp sand results in a gradual decrease in the

breaking strain of the testa, due in all probability to secondary causes such as bacteria and acid accumulation.

Changes in the Embryo.

The experiments already described having thus given no definite indication of a change occurring in the seed-coats such as would be sufficient to account for secondary dormancy, it became necessary to consider the possibility of some change occurring in the embryo.

To this end a comparison was made between the germination and growth of bare embryos removed from seeds showing secondary dormancy and bare embryos from normal untreated seeds respectively.

In these experiments the seed-coats were removed with special care and the bare embryos were placed on damp filter-paper dipping into water in a closed vessel. The filter-paper was in some cases hung horizontally and in other cases supported in a slanting position on glass, the embryo being placed about 1 cm. above the water-level. When the tap roots develop they grow down into the water. In the case of the control with

TABLE XVI.

Persistence of Secondary Dormancy after Removal of the Seed-coats.

Experiment.	*Number of seeds used.*	*Remarks.*	*Percentage of seeds ungerminated after—* 1 *day.*	4 *days.*	8 *days.*	12 *days.*	*Previous history of the seeds used in these experiments.*
No. 1	10	Control experiment with *fully swollen* uninhibited seeds.	0	0	0	0	
No. 2 *	8	Kept in the dark at 20° C.	100	50	0	0	21 days in inhibiting gas-mixture. Set 20 days after removal.
No. 3	10	„ „ 12·5° C.	100	10	0	0	19 days in inhibiting gas-mixture. Set 3 days after removal.
No. 4	10	„ „ „	100	40	10	10	Ditto.
No. 5 †	12	„ „ „	100	50	43	8	17 days in inhibiting gas-mixture. Set 14 days after removal.
No. 6	20	Kept in the light at 20° C.	40	5	0	0	24 days in inhibiting gas-mixture. Set 15 days after removal.
No. 7	7	Kept in the dark at 12·5° C.	85	15	0	0	19 days in inhibiting gas-mixture. Set 3 days after removal.
No. 8	10	„ „ „	50	0	0	0	Ditto.
No. 9	20	„ „ 16° C.	100	50	20	20	45 days in 28 % CO_2 10 % oxygen. Set immediately after removal.

* Cf. Plate XXIII, Fig. 1, A and B.
† Cf. Plate XXIII, Fig. 2, A and B.

normal untreated seeds, the seed-coats were removed after ten hours' soaking; germination and growth of the bare embryos proceeded immediately. Eight experiments were conducted with embryos from inhibited seeds, the results of which may be summed up as follows (see Table XVI). In all cases a certain number of seeds, usually about 50 per cent., started to germinate after removal of the testa with a short lag amounting to about twenty hours. Where germination did not ensue in this way almost immediately, a definite continuation of the dormant condition became apparent. The period of this dormancy varied in these experiments from three to thirteen days. The cotyledons and radicle enlarged to a considerable extent by inhibition of water, and, where exposed to light, the former assumed their green colour. The whole embryo, however, had the appearance of a fully mature organ without the capacity for growth. Neither the hypocotyl nor the radicle showed the least sign of elongation by growth in the normal fashion. The photographs (Plate XXIII, Figs. 1 and 2) show the marked contrast between the embryos in this dormant condition several days after the removal of the testa and those seedlings in which growth had started almost immediately.

With regard to the appearance and rate of growth after germination had once commenced, no marked difference was observed between the embryos of control untreated seeds and those of seeds showing secondary dormancy.

These experiments afford the second piece of positive evidence in this research with regard to the causes underlying the phenomenon of secondary dormancy in White Mustard. In the first section of this paper it was shown that changes in the embryo resulting in injury prohibited the occurrence of secondary dormancy. It now appears that in the case of seeds in which secondary dormancy has been successfully induced, changes occur which render them less sensitive to normal growth conditions than the tissues of a newly-swollen untreated seed.

The interpretation of secondary dormancy must now be, not that any change has occurred in the seed-coat, but that the power of the embryo to rupture the testa and germinate has been reduced. As has already been pointed out, the rupture of the seed-coat in *Brassica alba* results from a process of growth. In other words, the power of the embryo to respond to growth conditions and to germinate under the limitation of the seed-coats decreases during the primary period of inhibition in the presence of carbon dioxide.

On the other hand, the broad fact that by the removal of the seed-coats the germination of seeds showing secondary dormancy can be induced must not be lost sight of. In the first place, it is impossible to remove the seed-coats with a fine sharp needle, the method employed in our experiments, without almost certainly causing some injury to the superficial cells of the embryo, or, at any rate, giving the whole tissue considerable

mechanical shock by torsion and pressure. This in itself may be sufficient to upset the dormant condition of the embryo, and may account for the almost immediate germination of the majority of the embryos employed in the above experiments and for the fact that the dormant condition of the embryo itself had not been previously discovered in the many experiments in which the testa had been completely removed in order to induce germination of dormant seeds.

In the second place, by the removal of the testa, both the entrance of oxygen and the escape of carbon dioxide are facilitated. Thirdly, a further physical uptake of water occurs after the removal of the testa. In the following table the further swelling which occurs in the radicle of seeds showing secondary dormancy, after removal of the testa, is indicated. It is possible that the tissues are less stable in the fully swollen condition.

TABLE XVII.

Further Uptake of Water by Excised Radicles of Seeds showing Secondary Dormancy after Removal of the Testa.

Time.	*Percentage of elongation.*
5 min.	3·2 %
11 ,,	5·2 %
20 ,,	6·4 %
32 ,,	6·4 %
60 ,,	6·4 %
69 ,,	6·4 %
28 hrs.	42 % (growth now commenced).

Before the removal of the testa these seeds were soaked several hours under water.

Mean temperature of laboratory, 15° C.

§ 4. METHODS OF FORCING GERMINATION OF *Brassica alba* SEEDS SHOWING SECONDARY DORMANCY.

The present section proceeds to experiments dealing with the effect of various treatments in forcing the germination of *Brassica alba* seeds showing secondary dormancy.

Removal or Partial Removal of the Testa.

It has already been shown that complete removal of the testa induces germination; this fact has been discussed at length in the preceding section. It was found, however, in addition, that a considerable percentage of seeds germinated immediately after the removal of the outer testa only;[1] a similar result was obtained by the removal of a small sector of the testa over any part of the embryo. The following table summarizes the results of the experiments conducted:

[1] See Appendix.

TABLE XVIII.

Effect of Partial or Complete Removal of the Testa in forcing Germination of White Mustard Seeds showing Secondary Dormancy.

Treatment of inhibited seed.	*Percentage of germinated seeds after—*									
	1 *day.*	3 *days.*	4 *days.*	5 *days.*	6 *days.*	7 *days.*	9 *days.*	11 *days.*	13 *days.*	22 *days.*
Not removed to fresh sand.	0	4	4	4	4	12	12	12	12	16
Removed to fresh sand.	0	12	16	20	20	20	20	20	20	20
Outer testa removed with a needle.	35	70	75	75	75	75	75	75	75	75
Outer testa rubbed off with a towel.	40	40	40	40	60	60	60	60	60	60
Both testas removed.	100	100	100	100	100	100	100	100	100	100
Portion of the testa over the hilum removed.	24	60	60	60	60	60	80	80	80	80
Portion of the testa opposite the hilum removed.	30	80	80	80	80	80	80	80	80	80
Control: new uninhibited seeds.	4	100	100	100	100	100	100	100	100	100

Mean temperature of laboratory, 15° C.

The seeds which failed to germinate immediately as the result of either of these treatments remained dormant for an indefinite period. These facts, without being conclusive, indicate that the germinations induced result from the mechanical stimulus on the embryo of the treatment rather than from any change or weakening in the testa. The experiments in which a sector of the testa was removed would seem to rule out the factor of mechanical restraint as being operative for those seeds which subsequently continued dormant. This factor must have been practically reduced to a minimum.

Redrying.

It has been shown in a previous paper (1, p. 416) that by redrying seeds showing secondary dormancy, the capacity for immediate germination is restored. Further investigation during the course of this research showed, however, that a time factor is also involved. Thus, a sample of twenty-five seeds redried for three days in air at laboratory temperature gave only 25 per cent. germination during ten days when reset to germinate on wet sand. On the other hand, inhibited seeds redried for a month in air at the laboratory temperature gave 100 per cent. germination in two days.

In interpreting these results in view of the fact that no marked change in the mechanical resistance of the testa occurs (see Table XV), and also in view of the fact, which has already been demonstrated, that the seed-coats are extremely permeable both to oxygen and to carbon dioxide, we conclude that the redrying of the embryo destroys the dormant condition

which was found to be established in the embryo during secondary dormancy. The occurrence of a time factor is interesting since it indicates that the changes involved, by which dormancy is removed, are secondary to the changes involved in drying. In this connexion it should be remembered that an interval of time, pointing in all probability also to the occurrence of secondary changes in the saturated embryo, was found necessary for the production of secondary dormancy, as shown in section 1. When seeds are set on wet sand in the presence of inhibiting gas mixtures, growth and germination are inhibited from the beginning, but the condition of secondary dormancy is not established for several days.

It was found that the redrying of inhibited seeds has a further effect upon the embryo beyond that of destroying the condition characteristic of secondary dormancy. In comparing the germination of redried inhibited seed with that of control seed, the germination of the redried seed was invariably quicker and more vigorous in the initial stages than that of the control seed. Further experiments in which redried inhibited seeds were compared with control seeds, redried after fifteen hours' soaking, showed that this acceleration is a result of the redrying of the seeds and is not

TABLE XIX.

The Accelerated Germination of Redried White Mustard Seeds.

Experiment.	*Germinations after—*		
	26 *hours.*	48 *hours.*	70 *hours.*
Control; fresh seed.	0	8	10
Redried inhibited seed.	9	10	10
Redried non-inhibited seed.	9	10	10
Redried inhibited seed.	9	10	10

10 seeds used in each experiment. Temperature = 18° C. In light.

connected with previous inhibition or with secondary dormancy. The redrying of soaked seeds appears to act as a definite stimulus. In the case of control seeds redried after soaking, a varying proportion showed at the same time visible injury to the radicle. A second swelling and redrying increases this proportion. Further, if germination has occurred, redrying invariably kills the radicle. The case of redried inhibited seeds is in contrast to this. It is to be emphasized that when redried inhibited seeds are set to germinate all the radicles develop in a perfectly healthy and normal manner. Inhibition and secondary dormancy can be induced a second time. Redrying will again destroy the dormant condition. The radicles, however, still all remain healthy and normal. From these facts it may be concluded: (i) that redrying of fully swollen normal seeds at an early stage of development previous to germination results in an accelerated germination when the seeds are subsequently resown; (ii) that redrying of

fully swollen seeds at a later stage of development after the process of cell division has advanced results in injury; (iii) that both injury and acceleration of germination are due to changes of the same nature resulting from redrying; and (iv) that in the case of secondarily dormant seeds the effect of redrying, while not causing injury owing to the absence of cell-division, is not only sufficient to break up the dormant condition of the embryo, but also to cause acceleration of germination.

A series of experiments was conducted to test the reaction of redried inhibited seeds to carbon dioxide with regard to inhibition. It was found that a smaller concentration of carbon dioxide was required to initiate inhibition than that required in the case of normal seeds. The following table gives the results of one set of experiments in which the control non-inhibited seeds soaked and redried are compared with inhibited seeds which have been previously inhibited and redried during secondary dormancy:

TABLE XX.

The Increased Sensitiveness of Redried Inhibited White Mustard Seeds to the retarding and inhibiting Action of Carbon Dioxide.

Percentage of CO_2 (20 % *oxygen in each case*).		*Number of germinations after*— 46 *hours.*	97 *hours.*	120 *hours.*	After 120 hours the seeds which were still ungerminated were removed to air.	*Number of seeds still ungerminated after a further* 13 *days in air.*
0 %	A	19	20	20		
	B	20	20	20		
5 %	A	19	20	20		
	B	16	17	19		
10 %	A	18	19	19		
	B	5	12	12		4
16 %	A	17	18	18		
	B	0	3	3		9

20 seeds used in each experiment.
Temperature 17–19° C.
In light.
In each case seeds A and B were set side by side in the same flask.

A = control non-inhibited seeds soaked and redried.
B = inhibited seeds showing secondary dormancy redried.

Similar results were obtained when fresh control seed was used. The increased sensitiveness to the influence of carbon dioxide is clearly due to the fact of previous inhibition and not to the fact that the seeds have been soaked and redried. As will be seen also from the above table, secondary dormancy is again produced when redried inhibited seeds are submitted to the influence of carbon dioxide under germinating conditions a second time.

Temperature. The following table gives the results of experiments in which the endeavour was made to bring about germination of seeds showing secondary dormancy by exposure to high or low temperatures:

TABLE XXI.

The Effect of High and Low Temperatures in causing Germination of Brassica alba *Seeds showing Secondary Dormancy.*

Treatment of inhibited seeds.	*No. of seeds used.*	*Germinations after—* 1 *day*	2 *days.*	3 *days.*	4 *days.*	5 *days.*	6 *days.*	7 *days*	8 *days.*	9 *days.*	10 *days.*	*Percentage of germination after* 10 *days.*	*Remarks.*
50° C. for 3 hours, then returned to laboratory temperature.	20											0 %	All dead.
50° C. for ½ hour, then returned to laboratory temperature.	20	12	13			15	17	18				90 %	All healthy seedlings.
25° C. continuously.	20	0		2	2		4	5		5	5	25 %	
Control: inhibited seeds kept at laboratory temperature throughout.	20	0		3	4	5	5	5	5	5	5	25 %	
1–3° C. for 3 days, then returned to laboratory temperature.	20	0	0	0	0	0	0	0	0	0	0	0 %	
0° C. for 5 hours, then returned to laboratory temperature.	50	0	0	0	0	0	0	0	0	0	0	0 %	
−4° C. for 3 hours, then returned to laboratory temperature.	50				16			17			17	34 %	All healthy seedlings.
−7° C. for 5 hours, then returned to laboratory temperature.	25		10		10			10			10	40 %	The ungerminated seeds were killed.
Control: inhibited seeds removed to fresh sand at laboratory temperature.	50	0	0	0	0	0	0	0	0	0	0	0 %	

Mean temperature of laboratory, 16° C.

The above results indicate that germination can be induced by *short* exposure to the extremes of high or low temperature which just fall short of injury; exposure to intermediate temperatures, either for a short period or continuously, has no effect. Thus, exposure to 50° C. for half an hour was followed by 90 per cent. germinations, but exposure for three hours to the same temperature was fatal, while continuous exposure to 25° C. had no effect. In a similar way, exposure to −7° C. for five hours resulted in 40 per cent. healthy germinations, but the remaining 60 per cent. were killed.[1] Exposure to 0° C. for three days, on the other hand, had no effect in causing germination of inhibited seeds.

[1] In this connexion it is interesting to note that fully swollen normal seeds of *Brassica alba* submitted to the same temperature (i.e. −7° C.) in parallel with the above experiment showed marked injury without exception. The sharp contrast in this case between the 10 seeds which germinated in the normal manner and the 15 which did not germinate, and which when tested by removal of the testa proved to be killed, is probably to be accounted for by the fact that the injurious effect of low temperatures is due to the formation of ice-crystals which would occur at a critical point depending upon the concentration of the cell-sap.

Oxygen.

Experiments conducted by Crocker (7) [*Xanthium*], Shull (8) [*Xanthium*], and by Rose (9) [*Datura Wrightii* and *Martynia* sp.] have led these authors to conclude that with these seeds the factor limiting germination is the low permeability of the testa to oxygen. The results of Atwood (10) indicate a similar conclusion with regard to *Avena fatua*. As has been said above, a possible cause of the non-germination of White Mustard seeds after treatment with carbon dioxide may be either lack of oxygen or excess of carbon dioxide. It has been shown that the seed-coats do not appear to change with regard to their permeability to gases as the result of the treatment which produces secondary dormancy. But owing to the alteration in the condition of the embryo, already demonstrated, it is quite possible that the normal interference of the testa in gaseous interchange may act as a definite factor in the maintenance of secondary dormancy. It has been seen that the seeds showing secondary dormancy which have been redried are more sensitive to carbon dioxide. This increased sensitiveness in all probability exists previous to redrying.

The following experiments were conducted: Inhibited White Mustard seeds were placed in concentrations of oxygen up to 100 per cent. at one atmosphere pressure. No increase of germinations over the control in air resulted, and we may therefore conclude that the failure to germinate cannot be attributed to a need of oxygen on the part of the embryo.

TABLE XXII.

Negative Result of Treatment of White Mustard Seeds showing Secondary Dormancy with Increased Concentrations of Oxygen and of Treatment to Exposure to a Vacuum.

Treatment of inhibited seeds.	*Number of germinations on removal to air after—*									
	1 *day.*	2 *days.*	3 *days.*	4 *days.*	5 *days.*	6 *days.*	7 *days.*	8 *days.*	9 *days.*	10 *days.*
Control inhibited seeds in open flask.	0	3	4	5	5	5	5	5	5	5
50 % oxygen. 50 % nitrogen.	0	0	0	0	0	0	0	0	0	0
100 % oxygen.	0	0	0	0	1	1	1	1	1	1
Exhausted ½ hour; 75 mm. HgO_2 pressure admitted for two days.	0	0	0	0	0	0	0	2	2	2

25 seeds used in each experiment.
Mean temperature of laboratory, 15° C.

To test, on the other hand, the possibility of continued inhibition being due to an inhibiting concentration of carbon dioxide in the embryo owing to an increased sensitiveness to this gas on the part of the embryo, inhibited seeds were submitted to a vacuum for a short period and then

placed in an atmosphere of pure oxygen at 75 mm. Hg pressure, i. e. a tension of oxygen equal to half that in air. This treatment had no effect in stimulating the germination of seeds showing secondary dormancy and indicates that an inhibiting concentration of carbon dioxide in the tissues is not the cause of secondary dormancy.

Acids.

Crocker and Davis (5), working with dormant seeds of *Alisma Plantago*, concluded that the effect of acids in producing germination was due to their weakening action upon the seed-coat. Eckerson (11), however, showed that in the case of *Crataegus mollis*, *Pyrus malus*, &c., dilute acids also affected the internal factors controlling the length of the so-called after-ripening period, while Lehmann (12) found that the action of acids and of certain hydrolysing enzymes can replace the action of light in producing the germination of dormant light-sensitive seeds, and took the view that hydrolysis is the important factor, but in this connexion it should be pointed out that he does not clearly distinguish between the seed-coat and the embryo itself.

Experiments were conducted to test the effect of dilute acids upon White Mustard seeds showing secondary dormancy. Hydrochloric and propionic acids were used. It was found in result (Table XXIII) that the effect of acids in increasing concentration was as follows: With HCl, for example, the lowest concentration used, namely $\frac{N}{1,000}$, had little effect, whilst higher concentrations $\left(\frac{N}{100}\right)$ induced germination, but subsequently killed the primary root. Similarly, $\frac{N}{100}$ propionic acid caused 80 per cent. germination in two days, but subsequently killed all the seeds.

TABLE XXIII.

Treatment of the inhibited seeds.	*Number of seeds used.*	*Percentage of germinations after—* 1 *day.*	2 *days.*	3 *days.*	4 *days.*	5 *days.*	6 *days.*	7 *days.*	9 *days.*	10 *days.*
Water	25	0	0	12	16	20	20	20	20	20
$\frac{N}{1,000}$ HCl.	20	0	0	0	0	0	0	0	5	10
								All dead.		
$\frac{N}{100}$ HCl.	20	20	30	40	45	60	65	65	65	65
$\frac{N}{100}$ Propionic acid.	10	0	80	All dead.						
$\frac{N}{10}$ Propionic acid.	10	0	40	All dead.						

Mean temperature of laboratory, 16° C.

The results of these experiments with acids lead us to a conclusion similar in nature to that already stated with regard to the action of temperature, namely, that germination of dormant White Mustard seeds

can be induced by acid concentrations which are actually toxic to the growing radicle, or by concentrations which closely approximate to this. In other words, the conditions of acid concentration on the threshold of injury to the growing radicle will cause germination.[1]

In view of the results obtained with the above acids, and of the previous results in which it has been shown that following treatment with high concentrations of carbon dioxide secondary dormancy does not occur, experiments were conducted to test whether treatment with a high concentration of carbon dioxide would terminate the secondary dormancy of seeds inhibited by a lower concentration. Positive results were obtained. Treatment of seeds which had been inhibited by 25 per cent. carbon dioxide and 20 per cent. oxygen for twelve days, and which had lain dormant for two weeks subsequently in air, by an immersion for seven days in 100 per cent. carbon dioxide was followed by 95 per cent. germination in two days. The seeds not treated with 100 per cent. carbon dioxide remained dormant.

§ 5. Summary.

The presence of carbon dioxide inhibits the germination of seeds, and the concentration of carbon dioxide necessary is correlated with temperature and with oxygen supply as previously described (2).

In the case of *Brassica alba*, the primary effect of carbon dioxide in causing inhibition of germination is followed by a secondary effect of prolonged dormancy after the carbon dioxide has been removed. This phenomenon has been termed *secondary dormancy*.

Changes in the seed-coat occurring during the period of primary inhibition have been suggested as the immediate cause underlying the phenomenon of secondary dormancy in White Mustard seeds, namely, (*a*) a decreased permeability of the seed-coats to carbon dioxide or to oxygen occurring either as a result of a process of hysteresis in the colloids of the seed-coats (cf. Crocker, 3) or as a result of the specific action of carbon dioxide (Kidd, 1), or (*b*) an increase in the mechanical resistance of the seed-coats. No evidence was found in support of either of these hypotheses.

It was found, on the other hand, that the embryos of secondarily

[1] The interesting fact is to be recorded here that the ungerminated seed is far more resistant to acid injury than the growing radicle. Our experiments with carbon dioxide constantly demonstrated this fact; at ordinary temperatures (20° C. *circa*), any germinations that occur in the presence of 20 per cent. carbon dioxide (20 per cent. oxygen present) show marked injury to the radicle. Further, embryos, removed from their seed-coats, which can sprout in the presence of this concentration of carbon dioxide, invariably show injury after ten days' immersion. Again, the radicles of seeds which are just sprouting when placed in 20 per cent. or in higher concentrations of carbon dioxide (20 per cent. oxygen present) always suffer injury. In contrast, seeds inhibited in 20 per cent. carbon dioxide (20 per cent. oxygen present), when finally induced to germinate, show no signs of injury to the radicle. Concentrations of carbon dioxide up to 100 per cent., provided that the period of immersion does not exceed six days, and longer periods in the case of lower percentages, cause no injury to *ungerminated* seeds whether previously inhibited or not.

dormant seeds have become more stable and less responsive to the conditions under which growth is usually initiated. The bare embryos of secondarily dormant seeds continue dormant under appropriate conditions after the removal of the seed-coats. This change in the embryo appears to be due to the action of carbon dioxide during the period of primary inhibition, as it cannot be induced by restraining germination by other means, e. g. by submitting the seeds to low temperatures.

The embryo enclosed within the seed-coats is not completely swelled, but the rupture of the seed-coats when germination takes place, both in the case of normal seeds and in the case of seeds showing secondary dormancy, is due to a process of growth.

The dormant condition of the growing tissues of the embryos of seeds showing secondary dormancy is broken up by treatments which are injurious, but not fatal. Treatments which actually kill the meristematic cells of the root-tip whilst still enclosed within the seed-coats cause the adjacent cells of the hypocotyl to start growing, and germination, in which the root-tip is absorbed, results.

As a corollary, if the conditions during the primary period of carbon dioxide inhibition are injurious, either owing to lack of oxygen or to excess of carbon dioxide, secondary dormancy does not ensue. In consequence, a high percentage of secondary dormancy can only be produced by a limited range of carbon dioxide and oxygen mixtures (i. e. 20 per cent. to 30 per cent. CO_2 and not less than 15 per cent. O_2).

Embryos in their completely swollen condition still showing secondary dormancy after the removal of their seed-coats are more sensitive to growth stimuli than those still enclosed in their seed-coats and consequently not completely swelled. While in the latter case germination can only be induced by conditions which just fall short of producing visible injurious effects, in the former case it is only possible to maintain the secondary dormancy by removing the testa with the greatest care in avoiding as far as possible pressure torsion or abrasion.

It will be seen that the main interest of this communication centres round the causes underlying the initiation of growth rather than in the condition of dormancy. In considering this question of growth in the case of *Brassica alba* seeds, our experiments show clearly that there is no question of limiting factors. We have been able to trace no limiting factor responsible for the non-germination of White Mustard seeds showing secondary dormancy. We find ourselves rather in the presence of facts which emphasize a conception of stimulus. It has been seen that widely different treatments, quite unclassifiable in any feature other than that they all result in injury and death if carried too far, excite germination and growth of dormant White Mustard seeds. It appears to us probable that some return will have to be made to this conception of stimulus in plant physiology

generally, and that in any experimental analysis of the living plant, as a unit and in relation to its life-cycle, the idea of limiting factors, which has for so long dominated the minds of plant physiologists, will have to be modified.

§ 6. Conclusions.

Secondary dormancy in seeds of *Brassica alba* is not due either to increased mechanical restraint of the seed-coats or to decreased permeability of the seed-coats to gases. It is due to a stable condition of the embryo tissue, which becomes slowly established during the period of primary inhibition under the influence of carbon dioxide. This condition appears to be comparable to that of mature organs and of embryos maturing on the parent plant. The embryos of White Mustard in this stable condition (secondary dormancy) do not respond to the ordinary environmental factors under which germination and growth will proceed.

For the initiation of growth (by cell division) a *change in the state of tissue equilibrium* must occur, and this requires a definite stimulus. This change in the case of secondarily dormant White Mustard seeds is brought about by various treatments which cause injury and death when carried too far. The processes involved in the initiation of growth seem to be of the same kind as those which produce injury.

Imperial College of Science and Technology,
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May, 1917.

Appendix.

Structure and Microchemistry of the Testa.

The complete testa of the young green seed of *Brassica alba* consists of the following layers of cells: [1]

The layers numbered i–iv in Text-fig. 5, A and B, constitute the so-called *outer* testa or seed-coat, which, in the ripe seed, can readily be removed from the *inner* testa or seed-coat.

i. An epidermis of large cells, which, in the mature seed, undergo complete mucilaginous degeneration.

ii. A layer (1–3 cells in thickness) of large thin-walled cells which also undergo complete mucilaginous degeneration when the seed ripens.

iii. A layer of large thin-walled cells which, in the ripe seed, undergo partial mucilaginous degeneration. The cell walls of layers i, ii, and iii give the staining reactions characteristic of pectin.

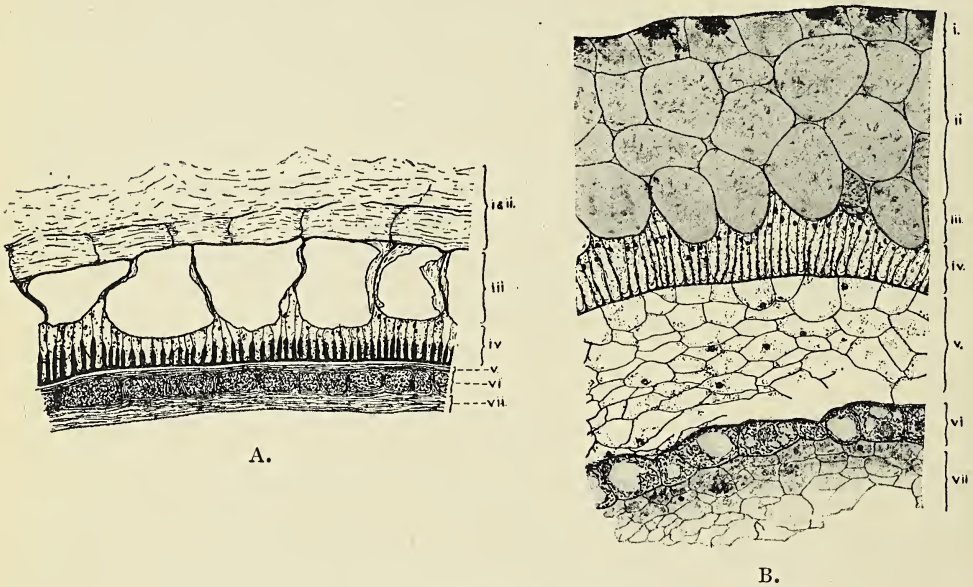
[1] For further details regarding the structure of the seed-coat in the genus *Brassica* the reader is referred to Holfert, J.: Die Nährschicht der Samenschalen, in Flora, Bd. lxxiii, 1890; and to Schroeder, J.: Untersuchung der Samen der *Brassica*-Arten und Varietäten, in Landw. Versuchs-Stationen, Bd. xiv, 1871.

iv. A layer of narrow radially elongated cells with characteristic highly refractive thickenings of the inner and radial walls (Text-fig. 5, A and B). These cells have coarsely granular contents and distinct nuclei.

The layers numbered v–vii in Text-fig. 5, A and B, constitute the so-called *inner* testa or seed-coat.

v. A layer, several cells in thickness, of delicate thin-walled tissue which appears totally collapsed and crushed in the ripe seed. The walls of these cells give the pectin reaction with ruthenium red.

vi. A layer of moderately large cells with dense granular contents and large nuclei. In the ripe seed these cells form a very



TEXT-FIG. 5.

characteristic compact layer (Text-fig. 5, A). The walls of these cells give the pectin reaction with ruthenium red and are insoluble in cuprammonia. The cell contents consist of proteid granules and oil.

vii. A layer, many cells in thickness, of delicate tissue, the cells of which appear totally collapsed and crushed in the ripe seed. The walls of these cells give the reactions characteristic of cellulose with congo red and with chlor-zinc-iodide. They do not stain with ruthenium red. Since, however, they are insoluble in cuprammonia, they probably consist of hemi-cellulose.

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DESCRIPTION OF FIGURES ON PLATE XXIII.

Illustrating Dr. Kidd and Mr. West's paper on the Controlling Influence of Carbon Dioxide.

Fig. 1. A & B. Eight embryos from dormant seeds photographed four days after removal of the seed-coats; four ungerminated.

B 2. The same four embryos photographed four days later, showing healthy development of the radicle.

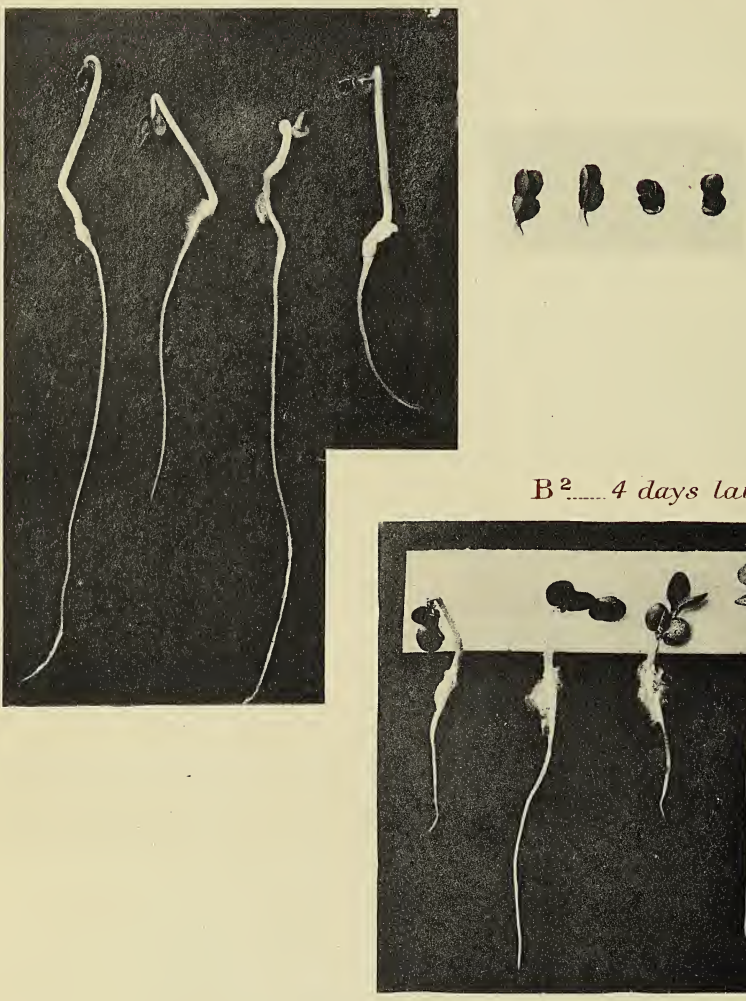
Fig. 2. A & B. Twelve embryos from dormant seeds photographed eight days after removal of the seed-coats; five ungerminated.

B 2. The same five embryos photographed three days later; one still ungerminated.

Fig. 1.

(Exper. 2, Table XVI.)

A ..... B ... *After 4 days.*



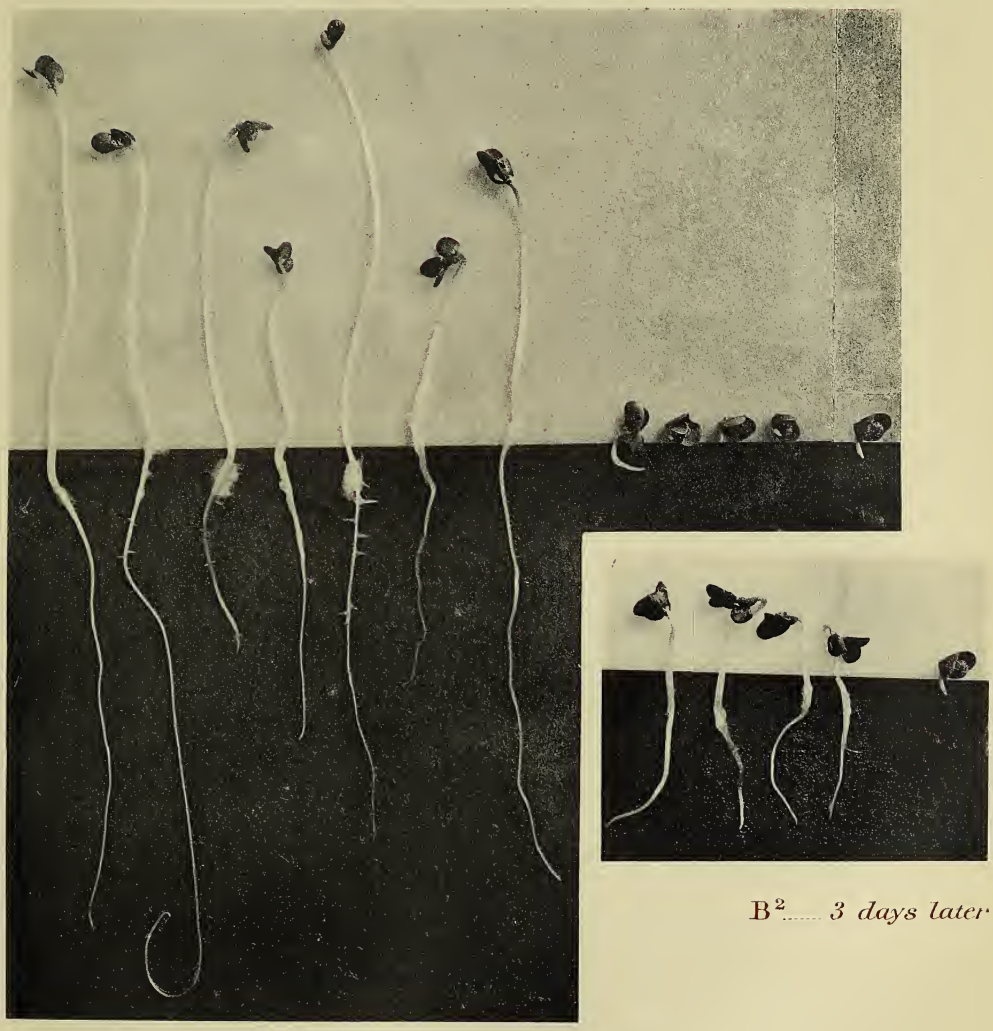
B^2 ..... *4 days later.*

KIDD AND WEST— BRASSICA ALBA.

Fig. 2.

(Exper. 5. Table XVI.)

A ..... B ..... *After 8 days.*



B^2 ..... *3 days later*

Studies in the Physiology of Parasitism.[1]

IV. On the Distribution of Cytase in Cultures of Botrytis cinerea.

BY

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IN the first of these studies a method was described by which a powerful enzymic solution could be obtained in reasonable quantities from fungal germ tubes of one day's age. It was claimed on *a priori* grounds that such an extract was more suitable for the study of the question of the nature of the active principle responsible for the breaking down of the host tissue in advance of the growth of the hyphae than the extracts which had been employed by previous investigators. The present paper relates to an investigation carried out with the object of determining in what way enzymic preparations obtained from the same strain of fungus may differ from each other. Though of a purely enzymological interest, these results have been incorporated in the present series, partly on account of the light which they throw on the nature of the extracts employed by previous investigators, and partly because of their bearing on the manner in which fungi secrete enzymes and on the technique of extraction.

It will add to clearness to tabulate first of all the factors which have been found to influence markedly the activity of the enzymic preparation obtainable from a fungal culture. These are:

1. The density of sowing of the spores.
2. The length of the period allowed for development.
3. The nature of the nutrient employed.

It must be emphasized at this point, in order to prevent misconception, that the first factor mentioned is not a factor *per se*. It will appear in the sequel that the essential factor concerned in the obtaining of a strong extract is the proportion of actively growing mycelium to that which has already ceased to grow. Variation of the density of spore-sowing, together

[1] For No. I of this series, see Ann. Bot., vol. xxix, 1915, p. 313; for No. II, see Ann. Bot., vol. xxx, 1916, p. 389; for No. III, see Ann. Bot., vol. xxx, 1916, p. 399.

[Annals of Botany, Vol. XXXI. Nos. CXXIII and CXXIV. July and October, 1917.]

with the variation of the length of the period allowed for development, is merely an experimental method by which the ratio of actively growing to non-growing mycelium can readily be altered while at the same time ensuring economy of space and labour. With this understanding, factors (1) and (2) will be considered as independent factors during the account of the experimental part of the work. Their exact bearing on the phenomena concerned will be set forth in the general discussion of results.

Factors influencing Activity of a given Enzymic Solution.

It was shown in the first paper of this series that the activity of an extract of germ tubes increased up to a certain limit with the amount of enzyme present and was reduced by the presence of certain other substances, e. g. salts, acids, &c. (except, in the case of acids, in the immediate neighbourhood of the neutral point). As therefore the enzymic activity is the resultant of two antagonistic factors, amount of enzyme and amount of retarding substances, it is possible for two extracts of equal activity to contain widely different amounts of enzyme. If we suppose, as seems legitimate, that deactivation of an extract by heating to 65° C. merely affects the enzyme (and it may be, some other colloidal substances which, however, are not likely to be of importance in this connexion), then it is possible to separate the two factors. The method of separation is as follows :

Suppose two extracts x and y are to be compared. Portions of x and y are heated to 65°; such deactivated extracts may be termed x' and y' respectively. If now 1 c.c. of x be mixed with 1 c.c. of y'—which mixture may be represented by the symbol xy'—and if similarly we prepare the mixture $x'y$, it is plain that the retarding factors (salts, acids, &c.) in both mixtures are equal in amount. We can therefore judge from any differences in the activities of xy' and $x'y$ as to whether x or y is richer in enzyme.

The method of estimating the activity of the extracts has been described in the paper already referred to (No. 1, p. 324). The discs employed were of 0·5 mm. thickness, and were of potato, turnip, or swede.

Notation. In the following account, estimations are made of the activities of preparations obtained by extracting the washed and dried hyphal mass of the fungus, and of preparations consisting of the medium in which the fungus has been grown. For convenience in description these will be referred to as H (hypha) and M (medium) preparations respectively. Furthermore, as preparations of different ages will be compared with one another, a suffix will be added to indicate the age (in days) of the preparation under consideration. Thus by H_2 is meant an extract of hyphae of two

days' age; by M_2, the medium in which the fungus has germinated and developed for two days, and so on.

Experimental Method.

In the case of thin sowings of spores, a quantity of the nutrient fluid (turnip extract) was infected in a flask with a small tuft of mycelium containing spores: for the thick sowings the method described in No. I of this series was adhered to. At the end of the period allowed for development, the nutrient medium was separated from the fungal mass by filtration through muslin. The fungal mass was thoroughly washed, dried, ground, and extracted in the manner already described.

Spores sown thinly. The general nature of the results obtained is indicated in the following experiment:

Three Erlenmeyer flasks, each containing 25 c.c. of turnip extract, were infected with a tuft of mycelium, and incubated at 20° C. Development in all three flasks appeared equal. Flask A was tested after 1 week, B after 2 weeks, and C after 3 weeks, the M and H preparations (0·1 grm. to 3 c.c. water in the case of the latter) being compared with standard extract. The activities of the various preparations are given in the following table, that of standard extract being taken as unity:

A (1 week).		*B* (2 weeks).		*C* (3 weeks).	
H_7	M_7	H_{14}	M_{14}	H_{21}	M_{21}
0·2–0·25	0·17	0·03–0·04	0·06	less than 0·02	trace (?)

In the case of both sets of preparations, the activity is seen to diminish continuously after one week, and at the end of the third week it is reduced to a negligible quantity in both cases. The further consideration of the H preparations will be taken up later. In the case of the M preparations, seeing that initially the enzymic activity is nil, it is plain that activity increases with time to a maximum and subsequently declines. The nature of this relationship is shown more clearly by the following more detailed figures. The dry weight of hyphal material derived from each culture is added:

Age in days	2	4	6	9	12
Activity of M prep.	<0·03	0·2	0·2	0·13	0·08
Dry wt. of mycelium	0·017 grm.	0·27	0·36	0·33	0·37

It will be observed that enzymic activity is slight on the second day; that the maximal activity is reached in the neighbourhood of the fourth to sixth days; also that about the latter time stagnation in the growth of the mycelium has been reached.

By applying the method described on p. 488, it is possible to determine the factor responsible for the reduced activities of the H preparations tabulated on p. 489. The table gives the enzymic activities of the various preparations indicated.

H_7, H_{14}, H_{21} are, as before, the preparations obtained by extracting hyphal material of 7, 14, 21 days' age respectively; H_7', H_{14}', H_{21}' are the corresponding deactivated extracts.
S = Standard extract.

S = 1	H_7 = 0·2	H_{14} = 0·03–0·04	$H_{21} < 0·02$
SS′ = 0·8	H_7S' = 0·16	$H_{14}S'$ = 0·04	$H_{21}S' < 0·02$
S′S = 0·8	$H_7'S$ = 0·8	$H_{14}'S$ = 0·8	$H_{21}'S$ = 0·8

The reduction of activity of S′S, $H_7'S$, $H_{14}'S$, and $H_{21}'S$, as compared with S, is about equal to that produced by dilution of S with an equal volume of distilled water. It is therefore plain that S, H_7, H_{14}, H_{21} all contain amounts of retarding substances too small to be recognized by the method. The weakness of the preparation H_7S' as compared with $H_7'S$ shows that the small activity of the H_7 preparation is due simply to the small concentration in it of the enzyme; and this is true *a fortiori* in the case of the H_{14} and H_{21} preparations.

Spores sown thickly. Spores were sown on circular glass plates in the ratio of 0·1 c.c. of spores to 10 c.c. turnip extract. The following table gives the data obtained as to yield of mycelium and activity of the corresponding H preparations:

Stage of Germination.	*Yield per Plate (dry wt.).*	*Notation.*	*Activity.*		
			0·1 grm. in 1·5 c.c.	0·1 grm. in 3 c.c.	0·1 grm. in 4·5 c.c.
Ungerminated	0·01 grm.	H_0	0·1	—	—
8 hrs. germination	0·02 ,,	$H_{\frac{1}{3}}$	0·19	—	0·12
1 day's ,,	0·14 ,,	H_1	1	0·75	0·6
2 days' ,,	0·17 ,,	H_2	1	0·75	0·6
4 ,, ,,	0·16 ,,	H_4	0·75	0·5	0·38
6 ,, ,,	0·14 ,,	H_6	0·25	—	0·17

The features brought out by the above table are:

1. The yield of dry material for a given quantity of spores sown reaches a maximum in the neighbourhood of the second day. Subsequently a slow decrease in dry weight takes place. This decrease is probably to be ascribed to respiration.

2. The activity of the extract obtainable from the dried mycelium reaches a maximum about the first or second day; on the fourth day it is distinctly, and on the sixth day very considerably, reduced.

In the above table, extraction was made on the basis of equal quantities of dry material. By combining the curves of activity and of yield, it is readily seen that when extraction is made on the basis of equal

quantities of spores originally sown, the curve of activity obtained is of the same kind, but grades off more rapidly on both sides of the maximum.

The material H_1 in these experiments is the same as the material employed for the preparation of standard extract (S). A comparison of H_0, $H_{\cdot 3}$, and H_6 with H_1 according to the method which has already been described showed the reduced activity of the extracts derived from these sources to be due to lack of enzyme and not to the presence of large quantities of retarding substances.

Spores sown thickly—M preparations. The activities of the various M preparations corresponding to the H preparations just described are given in the following table, that of standard extract being taken as unity:

$M_{\cdot 3}$ = trace	$M_1 = 1$	$M_2 = 1$	$M_4 = 0{\cdot}87$	$M_6 = 0{\cdot}25$–$0{\cdot}3$
	$M_1S' = 1$	$M_2S' = 1$	$M_4S' = 0{\cdot}87$	$M_6S' = 0{\cdot}25$–$0{\cdot}3$
	$M_1'S = 0{\cdot}17$	$M_2'S = 0{\cdot}2$	$M_4'S = 0{\cdot}2$	$M_6'S = 0{\cdot}15$

It is seen from the above table that the activity of the M preparations passes through a maximum which is reached on the first to second day. The activity is very considerably reduced by the sixth day.

A comparison of the figures in the second and third rows of the above table shows that the concentration of enzyme in M_1, M_2, and M_4 is much greater than in S; and that the same applies in a less degree to M_6. Nevertheless the activity of M_6 is less than a third that of S. The preparation M_6 therefore illustrates the case of an enzymic solution in which the presence of a large quantity of enzyme is masked by the presence of retarding substances so that the full activity of standard extract is not reached.

That the concentration of enzyme in S is relatively so small involves no contradiction, as a number of factors, such as degree of dilution, adsorption of enzyme on the fungal débris, loss on desiccation, might be expected to play a part in producing this result.

The high concentration of enzyme in the M preparations is also shown in the following ways:

1. While the activity of standard extract diminishes with dilution, that of such a preparation as M_2 remains unaltered up to a considerable degree of dilution. Thus by dilution to one-fourth the activity of S diminishes to a half (No. 1 of this series, p. 342), whereas dilution to one-eighth produces no appreciable diminution in the activity of M_2.

2. The M preparations are much less sensitive to the addition of salts and of plant extracts than is standard extract. The following table shows the retarding action of concentrations of KNO_3 upon M_2 and standard extract:

	Concentrations of KNO_3.				
	n/∞.	$n/128$.	$n/32$.	$n/8$.	$n/2$.
M_2	1	1	0·87	0·5	0·33
S	1	1 (−)	0·37	0·08	0·015–0·025

A comparison of the M preparations in the case where the spores are thickly and where they are thinly sown is instructive:

Manner of Sowing.	*Time in Days.*						
	0	1	2	4	6	9	12
Thick	0	1	1	0·87	0·25–0·3	—	—
Thin	0	—	< 0·03	0·2	0·2	0·13	0·08

The features of note are:

1. In both cases the activity of the M preparations passes through a maximum.

2. The effect of thick as compared with thin sowing is to increase the magnitude of the maximum, and to bring forward in time the date of its attainment.

Effect of medium. Various media may be expected to give different quantitative results in view on the one hand of the retarding factors which they introduce, and on the other of the vigour of the growth to which they give rise. Only a few experiments have been carried out in this connexion. The various media employed were dilutions of a strong turnip extract. The following table gives the activities of the M preparations in the various media after twenty-four hours' germination of the spores:

T = turnip extract.
m = a density of sowing equal to 0·1 c.c. spores in 10 c.c. nutrient.

Density of Sowing.	*Nutrient.*			
	T	*T*/10	*T*/100	*T*/1000
m	1	0·2	0·1	trace
m/10	0·2	0·2	0·1	trace
m/100	trace	—	trace	trace
m/1000	trace	—	trace	trace?

The results of the above table can be summarized thus: The activity of the M preparation obtained is reduced both by diminishing the strength of the nutrient and the density of sowing of the spores.

Further Comparisons between M Preparations and Standard Extract.

1. *Amount of retarding substances present.* The amount of these substances present in standard extract (and extracts of mycelium generally) has been seen to be negligible (p. 490). The amount present in the M preparations, on the other hand, is considerable, being only somewhat

less than that present in the original turnip extract. This is shown by the following table of activities:

$M_2 = 1$	$M_2S' = 1$	$M_2M_2' = 0{\cdot}75$	$M_2T = 0{\cdot}58$
$S = 1$	$SS' = 0{\cdot}8$	$SM_2' = 0{\cdot}2$	$ST = 0{\cdot}13$.

2. *Amount of crystalloids present.* This can be determined by estimation of the osmotic pressure. This was done by finding what concentration of a cane-sugar solution balanced the given solution on the opposite side of an osmometer. The osmometer consisted of a semi-permeable collodion thimble with capillary tube attached.[1]

The figures were:

Turnip extract	balances	0·43	molar	cane-sugar.		
M_2	„	„	0·29	„	„	„
S	„	„	0·03	„	„	„

General Discussion of above Results.

Enzymic preparations of two types can be obtained from a fungal culture, viz. (1) watery extracts of washed and triturated mycelium, and (2) the fluid in which germination has taken place. The activity of the preparation obtained has been shown to be dependent upon (1) the density of sowing of the spores, (2) the time allowed for germination and growth, and (3) the nature of the nutrient medium.

Fluid in which germination has taken place. With any given concentration of spores, the enzymic activity increases to a maximum with lapse of time, and subsequently falls off. When the spores are sown thinly, the absolute value of the maximum reached is diminished, and its attainment takes place at a longer interval from the time of sowing.

These enzymic solutions are characterized by the high concentration of enzyme present. They also contain an amount of retarding substances comparable with, but somewhat less than, that of the original nutrient medium.

Mycelial extract. In the case of thick sowings of spores, the curve of activity was shown to rise during the first day's growth, and then to decline after about the second day. In the case of the thin sowings in Erlenmeyer flasks, only the latter part of the curve was obtained. It is obvious

[1] An air-dried collodion thimble is placed in 50 per cent. alcohol in water for 24 hours, then washed in water till the alcohol is removed. The open end of the thimble is then dried in the air and sealed by means of a solution of collodion to the rubber cork through which the capillary tube passes. The junction is then allowed to dry completely in the air, when the osmometer is ready for use. This osmometer is permeable to simple salts like chlorides and nitrates of the monovalent metals, but not to sulphates or salts of bivalent metals such as barium chloride, &c. For a full account of the technique of preparing membranes of graded permeability, see papers by the writer in Biochemical Journal, vol. ix, 1915, p. 591, and vol. xi, 1917, p. 40.

on general considerations that the ascending part of the curve would also be found on the first day, but no experimental verification was attempted on account of the laborious processes which the preparation of the necessary amount of material would entail.

Enzymic solutions of this type are characterized by the presence of a medium amount of enzyme. They contain a negligible amount of retarding substances and of crystalloids generally.

The following practical rules may therefore be framed as bearing on the technique of preparing solutions of the enzyme under consideration :

(*a*) In order to obtain a very active enzymic solution from the nutrient medium, it is essential that simultaneous development of a large number of spores take place throughout the whole nutrient medium. Where a small number of spores develop on the surface of a relatively large volume of nutrient, the enzymic activity of the latter never reaches a high value.

(*b*) In order to obtain a strong mycelial extract, the germinated spores must be extracted while still young (one to two days old). Conditions of yield and of economy of space and labour necessitate as thick sowing of the spores as is consistent with their vigorous germination.

In the case of both types of extract, the maximum activity is attained at a time roughly coincident with that at which vigorous growth of the hyphae ceases.

The above results throw light on the relative merits of the extract employed in these studies and those employed by certain other investigators in the examination of the nature of the active principle concerned in the process of parasitic attack. The extract employed by the present writer shows the maximal activity obtainable from mycelial extracts, and is at the same time comparatively free from crystalloidal contamination such as occurs in the equally active preparations derived from the nutrient in which the fungus has developed. In Marshall Ward's experiments,[1] extraction of the hyphae took place too late (after three weeks' growth) to allow of a strong extract being obtained. The extracts of de Bary and Behrens were obtained from the nutrient medium and contained therefore crystalloidal contamination. It seems certain that the lethal effects of boiled extract in Behrens's experiments were due to the large amount of crystalloidal substances present, while the great age (six months) of his cultures precluded the presence of anything but the smallest traces of enzyme in his unboiled extracts.

[1] References to the papers cited here are given in No. 1 of the series.

General Remarks on the Secretion of Cytase by *Botrytis cinerea.*

In the case of the watery extracts of mycelium it has been shown that a weak enzymic preparation is obtainable from the mycelial film in the case where the spores were sown thinly, while in the case of the dense sowings on glass plates a much stronger extract is obtained. Now the outstanding difference between the two cases mentioned is that in the latter case the great proportion of the hyphal mass is in a state of vigorous growth, or, at any rate, has very recently passed through its growth period; whereas in the former, though the number of growing tips may be considerable, a large proportion of the fungal mass has ceased to grow. The small amount of enzyme obtainable in the latter case, as compared with the former, indicates that the source of enzyme is the growing region of the hypha. The older parts of the hypha do not contribute an appreciable amount of enzyme, but would probably tend to reduce by adsorption the amount going into solution during the process of extraction.

This view, that enzymic formation is confined to the growing apex, is further supported by the results obtained in extracting hyphae which have ceased growing. In the glass plate experiments it was found that there was a marked diminution in the amount of enzyme obtainable from plate sowings of four and six days' age as compared with that from plates one to two days old. As the plates were identical at the commencement, the older cultures must possess at least as many hyphal tips as the younger ones. The dry weight measurements show, however, that active growth ceases from about the second day. The reduced activity of the older cultures is thus to be set down in this case, not to a reduced number of hyphal tips in the extracted mass, but to reduction in the amount of enzyme in each hyphal tip, this reduction being correlated with the fact that these hyphal tips have ceased growth on account of the development of stale conditions in the culture.

The elaboration of cytase in the fungal hypha would thus appear to be a process bound up with the protoplasmic activity associated with growth in the hyphal tip. Whether this enzyme plays a direct part in the changes undergone by the cell wall during the period of growth it is as yet impossible to say. Again, the cause of the disappearance of this enzyme, both from parts of the hyphae which have ceased to grow and also from the medium in which growth has taken place, is not clear. It is known that its solution in water is not stable, and thus the disappearance of enzyme from cultures as time goes on may be due simply to this fact, though a process of actual resorption by the fungus is not impossible.

Summary.

1. Two types of enzymic preparations derivable from cultures of *Botrytis cinerea* are described. These are:

(*a*) Watery extracts of the ground mycelium.

(*b*) The media in which germination and growth have taken place.

2. The amounts of (*a*) enzyme, (*b*) enzyme-retarding substances present in these under various experimental conditions (density of sowing, age of culture, nature of medium) were determined in each case.

3. A discussion is given of the bearing of these results on the technique of enzyme extraction on the one hand, and on the process of enzyme excretion by fungi on the other.

On the Haustoria of Pedicularis vulgaris, Tournef.

BY

A. C. MAYBROOK.

With five Figures in the Text.

Introduction.

So far as it has been possible to ascertain, the only account of the haustoria of *Pedicularis* is due to Leclerc du Sablon,[1] who described, in 1887, the development and structure of these organs in *Pedicularis sylvatica*. The writer does not mention the existence of 'phloeotracheides' in the haustoria; indeed the conception of phloeotracheides in root haustoria did not arise till much later (1910), when Benson[2] demonstrated their presence in *Exocarpus*, and put forward the suggestion that they existed in all root parasites.[3] The following piece of work was undertaken with the object of ascertaining whether such elements existed in the haustoria of *Pedicularis*, and the results may therefore be of use in supplementing those of du Sablon.

Methods.

The material was fixed in acid alcohol, and was subsequently preserved in 70 per cent. alcohol. Microtome sections were used; the most satisfactory double stain for these was found to be cotton red and aniline blue.[4] Stains of suitable strength were found by adding 100 c.c. of 80 per cent. alcohol to ½ grm. cotton red, and 1,000 c.c. of 80 per cent. alcohol to 2 grm. aniline blue and 1 c.c. picric acid. The sections were placed for twenty-four hours in cotton red, washed with 80 per cent. alcohol, and then placed in aniline blue

[1] Leclerc du Sablon: Recherches sur les organes d'absorption des plantes parasites (Rhinanthées et Santalacées). Ann. des Sc. Nat. Bot., 7e sér., 1887.

[2] Benson, Margaret: Root Parasitism in *Exocarpus* (with Comparative Notes on the Haustoria of *Thesium*). Ann. Bot., vol. xxiv, 1910, p. 671.

[3] Benson (l. c.) describes phloeotracheides as lignified cells which are lined with protoplasm but contain no nuclei. Their end walls are absorbed as in open tracheides. Embedded in the matrix lining the walls are a number of granules which probably consist of hydrolysed cellulose which has been deposited as amylodextrin. Their function is to collect and act as a pathway for the hydrolysed products of solution of the host cells. In the case of *Exocarpus*, which is subject to drought conditions combined with much isolation, these elements are supposed to act as a filter, since, by the precipitation of the granules, the ascending sap is less charged with dissolved carbohydrate.

[4] The instructions for the use of this combination were kindly given me by Miss Bancroft.

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for fifteen to twenty minutes. By this method the walls of the xylem elements become clearly differentiated red, whilst dense protoplasmic contents of the parenchymatous cells of the roots, and the phloeotracheides, to be subsequently described in the haustoria, take up the blue stain; the nucleus stains deeply with the blue stain, but the nucleolus takes up the red stain, and appears as a bright red spot in the nucleus.

General Morphology of the Roots and Haustoria of *Pedicularis vulgaris*.

As du Sablon has not described the haustoria in any great detail, for the sake of simplicity a general account of their morphology and structure will be given, followed by a more detailed account of the histological nature of the conducting elements in the haustoria.

The material consisted of some roots of *Pedicularis vulgaris* preying on roots of *Calluna* and on its own roots. A typical root was from 4 to 6 cm. in length; at the upper extremity, adjacent to the stem, it was about 3 mm.

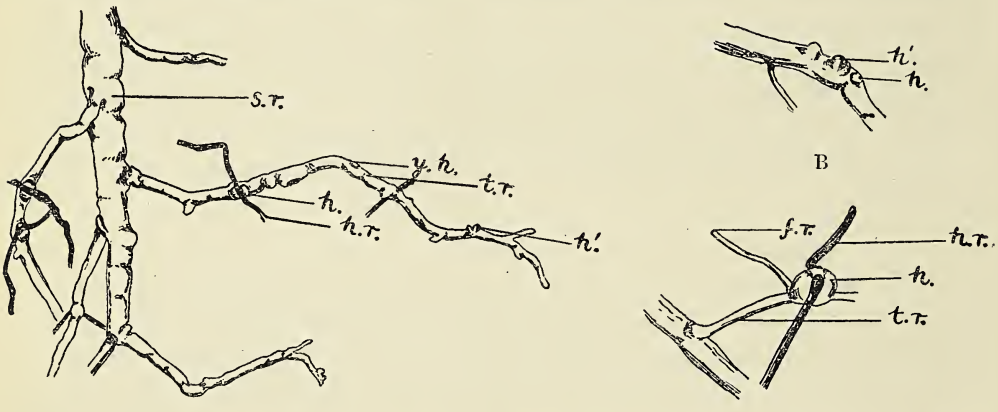
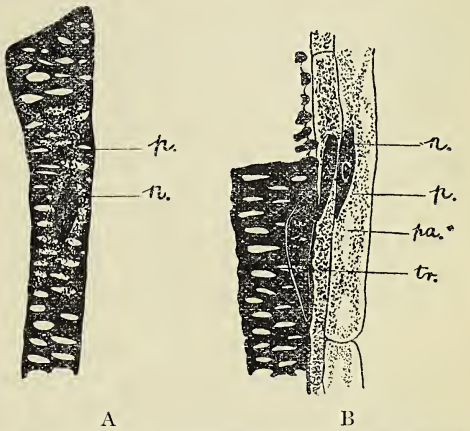


Fig. 1. A. Drawing of secondary root of *Pedicularis vulgaris*, bearing tertiary roots with haustoria. × 4. B. Portion of secondary root showing two-lipped appearance of haustoria. × 4. C. Drawing showing host root entirely embedded in tissue of haustorium. × 4. *s.r.* = secondary root of *Pedicularis*; *t.r.* = tertiary root of *Pedicularis*; *h.* and *h'.* = haustoria; *y.h.* = young haustorium; *h.r.* = host root; *f.r.* = fine rootlet.

in diameter, and tapered to a fine point at the penetrating end. The root was irregular in growth and in the method of branching; at the upper end adjoining the stem signs of shrinkage were distinctly obvious on the number of rings present. The haustoria were borne almost entirely on the finer rootlets, although signs of withered haustoria could be detected on the secondary rootlets, and even rarely on the primary root. This points to the parasitic habit being adopted even in the very young stages of the root's growth, although the life of these haustoria developed on the main roots is probably of short duration, since they die down and cease to be functional

with the production of the secondary and tertiary roots. Fig. 1, A, shows a secondary root *s.r.* of *Pedicularis*, which bears tertiary roots, *t.r.*, on which are borne the haustoria *h.* and *h'*. Fig. 1, B, shows a portion of a secondary rootlet bearing haustoria which have ceased to function. The haustoria are scattered irregularly on the roots bearing them (see Fig. 1, A), and have no definite order of development. They are first obvious as small hemispherical swellings on the rootlet (Fig. 1, A, *y.h.*). These increase in size and attach themselves to the host root in such a manner that the host root is always perpendicular to the portion of the parasitic root bearing the haustorium (see Fig. 1, A and C). The tissue of the haustorium grows up and partially encloses the host root; thus a groove is formed in the haustorium which is parallel to the host root and therefore perpendicular to the axis of the parasitic root at this point. In Fig. 1, A and B, *h'.*, the groove is distinctly visible owing to the host root being no longer attached—it has probably degenerated or become torn away—whilst in Fig. 1, A and C, *h.*, the haustoria are shown in connexion with the host roots *h.r.* The growth of the haustorium may sometimes be so vigorous that the host root becomes entirely enclosed in its tissues (see Fig. 1, C). The haustorium depicted here is borne on a tertiary root *t.r.*, and is also interesting in that it arises at the place of origin of a finer rootlet *f.r.*



A B

FIG. 2. A. Longitudinal section of tracheidal element of xylem of *Pedicularis*, showing protoplasmic contents and a nucleus. × 472. B. Longitudinal section of elongated parenchymatous elements which function as phloem. × 472. *tr.* = tracheide; *pa.* = elongated parenchymatous element; *p.* = protoplasmic contents; *n.* = nucleus.

Anatomically the roots of *Pedicularis* differ in two important details from the roots of normal Dicotyledons, namely in the complete absence of phloem and in the peculiar nature of the xylem elements. The xylem of the roots, both main and lateral, is composed entirely of tracheides and elongated parenchymatous cells, the latter being present in great abundance. The protoxylem consists of smaller tracheides, both spirally and annularly thickened. The other tracheides are all reticulately thickened, and, even in the oldest roots, still possess protoplasmic contents and often a nucleus. Fig. 2, A, is an old tracheide from a finer rootlet, and is typical of the tracheidal elements of the xylem. Much lignification of the walls of the element had taken place, yet it still showed abundant protoplasmic contents *p.*, and a large nucleus *n.* The place of the phloem in a normal root is taken by

a zone of elongated parenchymatous cells. All roots were very carefully examined for the presence of sieve-tubes, but no trace of any was discovered. Fig. 2, B, shows two of these elongated parenchymatous cells *pa.*, with their dense protoplasmic contents *p.*, and large nuclei *n.*, in longitudinal section. It is probable that transmission of organic food material takes place in *Pedicularis* root by osmosis, as Peirce [1] suggests for *Arceuthobium occidentale*, the complex materials passing from the elements of the host root through the thin walls of the parasitic parenchymatous elements by simple osmotic pressure. Now Benson [2] has suggested that the phloeotracheides of *Exocarpus* haustoria combine the functions of phloem and xylem. It seems probable that the tracheidal elements of *Pedicularis* root behave in much the same way, from the fact of their retention of protoplasmic contents.

This absence of typical phloem elements is not characteristic of all root parasites. Barber [3] has demonstrated the presence of sieve-tubes in the roots, though not in the haustoria, of *Olax scandens* and *Santalum album*; Peirce has shown that they exist in the roots and in the haustoria of *Arcenthobium occidentale*; and Benson has demonstrated their presence in the roots but not the haustoria of *Exocarpus*. Stephens,[4] however, failed to find any sieve-tubes in the roots of *Striga lutea* on which the haustoria were borne, and suggested that the transference of organic food might take place by osmosis. In the case of *Pedicularis* this seems to be most probable, especially in view of the nature of the xylem elements.

The Haustorium.

Du Sablon [5] has described briefly the anatomical development and structure of the haustorium, but for the sake of continuity and clearness a short account of the development will be given here. The haustorium arises in the hypodermal tissue of the cortex. The hypodermal cells of the cortex and the cells of the piliferous layer elongate enormously, and radial walls are formed in them. Tangential walls then follow rapidly in the elongated cortical cells, the cells of the piliferous layer, in contact with the host root, still continuing to divide by radial walls. Some of the outermost of these cells, that is, those farthest away from the surface of contact of the haustorium with the host root, elongate enormously, so that they have the effect of clasping the host root and acting as suctorial organs. Meanwhile division still continues in the cortical tissue, the endodermis even taking part in the division. Thus there

[1] Peirce, G. J.: Dissemination and Germination of *Arcenthobium occidentale*. Ann. Bot., vol. xix, 1905, p. 99.

[2] Benson, Margaret, l. c., p. 673.

[3] Barber, C. A.: Studies in Root Parasitism—The Haustoria of *Santalum album*. Mem. Dep. Ag. Ind., Bot. Ser., vol. i, 1906-7. Studies in Root Parasitism—The Haustoria of *Olax scandens*. Mem. Dep. Ag. Ind., Bot. Ser., vol. ii, 1907.

[4] Stephens, E. L.: The Structure and Development of the Haustorium of *Striga lutea*. Ann. Bot., vol. xxvi, Pt. II, 1912.

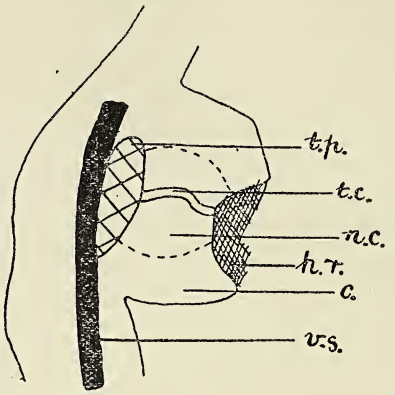
[5] Leclerc du Sablon, l. c.

is formed in the developing haustorium a mass of small-celled tissue, the 'nucleus', using the terminology of former writers;[1] this is bounded by the elongated palisade-like cells of the piliferous layer on the side adjacent to the host root, and by a cortex a few cells in thickness on its flanks. The haustorium penetrates the host root by means of the palisade-like piliferous cells, which grow straight forward into its tissue, dissolving the cortical cells through which they pass, and living on their contents, and eventually reaching the xylem elements of the root. After applying themselves by their tips to the elements of the host xylem, some of them become thickened in an annular or reticulate manner, still retaining their contents. Meanwhile a change has been taking place in the parenchymatous cells adjacent to the xylem elements of the mother root opposite the position of the haustorium. These become thickened in a reticulate or annular fashion and form a mass of tracheidal cells, which, however, still retain dense protoplasmic contents. Then a single row of the small cells of the central mass or 'nucleus', connecting the tracheidal penetrating cells with the mass of the tracheides adjacent to the xylem of the mother root, become lignified and thus tracheidal connexion is set up between the parasitic and host xylems. So much has been described by du Sablon, but little has been said by him about the internal anatomical structure of the mature haustorium, or the orientation of its internal conducting elements beyond mention of the fact that the penetrating cells are lignified in one plane only.

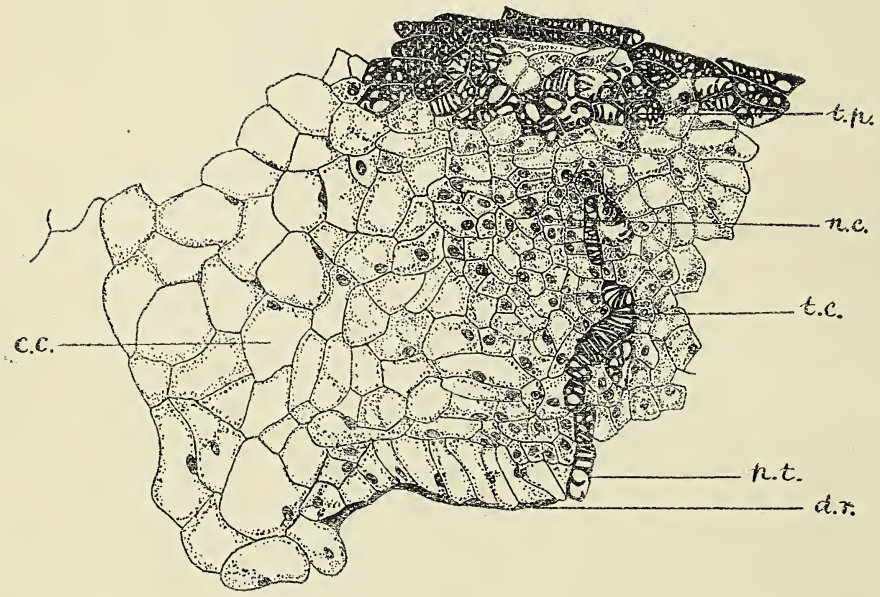
The orientation of the conducting tissue of the haustorium bears a definite relation to the orientation of the host root. It has already been said that the haustorium always attaches itself to the host root in such a manner that the latter is always perpendicular to the portion of the parasitic root bearing the haustorium (see Fig. 1). Hence if a median longitudinal section of the haustorium be made, in such a plane that a longitudinal section of the parasitic mother root is obtained, a transverse section of the attached host root will be made. Conversely, if a median longitudinal section of the haustorium be made in such a plane that a transverse section of the mother root is obtained, a longitudinal section of the host root will result. Always bearing in mind the orientation of the host root with regard to the parasitic root, and with the help of a few diagrams it is a simple matter to understand the internal structure of the haustorium. Fig. 3, A, represents a median longitudinal section of the haustorium taken in such a plane that a longitudinal section of the mother root and transverse section of the host root are obtained. The haustorium is seen to consist of a central mass of small-celled tissue *n.c.* Adjacent to the vascular strand *v.s.* of the mother root is the plate of tracheidal cells *t.p.*, and running directly from this to

[1] Barber, C. A., l. c.; Fraysse, A.: Contribution à la biologie des plantes phanérogames parasites. Montpellier: Société Anonyme de l'Imprimerie Générale du Midi, série A, No. 515, 1906; Benson, Margaret, l. c.; Stephens, E. L., l. c.

the host root *h.r.* is a single strand of tracheidal elements *t.c.* This strand may be one, two, or three cells in thickness when cut in this plane, but is rarely more, and most often consists of a single row of cells. Fig. 3, B, shows a portion of the same haustorium in detail ; the relative sizes of the cells *n.c.*



A

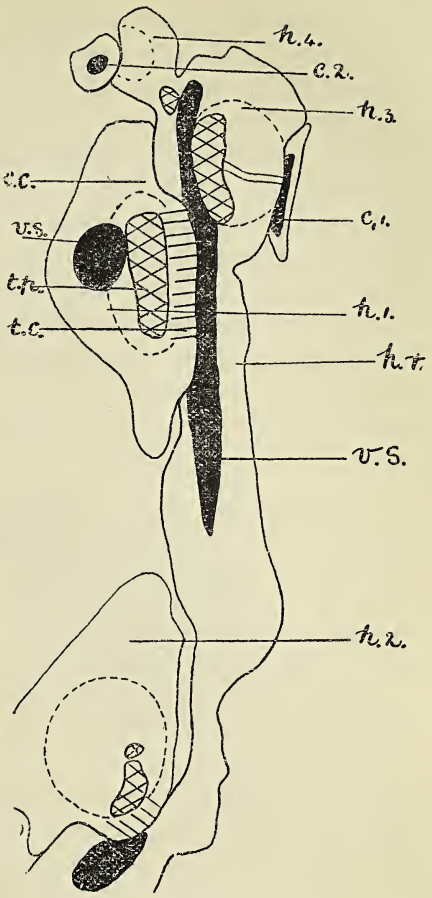


B

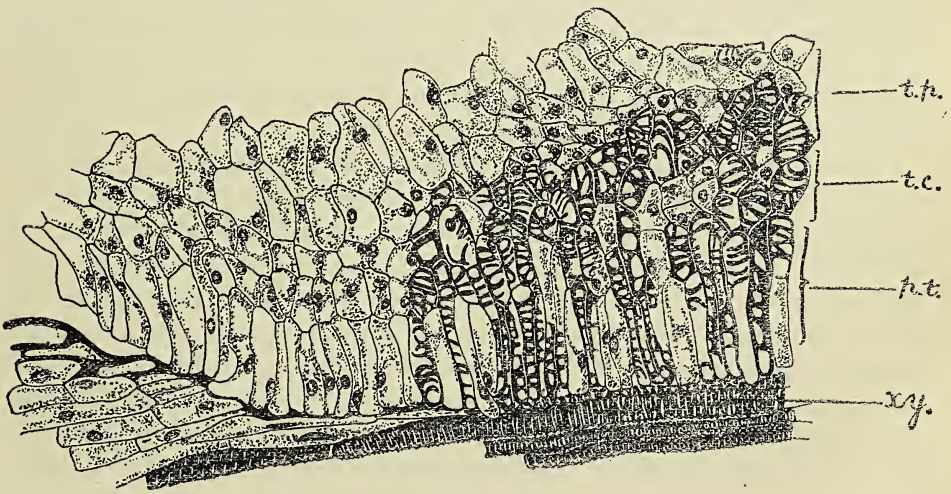
FIG. 3. A. Diagrammatic median longitudinal section of haustorium in a plane of a longitudinal section of the mother root and transverse section of host root. × 57⅓. B. Detailed drawing of a portion of haustorium represented in A. × 223⅓. C. Diagrammatic obliquely longitudinal section of root of *Pedicularis* attacked by two haustoria, *h* 1 and *h* 2, and bearing two haustoria, *h* 3 and *h* 4, which are attacking roots of *Calluna*, *c* 1 and *c* 2. × 44. D. Portion of haustorium, *h* 1 in detail. × 223⅓. *t.p.* = tracheidal plate; *t.c.* = tracheidal connexion; *n.c.* = cells of 'nucleus'; .= cortex of haustorium ; *v.s.* = vascular strand of parasitic root ; *h.r.* = host root ; *p.t.* = penetrating tracheides; *d.r.* = dead remains of host root ; *xy.* = xylem of host root.

of the 'nucleus', and those *c.c.* of the cortex, and the difference in density of their protoplasmic contents are shown. The cells of the tracheidal plate *t.p.* are in all stages of differentiation; some still have completely unthickened walls, whilst others have become lignified to a considerable extent. The strand of tracheidal cells *t.c.* connecting the thickened tracheidal cell *p.t.* of the penetrating piliferous layer with the elements of the tracheidal plate is not complete owing to the fact that the section was cut in a slightly oblique direction. The black line *d.r.* represents the crushed remains of the cortical tissue of the host root.

Fig. 3, C, *h.* 1 represents a median longitudinal section of a haustorium, cut in such a plane that a transverse section of the mother root, from which the haustorium arises, is obtained. In this plane the central mass of small-celled tissue is seen to be surrounded only by a very few layers of cortical cells, *c.c.*, and nearly all the cells of this central mass have become lignified



C



D

in a reticulate or annular manner. Similarly the majority of the penetrating piliferous cells have become lignified. The blackened circle *v.s.* represents the vascular tissue of the mother root, now cut in transverse section; the tracheidal plate is the cross-hatched portion *t.p.* and the portion marked by parallel straight lines represents the lignified elements of the 'nucleus' and penetrating piliferous layer, which connect the parasitic and host xylem. Fig. 3, D, represents half of this haustorium in detail; the tracheides *p.t.* which have been differentiated from the penetrating cells of the piliferous layer are at one end in direct communication with the xylem elements *xy* of the host root *h.r.*, and at the other with the elements *t.c.* of the tracheidal connexion which have been differentiated from the small-celled tissue of the 'nucleus'. These again are in direct communication with the elements *t.p.* of the tracheidal plate, and are practically indistinguishable from them, except for the larger size of the latter.

Before proceeding to construct the anatomy of the mature haustorium from the sections cut and just described, and from other serial sections, a fuller description of Fig. 3, C, will be given, as this represents rather a unique case of attack. The section is a slightly oblique longitudinal one of a root *h.r.* of *Pedicularis*, which is attacked in two places by haustoria, *h.* 1 and *h.* 2. Meanwhile it has itself produced two haustoria, *h.* 3 and *h.* 4, which are attacking two roots of *Calluna*, *c.* 1 and *c.* 2 respectively. The root *r.* of *Pedicularis* acts as host to the two haustoria *h.* 1 and *h.* 2, and hence, since it is itself cut in longitudinal section, these two haustoria are cut in the plane of section which gives a transverse section of the mother roots at the point of origin of the haustoria. Again, the root *h.r.* acts as the mother root of the two haustoria *h.* 3 and *h.* 4, whose host roots are respectively *c.* 1 and *c.* 2. Here, since the mother root *h.r.* is cut in longitudinal section, the two host roots *c.* 1 and *c.* 2 are cut in transverse section and the plane of section of the haustorium is the same as that shown in Fig. 3, A. Hence, here in one figure there are represented, typically by *h.* 1 and *h.* 3, the two planes of section of a haustorium, which give the two principal views of the anatomy just described.

From Fig. 3, A and C, *h.* 1, it can be seen that the differentiation of the palisade-like elements of the piliferous layer takes place in one plane only, the plane of section of the haustorium represented in Fig. 3, C, *h.* 1. This is also true of the cells of the 'nucleus' which become differentiated for conduction. The tracheidal plate, however, is not elongated in one direction, but is disc-like in shape, varying in thickness, but generally about three or four cells deep. Thus the mature haustorium consists of tracheidal elements arranged in a row, varying from one to three elements in thickness, and running in a direction parallel to the length of the host stem; these are surrounded by an elliptically shaped mass of small-celled tissue, the 'nucleus', the long axis of the ellipse being represented by the row of tracheidal

elements. The cells of the 'nucleus', and also the row of tracheidal elements, are in direct communication with the cells of the tracheidal plate, which is adjacent to the vascular tissue of the parasite, and all food material drawn from the host has to pass through this disc on its way to the parasitic root. The whole of this central tissue of the haustorium is surrounded by a cortex, which in most places is from four to six cells deep.

One of the haustoria examined exhibited a peculiar arrangement of the conducting tissue, which was interesting in so far as it showed a modification in the direction of economy of conduction elements. A median longitudinal section of a haustorium of *Pedicularis* had been cut in the plane giving a transverse section of the host root, hence the type of section illustrated in Fig. 3, A, was shown. One of the cells, Fig. 4, *p.t.*, of the piliferous layer, adjacent to a xylem element of the host root, had become thickened in the usual way, and still retained its contents. The cell *t.c.* 1 of the conducting tracheidal strand which was adjacent to this element *p.t.* was elongated to an abnormal extent, and in a direction perpendicular to that of the axis of the haustorium, and therefore perpendicular to the normal direction of elongation of these elements. The remaining elements of the tracheidal strand were of the normal form. The solution absorbed by the element *p.t.* from the host xylem element *xy* passes into the element *t.c.* 1, whence it can be passed into each one of the cells *n.c.*, and also the cell *t.c.* 2. Hence this phenomenon would appear to be a unique effort on the part of the haustorium towards economy of conducting material.

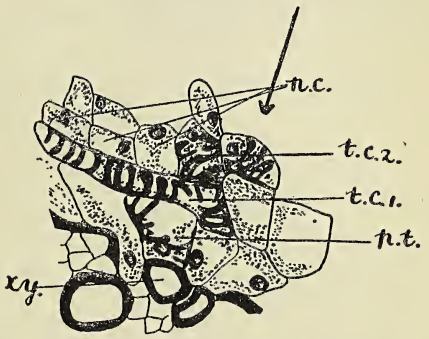


FIG. 4. Detailed drawings of a few cells at the penetrating end of a *Pedicularis* haustorium showing unique arrangement of haustorial tracheidal element *t.c.* 1. × 326⅔. *xy.* = host xylem element; *p.t.* = cell of piliferous layer tracheidally thickened; *t.c.* 1, *t.c.* 2 = cells of tracheidal connecting strand; *n.c.* = cells of 'nucleus' adjacent to elongated element *t.c.* 1. The arrow denotes the direction of the longitudinal axis of the haustorium.

It has already been mentioned that the parasitic root preys on other roots of the same or a different plant of *Pedicularis*. When such was the case no difference in the structure of the haustorium arose. Barber[1] has shown that where *Olax* attacks itself marked differences occur in the structure of the haustoria; all the vascular tissues of the mother root are continued down the entire length of the haustorium, and at the penetrating end enter into connexion with similar elements of the host root, the connexion being of the nature of a fusion. No such difference was found to exist in haustoria of *Pedicularis* in cases of self-attack, but the method of

[1] Barber, C. A.: Mem. Dep. Ag. Ind., Bot. Ser., vol. ii, 1907.

attack was precisely similar to that on *Calluna* root. As has been already mentioned, Fig. 3, C, represents in *h.* 1 and *h.* 2 two haustoria of *Pedicularis* attacking a root *h.r.* of the parasite. From the figure it can be seen that these two haustoria were similar in structure to the two haustoria, *h.* 3 and *h.* 4, which were attacking roots of *Calluna c.* 1 and *c.* 2.

There can be little doubt as to the morphology of the haustorium. That it is not a lateral root is evident, firstly because the haustoria arise indiscriminately, without any regard to sequence, on the surface of the lateral rootlets—in one case figured (Fig. 1, C) a haustorium had arisen actually at the point of origin of a lateral rootlet; and secondly, because the seat of origin is in the hypodermal layers of the root. They must therefore be regarded merely as superficial outgrowths of the roots formed for the purpose of extracting food material from foreign roots.

Minute Structure and Histology of the Tracheidal Elements of the Haustorium.

So far no account has been given of the minute structure of the elements composing the conducting tissue of the haustorium, beyond the fact that when lignification has occurred, the cells still retain their protoplasmic contents and nuclei. The penetrating cells of the piliferous layer differ from ordinary adult tracheides only in the possession of protoplasmic contents and a nucleus (Fig. 5, A, *p.t.*). Before lignification of the walls sets in, the contents of these club-shaped cells appear to diminish slightly in density, the protoplasm lying along the walls (Fig. 5, A, *p.c.*). No alteration in size of the nuclei is visible. That this slight diminution of protoplasm is not a stage in the complete degeneration of the protoplasmic contents, as would be the case in the development of an ordinary tracheidal cell, is demonstrated by the fact that in quite old haustoria these tracheidal elements are always seen to contain protoplasmic contents.

The elements of the tracheidal connecting strand and the tracheidal plate differ from each other only in size, the elements of the tracheidal plate being larger than those of the connecting strand. They both alike differ from the penetrating tracheidal cells of the piliferous layer, in that their protoplasm exhibits a number of granules, which stain bright blue with aniline blue. These elements, which will be called the 'phloeotracheides', differ only from those of *Exocarpus*, described by Benson,[1] in the possession of a nucleus. Otherwise they possess every characteristic of phloeotracheides. As Benson emphatically states that the phloeotracheides of *Exocarpus* were devoid of nuclei these cells were very carefully examined. In the majority of cases nuclei were found, and nowhere could any sign of degeneration of the nucleus be perceived. It is therefore concluded that the normal condition

[1] Benson, Margaret, l. c., p. 671.

in *Pedicularis* is for these cells to possess nuclei. Fig. 5, B, represents an optical section of a phloeotracheide which shows spiral thickening *l.*, and dense protoplasmic contents with granules *g.*, and a nucleus *n.* Fig. 5, C, shows two of these cells in transverse section, one of which has included the nucleus *n.*, and the other not. In Fig. 5, D, is depicted a fairly large phloeotracheide of the tracheidal plate in obliquely transverse section. The nucleus was not included in this section.

Previous records of these granules in tracheides have been made by Heinricher[1] and Benson.[2] Heinricher pointed out their presence in the con-

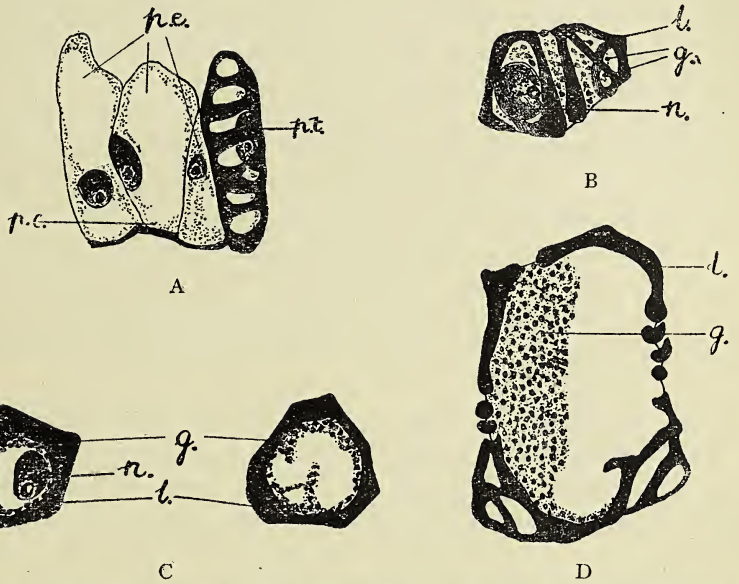


FIG. 5. A. Detailed drawing of cells of penetrating piliferous layer of haustorium, one of which, *p.t.*, has become lignified. × 472. B. Detailed drawing of optical section of cell of 'nucleus' which has become lignified. × 472. C. Transverse section of cells of nucleus which have become lignified. × 472. D. Obliquely transverse section of element of tracheidal plate showing protoplasmic contents and granules. × 472. *p.t.* = penetrating tracheidal element; *p.e.* = unlignified penetrating elements of piliferous layer; *p.c.* = protoplasmic contents; *g* = granules in protoplasm; *l.* = lignification; *n.* = nucleus of cells.

ducting cells of *Lathraea haustoria*, and figures some of these cells in Taf. IX, Fig. 7, and Benson describes them in *Exocarpus*, and considers that phloeotracheides are characteristic of all root haustoria, though they are arranged in different ways in haustoria of different plants. She considers the presence of the granules to be due to the deposition of the surplus of hydrated cellulose contained in the elements at the time of their lignification. In all the lignified conducting cells of this species of *Pedicularis* examined, not

[1] Heinricher: Anatomischer Bau und Leistung der Saugorgane der Schuppenwurz-Arten. Cohn's Beiträge z. Biol. d. Pflanzen, Band 7, ii, 1898.

[2] Benson, Margaret, l. c., p. 671.

only of the haustoria, but also of the roots, the persistence of the protoplasmic contents is a unique adaptation to the absence of phloem, the xylem elements being thereby enabled to conduct both organic and inorganic food material.

Summary and Conclusion.

1. *Pedicularis vulgaris* is a root parasite capable of attacking both the roots of other plants and of itself.

2. Haustoria of *Pedicularis vulgaris* were examined in detail and their anatomical structure has been described. The structure was found to be identical both in the case of attack of a foreign root and of itself. In this respect *Pedicularis* differs from *Olax*, which Barber has shown to vary in structure in the case of self-attack.

3. The haustoria were found to possess phloeotracheides, which, however, differed from those described by Benson for *Exocarpus* in being nucleated.

4. The xylem tracheides of the roots and of the piliferous layer of the haustoria were found to possess abundant protoplasmic contents.

5. Phloem was found to be absent from both the roots and the haustoria of *Pedicularis vulgaris*. This phenomenon was concluded to be correlated with the retention by the xylem tracheides of their protoplasmic contents. In the roots the place of the phloem is taken by elongated parenchymatous cells.

6. *Pedicularis vulgaris* being a root parasite is characterized by less highly differentiated haustoria than is found in stem parasites. The conducting cells are not specialized for the conduction of either organic or inorganic materials, but are capable of conducting them both indiscriminately.

The above piece of work was carried out in the Westfield College Laboratory during my tenure of research scholarships awarded by the Westfield College Council and by the London County Council. I wish to acknowledge the helpful advice and guidance I have received from Dr. de Fraine in this connexion.

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The Structure and Mode of Life of a Form of Hormidium flaccidum, A. Braun.[1]

BY

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With three Tables and six Figures in the Text.

1. Introductory.

In recent years a considerable amount of work has been published on the development and reproduction of Green Algae, but little attention has been paid as a rule to the way in which they vary in response to changes in the external conditions. Fritsch and Rich,[2] among others, refer to a special 'winter form' of *Cladophora*, produced by the cells of the ordinary filaments thickening their walls, darkening in colour, and accumulating abundant starch, besides often assuming an irregular, inflated shape. Few other data are to be found in the literature. It is to be supposed that terrestrial Algae, often exposed to extreme climatic changes, which they have frequently been noted to endure very successfully, should quickly and definitely respond to alterations in external conditions.

Oltmanns,[3] however, does not cite any records of special adaptation to drought occurring in such terrestrial Algae as *Pleurococcus*, *Hormidium*, and *Chlorella*. Nor does the recent summary by Petersen [4] of experiments on the power of various terrestrial Algae to withstand prolonged drought shed any light on the possible response of the Algae concerned to desiccation. On the other hand, Fritsch [5] has recently observed various effects of drought in *Zygnema ericetorum*, notably the special peripheral disposition of its 'fat' globules. With a view to throwing further light on the adaptation of

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[2] F. E. Fritsch and F. Rich: Biology and Ecology of the Algal Flora of Abbot's Pool, near Bristol. Bristol Naturalists' Society's Proceedings, Fourth Series, vol. ii, part ii, 1909, p. 40; and J. Comère: Observations sur la Périodicité du développement de la flore algologique dans la région toulousaine. Bull. Soc. Bot. de France, t. liii, 1906, p. 399.

[3] Oltmanns: Morphologie u. Biologie d. Algen, vol. ii, 1905, p. 352.

[4] J. B. Petersen: Studier over Danske aërofile Alger. Mém. Ac. Roy. d. Sc. et d. Lettres d. Danemark, 7e sér., t. xii, 1915, p. 353.

[5] F. E. Fritsch: The Morphology and Ecology of an Extreme Terrestrial Form of *Zygnema* (*Zygogonium*) *ericetorum* (Kuetz.), Hansg. Ann. Bot., vol. xxx, 1916, p. 143 et seq.

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terrestrial Algae to their conditions of life, the present observations were made on a form of *Hormidium flaccidum.*

The results set out below relate to one form of this species occupying the bare stretches of a piece of grassland near a pond at Woodford, Epping Forest. The characteristic features of the Alga may be summarized as follows: The filaments normally consist of a single row of cells (Fig. 1, A) and may be of considerable length (including sometimes as many as 1,400 cells); their width varies between 9 μ and 13 μ, the average obtained from a large number of filaments being 11 μ. The lengths of the cells range from

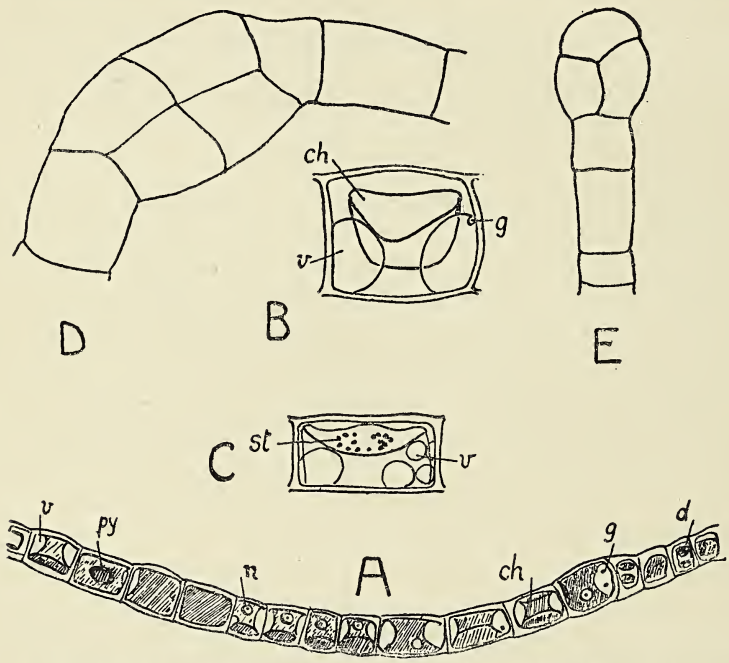

FIG. 1. A, portion of a typical filament of the Woodford form of *Hormidium flaccidum* growing on damp soil. B, cell containing two polar vacuoles. C, cell containing several small vacuoles in place of one of the polar vacuoles. D, E, parts of filaments showing abnormal cell-divisions. *n.*, nucleus; *ch.*, chloroplast; *py.*, pyrenoid; *v.*, vacuole; *st.*, starch; *g.*, granule; *d.*, dead cell. (A × 650; B × 2,000: C, D × 1,200; E × 850.)

two-thirds to two and a half times their width. A single plate-shaped chloroplast of a bright green colour lies just within the longitudinal wall of each cell, extending nearly the entire length and over about two-thirds of the circumference (Fig. 1, A); one or both of its longitudinal edges have frequently a curved outline (Fig. 1, C). The nucleus (Fig. 1, A, *n.*) lies near to the longitudinal wall, usually between the two edges of the chloroplast. There are often two large vacuoles in each cell, extending a variable distance from the ends towards the middle (Fig. 1, B, *v.*; Fig. 4, N); in short cells there is occasionally only one vacuole traversing the whole length of the cell. Numerous small vacuoles may replace the larger ones (Fig. 1, C).

Rarely, a cell becomes abnormally wide and subsequently divides by a longitudinal wall; two or three adjacent cells may behave in this manner (Fig. 1, D and E). A filament has never been observed more than two cells wide in the abnormal part, but the longitudinal walls may arise somewhat irregularly.

It will be seen from the above that of the three forms of *Hormidium flaccidum* enumerated by Heering [1] in his recent review, the Alga under consideration approaches the form *aquatica*, recorded as growing in stagnant water. It differs from the latter only in having a considerable proportion of short cells, of less length than breadth, and in habitat.

2. Mode of Growth.

At Woodford, the Alga occurs in isolated patches, colonizing some large, bare tracts which interrupt the continuity of the grassland. These tracts were evidently overgrown by grass at some previous time, since a layer of grass roots and rhizomes is habitually present beneath the *Hormidium* layer. The whole area is low-lying, there being a pond and system of small streams in the immediate vicinity, which, no doubt, help to keep the soil relatively moist. The matting of grass remains, referred to above, also tends to keep the surface layer of *Hormidium* damp by preventing water from quickly penetrating the soil. The latter is relatively light and remains continuously damp during the winter, but becomes very dry during the hot summer months.

There is a certain competition between the grass and the *Hormidium*, especially at the junction of the two zones. The grass cannot endure for any considerable period the intense dry heat which occurs from time to time in midsummer, and soon withers. The Alga, however, is able to resist desiccation for a longer period, and on the return of wet weather resumes its normal growth. It then tends to occupy former grass areas. At the approach of spring, however, the grass fruits, lying in the meshes of the tangle of old stems and roots, germinate, and the new plants overgrow the surrounding *Hormidium*, which tends to die away. Thus the edge of the grassland once again establishes itself.

The Alga grows as a thin layer scarcely 1 mm. thick over the surface of the ground. It is not definitely associated with the grass remains below, though occasional filaments grow downwards and then often coil round the latter.[2] Most of the threads, however, lie horizontally and interlace, producing a weft. The superficial filaments are much richer in the granules

[1] W. Heering: Ulotrichales, Microsporales, Oedogoniales, in A. Pascher, Die Süsswasserflora Deutschlands, Österreichs und der Schweiz, Heft vi, 1914, p. 46.

[2] It may be noted that the filaments of another form of *Hormidium flaccidum*, found on the thin soil covering some stones in Victoria Park, appeared to bend down in a knee-like manner here and there into the soil; the cells at the bends had usually lost their contents.

and refractive masses, described below, than those beneath the surface of the stratum.

Under the varying influence of wetness and dryness, cold and heat, the Alga preserves its general features. It thrives at Woodford during the winter, when, as already described, the soil is continuously damp. Experiments have, moreover, shown that many cells can resist considerable desiccation during cold weather. The *Hormidium* is rarely subjected, in its native habitat, to abundant rainfall simultaneously with high summer temperature, but the combination of such conditions experimentally produced in a greenhouse tends to destroy it. The filaments, on the other hand, can resist a remarkably long period of desiccation during warm weather, though the resistance of individual cells varies considerably. The intense insolation which occurs in midsummer may, however, cause the death of a large

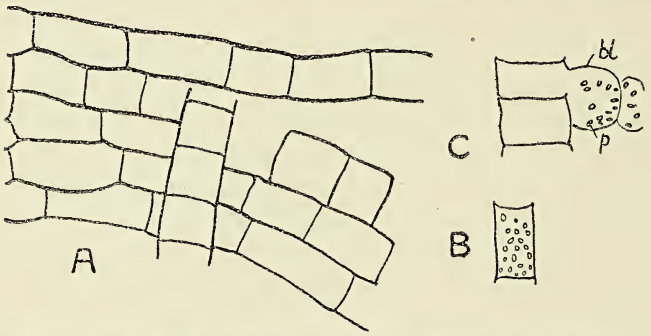

FIG. 2. A, false plate of cells formed by cohering filaments of *Hormidium* after drought. B, cell of a narrower form of *Hormidium flaccidum* infected by a parasite. C, shows escape of the parasitic organisms from the *Hormidium* cell. *p.*, parasite; *bl.*, enveloping vesicle. (A × 750; B × 1,200; C × 1,500.)

proportion of cells even after a few weeks, the algal layer then assuming a general dark greyish colour.

Dry filaments retain the same form as wet ones, but become narrower to the extent of about 2 μ. On access of water turgor is instantaneously restored and the cell-cavities widen; if desiccation has been prolonged, there is frequently a visible swelling of the longitudinal wall;[1] this seems to indicate a partially mucilaginous consistency. Neighbouring filaments, which usually cohere as plates or cords when dry (Fig. 2, A), move apart, and any existing curvature in a filament increases, unless it be due to hinging about a dead cell, in which case it decreases.

A close examination of filaments which have been subjected to desiccation shows that the latter produces definite changes in them. Some of the septa tend to become thickened so as to assume a biconcave form,[2] as described

[1] This was established by mounting the dry filaments first in alcohol and adding water subsequently.

[2] A considerable amount of dew may be necessary during the period of drought to cause this development, as it is not produced by rigid desiccation.

in detail below. Highly refractive granules and rounded masses generally accumulate in the cells (cf. p. 525), but the abundance and size of these depend on the food reserves available on the advent of drought, as well as on the degree of desiccation. Abundant starch is usually associated with the granules when numerous. Another effect of prolonged drought is the frequent contraction of the chloroplast away from the cell wall, the protoplasm remaining in contact with the latter; the chloroplast also tends to become a yellowish green. When the temperature is high, the outer layer of the cell wall, the 'cuticle',[1] cracks, and may become detached in small pieces.

In nature, *Hormidium flaccidum* may become infected by a parasite. This has not so far appeared in the Woodford Alga, but was found during the summer of 1915 in a form growing at Loughton, Epping Forest. Certain, usually scattered, cells were then observed to be filled with numerous minute ovoid organisms, which were perfectly colourless; the contents of the cells in question had either completely disappeared or become reduced to a thin peripheral vesicle often green in colour (Fig. 2, B). The cells of the parasite were frequently swarming, and would eventually escape through an aperture in the longitudinal wall (Fig. 2, C). They could subsequently be seen rotating about their longitudinal axis and swimming through the surrounding water. The organism is probably one of the Chytridiaceae, several of which are known to occur in the filaments of Ulotrichales.[2]

3. Splitting.

The splitting of the filaments at the transverse walls was recorded in *Hormidium* by A. Braun,[3] and has subsequently been examined in *H. dissectum* (*Ulothrix dissecta*) by Gay,[4] in *H. nitens* by Klebs,[5] and in *H. flaccidum* by Gay,[6] Klebs,[7] and Borzi.[8]

In *H. dissectum* Gay observed the middle lamella of the septum to dissociate from the periphery inwards, fission resulting. The separation proceeded in an unsymmetrical manner, being more marked at one side of the filament than at the other, so that the two resulting fragments were inclined to each other. According to Gay, *H. dissectum* dissociates actively

[1] This term, already adopted by Klebs and others, merely denotes the distinct, thin, external layer of the longitudinal wall. It has none of the characteristic properties of ordinary cuticle.

[2] Cf. Lemmermann: Die parasitischen u. saprophytischen Pilze der Algen. Abh. Nat. Ver. Bremen, xvii, 1901, p. 185 et seq.

[3] A. Braun: Rejuvenescence in Nature, Leipzig, 1851. English translation by A. Henfrey, p. 131, foot-note.

[4] F. Gay: Recherches sur le Développement et la classification de quelques Algues vertes. Thèse, Paris, 1891, p. 60.

[5] G. Klebs: Bedingungen der Fortpflanzung, Jena, 1896, p. 329 et seq.

[6] l. c., p. 63. [7] l. c., p. 341.

[8] Borzi: Studi algologici, fasc. ii, 1895, pp. 361–9.

when kept damp. In the case of *H. flaccidum* he found splitting to be somewhat rare under normal terrestrial conditions, but more abundant when growth took place in water. Borzi states that splitting sets in after the filaments have attained a certain length, and that dissociation into individual cells occurs on the advent of dryness and when the Alga is cultivated on gelatine.

Klebs,[1] working with *H. nitens*, found that splitting depended in no way on the number of divisions or the length of the threads. He states that it is occasional, mostly occurring at points of bending, when this Alga is cultivated in a solution of nutritive salts under conditions favourable to growth, but that a lack of nutritive salts, particularly nitrogen, phosphorus, and magnesium salts, or a lack of moisture causes a real breaking up of the threads into short few-celled pieces. He observed splitting in cultures on pure agar-agar. Regarding the direct cause of splitting, he suggests that on cessation of division thickening layers are no longer added to the cuticle connecting two adjacent cells; on the other hand, owing to the growth which goes on for some time and subsequently to the increase of turgor resulting from continued assimilation, the cuticle becomes stretched and ultimately tears. Thus the connexion between the cells is loosened here and there and the transverse septum then splits owing to the arching of its two constituent lamellae. In uniformity with Gay's[2] observation, Klebs found that separation occurs more rapidly at one side of a septum than at the other, an angle being formed between the separating portions, while the filament as a whole takes on a zigzag form.

The *process* of splitting in the Woodford *Hormidium* agrees with that outlined for other forms by Gay and Klebs. There is never a complete dissociation into single cells, though, under certain conditions, a small proportion of the filaments break into few-celled pieces. Usually splitting is rather occasional, and the fragments are of considerable length.

The only condition which has been found effective in producing splitting is desiccation followed by an abundance of water. When, during a fortnight in summer, material kept in the greenhouse was only watered at intervals of two or three days, so that it became quite dry between successive supplies, many filaments dissociated into few-celled fragments. Other experiments have shown that splittings arise in material already subjected to desiccation for several weeks within a day or two after watering, but are not obvious in dry material immediately after placing it in water. Again, splittings were found to be common in filaments growing out of doors, which were examined on the second day following a spell of nine dry days. It is not probable that drought alone can produce splitting, as the latter did not appear during the desiccation experiments described below (p. 525).

To throw more light on the direct causes of splitting in this form of

[1] l.c., p. 329 et seq. [2] l. c., p. 60.

Hormidium flaccidum, the parts affected, namely the longitudinal walls and the septa, were carefully examined. These investigations were made in the first place on material in which splitting had been abundantly produced by supplies of water alternating with periods of desiccation (p. 516), and which had been subsequently kept without water for six weeks.

The longitudinal walls were relatively thin, but one could usually distinguish a 'cuticle' of variable thickness, though undergoing disintegration here and there, especially in the neighbourhood of the septa. In curved filaments the cuticle appeared folded or corrugated along the concave surface of the bend (Fig. 3, A). Occasionally isolated bulbous thickenings were deposited on the inner surface of the longitudinal wall, while in dead cells a definite, though irregular, inner layer was frequent. The septa were commonly biconcave owing to the two component lamellae diverging before merging into the longitudinal wall, the region between them being occupied by a substance of different refractive index (Fig. 3, B). The latter often extended right across the septum so as to separate completely the two lamellae, and had occasionally become drawn out in the longitudinal direction so as to form a short cord connecting the two adjacent cells (Fig. 3, C, *m*.). Even when the thickening was much less pronounced the filaments were often slightly constricted in the regions of the thickened septa.

Thickened biconcave septa have been observed in desiccated material growing naturally out of doors in cold and warm weather. In such cases, however, the amount of damping produced by dew during the time of drought, as well as the exact external conditions preceding it, have not been properly known. The fact that thickenings did not appear in material subjected to continuous desiccation during many weeks when the dew was inconsiderable (cf. Desiccation Experiment I, p. 525) seems to indicate that drought is not the entire cause of this particular thickening of the septa. There may be a certain amount of intermittent moisture required, and possibly individual peculiarities of adjacent cells have some additional influence, as is suggested by the fact that septa in the same filament exhibit a great deal of variation in the degree of thickening, many remaining unthickened while others are considerably thickened.

Some indication of the chemical nature of the longitudinal walls and septa was sought by treatment of the above-mentioned material with various reagents and stains. Walls and septa were completely dissolved by concentrated sulphuric acid. Ordinary iodine solution, added to fresh filaments, caused the 'cuticle' to become very distinct as a dark line, which was thinner opposite the septa, the disintegrated portions staining blue. The remainder of the longitudinal wall, as well as the septa, assumed a pale blue colour. Methylene blue stained the disintegrating parts of the 'cuticle' (Fig. 3, D) and the débris of thickening substance often associated with

splitting septa; occasionally, the entire remaining portion of a partially split septum became blue, but usually, with these and intact septa, only a restricted area of the thickening substance within the fork of the lamellae was affected.

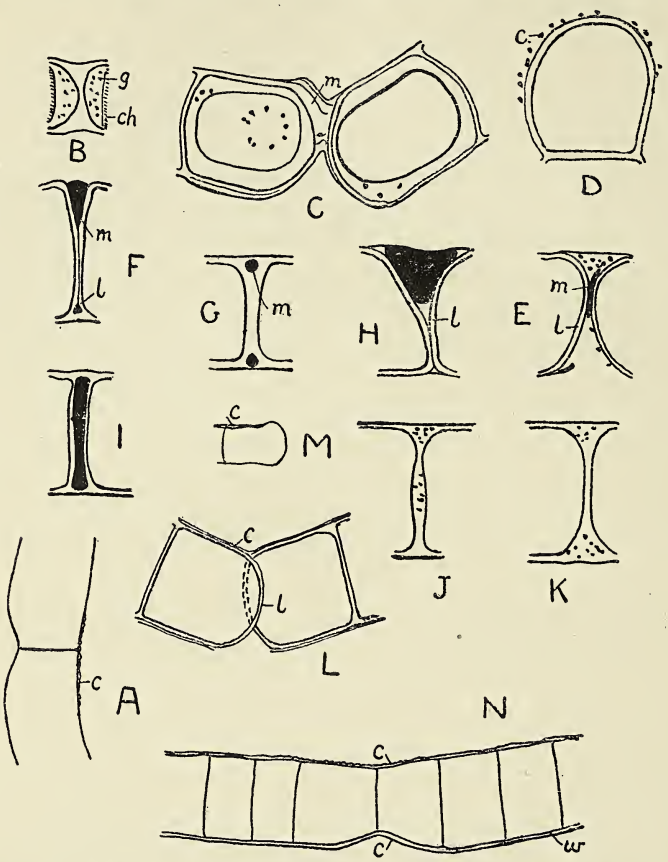

FIG. 3. A, bend in a filament, causing 'cuticle' at the concave surface to become corrugated. B, biconcave septum in optical section. C, thickening substance of septum drawn out between two cells. D, disintegrating 'cuticle' stained by methylene blue. E–I, septa stained by Ehrlich's haematoxylin, in optical section; E, F, splitting septa in which the middle layer of the intact part and the débris of thickening substance adhering to the dissociated part have taken up the stain; G, shows stained area within fork of the lamellae of the septum; H, shows stained thickening substance in a drawn-out septum. I, entire middle layer of an intact septum stained. J, K, septa from filaments growing in nutritive solutions stained with Ehrlich's haematoxylin. L, septum in which splitting is nearly accomplished. M, cell recently detached by splitting of filament, widened at its free extremity. N, curved filament which has contracted on becoming dry, showing the 'cuticle' dissociated from longitudinal wall on convex surface, and corrugated on the concave surface of bend. *c.*, 'cuticle'; *m.*, thickening substance of septum; *l.*, lamellae of septum; *w.*, longitudinal wall; other lettering as in Fig. 1. (A, B × 890; C–K × 1,500; L × 1,300; M × 480; N × 1,150.)

The longitudinal walls remained unstained. Treatment with Ehrlich's haematoxylin for about seven minutes, and rapid rinsing in water subsequently, produced results (Fig. 3, E and F) similar to those obtained with methylene blue, the longitudinal walls as a whole being still unstained, though the cell-contents had become deeply coloured, while usually the

same localized region of thickening substance within the fork of the lamellae of the septum became deep purple, and could be traced as a continuous girdle round the filament, embedded in the septum some little distance within the 'cuticle' (Fig. 3, G). The stained substance appeared in great bulk in drawn-out septa (Fig. 3, H). When splitting was in progress, as well as occasionally in intact septa, the whole middle portion of the septum, between two unstained lamellae, became deeply coloured (Fig. 3, E and I). A débris of stained particles was occasionally seen in contact with the new end cells of pieces of filament recently detached.

The results produced by the above stains show that in the material examined the cuticle was somewhat mucilaginous, especially where obviously disintegrated. The thickening substance in the septa was usually mucilaginous near the periphery, and in certain cases formed a continuous plate of mucilaginous material through the middle of the septum. Also, mucilaginous substance was generally associated with the separating lamellae of splitting septa.

For comparison, similar observations were made on material which had been growing for some weeks in nutritive solution and showed no indication of splitting. Here the 'cuticle' of the longitudinal walls was not distinguishable except opposite the septa, and there was no degeneration. The two distinct lamellae of the septa were often apparent, diverging a little before joining the longitudinal wall and often dissociated to a small extent in the central region, thus rendering the septum slightly biconvex (Fig. 3, J). There was always a thickening substance between the two lamellae, but this did not respond to methylene blue and Ehrlich's haematoxylin so readily as that in the material examined above. With both these stains, only small particles within the fork at the edge of the septum and scattered in the thickening of the central region became coloured (Fig. 3, J, K).

From the preceding data the following conclusions may be drawn: The 'cuticle' tends to undergo a certain mucilaginous degeneration and to become especially thin opposite the septa. There is normally secreted in thickening septa a mucilaginous substance, which may form a continuous layer throughout the septa. This substance is very generally associated with splitting septa and frequently with the end wall of newly separated pieces of filament.

An examination of partially split septa, which were numerous in the material described on p. 527, furnished the following details regarding the process of splitting. The latter was usually initiated by the disintegration of the 'cuticle' at a septum, but sometimes the lamellae of the latter first separated at their edges, and the 'cuticle' adapted itself to the new shape by stretching, until it ultimately broke across. Partial splittings were common in bent filaments, being restricted to the portions of the septa on the outside of the curve. They were, however, occasionally present in

straight filaments, and were then noted to occur usually between cells which contracted at different rates on loss of water; in these cases, too, they affected the entire circumference of the septum, without extending right across it. As the lamellae of a septum were gradually separated by the breaking down of the thickening substance between them, they became bulged in a convex manner as is usual with free end walls (Fig. 3, L).

Attempts to produce splittings by wetting dry filaments under the microscope, and to extend partial splittings by successively drying and wetting the filaments, were unsuccessful. The latter treatment, nevertheless, caused a swinging movement in curved filaments, and probably, where the rate of contraction was different in adjacent cells, a definite strain on the cuticle at the transverse walls. Dry filaments were, however, observed to shear at septa adjacent to dead cells, on becoming suddenly turgescent.

In a consideration of the causes of splitting, the 'cuticle' may be treated as a thin cylindrical shell subject to internal fluid pressure. This assumption appears to be warranted by the observation that cells separated by a split often widen at their free extremities (Fig. 3, M), where the 'cuticular' sheath has given way, this indicating that the latter constitutes a distinct strengthening layer of the wall. The 'cuticle' should therefore be especially capable of resisting a transverse tear.[1] When a filament is bent, a greater longitudinal stress is set up in the 'cuticle' at the outside of the curve, but splitting occurs also in straight filaments and then begins round the whole circumference of the filament. It would therefore seem that splitting at a septum, if occasioned by increase of turgor as described by Klebs, can only arise where there is a general or local weakening of the 'cuticle' at the septa. It has been shown above that a more or less general degeneration of the 'cuticle', frequently pronounced at the septa, does occur in filaments which are in process of splitting. Such a degeneration probably results from the folding and stretching of the 'cuticle' caused by curvature of the filaments, and from the separation of the 'cuticle' from the longitudinal wall, occasionally observed when a filament contracts owing to loss of water (Fig. 3, N). One reason for its prevalence in the regions of the septa may be the difference in the rate of contraction and possibly expansion, occurring among individual cells of the same filament, during changes in turgescence. On the other hand, splitting might result from a distinct longitudinal force being brought into play between the two lamellae of the septum, tending to separate them. A special weakening of the 'cuticle' adjacent to the septa must be produced by the development of the mucilaginous substance, and the continued extension of this substance possibly produces a longitudinal force which ultimately ruptures the 'cuticle'.

[1] The resistance of such a cylindrical shell to a transverse tear is double its resistance to a longitudinal tear. Cf. D. A. Low: Applied Mechanics, p. 76.

4. Aplanospores.

As appears in other sections of this paper, the Woodford form of *Hormidium flaccidum* has been grown under many different external conditions. Yet, apart from splitting, the only method of reproduction observed was the formation of aplanospores. The treatment by which

Fig. 4. A–K, stages in the production of free aplanospores. A, contraction of chloroplast to centre of cell. B, C, D, protoplasm contracted round chloroplast. E, development of surrounding membrane before liberation. F, G, liberation of aplanospore. H, empty parent cell, with aperture in wall. I, free aplanospore. J, aplanospore with surrounding wall. K, first stage in germination. L, cell showing distribution of refractive granules and masses in superficial protoplasm. M, customary distribution of granules, when numerous. N, granules arising in the vacuoles. *p.*, protoplasm; *w.*, cell membrane; other lettering as in Fig. 1. (A, B, D × 1,750; C, E × 1,360; F–K, N × 900; L × 1,500; M × 1,000).

Klebs[1] obtained zoospores in another form of *H. flaccidum*, viz. transference of threads from a nutritive solution into water in darkness, has not proved successful with this form.

The aplanospores of *H. flaccidum* have only been described in *forma typica*, where they are rounded and have a spinous wall resembling a

[1] l. c., p. 343.

Trochiscia.[1] The formation and liberation of aplanospores in the Woodford form takes place as follows: The chloroplast contracts and comes to lie in the centre of the cell (Fig. 4, A). Then the protoplasm retreats from the cell wall and closely envelops the chloroplast (Fig. 4, B, C). In cells containing granules, a certain number may be extruded during this process. The resulting aplanospore is generally slightly oval in shape, its average dimensions being 9 μ by 8 μ; in short cells it is frequently more pronouncedly oval, as though somewhat compressed longitudinally (Fig. 4, D). It usually remains naked until liberated, but occasionally develops a thin wall while still within the mother-cell (Fig. 4, E). The chloroplast is a bright green, and the pyrenoid often very distinct. Refractive granules frequently occur in the protoplasm. Occasionally, two aplanospores arise within a single cell (Fig. 4, E).

The aplanospore is liberated by the rupture of a papilla, which develops in the longitudinal wall (Fig. 4, F–I). The aplanospore is often seen to protrude in this direction before the cell opens.

The germination of these reproductive cells has not been followed in detail. In the normal habitat, in summer, they have been seen to elongate and then divide transversely. The same observation was made more rarely in cultures in 5 per cent. glucose solutions (Fig. 4, K). The width of such juvenile filaments is much more variable than that of adult ones, usually being less.

It is probable, however, that under certain conditions the aplanospores subdivide in two or three directions before germinating, after the manner of those of *forma typica*, since they have often been observed in pairs when free. Moreover, isolated packets of green cells, similar in size though of obscure structure, have been found in otherwise pure material producing aplanospores.

Many free aplanospores were found during the summer of 1916 in material growing at Woodford, and during three weeks of sunshine in the summer of 1915 (average daily range of temperature 53° F.–83° F.) in material growing in 2 per cent. to 5 per cent. glucose solutions. They have also appeared, though not in abundance, during damp winter weather. Aplanospores are not formed under conditions of drought; nevertheless, they can evidently resist considerable desiccation, since they were found to survive a dry period lasting one month, at low temperature (average range 31°–51° F.), and a short period of severe desiccation in midsummer.

5. Cell-contents.

Under certain conditions well-defined, white, refractive granules become conspicuous in the cell-contents of this *Hormidium*. These granules are

[1] Cf. Heering, l. c., p. 46, Fig. 49.

more or less spherical in form, though tending to assume an irregular shape when large. They vary considerably in size, being often scarcely visible under a magnification of 3,000 and passing through all gradations to a diameter of about 4 μ (Fig. 4, L). When present only in small numbers, they lie here and there in the surface layer of the protoplasm, most commonly near the septa. If exceedingly abundant, they are concentrated at the ends of the cells, but may also be numerous in the part of the protoplasm between the two longitudinal edges of the chloroplast (Fig. 4, M); occasional granules are always scattered in the surface protoplasm adjacent to the chloroplast. During the earlier stages of accumulation, the cells are usually vacuolar, as described above (p. 512), and the first-formed granules appear mostly at the surface of the vacuoles (Fig. 4, N). In the later stages, the masses of granules at the ends of the cells frequently preserve the outlines of the vacuoles and can be seen to have entirely occupied the latter. This suggests that they are produced as a result of changes occurring chiefly in the contents of the vacuoles (cf. p. 532).

Granules similar in appearance have been found in other forms[1] of *Hormidium flaccidum* distinct from the Woodford Alga, and in *Spirogyra*,[2] *Prasiola*, and *Pleurococcus*.

Gay[3] has recorded the accumulation of oil globules in *Hormidium dissectum* (*Ulothrix dissecta*) when growing in water, and Klebs[4] has stated with reference to *H. nitens* that the cells become filled with drops of fatty oil when subjected to slow desiccation. Klercker,[5] in *Stichococcus subtilis*, described droplets, which he called 'spherules', usually present in the polar vacuoles,[6] but sometimes 'accumulating in such abundance as to make an examination of the internal structure of the cell almost impossible'. Apart from their similar disposition in the cells, these bear a striking resemblance, as shown in the figures, to the granules of *Hormidium*. Klercker[7] states that they agree in many respects with the substances occurring in *Stigeoclonium* and other Chaetophoraceae. The globules of *Zygnema ericetorum* described by Fritsch,[8] West and Starkey,[9] and de Bary[10] are somewhat similar in form and appearance to those of *Hormidium flaccidum* and

[1] Viz. (i) a narrower form, with filaments of average width 5·7 μ, growing at Loughton, Epping Forest; (ii) a wider form growing on Hindhead Common. I have to thank Dr. Fritsch for the information regarding this Alga, and for material.

[2] Probably tannin-vesicles. Cf. Czapek: Biochemie der Pflanzen, vol. ii, 1905, p. 579; Hill and Haas: The Chemistry of Plant Products, 1912, p. 191. They take on a brown coloration with osmic acid similar to that produced by the latter in the granules of *Hormidium*.

[3] l. c., p. 62. [4] l. c., p. 340.

[5] Klercker: Ueber zwei Wasserformen von *Stichococcus*. Flora, vol. lxxxii, 1896, pp. 92, 93.

[6] These resemble, in form and disposition, the vacuoles of the Alga dealt with in this paper.

[7] l. c., p. 92. [8] F. E. Fritsch, l. c., p. 143.

[9] G. S. West and C. B. Starkey: A Contribution to the Cytology and Life-history of *Zygnema ericetorum* (Kuetz.), Hansg. New Phytol., vol. xiv, 1915, p. 197.

[10] de Bary: Unters. über die Familie der Conjugaten, Leipzig, 1858, p. 10, Pl. I, Figs. 16, 20.

Stichococcus subtilis; in the *Zygnema*, however, they commonly occur distributed in the whole peripheral layer of the protoplasm.

The refractive bodies of the Zygnemaceae (*Zygnema* and *Spirogyra*) have been referred to variously as tannin-vesicles[1] and fat-bodies.[2] According to Klercker,[3] those of *Stichococcus subtilis* have been constantly recorded as 'oil', but fail to show any distinct fat-reactions. As in the case of *Stigeoclonium*, he succeeded in staining them with Bismarck brown and aniline dyes, without killing the cells.

The frequent occurrence of white refractive granules in the cells of many different Algae suggests that there may be some product, common to their metatobic processes, which assumes this form under certain conditions. Such a view could only be corroborated by a large amount of experimental work, but the similarity in the appearance of the different granules and in their behaviour towards reagents,[4] as well as their accumulation under like conditions,[5] may indicate some such fundamental relationship between them.

The granules found in the Woodford *Hormidium* do not appear to be of the nature of ordinary fat, since they fail to dissolve in the usual fat-solvents (e. g. ether, benzole, carbon bisulphide) and are not saponified by treatment with a mixture of concentrated ammonia and caustic potash.[6] On the other hand, osmic acid stains them brown, though never black, and Sharlach R colours them light red, but not deep red as in the case of fats. They retain their form after treatment with boiling water.

Apart from the above, the granules show the following chemical properties: They are insoluble in concentrated sulphuric acid; are unstained by iodine; coloured green by chlorophyll,[7] and reddish-brown by a solution of iodine in potassium iodide with subsequent addition of dilute sulphuric acid.[8] These reactions, besides those with osmic acid and Sharlach R, are all more particularly characteristic of cuticle, and would appear to indicate that the granules are composed of some fat-like substance similar to that occurring in a cuticularized wall.[9]

In the case of large granules or masses, the interior appears to stain more deeply than the peripheral layer when the above reagents are applied. This differentiation is marked on staining with methylene blue,[10] the interior

[1] Van Wisselingh: Koninkl. Akad. v. Wetensch., Amsterdam, 1910.

[2] Cf. de Bary, l. c., p. 10; West and Starkey, l. c., p. 198, and Zimmermann, Botanical Microtechnique English translation by J. E. Humphrey, 1893, p. 234. [3] l. c., p. 93.

[4] This is not quite the same, however, in all cases. Cf. Fritsch, l. c., p. 144.

[5] Cf. Fritsch, l. c., p. 143, and Klercker, l. c., p. 94.

[6] Cf. Zimmermann, l. c., p. 73.

[7] When cells are heated in water or placed in alcohol, the granules absorb the chlorophyll from the chloroplast. The localization of chlorophyll which results is very noticeable, especially as it does not occur in non-granular cells present in the same filaments.

[8] As described by Strasburger: Practical Botany, English translation by W. Hillhouse, pp. 47 and 395. [9] Cf Zimmermann, l. c., p. 152.

[10] Recorded as a stain for fat by Lee: The Microtomist's Vade Mecum, 1893, 3rd ed., p. 80.

of the granule assuming a deep blue colour, while the thin external layer becomes merely light blue. The fact that the outer portion of the granules behaves rather differently from the interior towards reagents indicates that the structure of the granules is possibly vesicular, the contained substance differing somewhat from the bounding layer. Such a structure would be comparable to that recorded for the granules of Zygnemaceae (cf. p. 523).

Commenting on the special abundance of granules in a heath form of *Zygnema ericetorum*, and their formation into a dense peripheral layer in time of drought, Fritsch [1] observes that one might be disposed to associate the granular layer with the great power of resistance to drought possessed by this Alga. The question appeared of some importance in connexion with *Hormidium flaccidum*, which, though capable of considerable resistance to desiccation, shows none of the usual xerophytic adaptations, e.g. thick mucilaginous walls. Since granules are not habitually present in the cells, general observations were made to trace some relationship between desiccation and the appearance of granules, and also to reveal the power of resistance to drought possessed by granular material.

As a result, it was found that during periods of desiccation, both in summer and winter, granules always occurred in a large proportion of the living cells, but were frequently minute and scanty. When the Alga was receiving continuous supplies of water, the granules were sometimes prevalent in the cells, at other times localized in a small proportion of them. The latter variation apparently depended on the temperature and amount of sunshine. During winter, when the soil was continuously damp, the granules were often entirely absent, and, when present, were usually small and restricted to a minor proportion of the cells (25 per cent. and 46 per cent. in different samples collected in mid-winter). During warm, sunny weather,[2] they appeared in most of the cells, and were both large and abundant. These observations indicated that granules do arise as a result of desiccation, though at such times they may be small and scanty; but they also appear, often in abundance, under other circumstances, viz. warm, damp sunny weather.

To observe more accurately the resistance of the cells to drought and the effect of the latter in producing granules, experiments were set up to subject the Alga to continuous drought, the other conditions of growth being so far as possible untouched. A large patch of *Hormidium*, with a considerable thickness of underlying soil, was collected from the native habitat and examined at frequent intervals during the experiment.

In Experiment I, which lasted from the end of January to the middle of March, the piece of soil was laid on a glass Petri dish and kept out of

[1] l. c., p. 145.

[2] Observations were made on material transplanted, with considerable depth of underlying soil, to a greenhouse.

Table I

(First Desiccation Experiment.)

Time in Days.	*Temps.* °F. Max.*	*Min.*	*Dead Cells to Total Cells.* %	*Granular Living Cells to Total Living Cells.* %	*General Observations.* *Granules.*	*Substances stained with Sharlach R (cf. p. 532).*	*Starch.*
0	—		2	25	Very minute and sparse	Very abundant	Universally scanty
6	48	45	29	15	Still sparse but larger	Much less abundant	Scarce or absent
14	46	42	41	55	Rather more abundant	Rather less	Unchanged
18	42	37	40	98	Less in number and size	Less	—
28	47	38	51	100	Mostly minute	—	—
41	Snow		93	60	Very minute and sparse	Very scanty	—
51	—		Apparently all dead. Chloroplast generally still green and often rolled up in the centre of the cell. Occasional cells have minute granules.				
			After keeping re-watered from the 18th day.				
41	—		29	36	Always minute	Much more abundant than on the 18th day	—
			After keeping re-watered from the 41st day.				
66	—		77	—	Living cells isolated, or in long or short bands, often in process of division; many vacuolar.		

* Average temperatures for periods preceding counts.
— Indicates no record.

Table II

(Second Desiccation Experiment).

Time in Days.	*Temps.* °F. Max.*	*Min.*	*Dead Cells to Total Cells. %*	*Granular Living Cells to Total Living Cells. %*	*Granules.*	*General Observations. Substances stained with Sharlach R (cf. p. 532).*	*Starch.*
0	—		9	59	Not usually numerous	Fairly abundant	Fairly abundant
8	53	46	10	71	Larger, frequently numerous	Large increase	—
15	44	33	18	77	More numerous	Very slight decrease	—
23	54	40	21	86	No change	Unchanged	Much more abundant
31	52	40	20	89	More numerous	Marked decrease	—
36	52	40	18	90	Ditto	No change	—
41	52	41	32	95	Less numerous	Slight decrease	Decrease
54	68	45	49	87	Ditto	Decrease	—
62	63	49	46	95	Rather more numerous	Scanty	Further decrease
73	74	60	55	83	Decrease in many cells	—	—
81	71	58	60	82	Usually small and scanty In a few cells numerous	—	—
96	—		61	83	Ditto	—	—
106	—		69	86	Ditto	—	—
128	74	50	72	98	Ditto	—	—
142	82	58	87	99	Ditto	—	—
165	82	61	91	81	Ditto	—	—

* Average temperatures for periods preceding counts.
— Indicates no record.

doors under a large bell-jar. The dish containing the *Hormidium* rested on an inverted seed-pan having several large holes in its base. To allow the passage of air through the apparatus, the seed-pan was supported on two bricks, and a glass tube was led into the top of the bell-jar through a rubber cork. The tube was bent into two right angles, the free end being long and directed downwards. Thus, when the rubber cork had been thoroughly covered with vaseline and the bell-jar cemented to the seed-pan, the entry of rain was prevented. The bell-jar was weighted down to resist the wind.

The deposit of dew which might have occurred under these circumstances was negligible, since the temperature throughout the experiment was low and the diurnal range small (see Table I).

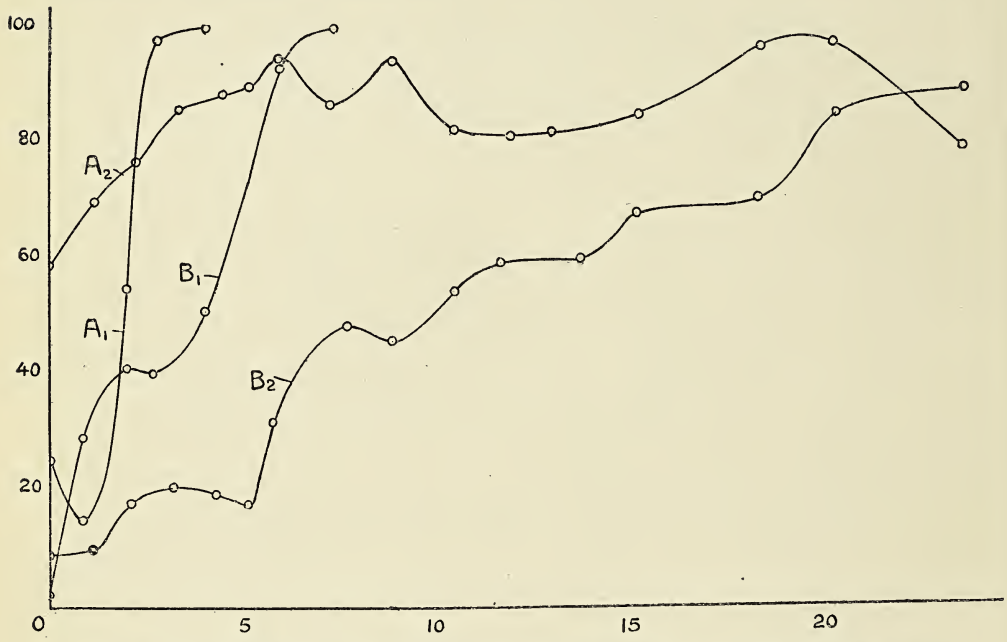

FIG. 5. Curves to show effect of desiccation. Abscissae indicate *time under drought in weeks*, ordinates indicate *percentages*. Curves A_1, A_2, show variation in the percentage of granular living cells to total living cells during Experiments I and II respectively. Curves B_1, B_2, show variation in the percentage of dead cells to total cells during Experiments I and II respectively (cf. pp. 526, 527).

In Experiment II, begun early in March and continued during the following six months, the *Hormidium* was simply placed in a deep glass bowl and protected from rain by a somewhat shallower covering dish, supported slightly above the first to allow the entry of air. The average temperature during the first two months was a little higher than in the first experiment, and increased subsequently; also, there was a greater diurnal range of temperature, especially during the last weeks of the experiment (see Table II).

These conditions of temperature tended to cause a greater deposit of dew than occurred during Experiment I, as did also the prevalence of much

less draught through the apparatus of the second experiment than through that of the first experiment. Direct evidence of the abundant humidity of the air inside the glass bowl was furnished by the condensation of drops of water on the inside of the covering glass during the first two or three weeks of this experiment. The process of desiccation to which the *Hormidium* was subjected in Experiment II was therefore much more gradual than in Experiment I, and this was shown by the soil remaining obviously damp during the first fortnight.

At intervals of about a week, the size and frequency of the granules in the cells were noted and records made of (i) the proportion of the living cells containing granules, and (ii) the proportion of dead cells. The results obtained during the course of the first and second experiments are shown in Tables I and II, respectively, and are plotted as curves in Fig. 5.

The question arises as to what reliance can be placed on individual observations. The chief errors may be conveniently divided into those due to observation and those due to variability among the filaments. To reduce the first to a minimum, great care was taken to secure good definition under the microscope, and a high magnification was used. The second were far more difficult to cope with. Filaments selected from different parts of an area of a few square inches were very variable, as were also, though to a less degree, even those within the limits of very small regions. An effort was made on each occasion to examine filaments representing the condition of the patch as a whole, and in addition records were made of a large number of cells; usually between 2,000 and 3,000.

To obtain some idea of the accuracy of the readings, counts were made, in two instances, on successive days; the variation of the readings on either day from the mean value, obtained by compounding the two days' counts, is shown in Table III below:

TABLE III

	Total Cells.	*Dead Cells.*	*Dead Cells to Total Cells.* %	*Living Cells.*	*Granular Living Cells.*	*Gran. Liv. Cells to Total Liv. Cells.* %
March 11	2263	149	7	2134	1415	66
March 13	3368	365	10	3003	1611	53
Compounded value for Mar. 11 and Mar. 13	5631	514	9	5137	3026	59
Greatest deviation from mean value			2			7
March 20	2609	203	8	2414	1621	67
March 21	2119	280	13	1837	1397	6
Compounded value for Mar. 20 and Mar. 21	4728	483	10	4251	3018	71
Greatest deviation from mean value			3			5

The fact that successive points on the curves are fairly consistent suggests that, in the worst case, errors are not likely to exceed those given in the above examples. In view of the present lack of information on the subject, no attempt has been made to smooth out the curves.

To return to the desiccation experiments themselves, both afforded the same general result. During the first few weeks of desiccation, the proportion of living cells containing granules increased till it reached a maximum of nearly 100 per cent., when it began to decrease, although this decrease was less marked in the second than in the first experiment. The proportion of dead cells increased rapidly at first, as though certain of the living cells had been surprised by the advent of drought. Then it remained roughly constant for a period which coincided approximately with the main production of granules, a fact which seems to point to this as the means by which the cells were coping with the desiccation. Finally, when the production of granules ceased, the proportion of dead increased.

There were, however, minor differences in the behaviour of the Alga in Experiments I and II. Thus, in the first experiment, an actual decrease in the proportion of granular cells occurred during the first week, so that it became reduced to 15 per cent.; the subsequent increase to the maximum was very rapid, being completed within the three following weeks; the final decrease was considerable. The granules in individual cells never became very numerous and were mostly small. The period of low mortality was brief, and the death-rate, both before and after, high; at the end of nearly two months not a single cell remained alive. In the second experiment there appeared no preliminary decrease of granular cells, and the increase to the maximum proportion was only reached after six to nine weeks.[1] During the latter part of the period of increase the granules were large and very abundant in the individual cells; big, refractive masses were also frequent. Only a small decrease in the proportion of granular cells followed, and in the succeeding few weeks there was no change. Eventually, during the fifteenth to twentieth weeks, the proportion of granular cells increased to another maximum, diminishing during the final stage of the experiment. The greater abundance and slower accumulation of granules in the second experiment are probably to be attributed to the increased temperature and sunshine and the more gradual desiccation. The mortality during the first five weeks of desiccation was slight and occurred, on the whole, at a slow rate during the remaining period of the experiment.

The accumulation of large granules in individual cells, together with the big increase in the proportion of living cells with granules, occurring when mortality is low, places beyond doubt the production of granules

[1] The actual readings give two maxima, one after six weeks and another after nine weeks. (See Fig. 5, A_2.)

during the first part of the period of desiccation. It is a more difficult matter to elucidate the converse process, an absorption of granules, which the curves indicate (cf. Fig. 5), after the proportion of living cells with granules has reached its maximum (e. g. after nine weeks in Experiment II), for even at this time the granules in a considerable number of the cells are small and scanty. Nevertheless, estimates of the proportion of living cells with *numerous* granules, though necessarily of an approximate nature, showed an increase during the first five weeks of Experiment II and a subsequent decrease, corresponding roughly with the similar variation in the *proportion of living cells with granules* shown by the curve (Fig. 5, A_2).

The falling off in the proportion of living cells with granules, which occurs in the latter part of both experiments, might be attributed to the death of granular cells. The latter were in fact frequently found among the dead. But in many cases at least, the increase in the number of dead cells was not nearly sufficient to account for the decrease in the above ratio. Thus, between the sixty-second and the seventy-third day of Experiment II, the percentage of granular living cells to total living cells decreased from ninety-five to eighty-three. The percentage of dead cells to total cells increased during the same period from forty-six to fifty-five. If this had been entirely due to the death of granular cells, there would only have resulted a decrease of 1 per cent. of granular living cells. Therefore an increase in the number of clear cells must be supposed to have occurred. This conclusion is borne out by the appearance of 40 per cent. clear cells in filaments, previously recorded as completely granular, between the fourth and sixth weeks of Experiment I.

The occurrence of a second maximum after twenty weeks in Experiment II, as well as the continued prevalence of granules in a large proportion of the living cells after the attainment of the first maximum (at the end of six to nine weeks), seems to indicate that the production of granules may extend over a long period, if the desiccation is gradual and the temperature and sunshine adequate.

A comparison of Experiments I and II shows that filaments which become stocked with large and abundant granules (as in Experiment II) are capable of much longer resistance to drought than filaments in which the granules disappear or become small and scanty (as in Experiment I). On the other hand, the continual dying of abundantly granular cells suggests that the granules themselves do not preserve the cells, but are indicative only of certain resources which the latter possess.

As described on p. 525, granules are very commonly present in normally growing cells, and it is therefore probable that they are primarily a by-product of metabolism. Results obtained from experiments on nutrition confirm this view. Thus granules are not formed in filaments, which, though growing under otherwise normal conditions, are deprived of

carbon dioxide, but, on the other hand, arise abundantly in filaments cultivated in 2 to ·5 per cent. glucose solutions. The results of the desiccation experiments indicate that the cells can absorb the granules. Additional evidence of this is furnished by the observation that a large decrease in the proportion of living cells with granules occurred in a sample separated from the material of Experiment I, after two and a half weeks of drought, when re-watered (cf. Table I). This was too large to be attributed to an increase of living cells by division, such as would account for the actual decrease in the proportion of dead cells.

It might be concluded that desiccation in itself does no immediate harm, but that it is the interference with the nutritive processes in the cells that causes death primarily. The presence of granules may mean the possession of adequate reserves, and hence such cells remain alive for a long period. On the other hand, *prolonged* drought may act directly on the protoplasm and bring about death, even in cells which are richly stocked with granules.

In summer, when all activities are at their maximum, such an excess of food is produced that enough is available both for growth and the accumulation of granules (cf. p. 525). In winter, however, there is not enough material to support more than growth, and it is only when the latter is inhibited by drought that granules make their appearance in any quantity (cf. Experiment I).

The production of granules appears to be associated with a definite substance in the cell, which is not affected by iodine but stains a deep red with Sharlach R.[1] This is almost habitually present, appearing when stained as larger or smaller drops distributed in the surface of the protoplasm (Fig. 6, A, *s.*). Under certain conditions it accumulates in the vacuoles at the ends of the cell, so that they stain as a whole (Fig. 6, B, *s.*), large drops being often discernible on a homogeneous ground (Fig. 6, C).

Granules have only been observed in abundance in normally growing cells when the latter contain a considerable quantity of the substance just mentioned. Such was the case with the greenhouse material referred to on p. 525, and especially with the filaments cultivated in glucose solutions (cf. p. 511). The granules produced during a period of desiccation are only large and numerous if the material contains a great deal of this substance on the advent of drought, and retains it for some time subsequently. This happened in the second desiccation experiment; in fact, as the desiccation set in very slowly, the soil remaining damp during the first fortnight, this substance showed a preliminary increase, possibly due to the continuance of assimilation after growth had ceased. A large proportion of the cells in the original material of the first desiccation experiment were primarily well

[1] In some cases the cells became black with osmic acid, and this reaction may be due to the same substance. Its general microchemical reactions have not been studied.

stocked with this substance, but absorbed the greater part of it during the first week of desiccation before the production of granules became apparent. Hence, possibly, the latter were small and sparse when they eventually appeared. In both experiments, as desiccation proceeded, the substance in question gradually diminished in amount, and had become exceedingly scanty by the time the granules attained their maximum abundance. This stands in contrast to the accumulation which occurred, simultaneously with the production of granules, in well-nourished cells receiving abundant water-supply.

It may be that in reality both ample nutrition and desiccation cause a concentration of this substance, which results in the formation of granules. This view receives some confirmation from the fact that small granules can be produced in cells in the right condition, by gradually withdrawing water

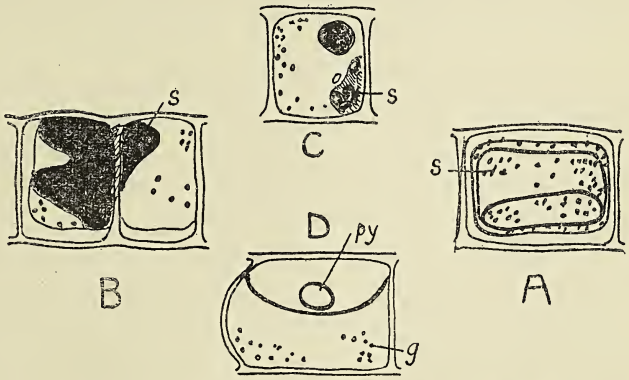

FIG. 6. A, cell in which the special substance stained by Sharlach R (cf. p. 532) is distributed as fine drops in the surface of the protoplasm. B, accumulation of the same substance in the vacuoles. C, ditto, the stained substance appearing partly in the form of large drops. D, small granules produced in a previously clear cell on the withdrawal of water from it by means of weak glycerine. *s.*, substance stained by Sharlach R; other lettering as in Fig. 1. (All figures × 2000.)

from them by means of weak solutions of glycerine, sugar, or salt (Fig. 6, D). It is possible that this substance is one of the primary products of photo-synthesis, while the granules constitute a secondary, reserve product.

A considerable interest centres round the relation between the production of starch and granules, since the granules also appear to be a reserve food. As a general rule, starch is scanty in cells poor in granules, and abundant in densely granular cells, so that both substances appear to accumulate under the same circumstances. For example, there was very little starch present in the material of the first desiccation experiment, but a great deal in many cells of the second desiccation experiment (see Tables I and II). Starch was abundant in the material cultivated in glucose solutions (cf. p. 531) and in the damp greenhouse material referred to above (p. 525). Nevertheless,

starch is frequently absent from granular cells. It is possible that there is a more direct relationship between the production of starch and the special substance referred to on p. 532. Thus they are usually either both abundant or both scanty. In the second desiccation experiment, starch remained in quantity as long as there was any appreciable amount of this substance, but tended to disappear as the latter became scanty. The relation between this substance, starch, and granules is, however, still obscure.

6. Summary.

A general account is given of the life of a form of *Hormidium flaccidum* in its native habitat. The survival of the vegetative filaments throughout successive seasons of the year is described, and the modifications to which they are subject during periods of drought, chiefly the accumulation of refractive granules, and changes in the longitudinal walls and septa, examined.

A detailed description is given of the two common methods of reproduction possessed by the Alga, viz. (i) transverse splitting of the filaments at the septa, and (ii) production of aplanospores. Regarding (i), a general breaking up of the filaments into isolated cells or few-celled pieces has not been observed, splitting occurring at points in a filament some considerable distance apart, though in favourable circumstances a minor proportion of the filaments have become divided into few-celled fragments. It is suggested that splitting is due to the effects of renewed turgor on desiccated filaments in which degeneration of the cuticle or weakening, caused by the development of mucilaginous substance between the two lamellae of the septa, has taken place. It is indicated that the production of aplanospores occurs in all seasons of the year, but is dependent on an ample supply of water. Cells giving rise to aplanospores usually contain an abundance of the special substance referred to on p. 532 and also granules.

A white refractive substance, which appears in the cells under certain conditions in the form of granules and rounded masses, is described. This is shown to arise chiefly in the region of the polar vacuoles, but also sparsely distributed in the peripheral protoplasm. Two conditions have been observed to favour its production, viz. (i) drought, (ii) a plentiful supply of carbohydrates, e. g. glucose. It appears to be associated with a second special substance in the cell (cf. p. 532) and is possibly formed as a result of concentration of this substance. Since, in suitable circumstances, the cells are capable of eventually absorbing the granules, they evidently function as a reserve food.

It is shown that during the first weeks of a period of drought the death-rate decreases, while the abundance of granules increases to a maximum. When growing in its native habitat, the Alga in all probability

rarely passes beyond this first stage of desiccation, as the spells of dry weather in temperate regions are comparatively short, and dew is continually deposited, especially in summer when the drought is most extreme.

I have pleasure in recording my thanks to Dr. F. E. Fritsch for his valuable help and suggestions throughout the progress of the work. My thanks are also due to Dr. E. J. Salisbury for helpful suggestions. I am glad of the opportunity to acknowledge that facilities for this research were provided by a scholarship of the Fishmongers' Company.

H. H. W. Pearson, F.R.S., Sc.D. (Cambridge).

BY

A. C. SEWARD.

HENRY HAROLD WELCH PEARSON was born at Long Sutton, Lincolnshire, on January 28, 1870, and died on November 3, 1916, at the Mount Royal Hospital, Wynberg, Cape Town. He had been ill for several weeks, and after recovering from the effects of an operation for haemorrhoids he contracted pneumonia, which caused his death: it is suggested by the author of an obituary notice in the 'South African College Magazine' that an attack of malaria in 1913 in Portuguese West Africa was the beginning of the undermining of his never very robust health. His widow writes: 'Most of us feel that the strain of the College and Garden work had been too much for him, and when pneumonia attacked him he had not sufficient strength to fight against it.' When Pearson was about eight years old his father went to live at Wickhambrook, Suffolk, where he kept a boys' school, and here the son received part of his education: he was for a short time a boarder at a school at Beccles. On leaving school it was arranged that he should begin his career as a chemist's assistant at Hawkhurst; the work was uncongenial, but his employer spoke of him as the best assistant he had ever had. By judicious use of his spare time Pearson was able to pass the London Matriculation examination, and shortly afterwards he was appointed assistant master at Mr. Waite's school at Eastbourne, where he remained about four years. In a recent letter to Mrs. Pearson the Head Master's sister wrote: 'He did a great deal of studying, and we were particular about the boys keeping quiet and away from his study during the evenings; we all just loved him.' He obtained a Clothworkers' Exhibition and entered the University of Cambridge as a Non-Collegiate student in 1893. He obtained a First class in both parts of the Natural Sciences Tripos (1896–7). In October, 1896, after taking his Bachelor's degree, he entered Christ's College and was elected Scholar in the following June. In the same year he went to Ceylon with the assistance of a grant of £100 from the Cambridge University Worts Travelling Fund, the additional cost of the visit being generously defrayed, as Pearson states in his account of the Ceylon work, by his very good friend the Rev. Herbert Alston, formerly Rector of Little Bradley, Suffolk. In 1898, in consequence of his election to a Frank Smart

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Studentship, he became a member of Gonville and Caius College. He was appointed Assistant Curator of the Cambridge Herbarium in 1898, and in 1899 he was awarded the Walsingham Medal for his work on the vegetation of the Ceylon patanas. He was appointed Assistant (for India) in the Herbarium of the Royal Gardens, Kew, in 1899, and two years later was transferred to the Director's office. In 1903 Pearson was elected to the Harry Bolus Professorship of Botany at the South African College, Cape Town, and took up his duties in April of that year. The relations between Dr. Harry Bolus and the first occupant of the chair bearing his name were always most cordial. The confidence felt by the older man for the younger, and expressed in many acts of kindness, was repaid by the respect and affection which he received in return. In a biographical sketch of Dr. Bolus Pearson described him as 'a man who, one would think, almost unknown to himself, has played a great part in the establishment of Botany as a science in South Africa', a description that would also apply to the writer of these words. In 1915, under the editorship of Pearson, the Cambridge University Press published the first part of the 'Annals of the Bolus Herbarium', a periodical devoted primarily to work inspired directly or indirectly by Dr. Bolus, and to investigations conducted in or in connexion with the Bolus Herbarium. He was also editor of the 'Journal of the Botanic Society of South Africa', a Society of which he was one of the most active members.

Before leaving England Pearson married Ethel, the youngest daughter of the late William Pratt, of Little Bradley, Suffolk. He took the Cambridge Sc.D. degree in 1907, and in 1916 was elected a Fellow of the Royal Society.

In the following sketch I have quoted freely from a series of letters in my possession in order to enable the reader to come into intimate relation with Pearson's singularly attractive personality. He combined the qualifications of the best type of student with the wisdom of the man of affairs; capable in organization, an original thinker, and a warm-hearted friend. He retained the enthusiasm and brightness of youth, and his uniform courtesy and tact made him popular with all who knew him. His influence as a botanist extended far beyond the ranks of his professional colleagues. It would be difficult to estimate at its true value the services he rendered to South Africa. He was a successful and stimulating teacher; his own keenness in research gave reality to his lectures, and through his influence several of his pupils came to Cambridge to continue investigations which he had suggested. The place he occupied in the hearts of his students is illustrated by the following extracts from letters written to his widow: 'He was our best and truest friend, for we had learnt to look to him for guidance and counsel in all matters of importance.' 'Those of us who had the privilege of working under him feel that it is an honourable

duty to help carry on the manifold activities which he had set in motion and bequeathed to the world.' 'I shall always count it one of my greatest privileges to have been one of his students.' 'We have known the full benefit of his happy knack of infusing energy into every one he inspired, and we have been brought by him into a truer, more idealistic appreciation of science for science's sake. A teacher-friend, we mourn an all-round loss.'

Pearson played a leading part in the foundation of the National Garden at Kirstenbosch, which in the hands of competent successors should become one of the finest in the world.

My first meeting with Pearson was in 1892 at Eastbourne as a student attending a course of lectures on 'Plant Life' which I was delivering as a University Extension Lecturer. During rambles on the shore in search of seaweeds he discussed with me the possibility of realizing his ambition to come to Cambridge, and from that time onwards it was my privilege to enjoy his friendship. In a letter written on April 4, 1916, after hearing of his election to the Royal Society, Pearson recalled the Eastbourne days: 'I was a little surprised to find myself in the list this year: that I was pleased needs no statement. Under any circumstances I should appreciate the honour immensely; under my particular circumstances I think I value it more than I should under some others—isolated here from the centre of the things that interest me, this distinction means a very great deal to me. I hope I deserve it. I am not quite sure, though I am grateful to you and others for saying and thinking that I do. . . . I regard this as an outcome of those . . . lectures you gave at Eastbourne—but for them where I should have been I do not know, but I certainly should not have been here and almost certainly the Council of the Royal Society would never have heard of me.'

Writing from Kew after his appointment to the South African chair he said: 'I have always been fond of teaching. I even liked it when we made our *Polysiphonia* excursions at Eastbourne. I have had very valuable experience here, for which I believe I am the better. I certainly know more of systematic, economic, and administrative work than when I left Cambridge, and if I have become a bit rusty in some of the other branches I think the defects will not be difficult to remedy.' His subsequent career demonstrated the value of his early training. He was always thoughtful of others, and expected those associated with him to do their share not only in teaching, but in the investigation of the numerous problems awaiting solution. In a letter asking for the names of possible men for an assistantship he added, 'He *must* do research work, for which he will have as much time as I have'.

At one time when the financial outlook in Cape Colony was far from bright and the College was compelled to reduce the staff and the stipends of professors, and because family affairs in England required attention, he

seriously thought of returning to England. He was an applicant for the Botanical chair at Leeds: 'My disappointment with regard to the Leeds post has been a thing of the past for some weeks. . . . That they should insist on seeing a man before appointing him is obviously reasonable, and were I on an appointment committee I should take the same line.... As long as I am here I can at least do spade-work in research' (July 30, 1907).

Pearson's first published paper deals with the anatomy of the seedling of the Queensland Cycad *Bowenia*, a straightforward piece of work carried out in the Cambridge Laboratory and undertaken, as I well remember, with characteristic keenness. He was always specially interested in the Cycads and contributed many important additions to our knowledge of the South African representatives of the family, not only directly by his own observations in the field, but by stimulating others more favourably situated geographically than he was for making regular records bearing on the phenomena connected with pollination, and by sending material to England. 'I have to-day', he wrote on June 11, 1906, 'got a little more light on the pollination question. One of my helpers, a lady living in the Native Territories, sends me a lot of notes she made on the growth of the male cone of *Encephalartos villosus*, which are of great interest in this connexion.' She noticed a horrible smell when the sporophylls were open and caught several beetles. 'Perinquey has identified the beast; he says it belongs to what is considered to be the most ancient group of the Coleoptera—which sounds fascinating, but may, of course, mean nothing. Excuse this long scrawl. People here don't care much about these things, and I must inflict them on somebody.' In a later letter he spoke of evidence of insect-pollination in *Encephalartos Frederici-Guilielmi*: 'the more I see of the wool-enveloped cones the more impossible it seems that it can be pollinated by any other agency. The cones are all infested by the same kind of weevil as that which inhabits *E. villosus*. The development part of the work is a tremendous business. It seems to me that I ought to go on fixing and section-cutting about ten years before I write.... I returned last night from the Karroo and Cycad country, having had a very successful time. I had a most interesting three days with *Encephalartos Frederici-Guilielmi*. It is coning freely now, and most cones when I was there were on the eve of pollination. As a result of a great number of observations I feel satisfied that in the case of a female plant cones are produced only once in six years, and very frequently a longer period intervenes. . . . As to coning, it looks as if the process taxes the plant very severely, so that it has to rest for some years before it can again attempt to produce cones. The first seed-bearing plants surely cannot have taken it out of themselves to this extent or they could never have left any descendants. Then it must be that these Cycads are a long way from the earliest Spermaphyta, and have developed on not very sound lines. One defect that suggests itself to me is that they produce

far too many seeds for the size of the plant; for instance, one of these *Encephalartos Fredericki-Guilielmi* plants produces easily more than 1,500 seeds in one season. When you think of the energy consumed in the rapid development of the cone . . . and the enormous quantity of carbohydrate stored away in the prothalli, you cannot wonder that the plant feels a bit done up when all is over. Is there any evidence of the existence of *herbaceous* Pteridosperms or Gymnosperms in Mesozoic times? If there have ever been such Gymnosperms, is it not strange that they have left no descendants of similar habit? . . . Yet the first vascular plants must have been herbaceous. What I have in my mind is the idea that the conditions which called forth the Cycads, for example, may have been entirely different from those at present prevailing and quite unsuitable for the existence of small perennials and annuals. Suppose, for example, a set of conditions which resulted in rapid and luxuriant growth. Then the Cycads of to-day might be the struggling posterity of a race of giants, preserving under adverse conditions the prolific reproduction of forbears which could afford such generous habits without endangering their existence thereby. But I must not worry you any more. These Cycads are most fascinating things, and they grow upon one. In the Eastern provinces I am rapidly acquiring a reputation for incipient imbecility, for I am told that there never before was any one in South Africa who would spend four days in the train in order to spend three days among the Palms.' Early in 1915 he wrote: 'I do wish you could see my Cycads. The slope [in the National Garden] which, when you were here [July, 1914], harboured one specimen of *Encephalartos Altensteinii*, now holds 300 plants, representing possibly all the known and one hitherto unknown South African species.' It was near to this slope that Pearson was buried on November 4, 1916.

Shortly after his arrival in Ceylon Pearson wrote: 'I am at work on the patana flora up country. It is far more interesting on the spot than on paper. The patana looks very uninteresting from a distance, as nothing shows but *Rhododendron arboreum* and a rough tussocky grass; but it really bears a fairly rich flora, composed almost entirely of xerophytic plants.' Several attempts had been made to explain the occurrence of a comparatively barren country occupied by the savanna-like patanas at approximately all altitudes over 2,000 ft., in the midst of a luxuriant subtropical vegetation; but much more information was needed. At the suggestion of the Director of the Peradeniya Garden (Dr. J. C. Willis), Pearson undertook the task of discovering the causes which led to the development of the flora. He concluded that the peculiarities of the climate have co-operated with periodically recurrent grass-fires in transforming an open forest into barren grassy plains. His results include an enumeration of the species collected, an account of their biological characters, and an interesting examination of the factors concerned in producing the xerophilous

character of both the wet and dry patanas, that is, the vegetation above and below 4,500 ft. In the joint paper with his friend Mr. J. Parkin the conclusions given in the first account, which were based on field observations, are tested by a thorough anatomical investigation. It was found that the peculiarities which usually characterize plants of insolated areas are not more strongly developed in members of the dry flora than in the plants of the wet patanas—a fact probably due to the influence of the powerful monsoon winds, the functional activities of the roots being also lowered by the humic acids in the soil. The Ceylon visit introduced Pearson to a new world which he thoroughly enjoyed, and by his apprenticeship to field-work in the wider sense he qualified himself for the later expeditions in South Africa which he conducted with conspicuous success.

A paper written at Kew deals with an inquiry, undertaken at the suggestion of Sir William Thiselton-Dyer, into the morphology and functions of the double pitchers of four species of *Dischidia*: this piece of work is exceedingly good considering that the material consisted entirely of herbarium specimens. He made full use of the time spent in the Herbarium to familiarize himself with the methods of systematic botany and, as the list of his publications shows, his output during that period was considerable. In later years he amply repaid the Royal Gardens by his gifts of material collected in South Africa. The collections sent to Kew included one of the finest series of succulents ever received in this country. The volume of the 'Botanical Magazine' published in 1910 is dedicated to Professor Pearson, 'as successful in his leadership of Botanical expeditions as he has been generous in distributing their fruits'. The Botanic Garden of his old University has also greatly benefited by his many contributions, and a word of acknowledgement is due to Mr. Lynch, the able Curator, for the skill with which he cultivated *Welwitschia* and other plants received from Pearson.

Soon after his arrival at the Cape Pearson set himself the task of continuing the investigation of *Welwitschia*, so splendidly inaugurated by Sir Joseph Hooker. In May, 1904, he wrote from Cape Town: 'A year and a week since I arrived in the land of sunshine, dust, and politics, and yet this is the first time I have set myself to write to you. . . . I started last year with eleven students. This year I have twenty-two. . . . The Council is putting me up a splendid laboratory which I shall not be ashamed to show you when you come out. . . . I thought of you a good deal in January last, when I spent two glorious days in the heart of the Damaraland desert in the company of the most magnificent array of flowering Welwitschias that ever man saw. You must know that when I was first appointed here I had a dream, the purport of which was that *Welwitschia* was delivered into my hands. And sure enough without any particular effort on my part I found myself in *Welwitschia*-land, and hoped to spend some weeks there. Fate, however, determined otherwise. The Hereros and the Germans came to

blows, and between the two of them I had a lively time of it. I was lodged in a German military station, which I had to quit in haste. I hear that a few days only after I left the station ceased to exist.' A second expedition was planned in 1906. He wrote in September of that year: 'The *Welwitschia* trip is, I hope, fixed up. The Governor of the territory seems quite keen on my going again.' In the same letter he made a suggestion which might with advantage be taken to heart by the governing bodies of Universities. 'I am expecting to take my year's leave in 1908. Unfortunately I have to come on half-pay and shall have to economize. I must come, however, by some means as I am getting stale. They give us the sixth year off as a favour, on half-pay. I lose no opportunity of pointing out that in their own interests they ought not only to make it easier for us to take the leave, but to insist that we do take it. I don't think one can stay here continuously for more than five years without deterioriating.' In February, 1907, he wrote: 'I and my collections landed this morning. I hope the trip has been entirely successful. Here I think I have established a record. The Swakop river-bed swarms with game, and therefore the leopard is fairly common at Hadjamchab—so common that the sergeant in charge of the station considered it quite unsafe to go out at night without arms, and indeed did not like me doing so during the day. So on two nights when I made expeditions to *Welwitschia* I was escorted by two men armed to the teeth, and you may imagine me sitting on the sand in the moonlight, with a bottle of chromacetic acid between my knees, dissecting female cones while the two warriors stood at attention behind me. . . . The German Government treated me with extraordinary kindness and generosity, they cabled instructions from Berlin to aid me as far as possible, and this they certainly did. . . . I have already told you they invited me to Windhuk.' In the latter part of 1915 he was invited to the same place by General Botha, and wrote on January 11, 1916, from the head-quarters of the Union forces: 'I arrived here to-day after a rather arduous journey of five weeks' duration through what is botanically an exceedingly interesting country. In the course of it I have seen what I have long wished to see, viz. the edge of the *Welwitschia* desert. In fact, I have been able to trace the change in the flora from the Kalahari plateau right into the desert. Until my collections are worked out I cannot quite see whither I am being led, but I think I can now more or less co-ordinate many odd facts I have been accumulating in the course of these journeys, and at least show the relation between the desert flora and those surrounding it. . . . The work this time has meant a journey through some most difficult places. Both my wagons broke, though fortunately we were able to bring them both to the end of the 410-mile journey. . . . On another occasion we had to travel all one night to reach the next water, and in the course of it we crossed the same rocky river-bed no less than twenty times. . . . However, it is something to feel that one

has been through it and has brought everything except two out of the team of thirty donkeys to the end of the journey. . . . I asked one of the Bastard Hottentots, who has now been under German government for years, what he thought of the Germans. He said they are "the worstest people under the sun"—and the Bastard has some reason for thinking so.' In a letter to Prof. Herdman (published in 'Nature', March 2, 1916), written after his return to Cape Town, on January 28, Pearson referred to his journey through the semi-independent territory of the Bastard Hottentots, adding, 'No German dare venture into it, but when the people found I was English they could not do enough for me'.

Perhaps the most interesting of the many results of his researches on *Welwitschia* is the discovery of the nature of the 'endosperm'. 'I am now more than ever certain that the plant [*Welwitschia*] stands at the top of a series—I fear on a giddy pinnacle whose sides are so steep that there is no telling how it got there.' In a later letter he says: 'I am nearly converted to your view of the possible relationship between the Gnetaceae and Angiosperms. The *Welwitschia* endosperm has quite altered my point of view. I am going to try and prove that the *Welwitschia* endosperm is homologous with that of the Angiosperm, and, further, that it belongs neither to the gametophyte nor sporophyte generation, but is a structure *sui generis*. . . . I must get hold of *Gnetum africanum*. If I can't get the money for the whole trip of which I sent you an outline scheme . . . I hope I shall at least be able to obtain, say, £100 to enable me to go to Quetta, where the *Gnetum* grows.' A substantial grant from the Trustees of the Percy Sladen Memorial Fund enabled him to carry out the scheme in 1908–9.

In the earlier stages of development the embryo-sac of *Welwitschia* contains numerous free nuclei: this condition is followed by partial septation, which produces a tissue of multinucleate compartments. In the upper part of the embryo-sac each 'cell' has 1–2 nuclei, and later as many as 5, while in the lower part of the sac each 'cell' has 2–12 nuclei. The 'cells' with 2–5 nuclei in the micropylar region of the embryo-sac produce embryo-sac tubes which grow up towards the descending pollen-tubes. On March 3, 1908, he discussed the question of nomenclature with regard to the 'endosperm': 'In *Gnetum* and *Welwitschia* the embryo-sac becomes filled with nuclei, *all of which* are probably capable of being fertilized, i.e. all are potential gametes. Of these a few are functional. The remainder, or most of them, fuse in groups of 7, 8, 9, 10, or more, and form a number of fusion-nuclei which I believe to be homologous with the definitive nucleus of the Angiosperm. These fusion-nuclei on division give rise to a tissue whose later growth is considerable, and is so highly organized that the tissue (endosperm) must be regarded as an organism. This organism is not in the direct line of the life-cycle and belongs neither to the sporophyte nor to the gametophyte. Now as the term endosperm is physiological (accord-

ing to its author's definition) and applies equally to the prothallus of Pines and to this endosperm of *Welwitschia* and the Angiosperm, I want a morphological name for my new organism. I propose to call it the Trophophyte.'

The *Welwitschia* work naturally led to an attack on *Gnetum*, and one of the main objects of the Expedition of 1908–9 was to obtain material of *Gnetum africanum* from Angola. The first part of this journey was made in company with the Magnetic Survey Expedition of the Carnegie Institute, under the leadership of his close friend and colleague, Dr. J. C. Beattie. On April 12, 1910, he wrote: 'I have both *Gnetum africanum* and *G. scandens* on the go, and I have cut about ten ovules of each. . . . While I am not yet able to prove it to the satisfaction of a sceptic I have myself no doubt that the endosperm [*Gnetum*] is formed as in *Welwitschia* (the attitude is, I am afraid, unscientific, but it is I think impossible to keep one's mind open until the proof is complete).' In November, 1915, he added: 'I have at last settled the question of the resemblance of the endosperm [of *Gnetum*] to that of *Welwitschia*; it is formed in exactly the same way, which pleases me mightily.'

Prof. Pearson consented to contribute a volume on the Gnetales to the 'Cambridge Handbooks', edited by Mr. Tansley and myself, and this work may, we hope, be far enough advanced to be published. In a letter dated April 20, 1916, he wrote: 'A large part of the book on the Gnetales is written, though it will need some revision. . . . As to the Gnetalean-Angiosperm alliance, there must be one, I think, but at present I cannot bring myself to believe that it is direct. If they are not connected that endosperm wants a lot of explaining. The trouble is that I cannot make head or tail of the flower, and the relation between *Ephedra* and the others is extremely puzzling. The latter is probably very simple if we only had the key—the former I dream about, so far unsuccessfully. As to *Bennettites*, I think I made far too much of the idea of a relationship in 1909, but I have not given it up yet. The more we know about the group the more difficult it seems to become.' In a later letter he made an interesting suggestion about the comparison of the Gnetales and the Bennettitales. 'I am bothered by my ignorance of *Bennettites*. I have never seen a section of a flower and the idea I have been harbouring for some time is very probably absurd. It is about that interseminal scale, the real nature of which, so far as I can make out, no one seems to understand. In my ignorance I have wondered whether it is really a "scale" at all.' He suggests that the interseminal scale 'may be something of the nature of the thick cushion of *Gnetum*. . . . If you have a large number of ovules arising in crowded whorls or spirals from an elongated conical axis, and sunk in the tissue of the axis, as are the young ovules of *Gnetum africanum*, it seems to me that transverse and longitudinal sections might give very much the appearance of such sections of *Bennettites*.'

Reference has already been made to Pearson's success as an explorer. The liberal grants from the Percy Sladen Memorial Trustees, who had complete confidence in his proposals, from the Royal Society, and from other sources led to results of exceptional importance—morphological, systematic, phytogeographical, and ecological. Pearson's success was due to his tactful persistence in the face of obstacles, his infectious enthusiasm, a sense of humour, his all-round training, and keen powers of observation. On all his journeys he took with him Lamb's 'Essays' and generally small volumes of Shakespeare's plays.

Before the final arrangements for his most important expedition were complete, Pearson wrote: 'On Saturday night I had a long talk with Dernburg in his bedroom at Government House. Did you ever discuss the situation with a European diplomatist in his bedroom? The [German] Imperial Government is most anxious that the country lying north of Windhuk . . . should be botanically examined with a view to its agricultural development. . . . I at once offered to go if the Government would organize the expedition and take me there and bring me back again. Dernburg accepted the offer on the spot. I asked for no remuneration and none was suggested.' Two months later he added: 'I am not quite sure, but I believe that the Dernburg proposal is off.' Shortly before leaving Cape Town Pearson wrote: 'The Sladen Trustees have most kindly left me free to take either route from Windhuk. Unless the obstacles are serious I shall adhere to the Angola plan. Through Sir Donald Currie I have received a most cordial letter of introduction from the Portuguese Minister of Marine and the Colonies to the Governor-General of Angola. Furnished with this and with a formal letter from Sir Hely Hutchinson I am certain to receive every possible attention (probably to an embarrassing extent) and assistance from the authorities.' He left Cape Town in November, 1908, and early in December reported progress from Calvinia: 'I have just removed the stain of twelve days' travel and had a more or less civilized breakfast. The first stage of the trip has been fairly arduous, as we have crossed the north-east corner of the Karroo, which this year is nearly as arid as the German desert. . . . I am sending nearly 300 species to Cape Town from here (you see I am in a fair way to become a real botanist and bundles of dried specimens are beginning to impress me greatly)—but seriously it is intensely interesting to follow the changes in the flora over a long-distance journey such as this.' From Seeheum in German South-West Africa he complained of suffering from 'another bout of hospitality which is if anything more killing than the English. . . . I estimate that since I started I have collected about 1,200 species, the majority of which are safely housed in Cape Town by this time. . . . By the way, I found living in a tent about 100 km. south of this place an old Irish lady who has a most vivid recollection of Galton, whom she saw at Rehoboth when he visited this country in the fifties.

She seemed quite confident that Galton will still remember her, and begged me to give him a message from her. Her name is Mrs. Bassingthwaite.... The flies and the temperature are both fairly beyond words. I suppose this country is destined to become a colony some day, but the native question must be solved first. I fancy that what is necessary is a thorough study of the subterranean watercourses throughout the whole of South Africa.'

Pearson had many stories of amusing incidents on his journeys. One of his colleagues in the South African College writes: 'It was a treat, for instance, to hear him tell in his unique way the story of the old storekeeper with whom he had stayed a couple of days and become friendly. This man had two sons and was obsessed with the necessity of fixing up their careers. He would like to consult the Professor. The elder was a really clever fellow; in his case there would be no difficulty, he naturally would go into business. But the trouble was the second. He was a very decent boy, but a bit dull—yes, dull; he would be no good at business. It is good to recall the twinkle in Pearson's eyes and the laughter in his voice when he described the old man's trouble and the evident beating about the bush, and finally his blurting out quite seriously, "Couldn't you tell me how to make him a professor like you?"'

The visit of the British Association to South Africa in 1905 was an event which gave great satisfaction to Pearson; he was justly proud of his department and enjoyed showing it to his visitors. He played no small part in contributing to the success of the meeting, which is associated in my mind with memorable days spent with him and his wife in their Kenilworth home.

Of the many services rendered by Pearson to Science perhaps in some respects the part he played in the establishment of the National Botanic Garden[1] is the greatest. It is a splendid memorial of a botanist whose explorations enormously extended our knowledge of the richness of the floras of South Africa, and who by his manifold activities and sound common sense succeeded in demonstrating to the layman the value of botanical research. There can be no doubt that the credit for this addition to the efficiency of the Empire is mainly due to Pearson, though he would not admit that he was entitled to more than a comparatively small share in the events which led the Government to take the final step. The project was first put forward in a concrete form in his Presidential address before Section C of the South African Association for the Advancement of Science in 1910. He advocated concentrated action by the South African Colonies with a view to the full development of their unrivalled botanical resources, and formulated a scheme worthy of a many-sided and far-sighted botanist. The Garden should be a centre of botanical activity in the widest sense; it should be concerned with

[1] For a fuller account of the Garden and its inception and establishment the reader is referred to the Kew Bulletin, 1913, p. 309, and to the Gardeners' Chronicle, August 30, 1913, p. 151.

botanical exploration, the cultivation of indigenous plants, and experimental work. He dwelt on the neglect of the native plants and stated that more South African species are cultivated in European gardens than at the Cape. The Garden should include a National Herbarium, a Museum of Economic Botany, a Library, and Research laboratories. He emphasized the importance of grasping the fundamental truth that the true springs of South African development are within and not oversea. The Garden should also be an 'expression of the intellectual and artistic aspirations of the new nation whose duty it is to foster the study of the country which it occupies, to encourage a proper appreciation of the rare and beautiful with which nature has so lavishly endowed it'. In an article on a 'State Botanic Garden', while advocating the importance of economic questions, Pearson urged the importance of pure science: 'problems which appear to be of merely academic interest to-day may be of unmeasured practical importance to-morrow.'

In reply to a request for a statement as to his share in the foundation of the Garden, Pearson wrote on March 18, 1914: 'I do not really think I can say what has been my share in the formation of these Gardens. I dare say they would not have been in existence just now but for my address to the South African Association in 1910. But since then a good many people have been prominent in the movement. . . . Having been fairly constantly in the fray for three years, I do not think I am well able to judge how much each of us contributed towards the result, and I fear that I should be more likely to exaggerate the importance of my own efforts than those of any one else.' On April 16, 1912, he wrote: 'I have got the Botanic Garden movement going strong just now. We have [to send] a deputation to the Prime Minister and he asks for a minutely detailed scheme. The site I have chosen (and which the Committee has approved) is a fine estate, 321 acres in extent, belonging to the Government (a part of the Rhodes estate), and now for many years derelict and unused. For the purpose of a Botanic Garden it could hardly be better suited.'

On October 6, 1912, after referring to a projected expedition, he adds: 'I am longing to get away for a time from this teaching grind, which is gradually wearing away my soul—this expression probably reflects but a passing mood. In any case, the top of the Karasberg cannot but be delightful.'

On May 22, 1913, he reported further progress with the Garden: 'I sent you a paper containing an account of the Botanic Garden debate [in the House of Assembly]. Since then Sir Lionel Phillips has kept things going, and to-day the end has been gained. . . . The success is entirely due to Phillips—a man of extraordinary energy and enthusiasm. . . . I am therefore to some extent identified with the scheme. A botanist must run it from the beginning if it is to be a success. At first it will be

H. H. W. Pearson in the Kirstenbosch Garden: Aloes in the foreground. A.C.S. Photo. July 20, 1914.

Temporary Office and Plant Nurseries, Kirstenbosch. A.C.S. Photo. July 20, 1914.

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impossible to provide a salary for such a botanist. There is no other one who is in a position to take charge of it without payment. If I don't do it the South African Botanic Garden collapses for the time being. Circumstances are therefore too much for me and, though at no little personal sacrifice, I must undertake it. It will not entail my giving up my own line of work (at least it shall not), but it must interfere with it to some extent. But my own view is that if I can establish a Botanic Garden here on a sound basis I shall do more for Botany than by writing an extra paper or two. It may not be so good for myself, but that is, I hope, beside the point. I have been carried on almost involuntarily and have persuaded myself that I am acting rightly. . . . Since I wrote to you the Council has given me a second Lecturer.' In July of the same year he wrote: 'As to the Directorship—there is at present no money available for a botanist, and I can do the place a greater service by doing what little I can in an honorary capacity than if I became a salaried officer. . . . I feel that the thing has come upon me unsought and I have no choice but to take it up. It will be a burden, but it is worth carrying if it never falls to me to exploit its contents.' The author of an appreciative and sympathetic article in the 'South African College Magazine' in describing Pearson's work for the National Garden writes: 'He never fully appreciated the value of his own enthusiasm and manner; and I remember the doubts which assailed him as the last attack—in a fight which he knew had been fought and lost many a time in the previous forty years—progressed and the unalloyed joy he experienced when at last he saw the Garden established.'

Pearson was appointed Honorary Director and began his duties on August 1, 1913. The last time I saw him was on July 20, 1914. The Director's house was nearing completion and the plant-nurseries (Plate) were well stocked with material received from various parts of the Dominion. From a wet glen in which were several plants of the South African Fern *Hemitelia capensis* we climbed up a rocky slope, on the summit of which Aloes were flourishing (Plate) in a habitat sharply contrasted with that below, and from a still higher point we looked across a forest of Silver-trees (*Leucadendron argenteum*) and beyond to the shores of the Cape Peninsula, a view that it would be hard to match in any other Garden.

The following notes, for which I am indebted to Mrs. Pearson, show how he spent his days as Director and Professor: 'He so arranged his work at the South African College that he was able to devote Wednesday, Saturday, and Sunday to the Gardens. On the other days he left home at 7.45 a.m. summer and winter, driving himself to the station in all weathers, a distance of $2\frac{1}{2}$ miles, and usually returned home at 5.30 p.m. After tea he walked round the Gardens inspecting work and arranging various matters with the Curator and Ranger. His evenings were spent in attending to

Garden correspondence, writing up research notes, reading scientific periodicals, and finishing the day with some book on the war. On Wednesday, Saturday, and Sunday he began the day with a ride before breakfast and devoted the rest of the morning to the Gardens. With the help of a coloured labourer he arranged and planted all the Cycads. The design and construction of all new work he thought out while wandering over the estate and imparted his ideas to the Curator who drew up plans for his approval.'

During his brief tenure of the Directorship he gave himself unsparingly to the work of construction, and the impression left on my mind after our tour of the Garden was that under his guidance Kirstenbosch would in course of time rival the best Gardens in the world. As the author of an obituary notice in the 'Cape Argus' says, 'he threw his whole soul' into the business of founding the National Garden and 'carried out a work which will live for ever and which may be regarded as one of the treasures of South Africa'. His burial-place, to quote from the 'Cape Times', 'faces the slope with the Cycad plantation, the one little section of his plans which, in the short time vouchsafed him, he was able to bring near completion'. The funeral service, in which the Archbishop of Cape Town took part, was held in the Protea church close to the Garden.

A few months after I saw Pearson in Cape Town he wrote to me about the condition of affairs in South Africa, showing his usual grasp of the political situation, and added: 'Botha's action has been magnificent and has had a great effect. Even I have volunteered for any work they choose to put so useless a person to.' And in April, 1915, 'I have felt a little easier in my mind since I volunteered for local defence. I am now enrolled as a mounted infantryman, my official title being "Trooper Pearson", which gives me some measure of satisfaction.' Pearson was one of the guard of honour when General Botha returned from the conquest of German South-West Africa.

The range of subjects illuminated by Pearson's researches is shown by an inspection of the Bibliography. His contributions to the morphology and reproduction of the Gnetales have a special significance both from the point of view of their great interest to botanists and as illustrating his skill as an investigator and his power of grappling with particularly difficult problems. As a systematist he held a high position: his earlier papers written at Kew deal with plants from many regions, and the fact that he was invited to describe the Verbenaceae and the Thymelaeaceae in the 'Flora Capensis' and the 'Flora of Tropical Africa' respectively shows that his ability in this branch of the subject received due recognition. His later papers on systematic botany are concerned chiefly with the rich material collected by himself. Pearson gave special attention to the examination of the desert flora of South-West Africa, particularly from the point of view

of its relationship to the floras of neighbouring regions. One of his aims in visiting the Karasberg range, which rises from the level plateau of the Kalahari desert, was to search for clues to the past history of the South African flora. He always arranged his routes according to a well-considered plan of attack upon the phytogeographical questions suggested by the different types of vegetation and the varied physical conditions of the countries through which he travelled. Had Pearson lived a few years longer there is no doubt he would have worked up his field-notes into a connected whole, and knowing how well qualified he was by training and by his ability to see things in their true perspective, one is able to realize to some extent how valuable such a digest of his knowledge and mature experience would have been.

His thorough treatment of the problems presented by the root-parasite *Striga lutea*, a Scrophulariaceous plant locally known as the Rooibloem or Witchweed, which causes serious loss to cultivators of Maize, and the valuable practical directions for dealing with the disease afford further evidence of his versatility and of his desire to demonstrate the importance of Botany as an applied science. With the assistance of Miss Stephens, one of his pupils, he studied the details of the haustorial structures and their connexion with the host; he also investigated the germination and dispersal of the small seeds and made many experimental trials of different methods of dealing with the pest. Previous attempts to germinate the seeds of *Striga* had been unsuccessful and Pearson proved that germination occurs only in presence of the host.

During a halt in Namaqualand Pearson made observations on the internal temperature of *Euphorbia virosa* and *Aloe dichotoma*. He found that *Euphorbia*, with its large chambered pith, responds more quickly than the *Aloe* to changes in the external temperature and attains higher maxima. He also investigated the effects of wounding: in *Aloe* the lowering of the internal temperature is due to evaporation at the surface of water conducted through the xylem, while in *Euphorbia* the lowering is due in part to surface evaporation, but also to the expansion of gases imprisoned in the pith.

In answer to a request for a few words about our friend the High Commissioner in London for British South Africa, the Right Hon. W. P. Schreiner, wrote as follows: 'I don't think that I can really add anything that is not better said by others about dear Pearson. I entirely associate myself with what is written of him in prose and poetry in an article in the "South African College Magazine" for November, 1916, where he is placed at the front of the Roll of Honour. My abiding impression of him is of a shining bright personality. His laugh was a tonic. He was the most cheery man at cheerful gatherings such as our remote Oxford and Cambridge dinners on Boat-race night, or at the annual College feasts at Cape Town. But

his work and purpose were never lost sight of, and he won his way not only to our hearts but also to our pockets for the big object which he accomplished. He was a boy among men, but a greatly respected man among his boys and girls at College. By none more than by his friends and students in South Africa is his loss deplored.'

Harold Pearson was a high-minded student and a loyal citizen whose short life was spent in the service of Science and who laboured to the utmost of his capacity for the good of the country of his adoption. By his devotion to duty and his suppression of self in his dealings with all sorts and conditions of men, he gained not only the affection of those with whom he was associated, but he exerted a very wide influence. His life recalls Hazlitt's words: 'When the pursuit of truth has been the habitual study of any man's life, the love of truth will be his ruling passion.' He enriched the world by deed and example; his pioneer work has made the path smooth for those who follow him, but it will be their responsibility and privilege to do their best to maintain the high standard represented by Pearson's work for Botany and for the common life of the Colony. As the author (W. Duncan Baxter) of an In Memoriam article in 'The Cape' truly says: 'The best memorial that can be raised to him is to see that his work at Kirstenbosch is carried on, and the National Botanic Gardens made what he pictured them in his mind's eye. That is the way to perpetuate his memory, for as long as Kirstenbosch exists, there will be linked with it the name of its founder—the scholar and gentleman, Harold Pearson.'

I cannot close this inadequate account of one of the most lovable men it has been my good fortune to know without a word of sympathetic reference to the devoted wife who shared his South African life. Though all botanists mourn the premature death of an able colleague and many are the poorer for the loss of a true friend, she, whose loss is the greatest, may derive some consolation from the knowledge that her husband's services were very widely and very sincerely appreciated.

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