EXPERIMENTAL INVESTIGATIONS OF THE SHOOT APEX OF *DRYOPTERIS ARISTATA* DRUCE

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[Plates 10–14]

CONTENTS

	PAGE
1. Introduction	344
2. Materials and methods	345
3. Observations on the partially isolated apical meristem	348
(*a*) Growth of experimentally treated plants	348
(*b*) Supply of nutrients to the apical meristem	348
(*c*) Development of isolated meristems	349
(*d*) Arrangement of new leaf primordia	352
4. Observations on partially isolated leaf primordia	353
5. The growth of the isolated lateral segments	356
(*a*) Development of buds	357
(*b*) Abnormal development of meristeles	361
(*c*) Medullated meristeles	365
(*d*) Polycyclic steles	365
(*e*) Histological observations	370
6. Discussion	371
(*a*) The isolated terminal region	371
(*b*) Isolated leaf primordia	373
(*c*) Isolated lateral segments	375
References	381
Explanation of plates 10–14	382

A method whereby the apical meristem of the fern *Dryopteris aristata* Druce can be partially isolated from the adjacent lateral organs and tissues is described. This procedure has been adopted as a means of investigating growth and morphogenesis at the shoot apex. The technique involves the severance of the incipient vascular tissue which originates immediately below the apical meristem; the isolated meristem is thus seated on a plug of growing medullary parenchyma. Leaf primordia can be similarly isolated.

Meristems treated in this way are capable of growth. They develop into short vasculated shoots bearing leaves. The nutrients sustaining this growth must reach the apical meristem from below by diffusing through medullary parenchyma at the base of the isolated terminal region. Above the parenchymatous region a solenostelic vascular system is present in the new axis; this is in marked contrast to the dictyostelic configuration of the parental shoot below. On the further growth of the isolated meristem leaves are produced and the stele becomes dictyostelic. The new leaves, of which as many as fourteen have been observed after 11 weeks' growth, show the normal phyllotactic arrangement, and this is continuous with that of the main shoot below. The procedure adopted has the effect of removing the physiological dominance of the apical meristem relative to the main shoot; thus numerous large buds develop on the lateral segments of the parental shoot but none on the isolated terminal region.

The growth of isolated leaf primordia is very limited. The vascular system develops as a solenostele, foliar gaps are not formed in the region of confluence with the shoot stele, axillary buds are developed, and the leaf apex becomes directed outwards. These several features are in marked contrast to the normal development.

The isolated lateral segments are also capable of further growth. The experimental procedure adopted involves the severance of the vascular tissues at various levels. An account is given of new and hitherto unrecorded morphological developments observed in these segments. Interesting features include the formation of large solenostelic buds, the solenostelic development of isolated meristeles, medullation of meristeles and the induction of a polycyclic stelar condition, in one instance by a process of cambium-like activity. These are all in marked contrast to the normal development of the intact shoot.

The data which have been obtained are discussed with special reference to the path of translocation of nutrients to the terminal meristem and to leaf primordia, morphogenetic processes at the shoot apex, the factors influencing the differentiation of the vascular system, and theories of shoot formation and constitution.

The results of these experiments give no support to phytonic theories but emphasize the difference in potentiality for development between shoot and leaf primordia. In this connexion the factors which determine the shape and system of segmentation of the apical initials of shoot and leaf are seen to require further investigation.

The hypotheses that lateral buds are inhibited by substances proceeding from the apical meristem, that the initial differentiation of vascular tissue can be attributed to the basipetal diffusion of a substance or substances from the actively growing apical meristem, and that under conditions of tensile stress incipient vascular tissue undergoes a parenchymatous development, are supported by the data of these experiments. The observations afford a clear indication of the diversity of the morphogenetic activity in the growing region. Nutritional, mechanical and other factors are seen to be important in influencing the distribution of tissues during development. The view entertained by comparative morphologists that the vascular system in ferns is of a highly conservative nature and therefore of great value in phyletic studies is to some extent opposed by the data of these experiments. But notwithstanding the several unusual vascular configurations produced as a result of the experimental treatment, there is eventually a return to the typical vascular arrangements of the normal shoot. There is thus a need for harmonizing the data of the causal and phyletic aspects. The more thoroughly the operation of morphogenetic factors extrinsic to the specific hereditary substance is understood, the more critical will be the selection of criteria of comparison for phyletic purposes.

1. Introduction

In leptosporangiate ferns the *apical meristem* consists of a superficial layer of meristematic cells of distinctive appearance and reaction, the centre of the meristem being occupied by a conspicuous initial cell—the *apical cell*. The apical meristem so defined (Wardlaw 1943*a*) is readily distinguished from the adjacent epidermal, cortical and vascular tissues. Leaf primordia, bud primordia and scales all originate from cells of the apical meristem; vascular tissue is differentiated immediately below the meristem; while the meristem itself is maintained by the regular segmentation of the apical cell.

As a general hypothesis it may be suggested that the development of the apical meristem—the morphogenetic region—affects, and is affected by, the growth and differentiation of the adjacent tissues and organs. Attempts have been made by the writer to devise methods by which this hypothesis can be tested and the progressive organization to be observed at the apex during the individual development more fully explored. In the present contribution an account is given of experiments in which the apical meristem of the fern *Dryopteris aristata* Druce is isolated, by intersecting longitudinal incisions, from the adjacent lateral organs and tissues and its further development observed. The procedure adopted involves an operation roughly comparable with the technique used in 'ringing' experiments in older regions of the shoot in that the incipient vascular tissue is severed. Similar observations have been made on isolated leaf primordia.

The first step was to ascertain the practicability of the operation. The manipulative difficulty lies in the fact that the treatment has to be applied to a minute region, the meristem, the tissue of which is soft, delicate and readily injured. Thereafter it remained to be seen whether a meristem so treated would prove capable of further growth.

The successful outcome of such experiments seemed likely to throw light on: (i) morphogenetic processes at the shoot apex and the development of the leafy-shoot type of organization; (ii) the problem of translocation of nutrients to the shoot apex, i.e. whether this takes place by way of the undifferentiated vascular tissue or by upward diffusion over the whole cross-sectional area of the shoot; (iii) factors influencing the differentiation of the vascular system, i.e. whether the effective stimuli proceed basipetally from the apex or acropetally from the older preformed parts; and (iv) the physiological dominance normally exercised by the shoot apex over lateral buds or bud primordia.

The progressive organization to be observed at the growing point during the normal ontogenetic development may be regarded as an expression of a specific reaction system under a particular set of conditions: under a different set of conditions, a different organization might result. Therein lies the interest of the experimental work described below. The observations recorded here are of a preliminary and exploratory character. Nevertheless, they afford clear indications of the extensive departures from the normal development which may be experimentally induced. Quantitative aspects and detailed histological observations will be considered in a later paper.

2. Materials and methods

For these experiments the common broad-shield fern, *D. aristata*, was selected, plants of all sizes being obtainable in abundance. Previous experience has indicated that this species possesses very considerable vitality under experimental treatment.

An apex of *D. aristata* as seen in longitudinal median section may for convenience be considered to include three regions in basipetal sequence: (i) the apical meristem which consists of a single layer of superficial cells, including the apical cell, of distinctive appearance and chemical reaction; (ii) the region of initial differentiation, in which leaf primordia, buds and scales originate superficially from cells within or on the margin of the apical meristem and in which stele, cortex and pith can first be distinguished internally; (iii) the region of subsequent differentiation which merges downwards with the fully matured tissue systems. Since the principal morphogenetic developments take place in region (ii), it is there that experimental treatments must be applied.

The first step is to have unimpeded access to the apical meristem. At first sight this does not appear to be a very feasible undertaking. Indeed, because of the large number of rolled leaves and leaf primordia in the terminal bud and the dense investment of scales, the apices of ferns have not generally commended themselves as providing favourable materials for experimental investigations. Nevertheless, it is a fact of experience that there is little difficulty in removing the scales and leaf primordia from stout shoots of *D. aristata* or *D. filix-mas* and thereby laying bare the apical cone. These operations are carried out by means of small pointed scalpels and forceps, the material being observed under a binocular microscope (Wardlaw 1944*b*). A downward view of the apical cone and of the associated leaf primordia is illustrated diagrammatically in figure 1.

To isolate the apical meristem from the adjacent superficial tissues the following procedure was adopted. The terminal region of a stout shoot, trimmed to a length of about 2–3 cm. and defoliated as described above, was observed under a binocular microscope at a magnification of fifty times. By means of a small, thin-bladed scalpel four vertical, i.e. longitudinal, cuts were made in such a way as to isolate the apical meristem, on a little square island of tissue, as indicated in figure 1. It will be seen that the operation involves the severance of the incipient vascular tissue. As seen in longitudinal section the isolated apical meristem, together with a thin layer of incipient vascular tissue, is seated distally on a cubical plug of developing medullary parenchyma (figures 2, 3).

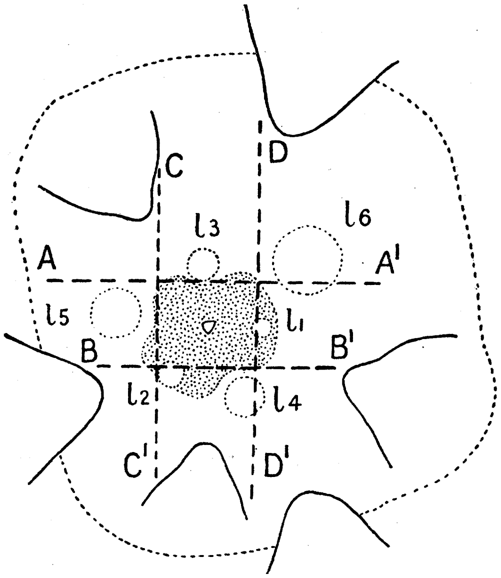


FIGURE 1. Downward view of a small apex of *D. aristata* showing the apical meristem (stippled) and a succession of young leaf primordia (l_1, l_2, etc.). The system of vertical incisions (AA^1, BB^1, etc.) by which the apical meristem is isolated from adjacent lateral tissues is indicated. (× 54.)

The system of incisions described above is obviously only one of many that may be applied. Thus, for example, the vertical cuts may be made on the margin of the apical meristem, within the margin, or outside it so as to include one or more leaf primordia, and so on. Again, leaf primordia of different ages may be isolated on separate plugs (figure 2). The writer has found that isolating by four vertical cuts is simple and least damaging to the apical meristem, but other patterns, e.g. by three cuts, are also possible. Radial cuts diverging from the margin of the apical meristem have also been made with interesting results. The procedure can be further varied by cutting more or less deeply into the tissue. In passing, it may be noted that the small size of the isolated meristem precludes the use of a cork-borer.

The experimental materials were planted in moist peat in a cool greenhouse. During the first week the apices were protected by pads of moist cotton-wool.

Terminology. For convenience, an apical meristem isolated laterally by downward incisions will be described as an 'isolated meristem', and a leaf primordium similarly treated as an 'isolated leaf primordium'. The growth development derived from an isolated meristem will be referred to as the 'isolated terminal region'. The dissected regions of shoot tissue situated outside the isolated meristem will be described as 'lateral segments'.

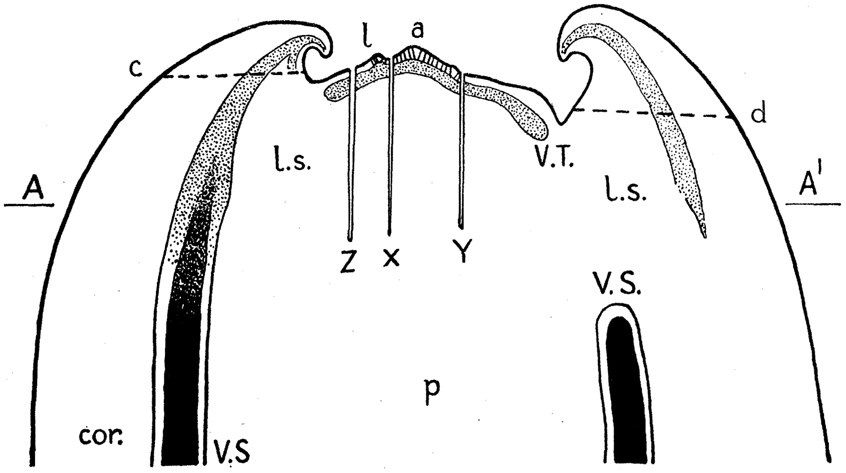


FIGURE 2. Longitudinal median section (diagrammatic) of a shoot apex of *D. aristata*, showing the apical meristem (*a*) and lateral segments (*l.s.*) isolated by intersecting longitudinal incisions, three of which are indicated (*x*, *y*, *z*). A leaf primordium (*l*) is isolated by the incisions at *x* and *z*, and by two other incisions at right angles. *c* and *d* indicate the levels at which leaf primordia would be pruned off in experimental shoots. *p*, pith; *cor*, cortex; *v.s.* vascular strand; *v.t.* incipient vascular tissue. AA^1, level in shoot at which the differentiation of phloem and xylem begins to be apparent.

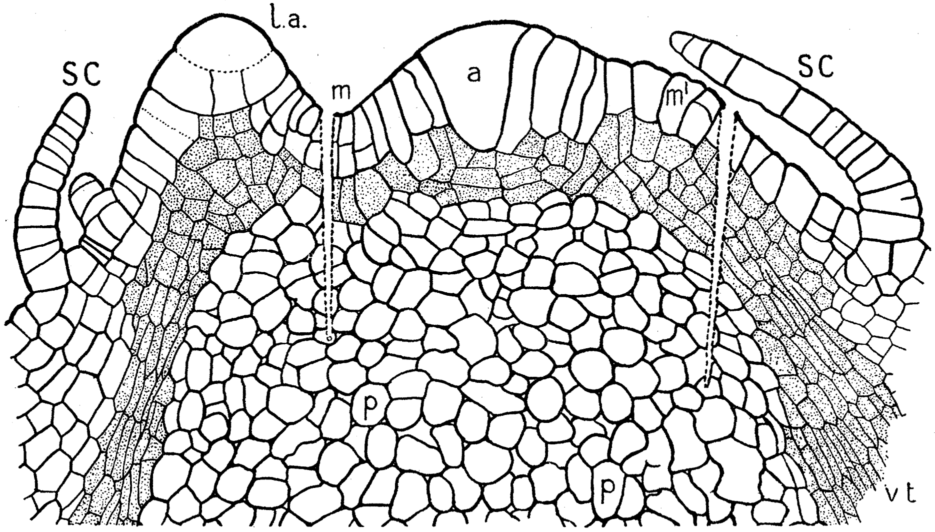


FIGURE 3. Longitudinal median section of a small apex of *D. aristata* showing the details of an isolated meristem. *m*–*m*¹, apical meristem, consisting of distinctive prism-shaped cells and large apical cell, *a*; *sc*, scale; *l.a.* leaf apex; *v.t.* incipient vascular tissue; *p*, pith. The vertical incisions are shown: the isolated meristem is continuous with a thin sheet of incipient vascular tissue and medullary parenchyma. (×158.)

3. Observations on the partially isolated apical meristem

(*a*) *Growth of experimentally treated plants*

The isolated meristem is capable of growth. A majority of the specimens survived the experimental treatment and showed evidence of growth within a fortnight. At the end of 4 weeks some specimens had developed 2–3 new leaf primordia; at the end of 11 weeks the isolated terminal region in one specimen consisted of a compact scaly bud containing fourteen leaf primordia.

Elsewhere the writer (Wardlaw 1943*b*) has shown that during active growth of the normal *Dryopteris* shoot lateral buds are not formed. Their development takes place either during normal periods of quiescence in the apical meristem or when the meristem has been removed, damaged, or temporarily affected by experimental treatment. Although isolated terminal regions with as many as fourteen new leaves have been obtained, no lateral buds have been observed in them. On the other hand, bud development in the lateral segments has been extensive (§ 5 (*a*)).

(*b*) *Supply of nutrients to the apical meristem*

It is perhaps not sufficiently realized that, as the apical meristem is the region of the shoot most remote from the sources of nutrients, special problems of translocation are involved in its maintenance. How, in fact, is the developing meristem maintained in an actively growing condition? There is support for the view that the extent of development of the leaf primordia is largely dependent on the nutritional status of the growing region (Wardlaw 1945*c*), and this will ultimately depend on supplies proceeding from below. Goebel (1900, 1908) considers that the primary leaves of the fern sporophyte are strictly comparable in structure with the adult leaves but that their development has been arrested in relation to limiting supplies of nutrients.

The supply of nutrients to the growing region though a problem of great importance remains almost completely unexplored. In the subterminal region of a shoot of *D. aristata* it can be seen (figures 2, 3) that although both cortex and pith are already well developed, the vascular tissue is still in the initial phase of differentiation, i.e. phloem and xylem cannot be distinguished (Wardlaw 1945*a*). It is therefore relevant to inquire if this incipient vascular tissue is the means whereby metabolites are translocated and distributed centrifugally and centripetally to cortex and pith respectively and upwards to the apical meristem, or whether, alternatively, the growing region is supplied by upward diffusion over the whole cross-sectional area. As an active meristem is a region of protein synthesis and as the sources of the constituent metabolites may be more or less remote, the movement of the comparatively large molecules involved to the distal region of the shoot may be indicated as a phenomenon of considerable interest.

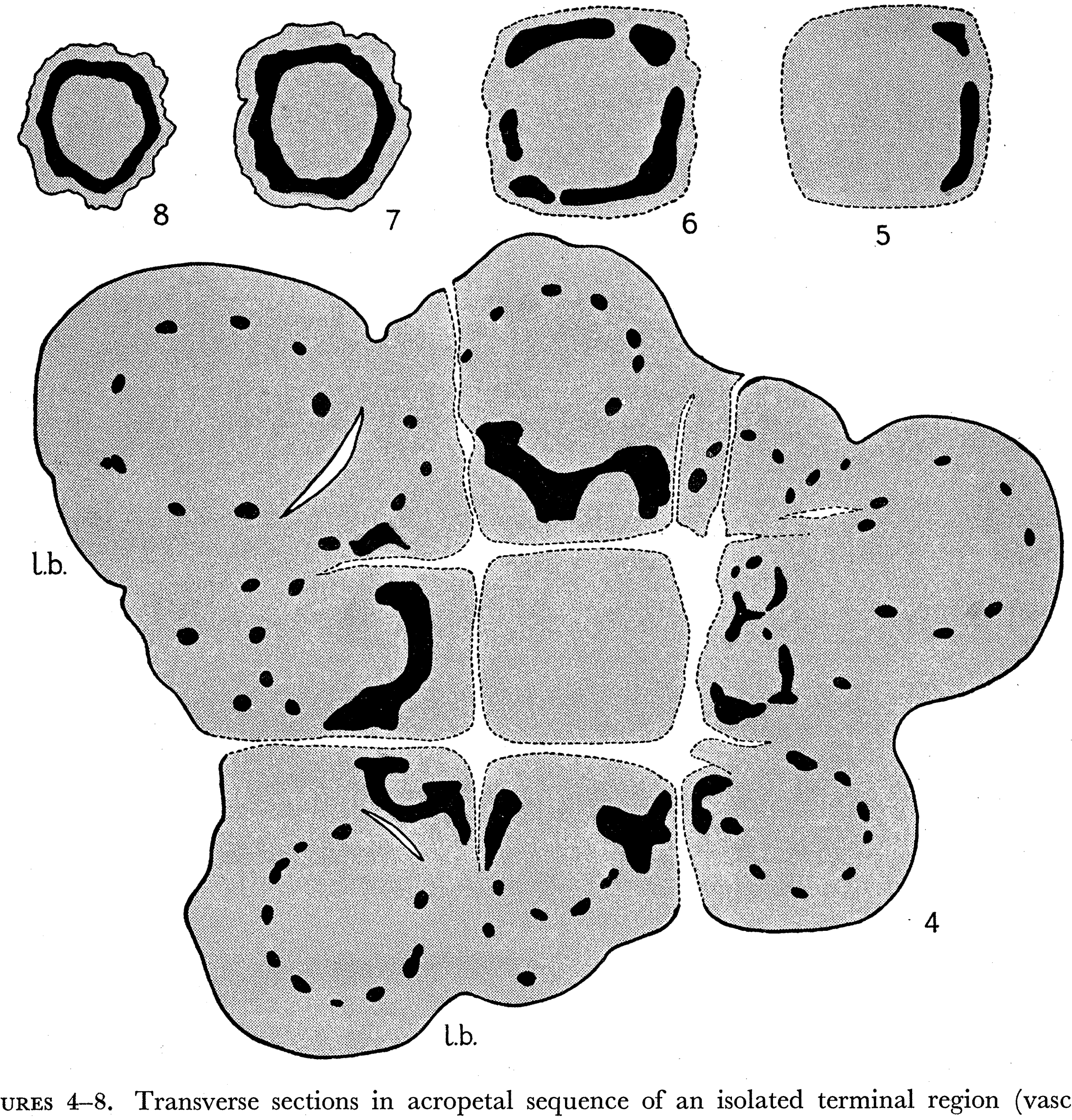
In the experimental materials under consideration the vascular system is completely severed. As a result, the lower part of the isolated terminal region consists entirely of medullary parenchyma (figure 77, plate 10). Since the meristem continues to grow actively and may produce fourteen or more new leaf primordia in the course of three months, it must be inferred that the nutrients utilized in this growth have diffused upwards through the medullary parenchyma. In completely defoliated shoots in which the apical meristem has not been isolated by dissection, the rate of formation of leaf primordia is approximately the same as that given above. Hence, it may be inferred that in the normal shoot the movement of nutrients to the apical meristem also takes place by upward diffusion over the whole cross-sectional area. The possibility that nutrients diffusing in a horizontal direction from the lateral segments across the knife incisions contributed to the growth of isolated meristems was eliminated in experiments in which the tissue on the outside of the incisions was removed, the meristem being left as a small island of tissue projecting above the general level. Such isolated meristems also continued to grow as already described.

(*c*) *Development of isolated meristems*

An isolated terminal region, after 4 weeks' growth, is illustrated in longitudinal median section in figure 77, plate 10; its lower region, which is continuous with the centre of the pith, is quite free from vascular tissue, but higher up, extending backwards from the apical meristem, the new development of vascular tissue can be seen. In figures 4–8 transverse sections in acropetal sequence of a comparable isolated meristem are shown diagrammatically. In this specimen the incisions went comparatively deeply into the pith. Figure 4 shows the normal dictyostelic vascular system of the shoot and the surrounding leaf-bases, each with several vascular strands. The shoot has been dissected in an approximately symmetrical manner, the central square of pith parenchyma being the base of the isolated terminal region. At a higher level in the latter, vascular tissue begins to appear on one side (figure 5). Still higher, more vascular tissue, interrupted by parenchymatous gaps, appears till finally it extends completely round the section (figures 6, 7). This continuous ring can be followed upwards to the region where it is differentiated immediately below the apical meristem (figure 8). In this specimen, the three or four new leaf primordia were still of very small size and so far no leaf-gaps had developed (Wardlaw 1944*b*, 1945*a*). Figures 78, 79, plate 10, show the solenostele observed in an older specimen, and the small gaps associated with the insertion of the new leaf primordia. Figure 81, plate 10, shows the dictyostelic shoot and leaf-bases of a still older specimen in which fourteen new leaf primordia had developed; figure 80, plate 10, is a section of the same shoot but taken lower down.

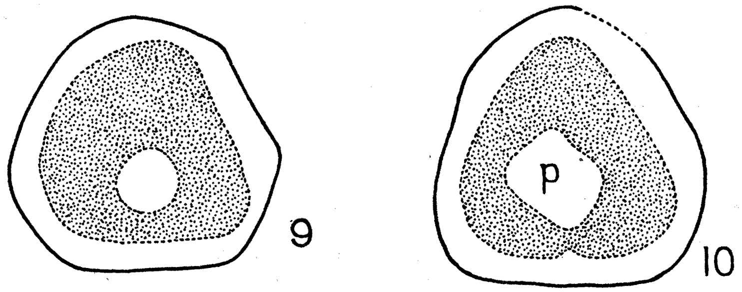
As shown in figure 6, the outer limit of the stele in the lower region of an isolated terminal region is parallel to the perimeter of the section. The stele, in fact, is of rectangular outline. In other instances where a triangular system of incisions was used (figures 9, 10), the stele was of triangular outline; and in yet others where a rectangular system was used, the stele was of rectangular outline. The shape of the stele, in fact, is directly related to the cross-sectional outline of the isolated terminal region. Higher up, where there is a return to the approximately cylindrical shape of the normal shoot, the stele is approximately circular in transverse section (figures 78, 81, plate 10). Figure 11 shows a transverse section of a shoot which had been dissected by six radial longitudinal incisions, the apical meristem being left intact. Here again it will be seen that the outlines of stele and shoot are in close agreement.

The rectangular or triangular outline of the stele in the lower region of an isolated terminal region is to be related, in the first instance, to the initial incisions, and secondly, to the fact that when the severed, incipient vascular tissue begins to grow, its outermost cells undergo a parenchymatous development. In other words potential vascular tissue becomes transformed into cortical parenchyma; the latter, at an early stage, can be seen to extend as radial rows originating from thin-walled vascular elements (figure **111**, plate **14**). At this level the new cortical parenchyma is of inconsiderable radial extent. So, too, at higher levels in an isolated terminal region, the ratio *radius stele : radius shoot* is greater than in the normal shoot. A comparison of the data relating to the relative development of pith, stele and cortex in experimental and normal plants may throw light on the distribution of nutrients in the growing region (Wardlaw 1945*a*).



FIGURES 4–8. Transverse sections in acropetal sequence of an isolated terminal region (vascular tissue shown in black). Figure 4, taken near the base of the region, shows the system of incisions whereby the apical meristem was isolated on a plug of non-vasculated medullary parenchyma. The dictyostelic vascular system and leaf-traces of the normal shoot are indicated. *l.b.* leaf-base, with leaf-trace. In figure 5, higher up, some vascular tissue is present at one side of the section; in figure 6 an interrupted vascular ring of square outline is shown. Still nearer the apex, figures 7 and 8, the vascular system is solenostelic. (× 11.)

Occasional specimens in which the isolated meristem had been damaged were grown on and kept under observation. Some of these have yielded data of considerable interest and importance because of the light which they throw on morphogenetic processes.



FIGURES 9, 10. Transverse sections, in basipetal sequence, of an isolated terminal region (incipient vascular tissue stippled). The apical meristem was initially isolated on a triangular panel of tissue: the outline of the vascular tissue is also approximately triangular. *p*, pith. (× 35.)

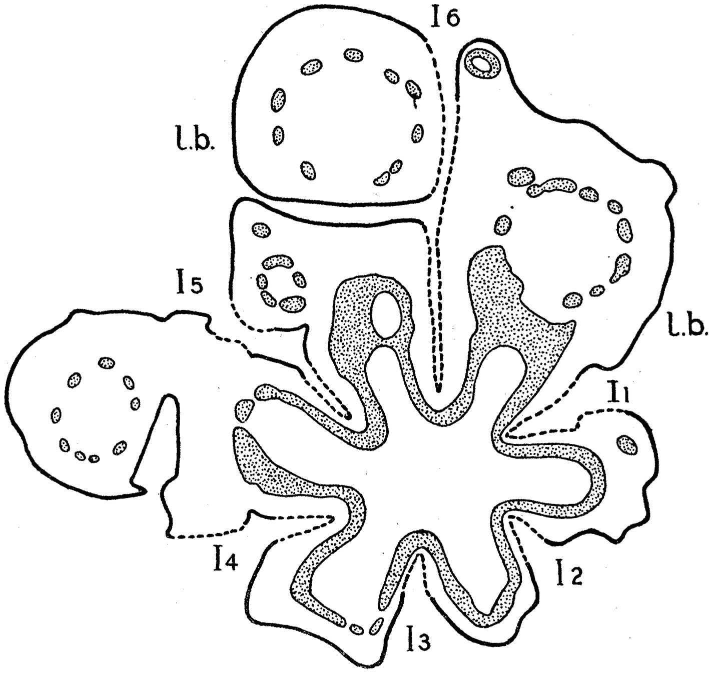
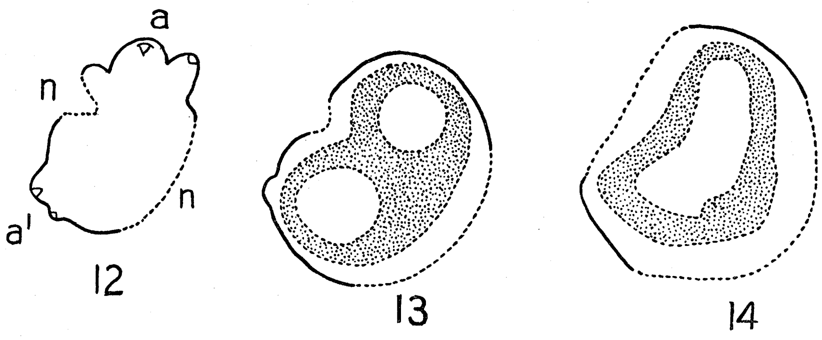


FIGURE 11. Transverse section near the apex of a shoot which received six radial incisions (I_1, I_2, etc.), the apex being left intact. The outline of the incipient vascular tissue (stippled) is parallel to that of the shoot. *l.b.* leaf-base, with leaf-trace. (× 14.)

In one specimen, in which the incisions were made within the margin of the meristem, the apical cell was damaged. This isolated meristem grew on and was fixed after 33 days. The terminal region showed what appeared to be an incipient dichotomous development, some necrosed tissue—the crushed remains of the damaged apical cell—lying in the angle between the two shanks. In other words, it appears that two approximately opposite regions of the apical meristem had developed as bud primordia. The configuration of the incipient vascular tissue at different levels is shown in figures 13, 14. Comparable data for another specimen in which the apical cell was damaged are illustrated in figures 83–85, plate 11. In this specimen the apical meristem had been isolated on a narrow rectangular panel of tissue. The solenostelic vascular systems (figure 83, plate 11) relating to the two buds could be traced downwards to the region of their conjunction (figure 84, plate 11) and beyond.

The incipient vascular tissue in isolated terminal regions eventually becomes differentiated into the normal stelar components (figures 80, 81, plate 10; figure 84, plate 11). In the incised region the endodermis, like the surrounding parenchyma, is derived from incipient vascular tissue. The endodermis may thus be considered to occupy a position of interaction between the stele and the cortex. Its position parallel to the periphery of the shoot also suggests that its formation may be related to critical partial pressures of carbon dioxide or oxygen, or both, in the tissue. The data show that the position of the endodermis and pericycle is not necessarily determined by a particular system of cell cleavages in the apical region. The histological details of the initial and subsequent differentiation of the vascular tissues will be considered in a later paper.



FIGURES 12–14. Pseudo-dichotomous condition observed in transverse sections of an isolated terminal region in which the apical meristem had been destroyed. In figure 12, the two new apices (a, a^1) are shown; *n*, necrosed tissue in region of damaged apical cell. Figure 13 shows the two fused solenosteles (stippled) relating to the two new apices. Figure 14 shows the single solenostele lower down. (×35.)

(*d*) *Arrangement of new leaf primordia*

In *Dryopteris* the incipient leaf-gaps in the shoot stele can be seen in the growing region below the apical meristem. Thus, while the last two, three or four leaf primordia to be formed show no gaps, small gaps relating to the next older group of primordia are present. Hence, in specimens where the vertical incisions are made outside the basiscopic margin of the apical meristem, incipient leaf-gaps may be cut through. During the subsequent growth of the isolated meristem, these incipient leaf-gaps undergo further development: hence the somewhat irregular distribution of vascular tissue seen in figures 5 and 6, the parenchymatous gaps being, in fact, the acropetal extension of gaps which were in the course of formation at the time of dissection. These gaps, therefore, indicate the positions of leaf primordia round the shoot apex prior to the growth of the isolated meristem.

Two questions relating to leaf arrangement may be considered here: (i) does the phyllotaxis of an isolated terminal region differ from that of a normal apex? and (ii) is the phyllotaxis in continuity with the arrangement in the parental shoot? In the preliminary experiments described here, the dissections were not made with the specific purpose of exploring the problems of phyllotaxis as were those of Snow & Snow (1931), though this aspect is now receiving attention. In a number of specimens, however, it was observed (*a*) that although the isolated terminal region is considerably reduced in cross-sectional area as compared with the parent shoot, the phyllotaxis is apparently identical with that of the normal shoot, i.e. there is a leaf divergence of 5/13; and (*b*) that the newly formed primordia are in continuity with the spiral arrangement of primordia in the original untreated shoot.

4. Observations on partially isolated leaf primordia

In the ferns as a class the leaves, or megaphylls, are large in proportion to the shoot on which they are borne. Indeed, it has been held that the leaves dominate the axis and, in some species, this view is apparently supported by the details of vascular anatomy. In phytonic as contrasted with axial theories of the leaf-shoot type of organization, it may even be denied that the shoot has any real existence apart from the leaves; that it is, in fact, no more than a structure derived from the fusion of decurrent leaf-bases. Although the concept of phytons has usually only a subjective significance, some investigators (e.g. Campbell 1921) have given it an objective meaning. Elsewhere, the writer (Wardlaw 1944 *b*) has shown that if all the leaf primordia are systematically removed from the shoot, the latter nevertheless continues its axial growth and develops a solenostelic vascular system. By means of the present procedure the development of a single leaf primordium, isolated on a plug or panel of medullary parenchyma, can be investigated.

In *Dryopteris*, each leaf primordium originates as a single superficial cell of the apical meristem; the further development of the leaf proceeds from the successive cleavages of this cell which thus functions as an apical cell. Young leaves are typically incurved and rolled as a result of the slower rate of growth of the adaxial tissues. As the relative development of leaf to shoot, the size and shape of the leaf during development, and its vascular anatomy, are among the characters by which a species is distinguished, the importance of experimental investigations of morphogenetic processes in leaves will be evident.

Bower (1935) has suggested, on the basis of comparative studies, that the fern megaphyll may be of cladode origin; in other words, that it has originated during the course of descent from an organization consisting of a bifurcating shoot, one shank of the dichotomy having acquired the dorsiventral symmetry and large photosynthetic expanse typical of the modern fern frond. From the standpoint of causal morphology it is relevant to inquire to what extent the form, structure and symmetry of the individual leaf are determined by the position of the primordium on the shoot; and how such features as the disrupted leaf-trace, which has been used as a criterion of comparison in phyletic studies (Bower 1923), can be interpreted in terms of the process of growth. These are problems of great complexity and, at best, only incomplete solutions are likely to be forthcoming.

Figure 15 illustrates diagrammatically the relation of a leaf primordium to the apical meristem and to the incipient vascular tissue of the shoot. The positions of the vertical incisions by which the meristem, leaf primordium and lateral segments are isolated are indicated. The isolated leaf primordium thus occupies the distal region of a plug of medullary parenchyma and immediately overlies a sheet of incipient vascular tissue. If now, after a period of growth, this material is sectioned transversely in acropetal sequence from the level AA^1 to the level CC^1, sections of characteristic appearance will be obtained. Near the base of the plug (AA^1) the section will consist entirely of medullary parenchyma. At BB^1 some vascular tissue will be present in the outer region of the section. On proceeding upwards, the vascular tissue will gradually spread across the section, till eventually, at the level of CC^1, vascular tissue will occupy almost the whole cross-sectional area. Above this level the configuration of the vascular system of the isolated leaf primordium will be disclosed. It has already been seen (§ 3 (*c*)) that when incipient vascular tissue is exposed to the atmosphere as a result of incision, the outermost cells undergo a parenchymatous development and a newly constituted endodermis closes the damaged stele. Hence, after growth, it may be anticipated that a section taken at the level CC^1 will show vascular tissue surrounded by 'cortical' parenchyma. Figures 16–24 show the arrangement of the vascular tissue at different levels in acropetal sequence in an isolated segment bearing a leaf primordium with an axillary bud. This experimental material had been kept in culture for several weeks. Below the level of figure 16 the entire cross-sectional area consists of parenchyma. In figure 16, two meristeles are present, the space between them being part of a foliar gap of a leaf borne lower down on the shoot. These meristeles converge at a slightly higher level and then the vascular tissue, in which xylem and phloem have been differentiated, is seen to spread across the section from the outer periphery towards the centre of the parent shoot as already described. Just at the base of the leaf primordium the vascular distribution is as shown in figure 18: no foliar gap connecting cortex and pith is present as in the normal development. Figure 86, plate 11, from a younger isolated primordium, shows undifferentiated vascular tissue occupying the greater part of the cross-sectional area at the base of a leaf primordium. This section is taken just about the level where the vascular tissues of the leaf primordium become confluent with those of the shoot. No leaf-gap is present. At a slightly higher level, i.e. in the basal region of the primordium, a small central pith makes its appearance (figure 87, plate 11). On proceeding upwards the pith enlarges and the vascular system of the isolated primordium is seen

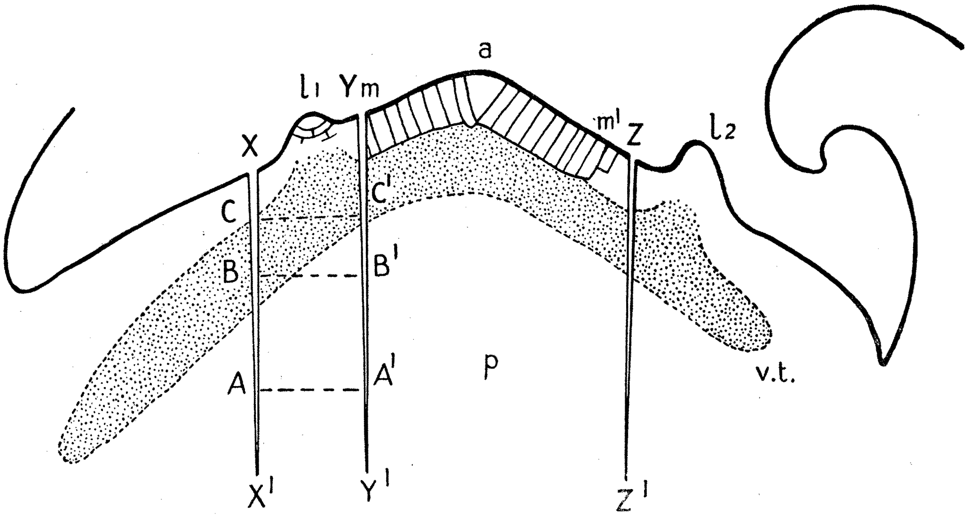
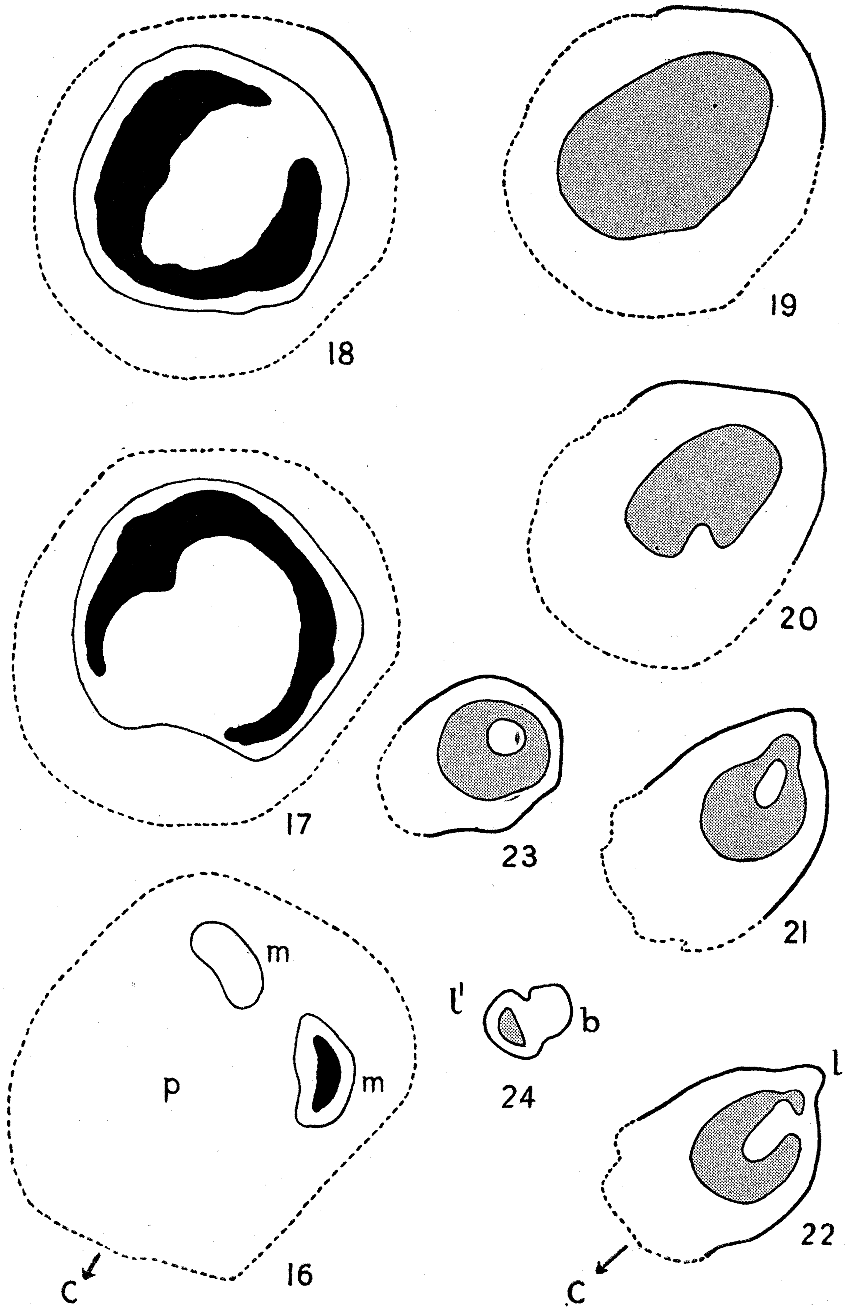


FIGURE 15. Longitudinal median section of the shoot apex of *D. aristata* (diagrammatic) showing the position of the longitudinal incisions XX^1, YY^1, ZZ^1, by which the leaf primordium (l^1) and the apical meristem (m–m^1) are isolated laterally. *a*, apical cell; *v.t.* incipient vascular tissue; *p*, pith; l_2, another primordium. Both the leaf primordium and the apical meristem occupy distal positions on plugs of medullary parenchyma. AA^1, BB^1, CC^1, levels of typical transverse sections discussed in the text.

to be solenostelic (figures 88, 89, plate 11). Such isolated leaf primordia undergo very little growth either in length or in girth, and at a slightly higher level than that shown in figure 89, plate 11, the leaf apex is cut through in an approximately longitudinal direction (figure 90, plate 11). But whereas in the normal development a leaf primordium at this stage of development would be curved in towards the centre of the parent shoot, in this experimental material it is directed outwards, i.e. away from the centre of the parent shoot,



FIGURES 16–24. *D. aristata* transverse sections, in acropetal sequence, of an isolated leaf primordium with an axillary bud (× 40). *m*, meristele; *p*, medullary parenchyma; *l*, near leaf apex; *b*, apex of axillary bud; l^1, leaf of axillary bud; *c*, direction of centre of parent shoot. Figures 16–18: xylem, solid black; phloem, etc., white. Figures 19–24, incipient vascular tissue, stippled.

as in a fully expanded old leaf. The disposition of the leaf apex, the solenostelic vascular system, the limited growth development, and the absence of foliar gaps have been consistent features of the isolated primordia so far examined.

Isolated leaf primordia typically develop axillary buds. These can be observed at an early stage under a binocular microscope. Figures 19 and 20 indicate the distribution of incipient vascular tissue at the base of a leaf primordium and figure 21 its solenostelic vascular system. In figure 22, higher up, the outwardly directed terminal region of the leaf is indicated. Above the level of figure 22 it will be seen (figure 23) that a solenostelic vascular system is present, this being the stele of the axillary bud. Figure 24, near the bud apex, shows the first leaf of the bud.

Mention may also be made of the fact that in other experiments in which a leaf primordium was isolated on the same panel of tissue as the apical meristem, the subsequent development of the leaf was very limited and compared closely with those described above. By contrast, the leaves which developed in the isolated terminal region and in lateral buds were of relatively large size.

5. The growth of the isolated lateral segments

Not only do isolated meristems continue to grow but the lateral segments (which are separated from each other and from the central meristem by the intersecting incisions) also undergo further growth during which new and hitherto unrecorded morphological developments take place. These departures from the normal development, which must be related to the new conditions introduced by the experimental procedure adopted, will now be described.

In figure 2 an apex of *D. aristata*, in which the apical meristem has been isolated by vertical incisions from the adjacent tissues, is shown in longitudinal median section. It may be noted that although the diameter of the shoot is approaching its adult size at the level indicated by the line A–A^1 the vascular tissue is still in the initial phase of differentiation (Wardlaw 1945*a*), i.e. the differentiation of phloem and xylem, etc., has not yet begun. Nearer the apex, the incipient vascular tissue has been severed by the incisions. Provided the more distal tissue does not become necrosed, the whole of its course of growth and differentiation, together with such further changes as may be induced by the development of lateral buds, lies before it. In the normal shoot the incipient vascular tissue, which consists of a continuous, unbroken sheet of small-celled tissue situated below the apical meristem, becomes expanded into an open meshwork, or dictyostele, in relation to the progressive basipetal enlargement of the leaf-gaps (Wardlaw 1944*a*, 1945*a*).

In the soft, plastic, growing region some tissues may be subject to compression as a result of the more rapid growth of tissues of either side, while tensile stresses may be developed in a slowly growing tissue by the more rapid growth of an adjacent tissue. The effect of the compressions and stresses sustained during growth will be reflected in the adult tissue pattern. Now, in a longitudinally incised shoot apex the growth and differentiation of the lateral segments must take place under mechanical conditions which are very different to those which obtain in the normal shoot. The analysis of such differences in internal structure as may result should contribute towards the elucidation of morphogenetic processes. The experimental materials may also indicate potentialities of the specific reaction system other than those revealed during normal development. The new and unusual features described here relate to the development of lateral buds and of the vascular system of the main shoot.

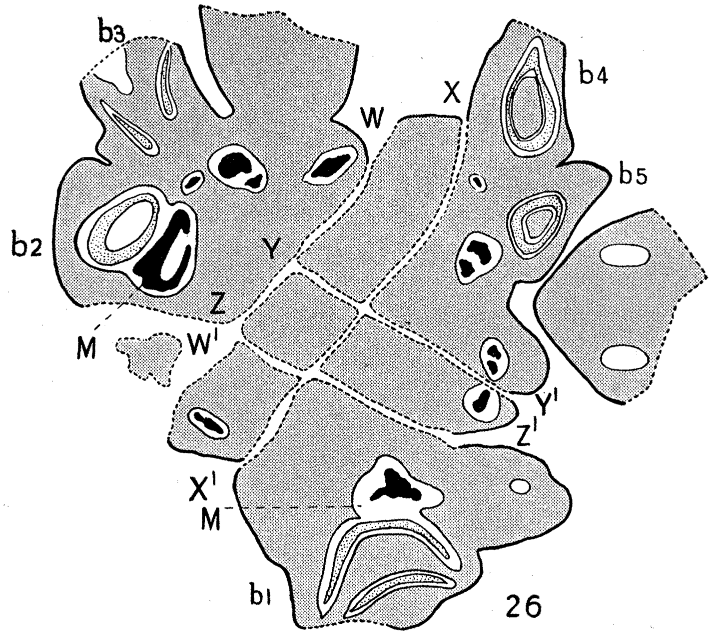
(*a*) *Development of buds*

Buds normally occur in association with a leaf-base, usually in a lateral position, or on the free surface of the shoot. These buds are axillary in origin but undergo considerable displacement during the growth of the shoot and leaf-bases (Wardlaw 1943 *b*). The bud stele as seen in the cortex of the parent shoot is typically small and protostelic and becomes confluent with the shoot stele in the region of conjunction of two meristeles (figure 25). There is evidence that the initial differentiation of the bud stele takes place basipetally, i.e. from the apex of the bud backwards towards the shoot stele (Wardlaw 1943 *b*, 1945 *c*).

In the materials under consideration the development of large solenostelic buds has been a conspicuous feature. Figure 26 shows an incised shoot in which five bud steles can be seen at the same level. These steles, in contrast to the small compact protostele illustrated in figure 25, consist of well-developed medullated protosteles or of solenosteles with a conspicuous pith. In serial sections each of these buds can be traced throughout its length from the point of its confluence with the shoot stele below to its distal region. Thus in figure 26 (and figure 93, plate 12) bud b_1 is seen near its point of emergence from the shoot, two leaf-gaps being present. Lower down the structural arrangements shown in figures 27–30 can be observed, the xylem of the bud stele becoming incompletely confluent with that of the shoot stele in the region of conjunction of two meristeles (figure 29). Figure 27 (and figure 94, plate 12) at a lower level, show a strand of bud xylem lying alongside the xylem of the conjoined meristeles, and in figure 28, still lower down, the bud stele is beginning to fade out. Observations such as these, which have been repeated in many different buds, confirm an earlier finding that where bud development is induced some distance away from the shoot apex, the junction of bud stele and shoot stele tends to be incomplete or may not be effected at all (Wardlaw 1943 *a*, *b*). It is difficult to see how such facts can be explained other than in terms of a basipetal initial differentiation of vascular tissue in the sense defined by Wardlaw (1944 *a*).

During the normal development, the bud stele may become conjoined not only with the shoot stele but also with a strand of the leaf-trace. Buds of *D. filix-mas*, though axillary in origin (Wardlaw 1943 *b*), are typically associated with the leaf-traces only. Figure 31 (and figure 91, plate 12) show a large solenostelic bud in longitudinal median section: its stele is conjoined both with a meristele and a leaf-trace, but in neither instance is there a confluence of the xylem strands. This can be ascertained by following the serial sections upwards and downwards. Figure 32 (and figure 92, plate 12) illustrate the relation of bud stele to meristele lower down in the shoot where the former is beginning to fade out. These facts again support the hypothesis of a basipetal development of bud vascular tissue. In figures 29 and 31 xylem of pericyclic origin can be seen.

Further observations on bud development and the relation of bud stele to shoot stele may now be considered. Figure 33 shows a transverse section of a shoot at a level where the incisions have almost disappeared. In the top right-hand corner a leaf-base with its numerous leaf-trace strands is shown; this level is also the region of conjunction of two



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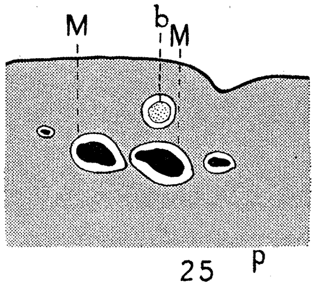

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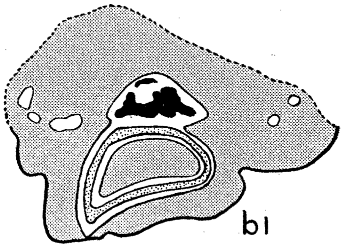

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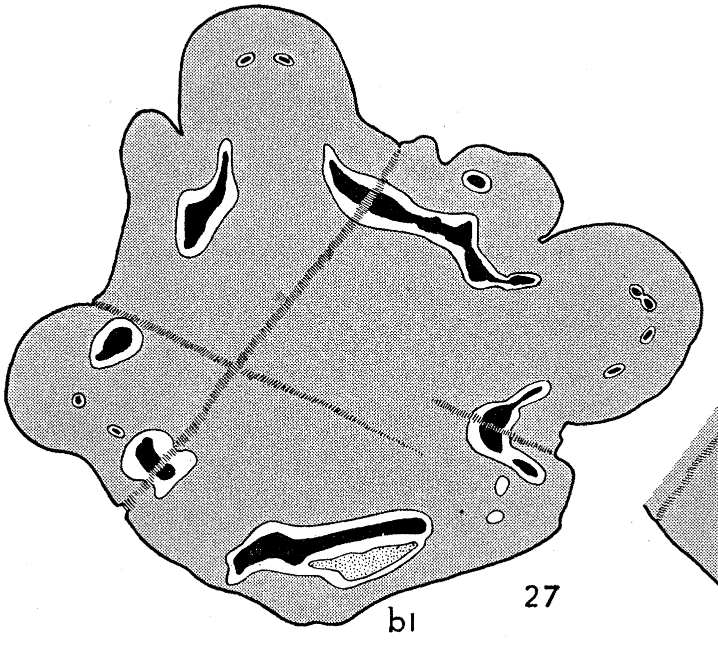

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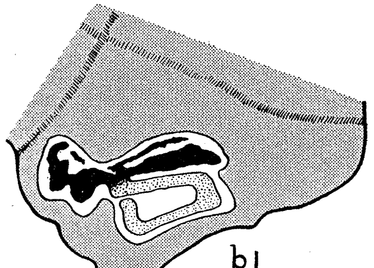

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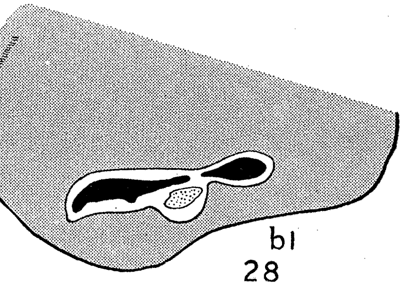

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FIGURE 25. Part of a normal shoot in transverse section showing the protostele of a bud (*b*) about to become confluent with two converging meristeles (*m*). *p*, pith.

FIGURE 26. Transverse section of a longitudinally incised shoot showing the incisions ww^1, xx^1, yy^1, zz^1. The solenosteles of five buds (*b* 1, *b* 2, etc.) are present. *m*, meristele; meristele xylem, solid black; bud xylem, stippled.

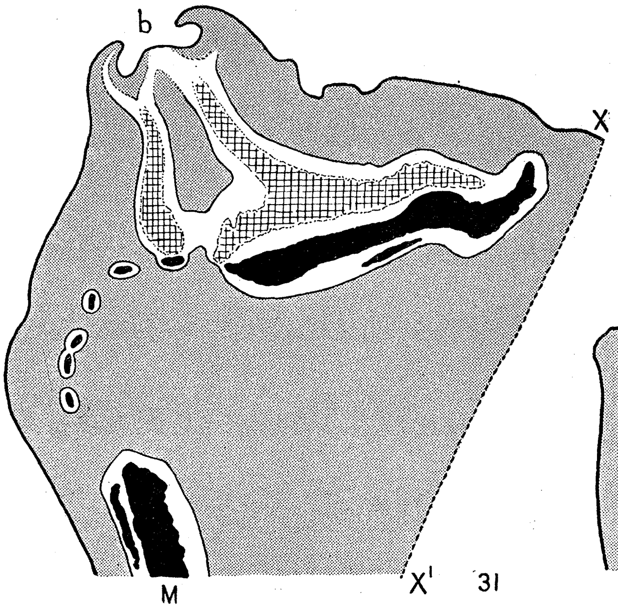
FIGURE 27. The same shoot, lower down. The incisions have almost disappeared. The xylem of bud (*b* 1) runs parallel to the meristele xylem but does not fuse with it.

FIGURE 28. The same shoot, still lower down. The bud stele has almost faded out.

FIGURE 29. The same shoot, above the level of figure 27. The bud protostele has become large and medullated. Bud and meristele xylem are conjoined at one point.

FIGURE 30. The same shoot, higher up than figure 29 but lower down than figure 26. The large bud stele lies on the outer face of the meristele. (All × 14.)

meristeles whereby the 'closing' is effected of the gap of the aforementioned leaf. Two masses of bud xylem, one with a small parenchymatous central region, are associated with the conjoined meristele. If, now, this lateral segment of the shoot is followed upwards, the relationship of the bud xylem to the shoot xylem, as illustrated in figures 33–37, can be observed. At the level of the leaf axil the vascular tissue, comprising bud and meristele components, has to accommodate itself to a restricted space and consists of a flattened solenostele (figures 34, 35). Higher up, the vascular complex, within the limits defined by the two incisions, undergoes further changes: the shoot stele separates into two strands (figures 34–36) which gradually fade out as the severed distal region is approached. These two strands form part of the solenostele, i.e. they have, in effect, been incorporated into the bud stele. At the level of figure 36 the meristele xylem can still be distinguished from the bud xylem by differences in the size of the tracheides and in their orientation, but eventually, about the level of the first bud leaves (figure 37), the meristele xylem fades out. It may be noted here that the bud occupies an axillary position; that the outline of its stele in transverse section is in close conformity with the outline of the isolated segment; and that where the incision at xx^1 (figure 33) has passed through a meristele, the two severed parts, *MA* and MA^1, have become closed by a newly constituted endodermis (see below).



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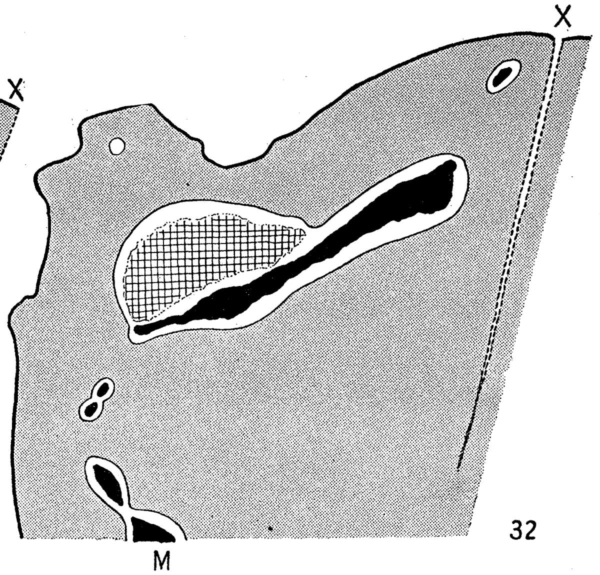

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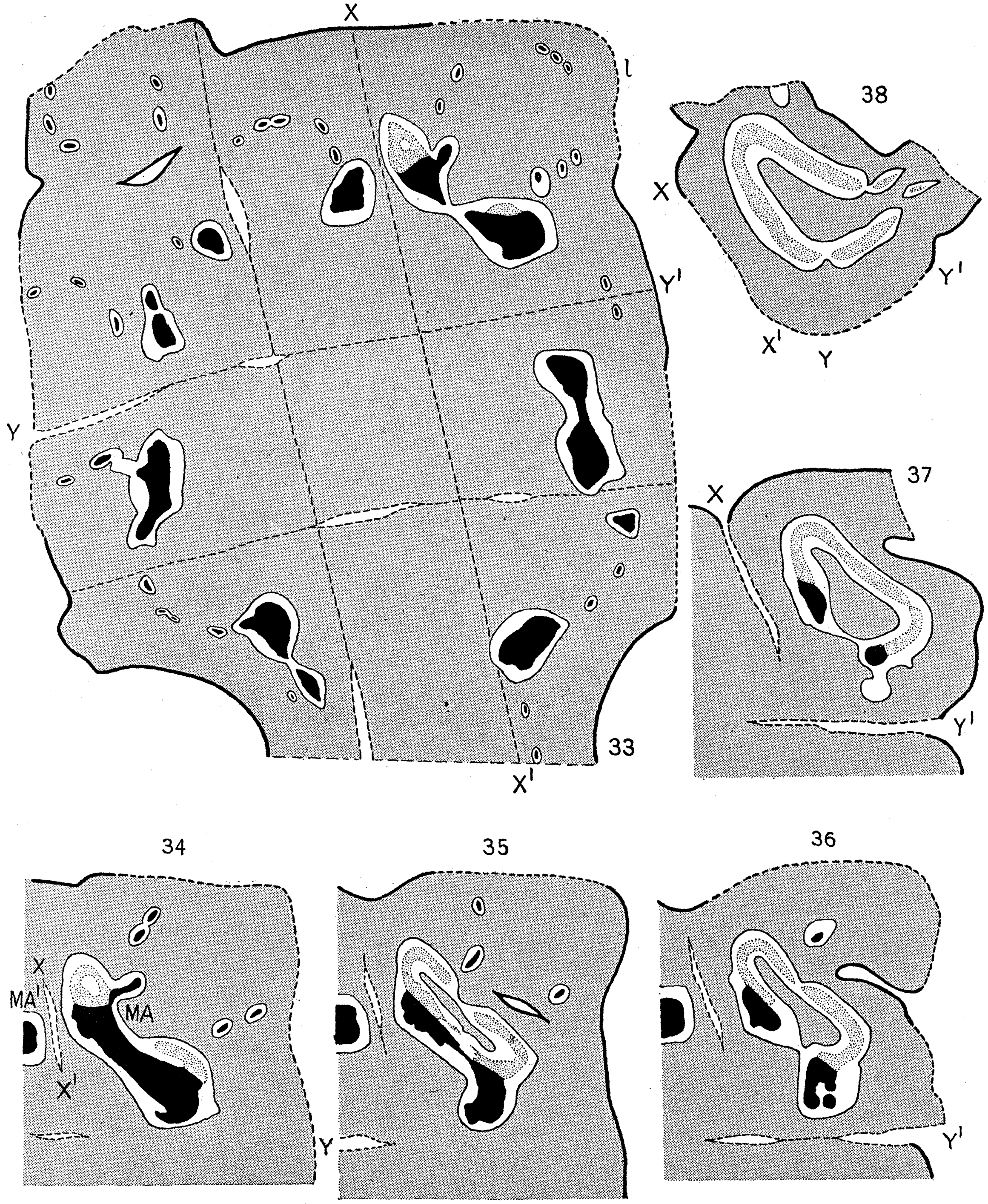
FIGURE 31. Transverse section of a shoot showing a large solenostelic bud (*b*) in longitudinal section. The bud xylem (cross-hatched) does not fuse with that of the meristele (black). Part of the bud stele is conjoined with one strand of the leaf-trace (on left) as well as with the meristele. The broken marginal line xx^1 represents the position of an incision. *m*, meristele.

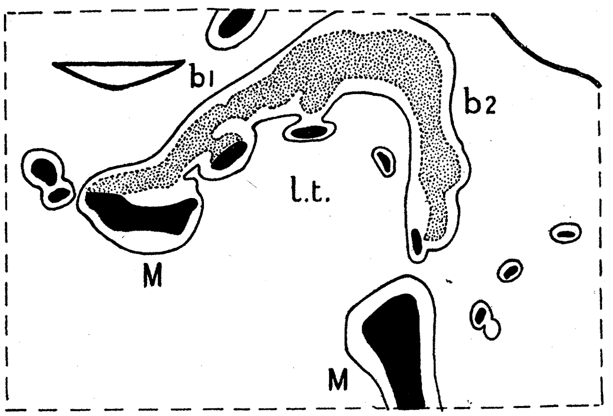
FIGURE 32. The same, lower down. The bud stele is fading out without becoming fully confluent with the meristele, e.g. there is no xylem-to-xylem connexion. (×26.)

The coalescence of developing bud steles with acropetally attenuating meristeles is a general feature of these experimental materials. Bud steles may also coalesce with each other, such data affording a clear indication of the diversity of morphogenetic activity inherent in the developing region. An example of the fusion of bud steles is given in figure 39



FIGURES 33–38. Transverse sections of an incised shoot showing the relation of a bud stele to a meristele at different levels. Meristele xylem, solid black; bud xylem, stippled. Broken marginal lines indicate incisions (xx^1, yy^1). The bud solenostele, with one leaf-trace, shown in figure 38, becomes confluent with the shoot stele and is seen at its lowest point in figure 33: two small strands of bud xylem, one with a central parenchymatous region (on left), can be seen. The vascular strands of the leaf (*l*), in the axil of which this bud has developed, are seen in section. The meristele tissue fades out acropetally, figures 34–37, and is replaced by bud tissue. MA, MA^1, a meristele longitudinally divided by the incision, xx^1. (× 14.)

(and figure 82, plate 10): the very extensive development of vascular tissue in the leaf-base, consisting of a broad curved ribbon encircling the leaf-trace strands and becoming incompletely conjoined with some of them, relates to two buds. Lower down the mass of vascular tissue diminishes greatly, till finally (figure 40) it can be seen that two bud steles are in fact involved: that on the left, belonging to the younger and larger bud, becomes confluent with the shoot stele at the level of the section illustrated in figure 40; the other, a small, non-medullated protostele typical of the normal development, becomes confluent with the shoot stele at a point of meristele conjunction lower down.



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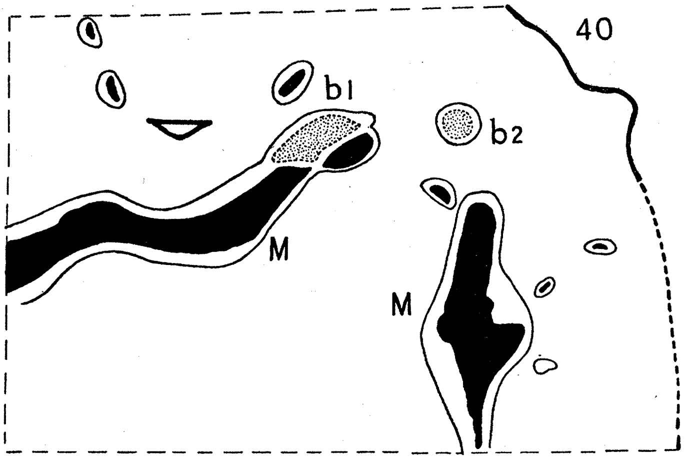

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FIGURE 39. Transverse section of part of an incised shoot showing the extensive development of two fused bud steles (*b* 1, *b* 2). Bud xylem, stippled; meristele xylem, black. *m*, meristele; *l.t.* strands of a leaf-trace.

FIGURE 40. The same shoot, lower down: the two separate bud steles (*b* 1, *b* 2) can now be seen. (× 14.)

(*b*) *Abnormal development of meristeles*

The normal shoot stele of *D. aristata* consists of an open meshwork or dictyostele. In other words, a diamond-shaped parenchymatous gap is present at the insertion of the trace or vascular strands of each leaf-base on the vascular cylinder of the shoot. The individual, strap-like vascular strands of the shoot stele which thus appear to separate and coalesce in an orderly pattern over the length of the shoot are known as meristeles. At the 'closing' of any leaf-gap, as seen in a series of acropetal transverse sections of the shoot, two meristeles will appear to converge and fuse margin to margin, giving a broad meristele. As this conjoined meristele is followed upwards its width decreases and it is seen to be a component of the meshwork of a leaf-gap obliquely above. It may also separate into two strands again. As the shoot apex is approached the meshes of the dictyostele become progressively smaller till finally, in the immediate vicinity of the apical meristem, they are not present at all.

In the experimental procedure adopted the stelar meshwork may be cut in different patterns in different isolated lateral segments, and this may affect the final structural development. Figure 41 shows part of a dictyostele as seen from the outside, and indicates the way in which the meristeles may be severed by the vertical incisions. At the exposed cut surface of an undifferentiated meristele the outermost tissue becomes parenchymatous. A thin layer of parenchyma, the outermost cells of which become corky, is thus interposed between the vascular tissue and the atmosphere. The new outer limit of the vascular strand consists of a layer of endodermis which did not form part of the original organization (see below). If, now, transverse sections are made of a lateral segment containing vascular tissue as illustrated in figure 41, the series read from the level AA^1 to BB^1 will show first a single broad ribbon, then a bifurcation of that ribbon, and finally the fading out of both ribbons, at slightly different levels. And the series read from CC^1 to DD^1 will show first no vascular tissue, then the presence of a small vascular strand at one side, then two vascular strands which coalesce and separate again higher up. In actual specimens not only are the more fully developed meshes severed, but the very small meshes just below the apex are also dissected. Particular interest attaches to the further development of the latter. It may be anticipated that the mechanical interaction of one developing tissue or another, for example, will be radically changed in these isolated segments.

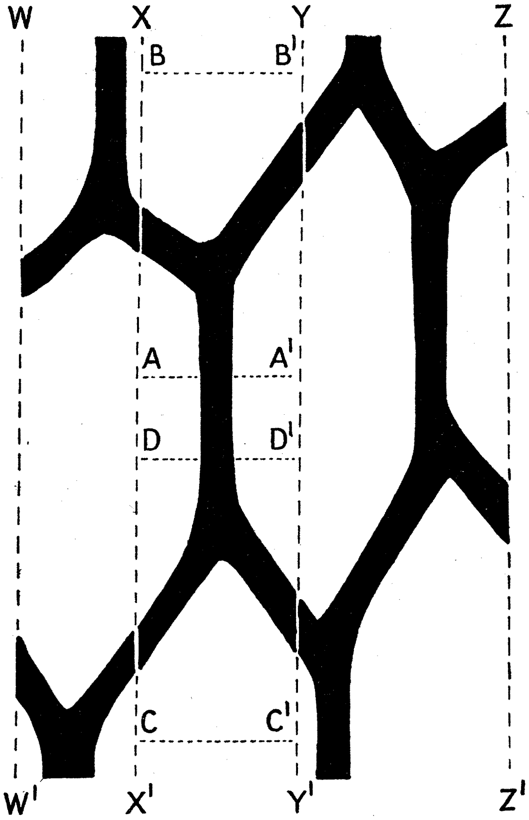
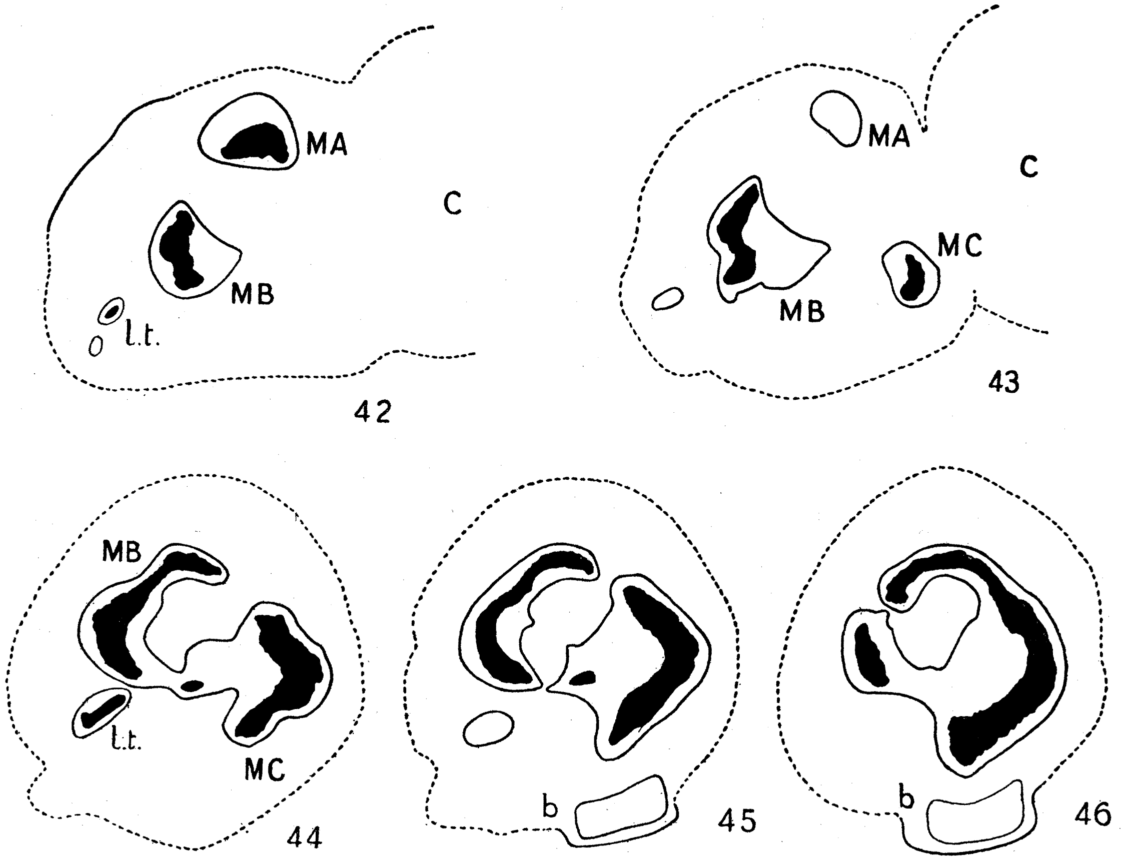


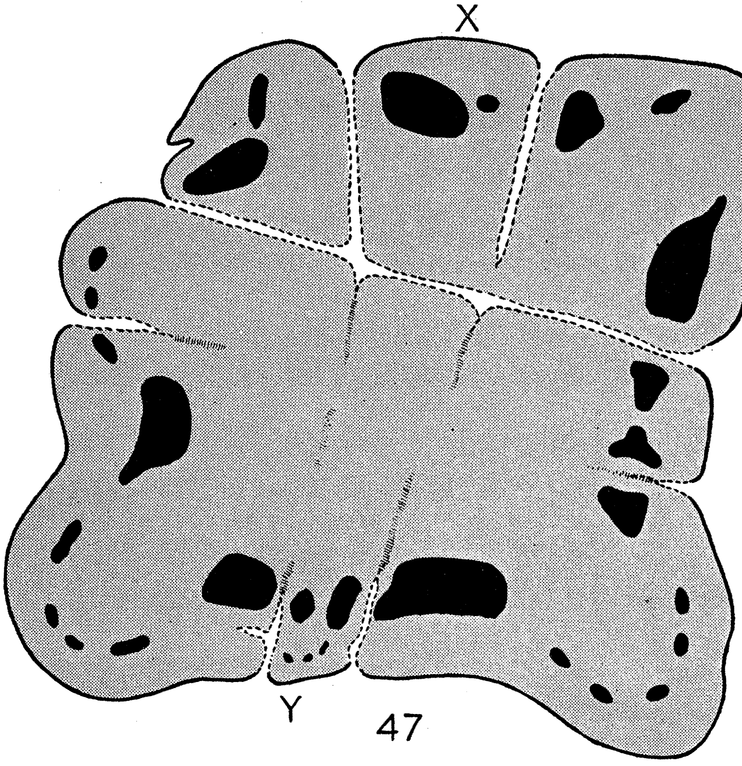
FIGURE 41. Diagrammatic representation of part of a dictyostele as seen from the outside. ww^1, xx^1, yy^1, and zz^1 indicate the positions of longitudinal incisions. (See text.)

In figures 42–46 transverse sections in acropetal sequence of a lateral segment are illustrated diagrammatically, incisions being indicated by broken lines. The two meristeles, *MA* and *MB*, shown in figure 42, are diverging in the upward direction. In figure 43 meristele *MA* is fading out, having been cut through, but another meristele, *MC*, is now present in the segment. *MB* and *MC* are, in fact, converging meristeles. They do not, however, occupy positions parallel to the epidermis, as in the normal shoot, but are parallel to the new perimeter defined by the incisions and thus occupy an approximately symmetrical position in the cross-sectional area. Towards the distal end meristeles *MB* and *MC* are seen to be conjoined and the vascular system is recognizably solenostelic (figures 45, 46). As already indicated (Wardlaw 1944*b*, 1945*a*) the meristeles do not actually fuse as an upward reading of the data would appear to suggest, the meristelic condition being itself due to the disruption of a sheet of vascular tissue initially continuous. In the material under consideration the sheet of incipient vascular tissue in the isolated segment is eventually of an approximately cylindrical shape as a result of the growth of the surrounding parenchyma. In these instances the tendency is for a figure of equilibrium to be produced. Near the upper limit of the segment the vascular tissue faded out.



FIGURES 42–46. Transverse sections, in acropetal sequence, of an isolated lateral segment. The initial positions of the meristeles (*MA*, *MB*, *MC*) have been modified during growth; in the distal region, figure 46, they have been moulded into an approximately solenostelic configuration. Meristele *MA* is fading out at the level of figure 43, whereas meristele *MC* has entered the segment between the levels of figures 42 and 43. Xylem, black; *b*, bud; *l.t.* leaf-trace strand. *C*, central segment of shoot. Broken perimeter lines indicate incisions. (× 14.)

That the configuration of the incipient vascular tissue in lateral segments tends to be affected by the pressure of developing parenchyma has been indicated by many observations. In some instances in which large solenostelic buds have become associated with severed meristeles, it has been difficult to determine where the meristeles end and where the bud stele begins. Figure 47 shows a dictyostelic shoot in transverse section near the lower limits of the several incisions. In figure 48 the same shoot is seen higher up: the central mass, terminated by the shoot meristem proper, is surrounded by the several lateral segments. A solenostele is present not only in the isolated terminal region, as previously demonstrated (§ 3 (*c*)), but also in most of the lateral segments. Figures 48–52 show the arrangement, in acropetal sequence, of the vascular tissue in segment *X* from a level slightly above that of figure 47: two converging meristeles are bent inwards to form a crescentic stele and eventually a solenostele is formed. This solenostelic development is due to the fusion of bud vascular tissue with the curved meristele. Each of the other lateral



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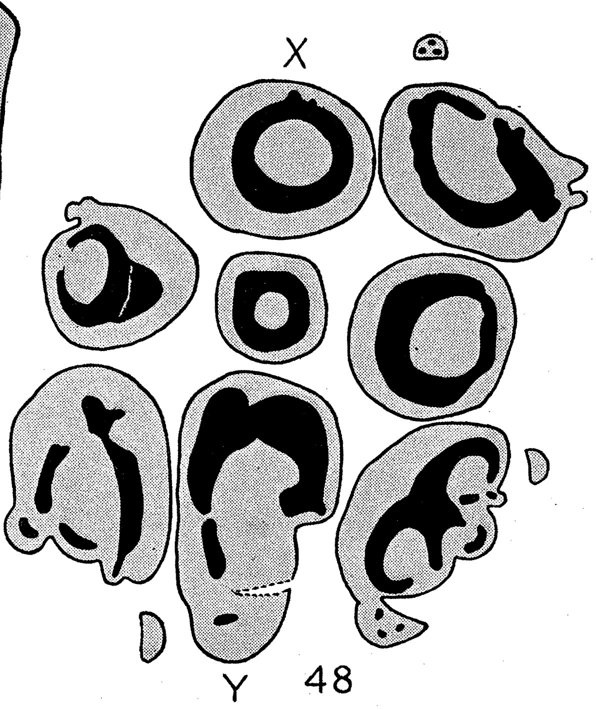

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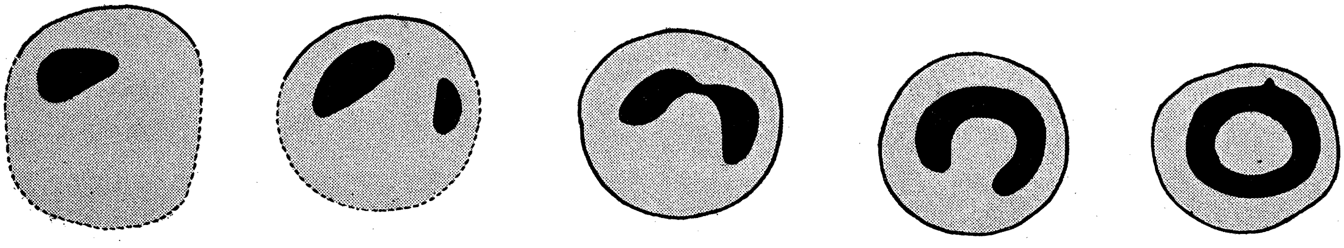

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FIGURE 47. Transverse section of an incised shoot near base of incisions, showing the normal dictyostelic vascular system. Meristeles, black. Broken perimeter lines indicate incisions.

FIGURE 48. The same shoot higher up, showing the isolated terminal region in the centre and the several lateral segments. All the segments at this or adjacent levels show solenostelic vascular systems. *X* and *Y* correspond to the segments X and Y respectively in figure 47.

FIGURES 49–53. Sections of segment *X* in acropetal sequence from above the level of figure 47. Two meristeles enter the segment, become conjoined, are moulded to conform with the shape of the segment perimeter, become confluent with the vascular tissue of a bud situated distally, and become part of a solenostelic structure. (All × 18.)

segments in figure 47 has yielded similar data. In each segment the developing bud stele becomes completely confluent with the severed meristeles. But whereas, in the instance cited (figures 47–53), the meristeles are curved inwards, in some other instances they are curved outwards. But in all, the developing vascular tissue eventually lies parallel to the perimeter of the segment, the final disposition being one of symmetry and equilibrium.

(*c*) *Medullated meristeles*

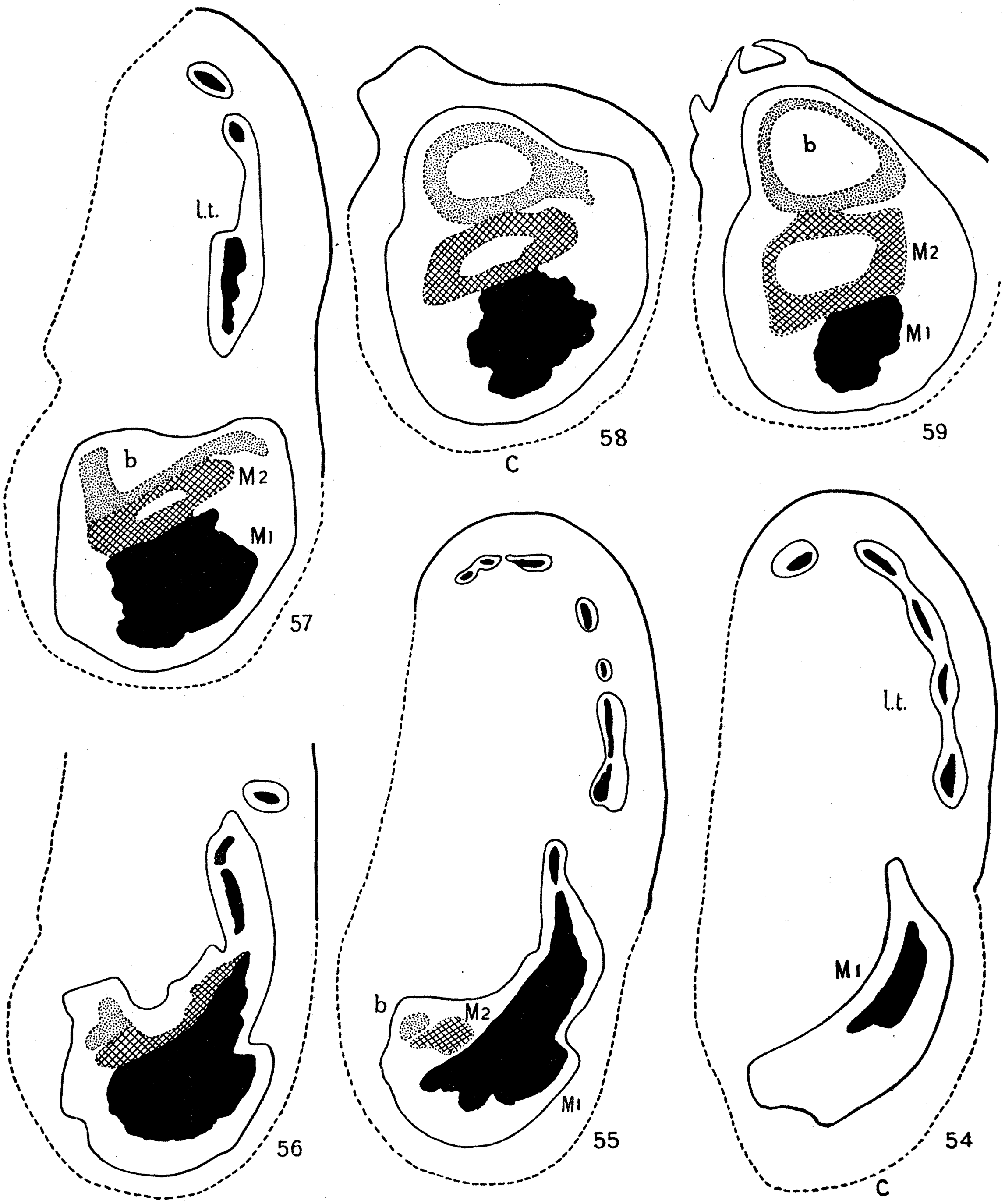
Some stelar developments of an unusual nature and, so far as the writer is aware, of a type hitherto unrecorded, may now be described. In one of the incised shoots the vascular tissue in a lateral segment was found to comprise two upwardly converging meristeles and a solenostele relating to a bud. The meristeles are seen to occupy abnormal positions, one lying external to the other on approximately the same radial plane. At a low level in the segment the xylem strands of the two meristeles lie side by side in contact (figures 54–56). Above this level the xylem of the outer meristele has enlarged, become elliptical in outline and has developed a small-celled, parenchymatous pith (figure 57). Higher up, this meristele is recognizably a medullated monostele (figures 58, 59) and can readily be distinguished from the inner meristele because of the slightly different orientation of the tracheides (figures 101, 102, plate 13). At these levels the bud stele is a solenostele. Towards the distal end of the segment the bud shoot becomes more conspicuous and the meristele complex fades out. Other instances of medullated meristeles have also been observed. Figure 97, plate 12, shows an isolated lateral segment in which one meristele of a converging pair of meristeles has become solenostelic; on the outer side a bud solenostele is present. In figure 108, plate 14 and figure 103, plate 13, medullation within the xylem layer of a shoot solenostele is illustrated, this stele having been produced experimentally by the systematic suppression of all leaf primordia (Wardlaw 1944*b*). Near a small leaf-gap (on the left) the xylem layer has developed a bulge (figure 108, plate 14). This is due to the development of an *intra-xylem* pith, internal endodermis and phloem (figure 103, plate 13), these tissues having no connexion with the outer tissues of the stele.

These observations indicate that the phenomenon of medullation, to be seen in the normal ontogenetic development of solenostelic and dictyostelic ferns, is not necessarily confined to the stele as a whole but, under certain conditions, may be exemplified by individual components of the stele.

(*d*) *Polycyclic steles*

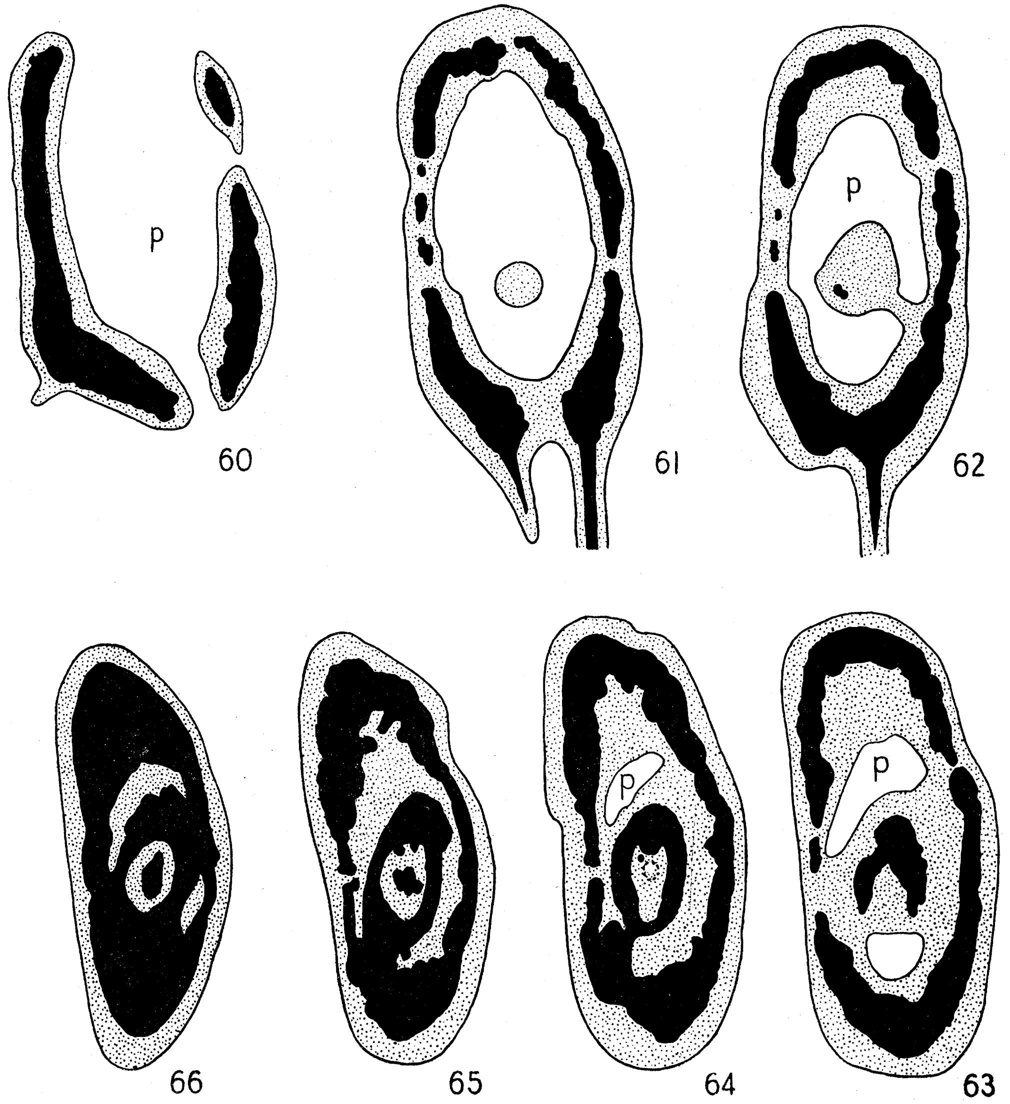
In three instances buds with a polycyclic vascular system have been observed in the course of these studies. Such materials afford further evidence of the morphogenetic 'plasticity' and potentiality for development of the growing region. A polycyclic stele may be defined as one in which more than one cylinder of vascular tissue is present, the several cylinders being concentric. In some ferns, e.g. *Matonia pectinata*, polycycly is present as a normal feature in plants of large size. In the present instance polycyclic steles were found in buds developing on isolated lateral segments.

Figures 60–71 illustrate the appearance of a bud stele at different levels in basipetal sequence. This bud had developed on one of the outermost lateral segments of a shoot which had been subjected to a considerable number of intersecting incisions, i.e. leaf primordia of different ages as well as the apical meristem had been isolated by vertical knife cuts. Below the apex the leafy terminal region of the bud is typically dictyostelic (figure 60), but lower down it is solenostelic and shows in addition a small, approximately central or medullary strand of vascular tissue (figure 61). On proceeding downwards this central strand enlarges and a few tracheides are seen to be present in it (figure 62). Below



FIGURES 54–59. Transverse sections in acropetal sequence of an isolated lateral segment, showing the medullation of a meristele. *l.t.* strands of a leaf-trace; xylem of bud (*b*) stippled; xylem of medullated meristele (M_2) cross-hatched; xylem of inner meristele (M_1), black; *c*, direction of centre of shoot. (See text.) (×40.)

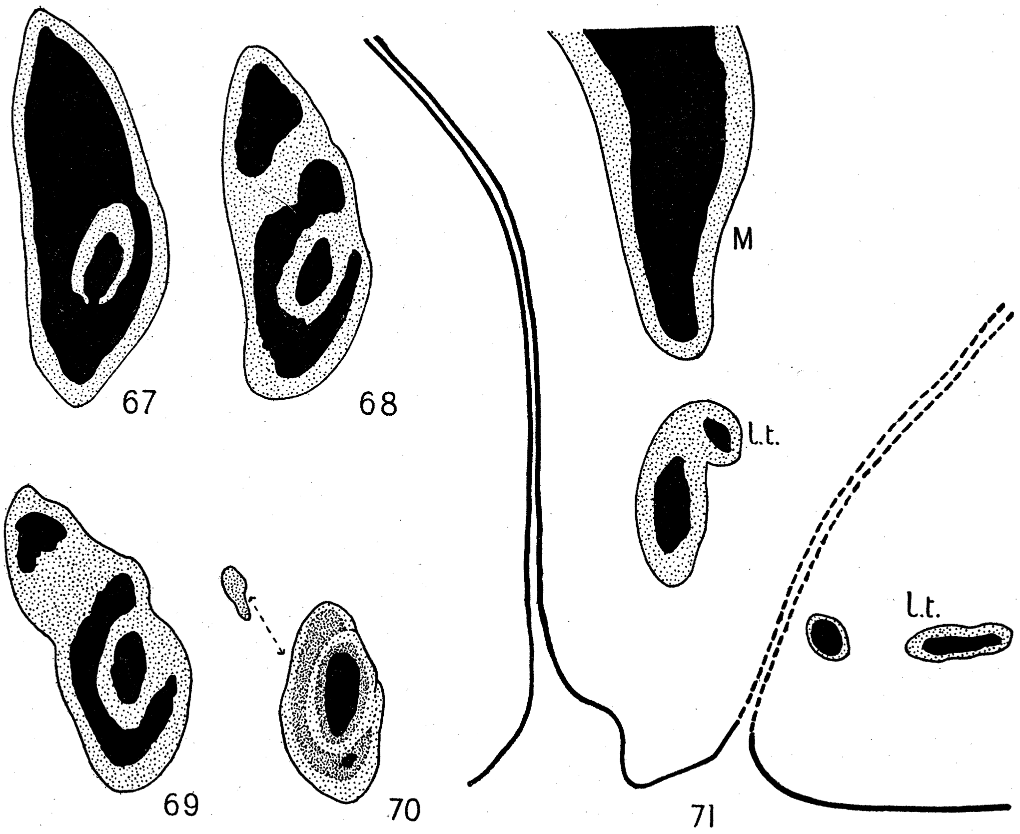
this level there is a central horseshoe-shaped mass of xylem surrounded by phloem (figure 63) and then a more or less complete inner solenostele (figure 64). The inner stele encroaches on the space which would otherwise be occupied by the pith of the outer cylinder (figures 95, 96, plate 12). Below the level of figure 64, the central pith, consisting of large parenchymatous cells, disappears: the stele now consists of two vascular rings and a central strand of xylem surrounded by phloem and small-celled vascular parenchyma. The cross-sectional area of the polycyclic stele is now seen to be diminishing basipetally: the two xylem rings are no longer completely separated and the central phloem and small-celled parenchyma are disappearing, but the central xylem strand is now more conspicuous (figures 66, 67). Finally, in the region where the small bud stele *appears* to become conjoined with a leaf-trace strand and eventually with a shoot meristele, the outermost vascular



FIGURES 60–71. Transverse sections, in basipetal sequence, of an isolated lateral segment, showing a polycyclic bud stele. *p*, pith; *M*, meristele; *l.t.* leaf-trace strands; broken lines (figure 71) indicate an incision; xylem, black; phloem, etc., stippled. (See text.) (× 35.)

tissues fade out (figures 68–70) leaving only the central xylem strand and its surrounding tissues. Figure 70 shows the central strand of tracheides, its surrounding phloem and vascular parenchyma and, on the left, a residual crescentic mass of vascular tissue; further out, there is an isolated mass of vascular tissue. Figure 71 shows the position of an incision: it will be seen that the bud had developed in a relatively restricted space.

In the present state of knowledge no adequate explanation of these structural developments is possible. Some factors in the situation may, however, be considered. In the first place, it should be mentioned that the inner strand of the polycyclic system is not a true component of the bud stele: it is, in fact, the stele of a root which has effected a more or less longitudinal penetration of the basal region of the bud stele. This root disappears about the level of the sections illustrated in figures 64 and 65 and figure 95, plate 12. The bud tissue, in this as in many other instances, is thus seen to end blindly in the outer cortex (figures 69, 70), and, apart from the presence of the penetrating root, is not conjoined with the meristele of the parent shoot. Space does not admit of a full account being given of the penetration of buds by roots, but in these and related experimental studies of *D. aristata* and *D. filix-mas* the phenomenon has been frequently observed. Briefly it may be said that the root nature has been ascertained (i) by the relation which the penetrative vascular strand bears to the meristele and (ii) by the observation of undeniable root apices actually penetrating buds. (Evidence is available in the writer's collection of slides and photographs.)



FIGURES 67–71. For legend see previous page.

Figure 72, also taken from the experimental shoot described above, illustrates a second example of polycyclic development. In this bud two cylinders of xylem are present, the bud stele being associated with an enlarged leaf-trace strand. The inner cylinder of xylem is of brief longitudinal extent: it first appears a little below and disappears a little above the level of figure 72.

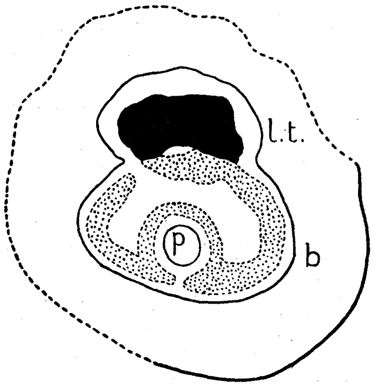
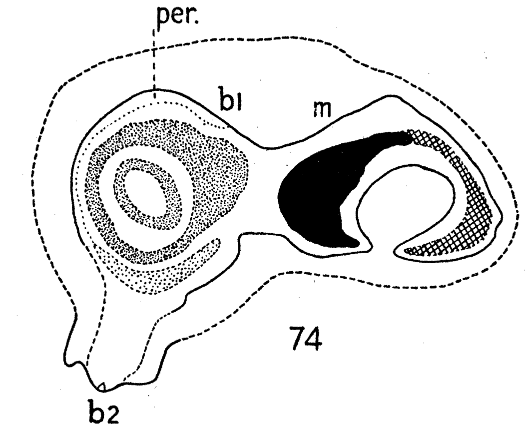
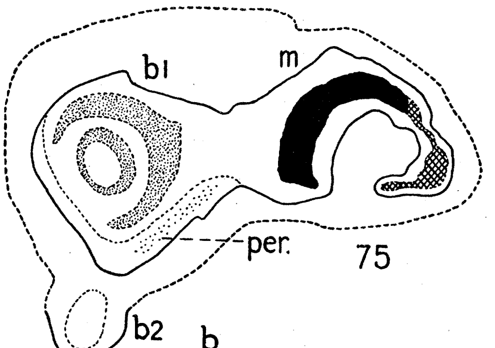


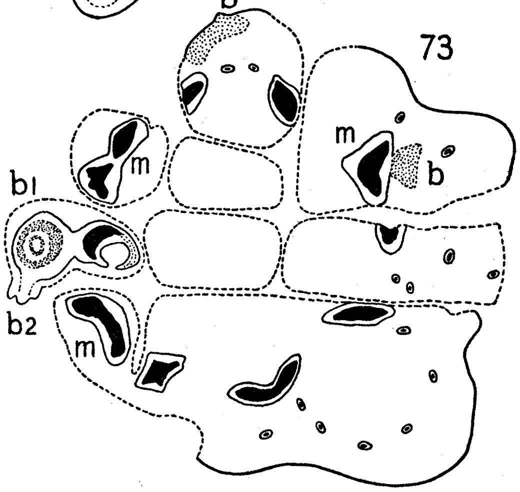
FIGURE 72. Transverse section of an isolated lateral segment showing polycyclic structure in a bud stele (*b*). This bud is associated with a modified strand of a leaf-trace (*l.t.*). Bud xylem, stippled; leaf-trace xylem, black; *p*, pith; broken perimeter lines indicate incisions. (× 27.)

The third instance of polycyclic development is of particular interest in that the inner vascular cylinder has been formed as a result of a cambium-like activity in the pith of the outer cylinder. Figure 73 shows an incised shoot in transverse section; the polycyclic bud occurs in one of the outermost lateral segments (left-hand side). In this instance the apex of the bud (b^1) had been damaged at some stage in the experiment. As a result, a bud of the second order had been induced to develop on the shoot of the first bud. Figure 74 (and figure 98, plate 13) illustrate the structure as seen in a transverse section of the isolated segment and of the first bud (b^1) at the level where the secondary bud (b^2) is cut in approximately longitudinal median section. It will be seen (i) that the associated shoot meristele is of almost solenostelic configuration, (ii) that the vascular system of the primary bud (b^1) is solenostelic, (iii) that there has been a conspicuous development of pericyclic tissue, (iv) that the vascular tissue of the secondary bud is confluent with the outer tissue of the primary bud, and (v) that a second vascular cylinder is present within the solenostele of the primary bud. Sections above the level of figure 74 show a gradual fading out of both vascular cylinders as the damaged apical region is approached. Figures 75 and 76 illustrate the structural organization at two successive levels below that of figure 74: the inner cylinder first of all disappears, and then the outer cylinder diminishes as it becomes incompletely conjoined with the shoot meristele. The maximum development of the inner cylinder thus takes place in the immediate vicinity of the secondary bud.

The state of differentiation of the tissues of the outer and inner cylinder indicates clearly that the latter is of later origin. Figures 99 and 100, plate 13, illustrate the structure and mode of inception of the inner cylinder. It has, in fact, been developed from parenchymatous cells which initially constituted the medulla or pith of the solenostele of the primary bud. Typical parenchymatous cells can still be seen in the centre of the inner cylinder, but in the peripheral region of the pith growth and division of the parenchymatous cells, mainly by tangential walls, have taken place and a cambium-like tissue has been formed (figures 99, 100, plate 13). The outermost cells of the radial cambiform rows have enlarged and are in the course of developing into tracheides. As a result, a layer of incipient xylem thus abuts on the inner phloem of the original solenostele. There are also indications that some of the intermediate or innermost cells of the cambiform rows may be developing into sieve-tubes. The inner cylinder of vascular tissue has thus resulted from a process of cell division and differentiation comparable with the formation of secondary tissues by cambial activity.







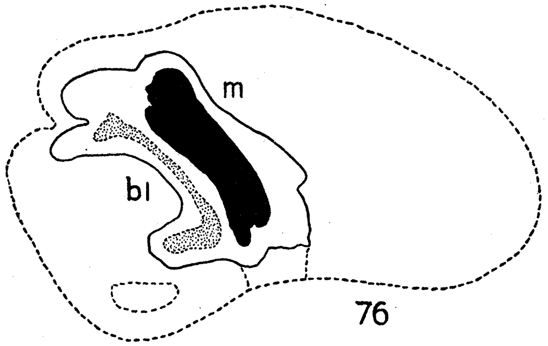


FIGURE 73. Transverse section of an incised shoot showing a polycyclic bud stele (*b* 1) and a lateral bud (*b* 2); *M*, meristeles, with xylem in black; *b*, buds. (×7.)

FIGURES 74–76. A segment of the same shoot, more highly magnified, at different levels. Figure 74 shows the approximately solenostelic meristele (*M*) with which bud *b* 1 is associated. The apex of this bud has been damaged and bud *b* 2 has developed. An inner ring of vascular tissue has been formed in the pith of *b* 1, a cambium-like activity being involved. Figure 75 and figure 76, successively lower down than figure 74, show the bud tissue fading out basipetally, no xylem-to-xylem connexion with the meristele having been effected. *per*, enlarged pericycle in which tracheides become differentiated; incipient meristele xylem, cross-hatched; incipient bud xylem, stippled. (×21.)

(*e*) *Histological observations*

A marked thickening of the pericyclic layer, due to an increase in the number of cells, is characteristic of meristeles in the experimental materials described here. The cells may be arranged in radial cambiform rows (figure 109, plate 14), or a less regular arrangement of the dividing cells may be observed. Individual short tracheides may develop in the pericycle (figure 110, plate 14); in some instances pericyclic xylem may be extensively developed and may be connected with the main xylem ribbon.

In earlier papers (Wardlaw 1944*b*, 1945*a*) it has been shown that when incipient vascular tissue is subjected to tensile stress, as during the formation of a leaf-gap, it becomes parenchymatous instead of developing into xylem, phloem, etc. In the present materials it has occasionally happened that a meristele has been partly split in the longitudinal plane. In such a meristele the following regions may be recognized in basipetal sequence: (*a*) the upper split region, (*b*) the region immediately below which has not been entered by the knife but which has been subjected to stress in relation to the thrusting apart of the two strips of the stelar ribbon, and (*c*) the unaffected region lower down. Data relating to such a situation are illustrated in figures 104–107, plate 14. These are based on serial sections from above downwards. In figure 104, plate 14, the split meristele is seen at a level just below the lower limit of the knife-cut; a secondarily constituted endodermis has been formed, from tissue initially and potentially vascular, along the adjacent sides of the two parts. The region between the meristele parts, M, M^1, is occupied by parenchyma. The mass of xylem, B, directed outwards relates to a bud of which the principal region of insertion is higher up. A little below this level the meristele parts converge and are enclosed in a common endodermis (figure 105, plate 14); within the meristele the two strips of xylem are separated by a region of parenchyma enclosed by endodermis, i.e. an intra-meristelic endodermis and pith are present. Some internal phloem is also present. Lower down the meristelic pith diminishes and finally disappears and the xylem becomes a coherent ribbon. At the levels illustrated in figures 106, 107, plate 14, some parenchymatous tissue can still be seen intermingled with the tracheides. Such evidence supports the view that incipient vascular tissue, when subjected to tensile stress, tends to be transformed into parenchyma.

6. Discussion

(*a*) *The isolated terminal region*

It has been demonstrated that the apical meristem of *Dryopteris aristata*, on being isolated laterally from the adjacent tissues by longitudinal incisions, is capable of further growth. The treatment has the effect of severing the incipient vascular tissue which leads up to the meristem from the sources of nutrition below. On *a priori* grounds this undifferentiated vascular tissue, which is longitudinally continuous with the fully differentiated vascular tissue below, might be considered to be the path of translocation of nutrients to the meristem. As isolated meristems show an approximately normal rate of growth, with the formation of many new leaf primordia in the course of a few weeks, and as the nutrients which sustain this growth must diffuse through a region of non-vasculated medullary parenchyma, it may be inferred that the incipient vascular tissue is not specifically the path of translocation of nutrients to the meristem.

Since the nature and mode of distribution of nutrients to the growing region seem likely to be important factors in morphogenetic processes, the problem under consideration is one which deserves further attention. With modifications the experimental method used here affords a means of obtaining data relating to the quantitative aspect; this is now being investigated.

In relation to the experimental procedure, metabolic and mechanical factors are modified and important morphological changes result. The growing region, in fact, shows considerable diversity in its morphogenetic activity. This is true of the lateral segments as well as of the isolated meristem. During normal growth at the apex, pressures and tensions are developed between the several enlarging tissue systems. As Priestley (1928) pointed out, the slowly growing incipient vascular tissue tends to be compressed between the more rapidly enlarging parenchymatous cells of the pith and cortex. Moreover, the existence of different rates of growth in adjacent tissues results in the development of tensile stresses in the more slowly growing tissue (Wardlaw 1944*b*, 1945*a*). The present studies have yielded evidence of the importance of this mechanical factor in morphogenesis. If the configuration of the incipient vascular tissue is, at least in part, determined by the pressure exerted on it by the rapidly growing medullary and cortical parenchyma, then it might be anticipated that, in the lower part of an isolated terminal region of square outline, the vascular tissue would also have a square outline; and so on for terminal regions of triangular outline, etc. This has been frequently demonstrated in these studies. But in such specimens, the 'cortical' parenchyma is derived from peripheral elements of the incipient vascular tissue exposed to the air by the downward incisions. A fundamental question therefore relates to the differentiation of this parenchyma.

In attempting to interpret the varied configuration of fern steles in terms of causal factors the first essential is to acquire an adequate knowledge of the facts of development in the growing region. Very little, indeed, is known of the factors which are causally related to the initial differentiation of vascular tissue, the absence of such knowledge being a serious obstacle to further progress. Lang (1915) suggested that among these factors may be included (i) functional stimuli, (ii) the inductive influence of the older differentiated parts on the developing region, or (iii) formative stimuli of unknown nature proceeding from the developing region. The relation of functional stimuli to morphogenetic processes is a problem of very great difficulty: it may be that no such direct relationship does, in fact, exist. In the normal development the second factor suggested by Lang—the inductive influence of older, preformed parts—may well be of considerable importance. This view, which has had many adherents, may now be considered in the light of the data given here. In the experimental materials the vascular tissue leading to the apical meristem from below is completely severed. Nevertheless, the thin layer of incipient vascular tissue which lies immediately below the apical meristem undergoes further development and becomes fully differentiated into phloem, xylem, etc. New vascular tissue is also developed in the isolated terminal region, and a solenostelic and dictyostelic vascular system is eventually developed. In short, vascular tissues are formed notwithstanding the fact that there is no organic connexion with the mature vascular tissue below, i.e. the influence of the older preformed tissues has been precluded. Such observations and the configuration of the pseudo-dichotomous specimens which have been described and illustrated give strong support to Lang's third suggestion, and to the present writer's hypothesis (Wardlaw 1944*a*), that in the pteridophytes the initial differentiation of vascular tissue is related to the downward diffusion of an unspecified substance (or substances) from the actively growing apical meristem.

Views relating to the fundamental nature of the shoot system are broadly speaking of two kinds, phytonic and axial (or strobilar) (Bower 1935; Wetmore 1943). In the former, the existence of the shoot or axis as an independent member is more or less explicitly denied, the plant being envisaged as a construction of phytons or segments of which the leaf-bases, or extensions thereof, unite to form the shoot. Although in some instances such theories—which usually but not invariably, e.g. Campbell (1921), have only a subjective significance—may appear to have a limited application or may actually possess value for descriptive purposes, they impress one as being artificial and divorced from the facts of physiology and embryology. The present observations, in which the further growth of an isolated apical meristem has yielded in the first instance a vasculated axis without leaves, give further grounds for rejecting phytonic theories of shoot construction. On the other hand, those who have supported axial theories—in which the shoot is accepted as a phyletically pre-existing axis or stem from which the leaves have subsequently developed—may be criticized on the grounds that their views are non-physiological in character. Moreover, they have taken for granted the existence of a morphological 'unit of construction', e.g. the axis, spindle, or leafy-shoot of Sachs, whereas the fundamental problem, as it appears to the writer, is to show how the axial type of construction, which is common to all vascular plants, can be related to the process of growth. When reduced to its simplest terms, an axis begins as a single embryonic cell, or as a superficial layer of meristematic tissue, in contact with a source of nutrition (Wardlaw 1945*c*). This, in effect, is the situation which is produced by the experimental technique adopted here; it is also typical of the detached meristems or bud rudiments in certain ferns (Wardlaw 1943*a*, *b*). Such meristems, when allowed to grow under suitable conditions, yield vasculated axes, and each axis acquires its characteristic external configuration and internal pattern during the process of growth.

(*b*) *Isolated leaf primordia*

A notable feature of these experiments—which are of a preliminary and exploratory nature—has been the very limited growth undergone by partially isolated leaf primordia. This is in marked contrast to the normal development. The incisions, which were made as close to the young primordium as possible, had the effect of curtailing the transverse area across which nutrients could move to the developing organ. Nevertheless, reduction in the size of the leaf-base seems scarcely adequate to account for the very restricted growth of the isolated primordium. It has been seen that under comparable conditions an isolated meristem may make considerable growth. The hypothesis that activating substances proceeding from the apical meristem are necessary for the growth of the leaf primordia—as distinct from their inception—would appear to be ruled out by the fact that leaf primordia isolated on the same panel as the apical meristem also undergo little enlargement. Snow & Snow (1937) have shown that if heteroauxin in lanoline is applied to a part of the stem apex in certain flowering plants, the growth of the existing leaf primordium and bud, or of those which subsequently arise, is promoted. When a leaf primordium is isolated from the apical meristem in *D. aristata* it may be that the restricted development is due to the absence of auxin proceeding from the meristem. In the case of a primordium isolated on the same plug of tissue as the apical meristem, the temporary cessation of apical growth due to the incision of the shoot may perhaps account for the restricted development of the primordium. An alternative hypothesis, that activating substances proceeding from older leaves are necessary for the growth of primordia, still awaits examination. In the normal development, leaf growth is much more rapid on the abaxial than on the adaxial side, i.e. it would appear that nutrient supply to the former is greater than to the latter. In these experiments one effect of the system of incisions is to preclude the upward movement of nutrients by way of the cortical and vascular tissues of the shoot to the primordium. In fact, the supply of nutrients may be held to have become approximately uniform over the whole cross-sectional area of the leaf-base—a condition which would in part account for the subsequent approximately symmetrical development of the isolated primordium. In studies of rye embryos, de Ropp (1945) has shown that excised stem tips, when placed in a culture medium, showed growth of the first leaf only. It was observed, however, that if any isolated stem tip developed a root, 'the entire growing point was stimulated to meristematic activity, and leaves normal in form and size developed'. Such an observation may have some bearing on the behaviour of isolated leaf primordia in *Dryopteris*, in that these are cut off from direct connexion with the roots which usually originate in the region of the leaf base. Such observations prepare the way for further investigations. In particular, the relation between distribution of nutrients and morphogenetic processes merits careful attention. In the view of Goebel (1900, 1908) the leaf primordia of the young sporophyte and those of the adult plant are strictly comparable, the difference in their subsequent development being a matter of nutrition, i.e. the characteristic morphology of juvenile leaves is due to their arrested development.

The base of the isolated leaf primordium is conjoined with a continuous sheet of vascular tissue, more or less differentiated according to the age of the primordium. As this sheet of vascular tissue lies across the path of nutrients diffusing upwards to the primordium from the parenchymatous cells of the pith, the possibility that it actually impedes the movement of nutrients cannot at present be excluded.

The poor development of isolated leaf primordia is further exemplified by the small pith which is present in its vascular system. The writer (Wardlaw 1945*a*) has shown that in ferns the active growth of a shoot, tuber or leaf-base is reflected in a more or less conspicuous enlargement of the medullary region, and conversely, in so-called 'starvation' experiments, that a marked reduction in the size of the pith is the rule. It has also been shown experimentally that the development of foliar-gaps in the shoot stele can be referred to the stresses set up by the large medullary development in leaf-bases (Wardlaw 1944*b*). In an isolated leaf primordium the insignificant pith development does not set up a tensile stress in the shoot stele and a foliar gap is not formed. This supports the earlier finding.

At an early stage in the development of an isolated leaf primordium an axillary bud makes its appearance, and tends to become the dominant organ of the isolated segment. This raises the question as to why a bud apex should develop in a situation where a leaf apex is inhibited. These and related observations indicate that a shoot meristem possesses potentialities for development which are not shared by a leaf meristem, but why this should be is still obscure.

In the normal development the vascular tissue of the leaf primordium is at first a minute, solid, approximately cylindrical strand. It soon becomes sickle-shaped or crescentic as seen in cross-section, the curvature being directed inwards towards the shoot. With increase in size, and the evident dorsiventral development of the primordium as a whole, the crescent of incipient vascular tissue also enlarges but fails to keep pace with the adjacent parenchymatous development, particularly in the tangential direction. As a result the curved sheet of vascular tissue, or leaf-trace, is subjected to tensile stress and becomes disrupted into separate vascular strands, with rapidly developing panels of parenchyma in between (Wardlaw 1945 *a*). Hence, in cross-section, the adult leaf-base of *D. aristata* characteristically shows 5–8 separate strands disposed in a deep crescent, with a large central pith. One effect of isolating the young leaf primordium has been to eliminate the possibility of the extensive tangential enlargement. The solenostelic vascular system which develops in an isolated leaf primordium is in marked contrast to the normal development and affords further evidence of the diversity of morphogenetic activity of the growing region. The data also indicate to what extent the configuration of the vascular system can be referred to mechanical, nutritional and other factors.

The leaf primordium in *Dryopteris* originates as a single cell of the apical meristem of the shoot and, at an early stage in its development, appears as a small conical papilla of radial symmetry. As development proceeds, this small cone enlarges and occupies a position progressively lower down on the subconical terminal region of the shoot. As a matter of spatial distribution, and having in mind that the rate of growth of tissues decreases as the apex is approached, and is therefore less on the adaxial than on the abaxial side of the leaf, it may be argued that the eventual dorsiventral symmetry of the leaf-base is an inevitable development. It appeared to the writer—as an idea to be tested—that if a leaf primordium could be isolated on its plug of tissue at a very early stage, the development of the characteristic dorsiventral symmetry might be precluded so that the further development of the primordium would be not as a leaf but as a shoot. It has been seen that although the vascular system of the isolated primordium is solenostelic and possesses radial symmetry, the foliar character of the primordium persists. It thus appears that once the apical cell of a leaf primordium has acquired its characteristic shape—and this takes place after one or two divisions of the leaf initial cell—it remains unchanged. In *Dryopteris* the leaf apical cell is two-sided, one of the edges being directed towards the shoot apex. The segments which result from the division of this apical cell thus form two rows right and left, i.e. tangential to the shoot. It is of interest that the experimental investigations described here should lead back to a theme of great interest to morphologists of past decades, namely, the relation between segmentation at the apex and the morphology of the tissues and organs derived from it. It is evident that any adequate account of morphogenetic processes in ferns will require a renewed investigation of the factors which determine the shape and mode of segmentation of apical cells in leaves and shoots.

(*c*) *Isolated lateral segments*

In incised shoots growth and development take place not only in the isolated apical meristem but also in the lateral segments, and, in particular, in the buds to which they give rise.

The external form, as also the internal structure, at any stage during normal development is the result of all the forces operating at that time and may be held to constitute, or to approximate to, a figure of equilibrium (D'Arcy Thompson 1942). Now, in the isolated lateral segments the distribution of metabolic substances and the incidence of mechanical and other factors are radically modified. Accordingly, the subsequent developments are of an unusual kind. The most notable general observation has been that, on further growth, isolated segments eventually return, or show a tendency to return, to the symmetry and internal organization of the normal shoot. During the attendant structural changes a considerable diversity of morphogenetic activity is manifested by the growing region. Evidence of this morphogenetic 'plasticity' has been seen in the development of an isolated segment and its bud as a single unit, and in many aspects of vascular construction, including the solenostelic development of severed meristeles, and solenostelic and polycyclic developments in bud steles.

During the growth of the normal shoot of *D. aristata*, lateral bud primordia and the occasional buds tend to remain inactive and undeveloped, their inception and development taking place either during periods of quiescence in the terminal meristem, or when it has been damaged or removed (Wardlaw 1943 *a*, *b*). The vascular system of such buds, as seen in the cortex of the parent shoot, is typically small and protostelic. From the conspicuous development of buds in the lateral segments, contemporaneously with the growth of the isolated terminal meristem, it may be inferred that the physiological dominance normally exercised by the shoot apex has been eliminated. By contrast, the isolated terminal region is devoid of buds.

Thimann & Skoog (1934) have shown in *Vicia Faba* that auxin, which is responsible for the inhibition of lateral buds—the phenomenon of correlative inhibition—is secreted by the rapidly growing leaves in and near the terminal bud; this substance also promotes the growth of the main shoot down which it passes. The question as to how auxin is causally related to these very different effects has given rise to several theories. These have been discussed and summarized in a valuable series of papers by Snow (1937, 1938, 1939, 1940) and by Ferman (1938). Thus, in the 'direct' theory of Thimann & Skoog (1934) the view is entertained that an excess of auxin is secreted by the terminal bud and that some of this auxin, on passing into the lateral buds, inhibits them by hindering their own production of auxin. Van Overbeek (1938) considers that the auxin in the main shoot finds its way into some conducting cells, e.g. the sieve tubes, in the bases of the lateral buds and in some way 'prevents these cells from functioning so as to supply the buds with substances necessary for growth. In the main stem the auxin is supposed not to obstruct the sieve tubes in this way, because they are much wider' (Snow 1939). In the 'diversion' theory of Went (1936) the correlative inhibition of buds is attributed (*a*) to the upward movement of special substances produced in the roots and cotyledons which are necessary for bud growth, and (*b*) to the downward movement of auxin from the terminal bud. It is held that the latter polarizes the main stem in such a way that nutrients move towards the source of auxin but do not move into the lateral buds, which, in consequence, do not grow. On the basis of a series of experiments Snow has given reasons for rejecting these several theories but for supporting, with qualifications, the views of Laibach (1933) and Loeb (1924). In what has been described as the 'indirect' theory of inhibition it is thought that when auxin moves downwards from its source—the actively growing terminal bud—it promotes the growth of the main shoot. As a result of this growth a second hormone, with the property of inhibiting lateral buds, is produced (Snow 1940). And whereas ordinary auxin, produced at the shoot apex, exercises its influence chiefly in the morphologically downward direction, the bud-inhibiting substance can exert its influence both in the upward and downward directions.

The theory of Ferman (1938) has an important point in common with Went's 'diversion' theory. In both it is held that regions of auxin production and those activated by auxin attract nutrient substances to themselves. In Ferman's view the difference in auxin production in the main apex and in lateral bud apices respectively is ascribed to a difference in their supply of an auxin precursor transported acropetally. The anatomical structure of the very young parts in their earliest stage of development determines which region shall receive most precursor. The region which receives the larger supplies in the initial phase of development will become the seat of greatest auxin production and therefore of growth and will thus tend to attract the major acropetal supplies of nutrients. 'So finally the anatomical structure of the tracks of transport in the young vegetation points (*sic*) determines the distribution of the precursor, predestinates the result of the next competition and therefore must be responsible for the external architecture of plants.' Ferman's account of the inhibition of lateral buds in experiments where heteroauxin is applied to decapitated shoots is less convincing.

The data of the present studies do not give particular support to any of these theories. They do run counter to the views of Van Overbeek in that in *Dryopteris* and certain other ferns (Wardlaw 1943*a*, *b*) the bud primordia may have no vascular connexion with the stele of the main shoot. Nor does the evidence suggest, in terms of the 'diversion' theory of Went, that the supplies of nutrients available locally, as in an isolated lateral segment, are inadequate for bud development. What the evidence does indicate is that, under suitable external conditions, the growth of lateral buds begins as soon as the physiological dominance of the terminal region of the shoot is curtailed, or eliminated, and that this influence is associated primarily with the apical meristem of the shoot rather than with the growing leaf primordia. Thus, in a shoot in which only the apical meristem is damaged but not the leaf primordia, numerous lateral buds are nevertheless developed. Some of Snow's experiments (Snow 1940) show that the movement of the bud-inhibiting substance is not necessarily confined to the vascular strands: it can also move through parenchyma and across protoplasmic discontinuities. In the present experiments the data suggest that the severance of the incipient vascular tissue may be important in excluding the inhibitive effect, but further evidence is required. The views of Ferman may have some application in this connexion.

Not only are lateral buds and bud primordia not inhibited, they show a conspicuous increase in size. This is exemplified by their medullated and solenostelic vascular systems. The enhanced growth may be referred to the increased supply of nutrients which is available in the absence of normal leaf development in the parent shoot.

The greater freedom for expansion in an isolated segment, as compared with the intact shoot, may also admit of the early development of the medullated or solenostelic condition in buds. In many instances the meristeles with which the bud stele becomes conjoined have been completely severed below: hence the nutrients used by the developing bud must be obtained locally from the isolated segment or must reach it from below by diffusing across parenchymatous tissue. It has already been seen that the isolated shoot apex is capable of considerable growth under similar conditions.

An earlier finding (Wardlaw 1943*b*) that *Dryopteris* buds are initially of axillary origin, though liable to be considerably displaced during the growth of the shoot, has been fully confirmed.

Although the growth of buds is the conspicuous feature of isolated segments, the parenchymatous and vascular tissues of the latter also undergo considerable development. In some instances this may be referred to the further development of partly differentiated tissues, but in others the presence of an actively growing bud in close proximity appears to be a factor in the situation, i.e. growth substances proceeding from the bud meristem stimulate further development in the meristeles.

The hypothesis that in pteridophytes the initial differentiation of vascular tissue is referable to the basipetal diffusion of a substance, or substances, produced during the active growth of the apical meristem (Wardlaw 1944*a*) is supported by data obtained in the present series of experiments: indeed, it is not evident how the details of development of the vascular system of the isolated terminal region and of buds can be explained otherwise than in terms of this hypothesis.

The further development of the incipient meristeles in lateral segments takes place under conditions very different to those which obtain in the normal intact shoot and more or less notable departures from the normal development may be observed during their subsequent differentiation. Vascular tissue exposed to the atmosphere by the incisions becomes differentiated as parenchyma and this, together with a newly constituted layer of endodermis, encloses the meristele. During the further development of the circum-stelar parenchyma (some of which is of cortical origin, some of medullary origin and some of vascular origin) the approximately square or rectangular cross-sectional outline of the lateral segment tends to become circular. Concomitantly, in relation to the development of parenchyma, the several strap-like meristeles, which were originally disposed parallel to the epidermis, eventually occupy a central position and together acquire an approximately cylindrical shape. In brief, the co-ordinated structural changes effected during the growth of a lateral segment are such as tend to produce a figure of equilibrium. The several series of illustrations presented here show how varied are the changes undergone by the severed meristeles in the isolated segments. In the distal region of segments in which only a single strap-shaped meristele is present, the meristele becomes differentiated like a protostele: it has a circular outline in transverse section and occupies a central position in a segment which is also of circular outline. In fact, a radially symmetrical structure is produced.

In the older literature the endodermis has usually been held to originate in a specific region of the developing shoot, i.e. either in the inner layer of the periblem or the outer layer of the plerome. It is true that, in the normal development, endodermis occurs with great regularity in a specific position, i.e. just outside the pericycle. Nevertheless, the present observations show that endodermis may be formed from incipient vascular tissue which otherwise would have developed into the several components of the stele. Hence it would appear that endodermis is not necessarily a tissue of fixed morphological origin but rather that it is typically differentiated in a position of interaction between the cortex and the incipient vascular tissue.

The medullation and solenostelic development in meristeles is of interest from several points of view. By the comparative morphologist the solenostele is accepted as being typical of ferns occupying an intermediate position in the phyletic series (Bower 1923). It is also recognized as being a transitional stage in the ontogeny of ferns possessing more elaborate stelar arrangements, i.e. it occurs between the protostelic stage and the dictyostelic stage. According to the Theory of Recapitulation, the historical development of vascular structure in dictyostelic ferns is reflected in the protostelic and solenostelic stages to be observed in the young plant. The vascular system has been regarded by morphologists as being highly conservative in its nature and therefore, of all the criteria of comparison, of greatest value in phyletic studies (Bower 1923). But Bower (1921, 1930) has also indicated that there are other ways of looking at the facts of stelar development, e.g. that the change from the protostelic to the solenostelic condition may be a necessary consequence of increase in size, and that the 'size factor', in conjunction with other factors, is causally related to the further transition from solenostely to dictyostely and polycycly. From such views it becomes evident that if structural complexity can be referred to causal factors, it necessarily loses value in phylesis. Ferns characterized by solenostelic structure, for example, are not necessarily closely related; nor does the dictyostelic condition necessarily indicate a higher evolutionary development than the solenostelic or protostelic conditions.

The evidence presented here shows that a solenostelic vascular system may not only be derived from a protostele, as in the normal development, but that it can also result, under certain conditions, from the further development of an undifferentiated meristele. From this it may be inferred that solenostely should not simply be regarded as an inevitable expression of the specific reaction system (or specific hereditary substance) at a certain stage in the individual development but that it should be referred to the action of nutritional, mechanical and other factors, as well as genetic factors. Notwithstanding the destructive effect of evidence such as that presented here on the findings of comparative morphology, it still remains true that the details of vascular structure in the pteridophytes do afford an important indication of affinity. In the present studies, due attention must therefore be given to the fact that, notwithstanding the several unusual vascular configurations produced as a result of the experimental treatment, there is eventually a return to the typical dictyostelic condition of the normal shoot. Existing knowledge of the differentiation of the vascular system in the ferns is still too inadequate to admit of a harmonizing of the data of the causal and phyletic aspects.

The presence of an actively developing bud in close proximity may play some part in the medullation of a meristele. The evidence is consistent with the view that the undifferentiated meristele has been stimulated to further growth by substances proceeding from the bud. With increase in cross-sectional area the meristele behaves like an enlarging protostele and becomes medullated and eventually solenostelic. The fundamental question thus relates to the causes which underlie the development of medullary parenchyma from incipient vascular tissue. It has already been ascertained that incipient vascular tissue which is subjected to tensile stress tends to develop as parenchyma. Further evidence of this phenomenon has now been presented. If the outer tissues of an enlarging protostele are growing more rapidly than those at the centre, the latter will be subjected to tensile stress and a condition which makes for the development of parenchyma will be established. Some such relationship may account for the initial medullation of solid protosteles. Once the differentiation of a parenchymatous pith is effected it will tend, by reason of its rapid growth, to exert pressure on the more slowly developing vascular tissue. It will also produce a condition of tension in the distal region of the medulla, i.e. at the junction of the young pith cells and the incipient vascular tissue below the apical meristem. The tendency will thus be for a progressive increase in the dimensions of the pith during the individual development. Elsewhere it has been shown to what extent the morphology of the pteridophyte stele, and the cross-sectional pattern of the shoot as a whole, is influenced by medullation (Wardlaw 1945*a*). Tensile stress is, of course, only one factor among many which may be causally related to the parenchymatous development of incipient vascular tissue.

In so far as it is not yet possible to give any adequate account of the development of a solenostele, the elucidation of the several examples of polycycly which have been recorded must be regarded as still more remote. Indeed, their chief interest at the moment lies (i) in the evidence they afford of the potentiality for structural development of the growing region, and (ii) in the fact that polycycly is not exclusively controlled by genetical factors. In *Todea barbara* the writer (Wardlaw 1946) has recorded the presence of a vascular strand in the pith of a shoot which had received experimental treatment. Examples of polycycly are normally seen in the Matoniaceae, Cyatheaceae, Marattiaceae, Dicksoniaceae and Pterideae but not in *Dryopteris*. As Bower (1923, p. 151) remarks: 'The occurrence is so sporadic that it can only have been initiated along a plurality of evolutionary lines.' Tansley (1907) regards polycycly as 'a response to a need for an increased vascular supply'. Bower supports this view and states: 'The need for ready conduction along the axis, as well as to and from the enlarged pith, appears a sufficient explanation of the occurrence of polycycly.' The writer (Wardlaw 1944*a*, *b*) has pointed out, however, that the interpretation of these structural developments in terms of supposed adult functional activities instead of in terms of the process of growth is liable to prove misleading. From the phyletic point of view 'these ferns which in the adult state are structurally nearest to the protostele are held to be relatively primitive in respect of that feature; those which have departed furthest from it are regarded as the most advanced' (Bower 1923, p. 159). Hence ferns with polycyclic steles would be regarded as advanced and derivative types, at least in respect of their vascular structure. But since the polycyclic stelar condition is found in ferns of very different affinity and at very different levels in the phyletic series, as judged by other criteria of comparison, polycycly has been regarded as the result of homoplastic development and of little value in phylesis. In the individual development of ferns with polycyclic steles there are clear indications of the existence of a size-structure correlation, although how this is brought about remains obscure. In the first instance of polycycly recorded here a bud stele of considerable size has undergone differentiation and development in an isolated lateral segment of limited cross-sectional area, and with the added complication that the base of the stele has been penetrated by a root. But to what extent these data are directly relevant to the observed polycycly cannot be decided at present. In the third instance of polycycly, a cambium-like activity in the pith has resulted in the development of an inner vascular ring. There seems little doubt that activating substances proceeding from an adjacent lateral bud provided the stimulus. Such stimulation of parenchymatous cells to growth and differentiation into vascular tissue, in proximity to an actively developing apical meristem, has frequently been observed (Wardlaw 1943*a*, *b*, 1944*a*, 1945*b*). The development of a cylindrical cambium-like layer, consisting of radial rows of dividing cells, is no doubt referable to the system of pressures set up within the cylindrical pith in which growth has been stimulated. In the material under consideration the possible action of a wound hormone, or similar substance, in stimulating the development of the inner vascular cylinder cannot be entirely ruled out. In other specimens, where the destruction of a bud apex has not been involved, but where all leaf primordia have been excised, a very remarkable cambiform enlargement of the meristele pericycle, with subsequent differentiation of tracheides, has been observed. In these instances substances from damaged leaf and bud meristems might also have been involved. Secondary thickening of the vascular system is of rare occurrence among living ferns. It appears in the young plant of several species of *Botrychium*, e.g. *B. Lunaria*, and reaches its most notable development in *B. virginianum* (Bower 1923). In these ferns the secondary zone, which may be extensive, consists of radial rows of tracheides separated by rays of parenchyma. The development in *Dryopteris aristata* described here is thus quite unique among ferns.

In conclusion, the data from these experiments direct attention to the extent to which characteristic structural configurations can be referred to factors which are not intrinsic to the hereditary or genetic constitution. It follows that the more thoroughly the operation of these factors is understood, the more precise and critical will be our selection of criteria of comparison for phyletic purposes.

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EXPLANATION OF PLATES 10–14

(All figures are from untouched photographs)

PLATE 10

FIGURE 77. Longitudinal median section of an isolated apical meristem after 5 weeks' growth. The meristem has grown on into a short axis. Two leaf primordia can be seen at the apex. The vertical incisions, the severed vascular strands, and the basal, non-vasculated parenchymatous tissue of the isolated terminal region are clearly shown. (× 20.)

FIGURE 78. The solenostelic vascular system of an isolated terminal region. (× 25.)

FIGURE 79. A section taken nearer the apex of the same shoot as figure 78, showing the interruption of the solenostele by two leaf-gaps; the small leaf-traces can also be seen. (× 25.)

FIGURES 80, 81. Transverse sections of an isolated terminal region which has given rise to fourteen new leaf primordia in the course of 11 weeks. Figure 80 shows the approximately solenostelic condition in the lower region of the axis and figure 81 the typical dictyostelic condition in the leafy region above. (× 15.)

FIGURE 82. Lateral segment of a shoot in transverse section showing two meristeles (*M*), and the vascular strands of a leaf-trace (*l.t.*). The broad ribbon of vascular tissue (*b*) which surrounds and partly fuses with the strands of the leaf-trace and with a meristele, consists of two fused bud steles. (× 80.)

PLATE 11

FIGURES 83–85. Transverse sections of a rectangular, isolated terminal region in basipetal sequence. The apical cell of this meristem was damaged. In relation to the two diametrically opposite buds which developed, solenostelic vascular systems have been differentiated (figure 83). The coalescence of these solenosteles is illustrated in figure 84, and a stage in the basipetal disappearance of the vascular tissue in figure 85. (× 25.)

FIGURES 86–90. Serial transverse sections, in acropetal sequence, of an isolated leaf primordium after growth. (× 80.)

FIGURE 86. At the base of the leaf primordium and in the region of conjunction with the vascular tissue of the shoot: incipient vascular tissue almost fills the cross-sectional area; no foliar gap is present. The centre of the shoot is below.

Figure 87. Just above the base of the leaf primordium: a small central pith has appeared in the leaf stele.

Figure 88. Higher up; the vascular system is seen to be solenostelic.

Figures 89, 90. Near the leaf apex, which is seen to be directed away from the centre of the parent shoot (below).

Plate 12

Figure 91. Lateral segment of a shoot in transverse section, showing a large solenostelic bud (*B*) (in longitudinal section) which is incompletely confluent with a shoot meristele (*M*) and a leaf-trace strand (*l.t.*). (× 30.)

Figure 92. The same bud lower down in the shoot; the broad meristele has separated into two meristeles; the bud stele, which is fading out, is situated on the outside of one of the meristeles. (× 30.)

Figure 93. Lateral segment of a shoot in transverse section showing a large solenostelic bud (*B*) attached to the outside of a meristele (*M*). (× 30.)

Figure 94. The same bud lower down in the shoot: the bud xylem has not become confluent with that of the shoot. The bud is 'inserted' in the region of conjunction of two meristeles (*M*). (× 30.)

Figure 95. Lateral segment of a shoot in transverse section showing a polycyclic bud stele. *X*1, outer cylinder of xylem; *X*2, inner cylinder of xylem; *r*, xylem strand of invasive root. See figures 60–71 in the text. (× 60.)

Figure 96. The same, higher up: the invasive root has not penetrated to this level. The two xylem cylinders, *X*1 and *X*2, are plainly shown. (× 60.)

Figure 97. Lateral segment of a shoot in transverse section, showing the solenostele of a bud (*B*) associated with a meristele (*M*1) which has become medullated and solenostelic; the conjoined meristele (*M*2), cut slightly obliquely, is fading out acropetally. (× 60.)

Plate 13

Figure 98. Lateral segment of a shoot in transverse section showing the shoot meristele (*M*) disposed in a sickle-shaped configuration. Attached to the meristele is the solenostele (*X*1) of a bud the apex of which has been damaged. Within the cylinder, in close proximity to a lateral bud (*B*) an inner cylinder of xylem *X*2 has been differentiated from the pith. (× 35.)

Figure 99. Same as above: part of the bud stele, more highly magnified. *p*, pith, cells of which are seen to be dividing to form a cambium-like layer. Cells derived from this layer are forming the xylem (*X*2) of the inner vascular cylinder; *ph*, inner phloem, and *X*1, xylem, consisting of tracheides (*T*) of outer vascular cylinder. (× 300.)

Figure 100. Same as above: part of the bud stele, showing clearly the pith (*p*), the actively dividing cambium-like layer and tracheides (*T*), which are being formed from it. (× 300.)

Figure 101. Lateral segment of a shoot in transverse section showing the solenostele of a bud (*B*) associated with two abnormally disposed meristeles, *M*1 and *M*2; the outermost meristele (*M*1) has become medullated. (× 40.)

Figure 102. A section of the same material higher up showing the well-marked medullation of meristele, *M*1; above this level the meristeles fade out. (× 40.)

Figure 103. Part of figure 108, plate 14, more highly magnified to show medullation, etc., within the band of xylem. (× 80.)

PLATE 14

FIGURES 104–107. Four transverse sections, in basipetal sequence, of a meristele which had been longitudinally divided into two portions (*M*, *M* 1), above the level shown in figure 33; *B*, vascular tissue of a bud which has become conjoined with *M* 1. The effect of tensile stress on incipient vascular tissue with concomitant development of parenchyma is illustrated. On proceeding downwards, as the stress diminishes, the development of parenchyma from incipient vascular tissue also diminishes. Figure 105 shows an intra-meristele pith and endodermis. (× 50.)

FIGURE 108. Part of a transverse section of a solenostelic shoot (with a leaf-trace below). Within the band of xylem a pith, internal endodermis and some internal phloem have become differentiated (position indicated by arrow); these having no connexion with the exterior. (× 18.) (See figure 103, plate 13.)

FIGURE 109. Transverse section of a meristele with a well-developed cambiform pericycle (*p*); *e*, endodermis; tracheides towards lower side. (× 300.)

FIGURE 110. Transverse section of a meristele with a well-developed but somewhat irregular pericycle. Tracheides (*T*) can be seen in the pericycle. *e*, endodermis. (× 300.)

FIGURE 111. Transverse section of an isolated terminal region showing the origin of 'cortical' parenchyma (above) from incipient vascular tissue (below). The outer cells in the region of the incision are dark and corky. (× 200.)


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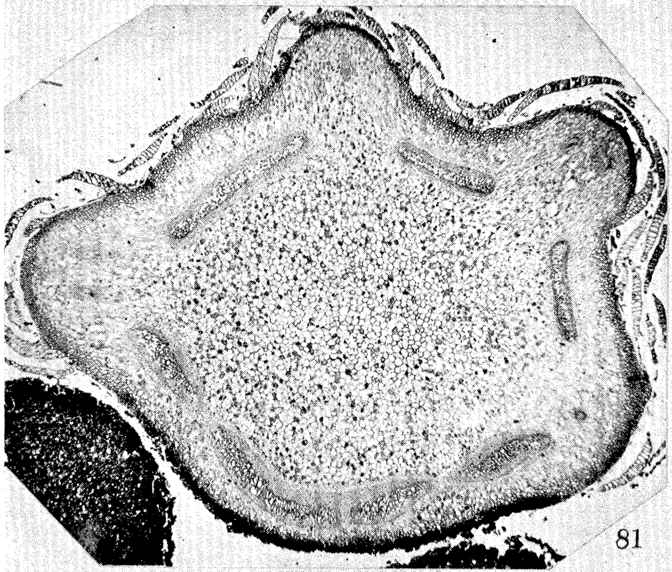
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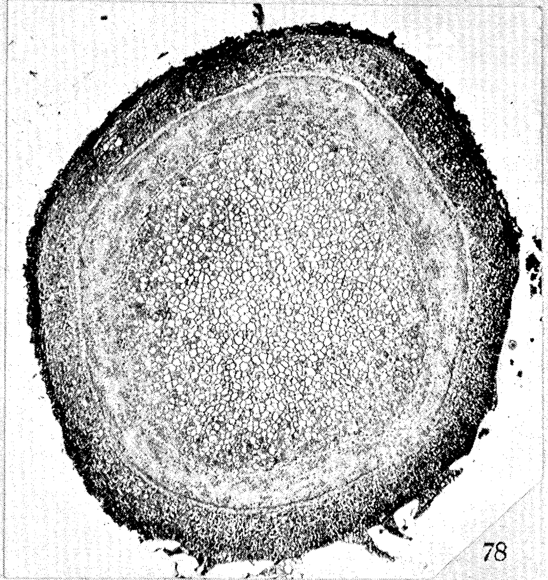
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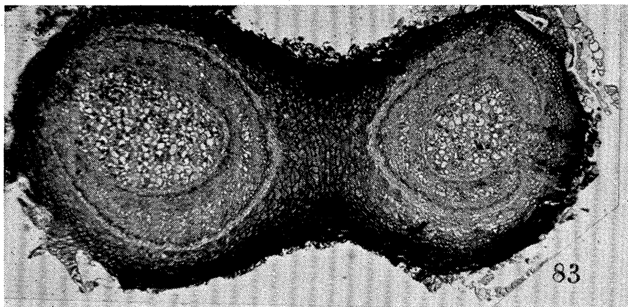
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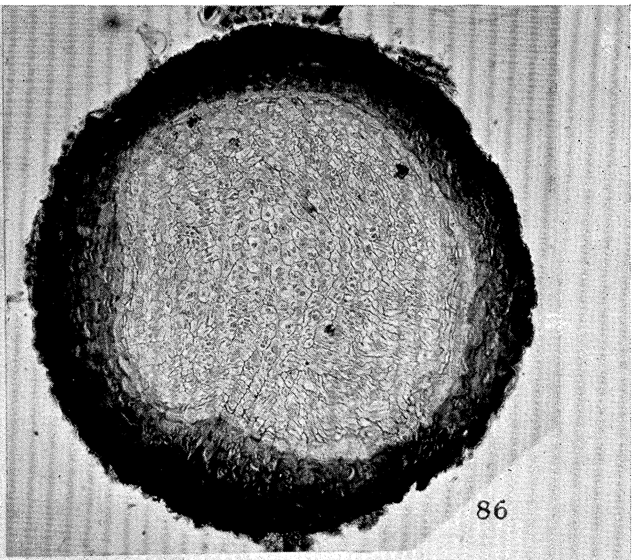
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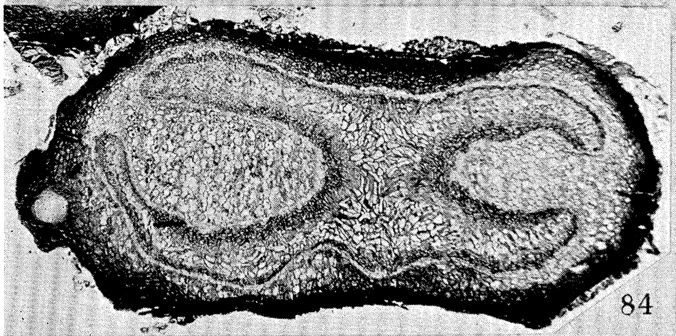
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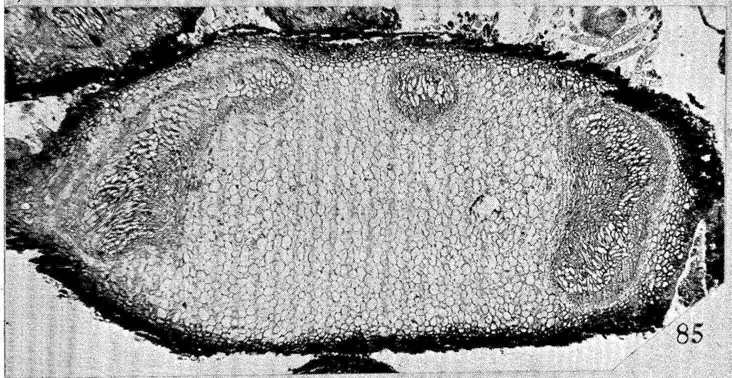
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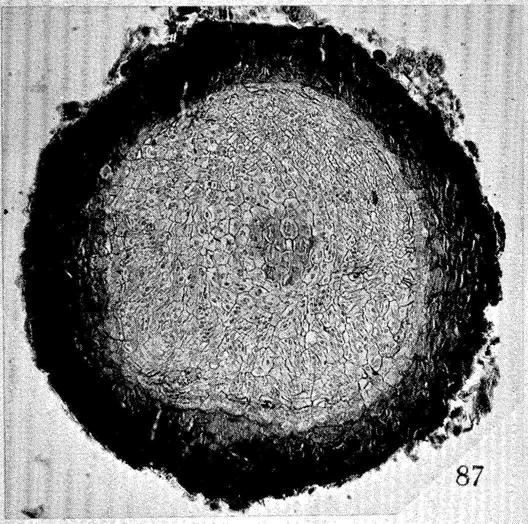
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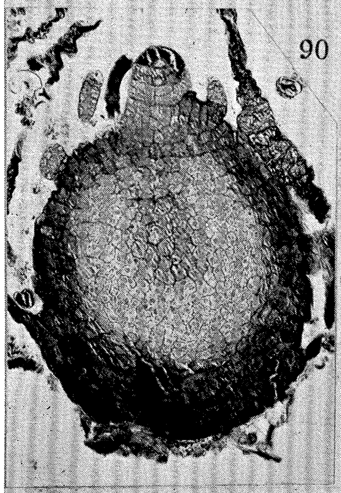
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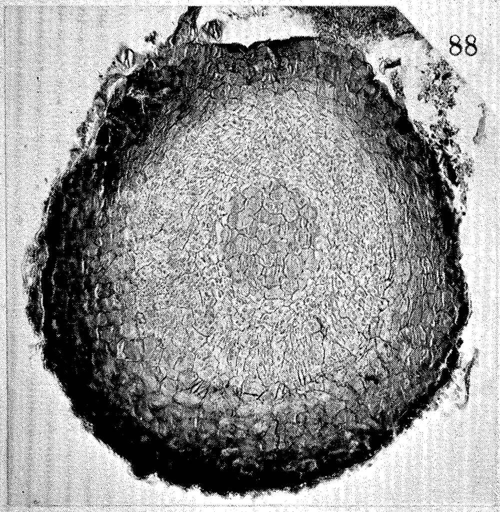
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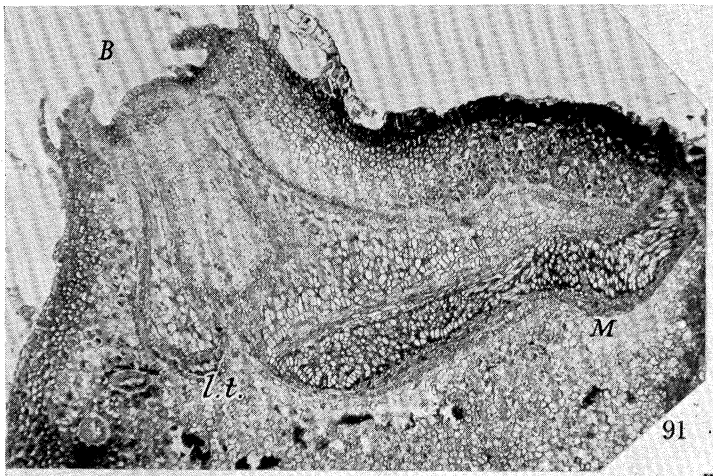

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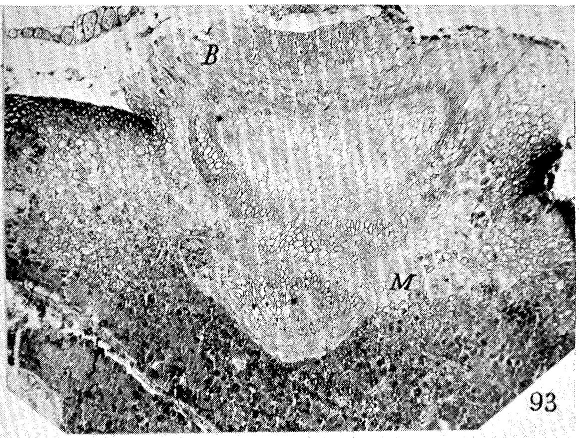

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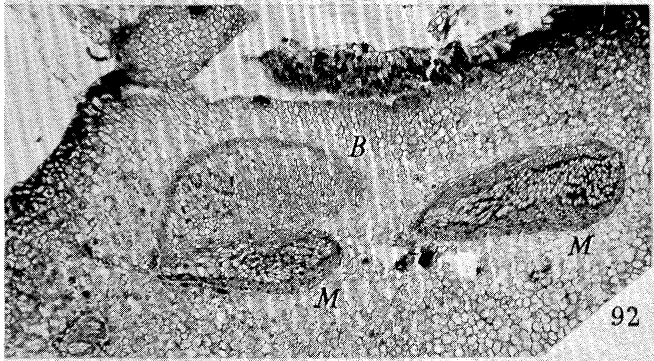

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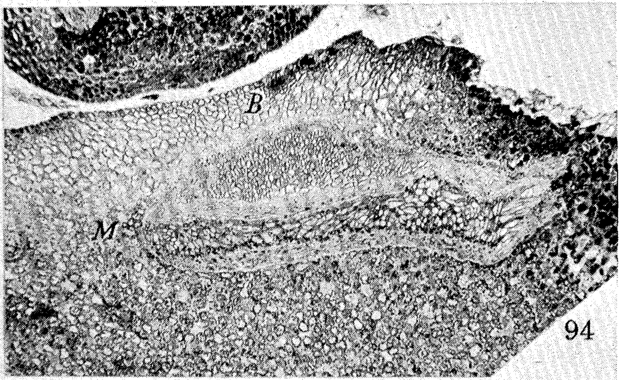

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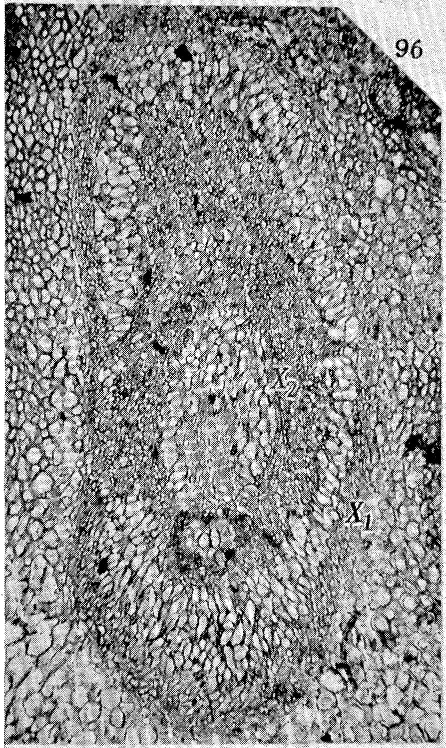

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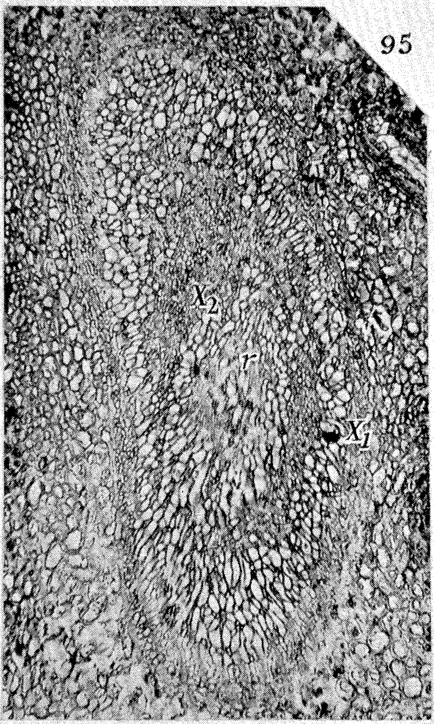

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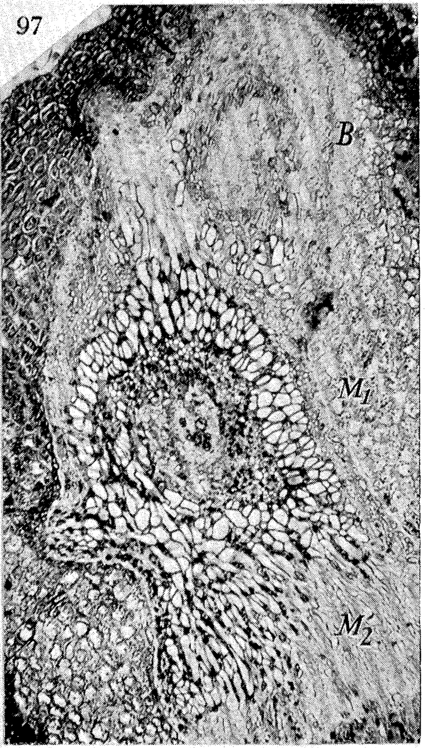

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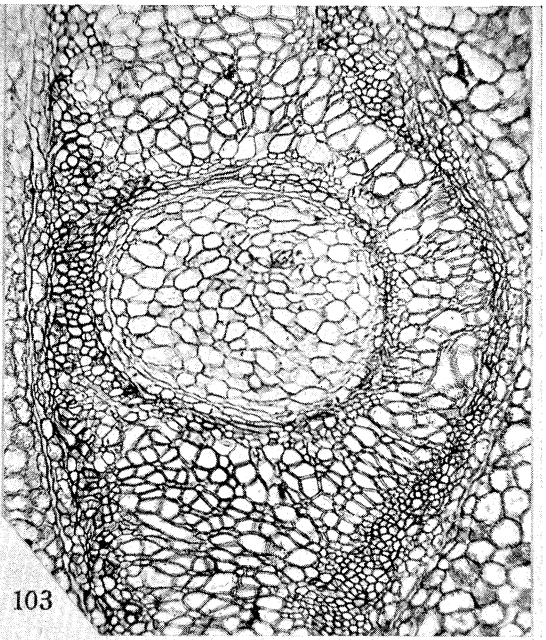

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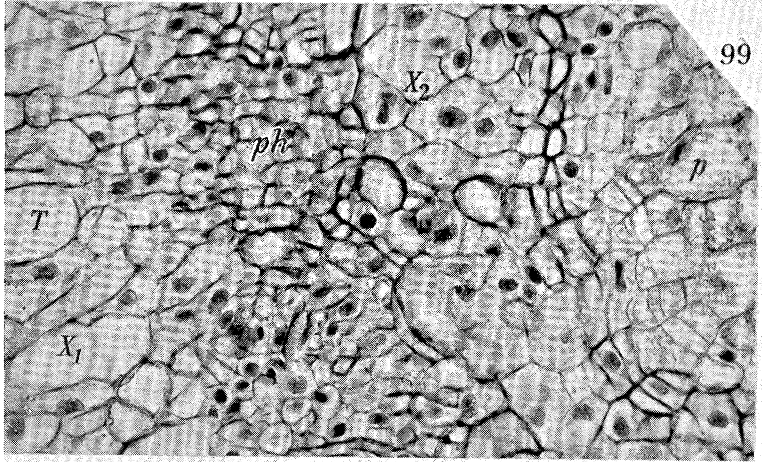

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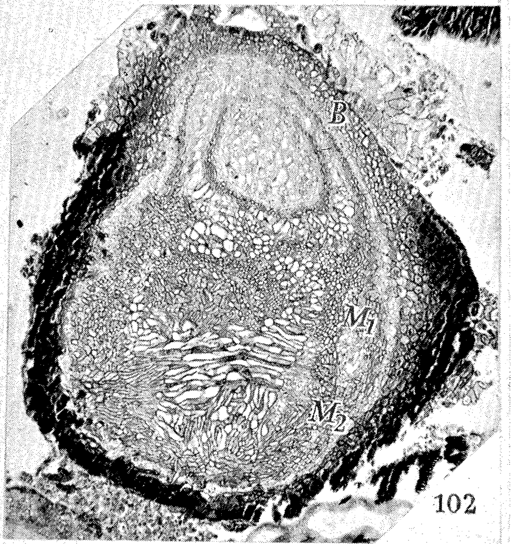

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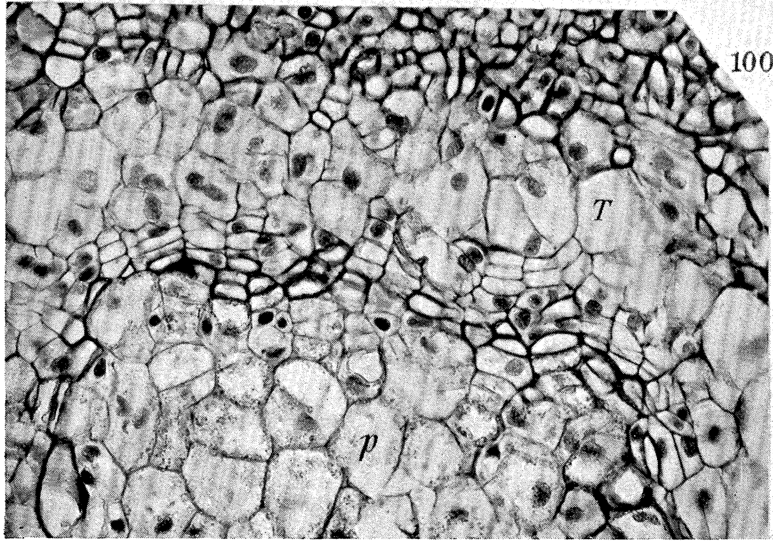

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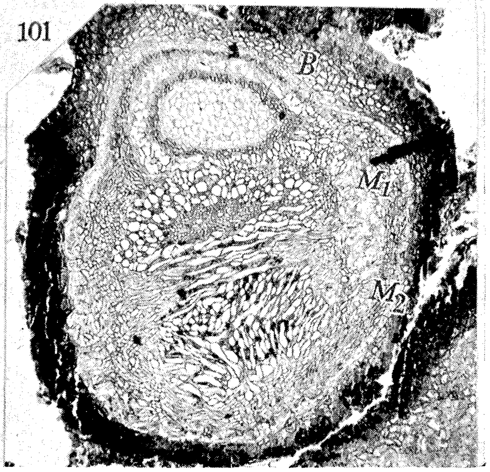

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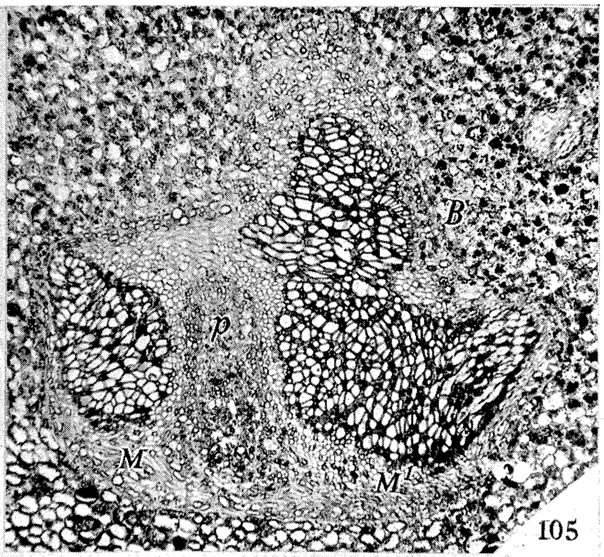

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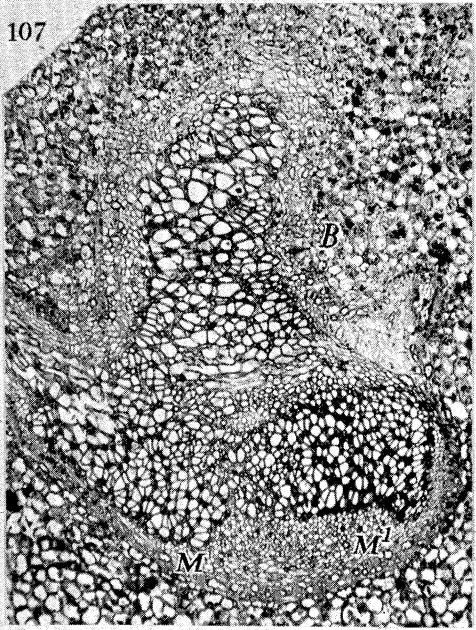

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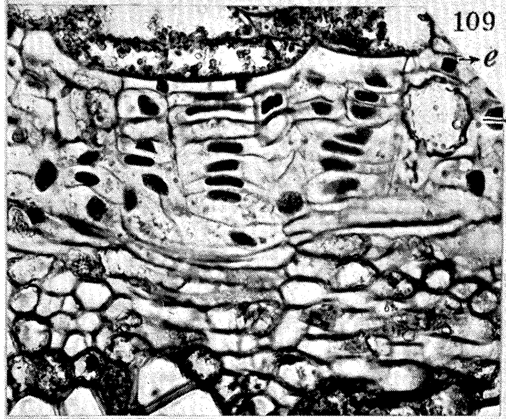

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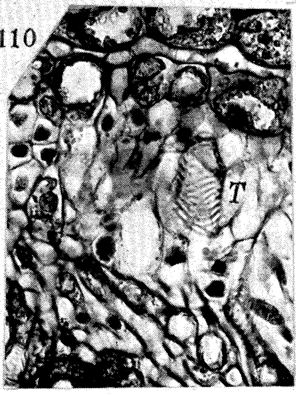

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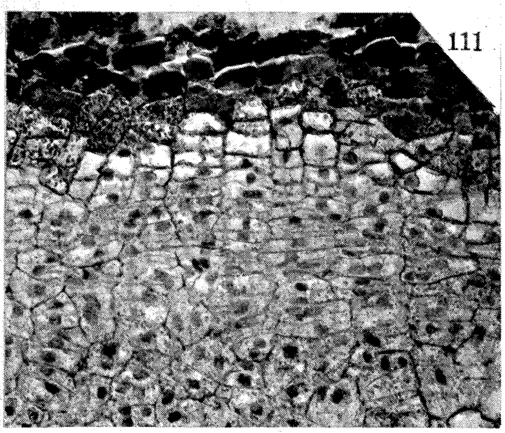

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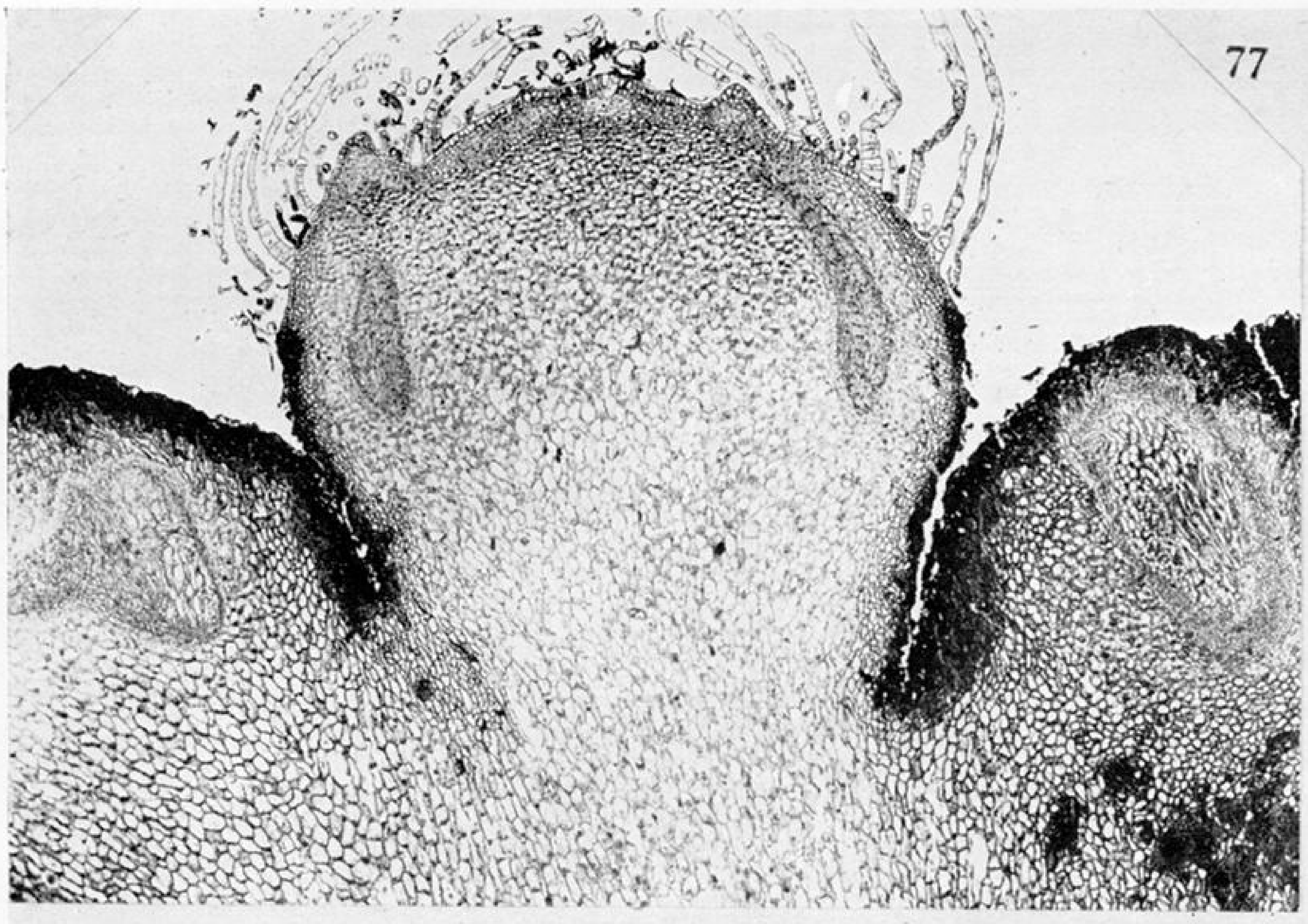
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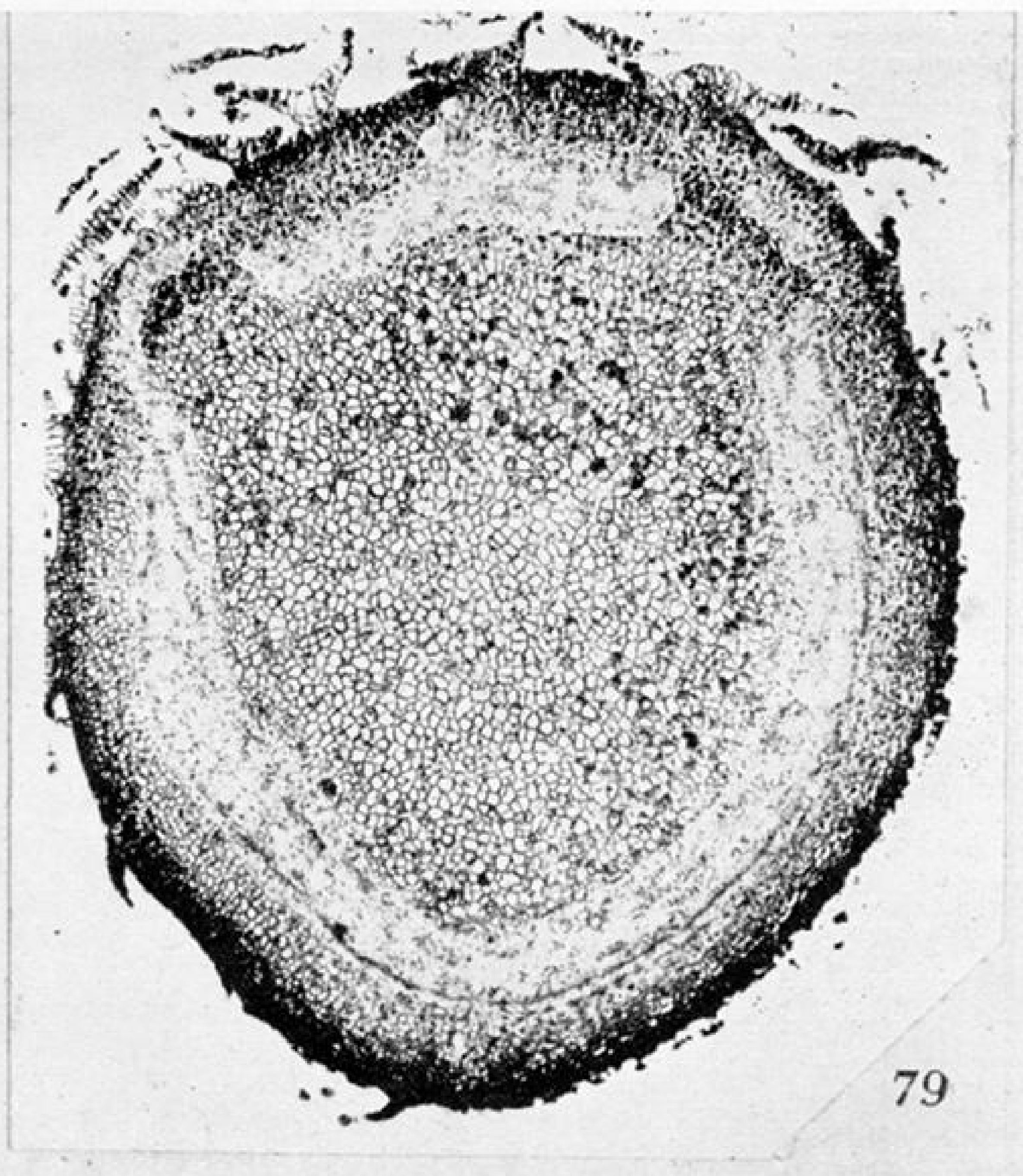
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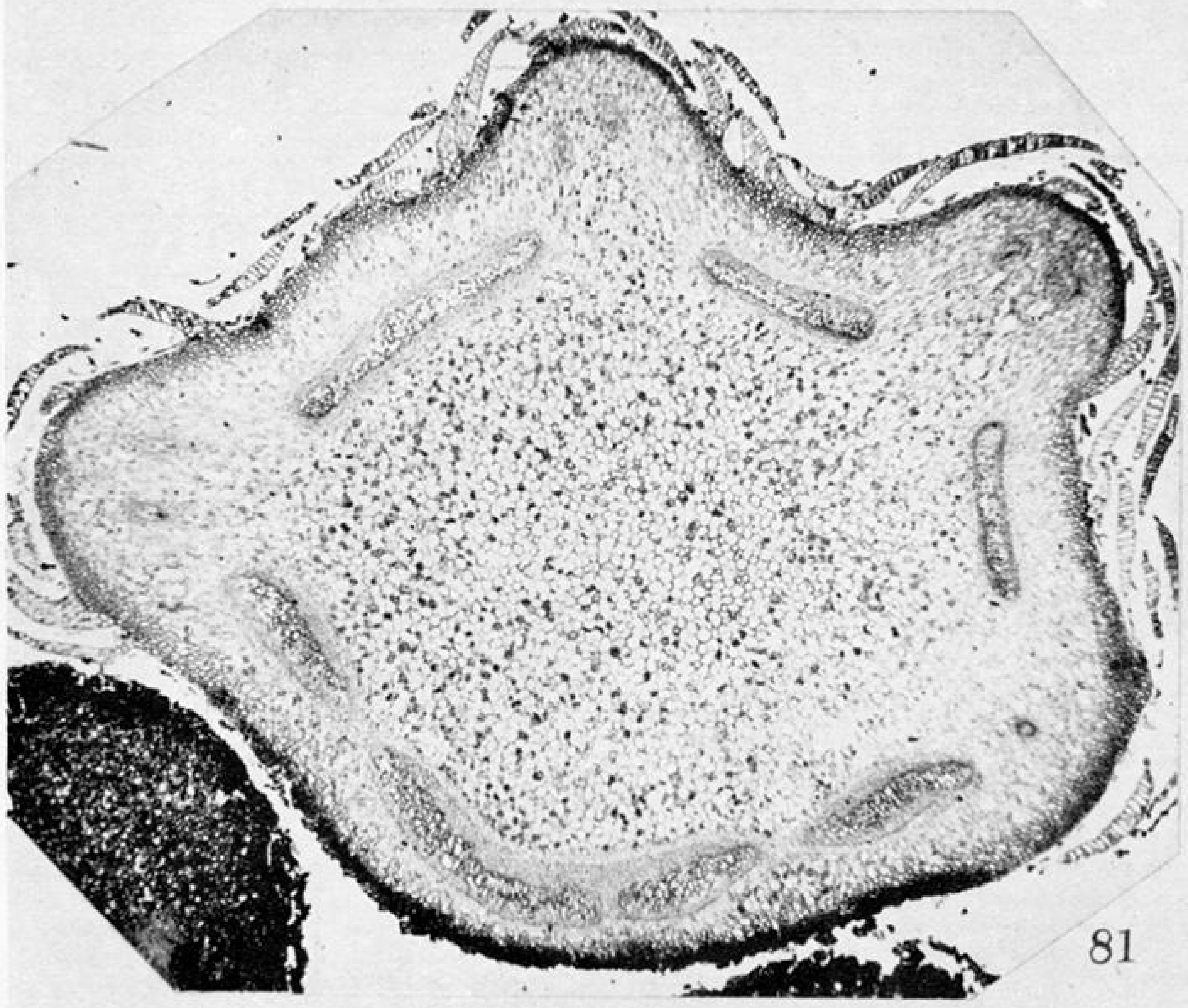
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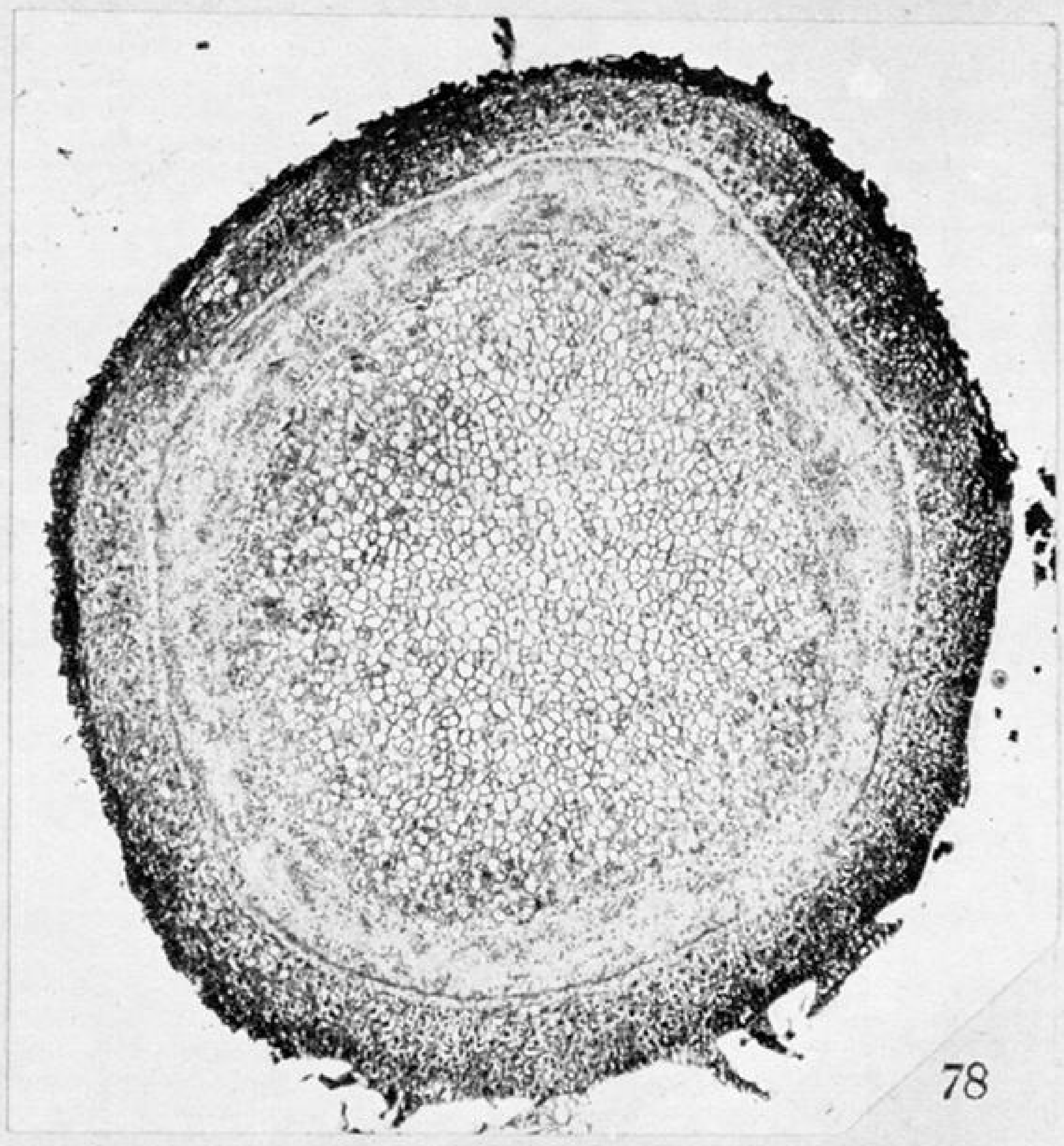
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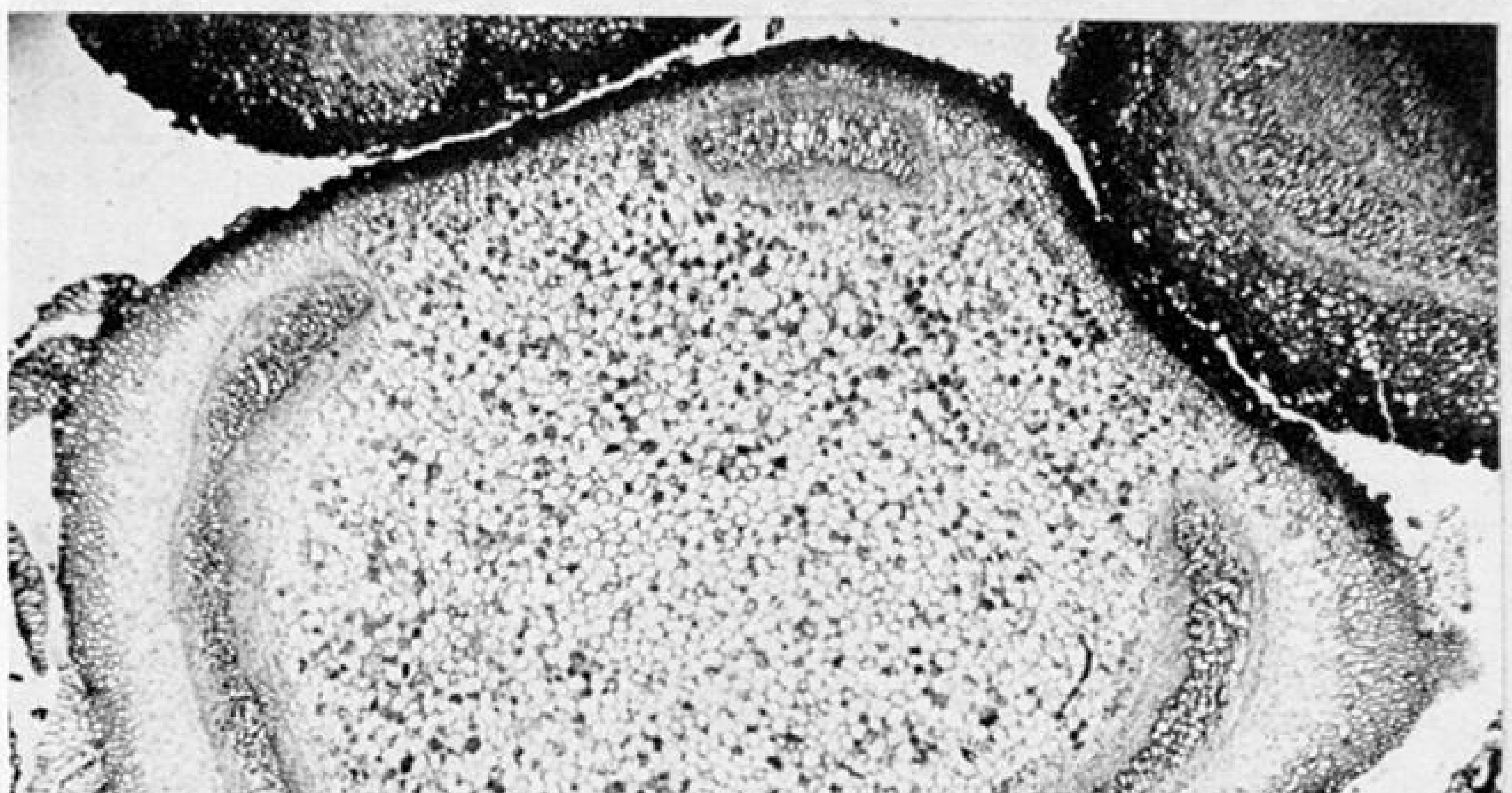
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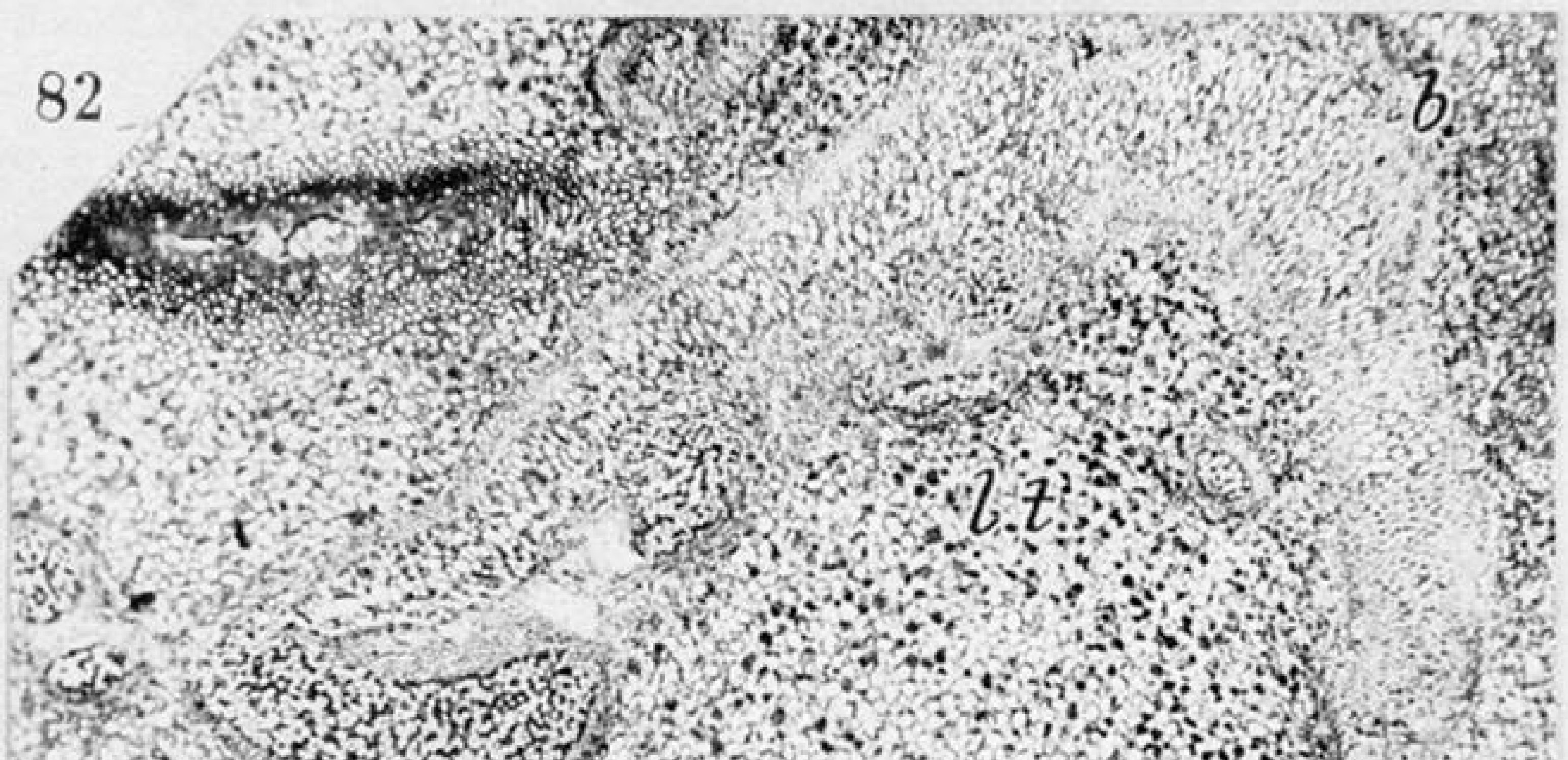
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PLATE 10

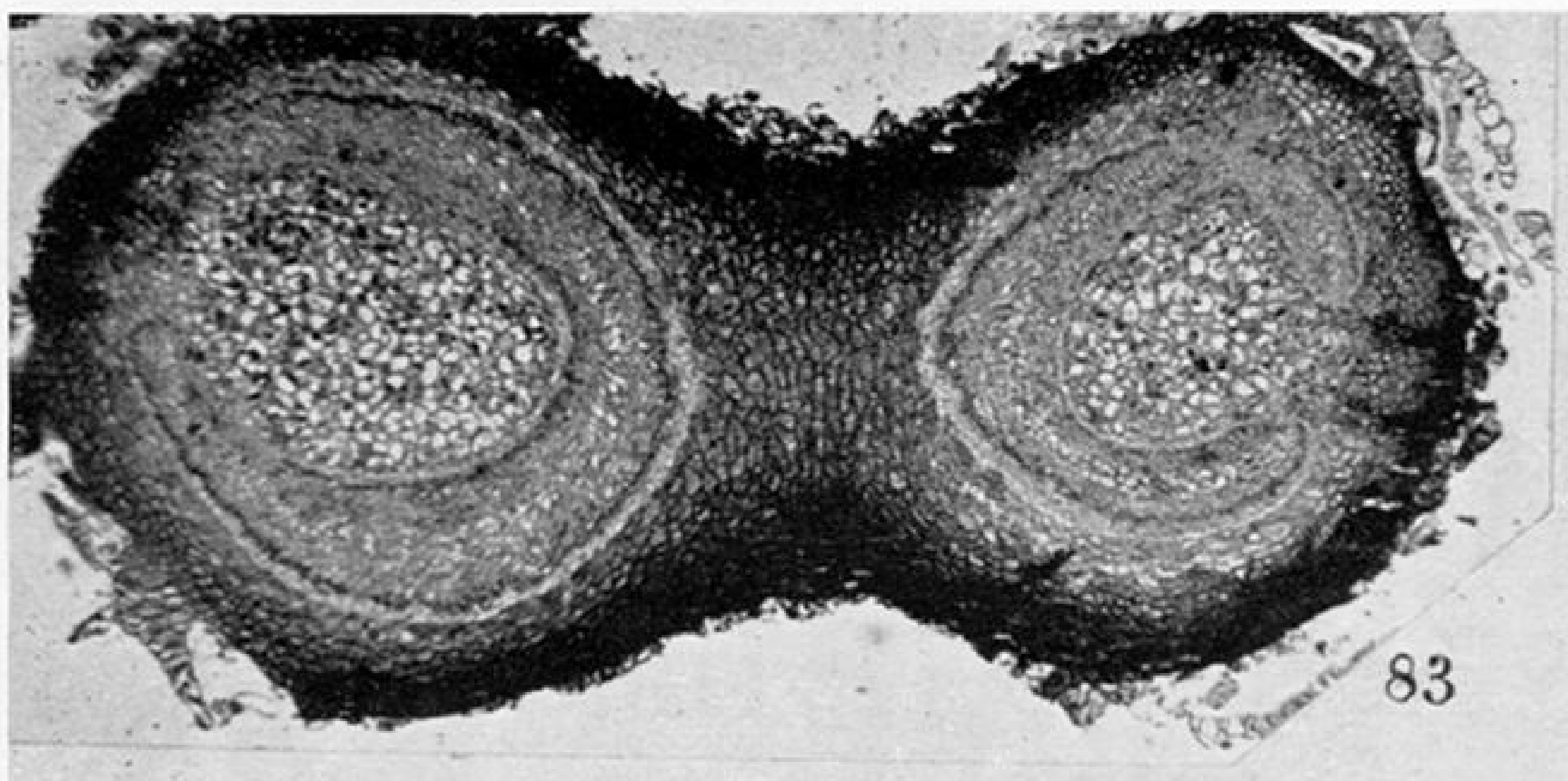
FIGURE 77. Longitudinal median section of an isolated apical meristem after 5 weeks' growth. The meristem has grown on into a short axis. Two leaf primordia can be seen at the apex. The vertical incisions, the severed vascular strands, and the basal, non-vasculated parenchymatous tissue of the isolated terminal region are clearly shown. (× 20.)

FIGURE 78. The solenostelic vascular system of an isolated terminal region. (× 25.)

FIGURE 79. A section taken nearer the apex of the same shoot as figure 78, showing the interruption of the solenostele by two leaf-gaps; the small leaf-traces can also be seen. (× 25.)

FIGURES 80, 81. Transverse sections of an isolated terminal region which has given rise to fourteen new leaf primordia in the course of 11 weeks. Figure 80 shows the approximately solenostelic condition in the lower region of the axis and figure 81 the typical dictyostelic condition in the leafy region above. (× 15.)

FIGURE 82. Lateral segment of a shoot in transverse section showing two meristeles (*M*), and the vascular strands of a leaf-trace (*l.t.*). The broad ribbon of vascular tissue (*b*) which surrounds and partly fuses with the strands of the leaf-trace and with a meristele, consists of two fused bud steles. (× 80.)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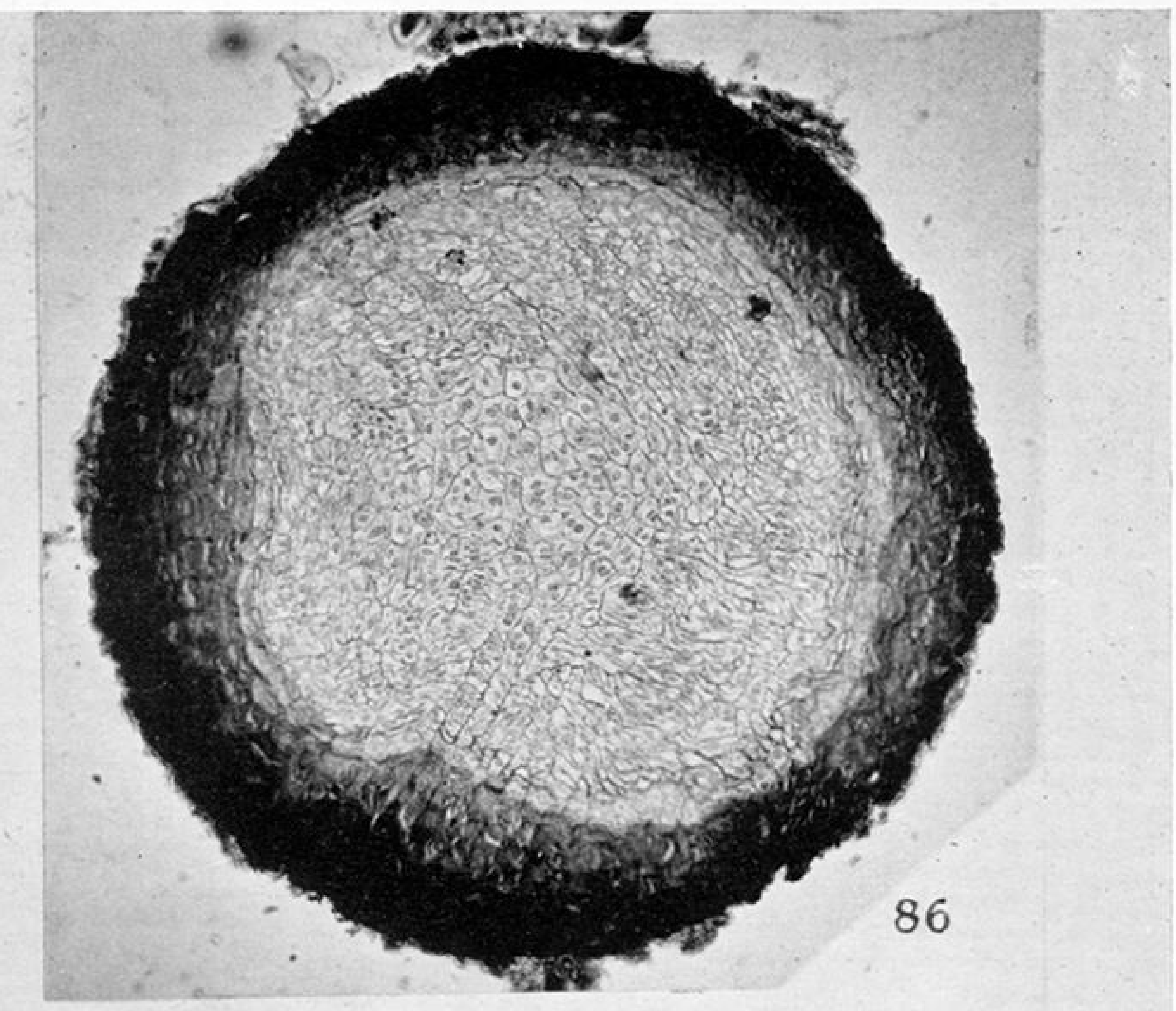
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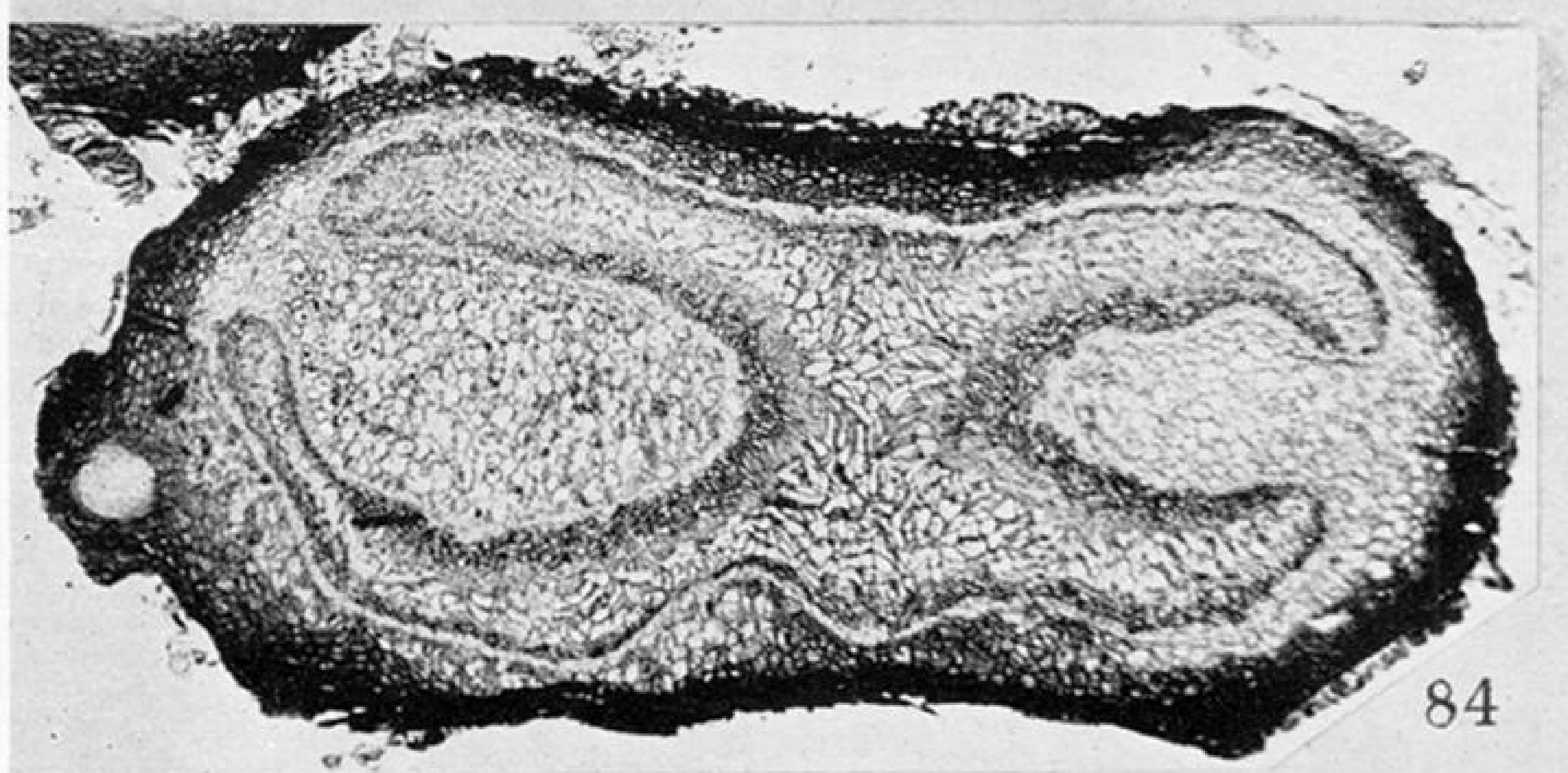
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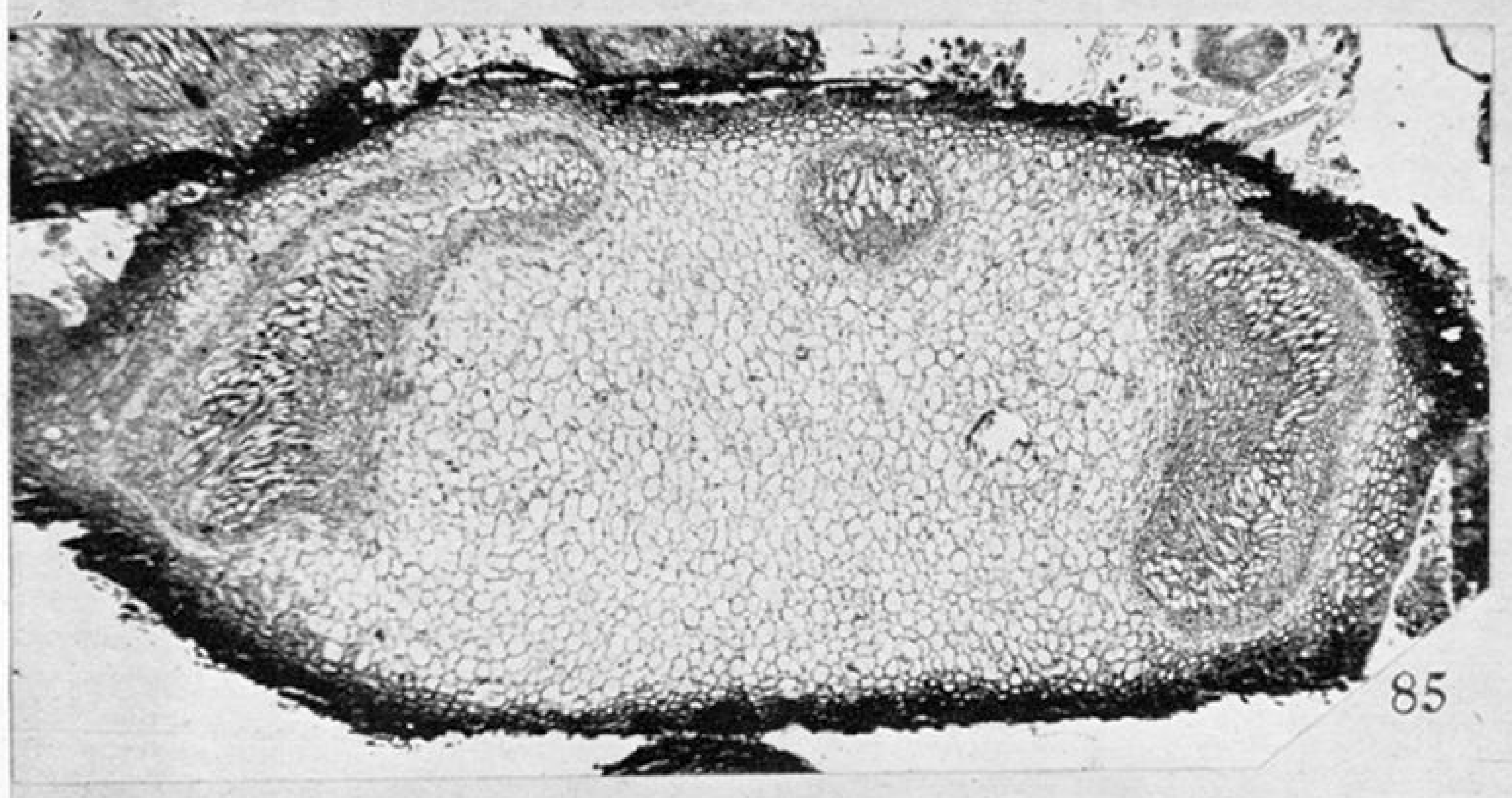
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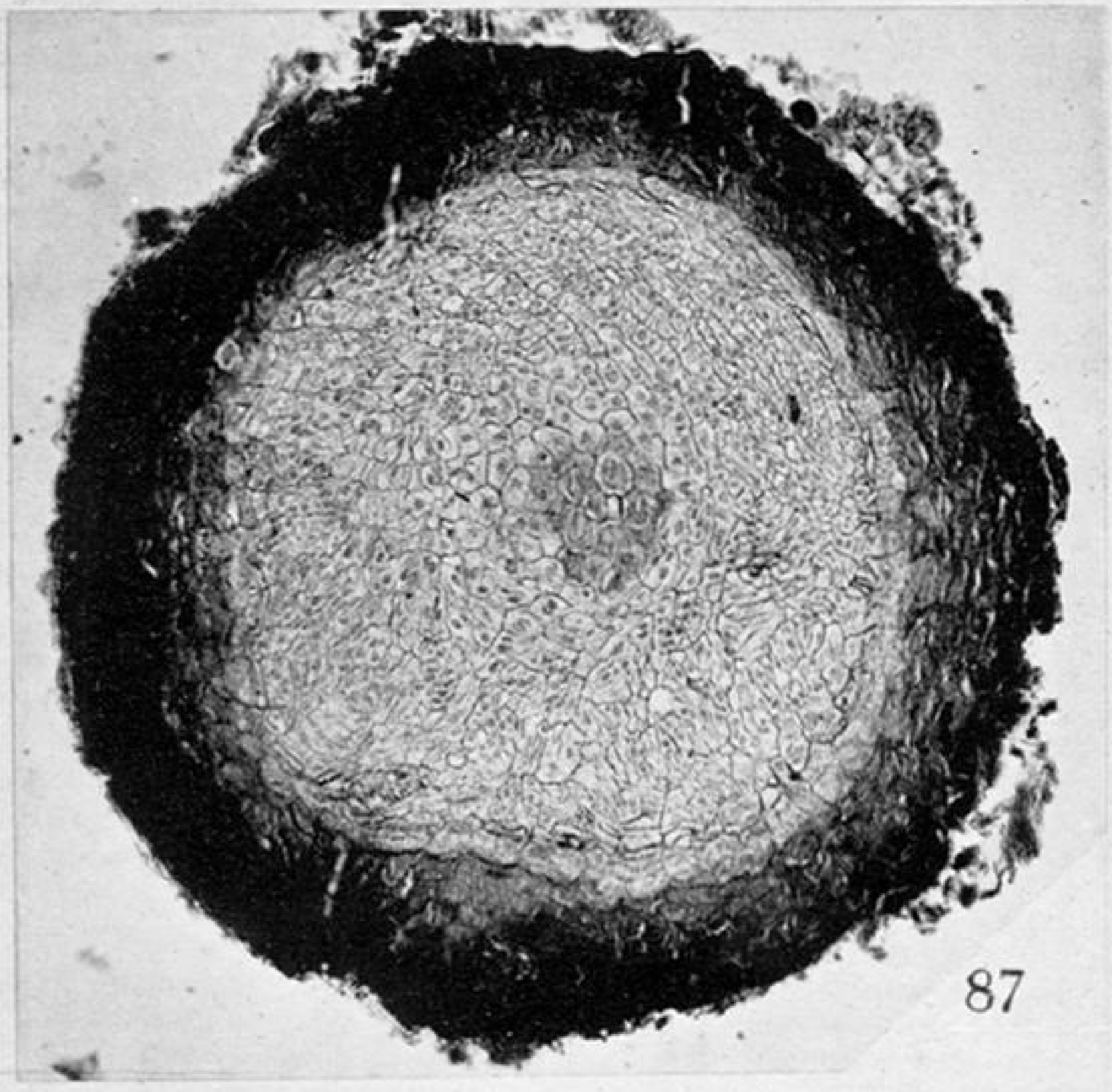
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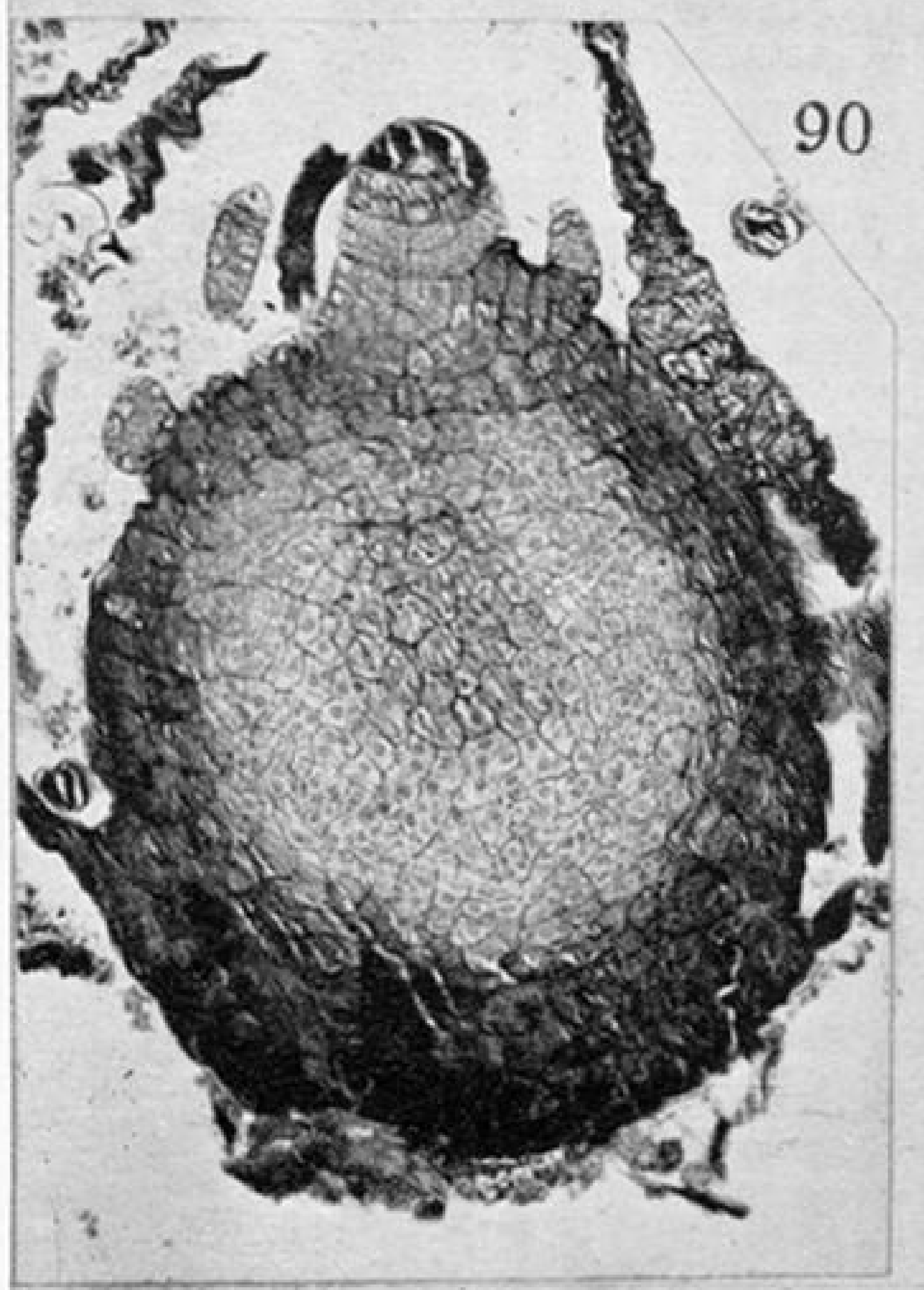
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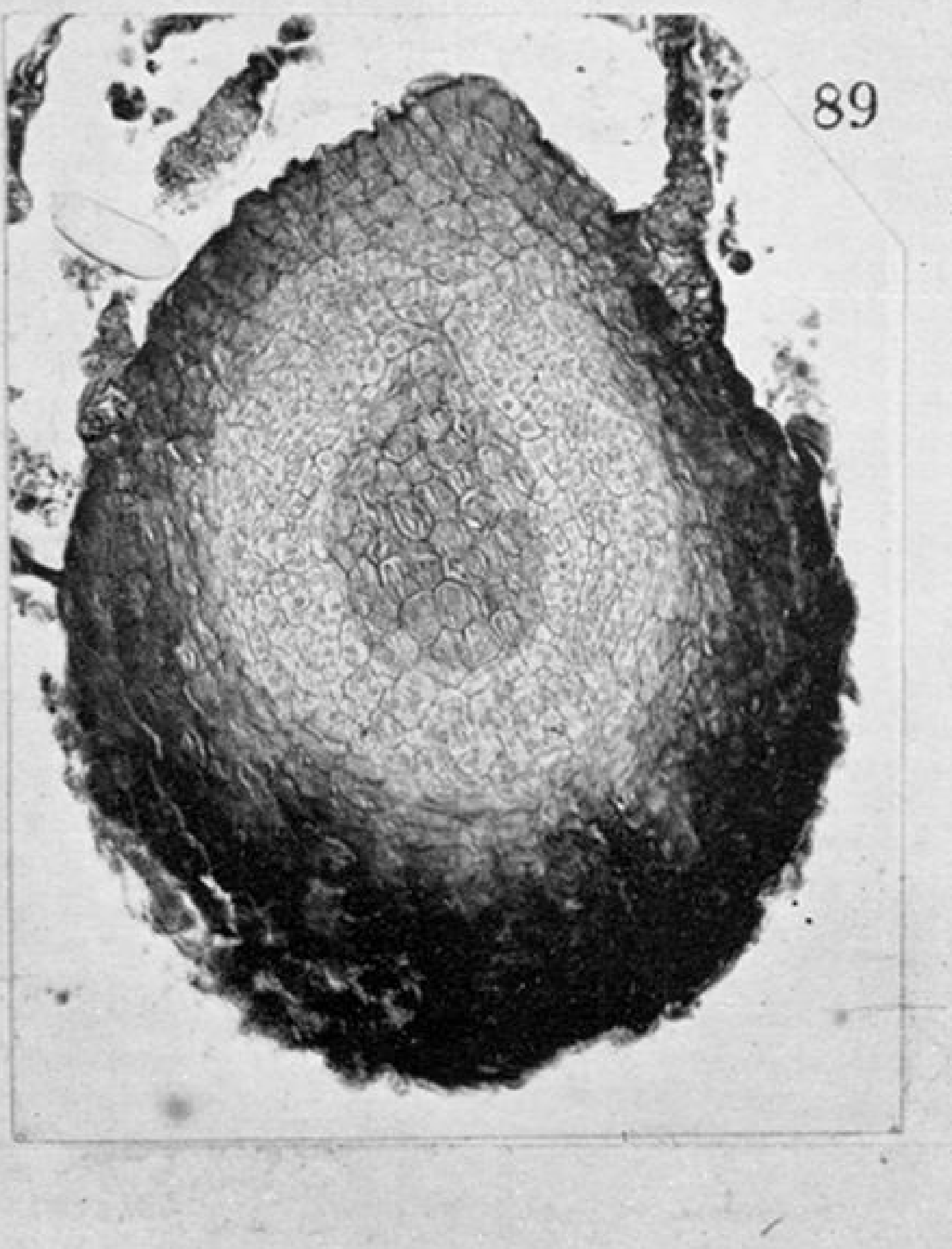
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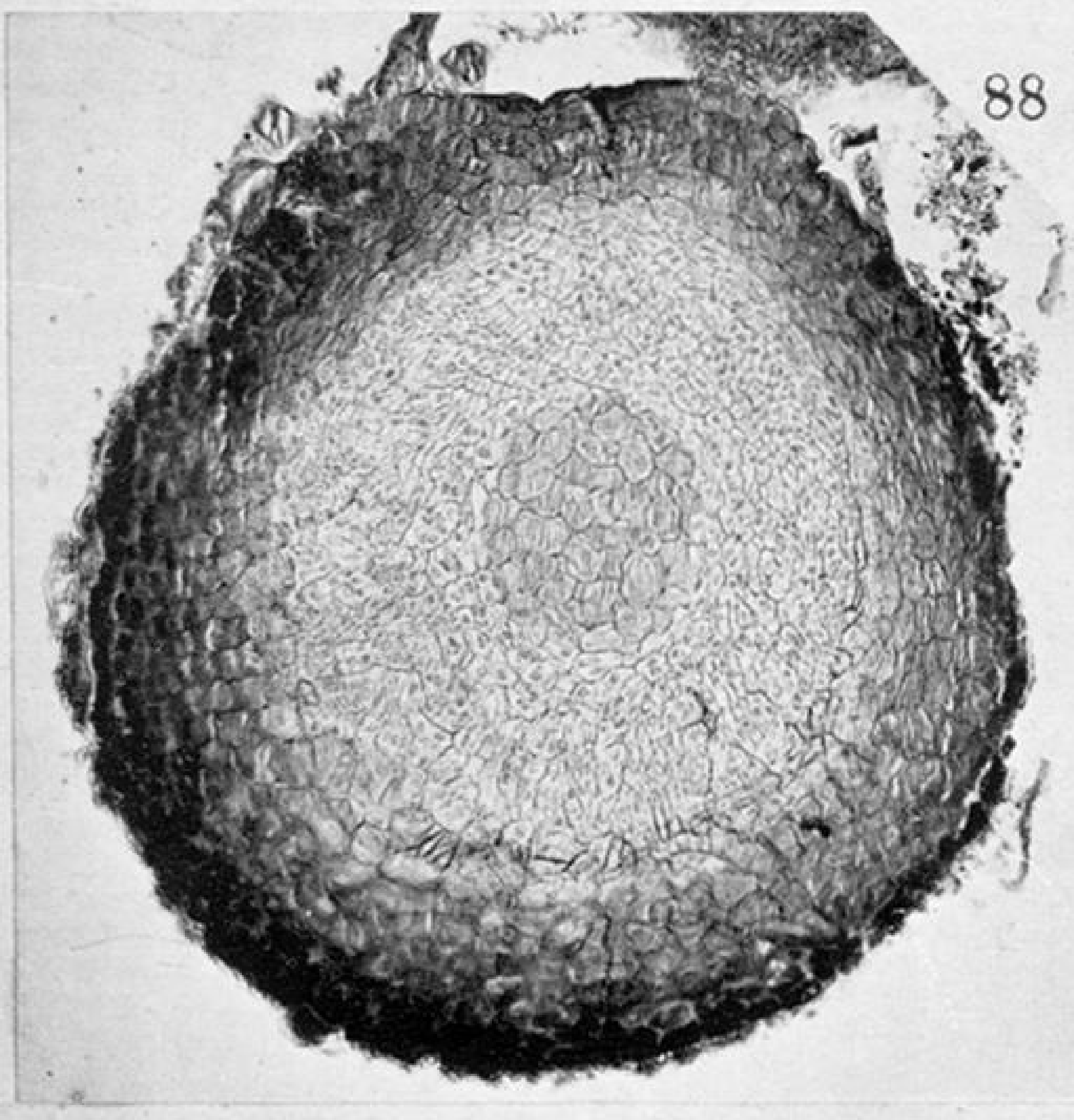
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Plate 11

Figures 83–85. Transverse sections of a rectangular, isolated terminal region in basipetal sequence. The apical cell of this meristem was damaged. In relation to the two diametrically opposite buds which developed, solenostelic vascular systems have been differentiated (figure 83). The coalescence of these solenosteles is illustrated in figure 84, and a stage in the basipetal disappearance of the vascular tissue in figure 85. (× 25.)

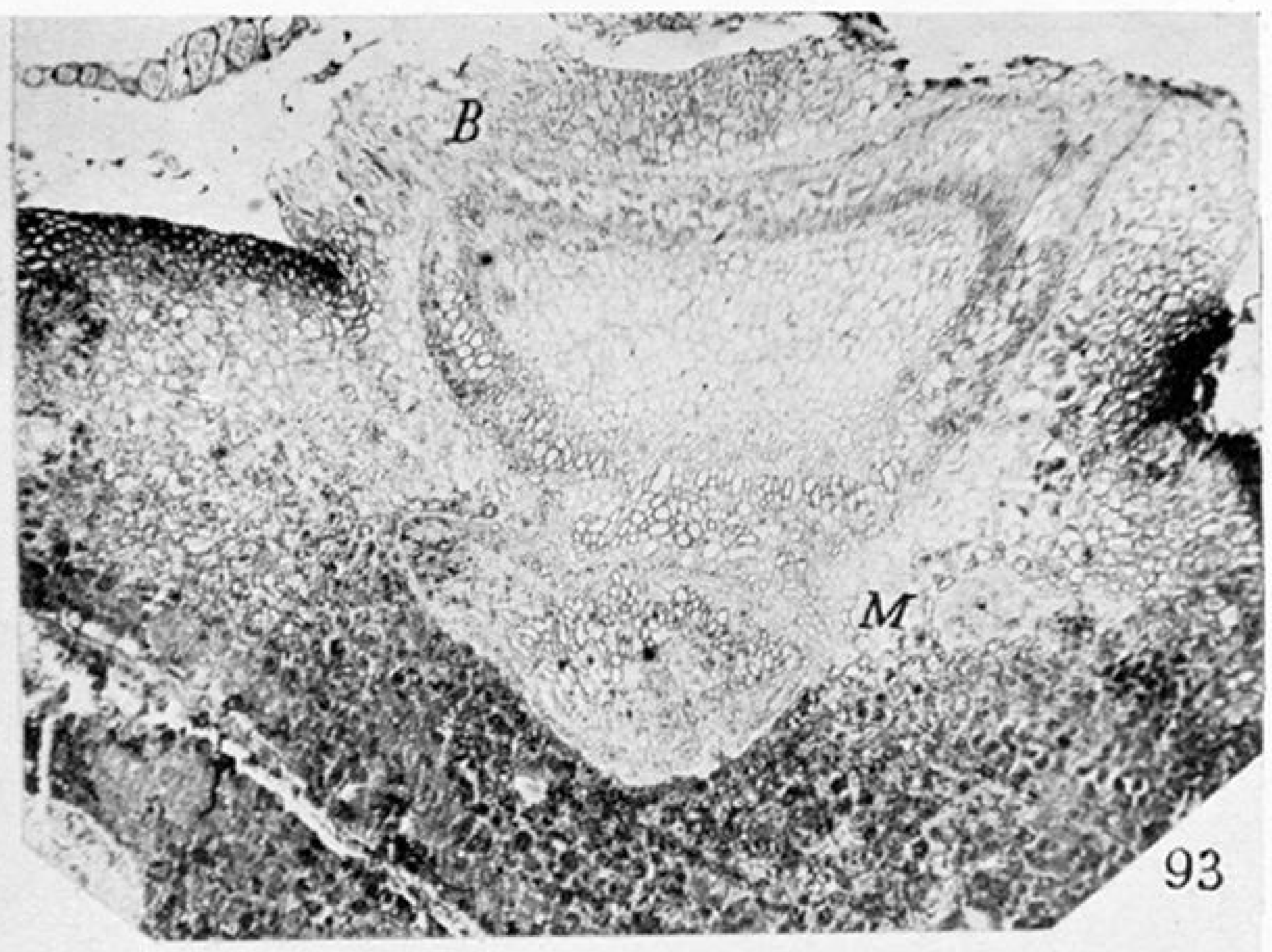
Figures 86–90. Serial transverse sections, in acropetal sequence, of an isolated leaf primordium after growth. (× 80.)

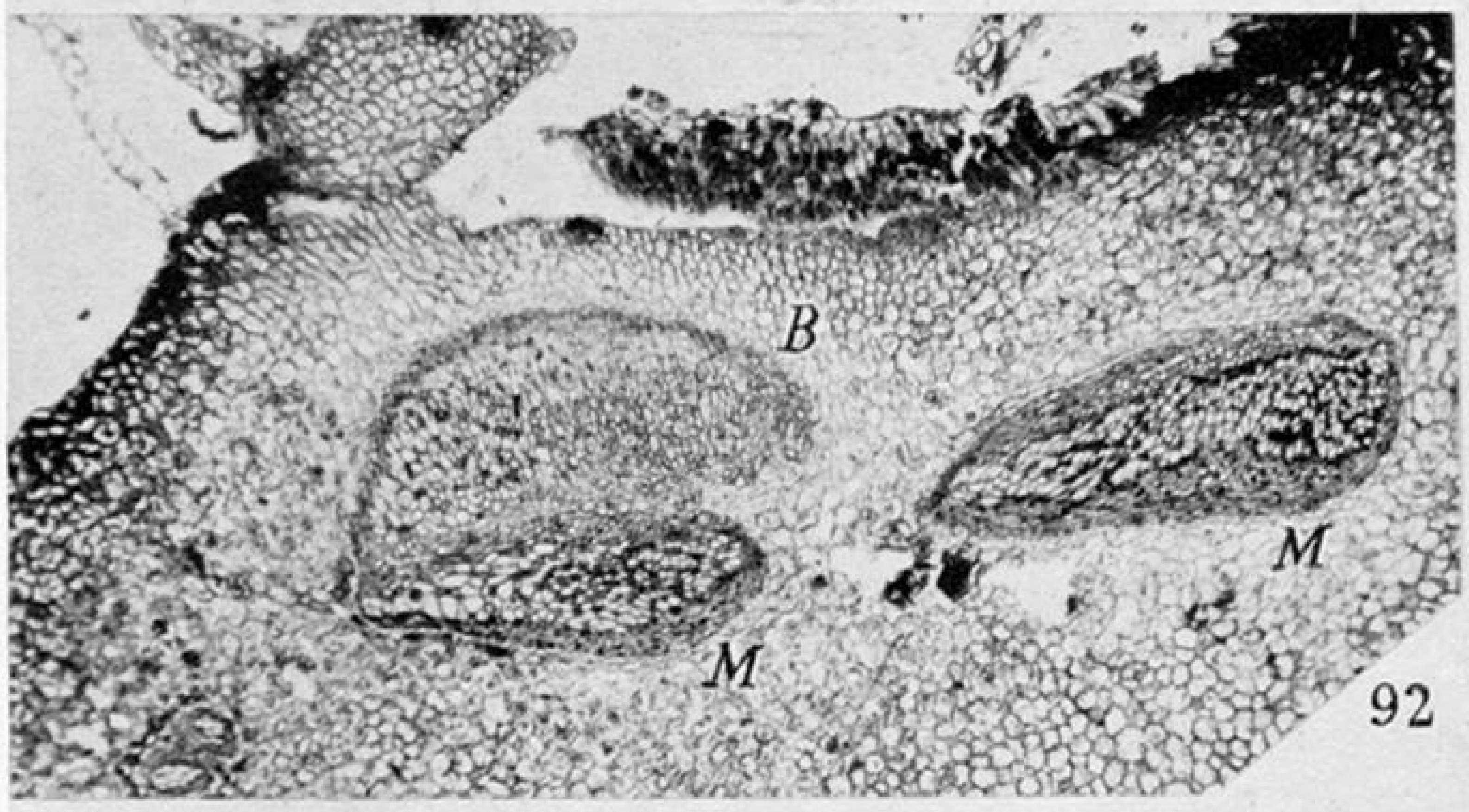
Figure 86. At the base of the leaf primordium and in the region of conjunction with the vascular tissue of the shoot: incipient vascular tissue almost fills the cross-sectional area; no foliar gap is present. The centre of the shoot is below.

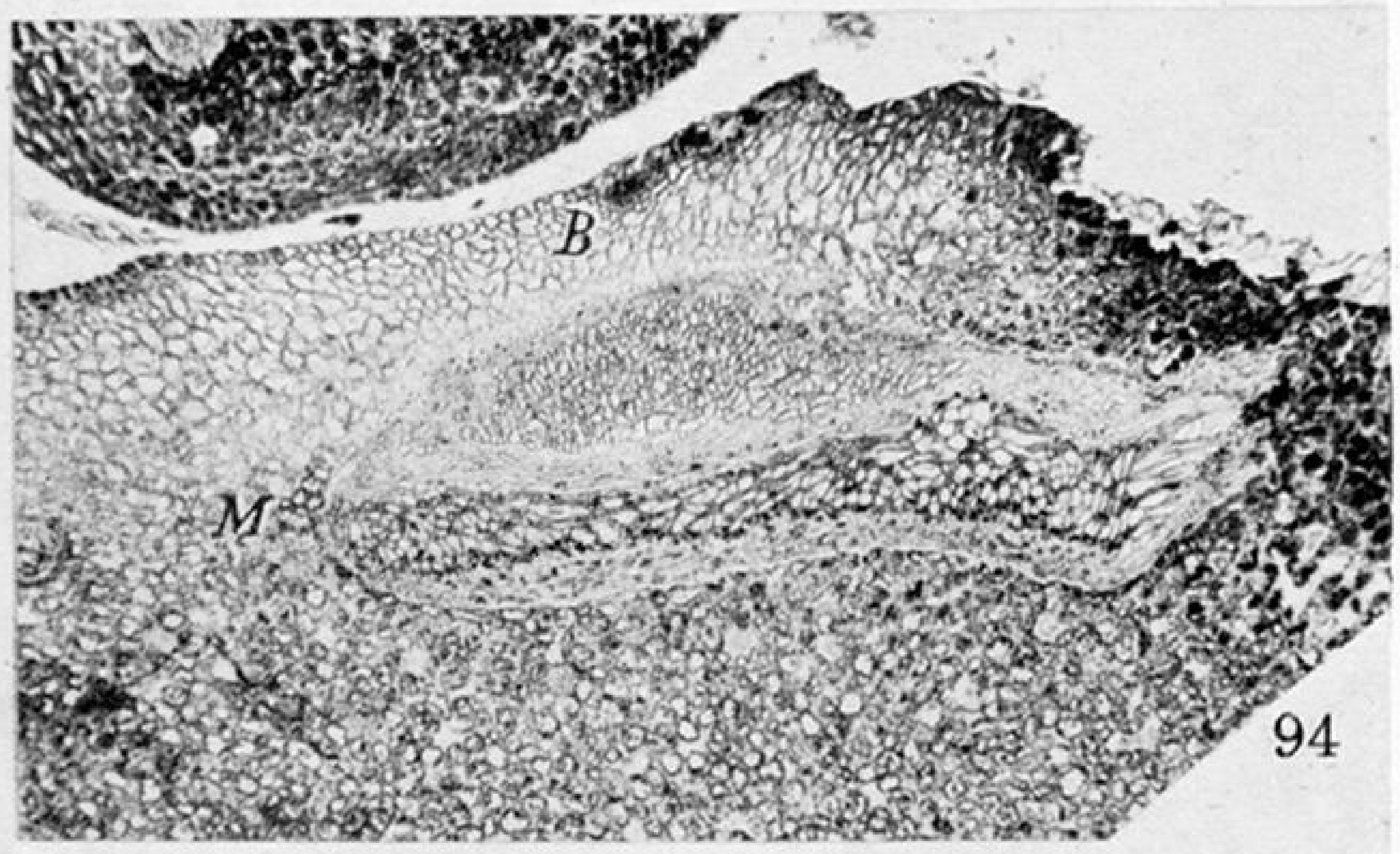
Figure 87. Just above the base of the leaf primordium: a small central pith has appeared in the leaf stele.

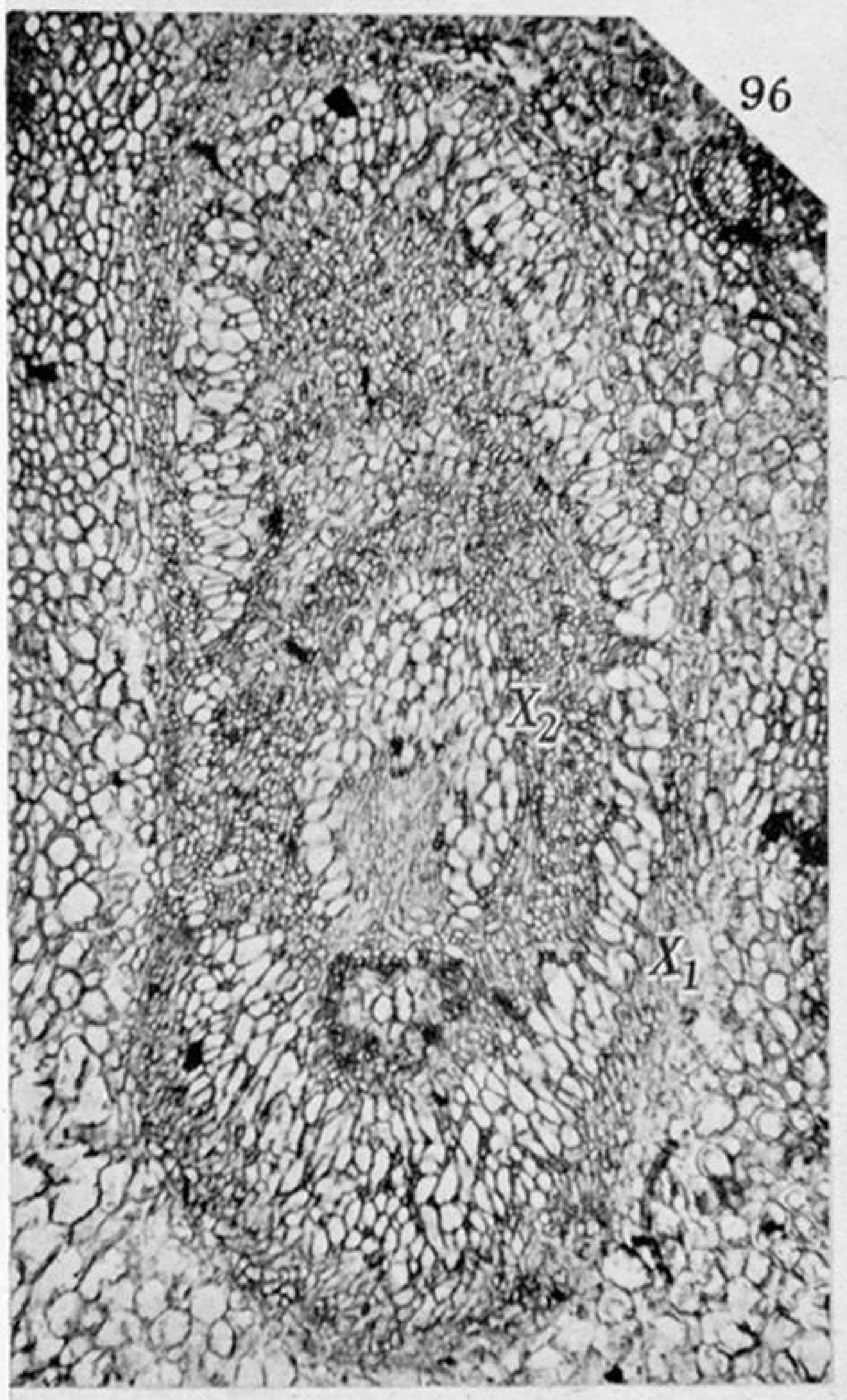
Figure 88. Higher up; the vascular system is seen to be solenostelic.

Figures 89, 90. Near the leaf apex, which is seen to be directed away from the centre of the parent shoot (below).









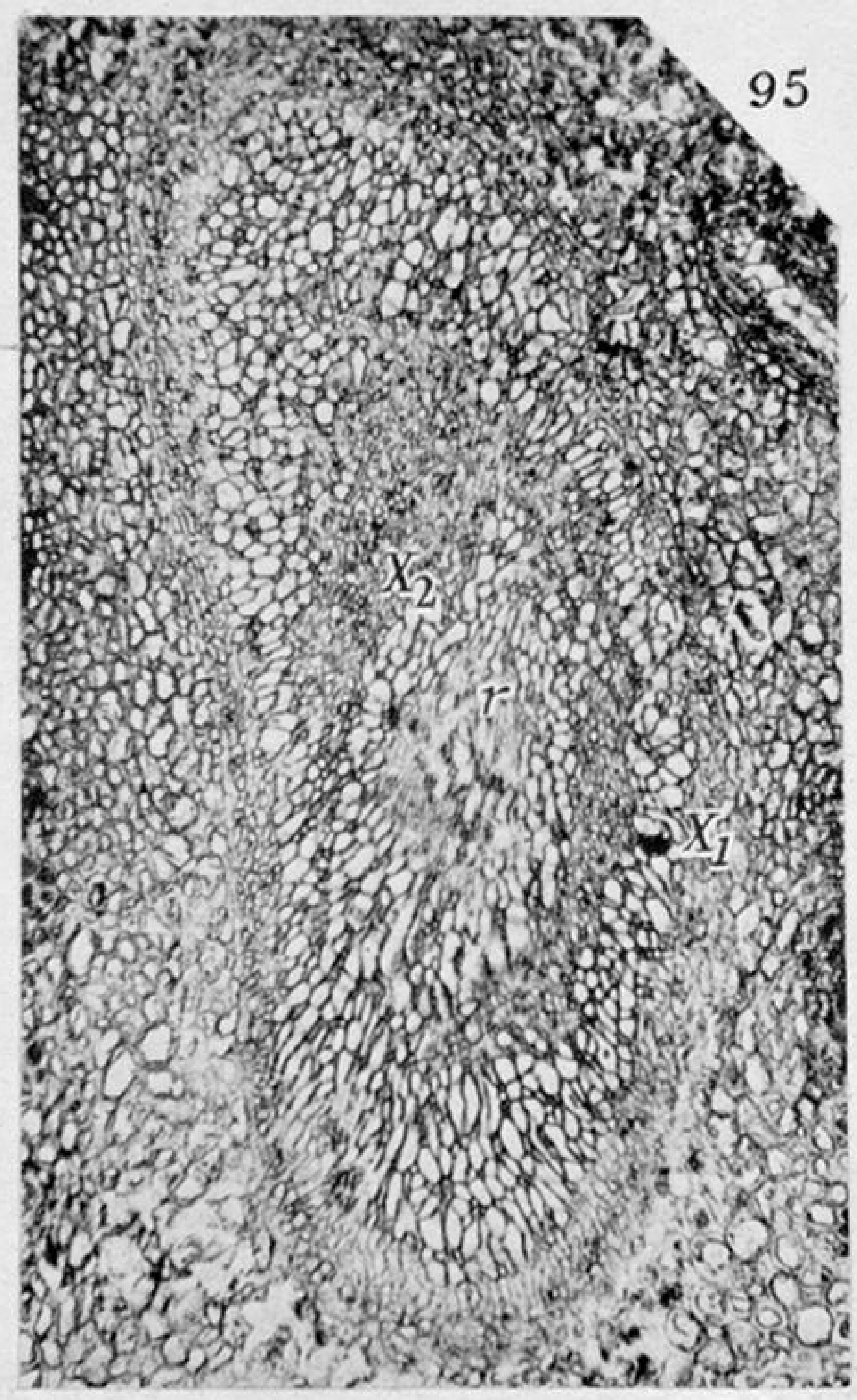


PLATE 12

FIGURE 91. Lateral segment of a shoot in transverse section, showing a large solenostelic bud (*B*) (in longitudinal section) which is incompletely confluent with a shoot meristele (*M*) and a leaf-trace strand (*l.t.*). (× 30.)

FIGURE 92. The same bud lower down in the shoot; the broad meristele has separated into two meristeles; the bud stele, which is fading out, is situated on the outside of one of the meristeles. (× 30.)

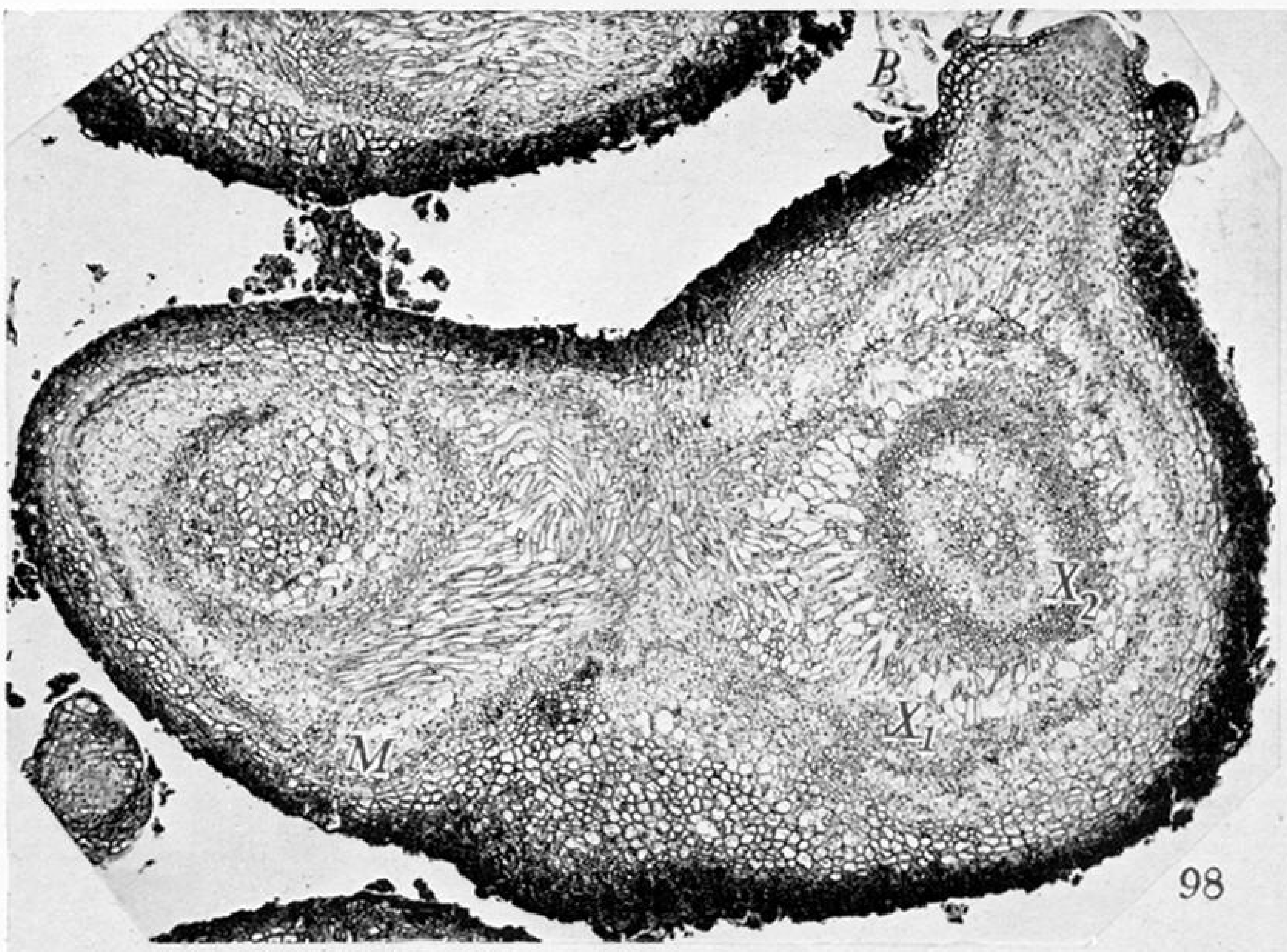
FIGURE 93. Lateral segment of a shoot in transverse section showing a large solenostelic bud (*B*) attached to the outside of a meristele (*M*). (× 30.)

FIGURE 94. The same bud lower down in the shoot: the bud xylem has not become confluent with that of the shoot. The bud is 'inserted' in the region of conjunction of two meristeles (*M*). (× 30.)

FIGURE 95. Lateral segment of a shoot in transverse section showing a polycyclic bud stele. *X*1, outer cylinder of xylem; *X*2, inner cylinder of xylem; *r*, xylem strand of invasive root. See figures 60–71 in the text. (× 60.)

FIGURE 96. The same, higher up: the invasive root has not penetrated to this level. The two xylem cylinders, *X*1 and *X*2, are plainly shown. (× 60.)

FIGURE 97. Lateral segment of a shoot in transverse section, showing the solenostele of a bud (*B*) associated with a meristele (*M*1) which has become medullated and solenostelic; the conjoined meristele (*M*2), cut slightly obliquely, is fading out acropetally. (× 60.)



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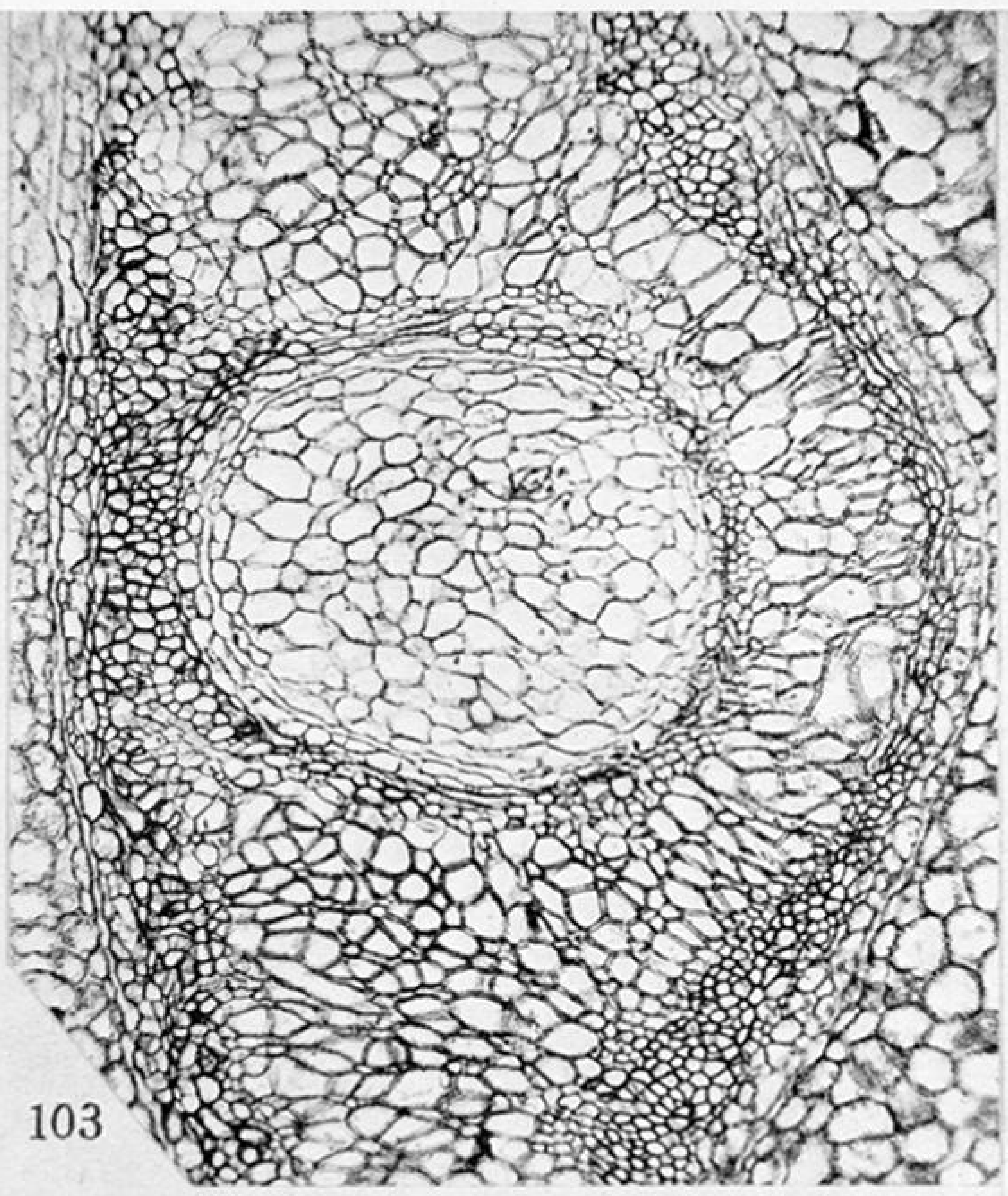

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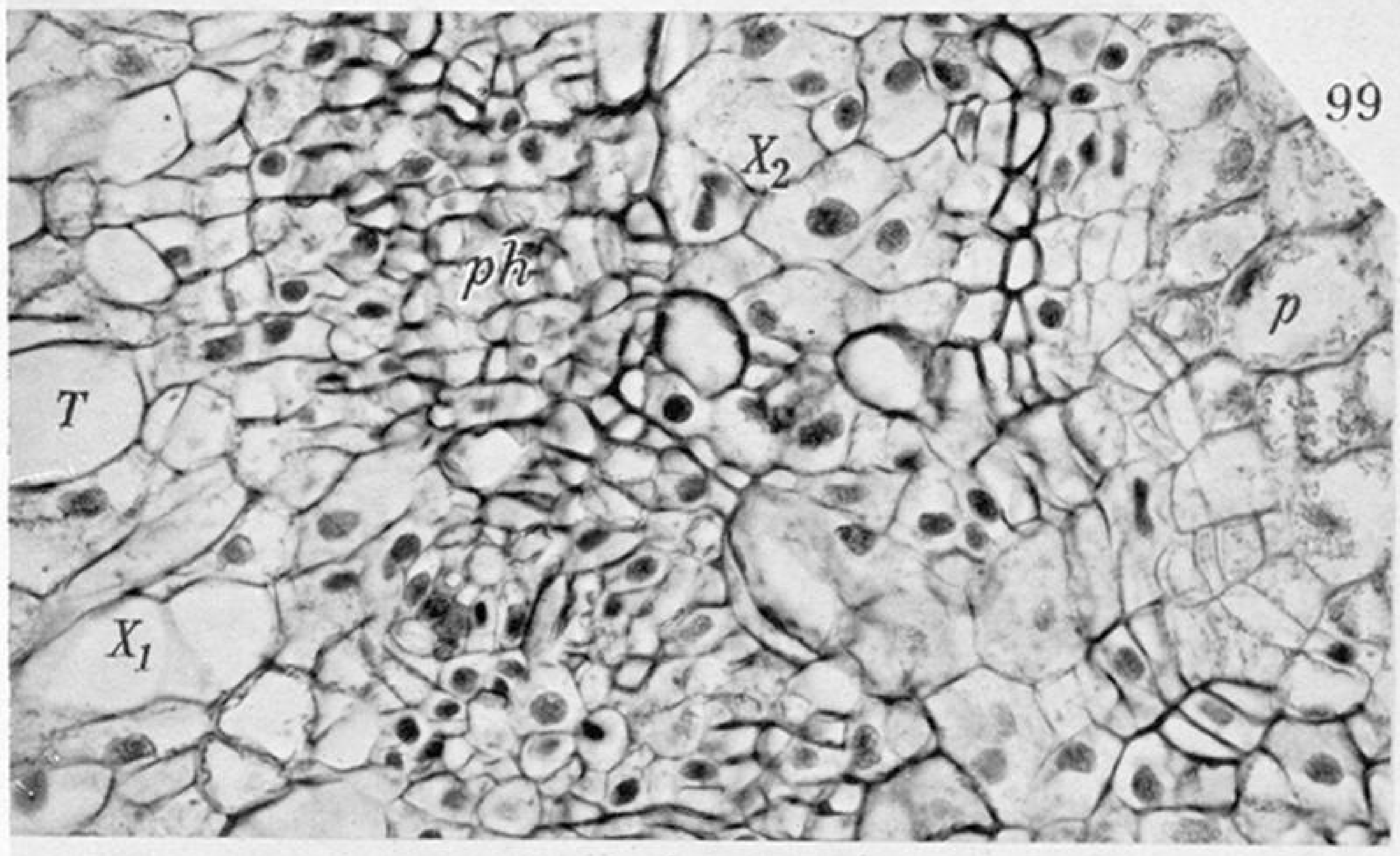

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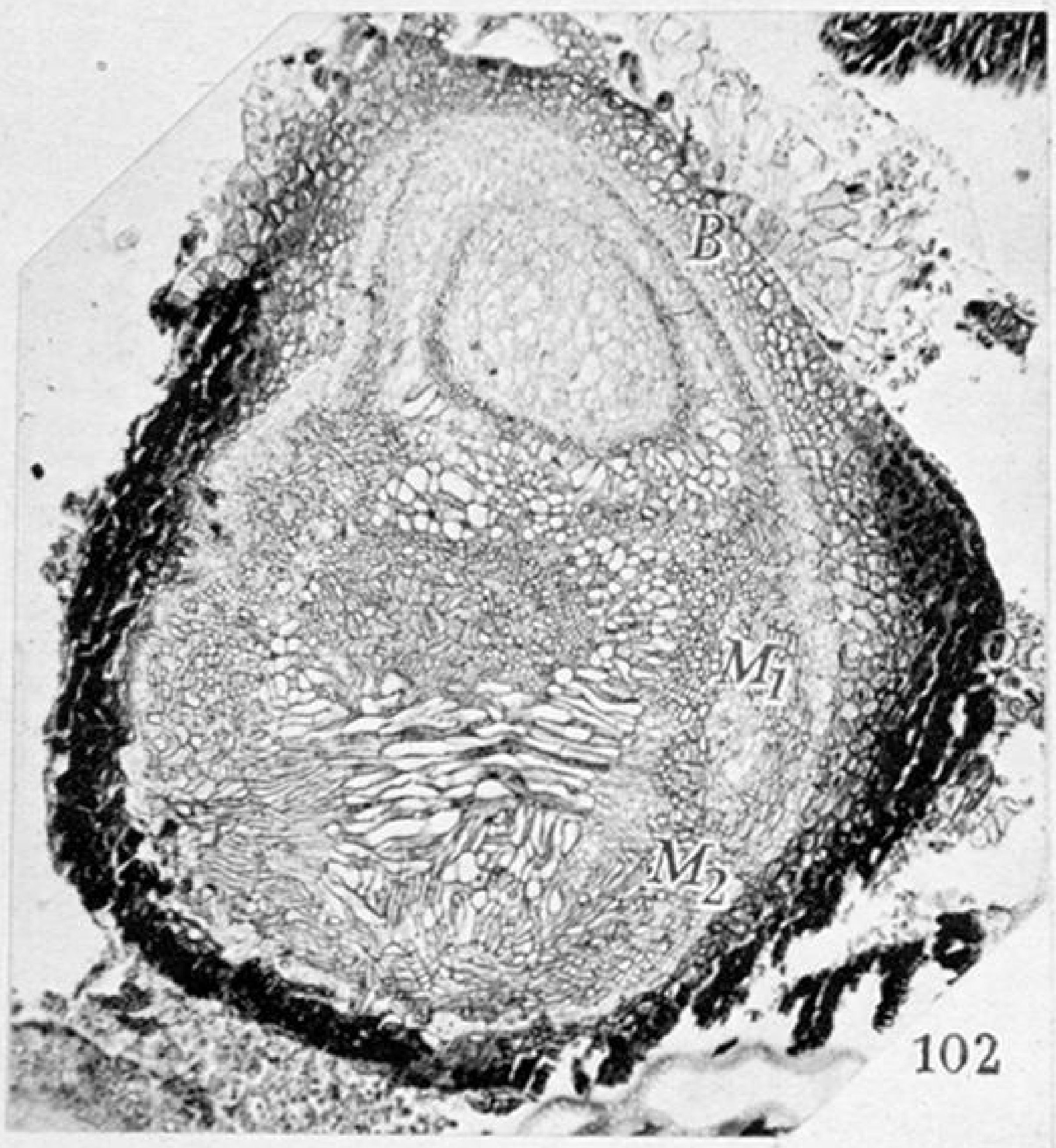

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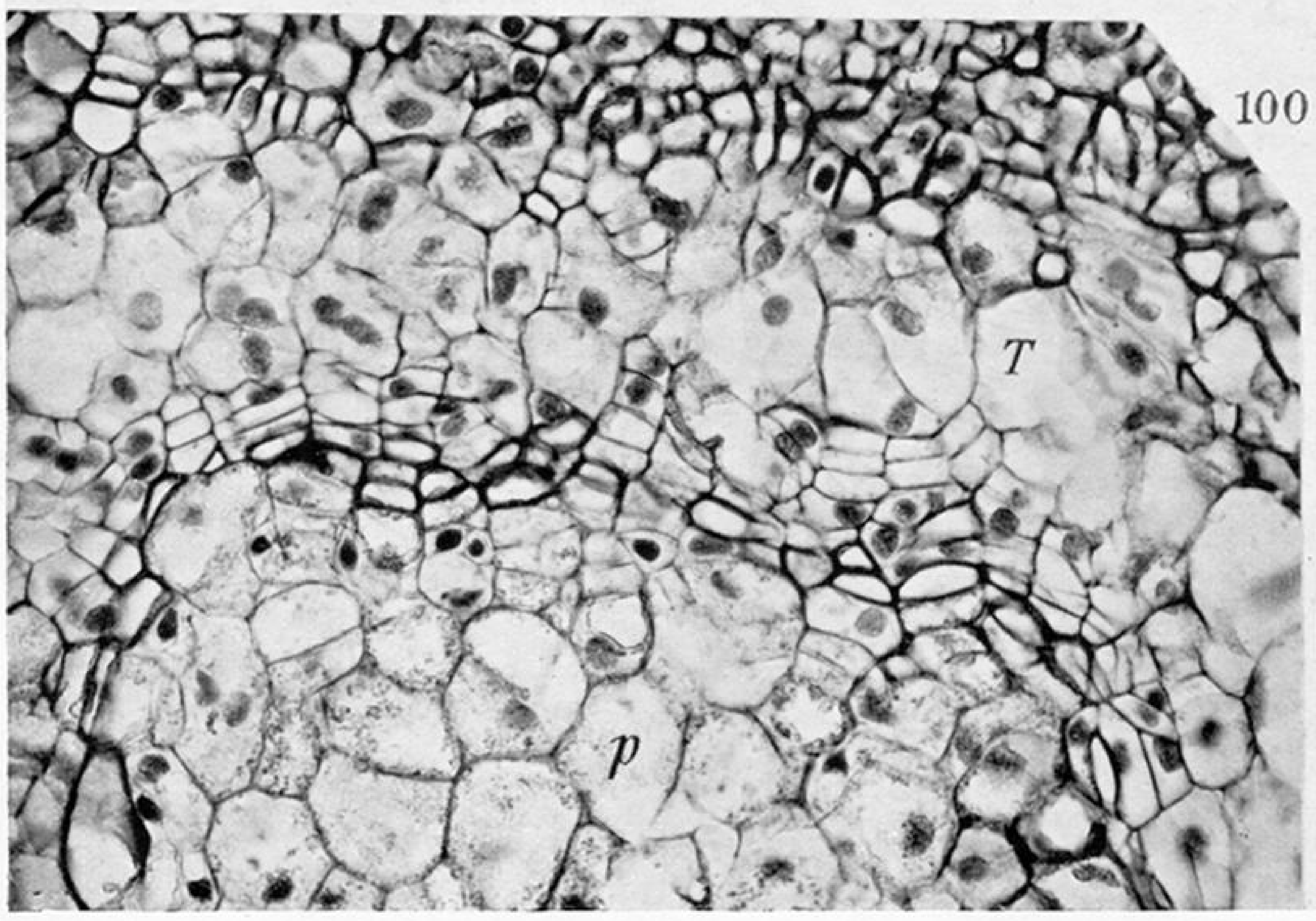

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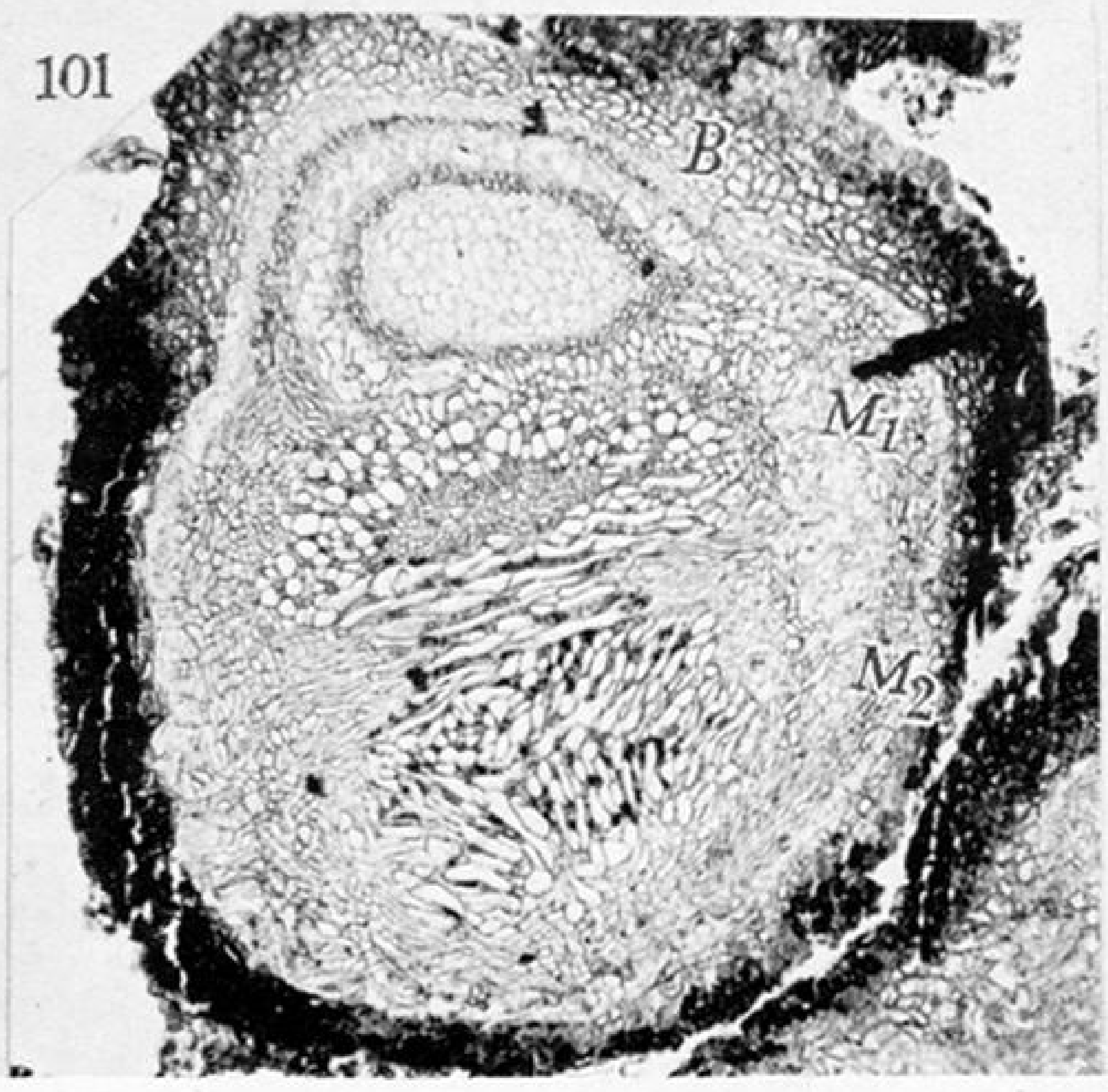

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PLATE 13

FIGURE 98. Lateral segment of a shoot in transverse section showing the shoot meristele (M) disposed in a sickle-shaped configuration. Attached to the meristele is the solenostele ($X1$) of a bud the apex of which has been damaged. Within the cylinder, in close proximity to a lateral bud (B) an inner cylinder of xylem $X2$ has been differentiated from the pith. (× 35.)

FIGURE 99. Same as above: part of the bud stele, more highly magnified. *p*, pith, cells of which are seen to be dividing to form a cambium-like layer. Cells derived from this layer are forming the xylem ($X2$) of the inner vascular cylinder; *ph*, inner phloem, and $X1$, xylem, consisting of tracheides (T) of outer vascular cylinder. (× 300.)

FIGURE 100. Same as above: part of the bud stele, showing clearly the pith (*p*), the actively dividing cambium-like layer and tracheides (T), which are being formed from it. (× 300.)

FIGURE 101. Lateral segment of a shoot in transverse section showing the solenostele of a bud (B) associated with two abnormally disposed meristeles, $M1$ and $M2$; the outermost meristele ($M1$) has become medullated. (× 40.)

FIGURE 102. A section of the same material higher up showing the well-marked medullation of meristele, $M1$; above this level the meristeles fade out. (× 40.)

FIGURE 103. Part of figure 108, plate 14, more highly magnified to show medullation, etc., within the band of xylem. (× 80.)


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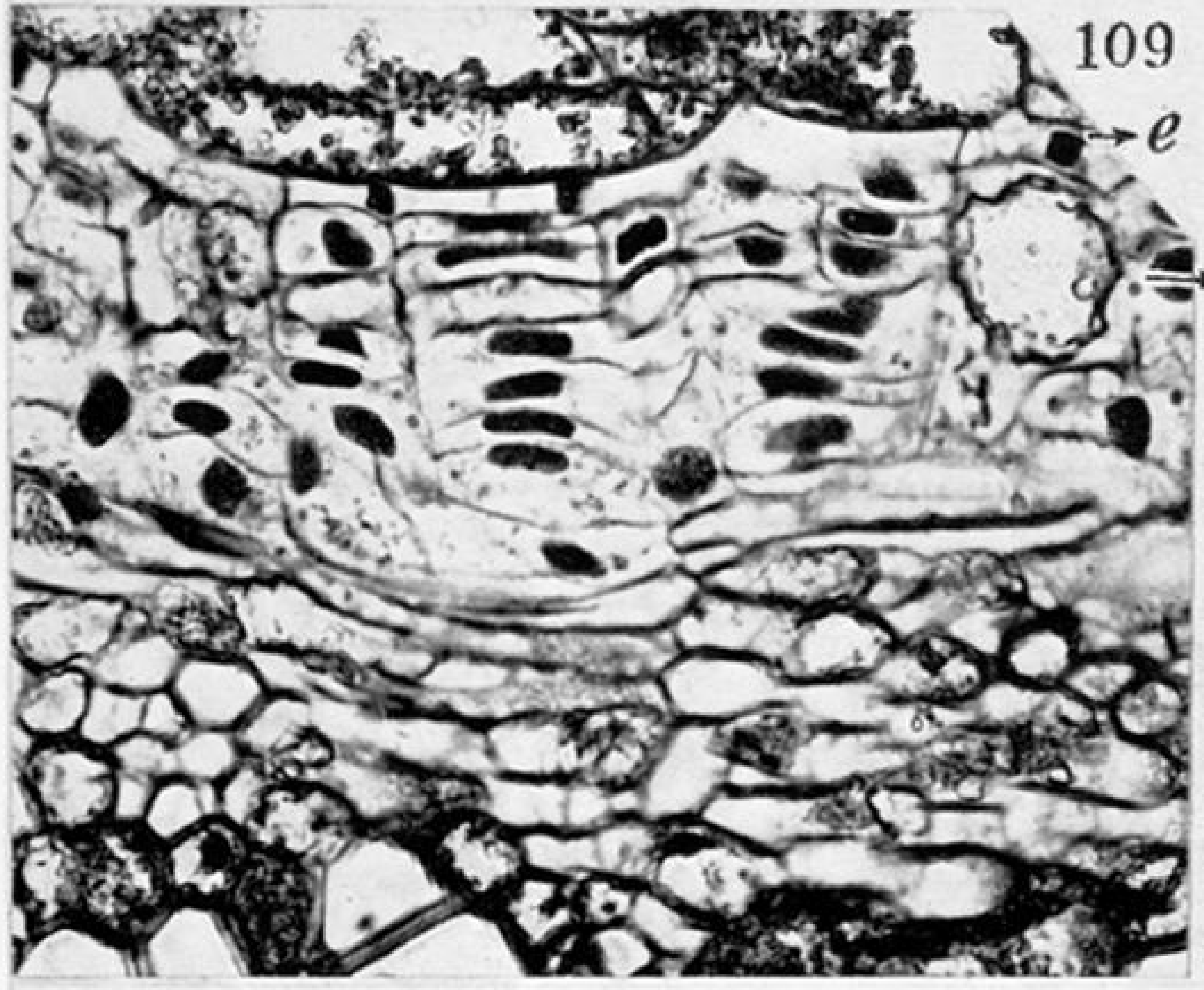
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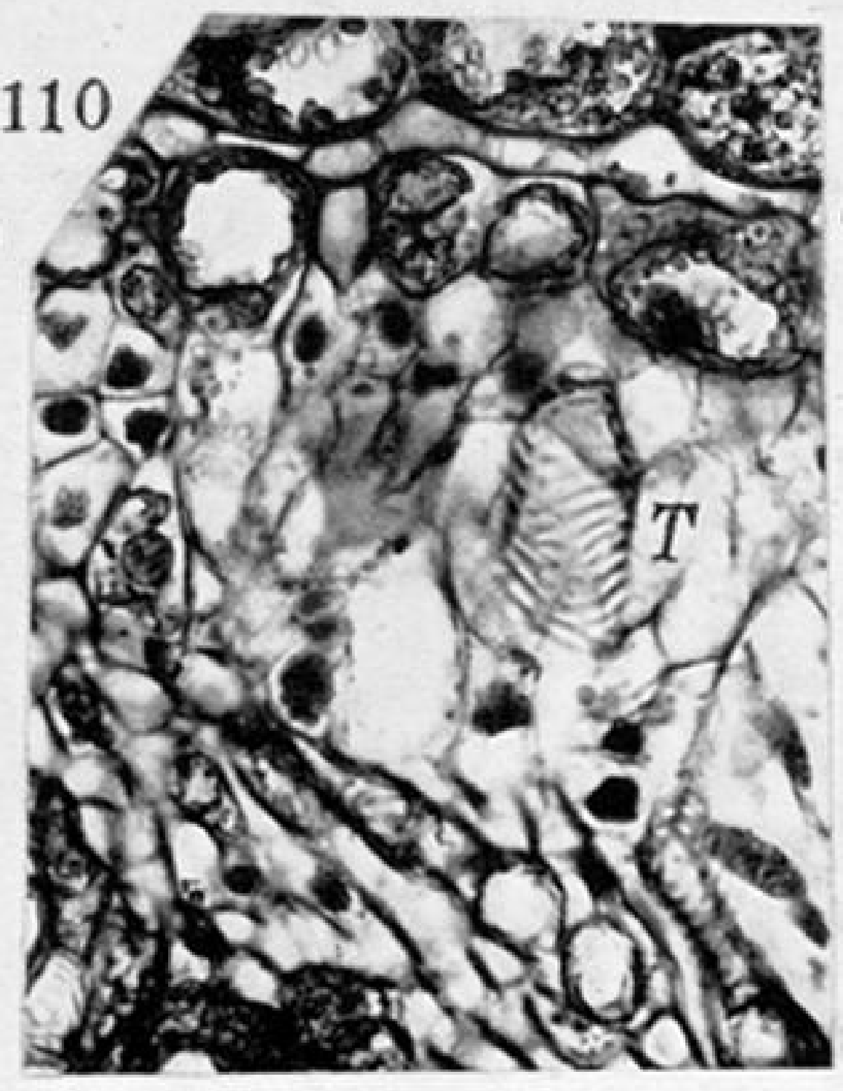
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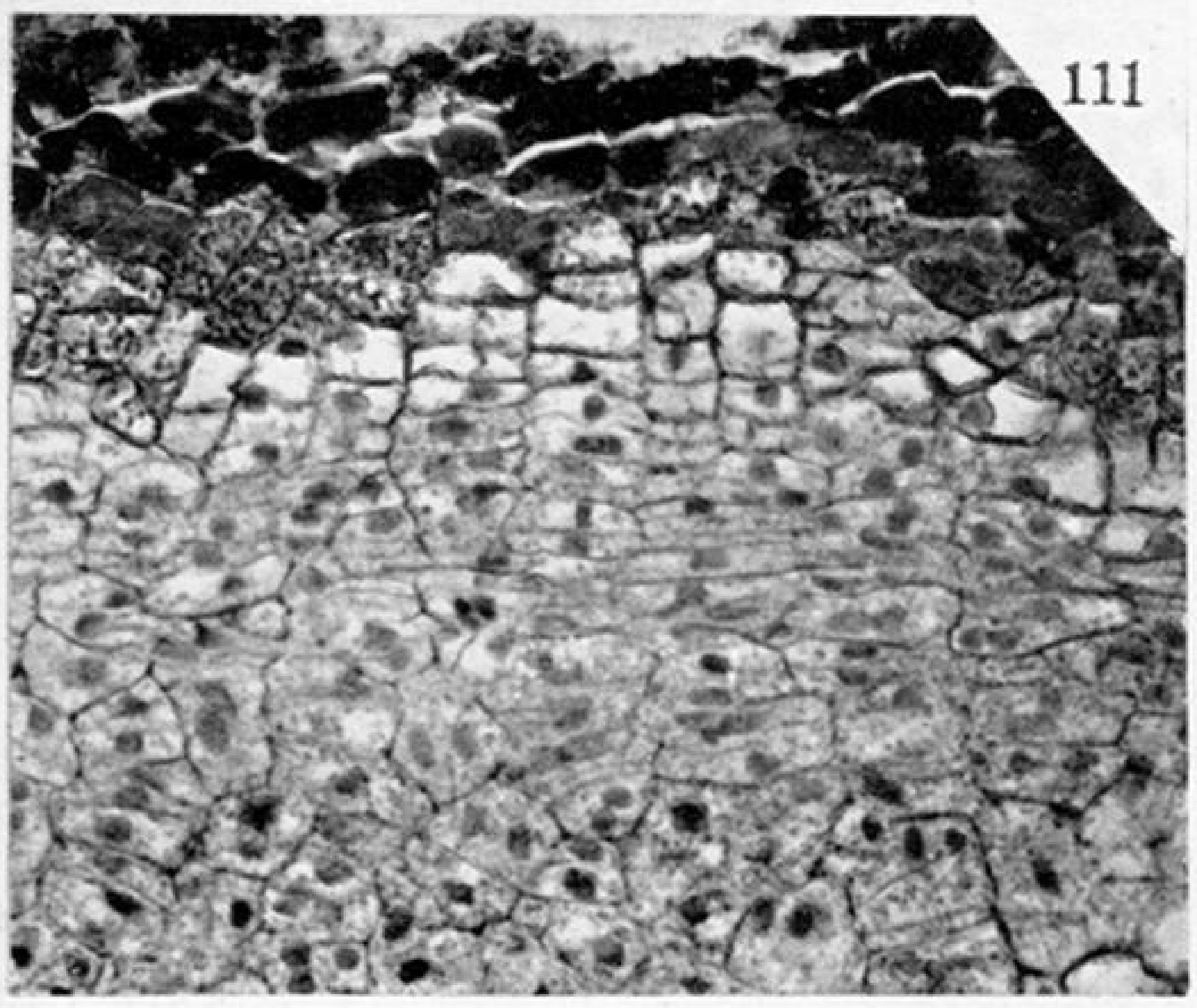
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PLATE 14

FIGURES 104–107. Four transverse sections, in basipetal sequence, of a meristele which had been longitudinally divided into two portions (M, $M1$), above the level shown in figure 33; B, vascular tissue of a bud which has become conjoined with $M1$. The effect of tensile stress on incipient vascular tissue with concomitant development of parenchyma is illustrated. On proceeding downwards, as the stress diminishes, the development of parenchyma from incipient vascular tissue also diminishes. Figure 105 shows an intra-meristele pith and endodermis. (×50.)

FIGURE 108. Part of a transverse section of a solenostelic shoot (with a leaf-trace below). Within the band of xylem a pith, internal endodermis and some internal phloem have become differentiated (position indicated by arrow); these having no connexion with the exterior. (×18.) (See figure 103, plate 13.)

FIGURE 109. Transverse section of a meristele with a well-developed cambiform pericycle (p); e, endodermis; tracheides towards lower side. (×300.)

FIGURE 110. Transverse section of a meristele with a well-developed but somewhat irregular pericycle. Tracheides (T) can be seen in the pericycle. e, endodermis. (×300.)

FIGURE 111. Transverse section of an isolated terminal region showing the origin of 'cortical' parenchyma (above) from incipient vascular tissue (below). The outer cells in the region of the incision are dark and corky. (×200.)