THE COMPARATIVE INVESTIGATION OF APICES OF VASCULAR PLANTS BY EXPERIMENTAL METHODS

By C. W. WARDLAW

Department of Cryptogamic Botany, University of Manchester

(*Communicated by H. G. Cannon, F.R.S.—Received* 23 *December* 1949—*Revised* 4 *March* 1950)

[Plates 31 to 35]

CONTENTS

	PAGE
1. Introduction	584
2. Methods	584
3. Experimental observations: ferns	584
(*a*) *Osmunda regalis* and *Todea barbara*	584
(*b*) *Angiopteris evecta*	585
(*c*) *Pteridium aquilinum*	587
(*d*) *Polypodium vulgare*	588
4. Experimental observations: primula	588
(*a*) Normal development	588
(*b*) Isolation of the apical meristem	591
(*c*) Effect of removing leaf primordia	595
5. Discussion	596
References	600
Explanation of plates	601

A considerable diversity of histological constitution is to be found in the apices of seed plants, and these again differ notably from those of eusporangiate and leptosporangiate ferns. Yet, in their growth and morphogenetic activity, the vegetative apices of all classes of vascular plants have much in common, i.e. they give rise to a vasculated axis with regularly disposed lateral members. Comparative investigations of apices by strictly anatomical methods have definite limits which are soon reached. In the present investigation the aim was to see if, when the same experimental treatments are applied to very differently constituted apices, closely comparable or divergent organographic developments ensue.

When the shoot apex in selected eusporangiate and leptosporangiate ferns and in species of *Primula* was isolated by vertical incisions from the lateral organs and tissues, with concomitant severing of the incipient vascular tissue or procambium, the apex continued to grow and gave rise to a short vasculated leafy shoot in which the normal anatomical pattern was soon reconstituted. Relevant data for *Dryopteris aristata* have appeared in earlier volumes of these *Transactions*. Comparable data for eusporangiate and other leptosporangiate ferns are now described and illustrated. In *Primula*, in and just above the region of the incisions, the vascular tissue of the new axial growth was in the form of an uninterrupted 'cylinder', conforming in outline with the triangular or rectangular contour of the isolated plug. The experimental materials have afforded clear evidence of a basipetal development of vascular tissue from pith cells, strands of the new vascular system eventually becoming conjoined with those of the parent shoot. In the leaf-bearing region of the new shoot the vascular cylinder was interrupted by leaf gaps as in the normal development in *Primula* and in ferns. A prevascular ring, with foliar gaps in the regions of leaf insertion, is present at the shoot apex in *Primula*, this being comparable with the arrangements in ferns such as *Dryopteris*. When all the very young leaf promordia were successively removed, the shoot was found to have an uninterrupted ring of vascular tissue, as in equivalent experiments with *Dryopteris* and other ferns. The experimental data so far obtained thus show that when the same treatments are applied to very differently constituted apices, closely comparable results are obtained. The implications of this finding are discussed in relation to (i) the importance of the cellular constitution of apices in organogenesis, (ii) the the diversity of apical constitution in vascular plants at large, (iii) the apex as a self-determining region, (iv) the inception and subsequent development of the vascular system in different classes of plants, and (v) the relative contributions of axis and leaves to the vascular system.

1. Introduction

In earlier papers the results of using a technique whereby the apical meristem in ferns can be isolated laterally from the adjacent organs and tissues have been described (Wardlaw 1947, 1949*a*). The method, which consists in making three or four vertical incisions near the base of the apical growing point, i.e. above, or through, the youngest leaf primordia, has the effect of severing the incipient vascular tissue which, in leptosporangiate ferns, lies immediately below the apical meristem. The isolated meristem thus continued its growth seated on a plug of pith parenchyma. This simple procedure has afforded a valuable means of investigating growth and morphogenesis at the shoot apex and has shed new light on such problems as the self-determining nature of the apical meristem, the distribution of growth in the apical and subapical regions, organographic features of leaf and bud formation, the inception of the vascular system and other aspects of stelar morphology. Other surgical treatments of the apex have yielded information on leaf formation and phyllotaxis, the morphological status of leaves and buds, and the regulated development of the leafy shoot (Wardlaw 1949*b*, 1949*d*).

In these investigations the shoot apex of a leptosporangiate fern, *Dryopteris aristata* Druce, was used. It seemed probable that by observing the results of these techniques when applied to other ferns, in particular to some of the primitive eusporangiate ferns, and to seed plants, comparative data on morphogenesis of considerable interest might be obtained. Already Ball (1948) has shown that the isolation technique can be applied to the apex of *Tropaeolum majus*. A considerable diversity of histological constitution is known among the apices of seed plants, and these again differ notably from those of leptosporangiate ferns. Yet in their main organographic developments both seed plants and ferns have much in common, e.g. the growing apex gives rise to a vasculated axis with regularly disposed lateral members. The comparative investigation of these apices by strictly anatomical methods has definite limitations. Whether or not comparative investigations by experimental methods will be attended by fruitful results and open up new lines of research is one of the objects of the present inquiry. In the present paper an account is given of experimental investigations of the apices of *Osmunda regalis*, *Todea barbara*, *Angiopteris evecta*, *Pteridium aquilinum* and *Polypodium vulgare*, as exemplifying a wide selection from the ferns, and of species of *Primula* from among the dicotyledons. The aim was to see if, when the same experimental treatments were applied to very differently constituted shoot apices, closely comparable or divergent organographic developments would ensue.

2. Methods

The methods are as already described (Wardlaw 1944*b*, 1947, 1949*a*), suitable cultural conditions being provided for the different species.

3. Experimental observations: ferns

(*a*) *Osmunda regalis* and *Todea barbara*

The Osmundaceae are considered to occupy a central position among primitive ferns. In the species under consideration the stele is a centrally placed medullated protostele.

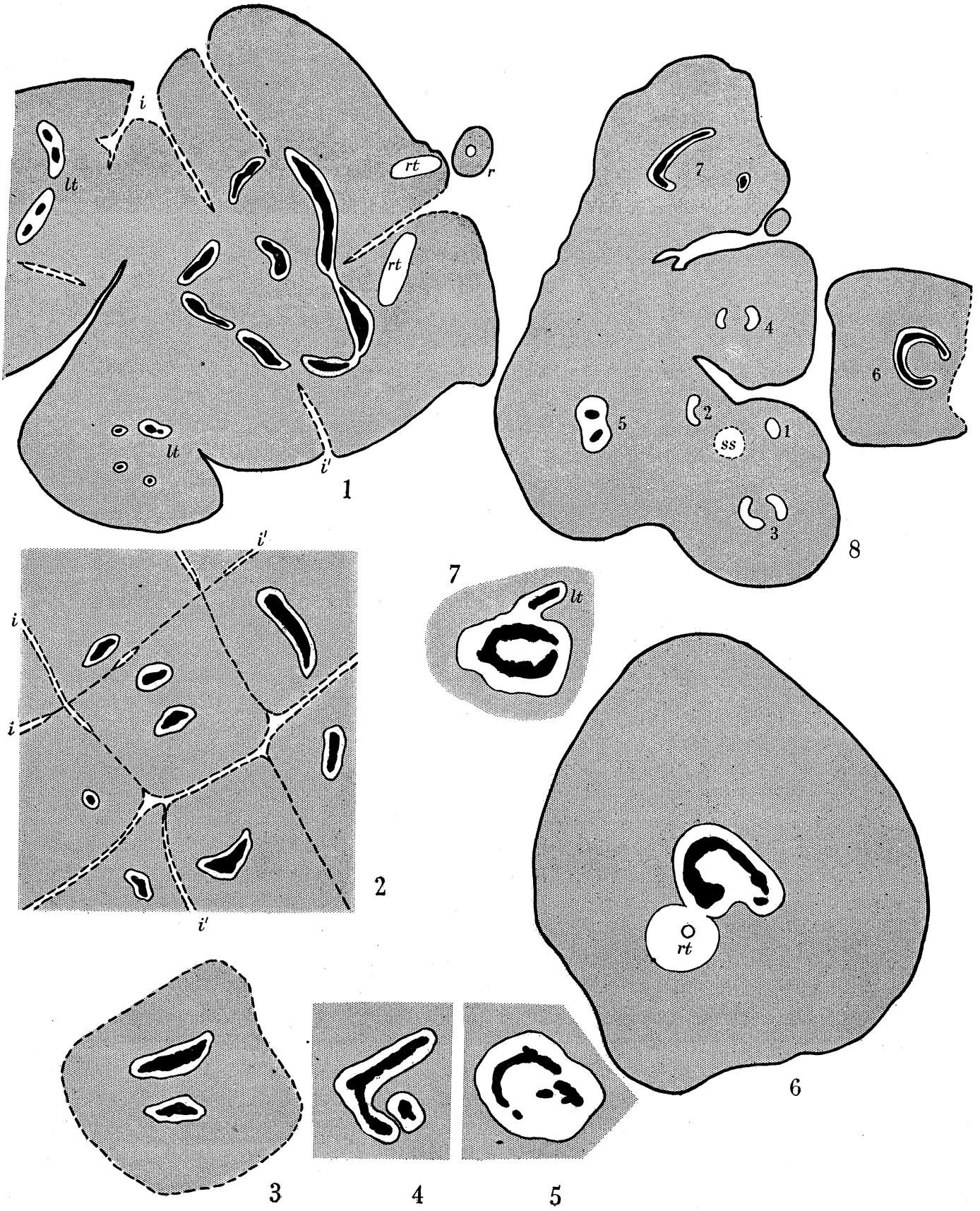
Relative to the large and accessible apices of *Dryopteris*, the apices of these ferns are small, delicate and difficult of access in that they are sunken in the midst of a dense assemblage of highly mucilaginous leaf bases. Moreover, when the apical meristem was isolated by vertical incisions above the youngest leaves, many specimens became necrotic. Several apices, however, grew on after being isolated and developed into short shoots bearing small, reduced, pinnate leaves (figure 28, plate 31).

In these species the normal adult shoot has a medullated protostele; the xylem is dictyoxylic, parenchymatous gaps being associated with the insertion of the leaf traces. Figure 29, plate 31, shows an incised shoot of *Todea* in cross-section, above the lower limits of the incisions. Figure 30, plate 31, shows the normal stele, below the experimental region, with a leaf trace and a root. In figure 31, plate 31, at a level above the base of the incisions, which had traversed the outer tissues of the stele, the pith and conducting tissues show a diminution in cross-sectional area. The outer stelar tissue, which was not fully differentiated at the time of the operation, has undergone a marked parenchymatous development. Still higher up (figure 32, plate 31) this parenchymatous tissue is seen to form the cortex of the new shoot, or isolated terminal region. In this leafless region, the tracheides tend to be disposed in a more or less uninterrupted cylinder, the effect being comparable to that produced by continuous defoliation (Wardlaw 1946*a*). Above this level, in the small shoot produced by the growth of the apex, leaves have been formed, and in cross-section the leaf traces are seen in relation to the small central stele (figure 33, plate 31). The anatomy of this region closely resembles that of a young sporophyte plant. although, as figure 28, plate 31, shows, the leaves are like small adult leaves rather than the juvenile leaves found on the young sporophyte. In *Osmunda regalis* and *Todea barbara*, then, the effect of isolating the apex is to bring about an immediate reduction in the size of the shoot and its anatomical complexity. Since in these ferns we are dealing with a centrally placed protostele with a small pith, the vascular supply to the apex is not severed by the experimental treatment.

(*b*) *Angiopteris evecta*

This species exemplifies the bulky eusporangiate type of fern organization. The young sporophyte is protostelic, but larger shoots are characterized by a complex dictyostelic and polycyclic vascular system. In laying bare the apex of this fern care has to be taken in dissecting away the overlapping stipulate leaf bases. The apical meristem is, however, large enough to admit of incisions being made within, i.e. on the adaxial side of, the youngest visible leaf primordia. Figures 34, 35, plate 32, show early stages in the growth of such isolated meristems in different specimens; the small size of the new growth is in marked contrast to the massive parent shoot with its fleshy leaf bases.

The experimental plants were propagated from stipular buds and had enlarged to the point where two vascular rings were present in the mature region below the apex. The isolated meristem showed active growth, so that by the time the specimens were fixed for sectioning small bulky plants had been formed. One specimen yielded the data illustrated in figures 1 to 8, and figure 38, plate 32. The appearance of the polycyclic shoot near the base of the cuts is illustrated diagrammatically in figure 1. As figure 2 shows, the two central or medullary strands (i.e. of the inner vascular meshwork) were included in the isolated central plug of tissue. On passing upwards in the shoot these meristeles are seen to


i
lt
rt
r
rt
lt
i'
1
7
4
6
5
2
ss
1
3
8
i'
i
i
i'
2
7
lt
rt
6
3
4
5

be conjoined (figures 4, 5), and a small medullated protostele, with a more or less complete cylinder of tracheides, is seen to be the effective vascular system of the new shoot (figures 6, 7, and figure 38, plate 32). At the time of fixing, this shoot had given rise to seven new leaves (figure 8), the small protostele (*ss*) being traceable as undifferentiated prestelar tissue to a level just below the apical meristem. As with *Osmunda* and *Todea*, a notable feature in *Angiopteris* was the reduction in the size and anatomical complexity of the new shoot which developed from the isolated apical meristem. In its morphological development the new growth, in fact, approximated closely to a young sporophyte plant or a young lateral shoot bud (Wardlaw 1946*b*). The other experimental materials of *Angiopteris* which were sectioned showed closely comparable developments, e.g. the polycyclic dictyostele was reduced to a medullated protostele (figures 36, 37, plate 32).

(*c*) *Pteridium aquilinum*

This leptosporangiate fern is characterized by a dorsiventral rhizome and by a vascular system of some complexity. The shoot apex is small, sunken and delicate and surrounded by mucilaginous hairs. Many of the apices which were incised above the region of the mucilaginous hairs became necrosed or rotted. However, in a small number of apices in which the incisions just avoided the delicate apical meristem, growth continued and a short length of shoot was formed.

The normal *Pteridium* stele has been described as a perforated polycyclic solenostele, i.e. the vascular network is not simply due to overlapping leaf gaps, as in the dictyostele, but to additional gaps or perforations not associated with leaf insertions. As in *Angiopteris*, the central meristeles were included in the isolated terminal region (figure 40, plate 32), which was taken near the base of the incisions. A notable feature is the very marked growth shown by the central region, suggesting that under normal conditions it is under considerable compressive stress. The new tissue was the result of growth of the central parenchyma and meristele tissue and was most extensive along the lines of intersection of the incisions (figure 40, plate 32). In the region of new shoot growth a transverse section showed two meristeles (figure 41, plate 32); higher up there was a return to the normal vascular system, an inner and an outer group of meristeles being present (figure 42, plate 32). These could be traced to the sunken apical meristem (figure 43, plate 32). This figure shows that in *Pteridium aquilinum*, as in other ferns in an actively growing condition, the incipient vascular tissue (or prestelar tissue) can be recognized as an undivided tissue immediately below the apical meristem. But a little lower down, in relation to the marked development of parenchyma in the pith

---

FIGURES 1 to 8. *Angiopteris evecta*. The apical meristem, isolated from the lateral organs and tissues by four vertical incisions (*i–i'*), has continued to grow and has given rise to a short vasculated axis bearing seven new leaves. Figure 1, transverse section near base of incisions, showing the positions of the incisions and the distribution of the vascular tissue. Figures 2, 3, taken successively above figure 1, show the isolated plug of pith with two meristeles; on proceeding upwards, the vascular system of the isolated region becomes a medullated protostele, figures 4 to 7. The stele of the new shoot (*ss*) can be traced upwards as incipient vascular tissue to a point just below the shoot meristem, figure 8. *lt*, leaf trace; 1 to 7, leaves, with their vascular strands, in order of increasing age; *rt*, root trace; xylem, solid black; other tissues, white; endodermis, continuous line round meristele; incised tissue, broken lines. (Magn. × 14.)

and cortex in the subapical region, and to the insertion of leaves, this incipient vascular tissue becomes divided into separate strands or meristeles. As incisions have not successfully been made to include only the delicate apex, i.e. above the youngest leaf primordium, it can readily be understood from figure 43, plate 32, how it is that the normal stelar pattern is regained in the growing distal region in these experimental materials. (Were it possible to make vertical incisions very close to the apical meristem, the resulting thin shoot might be expected to show vascular developments of an interesting kind.) The results given above are typical of six successful experiments of this series.

(*d*) *Polypodium vulgare*

The rhizome of this dorsiventral fern has a dictyostele with widely separated meristeles. The shoot apex is readily accessible but it is small and delicate, and experience so far has shown that if incisions are made so as to include only the apical meristem, it is invariably destroyed. However, if the incisions are made just outside the apical meristem, growth continues and a short leaf-bearing shoot is formed. A number of these have been obtained both from the main and from lateral shoots. In specimens of lateral shoots where the incisions were only 1 mm. apart (figure 39, plate 32), the whole of the dictyostele was present within the small cross-section of the isolated central plug.

4. Experimental observations: primula

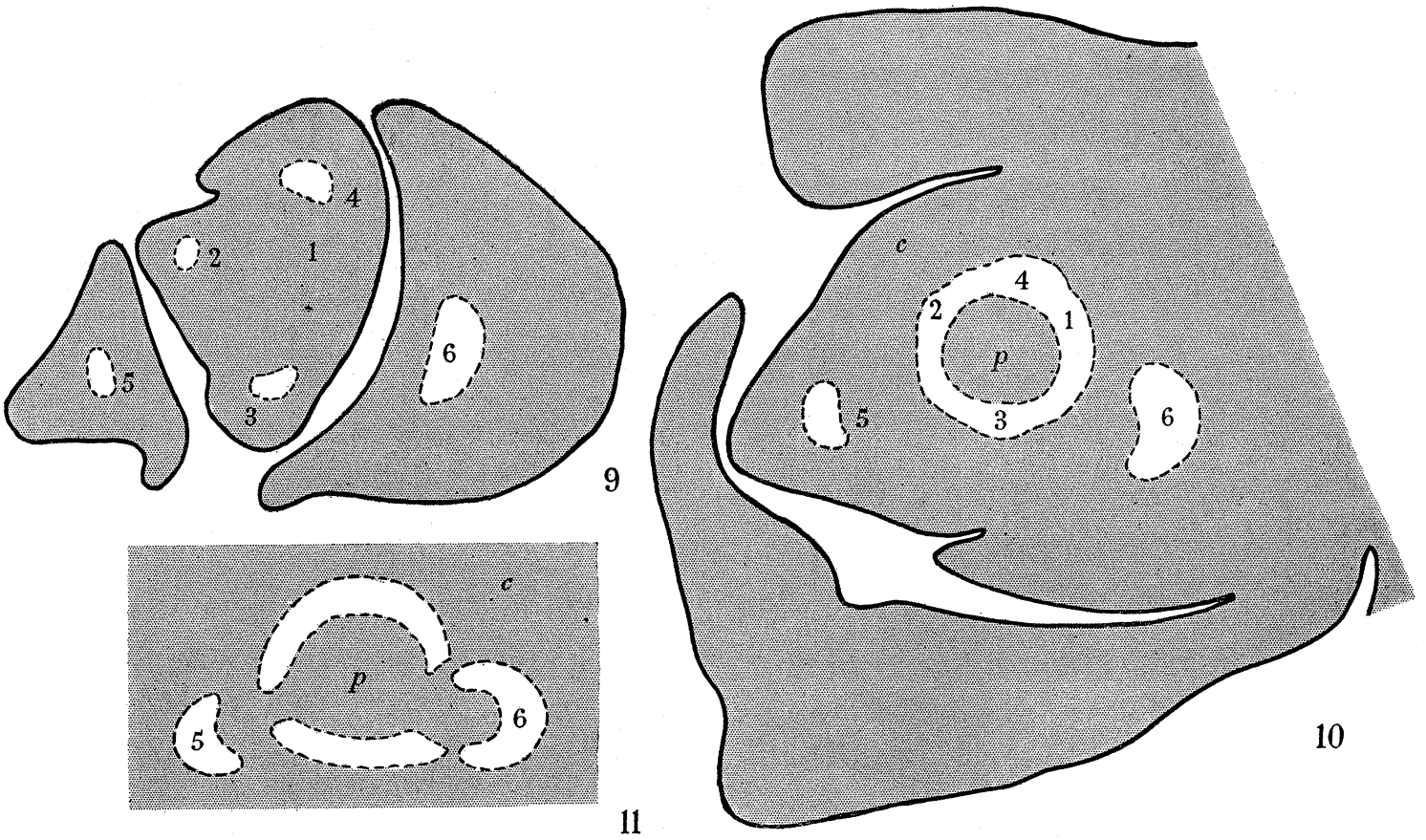
In these investigations, which are essentially of a preliminary nature, the genus *Primula* among flowering plants was selected for particular study. A more general survey of the response of flowering plant apices to experimental treatments will follow as a natural sequel to the present study. Vegetative and floral apices of *P. polyantha*, *P. Wanda* and *P. Auricula* have been used. This particular genus was selected because of its spiral phyllotaxis and certain general similarities between its vascular system and that of dictyostelic ferns. The apices of *Primula* species have proved favourable for experimental purposes in that they are sufficiently large, are easily exposed and remain viable after dissection.

(*a*) *Normal development*

When an actively growing vegetative apex of *P. Wanda* or *P. polyantha* is sectioned transversely in basipetal sequence, a ring of prestelar tissue* (or procambium) can be observed (figure 10, and figure 45, plate 33) a little below the extreme tip of the shoot. This is interrupted only by leaf gaps, the leaf traces being crescentic in outline and undivided (figure 11; figure 46, plate 33; and figure 56, plate 35). This ring cannot be distinguished right up to the apical meristem as in ferns (figure 9); it becomes perceptible just above the level of the insertion of the traces of leaf primordia P_4 and P_5. The youngest leaf primordium, P_1, which is situated close to the extreme apex of the shoot (figure 44, plate 33), consists of a mound-like outgrowth of meristematic tissue; at this early stage its incipient vascular

* The term *prestelar tissue* has been suggested (in discussions between Professor R. H. Whetmore and the writer) as a comprehensive term that may be applied to all vascular plants to indicate the uppermost limit of differentiating vascular tissue in the shoot. It would thus comprise the *incipient vascular tissue* recognized by the writer immediately below the apical meristem in ferns, and the *residual meristem*, the *provascular ring*, the *prodesmogen*, the *desmogen* and *procambium* as variously used in describing differentiation at the apex of seed plants.

strand cannot readily be seen in transverse sections (figure 9), i.e. it is either not yet differentiated or it is not visible with the staining techniques so far used; the position of the prestelar tissue at the apex is suggested in figure 27, p. 595. The leaf traces of primordia P_1 to P_4 form part of the prestelar ring, or they become confluent with it; part of the ring certainly does not originate from the leaf traces but is cauline or axial in origin. In the literature relating to the inception of primary vascular tissue in flowering plants a generally accepted view is that the vascular system of the shoot consists of decurrent strands from the

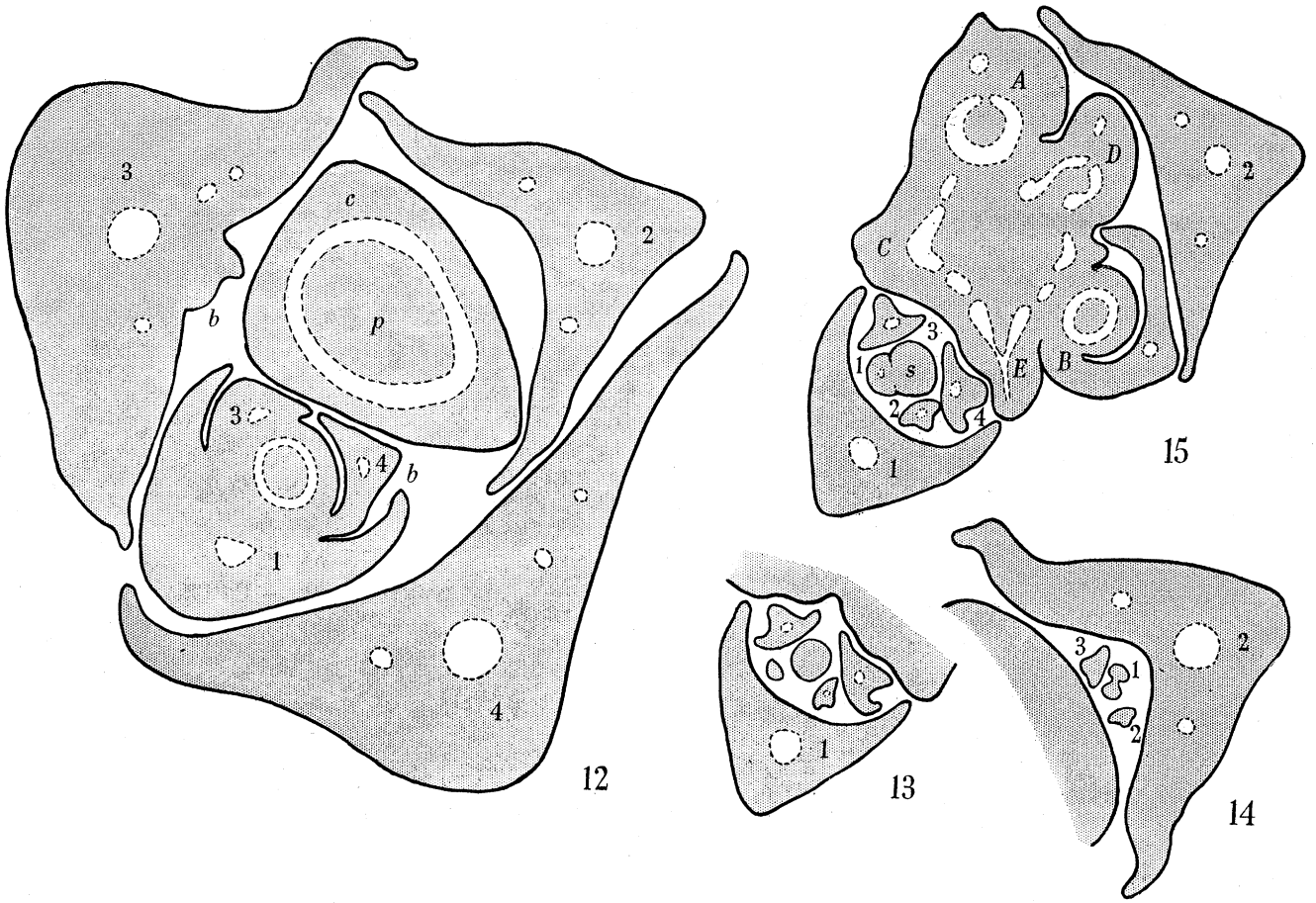


FIGURES 9 to 11. *Primula Wanda.* Transverse sections of a vegetative shoot apex in basipetal sequence. Figure 9, very close to the shoot apex, shows leaf primordia 2 to 6, each with its single vascular strand (white). The position of the youngest primordium (P_1) is indicated. Figure 10, a little lower down, shows the prestelar ring, situated between the cortex (*c*) and the pith (*p*); the vascular strands of primordia 2 to 4 have become confluent with the ring tissue. Figure 11, still lower down, shows the ring of incipient vascular tissue (or procambium) interrupted at the 'insertion' of the vascular strands (leaf traces) of primordia 5 and 6. (Magn. × 110.)

leaves, the presence of an axial stele, with some exceptions, being more or less explicitly denied. But in figure 10 it will be seen that prestelar tissue is present above the insertion of P_5 and P_6 although there are no leaf primordia immediately above these positions; and the same can be said of P_4. Two possible explanations suggest themselves: either the decurrent vascular strand from a primordium becomes extended tangentially in the shoot (or a chemical factor proceeding from the leaf primordium and causing differentiation spreads out tangentially) or vascular tissue is differentiated in the shoot just as it is in the leaf

primordia. And, indeed, unless we assume that there are fundamental metabolic differences between shoot and leaf apices, which seems improbable, there is no reason to suppose that there should not be axial as well as foliar prestelar tissue.

The successive stages in the development of the vascular system as shown in the basipetal sequence in figures 9 to 11, and figures 45, 46, plate 33, are not unlike those seen in *Dryopteris aristata* (Wardlaw 1945). Not all dicotyledons show the kind of differentiation illustrated here for *Primula Wanda*, but prestelar rings in the apical region have been demonstrated



FIGURES 12 to 15. *Primula polyantha*. Transverse sections of a shoot at the level of transition from the vegetative to the floral region. Figure 12, about the upper extremity of the vegetative region, shows a stele consisting of an uninterrupted cylinder of primary vascular tissue (white) surrounding a central pith (*p*); the last formed leaf (1) has a large axillary bud, also with a cylindrical stele; 2, 3, 4, older leaves. Figures 13, 14, illustrate the extent of bud development in the axils of leaves 1 and 2 respectively. Figure 15, transverse section of the axis in the floral region, showing the greatly divided shoot stele, and the bract and flower-pedicel traces (circular); *A* to *E*, flower buds and bracts, in the order of their appearance; *c*, cortex. (Magn. × 40.)

in a number of species by Louis (1935) and Kaplan (1937). In *Primula*, as in ferns, the interruptions in what would otherwise be a continuous vascular cylinder are due to the insertion of the crescentic leaf traces (figure 11, and figure 56, plate 35); above the insertion of a leaf trace a parenchymatous gap is typically present.

Observations of some interest were made on very young inflorescence apices, i.e. those which had recently passed from the vegetative state. Figures 12 to 15 show selected serial

sections from the vegetative to the floral region. For a brief period at the time of transition, the shoot apex produces no foliage leaves, and in this region the shoot is characterized by an uninterrupted cylinder of vascular tissue (figure 12). Higher up, the shoot stele is interrupted by the insertion of the traces of the bracts and their axillary flowers (figure 15). The floral pedicel is a shoot-like structure and has a cylindrical vascular system. In the region of transition from the vegetative to the floral shoot, buds of unusually large size have developed in the axils of the last formed vegetative leaves (figures 12 to 15). This suggests that there has been a diminution in the inhibitory effect normally exercised by the terminal apex on lateral shoot buds.

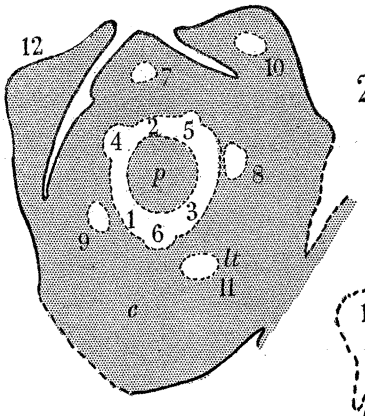
(*b*) *Isolation of the apical meristem*

Although the shoot apex in *Primula polyantha* (figure 27, and figure 44, plate 33) is small by comparison with that of *Dryopteris aristata*, it is, nevertheless, easily laid bare, and its isolation by vertical incisions above or through the axils of the youngest leaves was achieved in a number of specimens. Where the last-formed primordium (P_1) was very young, inconspicuous and close to the shoot apex, it would lie *within* the incisions. The external appearance of one of the experimental apices after some growth had taken place is illustrated in figure 50, plate 34. Some materials for sectioning were fixed after 2 to 3 weeks' growth and others after longer periods.

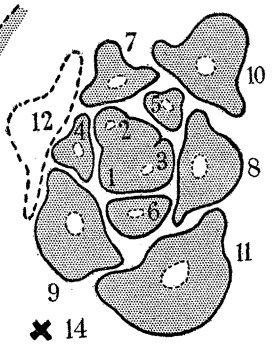
As in the *Dryopteris* experiments, the incipient stelar tissue, which may be present below the apical meristem, would be severed by the vertical incisions, i.e. the isolated apex grows out from a basal region of pith parenchyma. In a majority of the specimens the apex continued its growth and formed a short length of shoot bearing leaf primordia (figures 16 to 22, 24 to 26, and figures 51 to 55, plate 34). In specimens which were fixed after 2 to 3 weeks a continuous ring of prestelar tissue was perceptible a short distance below the apical meristem, i.e. no leaf primordia were present in this region and there were no leaf gaps. Where the apical meristem had been isolated by four incisions, the vascular tissue in the experimental region conformed with the outer contour of the plug and was rectangular in cross-section (figure 23, and figures 57, 58, plate 35). Where the apex had been isolated by three incisions, the vascular system had a triangular conformation in the experimental region (figure 18, and figures 53, 54, plate 34). As the shoot grew on, however, and leaves were formed, there was a return to the normal radial symmetry (figures 20 to 22 and 24 to 26).

As in similar experiments with *Dryopteris* (Wardlaw 1947, 1949*a*, 1949*c*), the leaves which were formed by the isolated apex were in normal or approximately normal phyllotactic sequence with the older leaves present at the beginning of the experiment (figures 18 to 22 and 24 to 26). This was ascertained by examining serial sections in basipetal sequence, the vascular ring just below the region of new growth being interrupted periodically by the gaps of the leaves close to the incised apex.

In figure 22, the first leaf to arise on the isolated shoot apex was leaf *12* (figure 20); this may have been present at the beginning of the experiment as an invisible but incipient primordium. Isolated leaf primordia, with wound scars on their adaxial sides, were present in the positions labelled *13* and *14* in figure 22; there were also small leaf gaps relating to these leaves. The third leaf primordium to be isolated by incision of the apex, leaf *15*, had developed an axillary bud; the corresponding leaf gap is shown in figure 19;

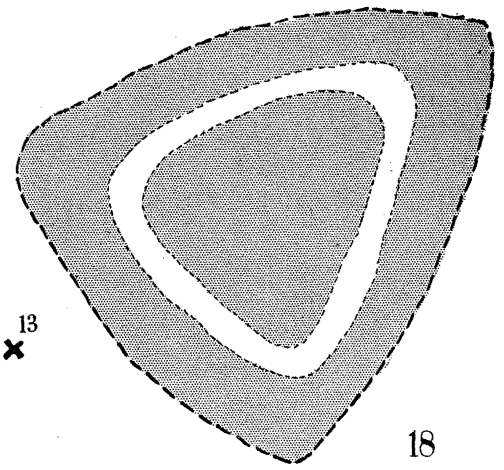


21



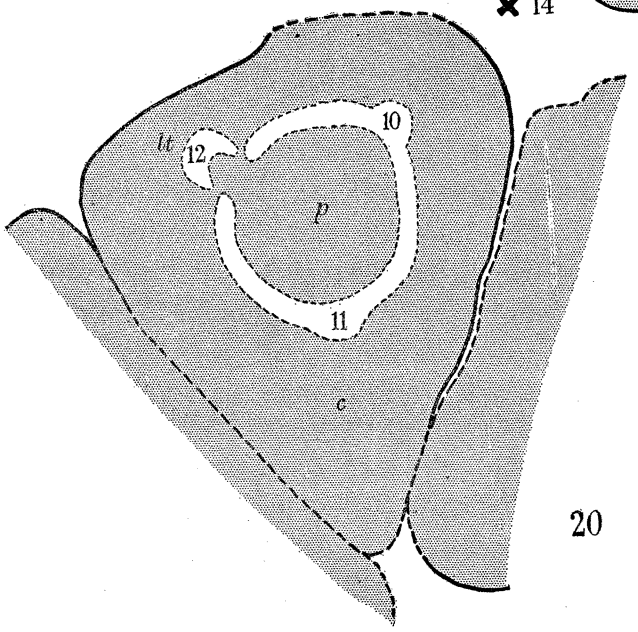
✖ 14

22

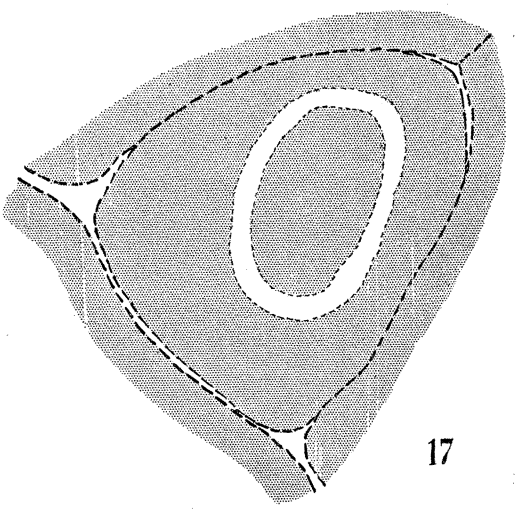


✖ 13

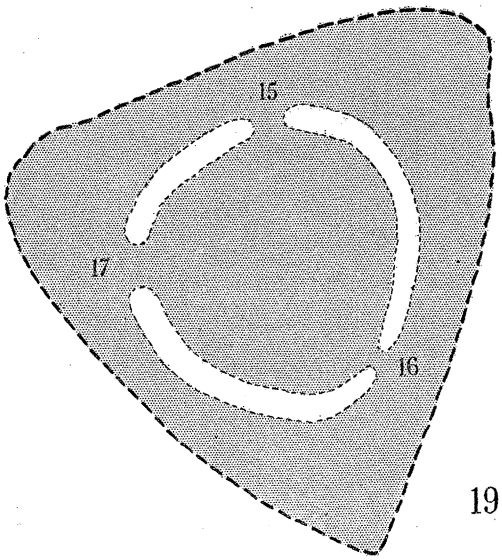
18



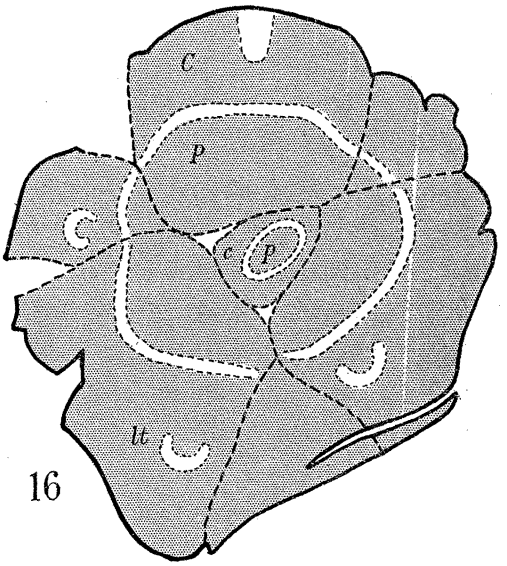
20



17

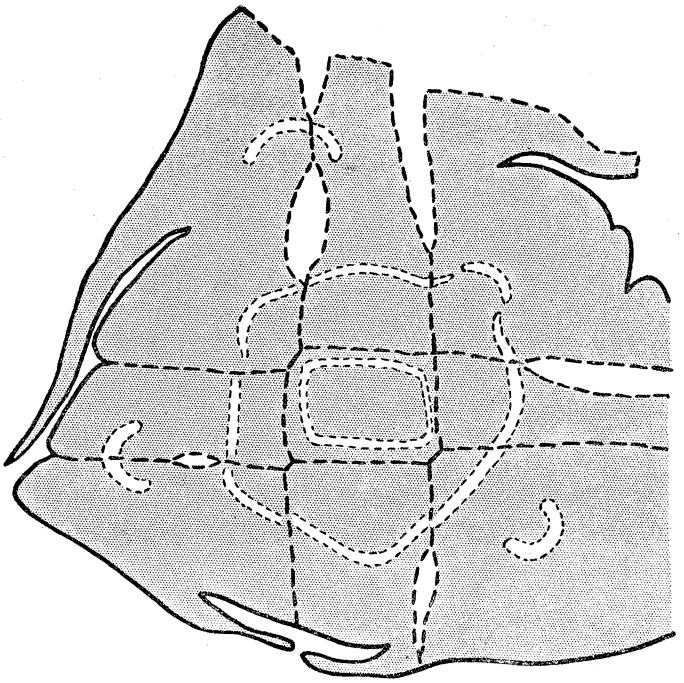


19



16

the gaps relating to leaves *16* and *17*, both with axillary buds, can also be seen at this level. In this specimen, when some allowance is made for displacements of primordia either at their inception or during the growth of the central and lateral regions of the shoot, it is seen that the new leaf primordia are in approximately normal phyllotactic sequence with the older



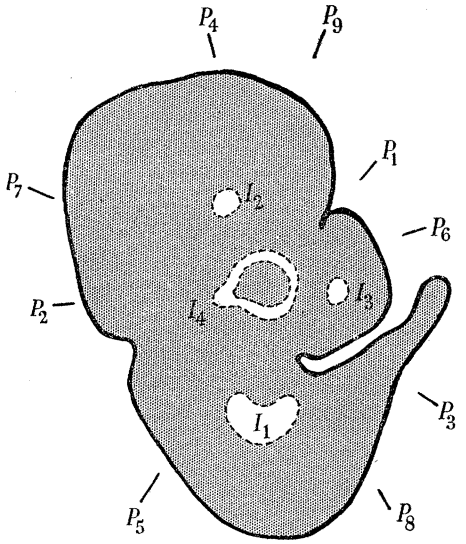
23

FIGURE 23. *Primula polyantha.* An apical meristem isolated by four vertical incisions: two stelar cylinders are present, that in the centre conforming in its outline with the cross-sectional outline of the isolated pith block. (Magn. × 20.)

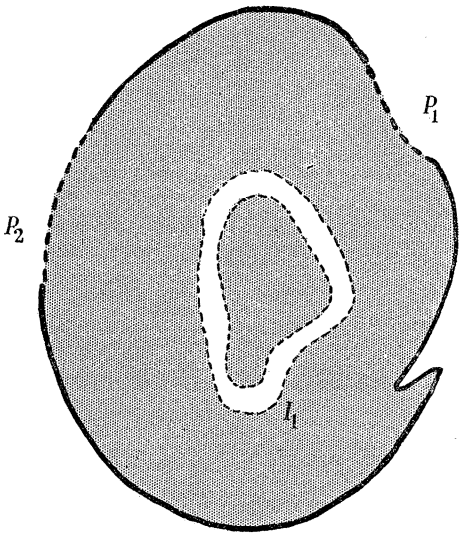
leaves. Figures 24 to 26 illustrate comparable developments in a shoot in which the apex was isolated by four incisions above the youngest visible leaf primordia. I_1 to I_4 are the new primordia in the order of their appearance (figure 24). Figure 25, a little lower down, shows the leaf trace of I_1, the oldest primordium, becoming conjoined with the cylindrical shoot stele, and also the scars where the youngest primordia at the beginning of the experiment,

---

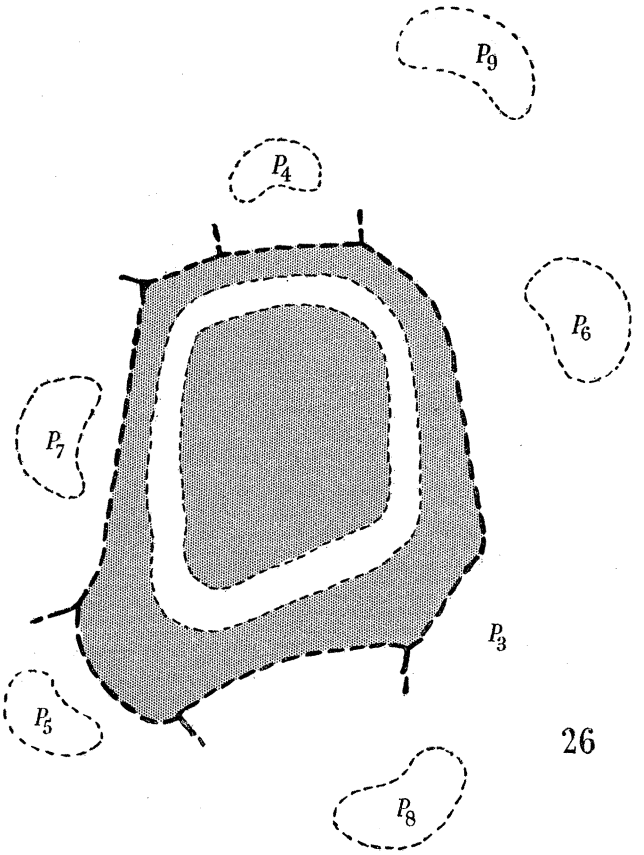
FIGURES 16 to 22. *Primula polyantha.* An apical meristem (isolated by three vertical incisions above the youngest leaf primordia), figure 16, has continued to grow and has yielded a short shoot, with the vascular arrangements shown in acropetal sequence in figures 18 to 21. Figure 19 shows gaps in the vascular cylinder of the shoot which are associated with the leaf primordia present at or near the apex at the time of the operation. Figures 20 to 22 show that twelve new leaf primordia have been formed in approximately normal phyllotactic sequence with the older primordia (13 to 17) present at the beginning of the experiment. Leaf 12, which may have been present on the isolated shoot apex as an invisible but incipient primordium, is the first leaf of the new shoot. Leaves 13 and 14, isolated by the incisions, can be seen in other sections of this series: their positions are indicated in figure 22. Figures 18 and 17 (and 16), in basipetal sequence, show the basipetal or downward differentiation of an uninterrupted cylinder of vascular tissue in the pith of the parent shoot *c*, cortex, *p*, pith, in the isolated central plug; *C*, cortex, *P*, pith of parent shoot; *lt*, leaf trace; vascular tissue, white. (Magn. × 14.)



24



25



26

FIGURES 24 to 26. *Primula polyantha.* An apical meristem isolated by four vertical incisions above the youngest leaf primordia. Figure 24 shows the positions of the four new leaves (I_1–I_4, in the order of their appearance) to which the isolated meristem has given rise on further growth. The positions of the leaf primordia present at the time of the incisions (P 1, 2, 3, 4, etc., in order of increasing age) are indicated, on the assumption that the new primordia I_1 to I_4 are in approximately normal phyllotactic sequence with them. Figure 25, some distance below figure 24, shows the vascular trace of I_1 becoming conjoined with the cylindrical stele of the shoot and the scars where leaf primordia P_1 and P_2, present at the beginning of the experiment, were isolated from the shoot apex by the incisions. Figure 26, considerably lower down than figure 25, shows the isolated central region, with its vascular cylinder, and the positions of the vascular traces of leaves 4 to 9. No trace relating to leaf 3, at the region of intersection of two incisions, could be observed. (Magn. ×40.)

i.e. P_1 and P_2, were incised. In figure 24, the positions in which the older primordia, in sequence with I_4 to I_1, would be expected to occur are indicated, while figure 26, taken lower down, shows the central plug with its induced vascular column, and the surrounding leaf traces. P_3 was apparently obliterated by the intersection of two of the incisions, but it will be seen that P_4 to P_9 occur in approximately the expected positions.

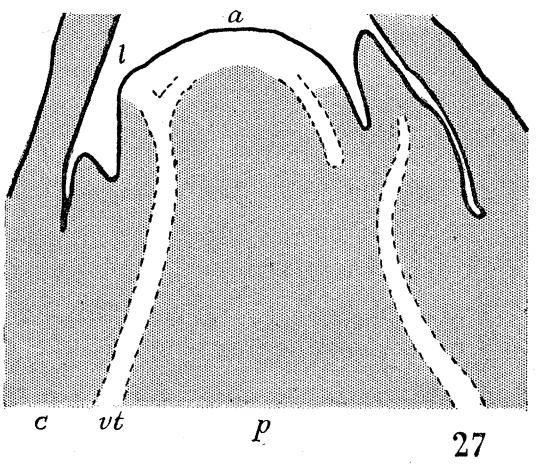


FIGURE 27. *Primula polyantha.* Longitudinal median section of a vegetative shoot apex, passing through the youngest leaf primordium (*l*). The incipient vascular tissue (*vt*) can be traced up to primordium *l*: the relationship of this tissue to the apical meristem (*a*) is suggested; *p*, pith; *c*, cortex (tracing of a photograph). (Magn. × 100.)

Below the region where leaf gaps could be seen, particularly in older specimens, the stele extended downwards, at first as a continuous ring (figures 16, 17), and still lower down as an interrupted ring of discrete strands, unlike any seen in the normal development (figures 54, 55, plate 34, and figures 48, 49, plate 33). In experimental materials fixed after 2 to 3 weeks, these strands faded out in the pith, but in older materials some of them continued their basipetal differentiation and eventually became conjoined with the vascular system of the parental shoot below the base of the incisions. Occasional strands were also observed in the pith.

(*c*) *Effect of removing leaf primordia*

In some specimens the apical meristem was isolated by four vertical incisions, and thereafter the very young leaf primordia which appeared were removed. On further growth the defoliated region of the shoot became a cylindrical column, topped by the 3 to 4 primordia which were eventually left to develop. Sections through the defoliated region of the shoot showed an uninterrupted ring of vascular tissue (figures 57 to 61, plate 35, and figures 47 to 49, plate 33). In other experiments where the apex was continuously defoliated for some time (but not isolated) an uninterrupted cylinder of vascular tissue was also obtained. In some instances, where rather deep incisions had been made on removing the leaf primordia, parenchymatous gaps were present in the vascular tissue. These gaps were due to the formation of wound parenchyma from procambial tissue. Only quite short lengths of shoot have so far been obtained in these defoliation experiments, the apex becoming small and attenuated by the time some 5 to 6 leaf primordia had been removed. But the general result is clear; in *Primula*, as in *Dryopteris*, a vascular column is formed in the shoot in the

absence of developing leaf primordia, this column being typically cylindrical and with no parenchymatous gaps. Figures 47 to 49, plate 33, are cross-sections in basipetal sequence of a shoot in which the meristem was isolated by vertical incisions and all young leaf primordia removed as soon as they were formed; in the upper part of the experimental region a continuous, uniform ring of prestelar tissue is present; in the more mature and fully differentiated region lower down, discrete vascular strands have been differentiated in the ring tissues, the strands, surrounding a wide pith, being separated by narrow rays of parenchyma (figure 47, plate 33). Still lower down, a basipetal differentiation of vascular tissue has taken place in what was originally pith parenchyma; here the discrete nature of the vascular strands is still more evident (figure 48, plate 33). Moreover, in this region, some strands have also been differentiated in the pith of the experimentally induced stele. At a still lower level, the basipetally differentiating vascular tissue is seen to consist of a compact group of discrete strands round a very small central pith (figure 49, plate 33). No comparable stelar configuration is to be observed at any level in the normal shoot. This anomalous stele eventually faded out in the pith of the original shoot.

5. Discussion

In the studies described and illustrated here (and in earlier papers) the apices of selected eusporangiate and leptosporangiate ferns and of the dicotyledonous genus *Primula* have been subjected to the same experimental treatments, viz. (i) the isolation of the apical meristem by vertical incisions from the lateral organs and tissues, and (ii) the continuous removal of very young leaf primordia at the shoot apex. Notwithstanding the differences in the histological constitution of the several apices the results of the first treatment have been closely comparable throughout; the isolated shoot apex has continued to grow and has given rise to a vasculated shoot with leaf primordia. In *Primula*, as in *Dryopteris* and *Angiopteris*, the experimental data indicate that the inception of the vascular tissue is due primarily to the activity of the apical meristem, i.e. there is a basipetal movement of a substance (or substances) from the active meristem which brings about the initial differentiation of vascular tissue (or, in more general terms, this tissue is in some way determined by the active apical meristem). This finding is supported by the data of other experimental and analytical studies, e.g. those of Helm (1932), Jost (1940), Ball (1948) and Hegedüs (1949). When very young leaf primordia were removed from apices of *Primula*, so that the formation or further development of foliar vascular strands was precluded, an uninterrupted cylinder of vascular tissue was formed in the shoot. These data for *Primula* spp. are thus closely comparable with those already obtained for *Dryopteris aristata* (Wardlaw 1944*b*, 1947, 1949*a*), for *Osmunda* spp. and *Todea barbara* (Wardlaw 1946*a*) and *Angiopteris evecta* (Wardlaw 1946*b*).

Helm (1932) found that by removing young leaf primordia from the apices of several dicotyledonous plants the formation of the procambial strands relating to them was impeded or precluded. In some of his illustrations, gaps are present in the stele in proximity to leaf bases which had been dissected off; these gaps show active cell divisions, and the transformation of prestelar or procambial tissue into parenchyma. This is closely comparable to what the present writer has observed in *Primula* spp. where incisions to remove leaf primordia had gone rather deeply into the tissue of the shoot.

Whereas the vegetative apices of many flowering plants are characterized by more or less well-defined histogenic layers, without a distinctive apical cell, or group of initial cells, the ferns, both eusporangiate and leptosporangiate, have a well-defined superficial meristem which originates from a small group of conspicuous initial cells or from a single very conspicuous apical cell. But many intermediate conditions are known; the shoot apices of vascular plants at large exemplify a wide range of construction including those with (i) a single conspicuous apical or initial cell, (ii) several rather less conspicuous initial cells, (iii) inconspicuous but definitely superficial apical cells, (iv) a weakly zoned or layered construction, (v) a fluctuating layered construction, and (vi) a highly definite layered construction. In the cellular constitution of apices as thus set out, some observers may see an evolutionary trend. That the particular histological constitution of an apex is directly or indirectly due to the genetic constitution of the species can hardly be doubted; nevertheless, the shoot apices of all classes of vascular plants are closely comparable in their general mode of growth and in their morphogenetic activities. Indeed, if our observations on different classes of vascular plants were restricted to their external morphology, we should have little reason to suspect the underlying histological diversity of their apical growing points. It thus appears (as de Bary, Sachs and others so clearly saw) that it is the plastic apical region as a whole that is of primary importance in morphogenesis and not its cellular construction, though, on a further analysis, it is seen that the two aspects are probably reciprocal. The plastic apical region comprises the relatively slow-growing region at the extreme tip of the shoot and the more rapidly growing region below it—the subapical region. In view of the close similarity between the apices of ferns and seed plants in respect of growth and morphogenetic activity, the presence of a distinctive apical meristem in the ferns suggests that there is probably a physiologically comparable region at the apex of seed plants, even although its histological basis is less apparent.

In support of this view, which some observers will doubtless consider to be highly problematical, mention may be made of some facts emphasized by Foster (1939, 1949): (i) 'that the distinctness and, to some extent, the type of zonation in the shoot apex vary with the phase of activity of the meristem', and (ii) 'the the number of tunica layers varies widely between species and genera and the sharpness of the boundary between tunica and corpus may fluctuate even within the same species at different phases of its ontogeny' (1949). Moreover, just as Van Fleet (1948) has shown that apparently similar parenchymatous cells of the cortex may have very different physiological reactions, so, too, it may be assumed, as a working hypothesis, that the equidimensional meristematic cells in different regions of the tunica are not identical in their physiological properties. In this view the superficial cells at the summit of the dicotyledonous shoot would be regarded as having distinctive properties, as in the ferns. To this may be added the fact that some investigators have rejected the tunica-corpus theory as not affording an adequate account of particular apices (Reeve 1948). It may, indeed, be said that until the tunica-corpus concept has been elaborated and explored by the methods of physiology and experimental morphology, it must be regarded as being still somewhat formal in character.

The distinctive arrangement of cells in the apical meristem and adjacent regions is determined by the genetical, physiological and physical factors which are at work there, due attention being also paid to the changing geometrical relationships in any enlarging

system. The presence of a large and distinctive apical cell in leptosporangiate ferns, for example, must, in some way, be due to forces present in the growing point; and so, also, for the layered apex in flowering plants. Such ideas are in accordance with the classical view of de Bary that the plant or organ determines the tissue pattern; or, in the words of D'Arcy Thompson (1942), the growing-point might be regarded as 'a comprehensive field of force ...somehow shaping the whole organism independently of the number, magnitude and form of the individual cells', the formation of the apical cell being a particular and necessary expression of this force in certain plants. Wilson (1925), too, has said that 'the physiological anatomy of the individual cell falls into the background...and the apparently composite character which the multicellular organism may exhibit is owing to a secondary distribution of its energies among local centres of action'. Earlier, both Hofmeister and Sachs had maintained that organ growth in plants is the primary fact and cell formation only of secondary significance, while Whitman (1893), supporting this view, pointed out that 'for the same purpose', e.g. the formation of an organ, one, several, or many cells may be used. The truth of this statement becomes apparent when the apices of different classes of vascular plants are compared. But while it may be maintained that the apex functions as a whole, the terminal apical meristem, with its initial cells or layer of cells, is indispensable to the harmonious and orderly (i.e. the 'normal') development of the axis and its appendages.

The arguments outlined above suggest that the differences between the shoot apices in seed plants and ferns may not be so great as is sometimes thought; there is, apparently, a common pattern of growth. The histological differences may be attributed to those genetical factors which affect cell size and growth and to physiological and other factors which are evoked during development; but on those aspects of apical activity where genic factors are involved, practically everything remains to be discovered. A knowledge of how genes control the distribution of growth in the apical and subapical regions, for example, would contribute to a solution of many of the problems that have been raised here.

In actively growing ferns the incipient vascular tissue at the apex is conspicuous in suitably stained preparations. In many seed plants, on the other hand, such a tissue is not easily demonstrated, if it is indeed present. In fact, the vascular system in flowering plants is regarded by some investigators as being entirely of foliar origin. But there are some flowering plants, e.g. *Primula*, where a prestelar ring can be observed close behind the tip of the shoot; a small acropetal extrapolation of this ring would indicate the region of its inception to be just below the meristematic cells of the tunica at the extreme summit of the shoot. It is because of the visible continuity of distal meristem and prevascular tissue in some species that the vascular tissue has been described as originating from 'residual meristem'. In the ferns, the prevascular tissue is not simply 'residual meristem' but is a differentiated product of the superficial apical meristem. In some flowering plants the inception of vascular tissue at the apex is closely comparable with what is found in the ferns. Thus the published illustrations of *Opuntia* (Boke 1941) and of *Hippuris* (Louis 1935) show axial prestelar tissue above the level of the youngest leaves and in close proximity to the most distal group of cells. Investigations by new microchemical techniques may well show that this is a considerably more general phenomenon than has hitherto been recognized. Furthermore, when a fern apex is in an inactive state, the presence of incipient vascular tissue is difficult to demonstrate. If we suppose that the shoot apex in flowering plants is

relatively inactive compared with the rapidly growing leaf primordia, then the vascular traces relating to the latter would be conspicuous, whereas the contribution of the former to the vascular system might be inconspicuous or negligible. Yet the basic phenomenon of apical determination of vascular tissue would remain. Moreover, it has been shown in the ferns that the development of the leaf bases leads to an actual transformation of potential axial vascular tissue into parenchyma. A study of shoot development in *Primula* suggests that this may be true of seed plants also. Now, if this transformation of potential vascular tissue into parenchyma is evident in species with a well-developed prevascular ring, it becomes understandable that in a relatively inactive apex, where no axial prestelar tissue is evident, the whole vascular system of the shoot will appear to be of foliar origin. Even though it cannot be seen, the prestelar tissue in seed plants would appear to originate in the region of conjunction of the tunica and the corpus.

The experimental results recorded here have been of sufficient interest to suggest that this work may be extended with profit to the many different types of apex to be found among gymnosperms, dicotyledons and monocotyledons. While proposing an extension of work in this field it is realized that the methods used have distinct limitations; for, in a further analysis, since the changes which take place after experimental treatment are brought about during growth, it is to an analysis of the factors of growth, together with other factors which become incident (including a consideration of spatial relationships), that we must look for a fuller understanding of the formative activities of the apical region. In a recent paper, Woodger (1946) has pointed to the need for a new outlook on problems of embryology. The underlying idea is that although the structural plan ('Bauplan') in two adults of different systematic groups (which are being compared) may appear to be widely divergent, yet, if studied from a different angle, they might be seen to have a Bauplan in common; and, given an adequate theory of cell organization, it might be seen that the Bauplan determining the features of one type was but little different from that determining the features of the other. Woodger contends that a new theoretical approach to the problem of zygote structure and, more generally, of embryonic development, is required; when such a theory is devised, a time will come when taxonomy and morphology will be completely transformed. The data of the present studies may contribute to this new phase of biological work.

In *Dryopteris*, the vascular tissue formed below the isolated apical meristem has never been observed to become conjoined, by a downward differentiation, with the vascular tissue of the parent shoot; in *Primula* spp. this has been regularly observed in older specimens. These observations point to a probable difference either in the intensity of production of the effective substance in *Primula* as compared with *Dryopteris*, or of its greater ability to move, or of its effect to be transmitted, in the dicotyledon axis. Comparable observations have been made by Ball (1948) in experimental studies of *Lupinus* and *Tropaeolum*. The evidence affords clear proof that a basipetal differentiation of vascular tissue does take place—a point on which there has been much controversy among anatomists investigating vascular structure in seed plants. A full discussion of the relevant literature has been given by Esau (1943). The *Primula* materials have also yielded some further points of anatomical interest. As the downward differentiation of vascular tissue proceeds in the pith of the isolated region, the ring of prestelar tissue is not all transformed into vascular tissue, but

a number of more or less discrete vascular strands are differentiated, separated by rays of parenchyma. A prestelar ring is definitely the precursor of these vascular strands—which are in no way related to the insertion of leaf traces on the vascular column of the shoot. This kind of development has also been seen in the normal development in other flowering plants with a prestelar ring (for details, see Esau's review).

In a recent study of the ontogeny of the vascular tissue in the shoots of flowering plants Hegedüs (1949) holds that the inception of vascular tissue at the apex is probably due to the action of a basipetally moving auxin and possibly another hormone also (see also Wardlaw 1944*a*; Camus 1944, and others). In selected dicotyledons, *Aristolochia clematitis*, *Vicia faba* and *Pelargonium zonale*, all of which have opposite alternate leaves and a prevascular ring at the shoot apex, Hegedüs observed that the fate of this ring during development is very different: in *Aristolochia*, no interfascicular cambium develops; in *Vicia*, the interfascicular parts of the ring become cambium which forms only ground parenchyma, and perhaps some vascular bundles; in *Pelargonium*, the interfascicular ring tissue gives rise to a cambium and the latter to a continuous ring of vascular tissue. Furthermore, in *Lycium halimifolium*, with $\frac{3}{8}$ phyllotaxy on the main shoots and $\frac{2}{5}$ on the lateral branches, as described by Hegedüs (1943), and in *Centaurium umbellatum* with decussate phyllotaxy, as described by Halmai (1935), a continuous vascular ring is formed below the apex. From this survey Hegedüs concludes that 'the course of development of the vascular system of stems does not depend alone on the pattern of phyllotaxy. It is also clear that, in contrast to the opinion of Kostytschew (1924), there is no necessary relation between the manner of procambial differentiation and that of secondary thickening.'

Another feature of the experimentally induced steles in *Primula* is the presence of isolated vascular strands in the pith. This is comparable, in some ways, to the 'mixed pith' induced in certain ferns which have been wounded or subjected to experimental treatment (Bower 1923; Wardlaw 1946*a*).

The writer has pleasure in acknowledging the assistance received from Mr E. Ashby in microscope preparations and photographic illustrations.

References

Ball, E. 1948 *Exp. Biol. Symp.* 1947. Cambridge University Press.
Boke, N. H. 1941 *Amer. J. Bot.* **28**, 656.
Bower, F. O. 1923 *The ferns*. Cambridge University Press.
Camus, G. 1944 *C. R. Acad. Sci., Paris*, **219**, 34.
Esau, K. 1943 *Bot. Rev.* **9**, 125.
Fleet, D. S. Van 1948 *Amer. J. Bot.* **35**, 219.
Foster, A. S. 1939 *Bot. Rev.* **5**, 454.
Foster, A. S. 1949 *Practical plant anatomy*. New York: Van Nostrand.
Halmai, J. A. 1935 *Bot. Közl.* **32**, 115.
Hegedüs, A. 1943 *Bot. Közl.* **40**, 320.
Hegedüs, A. 1949 *Bot. Gaz.* **110**, 593.
Helm, J. 1932 *Planta*, **16**, 607.
Jost, L. 1940 *Z. Bot.* **35**, 114.
Kaplan, R. 1937 *Planta*, **27**, 224.

Kostytschew, S. 1924 *Bot. Zbl.* **40**, 295.
Louis, J. 1935 *Cellule*, **4**, 87.
Reeve, R. M. 1948 *Amer. J. Bot.* **35**, 65.
Thompson, D'Arcy W. 1942 *On growth and form.* Cambridge University Press.
Wardlaw, C. W. 1944*a* *Ann. Bot., Lond.*, N.S., **8**, 173.
Wardlaw, C. W. 1944*b* *Ann. Bot., Lond.*, N.S., **8**, 387.
Wardlaw, C. W. 1945 *Ann. Bot., Lond.*, N.S., **9**, 217.
Wardlaw, C. W. 1946*a* *Ann. Bot., Lond.*, N.S., **10**, 97.
Wardlaw, C. W. 1946*b* *Ann. Bot., Lond.*, N.S., **10**, 223.
Wardlaw, C. W. 1947 *Phil. Trans.* B, **232**, 343.
Wardlaw, C. W. 1949*a* *Phil. Trans.* B, **233**, 415.
Wardlaw, C. W. 1949*b* *Nature*, **164**, 167.
Wardlaw, C. W. 1949*c* *Ann. Bot., Lond.*, **13**, 163.
Wardlaw, C. W. 1949*d* *Growth* (Supplement), **13**.
Whitman, C. O. 1893 *J. Morph.* **8**, 653.
Wilson, E. B. 1925 *The cell in development*, 3rd ed. London: Macmillan.
Woodger, J. H. 1946 *Essays on growth and form.* Cambridge University Press.

Description of plates 31 to 35

(All figures are from untouched photographs)

Plate 31

Figure 28. *Todea barbara.* Downward view of the leafy shoot which developed from an apical meristem isolated by vertical incisions. The axis, greatly reduced in size, bears small adult leaves showing various stages of reduction. (Magn. × 4.)

Figure 29. *Todea barbara.* Transverse section of a shoot of which the apical meristem had been isolated by four vertical incisions. The modified shoot stele is seen in the isolated central plug. (Magn. × 20.)

Figures 30 to 33. *Todea barbara.* Figure 30 shows the normal shoot stele—a medullated protostele—with a leaf trace and a root trace, below the level of the incisions. Figure 31 shows the greatly modified stele in the incised region; the outer tissues of the stele have been transformed into a 'cortical' parenchyma. Figure 32, taken higher up than figure 31, shows a cylindrical protostele, of reduced size, as compared with figure 30, with a 'cortex' formed from the peripheral stelar tissue. Figure 32, near the apex of the new axial growth, shows a small protostele, like that found in the young sporophyte, with the traces of the new leaves. (Magn. × 50.)

Plate 32

Figure 34. *Angiopteris evecta.* On the right, a shoot apex (as seen from above, after some growth has taken place) which had been isolated by three vertical incisions. The apical growing point and three new leaf primordia can be seen; on the left, one of the original leaf primordia which had also been isolated by vertical incisions. (Magn. × 15.)

Figure 35. *Angiopteris evecta.* A shoot apex (as seen from the side after some growth has taken place) which had been isolated by vertical incisions. (Magn. × 15.)

Figures 36, 37. *Angiopteris evecta.* Sections of the specimen illustrated in figure 34, after further growth. Figure 36, transverse section of the shoot below the incisions, showing the distribution of vascular tissue. Figure 37, the shoot (right), with its small protostele, which developed from the isolated apical meristem; left, a leaf, with a very reduced vascular trace, which developed from an isolated primordium. (Magn. × 4.)

FIGURE 38. *Angiopteris evecta.* Transverse section of the medullated protostele in a shoot which developed from an isolated apical meristem: the parental shoot had a polycylic dictyostele as in figure 36. (Magn. × 35.)

FIGURE 39. *Polypodium vulgare.* Transverse section of a lateral branch of which the apical meristem had been isolated by four vertical incisions. (Magn. × 20.)

FIGURE 40. *Pteridium aquilinum.* Transverse section of a rhizome, the apex of which had been isolated by four vertical incisions. Some proliferation of the central tissues has taken place. (Magn. × 16.)

FIGURES 41, 42. *Pteridium aquilinum.* Figure 41 shows the vascular arrangements in the plug of tissue below the isolated apical meristem. Figure 42 shows the reconstituted vascular system in the shoot which developed from the isolated meristem: the apex of the new axis lies in a small groove (top). (Magn. × 16.)

FIGURE 43. *Pteridium aquilinum.* The apex of the new shoot illustrated in figure 42. The incipient vascular tissue (dark) is seen to be a coherent tissue immediately below the apical meristem. (Magn. × 100.)

PLATE 33

FIGURE 44. *Primula polyantha.* A vegetative shoot apex in median longitudinal section, showing the two-layered tunica and the last-formed leaf primordium (on left). (Magn. × 225.)

FIGURES 45, 46. *Primula Wanda.* Transverse sections, in basipetal sequence, just below the apex, of a vegetative shoot, showing the prevascular ring interrupted by the gaps of leaves 5 and 6 (see figures 10, 11 in text). (Magn. × 150.)

FIGURES 47 to 49. *Primula polyantha.* Three transverse sections, in basipetal sequence, of the shoot formed from a vegetative apex which had been isolated by vertical incisions and then defoliated as new leaf primordia appeared; a continuous vascular ring is present (figure 47), the vascular strands becoming more discrete and separated by ray parenchyma in the downward development of the vascular tissue. Below the level of figure 49 the vascular strands faded out in the pith of the parent shoot. (Magn. × 80.)

PLATE 34

FIGURES 50 to 55. *Primula polyantha* (see also figures 16 to 22 in the text).

FIGURE 50. Downward view of an apical meristem which has been isolated by three vertical incisions. (Magn. × 34.)

FIGURE 51. The apex of the isolated terminal region in transverse section after further growth: twelve new leaves have been formed. (Magn. × 40.)

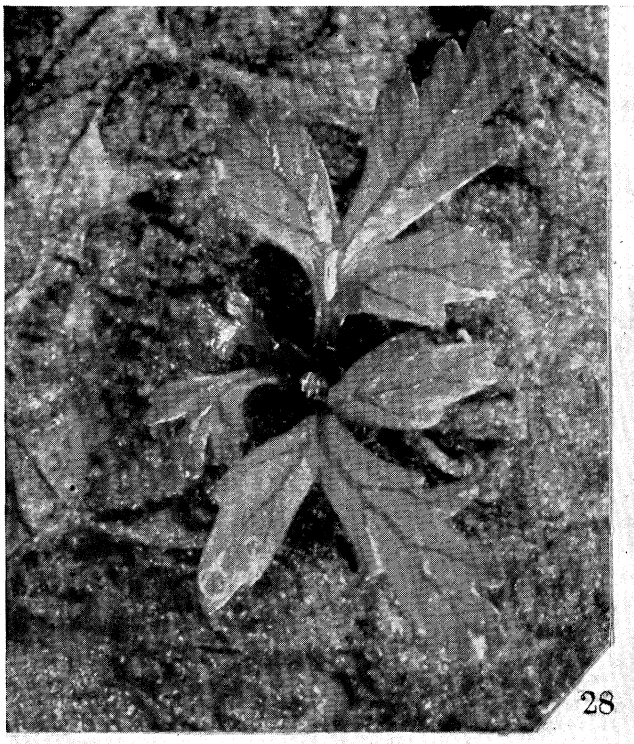
FIGURE 52. Section of the parent shoot, showing the vascular system and a cylinder of vascular tissue which has been differentiated in the centre of the pith, isolated by the incisions. (Magn. × 20.)

FIGURES 53 to 55. Three sections in basipetal sequence, showing the differentiation of vascular tissue in the isolated plug of pith. (Magn. × 40.)

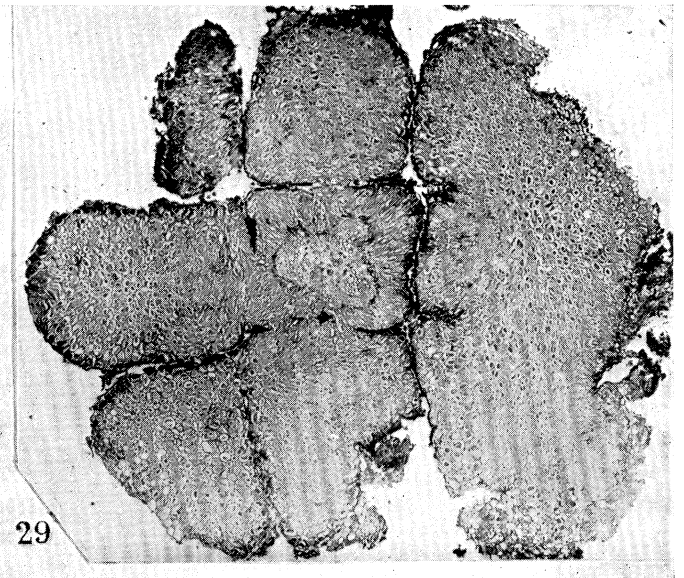
PLATE 35

FIGURE 56. *Primula Wanda* (see figure 11 in the text). Shows the trace of leaf 6 and its gap, close to the shoot apex. (Magn. 150.)

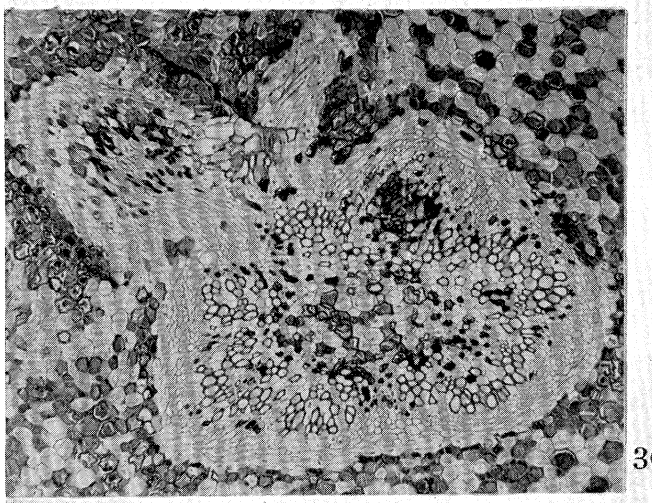
FIGURES 57 to 61. *Primula polyantha.* The apical meristem was isolated by four vertical incisions and the new shoot was defoliated; figure 57, a stele of rectangular outline in cross-section has been differentiated in the central pith. (Magn. × 20.) Figure 58 shows the structure of the induced central stele, the position of the incisions and the cylindrical stele of the parent shoot. (Magn. × 50.) Figures 59 to 61, three sections of the defoliated axis in acropetal sequence, showing an uninterrupted cylinder of vascular tissue. (Magn. × 50.)



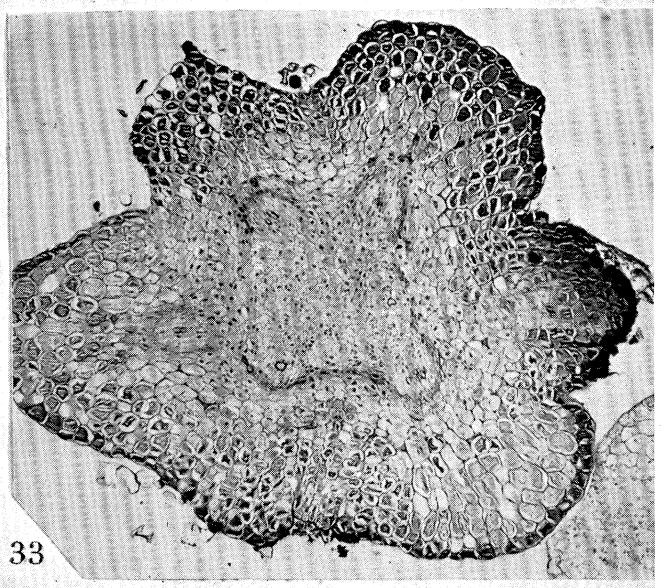
28



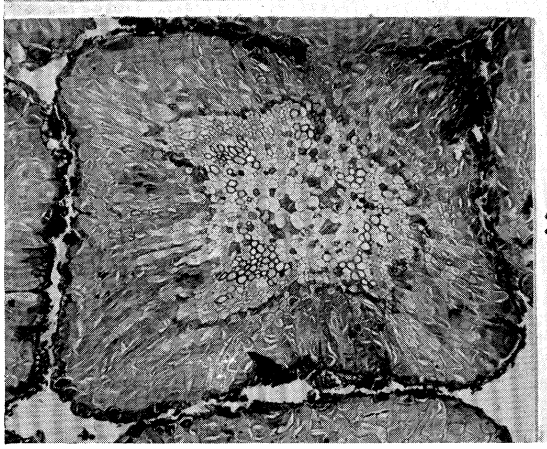
29



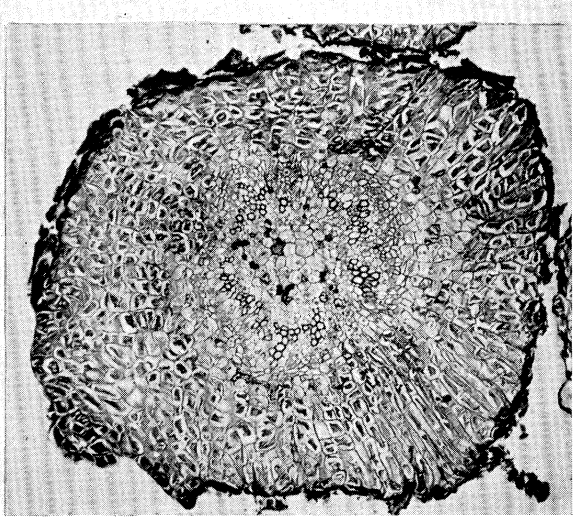
30



33

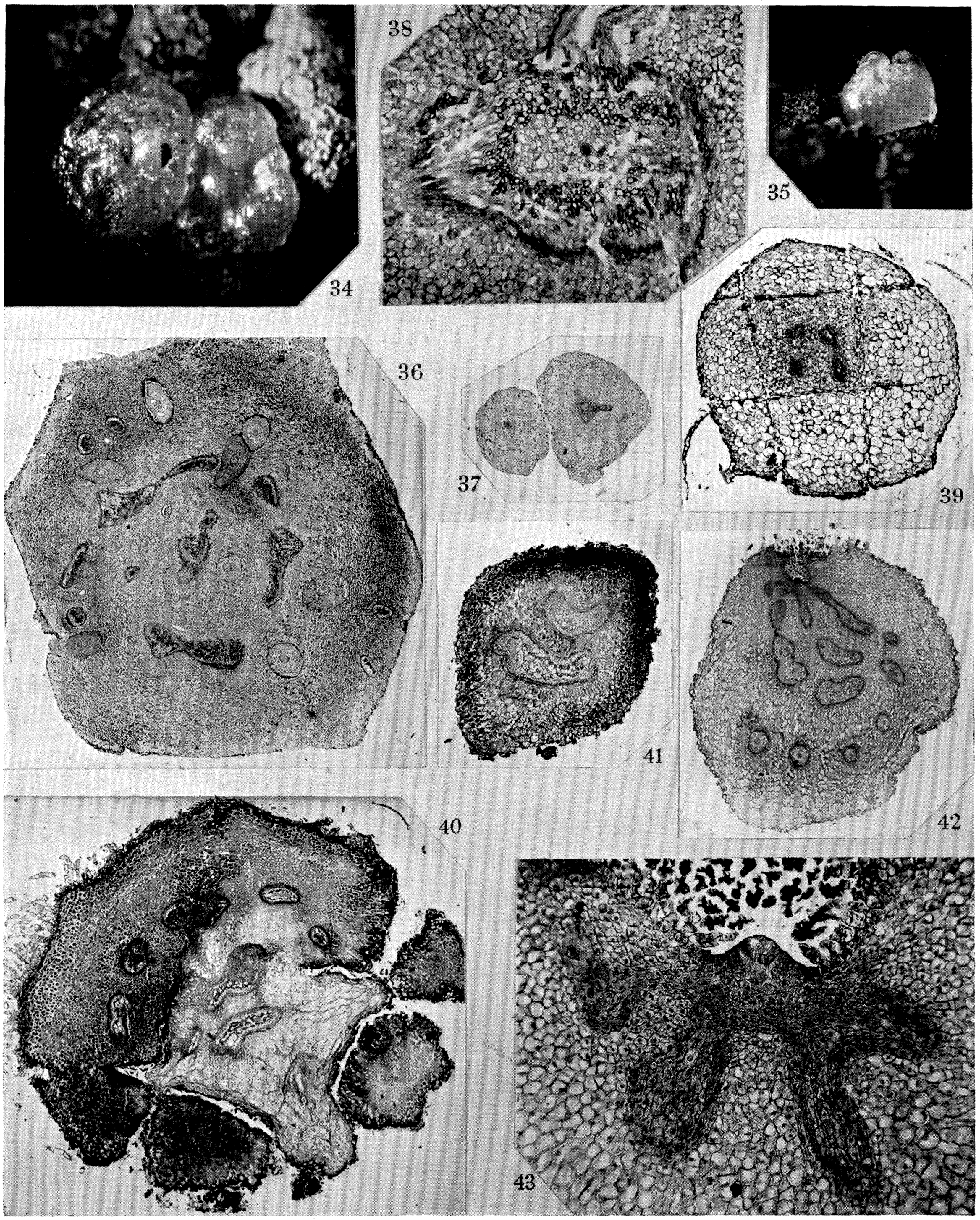


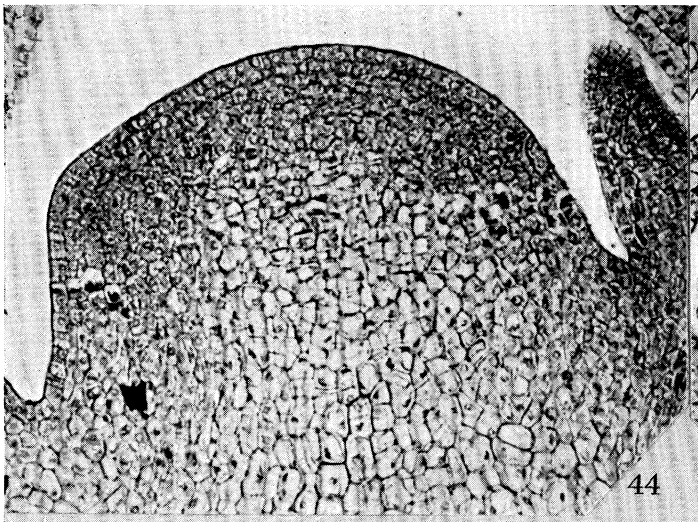
31



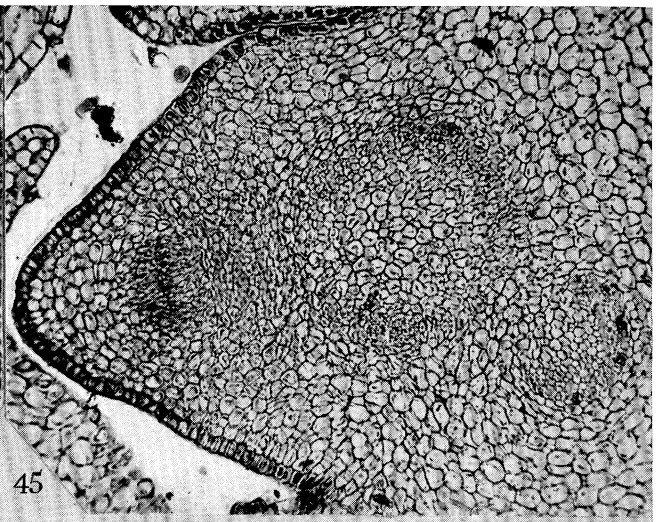
32

(*Facing p.* 602)

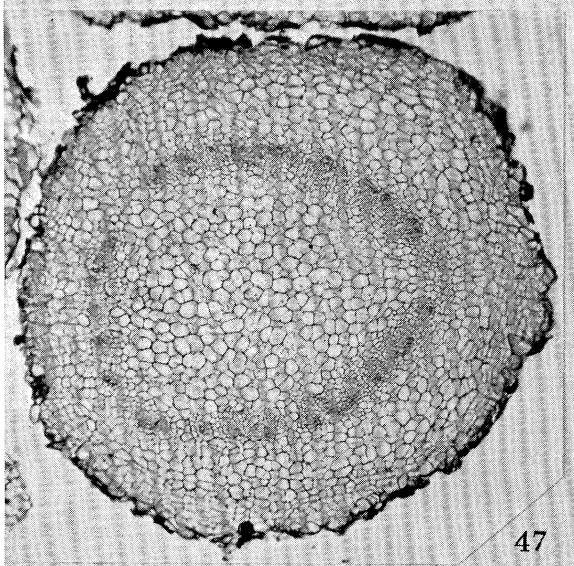

34
38
35
36
37
39
41
42
40
43



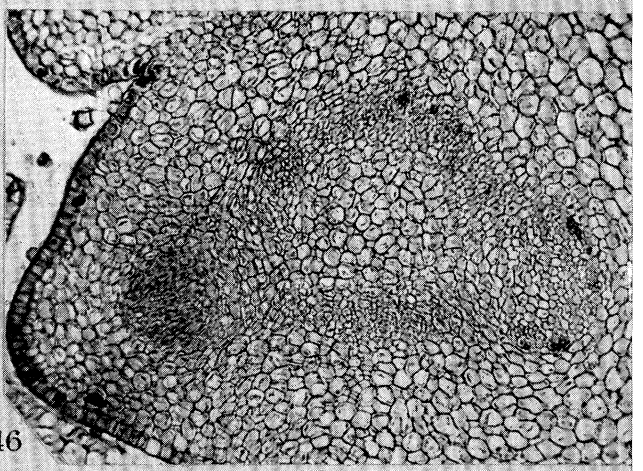
44



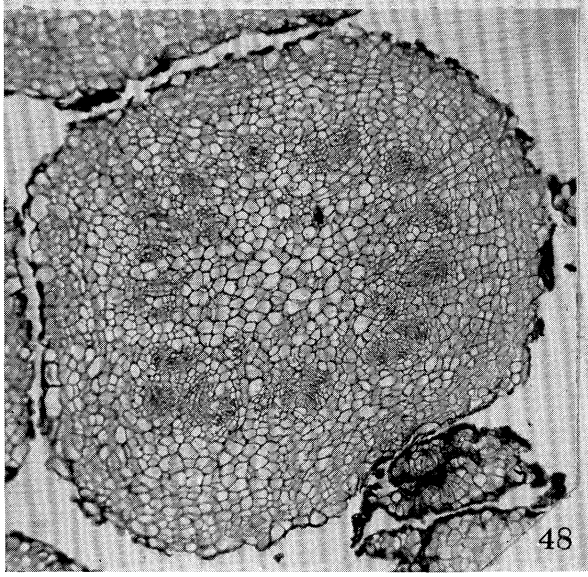
45



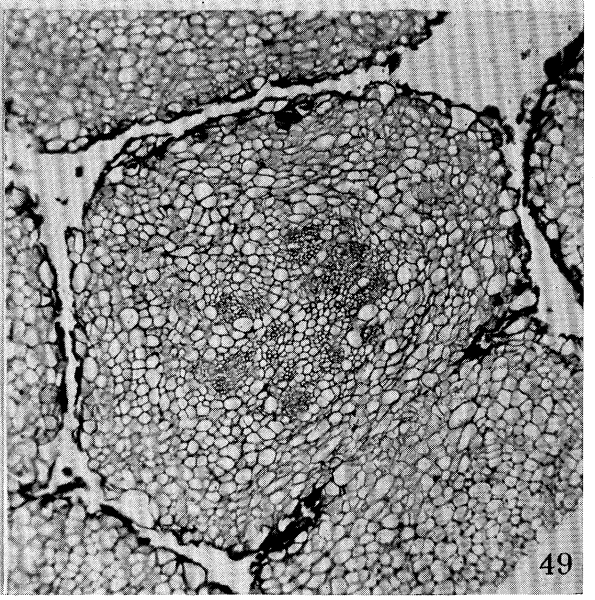
47



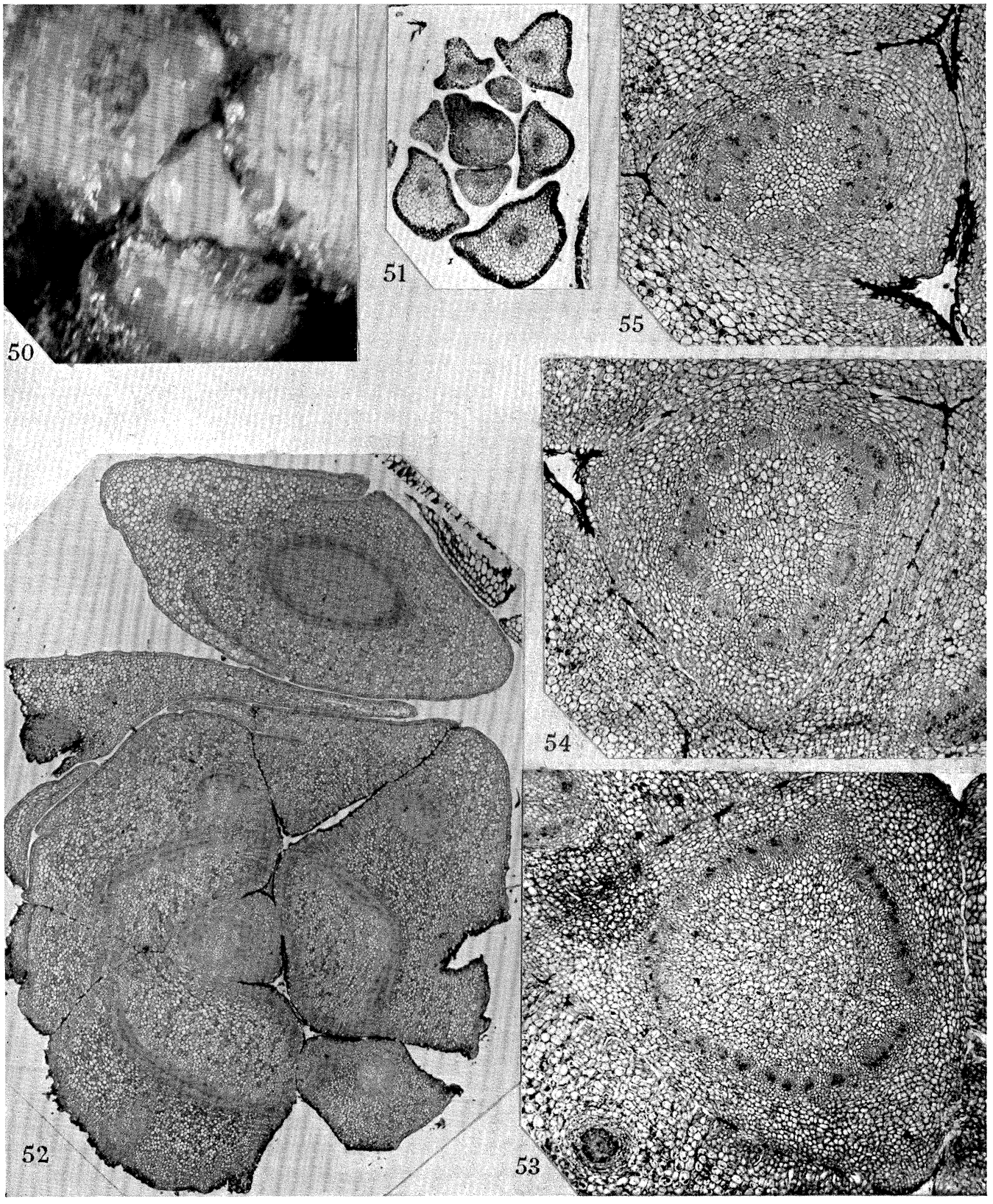
46

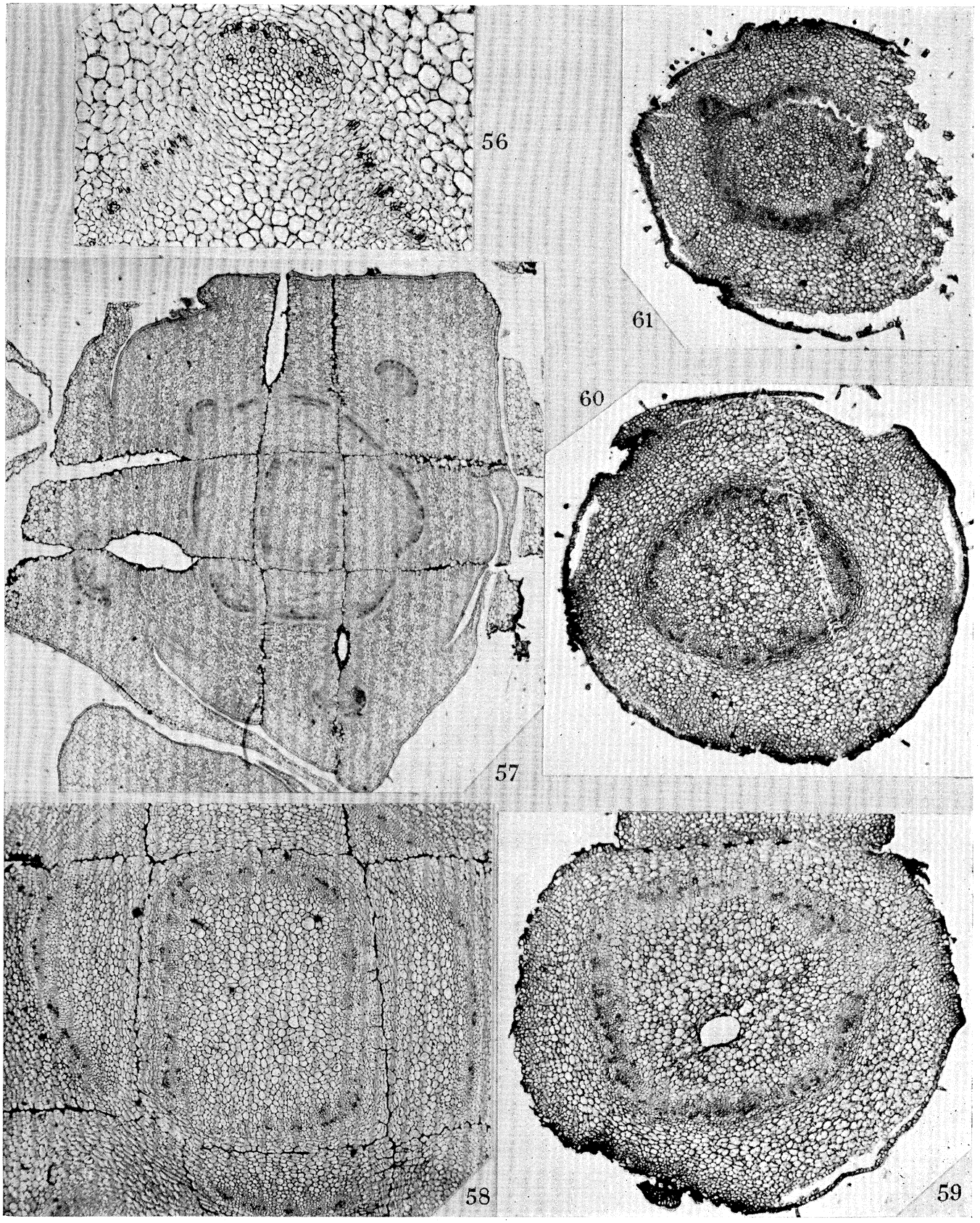


48

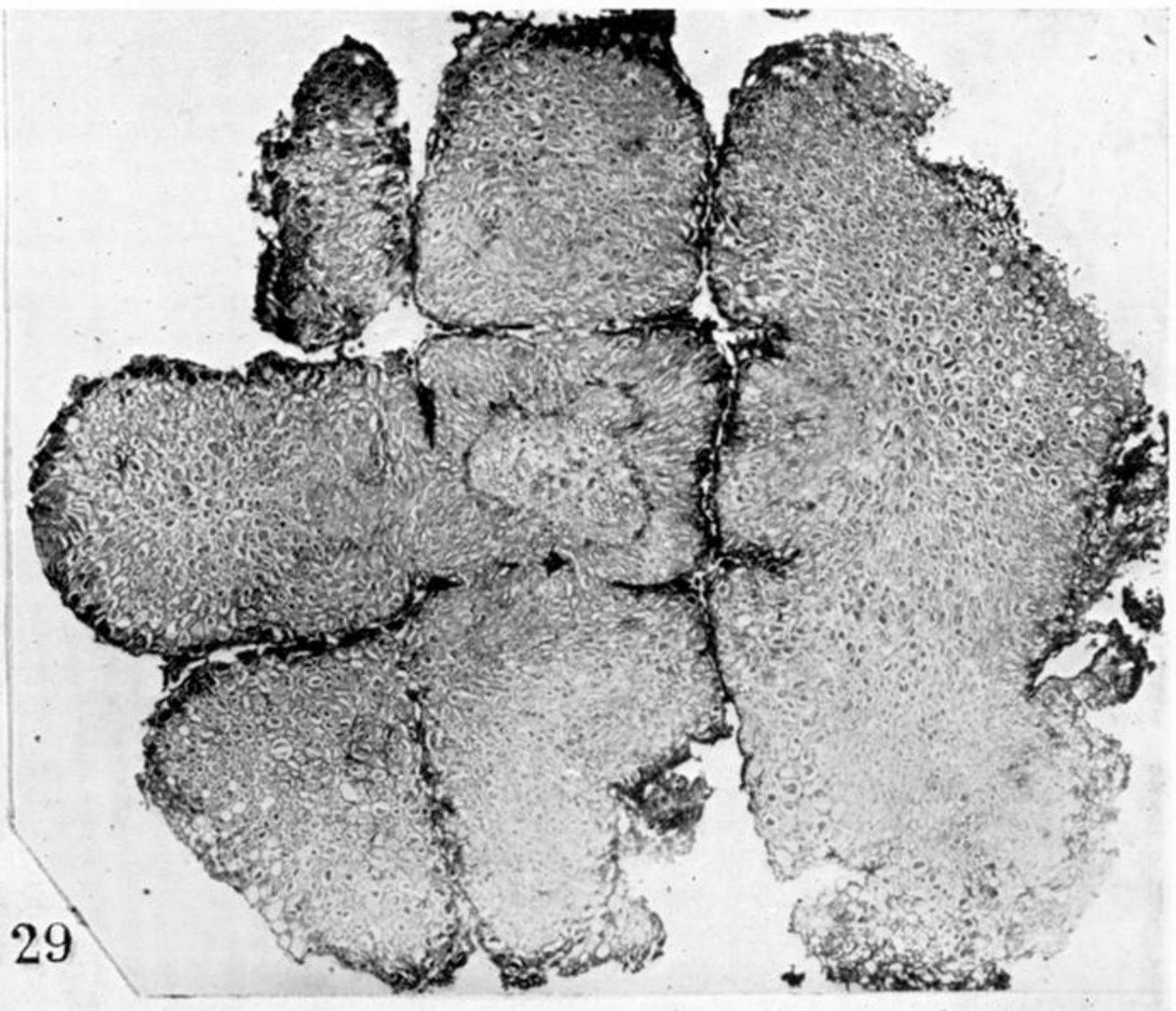


49

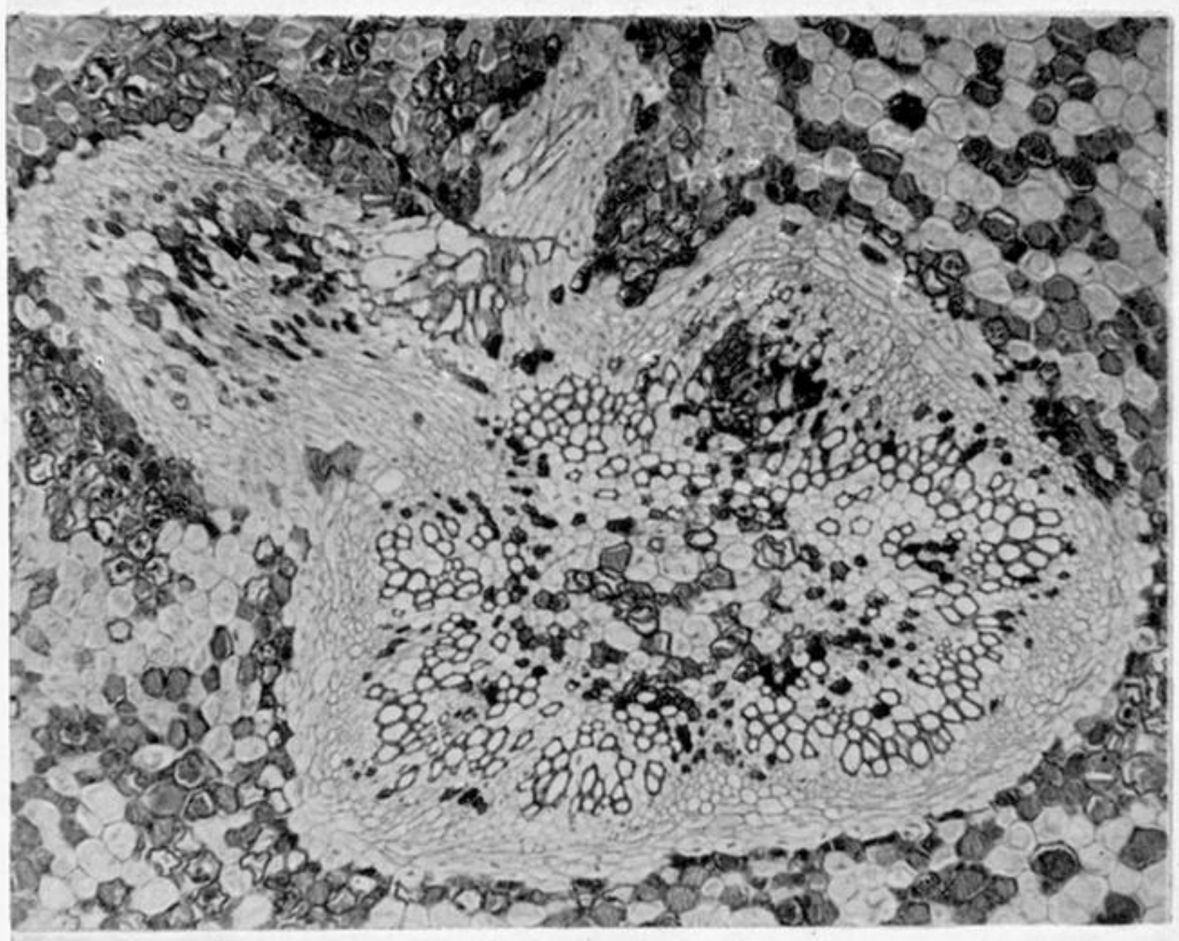

50
51
55
54
52
53


56
61
60
57
58
59

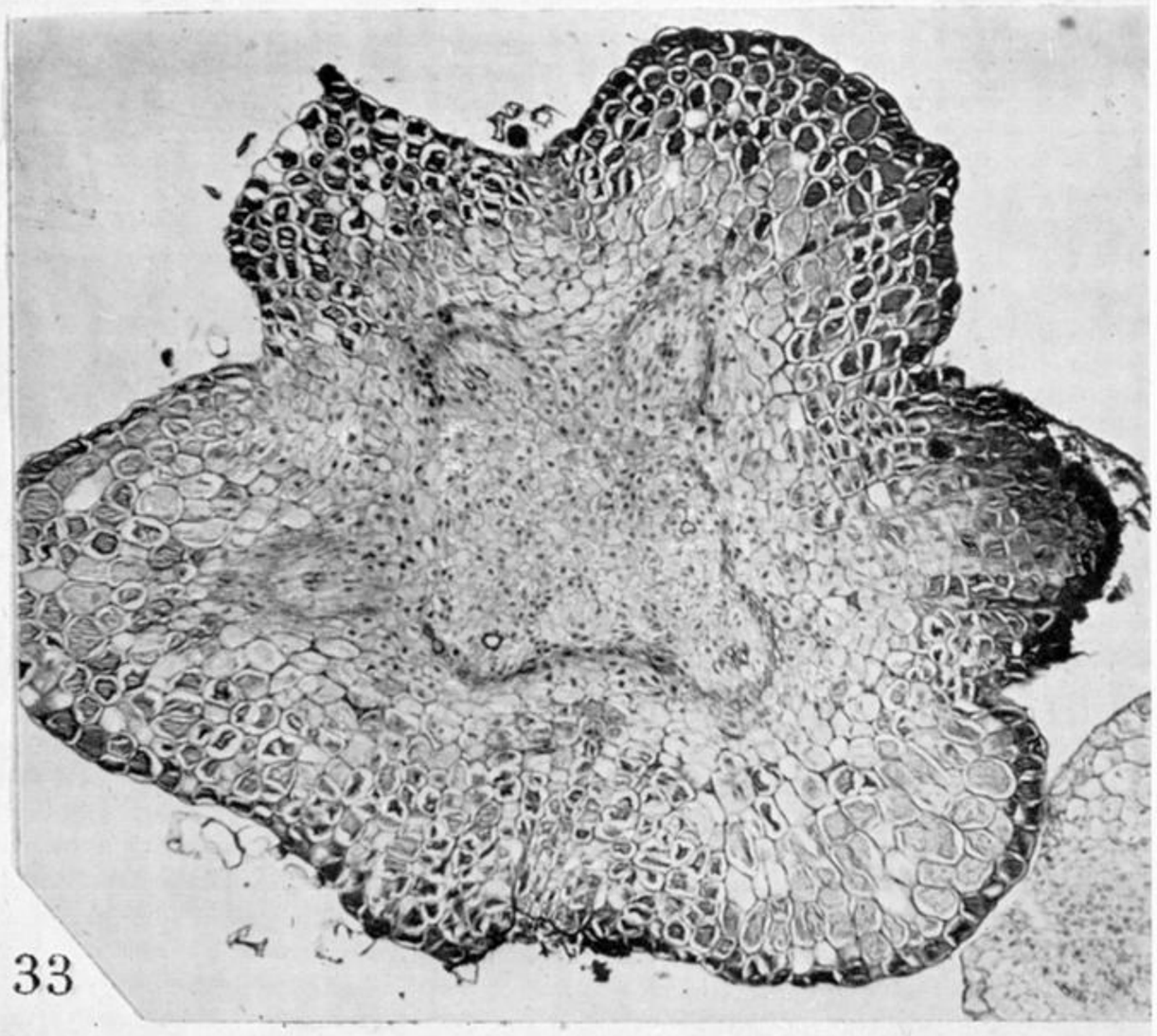
28



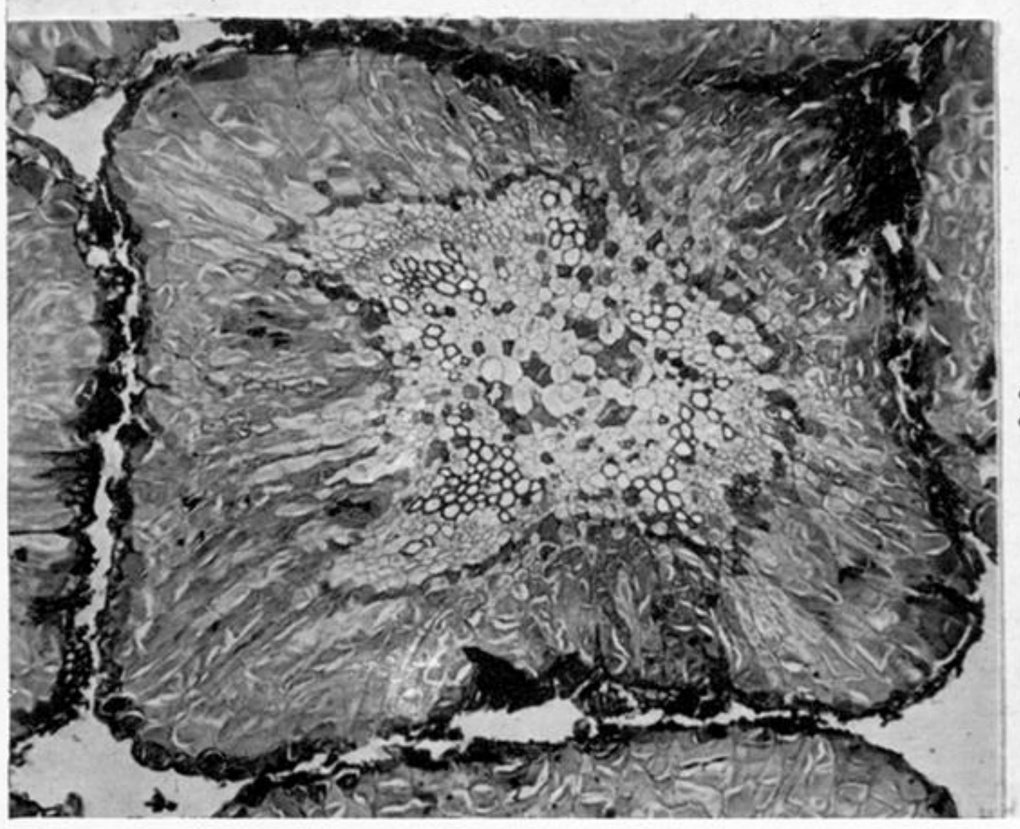
29



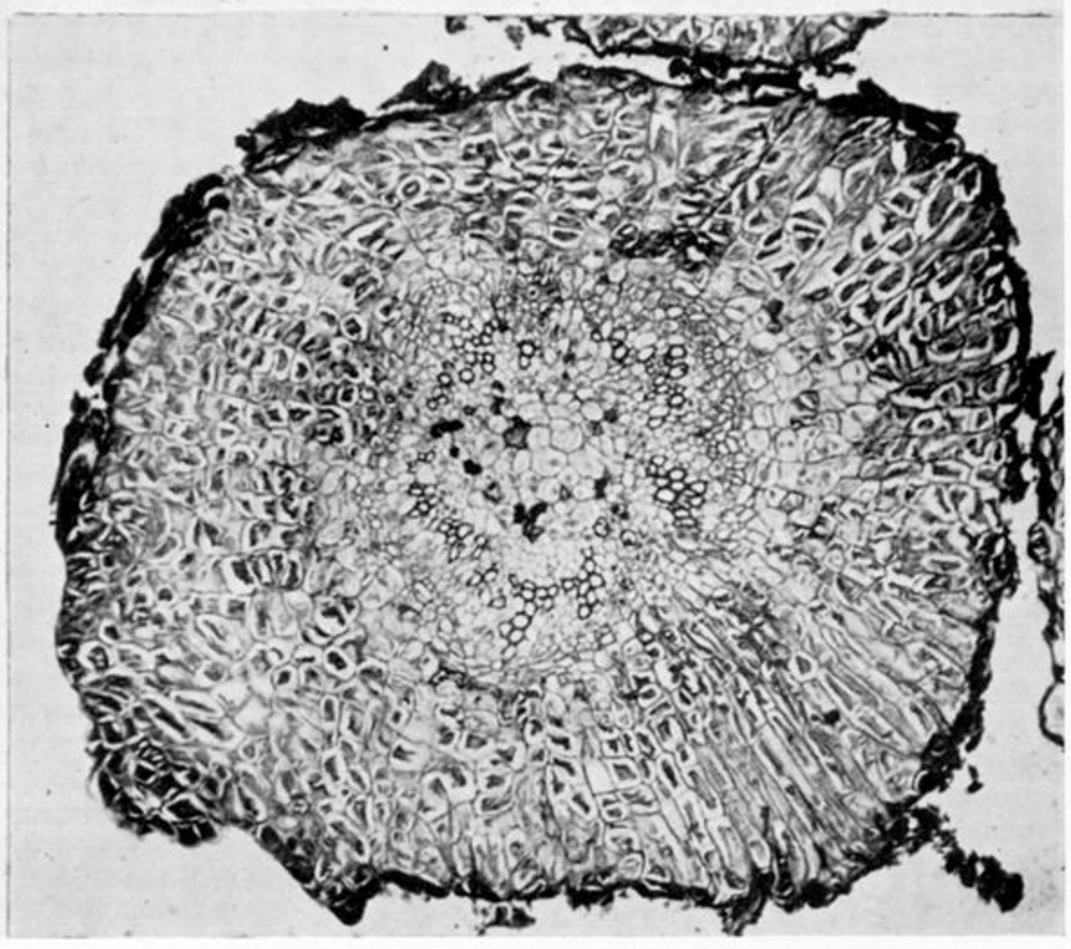
30



33



31



32

Plate 31

Figure 28. *Todea barbara.* Downward view of the leafy shoot which developed from an apical meristem isolated by vertical incisions. The axis, greatly reduced in size, bears small adult leaves showing various stages of reduction. (Magn. × 4.)

Figure 29. *Todea barbara.* Transverse section of a shoot of which the apical meristem had been isolated by four vertical incisions. The modified shoot stele is seen in the isolated central plug. (Magn. × 20.)

Figures 30 to 33. *Todea barbara.* Figure 30 shows the normal shoot stele—a medullated protostele—with a leaf trace and a root trace, below the level of the incisions. Figure 31 shows the greatly modified stele in the incised region; the outer tissues of the stele have been transformed into a 'cortical' parenchyma. Figure 32, taken higher up than figure 31, shows a cylindrical protostele, of reduced size, as compared with figure 30, with a 'cortex' formed from the peripheral stelar tissue. Figure 32, near the apex of the new axial growth, shows a small protostele, like that found in the young sporophyte, with the traces of the new leaves. (Magn. × 50.)

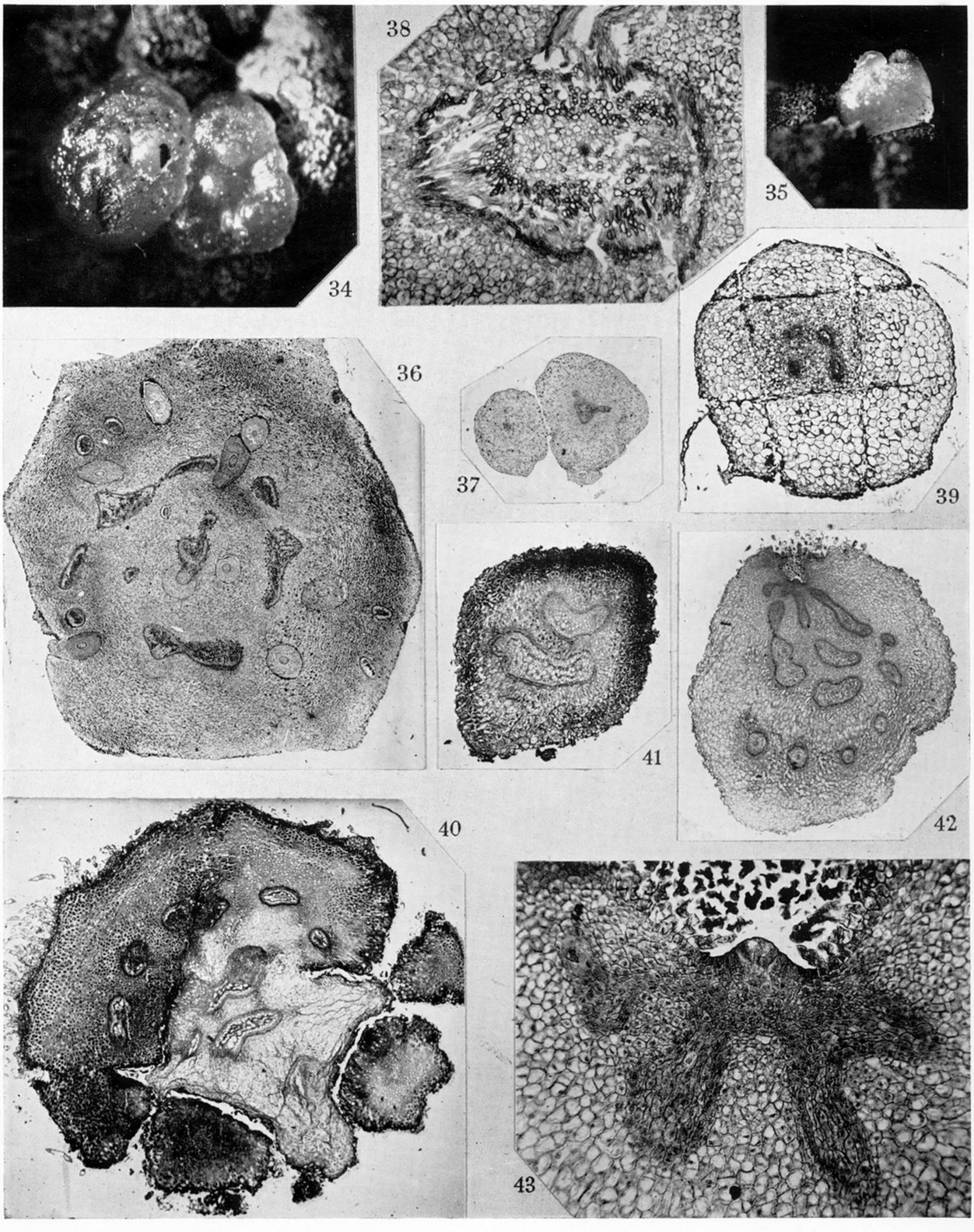


PLATE 32

FIGURE 34. *Angiopteris evecta.* On the right, a shoot apex (as seen from above, after some growth has taken place) which had been isolated by three vertical incisions. The apical growing point and three new leaf primordia can be seen; on the left, one of the original leaf primordia which had also been isolated by vertical incisions. (Magn. × 15.)

FIGURE 35. *Angiopteris evecta.* A shoot apex (as seen from the side after some growth has taken place) which had been isolated by vertical incisions. (Magn. × 15.)

FIGURES 36, 37. *Angiopteris evecta.* Sections of the specimen illustrated in figure 34, after further growth. Figure 36, transverse section of the shoot below the incisions, showing the distribution of vascular tissue. Figure 37, the shoot (right), with its small protostele, which developed from the isolated apical meristem; left, a leaf, with a very reduced vascular trace, which developed from an isolated primordium. (Magn. × 4.)

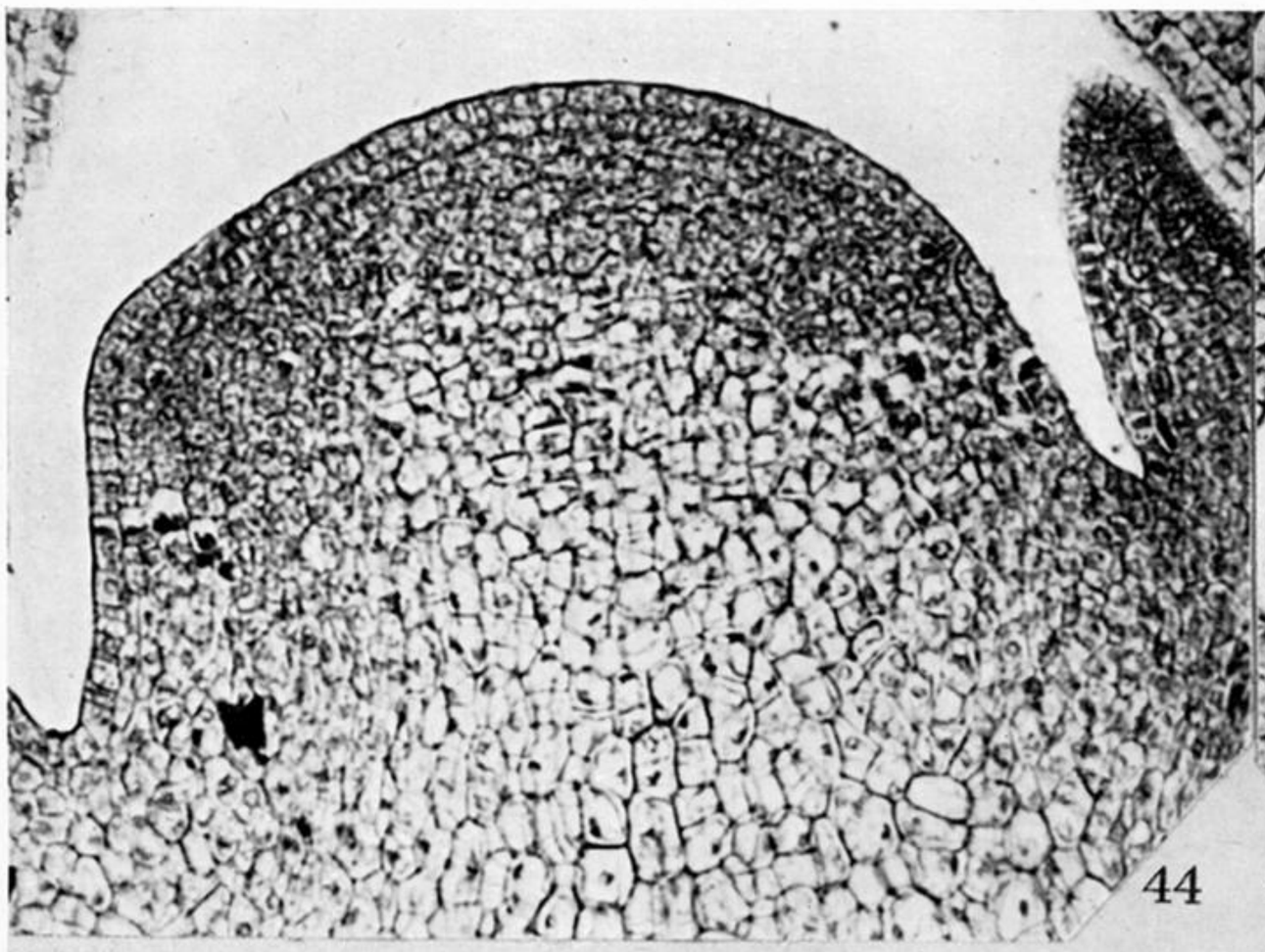
FIGURE 38. *Angiopteris evecta.* Transverse section of the medullated protostele in a shoot which developed from an isolated apical meristem: the parental shoot had a polycylic dictyostele as in figure 36. (Magn. × 35.)

FIGURE 39. *Polypodium vulgare.* Transverse section of a lateral branch of which the apical meristem had been isolated by four vertical incisions. (Magn. × 20.)

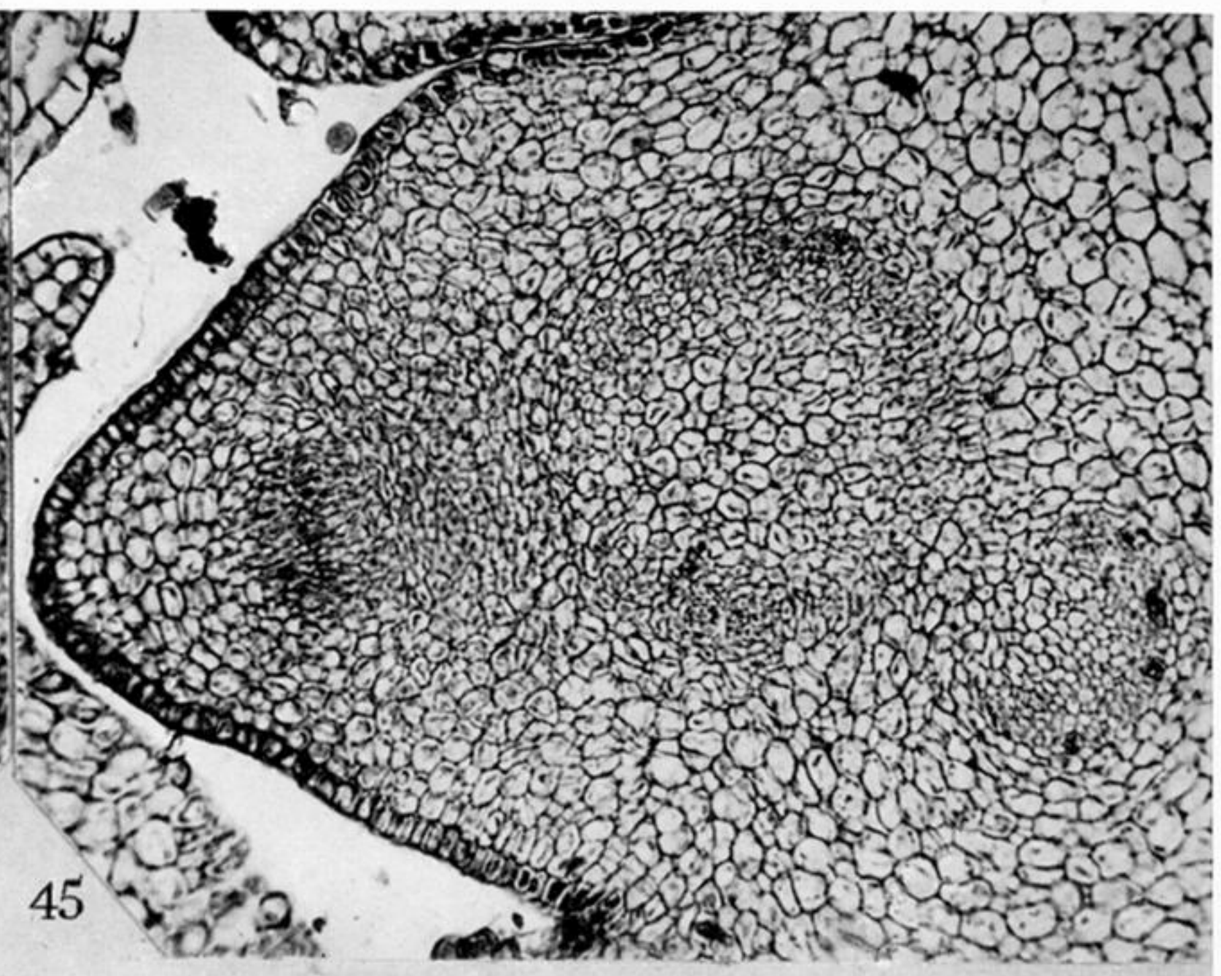
FIGURE 40. *Pteridium aquilinum.* Transverse section of a rhizome, the apex of which had been isolated by four vertical incisions. Some proliferation of the central tissues has taken place. (Magn. × 16.)

FIGURES 41, 42. *Pteridium aquilinum.* Figure 41 shows the vascular arrangements in the plug of tissue below the isolated apical meristem. Figure 42 shows the reconstituted vascular system in the shoot which developed from the isolated meristem: the apex of the new axis lies in a small groove (top). (Magn. × 16.)

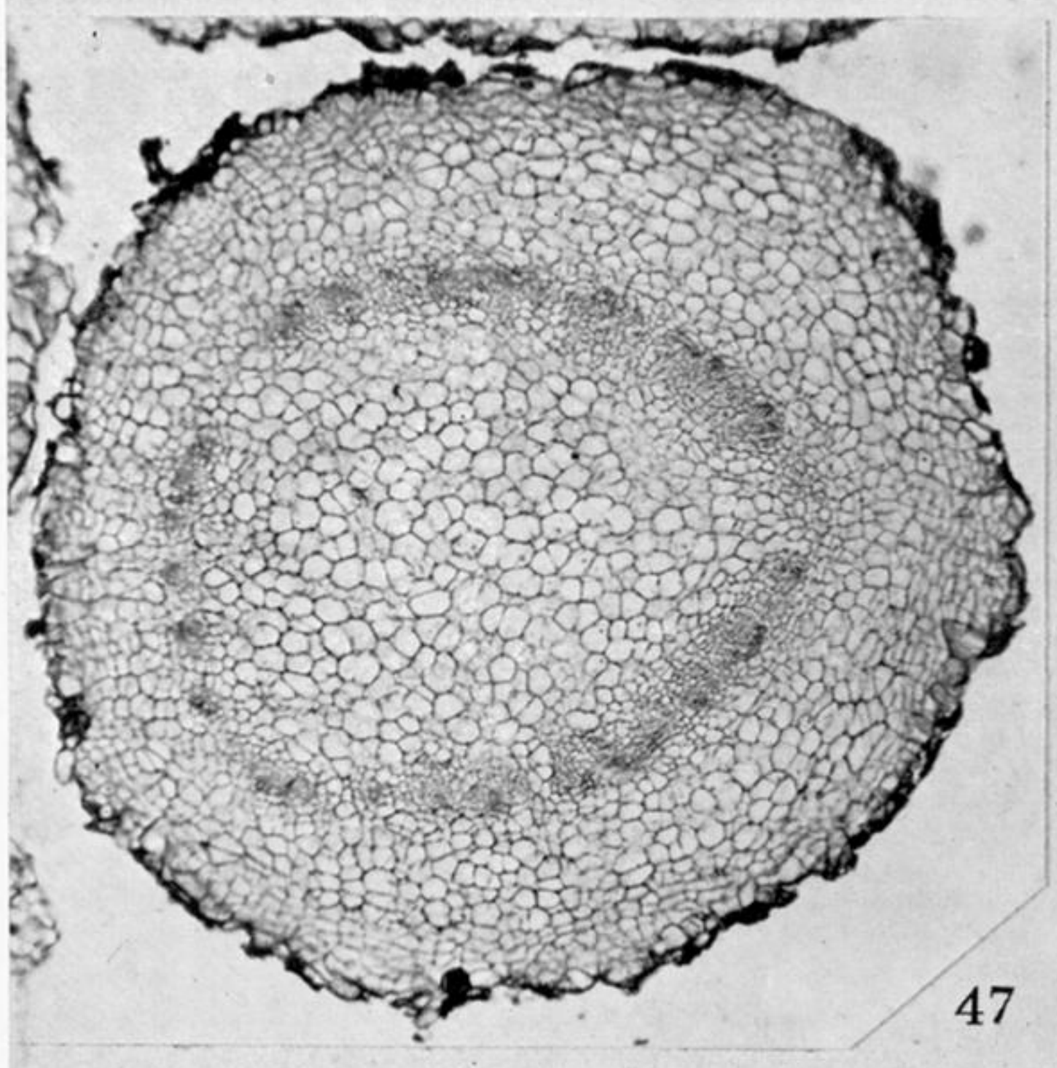
FIGURE 43. *Pteridium aquilinum.* The apex of the new shoot illustrated in figure 42. The incipient vascular tissue (dark) is seen to be a coherent tissue immediately below the apical meristem. (Magn. × 100.)



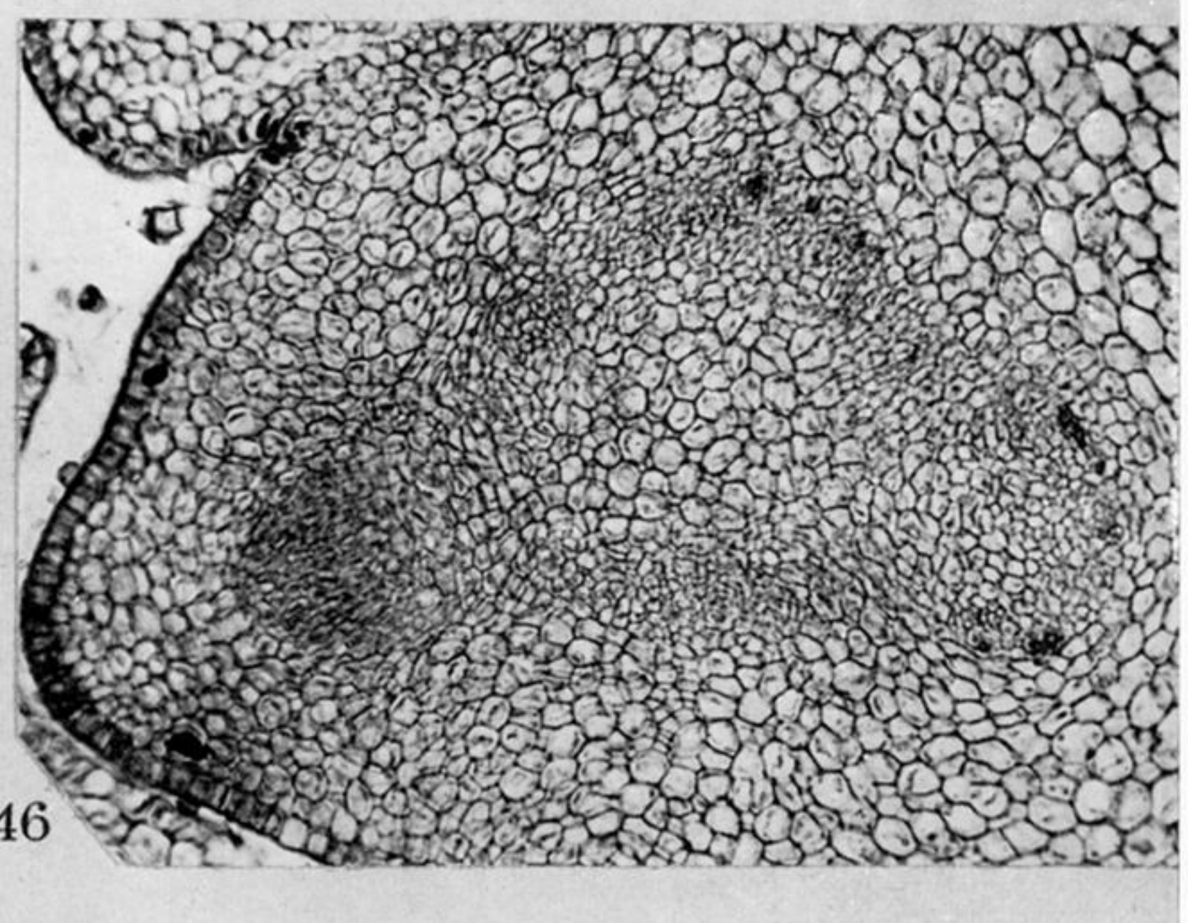
44



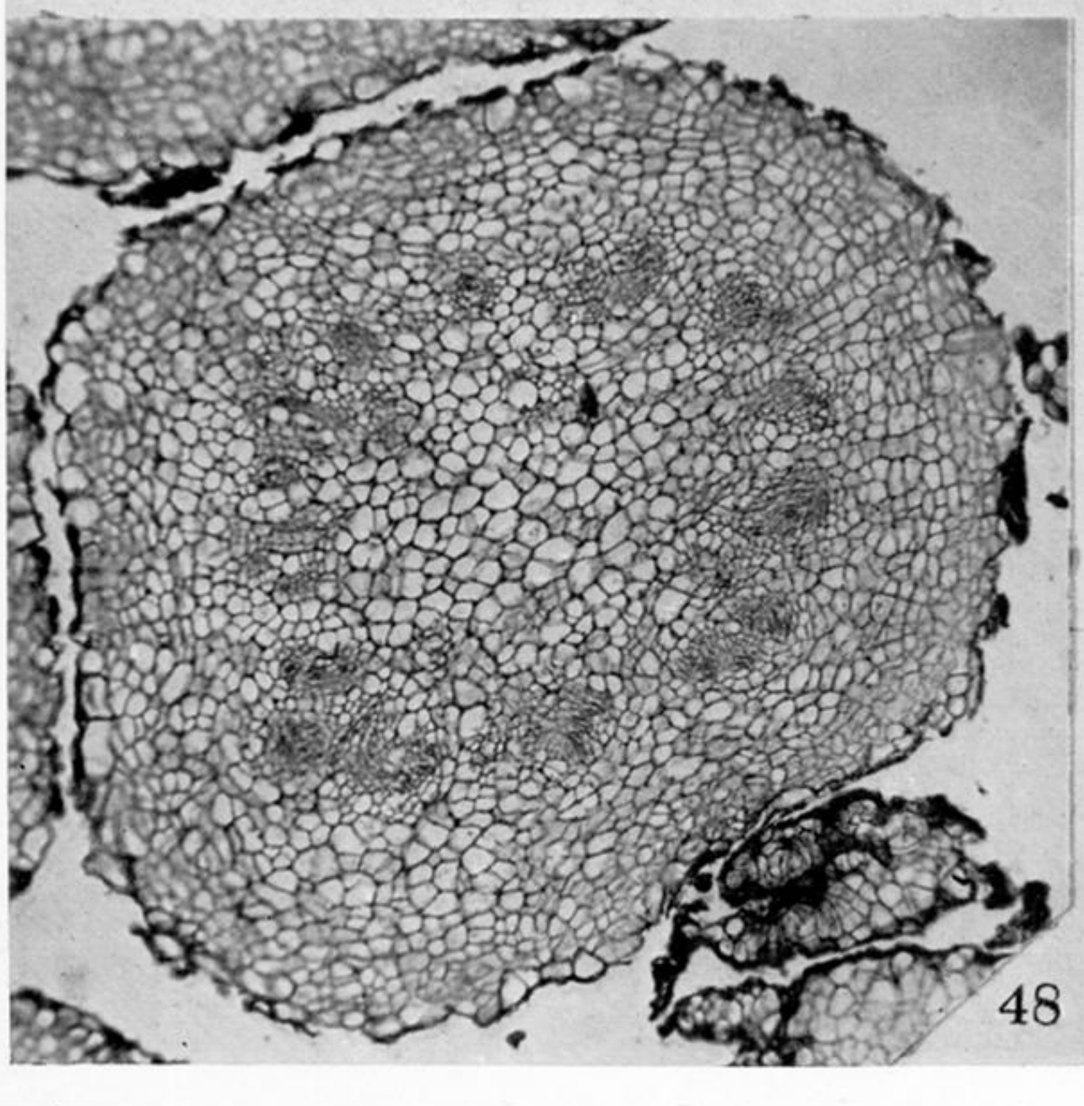
45



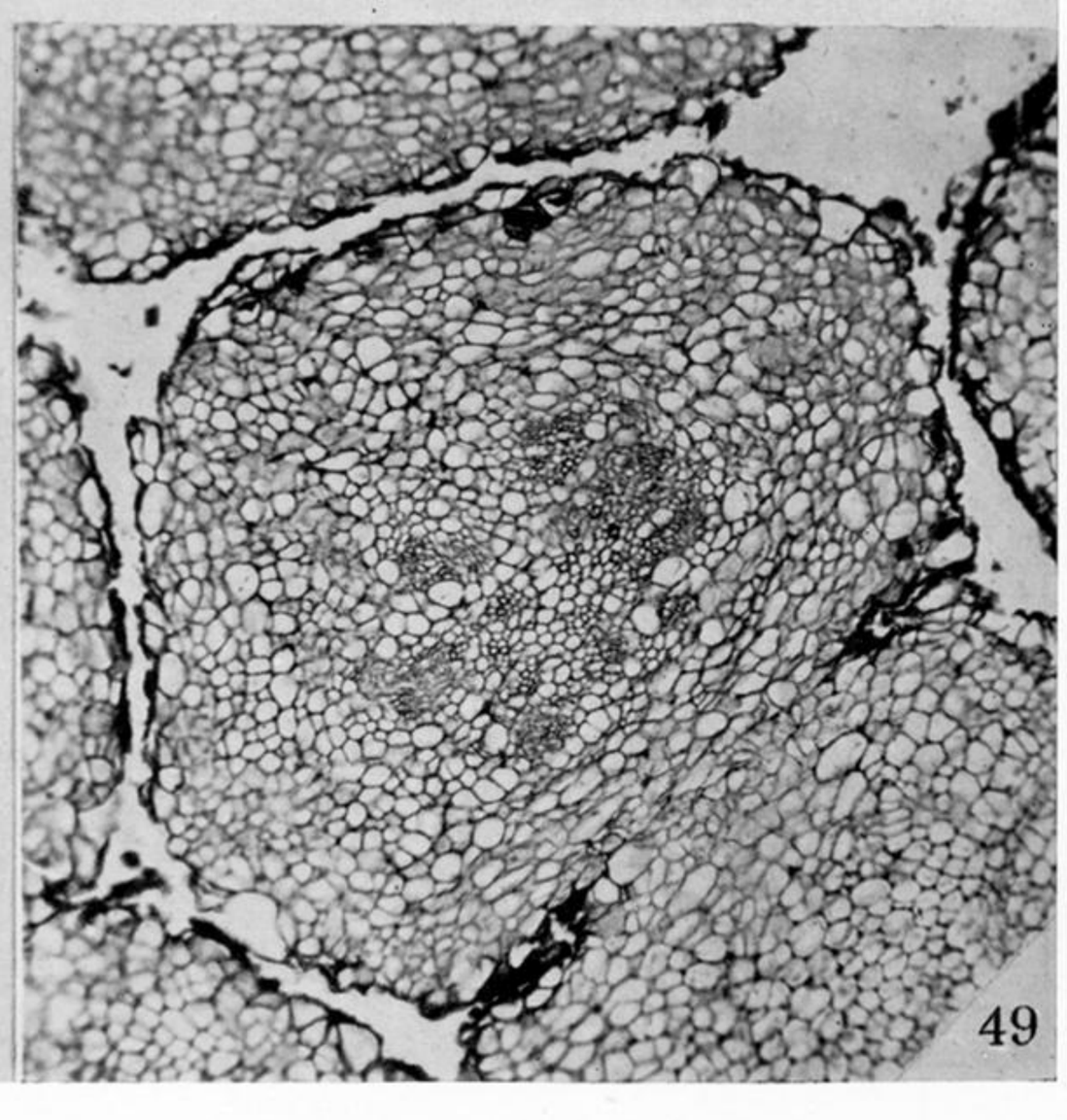
47



46



48



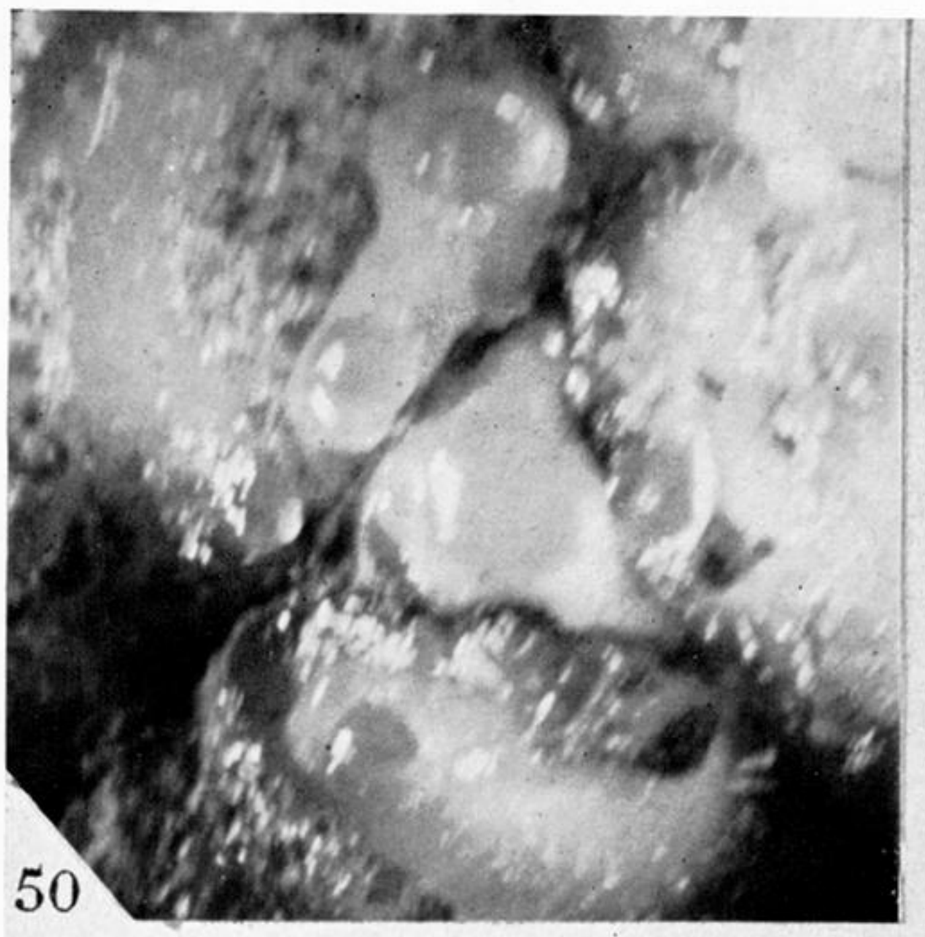
49

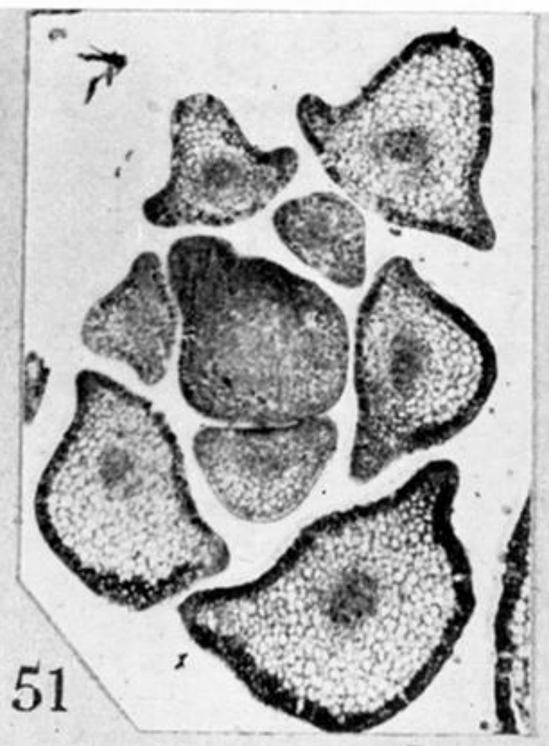
Plate 33

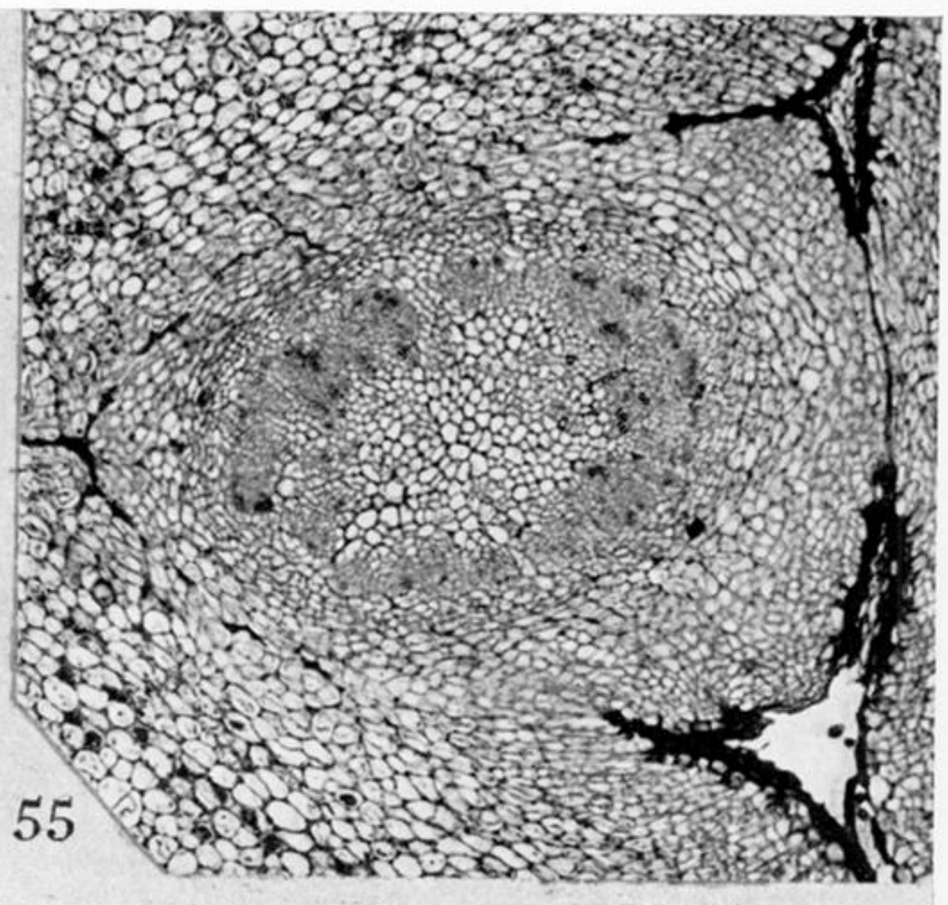
Figure 44. *Primula polyantha.* A vegetative shoot apex in median longitudinal section, showing the two-layered tunica and the last-formed leaf primordium (on left). (Magn. × 225.)

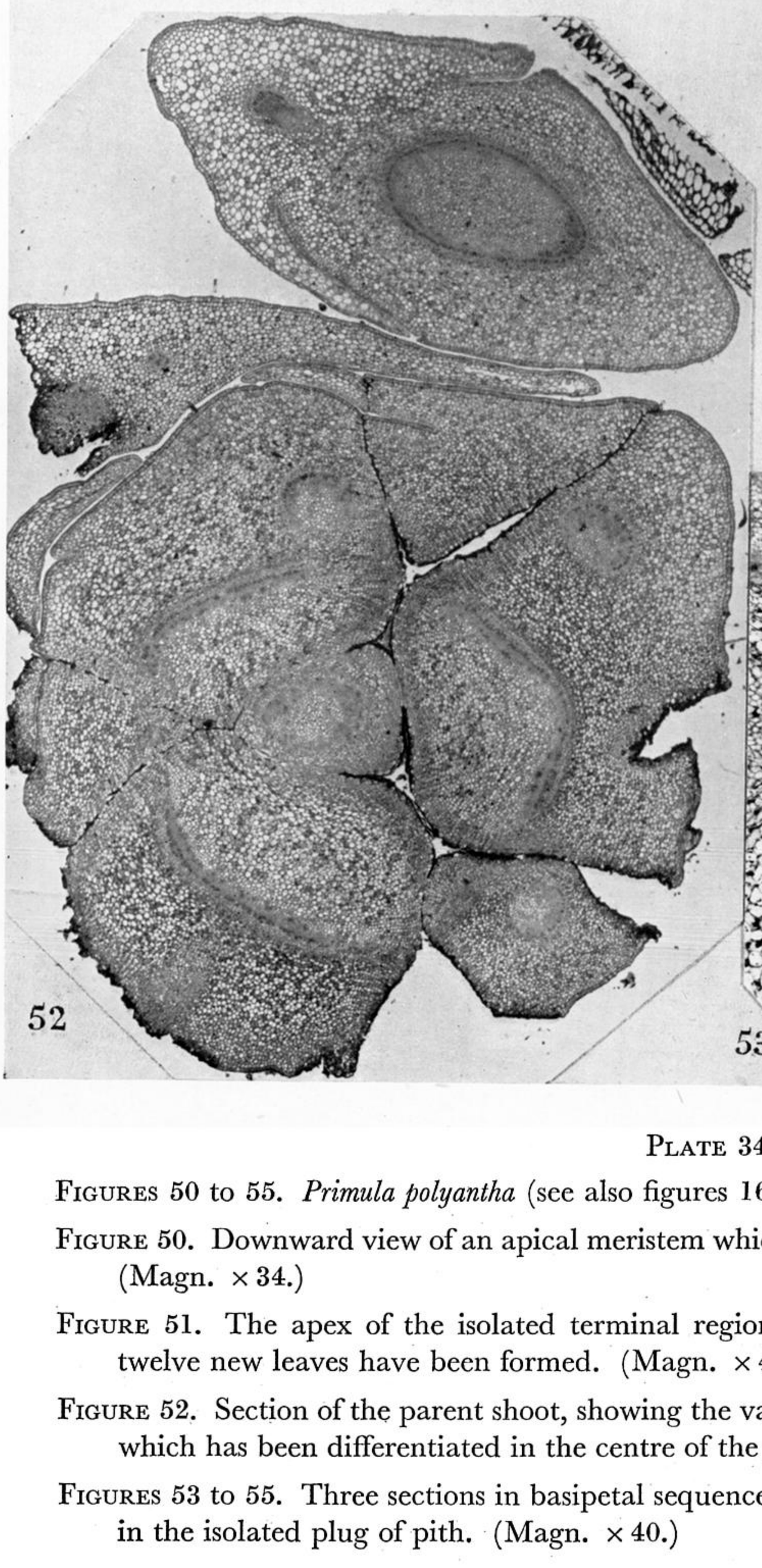
Figures 45, 46. *Primula Wanda.* Transverse sections, in basipetal sequence, just below the apex, of a vegetative shoot, showing the prevascular ring interrupted by the gaps of leaves 5 and 6 (see figures 10, 11 in text). (Magn. × 150.)

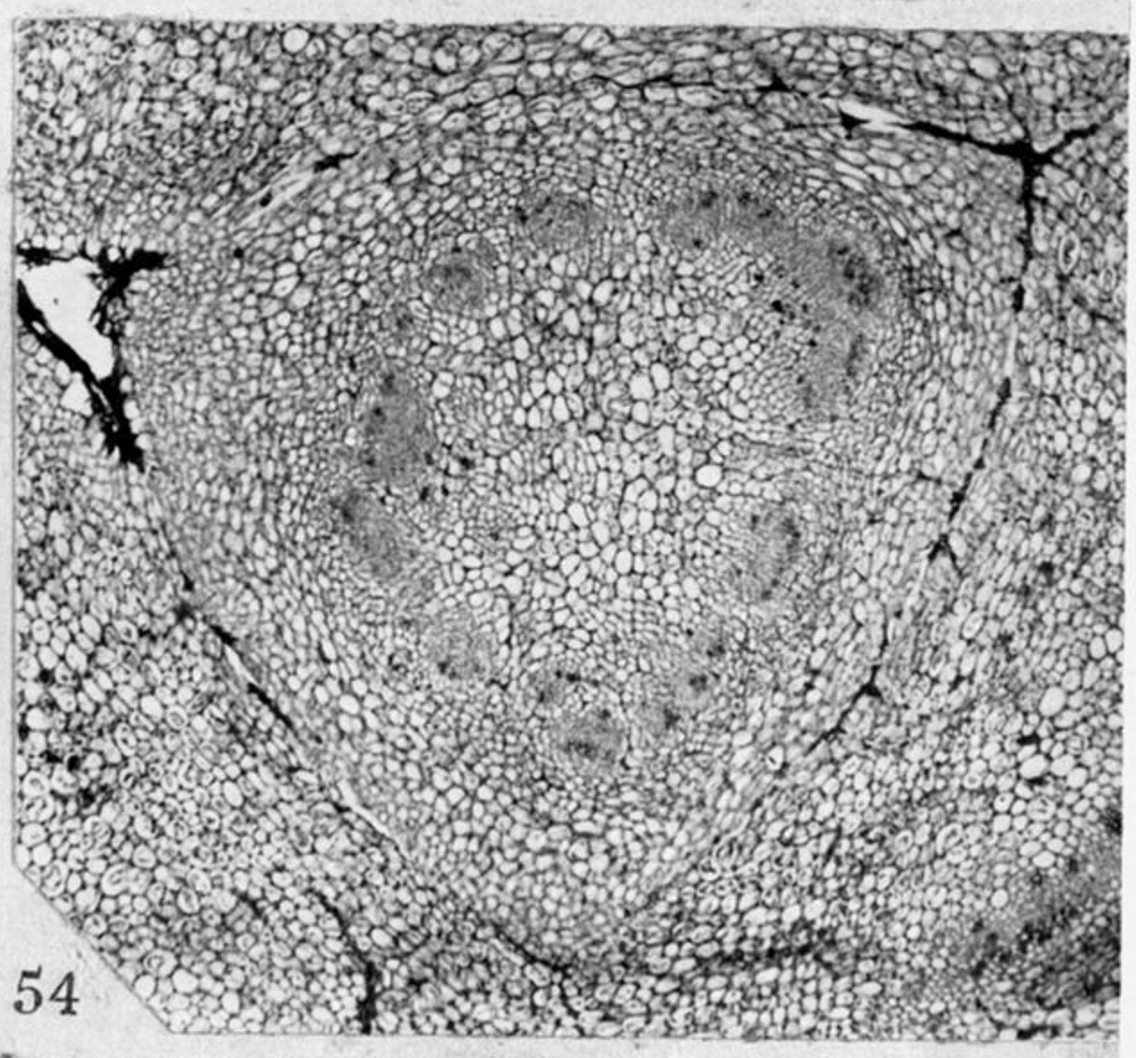
Figures 47 to 49. *Primula polyantha.* Three transverse sections, in basipetal sequence, of the shoot formed from a vegetative apex which had been isolated by vertical incisions and then defoliated as new leaf primordia appeared; a continuous vascular ring is present (figure 47), the vascular strands becoming more discrete and separated by ray parenchyma in the downward development of the vascular tissue. Below the level of figure 49 the vascular strands faded out in the pith of the parent shoot. (Magn. × 80.)


50


51


55


52


54

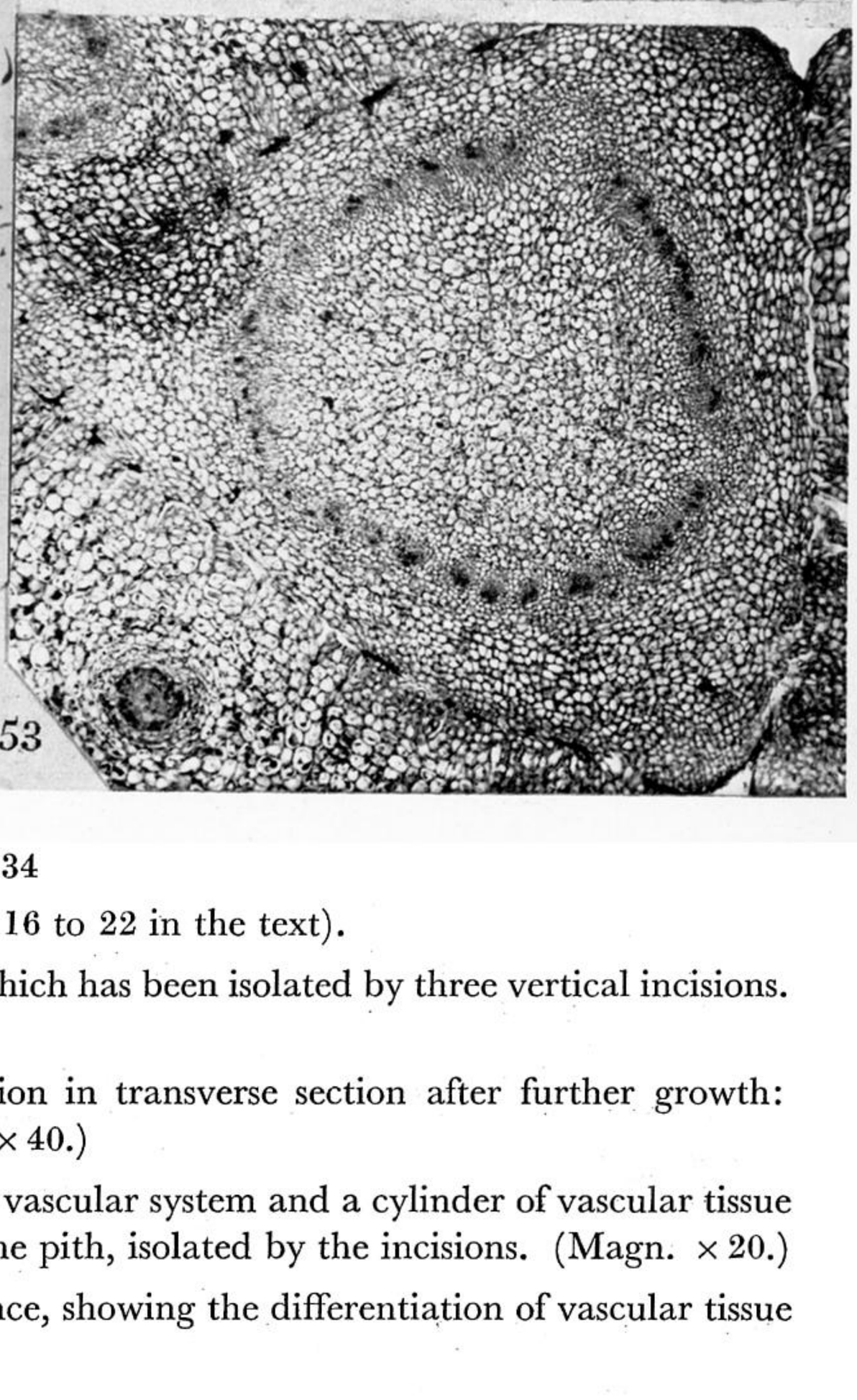

53

Plate 34

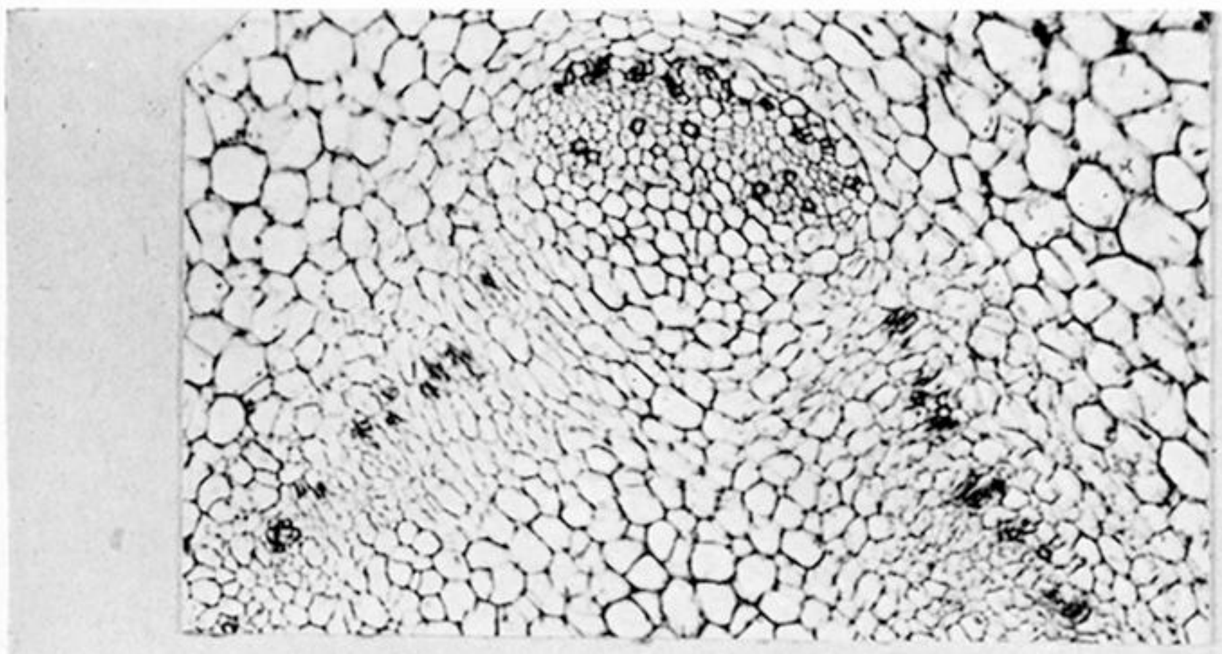
Figures 50 to 55. *Primula polyantha* (see also figures 16 to 22 in the text).

Figure 50. Downward view of an apical meristem which has been isolated by three vertical incisions. (Magn. × 34.)

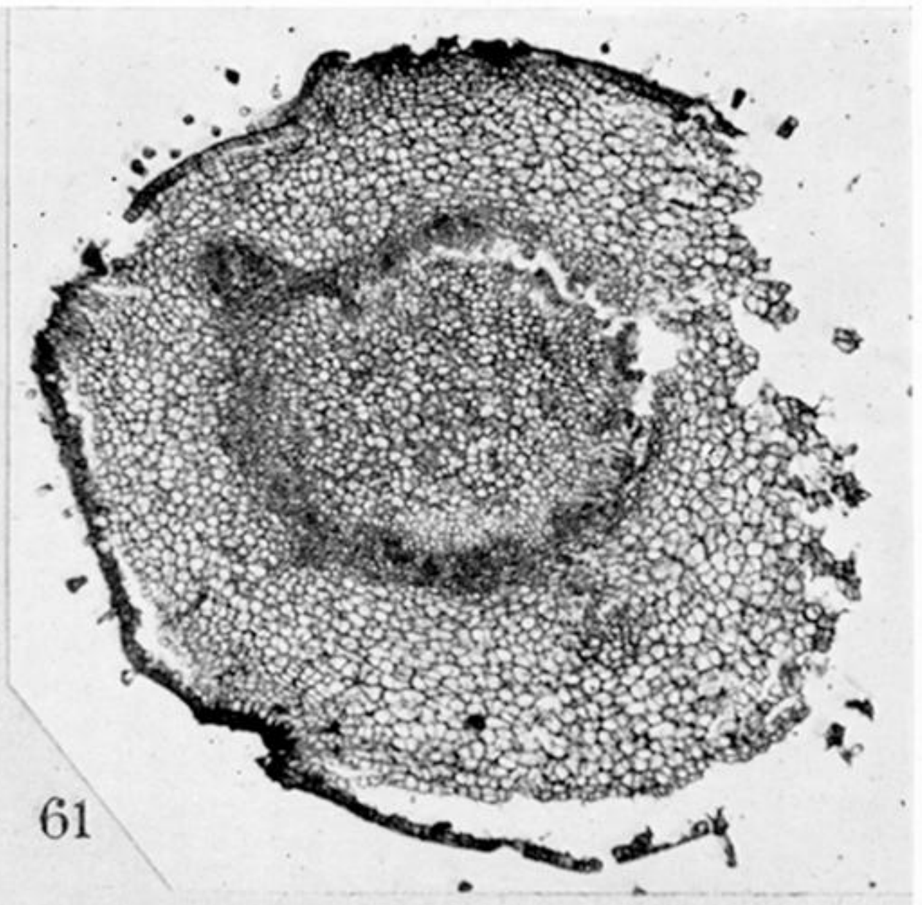
Figure 51. The apex of the isolated terminal region in transverse section after further growth: twelve new leaves have been formed. (Magn. × 40.)

Figure 52. Section of the parent shoot, showing the vascular system and a cylinder of vascular tissue which has been differentiated in the centre of the pith, isolated by the incisions. (Magn. × 20.)

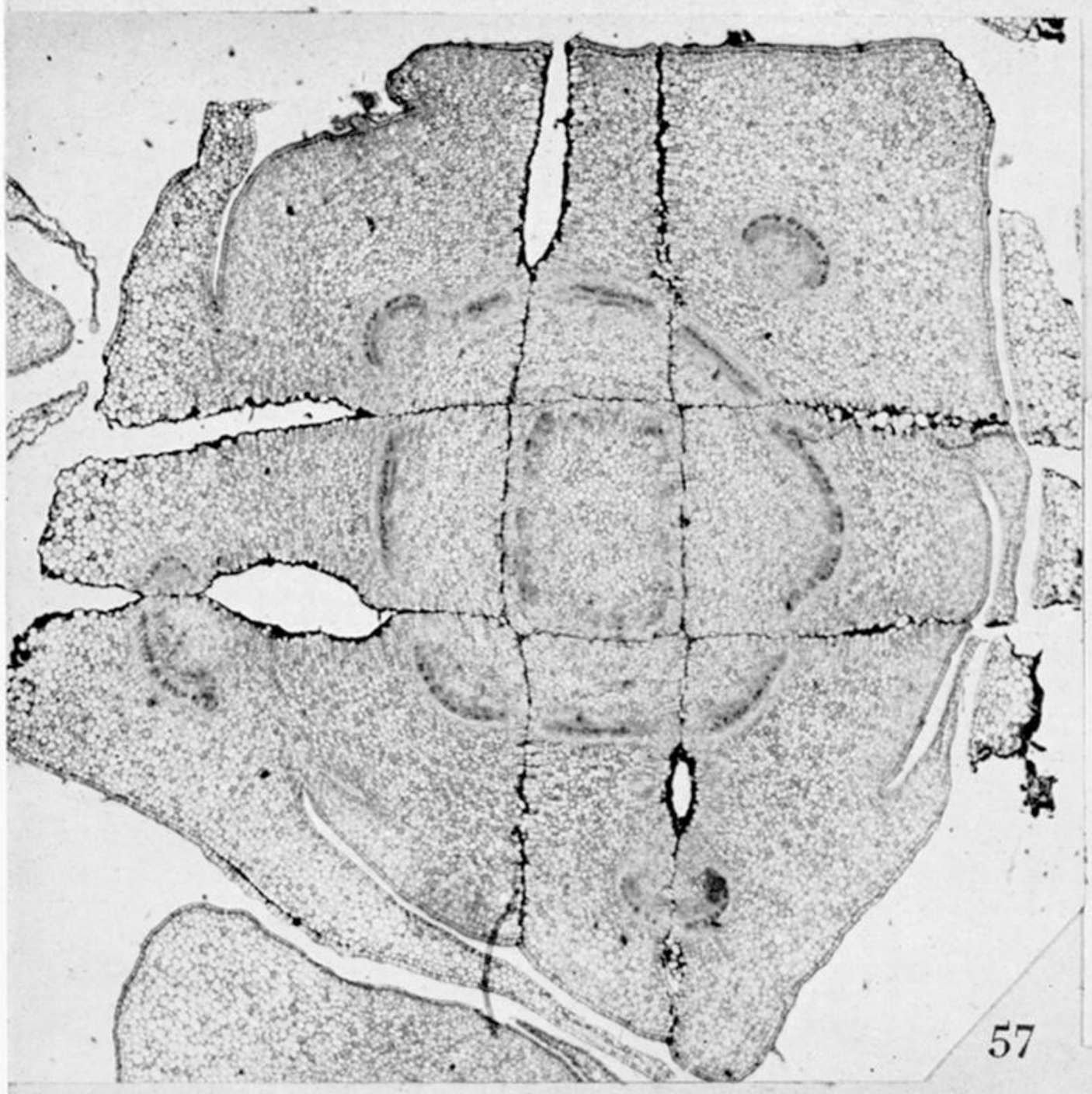
Figures 53 to 55. Three sections in basipetal sequence, showing the differentiation of vascular tissue in the isolated plug of pith. (Magn. × 40.)



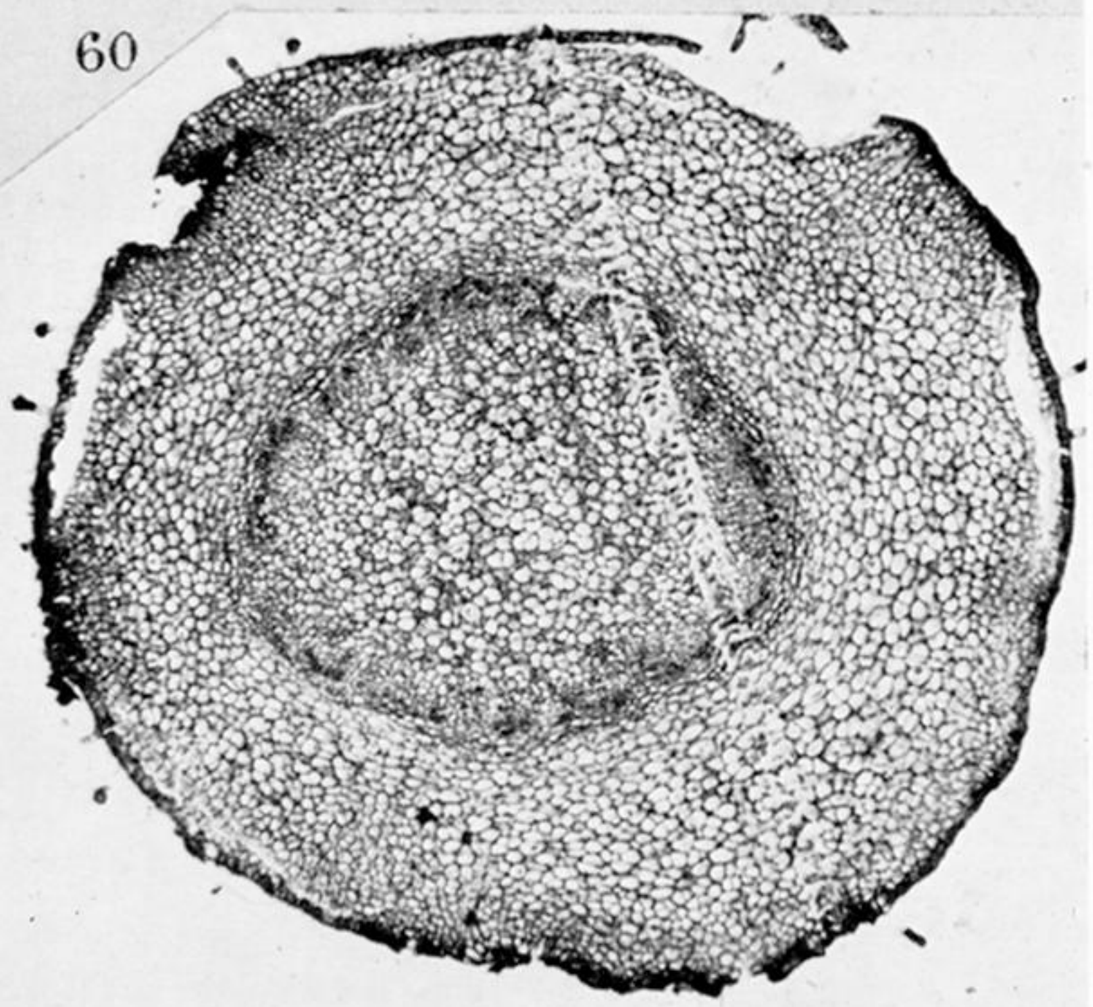
56



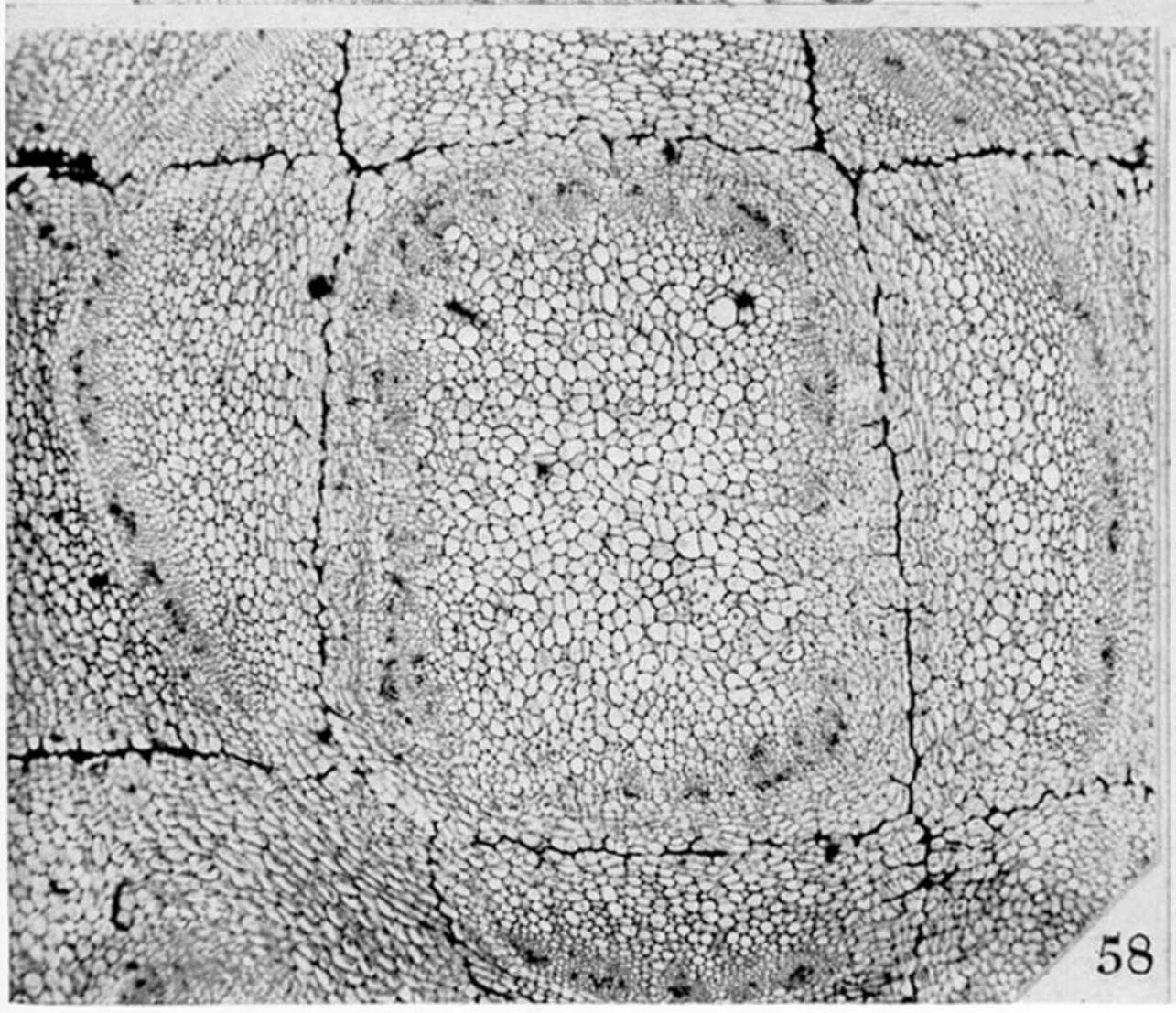
61



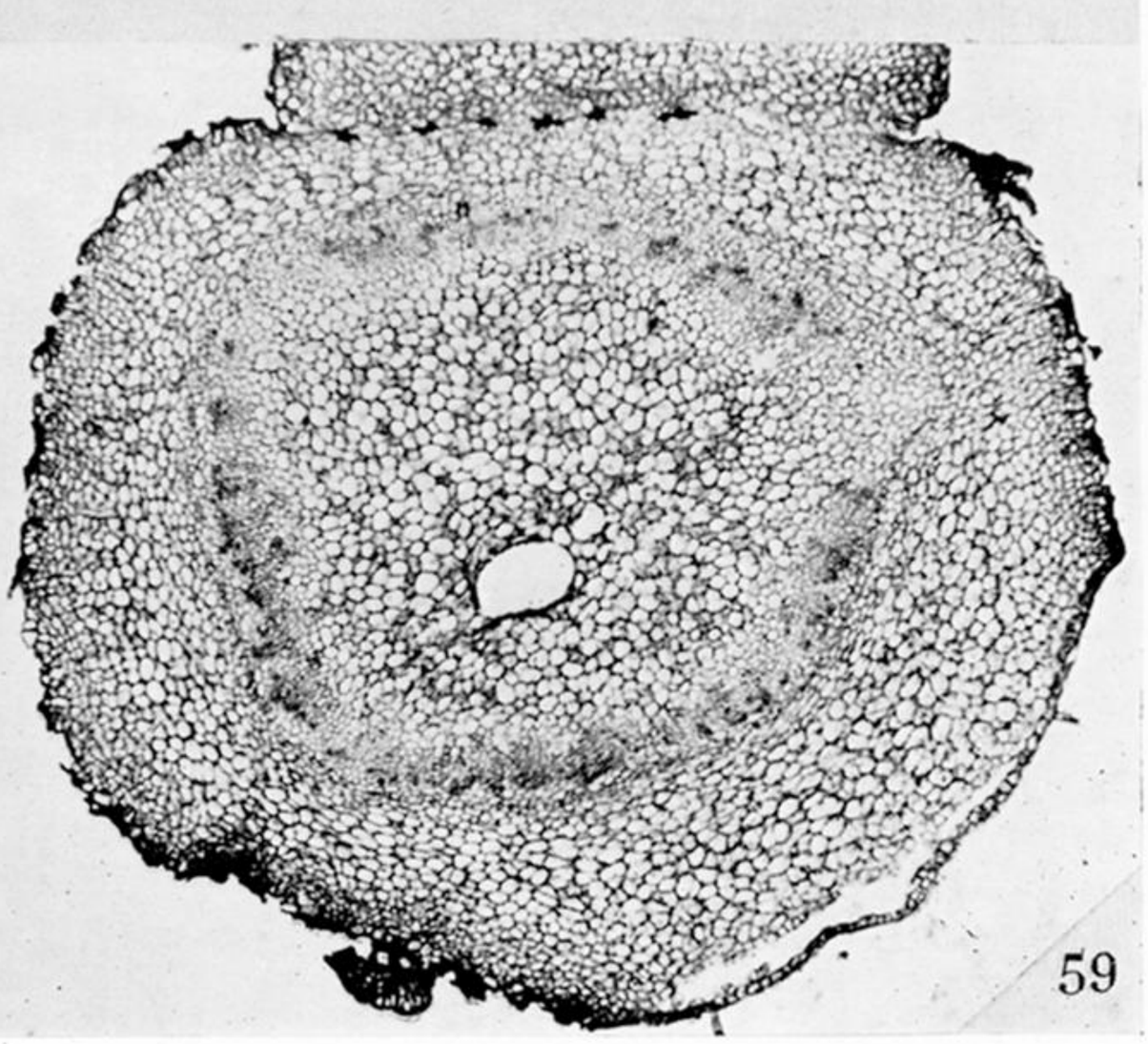
57



60



58



59

PLATE 35

FIGURE 56. *Primula Wanda* (see figure 11 in the text). Shows the trace of leaf 6 and its gap, close to the shoot apex. (Magn. 150.)

FIGURES 57 to 61. *Primula polyantha.* The apical meristem was isolated by four vertical incisions and the new shoot was defoliated; figure 57, a stele of rectangular outline in cross-section has been differentiated in the central pith. (Magn. ×20.) Figure 58 shows the structure of the induced central stele, the position of the incisions and the cylindrical stele of the parent shoot. (Magn. ×50.) Figures 59 to 61, three sections of the defoliated axis in acropetal sequence, showing an uninterrupted cylinder of vascular tissue. (Magn. ×50.)