On Isoetes lacustris, L.

BY

J. BRETLAND FARMER, M.A., F.L.S.

Fellow of Magdalen College, Oxford.

With Plates V and VI, and Woodcut 1.

FEW genera amongst the higher series of plants have attracted more general attention and interest than that which has for so long been centred round *Isoetes*. Regarded as a study in taxonomic research, the numerous points of contact which it displays with families which are otherwise sharply severed from each other in affinity, are sufficient to justify its claims on the comparative morphologist, whilst at the same time the anomalous character of its structure has long afforded ample and fruitful material for histological investigation.

The object of the present paper is to attempt the elucidation of some of the numerous points of development and organogeny which have hitherto, in spite of the numerous workers in the field, baffled or escaped explanation, and also as far as possible to give a connected account of the oophyte whose development has long remained obscure, in the hope that further light may thereby be thrown on the difficult question as to the position of *Isoetes* in the natural system.

Von Mohl[1], who may be said to be almost the first person who investigated carefully the structure of *Isoetes*, drew attention to its more important peculiarities, such as the arrange-

[1] V. Mohl, Ueber den Bau des Stammes von *Isoetes lacustris*, Linnæa, 1840; also Vermischte Schr.

[Annals of Botany, Vol. V. No. XVII. December 1890.]

ment of the roots and the character of the cambium, and secondary thickening. Wahlenberg[1] seems to have been the first to notice the peculiar mode of thickening characteristic of this plant. His remarks are as follows: 'Caudex radicis constituitur taleola satis crassa quam nux avellanae saepe majore *in centro vegetante et ad ambitum moritura.*' But without doubt to Von Mohl belongs the credit of having pointed out the fundamental differences existing between the stem of *Isoetes* and those of the other vascular cryptogams which were familiar to him.

Alexander Braun[2] further explained the relation of the leaf-arrangement to the conformation of the stem; and he also correctly described the branching of the roots as dichotomous, although he does not seem to have grasped so clearly as Mohl their connection with the stem.

The chief source of our information on the genus is to be found however in the classical memoir of Hofmeister[3], in which the external morphology as well as microscopic structure and development, as far as his opportunities admitted, are for the most part dealt with in a masterly manner. He was, it is true, in error in his statements as to the character of the apices of the stem and root, in which he believed he had found apical cells; going so far, in the case of the stem, as to correlate the mode of segmentation with the 2- or 3-forking of the stem. Naegeli and Leitgeb[4] also came to the conclusion that the root-apex was dominated by a 2-sided apical cell, although they confess that their view is based on negative rather than on positive evidence, and is arrived at after rejecting other apparently possible explanations. Their figures, like Hofmeister's, were wonderfully accurate, and later observers have not always paid them the attention which they deserve. The results of my own investigations will, I hope,

[1] Wahlenberg, Flora Lapponica, p. 264.

[2] Alex. Braun, Weitere Bemerkungen ueber *Isoetes*, Flora, 1847.

[3] Hofmeister, Beitr. zur Kenntniss d. Gefässkryptogamen, Abh. Math. Phys. Kl. d. Kön. Sächs. Gesell. d. Wissenchaften, 1857.

[4] Naegeli u. Leitgeb, Entstehung u. Wachst. d. Wurzeln, Beitr. zur Wiss. Bot. iv. Heft, 1868.

show the source of their misconception of the structure of the apex.

The structure and development of the Stem.—As far as the structure of the stem is concerned, however, it is much more difficult to understand on what grounds it was possible for so careful an investigator as Hofmeister to describe its growth as regulated by an apical cell. At no time is any cell-arrangement perceptible which would warrant this conclusion, and if the appearance presented by sections cut parallel to the furrow of the stem occasionally seems to point to it, more careful study of a series invariably leads to an opposite result, and clearly shows that the mistake is referable either to the obliquity of the section or to the fact of its having passed through the base of a leaf-rudiment. Comparison of transverse with longitudinal sections fully confirms this observation (Pl. V. Figs. 11, 13), and further affords proof that the apical meristem is even less regular than is the case in the Lycopodiaceae, judging at least from the figures published by Strasburger[1] and others. The entire apex of the stem is covered by a columnar layer of cells which divide chiefly anticlinally, periclinal divisions only occurring at rare intervals, a fact which is to be connected with the slight increase in length of the stem. I endeavoured to find out if the frequency of the periclinal divisions bore any relation to the number of leaves formed, but have been unable to ascertain that any such connection exists. The cells which lie beneath the superficial layer, though irregular in size and shape, indicate, in their general arrangement, a conformity with Sachs' law.

Hegelmaier[2] and Bruchmann[3] have regarded the bundle of the stem as consisting partly of a sympodium of leaf-traces, and partly of a cauline portion, represented by xylem-elements derived from a supposed plerome-tissue which surmounts the woody portion of the bundle. A comparison of a great

[1] Strasburger, Coniferen u. Gnetaceen, Taf. xxv. figs. 29, 30.

[2] Hegelmaier, Zur Kenntniss einiger Lycopodinen, Bot. Zeit. 1874.

[3] Bruchmann, Ueber Anlage u. Wachst. d. Wurz. bei *Lycopodium* u. *Isoetes*, Jen. Zeitsch. für Naturwissenschaften, VIII.

number of sections has however convinced me that Hofmeister's view is more in accordance with the facts, and that the axile bundle is really made up of leaf-traces, a view in which De Bary[1] also concurs. But in this case, like the alleged exogenous nature of the first root, to be considered subsequently, there is represented just one of those transitional stages where distinctions lie rather in the mind of the investigator than in the actual object before him. In this particular instance it is exceedingly difficult to draw a limit between a possible cauline and a common bundle in the older stages, although in young plants it is perfectly obvious. The cause of the discrepancy existing between the accounts of the various writers possibly lies in the great difficulty, without a complete series of sections, in reconstructing the entire vascular system with any degree of accuracy, and the difficulty is further increased by the great number of leaves which arise at almost exactly the same level on the stem.

About a year after germination, when the first few leaves are fully formed, the parenchyma around the vascular bundle of the stem (the *pericycle* of Van Tieghem) begins to divide periclinally to form the 'cambium.' The divisions extend around and above the axile bundle of the stem, but not so far as the youngest leaf-traces. And thus, whilst agreeing with Hegelmaier in the main features, I cannot but think that his description is misleading when he states that, whilst the cambium extends above the bundle in the form of a barrel, those cells which take part in the formation of a leaf-trace change their direction of division to one at right angles to that of the surrounding cells, in order to contribute to the trace. This statement is however only true of the appearance presented by older leaf-traces, which have become surrounded by, and enclosed in, the cambial zone. Originally, as I have said, the particular direction of their division is determined *before* the cells in their vicinity assume the freshly active merismatic condition.

The leaf-trace originates in the division of a row of cells, in

[1] De Bary, Comp. Anat. p. 280 (Engl. Trans.)

an upward and outward direction, which more or less irregularly connect the base of the leaf-rudiment with the central part of the stem, at the apex of the woody portion of the bundle. Thence the division extends upwards into the leaf and downwards into the stem. In all cases where I have been able to observe accurately, the tracheids of the trace are seen to join the axile strand in such a way as to afford conclusive evidence that they, with the tracheids of the neighbouring traces, form the entire mass of wood. No xylem-elements, so far as I could determine, are ever present *above* the insertion of the last leaf-trace.

The elements of the wood in the vascular cylinder of the stem are very short as compared with the corresponding cells of the trace, and are largely mixed with conjunctive parenchyma. At a short distance below its summit, the axile cylinder of vascular tissue in the stem shows signs of irregular splitting, owing to the disruption of its cells, and the whole structure rapidly becomes full of intercellular spaces. This result is indirectly brought about by the activity of the cambium. The traces of the roots and leaves which pass through this zone to join the axile vascular cylinder are subjected to great strain by the fact that whilst the cells round them are growing, and, so to speak, travelling outwards, they themselves are stationary, and can only increase in length by extension. This takes place to a limited extent, and effects a great distortion of their constituent cells, but finally the loose central tissue gives way, and they are partially relieved of the strain at the expense of the cohesion of the inner tissue. That such really represents the actual state of the case may be ascertained by inspecting longitudinal sections through parts of the stem where the process is being vigorously carried on, and it is indicated by the character of the cells in the photograph (Pl. VI. Fig. 27). It is difficult to assign any other reason for the occurrence, mere extension of the cells of the stem will at any rate give no explanation.

The cambium formed as above described, besides giving off cells peripherally, produces also the so-called *prismatic cells* at its interior, which are continuous above with the phloem of the leaf-traces. Russow[1], partly for this reason, has regarded the prismatic layer as representing a bast or phloem, but quite apart from the fact that it is produced internally to the cambium, and would thus, from the point of view of its position, be anomalous, its structure is remarkably complex and heterogeneous. Hegelmaier[2] briefly indicated the zones observable in the tissue under consideration and assigned the cause of the appearance to alternating layers of clear and starch-filled cells. Transverse sections taken through the central part of the stem show that the prismatic layer which surrounds the central xylem is a band of varying thickness, being especially interrupted by the bundles which pass out to the roots. The zone-like arrangement consists in alternations of tubular thin-walled cell-rows of varying thickness, whose cell-contents are clear and watery, with others, whose cells are wider in the radial direction, and filled densely with starch. Occupying a middle position in the latter zone, is embedded an irregular ring of cells whose walls are thickened like those of the tracheids, but these too, unlike the latter, often contain protoplasm and starch. (The dark bands in Pl. VI. Figs. 26 and 27.) The number of the zones or bands varies with the thickness of the stem, and therefore presumably with its age, and also with the height at which the section is cut. The longitudinal section is especially instructive in explaining the relation which exists between the number of the zones at different heights. The bands are seen to converge towards the axile xylem in the order of their position; the outermost one being of course inserted highest. Their relative position naturally suggests some connection between their number and the periods of active growth, but I am unable to say if such a connection really exists. Fig. 27, which is a photograph of a section of *I. velata*, for the material of which I am indebted to the kindness of Prof. Pirotta, shows the longitudinal arrangement of the bands through part of their length.

[1] Russow, Vergl. Unters. p. 139.
[2] Hegelmaier, loc. cit.

The cells lying between the apical merismatic layer of the stem and the xylem of the vascular bundle evince, as has been already said, a general convergence towards the apex, and it is at the periphery of this mass that the cambium is first differentiated, and it is gradually pushed outwards below by the products of its own activity. Hence, as in the higher plants, radial divisions are essential to admit of the attainment of the increased circumference which the zone occupies. There are two methods whereby these radial divisions are effected: (1) Either, a cell of the cambium divides into two equal halves

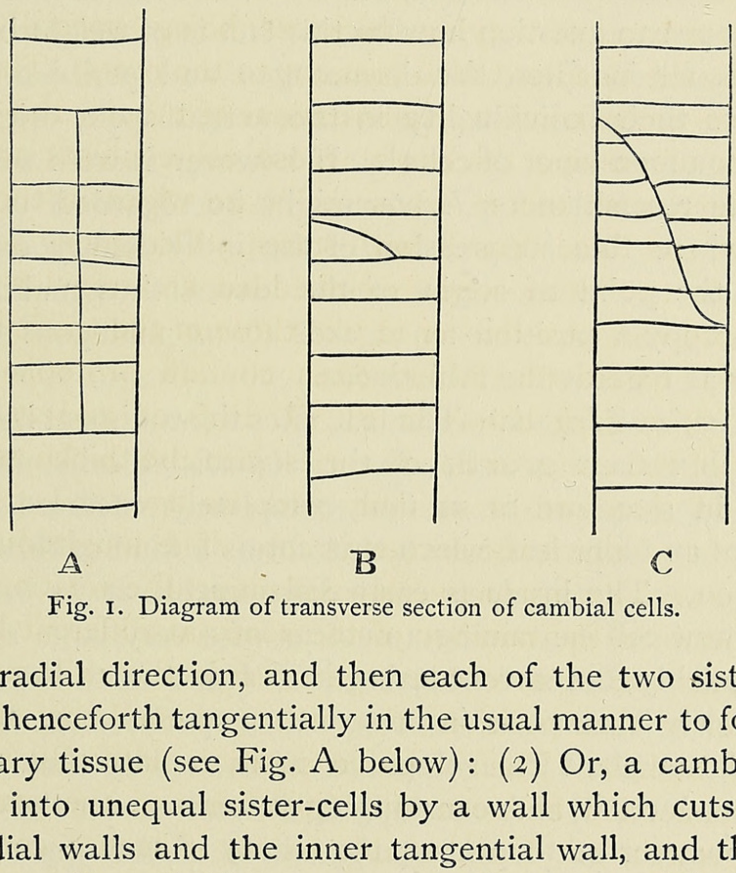


Fig. 1. Diagram of transverse section of cambial cells.

in the radial direction, and then each of the two sister-cells divides henceforth tangentially in the usual manner to form the secondary tissue (see Fig. A below): (2) Or, a cambial cell divides into unequal sister-cells by a wall which cuts one of the radial walls and the inner tangential wall, and then the first succeeding divisions are not quite tangential, but are curved to meet the rounded cell-wall; but finally the result is the same as in the first case, though the products can be more easily traced, at any rate inwards, on account of the pointed end by which such a cell-row is always characterised (Figs. B, C, above). These two modes of division are however only extreme forms of the same process, and all conceivable transitional stages may be met with in good preparations, that is, the radial wall of (1) may incline more or less from the truly radial direction, until it passes over into the form of (2).

Around the apical meristem of the stem are situated the young leaves which originate as horseshoe- or crescent-like protuberances from the apical tissue. As is well known, they arise exclusively as outgrowths of the superficial layer of cells, and this point has been noticed as in some degree weakening the suggested resemblance to the Cycads, in which the periblem also enters into the formation of the leaf. It is however, I think, open to question how far this fact is of weight in either direction, for in *Cycas* the dermatogen and periblem of the stem lose their individuality in the actual apex, originating from a common layer of cells[1]. If however it were wished to press the resemblances, *Isoetes* might be regarded as an instance where this suppression of the individuality extended beyond the point of origin of the leaves, thus making this genus occupy a position as regards *Cycas* analogous to that of *Cycas* as regards the Abietineae.

The Leaf and Ligule.—The leaf, after the rudiment is formed, by the luxuriant growth of the superficial cells, increases rapidly in size, and is at first completely enclosed by the sheath of an older leaf, which thus forms a kind of tube for its protection. The ligule is early discoverable at its base as a protuberant cell containing a nucleus of a very large size. It grows quickly, but never attains, in *I. lacustris*, to the proportions which it assumes in some other species, e.g. *I. velata*. In this latter plant it develops far more rapidly than the leaf which produces it, and, overtopping it, curves over the apex in such a manner as to protect the whole of the upper part of the leaf. The marginal cells of the expanded upper portion of the ligule are prolonged into short finger-like processes which not improbably contain a mucilaginous substance, and thus serve as additional means of protection against possible periods of dryness. In *I. lacustris* the ligule is, comparatively

[1] Strasburger, Coniferen, etc., pp. 335–6.

speaking, but little developed, and there is moreover in this plant no apparent need of special protection, especially as the older leaves so securely shelter the younger ones. It is only in exceptional cases that I have met with any prolongation of a few of its terminal cells at all comparable with that in *I. velata*, and the whole structure is smaller and simpler than in that plant. It is of course known that the slow-growing leaves of Ferns are commonly provided with some protective structure, and they may be regarded as representing the means for satisfying in a simple manner those requirements which in the higher plants involve the modification of potential foliage-leaves[1], stipules, etc. into scale-leaves. Without desiring to press the point too far, it may be noticed that these modifications are especially characteristic of the highest orders of plants, i.e. Phanerogams, and that in the lower members, such as *Cycas*, in which the early steps may be perhaps supposed to be observable, the arrangement is not such as to suggest that economy of material which is commonly to be seen in adaptive modifications. The stipules of *Marattia* and the curious structures described by Holle[2] for the Ophioglossaceae are familiar examples of the more complicated kinds of protective structures met with amongst the Vascular Cryptogams, whilst the simpler forms of the series are represented by the *ramenta* of so many Ferns. Much has been made of the ligule in *Isoetes* as indicating some affinity with *Selaginella* in which a similar structure occurs, but the two are in reality very different; that of *Selaginella* arises not from one cell, as in *Isoetes*, but as a multi-cellular protuberance; the mature structure is, moreover, not nearly so complete as in *Isoetes*, especially as regards its insertion. The point, however, which specially weakens the grounds for basing any theories of affinity on the common possession of the ligule by the two genera, is the much later development of this structure in *Selaginella* than in *Isoetes*. And even if this were not the case, outgrowths of a similar nature and belonging to the same category are so

[1] Goebel, Beitr. z. Morph. u. Physiol. des Blattes, Bot. Zeit. 1880.

[2] Holle, Ueber Bau u. Entw. d. veg. Organe d. Ophioglosseen, Bot. Zeit. 1875.

numerous in plants otherwise widely distinct, that it appears scarcely legitimate to regard them as affording any other but confirmatory evidence in establishing a theory of relationship which has been already arrived at on broader grounds. It is of course well known that outgrowths of a somewhat analogous nature are found to be often remarkably constant in certain circles of affinity, such for instance as the stipules of Rosaceae and of Cupuliferae. But this fact really strengthens the case against the employment of the ligule of *Isoetes* as affording any evidence of affinity with *Selaginella*, for no one will refuse to admit that the relationship between the two is at the best but very remote, so different are they in all other important characters; to endeavour therefore to unite them on account of the presence of a ligule in each of them, even if this structure were more similar in the two plants than as a matter of fact is the case, is like an attempt to establish an affinity between the Rosaceae and Cupuliferae on the ground that stipules are common to both orders.

Development of the Leaf.—In describing the development of the leaf, it will be convenient to consider in the first place its growth in length. After its rudiment has become well pronounced as a flattened and conical papilla, further cell-division is chiefly restricted to a zone situated at or near the base of the young leaf. In plants in which sporangia have begun to be formed, the cells *below* the insertion of the ligule remain for some time merismatic, and thus space is provided for the large sporangium, and at the same time the zone, including the insertion of the ligule, is raised up. If, on the other hand, the leaf belongs to a plant which has not as yet begun to bear sporangia, the merismatic tissue is localised in the part of the leaf immediately *above* the ligule. This position is ultimately taken up by the merismatic tissue of all leaves, and it is to the activity of cell-division in this region that the greater part of the mature supraligular portion owes its existence. The cells which are thus formed differ in their subsequent growth, and thus differentiation is at an early period perceptible in the leaf above the meristem. Whilst the middle cells, which form the vascular strand, grow mainly in length, and remain of small diameter, the parenchymatous cells which immediately surround them as a double or triple layer divide rapidly in the transverse direction. The same is also true of the two rows at the periphery of the leaf, although in this respect they exhibit certain minor differences (see Pl. V. Fig. 16). If the section has passed through the leaf in a direction other than that of the median plane, or one at right angles to it, the cells which occupy the space between the central strand and the periphery are seen to grow quite differently from the tissues on each side. They divide freely whilst in the merismatic region, but after emerging from it they scarcely increase in size at all and their nuclei are very small, a fact which is probably to be correlated with their arrested growth and extremely thin walls. Owing to this condition, and to the fact that the surrounding cells are largely increasing in length, the stationary cells are torn asunder in the longitudinal direction, cohesion being first lost at the centre, whilst the peripheral cells remain permanently attached by their transverse walls: in this way are formed the diaphragms which cross the four air-chambers found in the mature leaf of the plant. The diaphragm is thinnest at its centre, i.e. is of double concave lens-shape, and consists usually of only one cell-layer at this point, becoming thicker at the edges where it is suddenly inserted (*sit venia verbo*) on the outermost layer of the cells which were concerned in its formation (Fig. 16 in Pl. V). The earlier periclinal divisions which occur in the leaf are better seen in transverse than in longitudinal sections.

In transverse sections of young leaves in which the central strand has not as yet reached its final stage of differentiation, but which is, however, perfectly distinct, the outer cells of the leaf are seen to be increasing rapidly by periclinal or tangential divisions, which occur very regularly, and for the most part centrifugally. This is well illustrated by the photograph (Pl. VI. Fig. 22), in which the slight difference which exists between the development of the inner and the outer surfaces is already visible. It is only at a later stage that the diaphragm-cells become clear and marked off from the four rays of parenchyma which always connect the central bundle with the periphery of the leaf (Pl. VI. Fig. 23). Division is not so regular in the lower part of the leaf, where the sheathing portion is formed as a wing-like outgrowth, which is rendered more evident as the base of the leaf becomes elevated above the level of the stem. The bundle of the leaf in its subulate portion pursues a straight course, and, bending out behind the insertion of the ligule, where also its character slightly changes, it finally curves inwards again below it, and passes as a trace into the stem. It is rudimentary in structure, but of very constant form, and is collateral, both in its course in the leaf and also after entering the stem, thus indicating a relationship with the higher Ferns and with the Phanerogams.

In the portion of the leaf near the apex, the lignified portion of the xylem is reduced to a single tracheid, which is surrounded by six or eight parenchymatous cells, representing the rest of the xylem, which occasionally suffer more or less complete lignification, especially in the lower part of the leaf. I am unable to confirm Russow's statements as to the development of the xylem ; I do not find, at least in *I. lacustris*, that there is any definite direction in which the fresh xylem-elements are produced. Sometimes they are formed centripetally, sometimes in the opposite direction, and occasionally they spread unequally in the lateral direction.

The phloem is represented by a few cells which occur at the outer flanks of the xylem, and the protophloem tissues, where they can be distinguished, occur as distorted elements at the outer side of the wood. This slight tendency to encroach around the xylem is of interest, as pointing to an approach to the concentric type of the Fern-bundles proper ; it is shown in Pl. V. Fig. 15, which was drawn with the camera lucida. I failed entirely to discover any sieve-plates in the phloem either of *I. lacustris* or *I. velata* ; possibly, however, they might be found in a more pronounced terrestrial form. The elements of the xylem become more numerous as the bundle bends out behind the ligule, and at the same time much shorter ; a fact which is to be accounted for by the slight increase in length of the cells of this region. The arrangement both of xylem and phloem is also here obscured, and the curvature makes it almost impossible to determine their exact relation, though it is improbable that they differ from that already described for the upper part of the leaf.

The Sporangium.—The development of the sporangium has been so admirably worked out by Goebel[1], who has corrected and extended the observations of other investigators, that I have nothing to add to his results, which all my own work has confirmed. A comparison with some of the younger stages of *I. velata* and *I. Hystrix* has not yielded any new matter of importance, though it is not improbable that a more extended comparative research may throw light on the question as to the uni- or multi-locular nature of the sporangium in this genus. The facts are, briefly, that each hypodermal archesporial cell has a separate and individual growth ; that certain of these form the sterile trabecular tissue, whilst the others form the sporogenous tissue. The exact development of the latter varies according to the macro- or micro-sporangial nature of the body in question. The tapetum in both cases is derived partly from the sporogenous archesporial cells, partly from the trabeculae, and, according to Goebel, partly also from the cells at the back of the archesporium: my observations have made me a little uncertain on the latter point, but it is in any case of no great morphological importance, as is shown by the variation which exists in this respect in Phanerogams. The trabeculae, then, really appear to serve two functions ; first to provide a large amount of the tapetal nourishment, and secondly to provide a support for the outer wall of the somewhat bulky sporangium, and indeed from the close manner in which the leaves are packed together on the stem and the mutual pressure they must exert, it is probably a matter of some importance that some such protection should be supplied. The formation of sterile tissue, which is homologous with the actual sporogenous tissue, is well known in the

[1] Goebel, Beitr. z. vergl. Entw. d. Sporangien, Bot. Zeit. 1880.

Hepaticae, and it reaches its highest expression in forms like *Dendroceros*, in which there is a distinct resemblance to *Isoetes*. Of course, the sporangia of the Marattiaceae naturally suggest the explanation that the trabeculae represent the sporangial walls, but the inspection of a tangential section through a half-matured sporangium, especially of a micro-sporangium, tends to weaken the value of such a comparison. In any case, however, it must of course be admitted, that if *Isoetes* is actually allied, as I am convinced it is, with the Eusporangiate Ferns, the exceedingly common occurrence in them of numerous sporangia in close proximity would offer a contrast to *Isoetes* with its single sporangium. Such divisions in the sporangium as are formed by the trabeculae are not unknown in Phanerogams—in certain Onagraceae, for example—but I cannot regard this fact as in any way tending to establish the multilocular nature of the body in *Isoetes*.

The Root.—As regards the root, hardly two writers agree in their accounts of its structure and development. The facts that its apex ceases at an early age to remain in the merismatic condition, and also that the dichotomy of the apex takes place repeatedly while it is still enclosed in the original root-cap, serve to render the task of understanding its structure one of considerable difficulty.

Hofmeister, as is well known, regarded its growth as taking place by means of an apical cell, and Naegeli and Leitgeb also assumed its presence, although on negative rather than positive grounds. It is difficult to see how they missed the more correct explanation: their figures are extremely good, and they actually succeeded in unravelling some of the more difficult points, one of which, the nature of the plerome-initial, it is not easy to reconcile with any apical cell hypothesis such as they put forward: 'Der Cambiumcylinder ist anfangs immer einzellig[1].' Bruchmann[2], who studied the root in detail, believed he had succeeded in demonstrating three

[1] Naegeli u. Leitgeb, Enst. u. Wachst. d. Wurzeln, Beitr. z. wiss. Bot. 4. Heft, p. 134.

[2] Bruchmann, Ueber Anlage u. Wachst. d. Wurzeln bei *Lycopod.* u. *Isoëtes*, Jen. Zeitsch. für Naturwiss. VIII. 1874.

histogenic layers at the apex, a calyptro-dermatogen, a double cortex or periblem, and a plerome. He practically confirms Naegeli and Leitgeb's previous description, when he says, 'Das Plerome wächst im wahrsten Sinne des Wortes mit einer Scheitelzelle,' although the investigators just mentioned had not apparently foreseen the conclusion to be drawn from their own observations. Kienitz-Gerloff[1], on the other hand, after criticising the views of his predecessors, arrives at the singular conclusion that the apex is occupied by an undifferentiated meristem: 'Am naturgemässesten scheint es mir . . . das Gewebe der Wurzelspitze von *Isoetes* als ein völlig indifferentes Meristem zu betrachten. In dem durch dieses Meristem abgeschiedenen Gewebe sondern sich dann später die verschiedenen Gewebesysteme aus' (p. 793). He was probably led into this error by confining himself to a study of optical sections of cleared apices, otherwise his account can hardly be explained at all. His figures do not give the correct relations which exist between the different tissues: the derivatives of the plerome-initial never appear to run straight down the root, as he represents them, except when seen in a plane at right angles to that in which it is alone possible to draw any correct inference as to their origin. And even some of Bruchmann's figures are open to question on this account. The latter investigator does not seem to have paid sufficient attention to the stronger roots of older plants, and I find myself unable to agree with him in his attempt to draw a sharp line of demarcation between the periblem and the meristem which lies externally to it.

I have invariably found the sharpest distinction to prevail between the inner and outer cortex (see Pl. V. Figs. 9, 10; Pl. VI. Fig. 30), while the outer layer of periblem-initials are in most cases not clearly separable from the layers giving rise to the epidermis and root-cap. The variation which seems to exist on this point is suggestive, taken in connection with the relation existing between the dermatogen and periblem

[1] Kienitz-Gerloff, Ueber Wachst. u. Zelltheil. und d. Entwickl. d. Embryo von *Isoetes lacustris*, Bot. Zeit. 1881.

in the Gymnosperms, in *Cycas* or *Pinus*, for example. The plerome, in all cases where I have been able to trace it clearly, grows certainly from a single initial cell, but from the excentric course which the pro-cambial cylinder pursues in the root, it is by no means easy to secure sections which pass through it in the right plane. The cells which are cut off behind the irregularly shaped cell at the apex are readily distinguishable on account of their large nuclei and protoplasmic contents, as well as their large size as compared with the surrounding cells. The cells thus forming a row, divide peripherally and longitudinally, leaving a much larger central cell, from which further cells are cut off longitudinally; this is figured by Hofmeister[1], and also by Naegeli and Leitgeb[2], and in both cases with remarkable accuracy. Hofmeister explained the large cell as the apical cell, but a series of transverse sections shows this view to be untenable (see Pl. V. Figs. 5, 6, 7, 8), and longitudinal sections (Figs. 9, 10, 30) also demonstrate clearly the actual state of the case. Hofmeister's sections were cut, as a matter of fact, below the actual apex of the plerome, and show the early stages of the development of the vascular bundle. The photograph (Fig. 30) shows how large the central cells are in proportion to those surrounding them, and also how they retain the dense protoplasmic contents and large nuclei which are commonly associated with merismatic structures. The xylem-elements of the monarch vascular bundle only make their appearance at a relatively late period. The two photographs (Figs. 28, 29) show pretty clearly the stages by which this is brought about; and they also show how the phloem-portion of the bundle is well differentiated before the xylem, which is not as yet formed in either, although in Fig. 29 its general future position is distinguishable. There is no need to further discuss the structure of the mature portion of the root, it having been sufficiently described by other investigators, and the split in the cortex has also been correctly explained by Bruchmann. As to the origin of the lateral roots from the stem, I have as yet been unable to

[1] Loc. cit. Plate XIII. Figs. 3, 4.

[2] Loc. cit. Plate XIX. Figs. 3, 6.

investigate this point independently, although the statements made in reference to it by Van Tieghem and Douliot[1] leave the matter in some doubt. I cannot, however, agree with these authors in regarding the cortex of the root as referable to a single layer of periblem-cells, nor do I find their figure (Pl. XL. Fig. 583) at all convincing. It does not represent anything like that which I have seen in perfectly median sections where the distinctions of the different tissues are much more clearly marked than in their figure, in which the plerome is very much larger in proportion to the size of the whole root than in any preparation I have met with.

I have been unable to follow out all the stages of the dichotomy of the root, but so far as my observations extend, they confirm Bruchmann's statements on these points.

As regards the exogenous character of the primary root my results are, in the main, in agreement with those of other writers; the first divisions certainly occur in the superficial layer of cells, and the inner of the two daughter-layers formed by this periclinal division again divides, giving off fresh root-cap-cells at its exterior. The inner meristems are, however, formed from cells within this layer, which only forms the outer cortex, as is partly indicated in Pl. V. Figs. 2, 3; and Kienitz-Gerloff also has arrived at the same result. The outer layers of the root are as a matter of fact exfoliated, and relying on this fact, the author just cited argues against the claims of the root to be regarded as exogenous. The difference between exogeny and endogeny in a structure such as this is, however, somewhat shadowy, and perhaps it would be well to regard it as representing actually a transition between the two. Of course many (comparatively speaking) exogenous roots are now known, amongst which may be quoted that of *Phylloglossum*. Van Tieghem and Douliot have attempted to correlate the relative time of development with the exo- or endo-geny, but I think that the instance of *Isoetes* shows that this principle, useful as it is in many cases, may be carried too far. The root

[1] Recherches comparatives sur l'origine des membres endogènes dans les plantes vasculaires, An. Sci. Nat. (Bot.) 1888.

of *Isoetes* is not perhaps, strictly speaking, a primary structure, and I incline rather to consider it as an early type of adventitious root. It is formed relatively much later than in the true Ferns, and yet it is far more exogenous in character than in them.

The Oophyte.—The development of the oophyte has long presented difficulties to those who have attempted its investigation, partly owing to the ease with which the contents of the spore are injured, and partly to the hardness and thickness of its coats.

Mettenius[1], who described the structure of the macrospore, correctly distinguished its three principal layers or coats. The outer glassy epispore or perinium is extremely hard and brittle, and its surface is beset with numerous prominences. It is without doubt derived from the plasma of the sporangium formed by the breaking down and solution of the tapetal cells (and probably the mother-cell walls), and it stains faintly with safranin. The exospore, a dark brown band of somewhat fibrous appearance, is clearly resolvable into a double layer, and the outer one of the two frequently splits again. The endospore is a rather thick cellulose-wall, of irregular thickness, and it is characterised by a brightly refractive appearance, recalling that presented by collenchymatous walls.

The contents of the spore are rendered obscure by the enormous quantity of oil, the removal of which can however be easily effected by turpentine or ether, when the protoplasm appears as a granular and reticulated structure. The granularity is caused by the numerous small proteid bodies which are embedded in its substance, whilst the reticular structure is due to the withdrawal of the oil which formerly filled the meshes. Starch-grains are also freely present, so that the protoplasm is thus very richly supplied with nutritive reserve material. At the upper end of the spore lies the nucleus, which is of very large dimensions, and contains a variable number of nucleoli of different sizes. These points are illustrated by the photographs (Figs. 24, 25). The nucleus, which

[1] Mettenius, Ueber *Azolla*, Linnaea, 1847, p. 269.

is separated from the cytoplasm by a well-marked membrane, is of a very watery consistency, and is so poor in chromatin as to be scarcely stained at all by reagents more especially used for nuclear structure, as methyl-green, safranin, or haematoxylin, although the nucleoli are rapidly and intensely coloured; and it is to these facts that the great difficulty of following out the changes which take place in the nucleus during the earlier stages of germination is to be attributed.

In the mature spore the apical portion (in which the nucleus is imbedded) is clearly separable from the remaining larger part by the dense character of its protoplasm, and the comparative absence from it of the reserve stores of starch and oil; and this differentiation becomes more and more obvious as the formation of cell-walls approaches. I have not, in spite of careful search through many hundreds of spores, succeeded in recognising the nucleus at this stage; probably the nucleoli, during its division, may suffer disintegration, and diffusion into the cytoplasm as the result of the heat necessarily employed in parafin-embedding.

But although the changes in the nucleus remain obscure, the other processes attending germination are clear enough. The mass of protoplasm already mentioned as occupying the apex of the spore becomes traversed by fine cracks coinciding in their general directions with the positions ultimately taken up by the young cell-walls. I have no doubt that this splitting is subsequent to, and perhaps conditioned by, the division of the spore-nucleus, though direct evidence is wanting on this point. After cell-formation has begun, it proceeds with great rapidity, though in a manner differing in the two regions of the spore already alluded to. Figure 17 exhibits one of the earlier stages, and is intelligible when it is borne in mind that the section has passed obliquely below the spore-apex. Figure 18 shows clearly the differences in the process of division prevailing in the upper and lower part of the spore, the extreme slowness with which the process is conducted in the lower part being strongly contrasted with the rapid cell-increase in the upper portion. The divisions in the latter region lead to the formation of the archegonia, and so similar are the earlier formed cells of the outer layer all through this upper portion of the prothallium that it is impossible, until quite late, to predict which are destined to give rise to archegonia; all are apparently, and to an equal extent, potential reproductive-organ-forming cells. The archegonia are formed by the periclinal division of an outer cell into two daughter-cells, the outer of which forms the four (sometimes three) stories of neck-cells, whilst from the inner one are cut off successively the neck-canal- and ventral canal cells, leaving the oosphere at the base. While these divisions are taking place the surrounding cells divide into a small-celled tissue in which the archegonium is buried, only its outermost neck-cells being free. The venter is thus formed, as in the highest Vascular Cryptogams and Gymnosperms, not from the mother-cell of the archegonium, but from the cells of the surrounding tissues. The neck-cell, when first cut off, divides into four cells arranged cross-wise, and then each of the four is divided into two cells transversely, and each of these again divides, once more forming the four stories of which the mature neck is commonly composed (Pl. V. Figs. 20, 21). The neck-canal-cell grows in between the neck-cells, thrusting them apart, and establishing, eventually, a connection between the exterior and the inner part of the archegonium. The neck-cell and the ventral canal-cell all finally become mucilaginous as in other Cryptogams.

Whilst these changes are rapidly proceeding in the upper part of the spore, the larger basal portion also undergoes changes. Its protoplasm is never so abundant as that of the upper cell-tissue, but the reserve material stored up in it is far more plentiful. It forms a cellular mass, but comparatively slowly, beginning at the periphery, and fortunate preparations show the large peripheral cells enclosing a central mass of protoplasm, as yet undivided, but in which are lying several free nuclei (Fig. 19). The whole appearance recalls the free cell-formation taking place in an embryo-sac during the formation of endosperm, though there is nothing more than analogy between the final products in the two cases. The lower portion of the spore eventually becomes filled with cells which are always distinct from those of the upper portion, both on account of their size and their contents, and moreover they never form archegonia. The prothallium thus formed lies within the spore, but not connected with it, as its outer cells have their own external wall distinct from the endospore.

The formation of the prothallium in *Isoetes* presents certain striking features of resemblance with that of *Selaginella* as described by Pfeffer [1], and may perhaps help to explain some of the peculiarities which render the oophyte of the latter plant so remarkable. Pfeffer states, and I am able to confirm his results in their chief points, that the spore is first divided by a wall (diaphragm) into an upper portion in which cell-division goes on rapidly, and a lower and much larger portion in which cell-formation is long retarded, and of which the protoplasm contains large quantities of food-material. The 'Prothallium' (of Pfeffer) makes its appearance before the spore has reached its full size, and is only followed later, after sowing the spores, by a free cell-formation in the infradiaphragmatic portion. Cell-division is brought to a close here apparently through the gradually increasing inability to complete the process, the lower part, as I have sometimes seen in *S. Braziliensis*, still remaining undivided into cells when an embryo was already growing in the spore. Pfeffer has endeavoured to make a morphological distinction between the two kinds of tissue thus described, regarding the meniscus of small-celled tissue as the prothallium, and comparing the lower and looser mass to the endosperm of Angiosperms. I venture to think, however, that such a position is untenable, and that the facts are to be better explained in another way, especially when *Isoetes* and *Selaginella* are compared in respect of their sexual generation. In both there is a clearly marked upper portion in which cell-division proceeds with great rapidity; and a larger basal portion in which cell-formation takes place slowly. That this production of cells proceeds centripetally in *Isoetes*

[1] Pfeffer, Die Entw. d. Keimes d. Gattung *Selaginella*, Hanstein's Bot. Abhandl. Bd. I.

and basipetally in *Selaginella* I do not regard as a fact of great importance. The essential point is, that in each, a specially *reproductive* portion is separated from a specially *vegetative* and nutritive portion ; the chief difference between the two cases lying in the greater completeness with which the differentiation is carried out in the case of *Selaginella*. It is natural to expect that a tissue which is required rapidly to give up its substance to a growing organism would not form a small-celled tissue. This cell-wall formation would involve an uneconomical expenditure, and its occurrence in the endosperm of Angiosperms may perhaps be correlated with the comparatively slow growth of the embryo which obtains in these plants.

Evidence is not wanting to show that a localisation of the reproductive organs of the oophyte, such as is required to support the suggested explanation of the structure in the two cases under discussion, is exactly the rule in a great number of widely different forms. This is especially true of the archegonia, with which we are here more directly concerned, and whose restriction to the 'cushion' of many Fern-prothallia, to the 'saddle' of *Salvinia*, to the central part of a *Pilularia*-prothallium, and to the more specially modified archegoniophores of many other plants, in each instance points in the same direction, and still further support is given by the Equisetaceae and the Lycopodiaceae.

It might have been expected that Gymnosperms would evince a more decided distinction, if these conclusions are to hold good, between the reproductive and vegetative portion of their prothallia; but apart from the fact that our knowledge on the early history of this structure in these plants is not very detailed, it may be pointed out that apparently the reduction has become so advanced, so far as the reproductive bodies are concerned, in the higher forms at least, that not more archegonial rudiments are formed than can be brought to maturity. Further investigation in this direction would probably yield interesting results.

I think that the above facts and considerations show plainly that Pfeffer was in error in regarding the 'Endosperm' of *Selaginella* as representing anything more than the vegetative or specially nutritive portion of the prothallium : the differences in the character of the cell-division in the two regions arising from the fundamental difference between the respective protoplasms (in the widest sense of the word) which so modify the manner of cell-formation as to suit the ultimate requirements of each.

I believe these conclusions may serve further to throw light on the question of the significance of the changes which occur in the embryo-sac of Angiosperms up to the period when the oosphere is ready for fertilization. Just as in *Selaginella* and *Isoetes* the first divisions of the spore separate the reproductive from the vegetative protoplasm, each of which thenceforth develops on different lines, so also the first division of the nucleus of the embryo-sac produces two daughter-nuclei, whose further products are perfectly dissimilar, resulting finally in the production of the 'egg-apparatus' at the one end, and the antipodal cells at the other, with the definitive nucleus half-way. The antipodal cells do not, I believe, represent the whole prothallium, as they are usually considered to do, but only the reduced vegetative portion of it. And it would seem that the reduction has progressed so far that the original vegetative part is unable to, or at least does not, fulfil the needs of the growing embryo as regards nutriment, and hence the coalescence of the two nuclei, one from the *reproductive* (micropylar) and one from the *vegetative* (antipodal) part of the prothallium.

The definitive nucleus, formed in this manner, may probably be regarded as the result of a coalescence of nuclei analogous to that which obtains in actual fertilization, but with this difference, that in this case it is not an oosphere, but probably the *second polar body*, which descends from the generative apparatus and fuses with a vegetative nucleus which is derived from the opposite end of the spore. The union does not confer great power on the resultant nucleus: merely that of forming, under certain conditions, a number of similar cells—the endosperm. It may be that the one nucleus contains sufficient histogenic plasma (to borrow Weismann's expression) to enable, under suitable circumstances, the 'reproductive substance' provided by the other nucleus (the *sister-nucleus* of the oosphere) to go through that simple form of segmentation which characterises the endosperm of Angiosperms.

This view of the matter makes the micropylar nucleus, before it divides into the four nuclei which compose the original 'egg-apparatus,' the equivalent of the 'central cell' of a Fern-archegonium; it cuts off successively two polar bodies, of which the first gives rise to the synergidae by further division, just as the neck-canal-cell of Ferns commonly divides further, after its separation from the central cell; and the second one, which I regard as representing the ventral canal-cell of the Fern, forms half the definitive nucleus. It is true the existence of a ventral canal cell has been denied in the case of some plants, but the point requires renewed investigation. And there is nothing surprising in such a reduction of the generative apparatus in the highest plants; such a process is indicated all through the various lines of descent of the different branches of Vascular Cryptogams, as well as of the Gymnosperms. In the Angiosperms indeed, bearing in mind how the tissue for the support of the growing embryo is not even capable of developing unless there is a chance of its being used, and also how this principle of saving material is carried yet further in the case of ovules of many Orchids, such a reduction as actually occurs in the embryo-sac is not only possible but inherently probable.

If these conclusions be accepted, *Isoetes* becomes additionally interesting as throwing light on some of the most obscure phenomena prevailing in Angiosperms. Its connection with *Selaginella* is equally interesting, as serving to explain the peculiarities of the sexual generation of this plant, but I do not regard the approximation in the character of the two oophytes as affording any weighty arguments for placing them near each other in the natural system. Rather we have an indication of one of those 'parallel developments,' which are hinted at in so many other forms. The reduction of the oophyte has advanced along similar lines, wherever it has occurred, in the Vascular Cryptogams, and in the direction indicated above; and it is from broader and more general comparative and morphological characters that the criteria of affinity must be derived.

It is acknowledged by all modern writers that in reality *Isoetes* presents but little affinity with *Selaginella*. Goebel has even stated his belief that the ligule is perhaps the only important feature which they have in common, and I have attempted to show that the admitted community in this respect does not really advance the question at all. I myself entirely admit the force of Prof. Vines'[1] arguments in support of the affinity with the true Ferns, and I think that the general results contained in this paper do not in any way oppose such an alliance, although they show that it is not perhaps very close. This is however not a matter for surprise; the heterosporous character of the genus clearly indicates a great advance on the homosporous condition of the Filicinae.

[1] Annals of Botany, vol. ii.

EXPLANATION OF FIGURES IN PLATES V AND VI.

Illustrating Mr. Farmer's paper on *Isoetes lacustris*.

(N.B.—Figures Nos. 26 and 27 refer to *I. velata*.)

PLATE V.

Figs. 1, 2, 3. Sections through the root of the embryo. The lines through the small diagrams accompanying Figures 1 and 2 represent the direction of the section, which lies in the plane of the paper in Fig. 2.

Fig. 4. Transverse section through the vascular bundle at the base of an old root.

Figs. 5, 6, 7, 8. Transverse sections through young root-tips at successively greater distances from the actual apex.

Fig. 9. Longitudinal section through young root apex. *P* = plerome.

Fig. 10. Longitudinal section through another root apex. *P* = plerome, *E* = dermatogen.

Figs. 11, 12. Transverse sections of stem-apex. Fig. 11 shows superficies; xxxx the meristem; *L* a leaf rudiment. Fig. 12 from the same stem, but the plane of the section lies deeper than in Fig. 11.

Figs. 13, 14. Longitudinal section of stem with young leaf.

Fig. 15. Vascular bundle of leaf. *x* = xylem, *Ph* = phloëm, *s* = sheathing cells of the xylem.

Fig. 16. Longitudinal section through half a leaf. *E* = epidermis. The large cells on the left abut on the vascular bundle which is not shown.

Figs. 17, 18. Stages in germination of macrospore.

Fig. 19. Section through a spore cut parallel to the base.

Figs. 20, 21. Archegonia.

PLATE VI.

Fig. 22. Transverse section through young leaf, *L*. This is enclosed in the ligule (*Li*) of the next oldest leaf, and this again by that of a still older one. *x* = tracheid.

Fig. 23. Transverse section of leaf. *x* = tracheid.

Fig. 24. Section through a spore. *n* = nucleus, *k* = nucleolus, *s* = starch.

Fig. 25. Another spore, with well-marked nucleus and nucleoli.

Fig. 26. Transverse section of stem of *Isoetes velata*.

Fig. 27. Longitudinal section of the same.

Fig. 28. Transverse section of root of *Isoetes lacustris* just beneath the apex.

Fig. 29. Transverse section through the same root, separated by the thickness of one section.

Fig. 30. Longitudinal section through root. *P* = plerome.

Fig. 1.

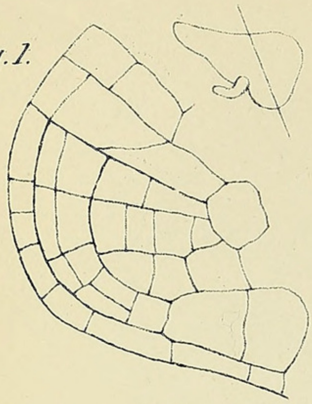


Fig. 2.

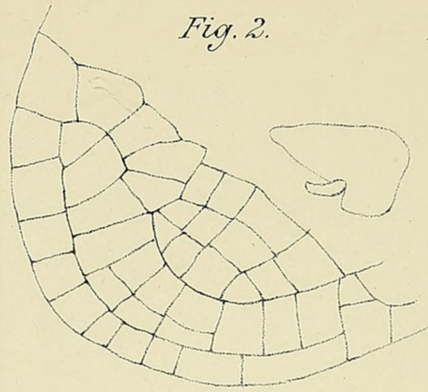


Fig. 3.

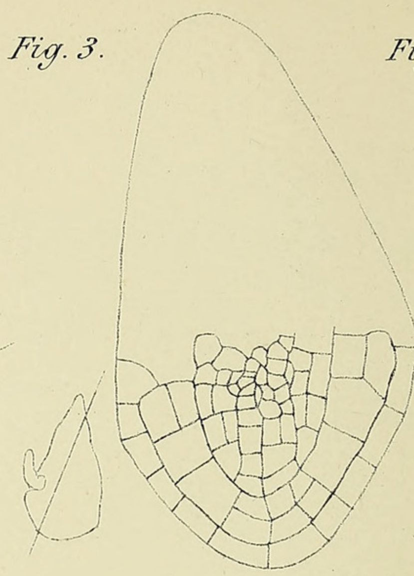


Fig. 4.

Fig. 7.

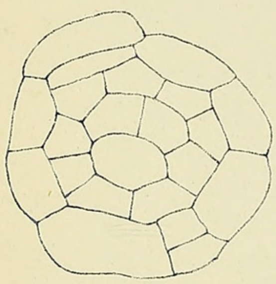


Fig. 8.

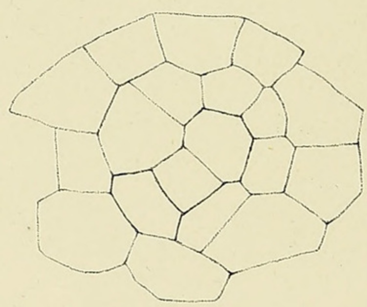


Fig. 9.

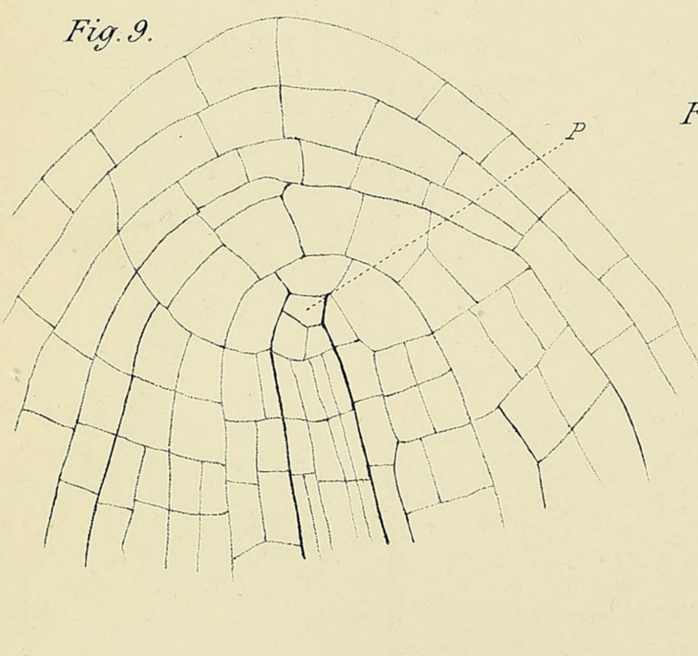


Fig. 10.

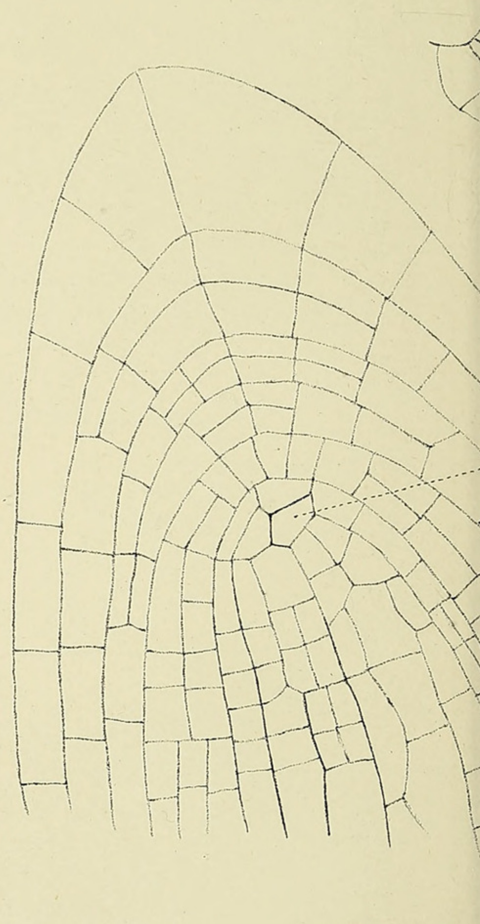


Fig. 18.

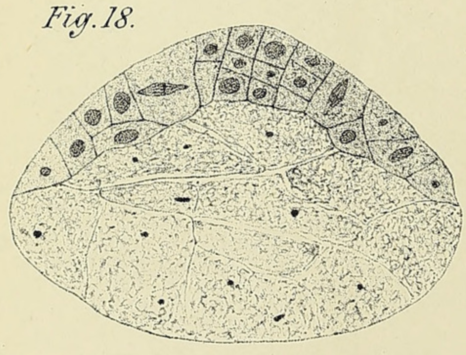


Fig. 19.

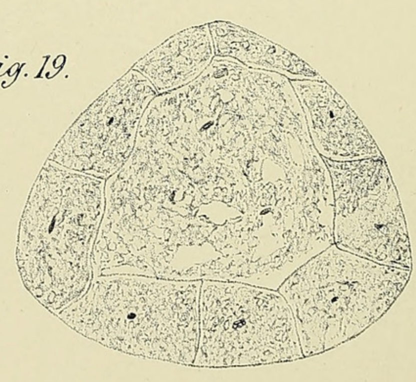


Fig. 20.

Farmer del.

FARMER.—ON ISÖETES.

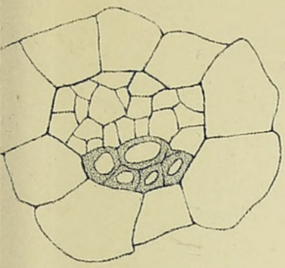


Fig. 5.

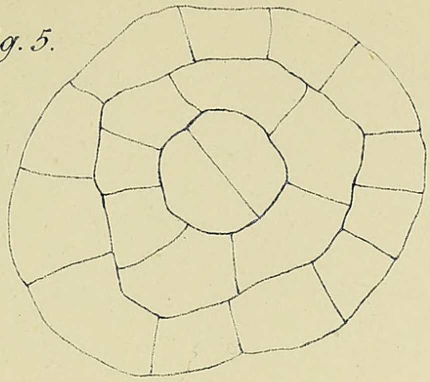


Fig. 6.

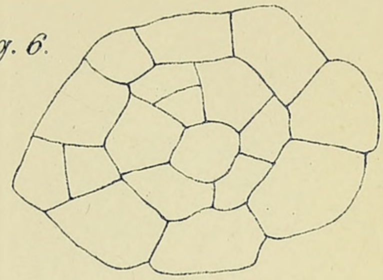


Fig. 11.

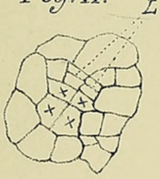


Fig. 13.

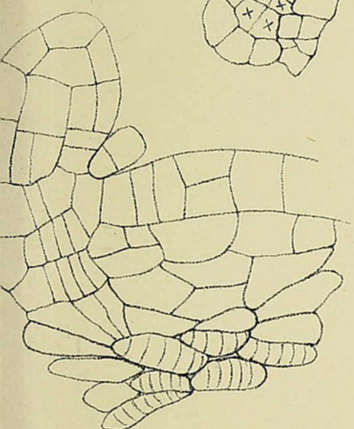


Fig. 12.

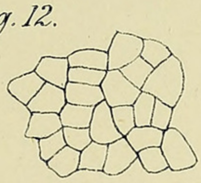


Fig. 14.

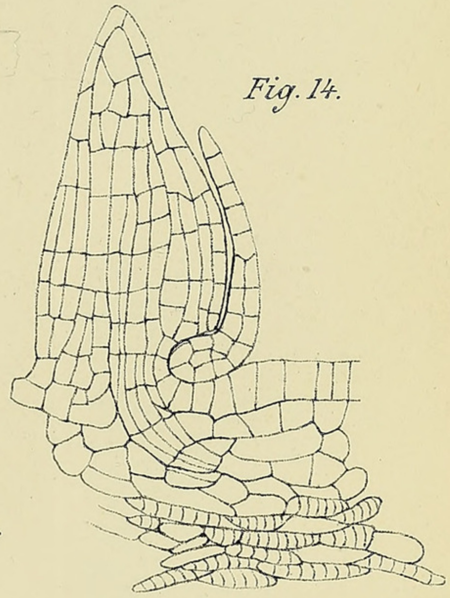
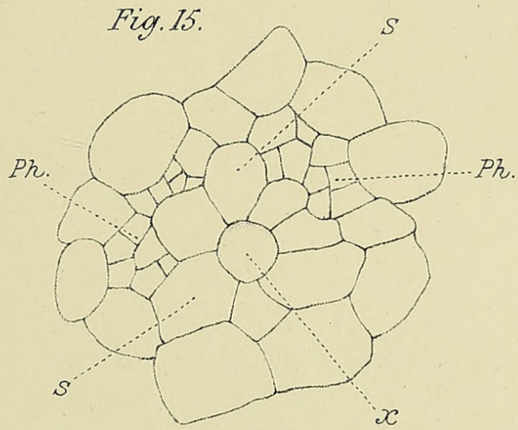


Fig. 15.



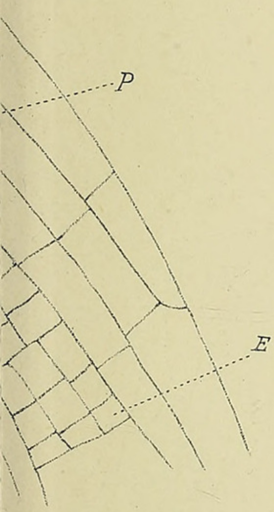


Fig. 16.

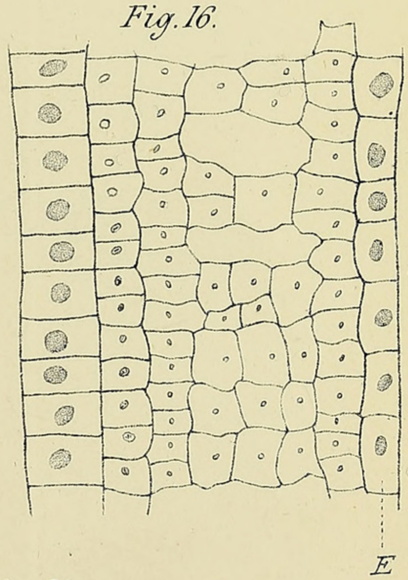
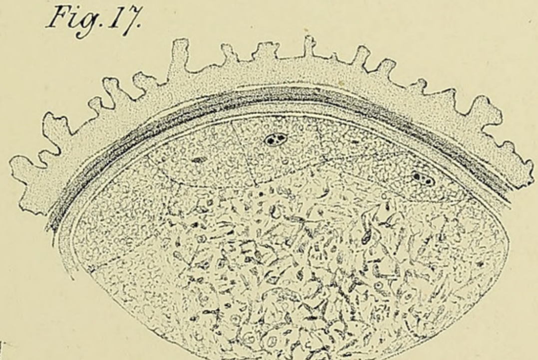


Fig. 17.



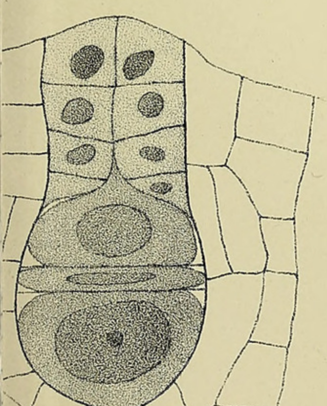
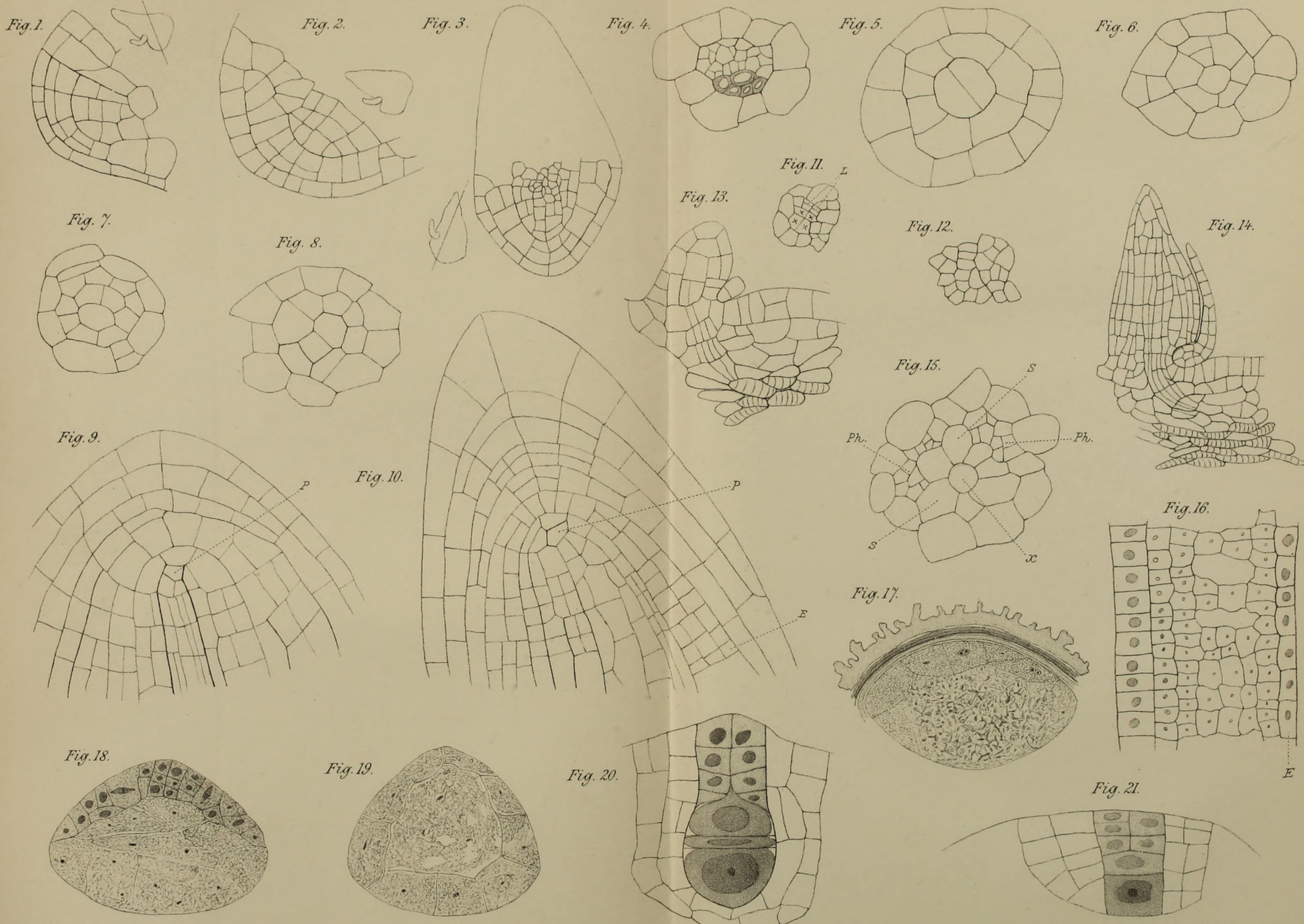


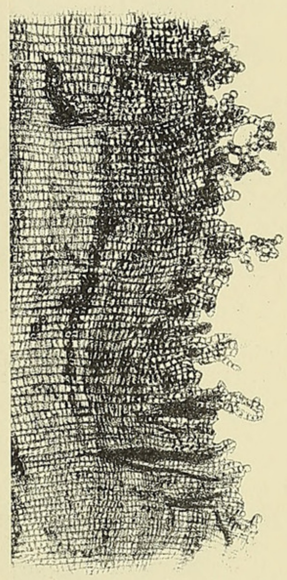
Fig. 21.

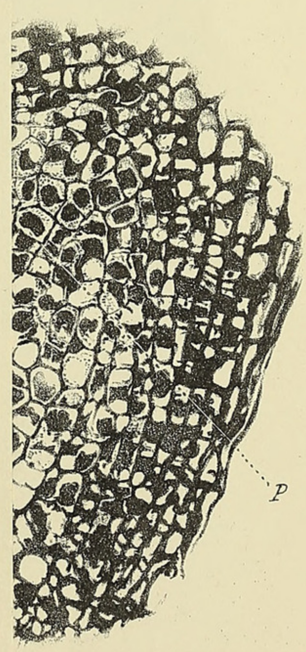
University Press, Oxford.



Farmer del. University Press, Oxford.

FARMER.— ON ISOËTES.





University Press, Oxford.

Fig. 22.

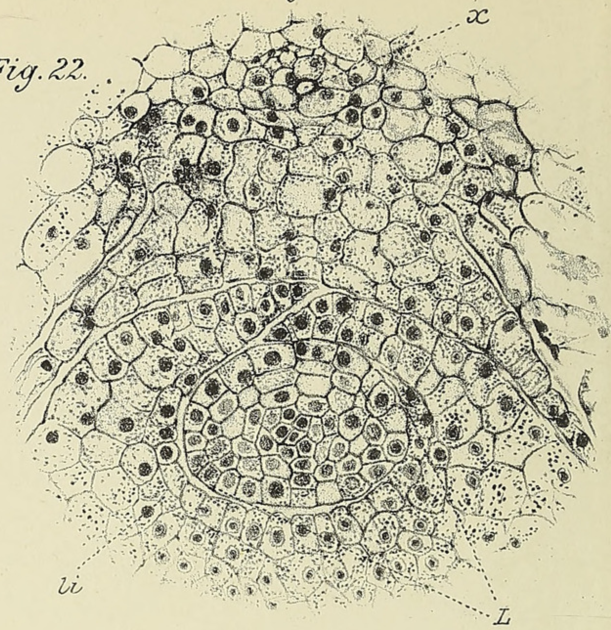


Fig. 25.

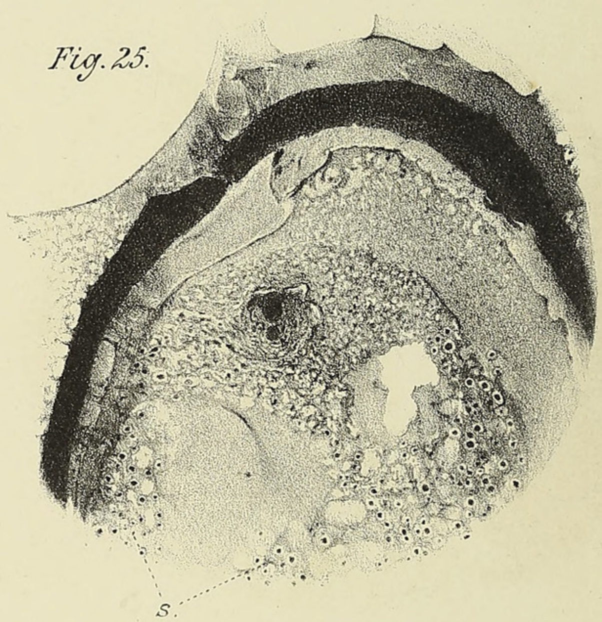
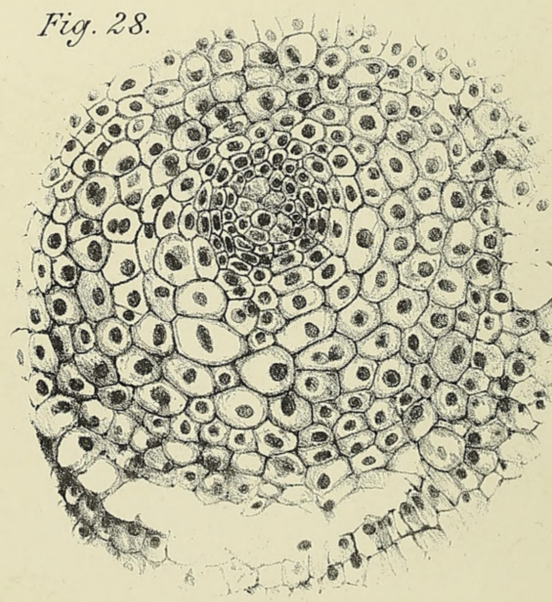


Fig. 28.



From Photo.[s]

FARMER. — ON

23.

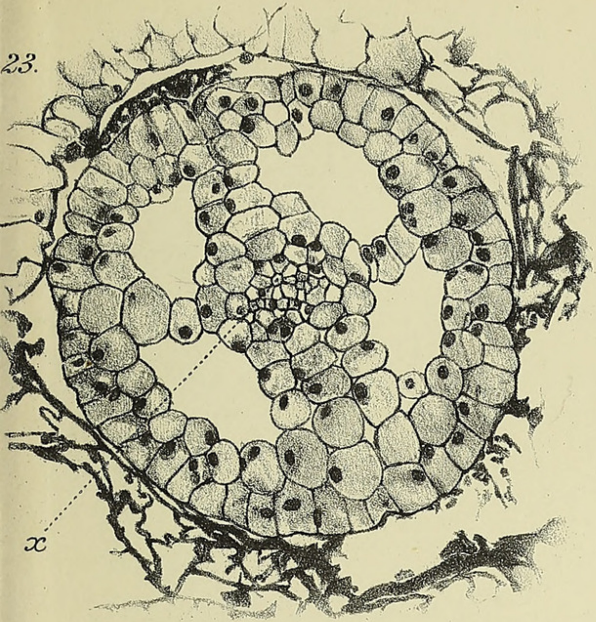


Fig. 24.

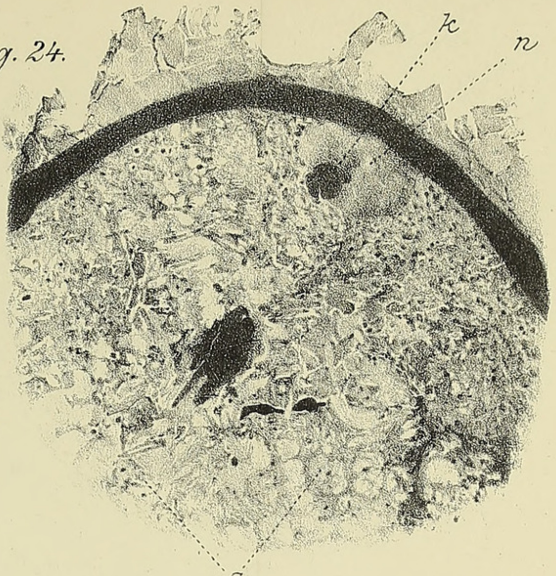


Fig. 26.

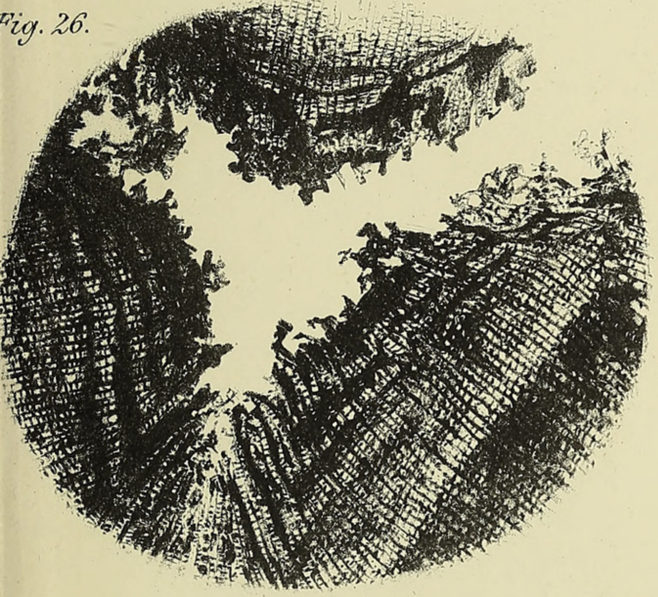
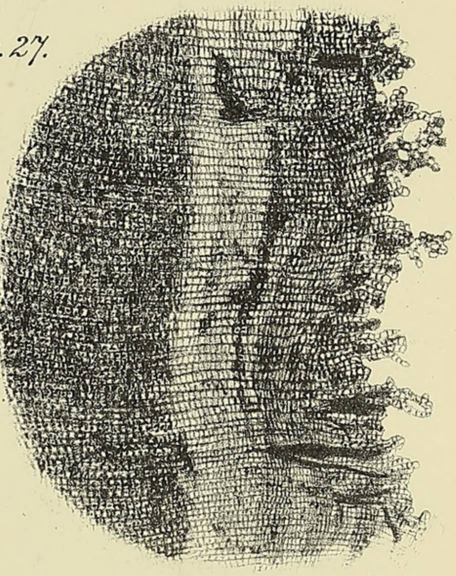


Fig. 27.



29.

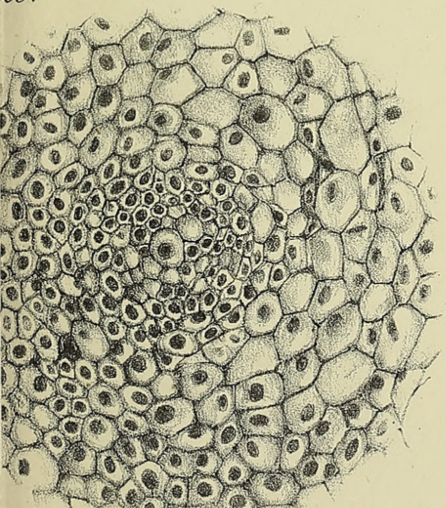
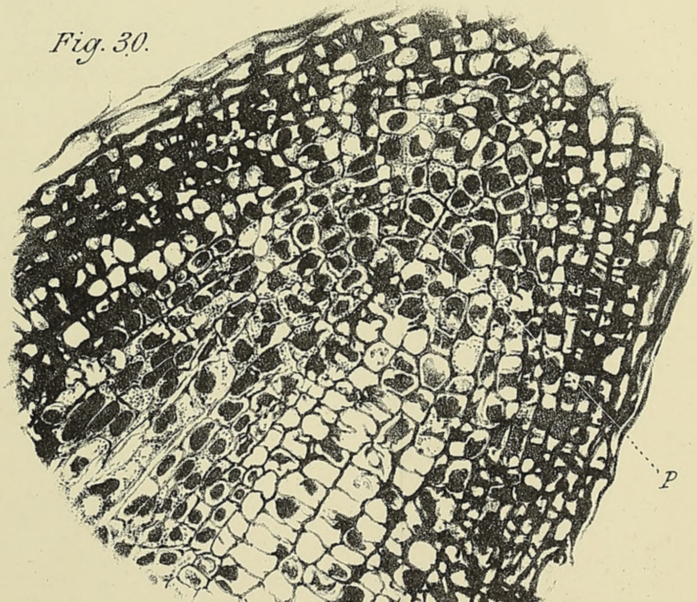


Fig. 30.



University Press, Oxford.

ISÖETES.

Fig. 22.

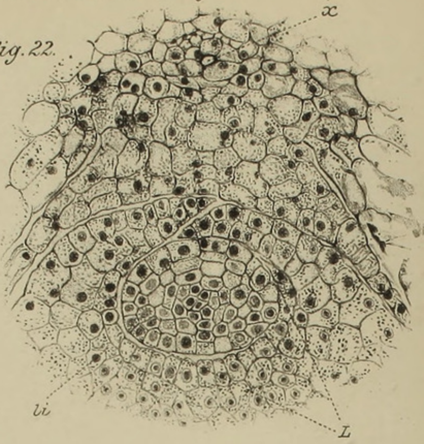


Fig. 23.

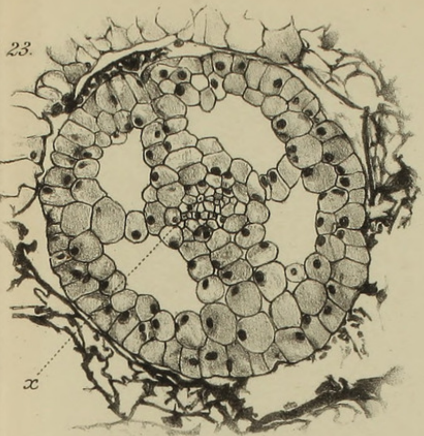


Fig. 24.

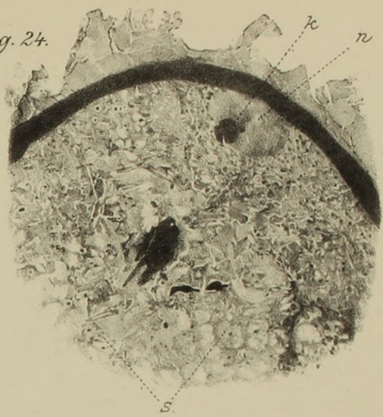


Fig. 25.

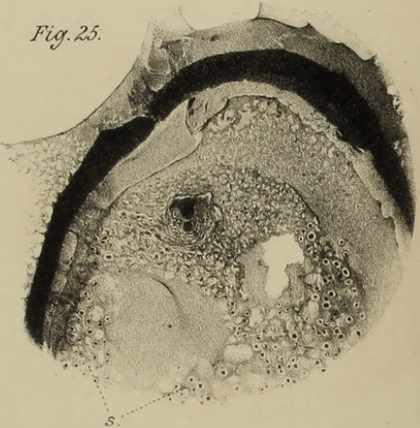


Fig. 26.

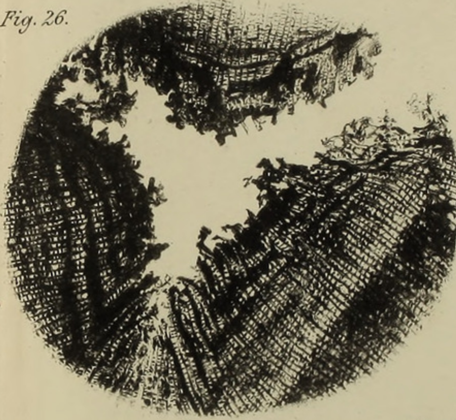


Fig. 27.

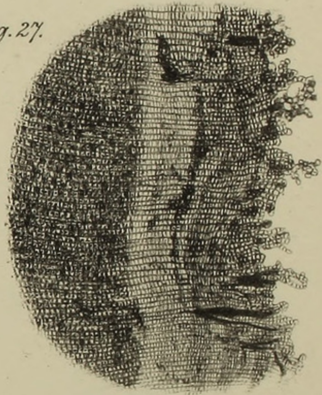


Fig. 28.

Fig. 29.

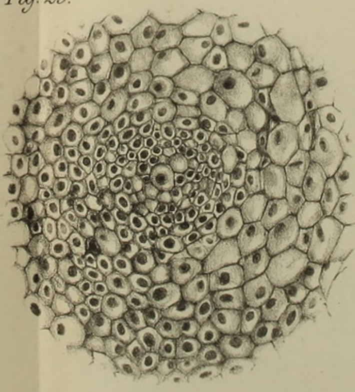
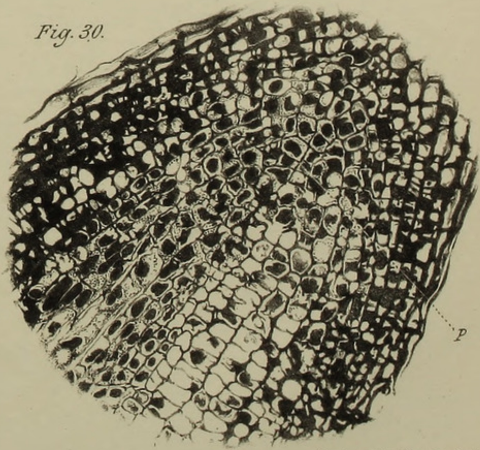


Fig. 30.



From Photo[s]

University Press, Oxford.

FARMER.— ON ISÖETES.