FURTHER EXPERIMENTAL OBSERVATIONS ON THE SHOOT APEX OF *DRYOPTERIS ARISTATA* DRUCE

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[Plates 24 to 30]

CONTENTS

Investigations of the distribution of growth in normal and experimentally treated apices of *Dryopteris aristata* Druce, and of factors which determine the form and structure of leaves, buds and scales, are described and discussed. The conclusion is reached that some of the classical views on the organization of the leafy shoot are not compatible with the data now available.

Growth is considerably more rapid in the subapical region than at the apex. Leaf primordia grow actively, but growth is slow in their axils and in certain interfoliar positions. As a result a system of stresses is induced in the meristem; this may be a factor determining the positions of new leaf primordia. Shoots from which all primordia, leaves and roots had been removed became attenuated, but leaf primordia and scales continued to be formed at approximately the normal rate. The primary morphogenetic processes at the apex are thus apparently independent of the presence of primordia, leaves and roots. When the apical meristem was isolated by deep vertical incisions it grew slowly and decreased in size; but lateral buds formed in close proximity soon developed to large size. When the apical cell was damaged, the growth of the isolated terminal region was carried on by one or more buds which developed from cells of the meristem. Apical meristems, three-quarters ringed, with concomitant severing of the incipient vascular tissue, developed symmetrically; hence it appears that the upward movement of nutrients to the meristem takes place over the whole cross-sectional area of the shoot and not specifically by way of the incipient vascular tissue.

When incisions were made very close to the apical meristem a protostelic shoot, with a solid core of tracheides, was formed, i.e. the dictyostele was reduced to a protostele by a single operation. In many of the large buds which developed in the subapical region a solenostelic vascular system was differentiated, there being no antecedent protostelic stage as in the normal development. This finding is of interest in relation to classical views on the nature and evolution of the fern stele.

When the apical cell was punctured, leaf primordia continued to be formed, usually in normal phyllotactic sequence, until all the space on the apex had been utilized. Bud primordia were formed in the subapical region and lower part of the apical meristem, their formation being subsequent to that of the leaf primordia. Scales developed on the meristem round the margin of the necrosed tissue. The formation of leaf primordia is thus independent (*a*) of the apical cell, although the presence of the latter is necessary for the continued growth of the meristem, and (*b*) of older primordia and leaves, though the possibility that these members may exercise an indirect effect is not excluded. Buds are not inhibited by leaf primordia, but they are by substances proceeding from the apical cell and possibly its immediate segments. The view is advanced that the chief differences between leaf and bud primordia are referable to the positions which they occupy at the time of their formation.

A re-examination of leaf formation has shown that the very young primordium originates *not* from a single superficial meristematic cell, as described in the literature, but from a group of meristematic cells. At an early stage, however, one of the more centrally placed cells begins to enlarge and becomes the conspicuous apical cell of the primordium. The underlying cells also undergo rapid division. These data have a particular interest in that they show that leaf formation in ferns and flowering plants is in the main essentials closely comparable.

Leaf primordia of different ages, isolated by vertical incisions, undergo some further growth: the older primordia may form pinnae but the younger ones terminate in a straight or outwardly-curved, awl-like structure; in the latter, the apex loses its meristematic character and becomes parenchymatous and the vascular system gradually fades out. According to the size of the primordium at the time of isolation, a foliar gap may or may not develop in the shoot stele; the leaf trace may develop as a solenostele, it may remain coherent and crescentic in cross-section, or it may become disrupted into meristeles. In a majority of isolated leaf primordia, axillary buds develop and become the predominant feature; these buds may be solenostelic from the outset; lateral and abaxial buds may also occur. Even when very young leaf primordia are isolated, the typical dorsiventral symmetry, which is evidently established at a very early stage, is retained.

In the formation of plant organs, factors in the genetic constitution, physical factors of various kinds, and factors in the environment are all involved. In practice, these are difficult to distinguish. From the writer's analysis it appears that many of the major morphological features of vascular plants are to be related to extrinsic rather than to genetic factors; the two kinds of factor, of course, work in conjunction. It is a matter of general interest to botanists to know which aspects of morphological development are due primarily to extrinsic factors and which to intrinsic or genetic factors; in investigations into causality our primary concern is with the former, in phylogenetic studies it is with the latter. Reasons are given for the view that the shoot type of organization in different classes of vascular plants is probably the result of parallel evolution, i.e. homoplastic development. Furthermore, it is suggested that the lateral members are not necessarily determined by specific factors in the hereditary constitution. Thus, in a plant with the potentiality for producing lateral members, the form and structure of a lateral organ, be it leaf, bud or scale, depend primarily on the position in which it is formed at the apex; i.e. given a certain specific hereditary constitution, it is to the mechanics of growth in the plastic distal region of the shoot that we must look for explanations of the characteristic symmetry, form and structure of the several lateral organs. But ultimately, in every investigation of morphogenetic processes, the hereditary constitution of the organism must be considered and an attempt made to understand how the several kinds of factors, by their action and interaction, determine specific form and structure.

1. Introduction

What has been described as the leafy shoot, the shoot with appendages, or the shoot type of organization, is characteristic of pteridophytes and seed plants. Whether the vasculated shoot has been evolved independently along several lines of descent, or whether it affords evidence of community of origin of vascular plants, has been a matter of much conjecture. What is evident is that all vascular plants have much in common: their axial development, the formation of their lateral members (leaves, buds and scales), and the differentiation

of their tissue systems, all result from the activity of an apical growing point. This dynamic region, though of paramount importance, is one of which our knowledge is still quite adequate. Extended morphological, physiological and genetical investigations are required for the elucidation of the problems of organization and formative activity at the shoot apex. Whatever views may be held as to the phylogenetic origin of the shoot and its lateral members, the formation of each and every organ in the individual plant is the result of factors which are at work during the process of growth. Studies of organ formation under both normal and experimental conditions are among the ways by which our knowledge of the apical meristem and the shoot type of organization may be advanced. The purpose of the present paper is to give an account of (*a*) the apical meristem of leptosporangiate ferns and the organs and tissues to which it gives rise, (*b*) hypotheses which have been advanced in explanation of the observed developments, and (*c*) experiments undertaken to test these hypotheses and to increase our knowledge of the apical region and of the shoot type of organization to which it gives rise.

2. The apical meristem and organogenesis

The shoot *apex* (*apical growing point* or *apical cone*) in adult plants of *Dryopteris aristata* consists of a centrally placed, conical protuberance on the rounded distal region of the shoot (figure 45, plate 24). Each new leaf primordium is formed a short distance above the base of this cone. During growth the primordium enlarges and recedes from the apex; the older primordia and young leaves thus occupy positions on the *rounded subapical region*. The *apical meristem*, as defined by Wardlaw (1943*a*), consists of a superficial layer of *prism-shaped cells* of distinctive appearance and chemical reaction, the summit of the cone being occupied by the tetrahedral apical cell. The prism-shaped cells, which are derived from the apical cell, extend down the sides or flanks of the cone for a variable distance, but do not extend to its base (figure 1). The apex, though covered in by scales, is itself devoid of scales; these are formed round its base, i.e. in the region where the cone becomes laterally distended to form the broad subapical region. The scales form a close investment round the developing leaf primordia but very young primordia have no associated scales; scales first appear on the abaxial side of the primordium and then all round it. In the normal apex, lateral buds are never observed on or near the apical meristem, but they can be induced to develop there (Wardlaw 1943*b*). In relation to the distribution of growth in the apical and subapical regions, small areas of the original apical meristem become separated off or detached, and persist in characteristic positions along the shoot. These *detached meristems* or *bud rudiments* may be described as occupying axillary or interfoliar positions; they are sometimes considerably displaced by the extensive growth of the leaf bases (Wardlaw 1943*a*, *b*). Each of the three kinds of lateral organ thus originates in a characteristic position and at a characteristic level on the apical cone, while their spacing is such as to constitute a regular pattern, e.g. the leaves are formed in a regular phyllotactic sequence.

3. Views on the organization of the leafy shoot

It has been suggested by Bower (1923, 1935) from studies of land plants, and by Fritsch (1945) from studies of the algae, that the prototype of the pteridophytes was probably an organism of considerable simplicity, consisting of a rootless, leafless axis, with a simple

vascular strand, and terminated by a sporangium. Relative to such a plant, a leptosporangiate fern with its dictyostelic shoot, large leaves, scales and roots and sori of sporangia, is an organism of considerable complexity. These several elaborations of external form and internal structure are held to be the result of progressive change during descent, and adherents of comparative morphology, from studies of fossil and living plants, have attempted to give some reasoned account of the course of events. The adherents of the general or causal morphology of Hofmeister (1868), on the other hand, have been more concerned with problems relating to the elaboration of form and structure in the development of the individual plant. It is not enough to say that an organism develops as it does because of its hereditary constitution; for, to quote His (1888): 'To think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism.' The shoot type of organization has thus a twofold interest.

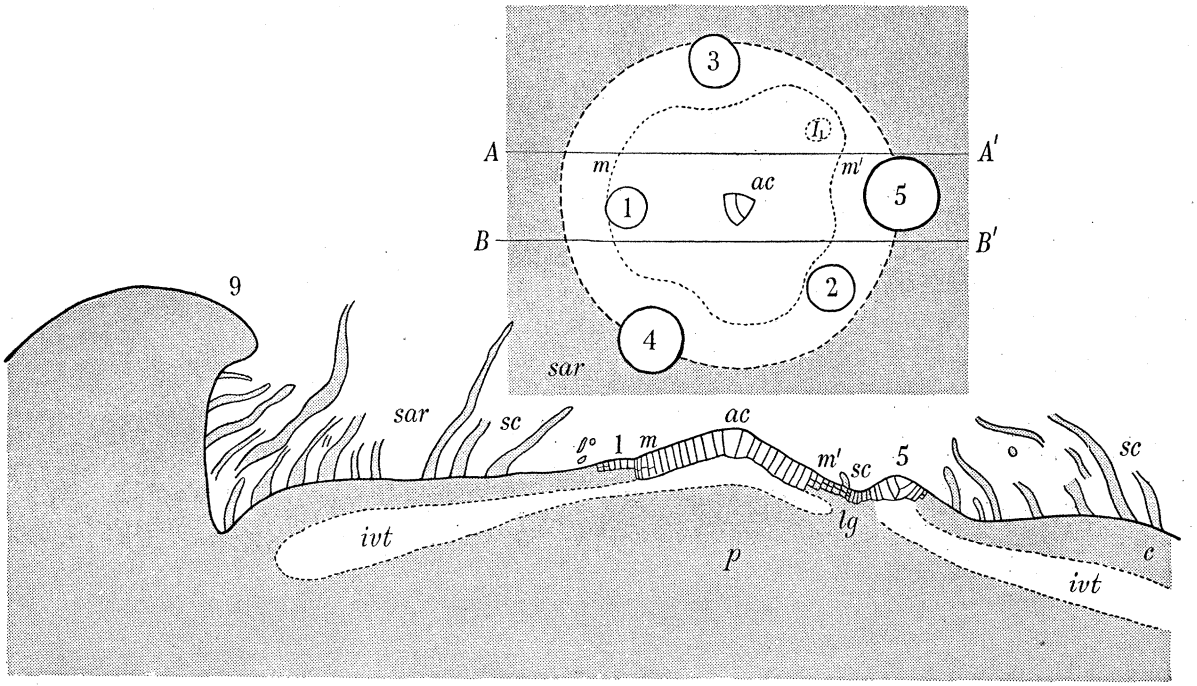


FIGURE 1. *Dryopteris aristata:* the apical cone as seen from above and in median vertical section (*AA'–BB'*). The interrupted line separates the base of the cone from the subapical region (stippled). Leaf primordia, 1, 2, 3 etc. in order of increasing age; I_1, position of next primordia to be formed; *ac*, apical cell; the apical meristem (*m–m'*) does not cover the whole surface of the apical cone but extends a variable distance down its sides; *ivt*, incipient vascular tissue below apex; *sar*, subapical region; *c*, cortex; *p*, pith; *sc*, scales; *lg*, leaf gap. (Semi-diagrammatic, × 30.)

Different views have been expressed as to the nature of the shoot and its lateral members during the last two hundred years. Many of these, products of strictly comparative studies, suffer from the defect of being more or less completely non-physiological in conception. Yet they have held an important place in the development of botanical thought. It is therefore a matter of interest to consider to what extent they are compatible with the data of experimental studies.

It was Wolff (1759) who first stated that growth in plants proceeds from an undifferentiated growing point and that he saw nothing ultimately in the plant but leaves and stem,

including the root in the stem (Sachs 1890). In Goethe's *Theory of Metamorphosis* (1790) the shoot only received attention as the axis on which the leaves were disposed, while the root was almost completely neglected. To Sachs (1875), a fully developed vascular plant consisted of parts belonging to certain fundamental categories: the *caulome* or shoot, the *phyllome* or leaf, the *rhizome* or root, and the *trichome* or hair. These parts were combined or integrated to form the rooted, leafy shoot which was the real unit. Though modified in his later work (1887), Sach's view implied, to some extent at least, that the construction of the leafy shoot was based on some ideal plan. As Bower (1947) has pointed out, no separate category was established to include the sporangia, since these were held to be metamorphosed vegetative parts, a conception which is traceable back to Goethe.

Current views relating to the leafy shoot are broadly speaking of two kinds, *phytonic* and *axial* (or strobilar) (Bower 1923, 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 are the fundamental units (Gaudichaud 1841; Chauveaud 1921). Although 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 are artificial and unrelated to the facts of physiology and embryology. The telome theory of Zimmermann (1930), as it seems to the present writer, is open to similar criticisms.

Supporters of axial theories regard the shoot 'as a phyletically pre-existing axis or stem from which the leaves may have arisen by enation' (Lang 1915). A view similar to that of Sachs, i.e. that the leafy shoot is the real unit, has been adopted by Bower (1935) where he observes that 'axis and leaves act together as a physiological whole and are so initiated in the embryology: also, in evolutionary history, as based on comparison of early fossils, such as the Psilophytales. The shoot unit of Sachs is the natural, that is the developmental and evolutionary unit.' Earlier, Bower (1922) had pointed out that the young vascular plant consists essentially of a simple spindle or axis, with a distinction of apex and base. Given such a unit, he has elaborated the view that plant bodies progressively more elaborate in construction may result from dichotomy; and this in turn 'may pass over to monopodial branching, thus producing lateral appendages' (Bower 1935). These appendages may include: (*a*) *lateral branches* in which the shoot structure is repeated; and (*b*) *megaphylls*, like the large leaves of ferns, which are thus regarded as being of cladode origin. Other lateral appendages may originate by *enation of parts* from shoot surfaces 'not previously tenanted' (Bower 1935, p. 540); such appendages would be exemplified by the *scales* of ferns and the *microphylls* of the Lycopodiales. These instances make it evident that Bower regards the fundamental unit as being the shoot, axis or spindle, and not the leafy shoot of Sachs. But in all these morphological discussions, the so-called fundamental unit, whether filament, axis or leafy shoot, is taken for granted as a unit ready-made. From the standpoint of the experimental morphologist nothing should be taken for granted, but the potentiality for growth of the zygote, the meristematic cells of a bud rudiment, or a tissue culture of the plant selected for investigation. The task is then to answer the question: During the growth of the zygote or of a group of meristematic cells, what factors determine the characteristic form and structure of the axis and its lateral members?

4. Materials and methods

Apices of stout shoots of *Dryopteris aristata* were laid bare as already described (Wardlaw 1947*a*), all the older leaves and primordia except those immediately surrounding the apex being excised and the scales removed (figure 45, plate 24). For operations such as puncturing the apical cell a needle ground to a fine tapering point was used. Scales were removed by means of a similarly sharpened needle bent at the point. Small incisions were made by means of a cataract knife. Information on the distribution of growth at the apex was obtained by applying a suspension of lamp-black (in a 10 % aqueous solution of gum-arabic) and observing its dispersal. The experimental materials were kept in moist peat in a cool greenhouse.

Terminology. In addition to the terminology given in §2, the writer has followed that of M. and R. Snow (1931, 1933, 1947, 1948) in indicating the leaf primordia. Thus P_1, P_2, P_3, etc., indicate the leaf primordia which are *visible* at the apex, in order of increasing age. I_1, as yet *invisible*, is the next primordium to be formed; I_2, the next again, and so on. Here the writer has used I_1, I_2, etc., to indicate positions to be occupied by primordia, or, where an apex was under observation for a considerable time, for the new primordia which in due course had actually appeared. Leaf primordia may occur either in right-handed or left-handed spirals, an angle of divergence between primordia of 138·5° (5/13 phyllotaxis) being general.

5. Observations on growth at the apex

(*a*) *Normal distribution of growth*

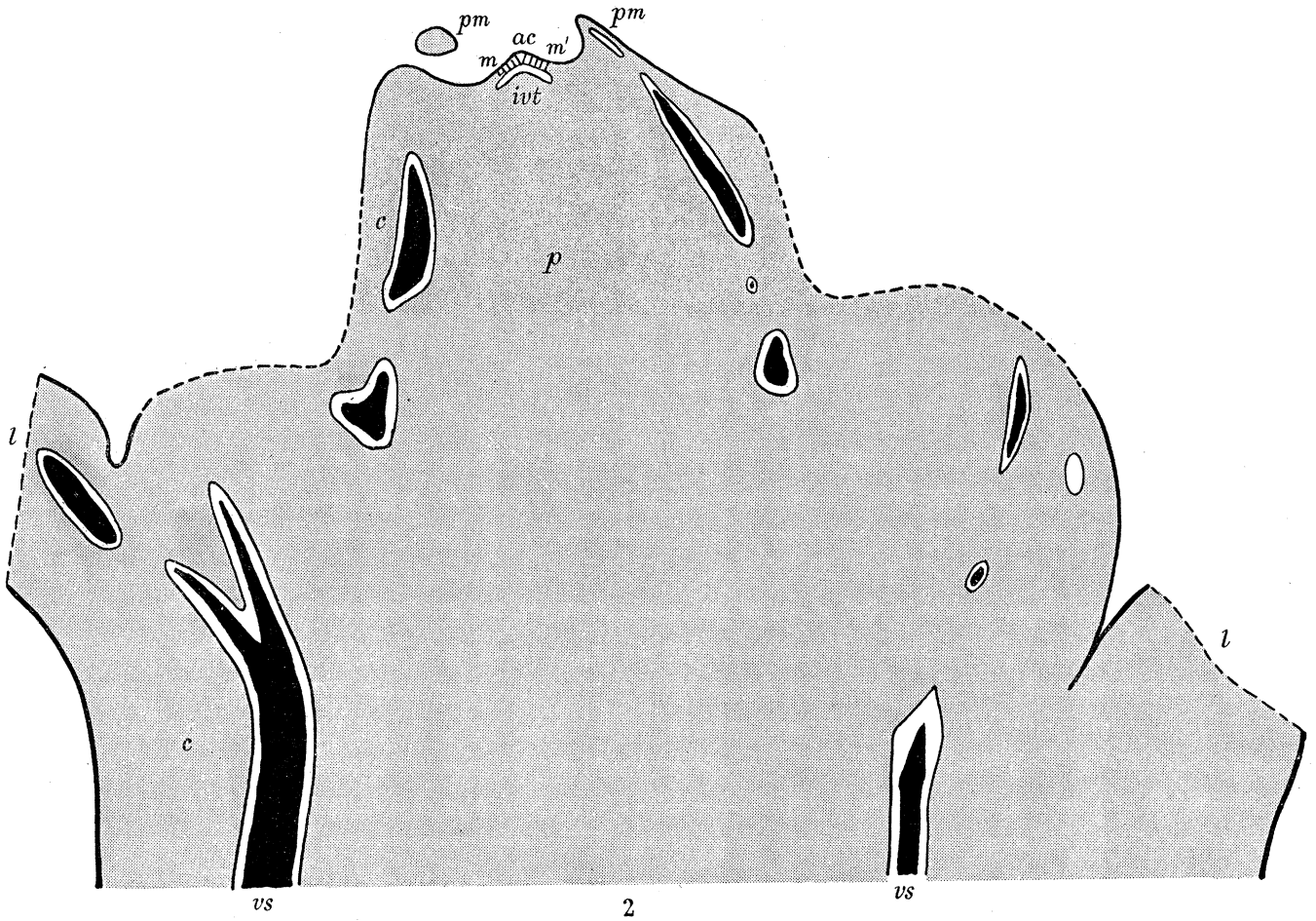
Figure 46, plate 24, illustrates the appearance of an apex of *D. aristata* after the application of a suspension of lamp-black in gum-arabic solution. In the course of a week in a cool greenhouse scales began to appear through the film of lamp-black. During the next few weeks, some of the older primordia situated just outside the apex began to appear through the black deposit as shining mounds of green tissue. The younger primordia at the base of the apical cone, i.e. P_1 to P_5, also became evident after some time. But whereas the actively growing leaf primordia were thrusting aside the lamp-black, no comparable growth was evident in the axils of the younger primordia. The deposit, in fact, persisted in the axil until the primordium had receded from the apex; thereafter it became dispersed. An early dispersal of the lamp-black on the abaxial side of primordia was general. Again, whereas from an early stage there was evidence of active dispersal of the deposit in the subapical region immediately adjacent to the base of the apical cone, the apex, except in the region of P_4 and P_5, still showed a dense deposit. That some growth was taking place, in the apical cone was, however, indicated by the fact that the black deposit slowly became broken up into a mosaic-like pattern; with the passage of time a gradual dispersal of the lamp-black on the apex, from the base upwards, took place. It could then be seen that the deposit lay farthest down the sides of the cone in the positions of I_1 and I_2. Residual black areas were also present in interfoliar positions just outside the base of the apical cone. After several weeks, only a light peppering of lamp-black remained in the region of the apical cell and this eventually disappeared. Some of these points are illustrated photographically in figures 47, 48, plate 24. From these preliminary observations it is apparent that there is a sharp increase in the rate of growth below the apical cone.

(*b*) *Growth of defoliated shoots*

In shoots from which all the old leaves and primordia have been removed new scales appear in the course of a week and new leaf primordia in 1, 2 or 3 weeks. The formation of lateral buds may also begin in the subapical region, but these soon become inhibited if the main shoot apex is active. During the growing season new leaf primordia are formed at the rate of one per week approximately. After 9 to 10 weeks the new growth has the appearance of a small scale-invested bud situated on the broad distal region of the shoot, i.e. there is a sharp transition from the older normal region of the shoot to the recently developed region (figure 2). In some specimens the new region of the shoot had sensibly decreased in diameter, each of the several tissues (cortex, vascular tissue and pith) being affected. The apical meristem and developing leaf primordia of the new growth were also of smaller size than in a normal untreated shoot (figure 3). Thus, while there are sufficient reserves in stout shoots to admit of continued and even considerable apical growth, the general effect produced is one of attenuation. Exceptions to the kind of development described above have been noted (*see* Wardlaw 1944*b*, figure 3, plate IV).

(*c*) *Growth of laterally isolated meristems*

In these experiments the apical meristem was isolated laterally from the adjacent organs and tissues, usually by four vertical incisions. This technique, which involves a complete severing of the incipient vascular tissue, has already been described (Wardlaw 1947*a*), together with a preliminary account of the growth developments which take place. It has been shown that the meristem thus isolated is capable of further growth; that it forms a short shoot with a solenostelic vascular system, the latter being in no way connected with the vascular system of the older region of the shoot below; that new leaf primordia are formed in normal phyllotactic sequence with the older primordia, the shoot vascular system becoming dictyostelic once again; and that eventually roots are also formed. In the present series of experiments deep incisions were made close to the meristem (figures 49, 51, 52, plate 25). The meristem was thus isolated on a long thin column of pith parenchyma. In these materials numerous lateral buds developed, some in close proximity to the apical meristem. It was thus possible to compare the growth of the latter with that of the immediately adjacent buds. Figure 4 illustrates diagrammatically a longitudinal median section of a typical specimen; a notable feature is the great reduction in the size of the apical meristem in the isolated terminal region. The amount of growth at the shoot apex—the isolated terminal region—has been small as compared with that of the lateral buds (figures 49, 51, plate 25). Moreover, whereas the shoot apex was already an organized growing system at the beginning of the experiment, the lateral buds were present only as rudiments. Figure 5 illustrates these points in another specimen as seen in transverse section; the isolated terminal region is smaller in every respect than the adjacent lateral bud and is less advanced in the differentiation of its tissues. The bud illustrated developed in the axil of the leaf primordium recorded as P_4 at the beginning of the experiment. Buds also developed in the axils of the next two younger primordia (P_3 and P_2); these were progressively smaller than the bud of P_4, but were of larger cross-sectional area than the isolated terminal region. From these data it appears that the rate of growth of a bud depends on its position, those nearest to the centre of the shoot growing most slowly.



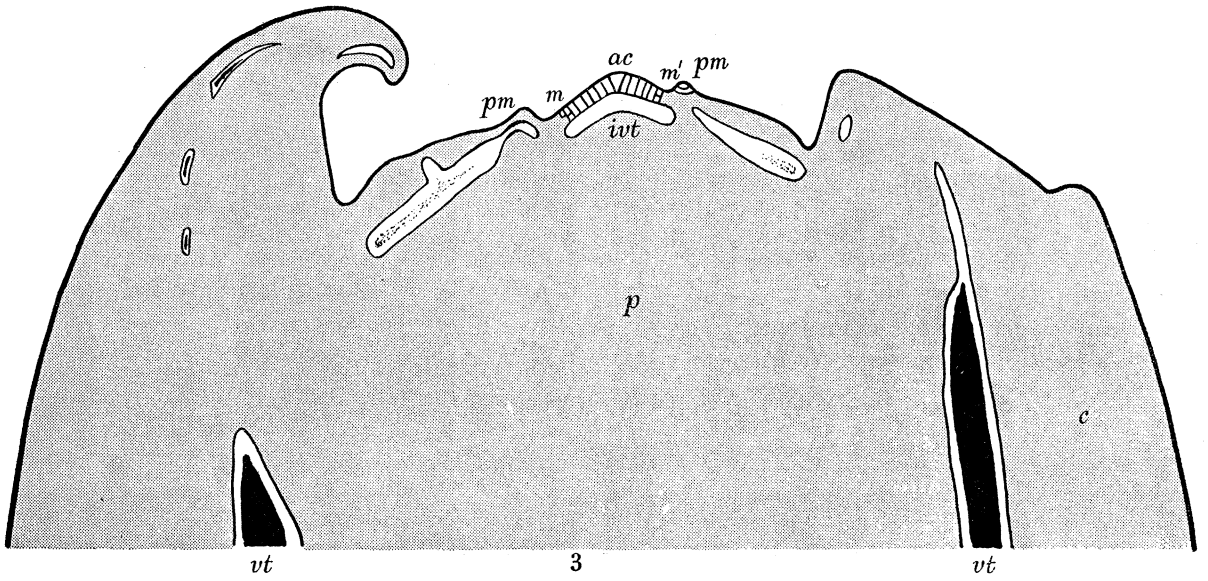


FIGURE 2. Defoliated shoot after nine weeks' growth, in longitudinal median section (scales are omitted). The diameter of the new region of the shoot has decreased and the apical meristem has become smaller, but new leaf primordia and scales continue to be formed at approximately the normal rate. *ac*, apical cell; *m–m'*, apical meristem; *pm*, leaf primordium; *l*, leaf base; *ivt*, incipient vascular tissue; *vs*, vascular strand, with xylem in black; *c*, cortex; *p*, pith. The broken line indicates where leaf primordia and leaves have been excised. (Semi-diagrammatic, × 15.)

FIGURE 3. A normal apex, in longitudinal median section, for comparison with figure 2. Lettering as in figure 2. (Semi-diagrammatic, × 15.)

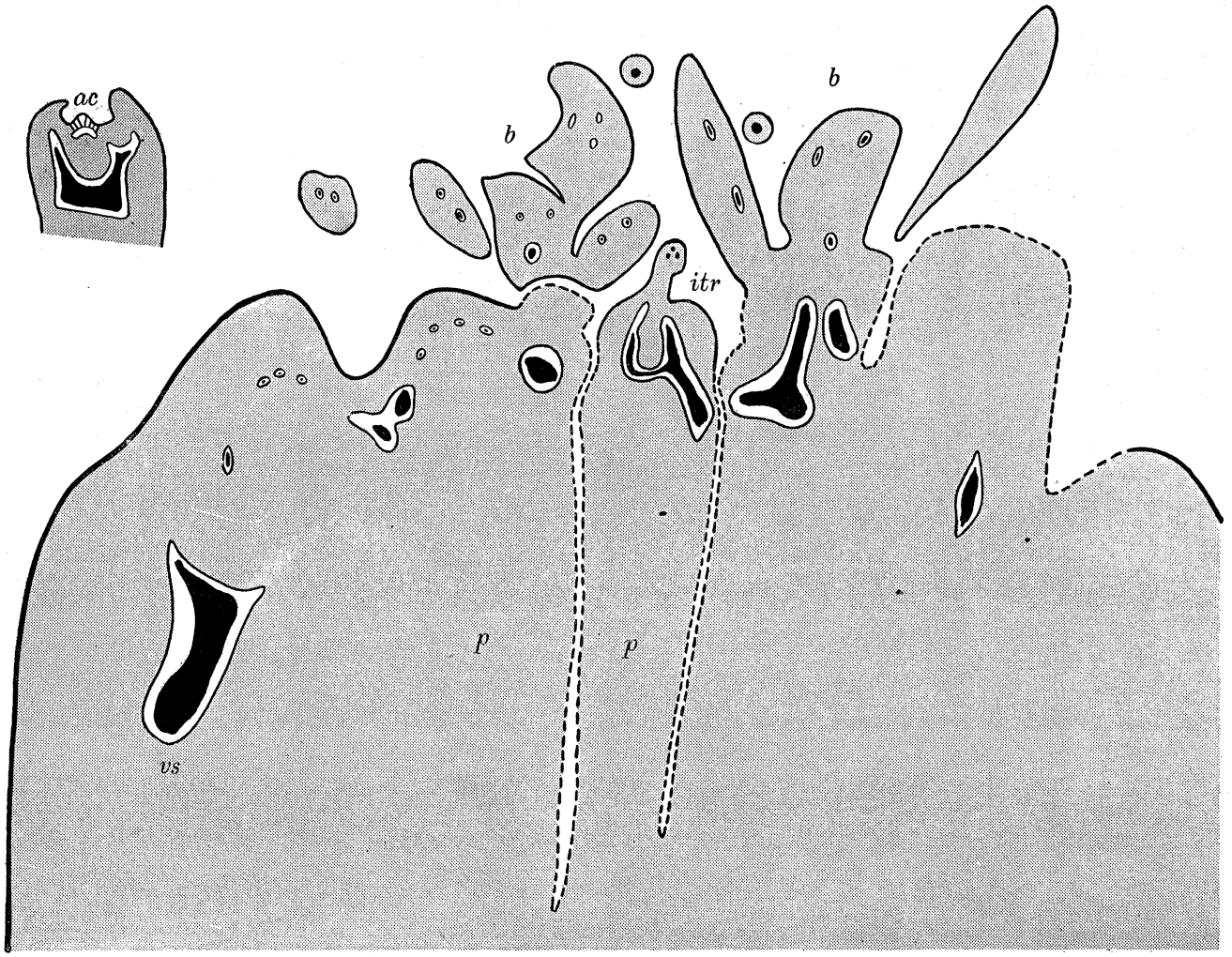


FIGURE 4. An apex, in longitudinal section, in which deep incisions were made close to the apical meristem. The isolated terminal region *itr* has grown slowly as compared with the lateral buds *b*, and its apical meristem (top left) has greatly decreased in size. *p*, pith; *vs*, vascular strand, xylem in black. (Semi-diagrammatic, × 15.)

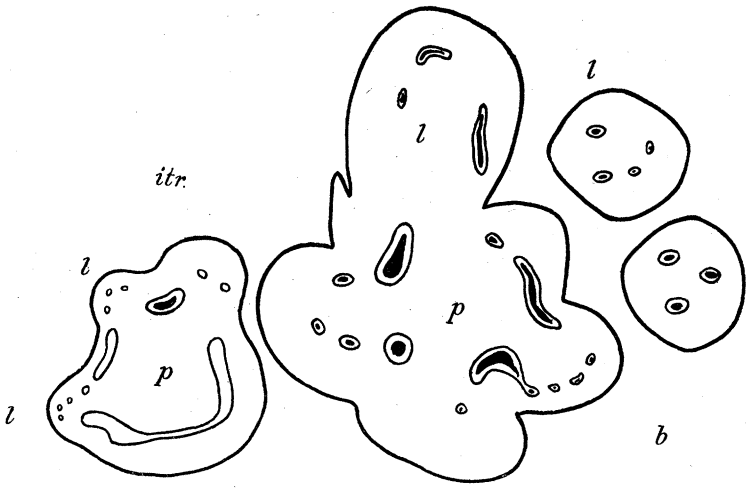


FIGURE 5. A specimen similar to that illustrated in figure 4 as seen in transverse section. The isolated terminal region *itr* is distinctly smaller than the adjacent lateral bud *b*; *p*, pith; *l*, leaves and leaf-bases; meristeles and leaf-traces with xylem in black. (Semi-diagrammatic, × 15.)

In another specimen the numbers of new leaf primordia formed on the isolated terminal region and in the newly developed lateral buds were compared. It was found that 6 had been formed in the former and 6, 7 and 8 in the buds of P_4, P_5 and P_6 respectively. In another specimen in which the apical meristem was isolated on a plug of considerable cross-sectional area, the isolated terminal region after several weeks' growth still overtopped the lateral buds (figure 6).

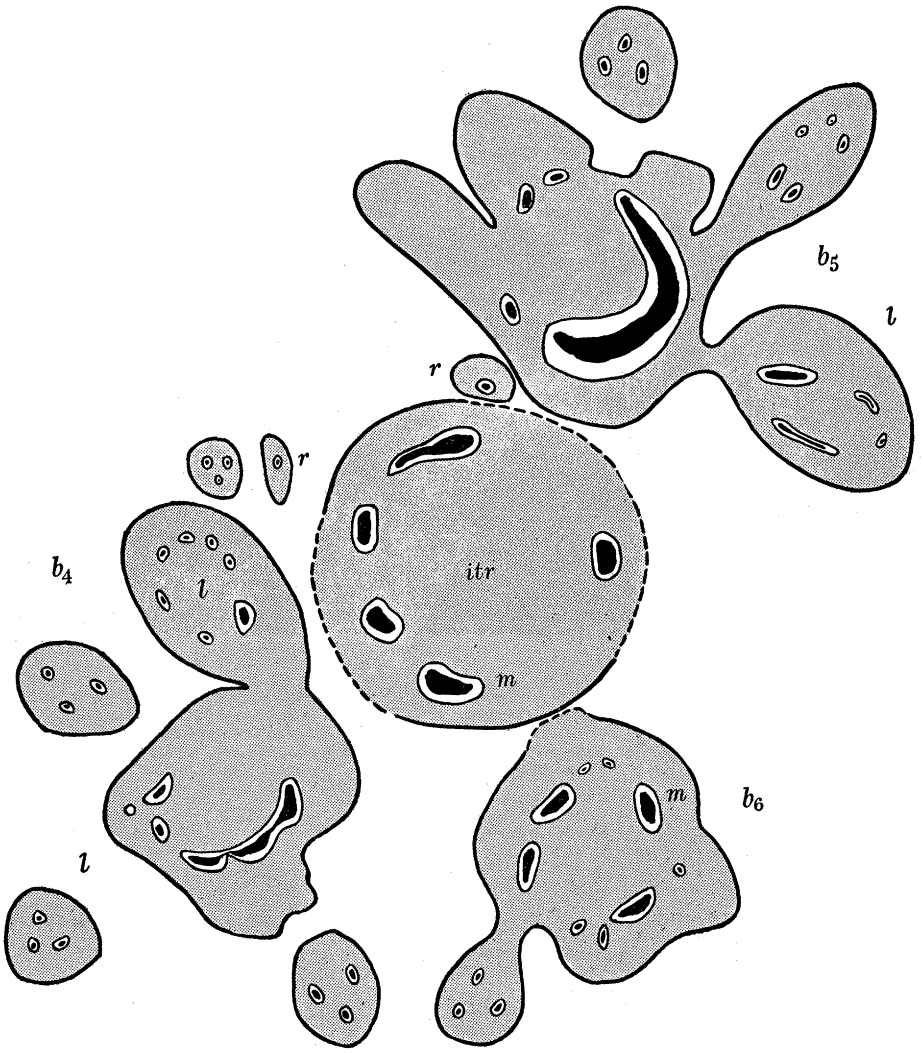


FIGURE 6. Transverse section of a specimen in which the apical meristem was isolated on a plug of considerable cross-sectional area. The isolated terminal region *itr* was larger and still overtopped the lateral buds b_4, b_5, and b_6 which were formed in the axils of P_4, P_5 and P_6 respectively. *m*, meristele; *l*, leaves and leaf-bases; *r*, root. This section was taken below the solenostelic region of the isolated terminal region, broken lines indicating incised tissue. (Semi-diagrammatic, × 15.)

In these materials, although a considerable column of pith parenchyma was isolated, no vascular strands, or isolated masses of vascular tissue became differentiated in the lower region of the parenchymatous mass (figures 49 to 52, plate 25). *Vascular tissue was formed only in close proximity to actively developing meristems*, as postulated by the writer (Wardlaw 1944*a*). This is specially mentioned here because in some tissue cultures isolated vascular tissue has been observed among the parenchymatous cells (White 1943; Gautheret 1945).

As described in the earlier memoir, the leaf primordia which developed on the isolated terminal region were in normal phyllotactic continuity with the older leaves below. This has been verified by a close analysis of a number of specimens. The relevant data are considered elsewhere (Wardlaw 1948*c*).

Starch was relatively abundant in the newly formed lateral buds and in the cortex in the subapical region, but was not present in the isolated terminal region, i.e. the original reserves of starch in the pith parenchyma had been utilized. Whether the carbohydrate supply in some instances is limiting and is directly responsible for the small size of the leaves in the isolated terminal region is a matter which requires further investigation.

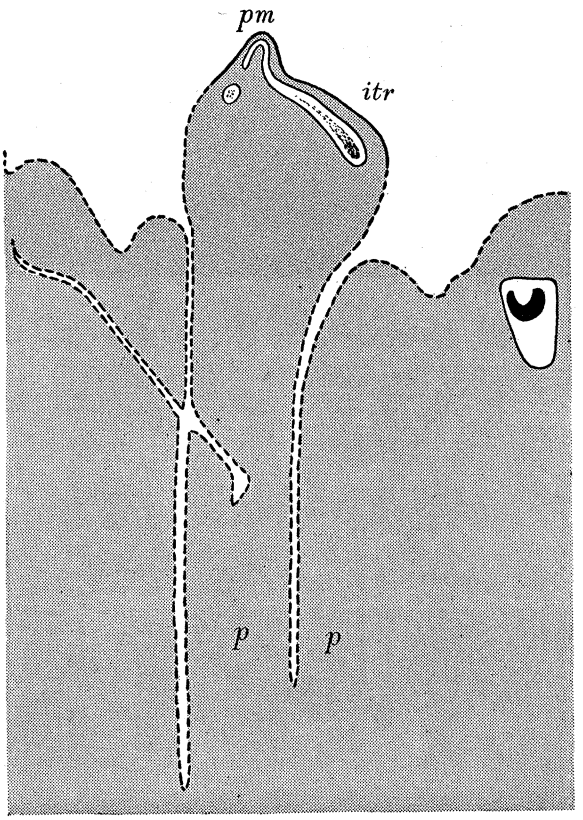


FIGURE 7. Longitudinal section of a specimen in which deep incisions have been made close to the apex and the surrounding tissue removed. Broken lines indicate incised tissue. The isolated terminal region *itr* has continued to grow and form leaf primordia *pm* and has enlarged transversely. *p*, pith. (Semi-diagrammatic, × 15.)

In some specimens the tissue surrounding the isolated meristem was removed (figure 7; and figure 50, plate 25); in others, the incisions were made at such an angle as to undercut the apex (figure 8; and figures 53, 54, plate 26). In such materials there can be no doubt as to the path of translocation to the apical meristem; it must be by way of the pith parenchyma. In both of the experiments the isolated apical meristem continued to grow and formed scales and leaf primordia. Figure 7 shows that the growing region has become transversely enlarged. Figure 8 shows that a considerable meristem is being maintained through a comparatively narrow column of parenchyma.

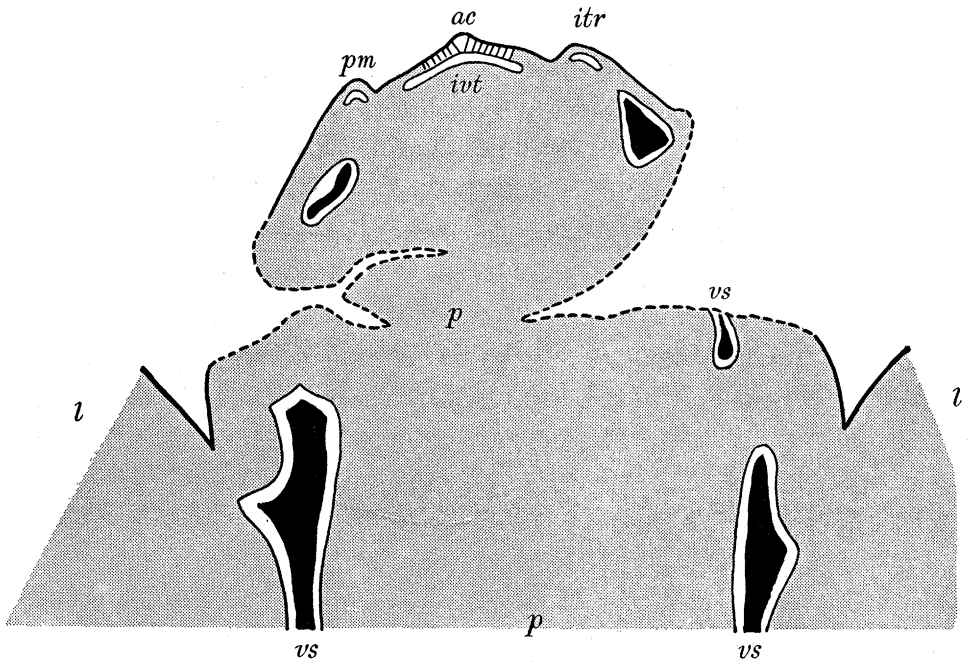


FIGURE 8. Longitudinal section of an apex which has been undercut. Broken lines indicate incised tissue. *itr*, isolated terminal region; *ac*, apical cell; *pm*, leaf primordium; *ivt*, incipient vascular tissue; *vs*, vascular strand, xylem in black; *l*, leaf-base; *p*, pith. (Semi-diagrammatic, × 15.)

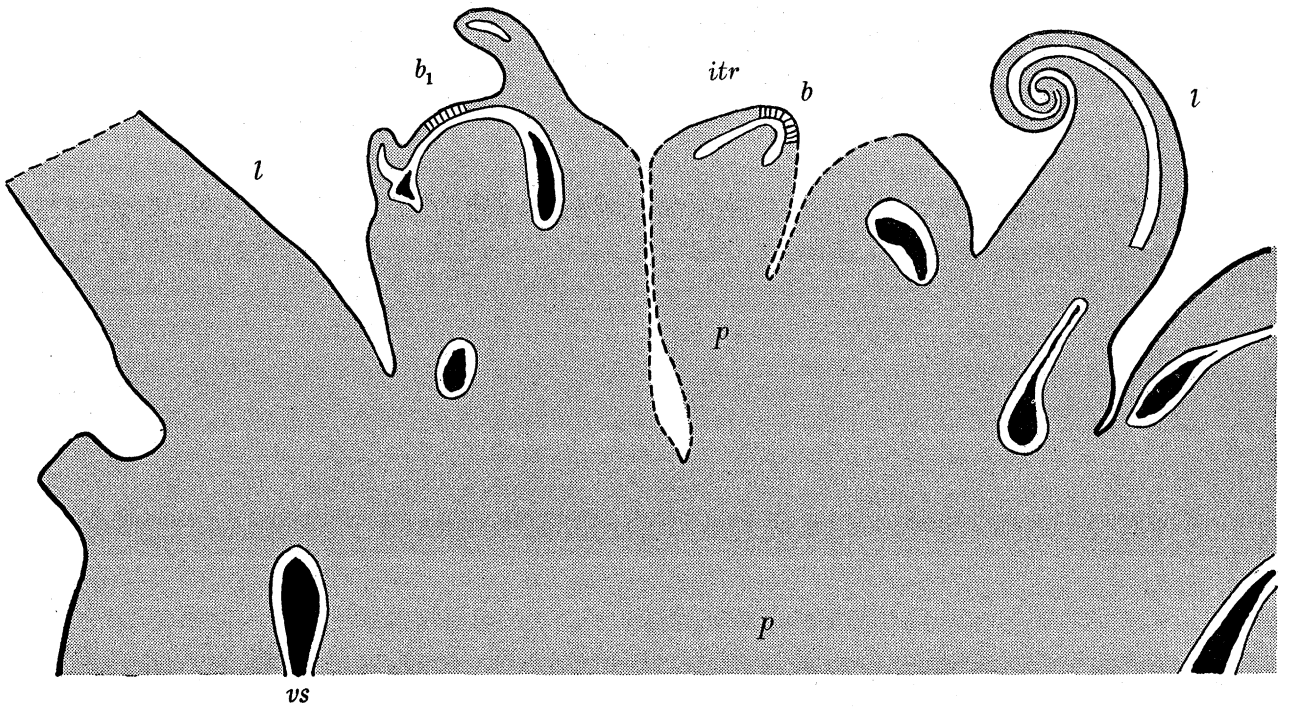
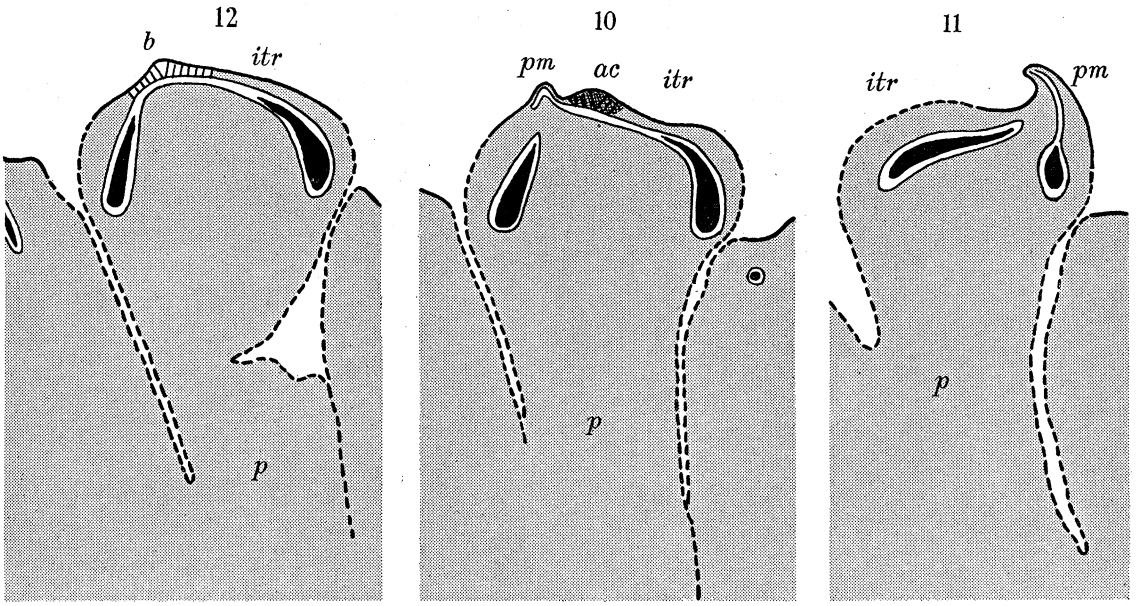


FIGURE 9. Longitudinal section showing an isolated terminal region *itr* in which the main apex has been damaged, growth being continued by means of a lateral bud *b*. The broken lines indicate incised tissue. A large bud b_1 has developed in proximity to the isolated terminal region; it is situated in the axil of a leaf *l*; *p*, pith; *vs*, vascular strand, xylem in black. (Semi-diagrammatic, × 15.)

Damaged apices. In some of the incised specimens the apical cell was damaged and together with adjacent cells became necrosed. The remainder of the apical meristem was, however, still capable of growth, one and sometimes two lateral buds being formed. These continued the growth of the isolated terminal region. A typical example is illustrated in figure 9, the active apex of the isolated terminal region being of small size and occupying a lateral position. The uninjured leaf primordia of the original shoot apex also continued to develop, but their vascular systems did not stand in a direct relation to the vascular system of the developing bud. Figure 10 and figures 55, 56, plate 26, show the necrosed apical region and an adjacent leaf primordium in medium longitudinal section; figure 11 shows



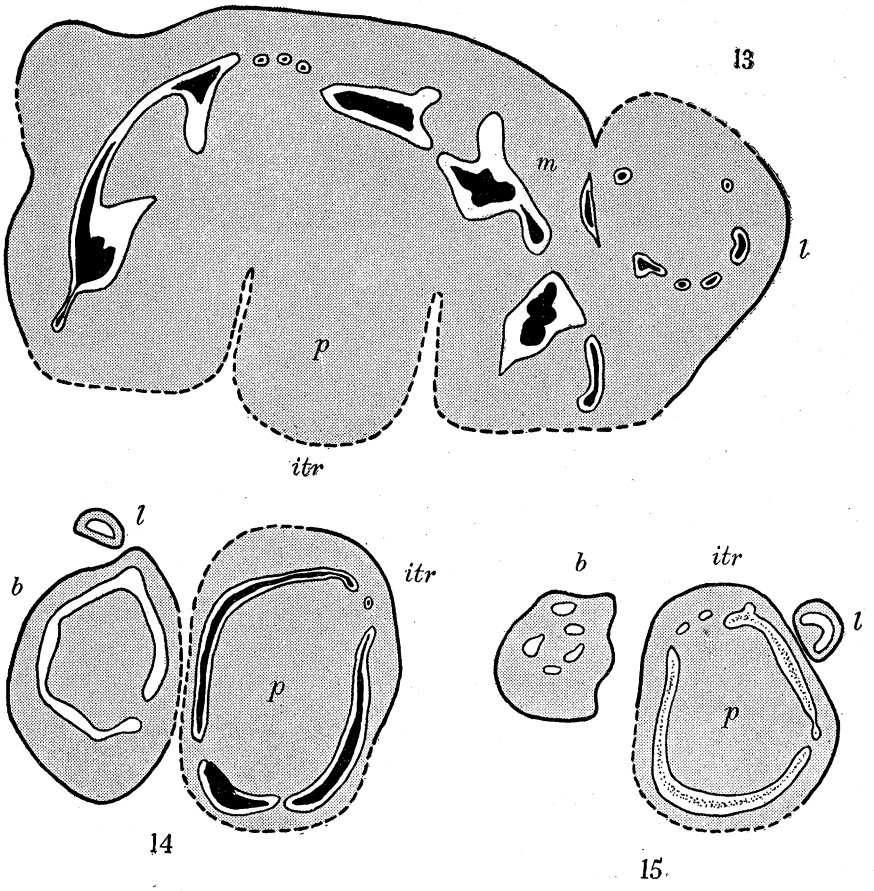
FIGURES 10 to 12. Longitudinal sections of an isolated terminal region *itr* in which the apical cell (*ac*, figure 10) has become necrosed, growth being carried on by means of a lateral bud (*b*, figure 12). Leaf primordia of the original apex (*pm*, figures 10 and 11) have continued to grow, but their vascular strands do not stand in any direct relation to the new lateral bud. *p*, pith. The broken lines indicate incised tissue. (Semi-diagrammatic, × 15.)

an older leaf primordium of the original apex; while figure 12 shows the apex of the new lateral bud. This bud apex was considerably smaller than the original apex, as were also its constituent cells. In fact, these bud apices resemble those of young sporophyte plants. In proximity to the necrosed apical cell, the tissues had undergone a characteristic parenchymatous development.

(*d*) *The effect of three-quarter ringing*

For comparison with the materials described above, the apex in some specimens was only three-quarters ringed, i.e. two parallel vertical incisions and a third vertical incision at right angles were made. The apical meristem had thus continuity of tissues, including vascular tissue, on one side. The data for one specimen are illustrated in figures 13 to 15. The isolated terminal region showed more development than the immediately adjacent lateral bud. Whether or not this was due to the inhibition of the latter by the former after

a certain amount of growth had taken place cannot be decided on the evidence available. In the isolated terminal region there was no evidence of greater growth on the non-incised side, where the vascular tissue had not been severed. There was, in fact, a slight tangential enlargement on the incised side (figure 15), this being probably due to the absence of compression from adjacent tissues.



FIGURES 13 to 15. Transverse sections of a shoot in which the apical meristem had been three-quarters ringed.

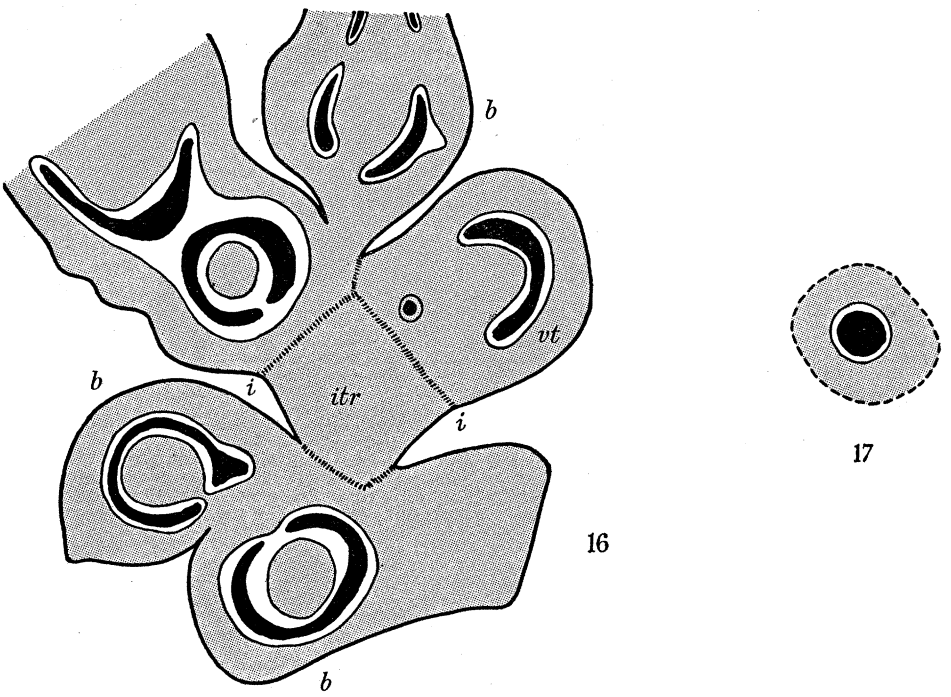
FIGURE 13. Taken near the base of the incisions: shows the incised tissue (broken lines) and the position of the isolated terminal region *itr* in the centre of the pith. In figure 14, taken higher up, the isolated terminal region, with its approximately solenostelic vascular system, and an adjacent solenostelic bud *b* are shown; the former does not show any difference between the incised and non-incised side (below and above respectively). In figure 15, still higher up, the isolated terminal region, which is somewhat wider on the incised side (below), overtops the lateral bud *b*; *l*, leaves or leaf-bases; *m*, meristele, xylem in black; *p*, pith. (Semi-diagrammatic, × 15.)

(*e*) *Position of lateral buds*

These materials have afforded abundant evidence on the essentially axillary or interfoliar position of the lateral buds of *D. aristata* (Wardlaw 1943*b*) (figures 79 to 85, plate 30). This is often not apparent in the normal development because of the extensive displacement of buds during the growth of the shoot and of the large distended leaf-bases (see also §8 (*c*)).

(*f*) *Induced protostely and solenostely*

The incipient vascular tissue in leptosporangiate ferns can be observed as an uninterrupted layer situated immediately below the apical meristem, no leaf gaps being present (Wardlaw 1944*a*, 1947*a*). When the apical meristem is isolated by vertical incisions the incipient vascular tissue is severed and no vascular connexion with the stele below is established during the subsequent growth. In the lower region of the new shoot growth, the vascular system is typically solenostelic. This development was predictable from a knowledge of the disposition of the tissue systems at the shoot apex (Wardlaw 1947*a*). Since the amount of radial growth at the centre of the shoot is very small it could further be predicted that if the incisions could be made sufficiently close to the apical cell the resulting thin shoot would be protostelic. In practice, this operation is difficult to carry out satisfactorily as the apical cell tends to collapse and becomes necrotic. However, in the course



FIGURES 16, 17. Induced protostely.

FIGURE 16. Transverse section of an incised shoot near the base of the incisions, *i*; the isolated terminal region *itr* consists of pith parenchyma at this level. The outer region of the shoot consists of the bases of large solenostelic buds *b*; *vt*, vascular tissue, xylem in black.

FIGURE 17. Transverse section of the isolated terminal region showing the protostelic condition of the vascular system; xylem in black; see also figures 59 to 61, plate 27. (Semi-diagrammatic, × 15.)

of these experimental studies, some very small terminal shoots have been obtained, and in one of these the protostelic condition has actually been realized (figures 59 to 61, plate 27) (Wardlaw 1947*c*). Figure 16 shows a cross-section of an incised shoot of *D. aristata* near the base of the incisions; the outer region of the shoot is almost completely occupied by the bases of large solenostelic buds which have developed as a result of the experimental treatment. Figure 17 and figure 61, plate 27, taken higher up, show a cross-section of the small protostelic shoot which has developed from the further growth of the isolated apical

meristem. Near the base of the vasculated region the stele is a small solenostele; higher up it becomes a medullated protostele (figure 59, plate 27); and still higher up a solid protostele with a central core of xylem (figure 61, plate 27). This shoot became attenuated and later stopped growing; the meristematic character of the apex was lost and the distal region of the shoot became parenchymatous. Sections taken progressively nearer the distal region showed a decrease in the cross-sectional area of the protostele (figure 60, plate 27) until eventually it faded out, the whole cross-section of the shoot being occupied by parenchyma. The attenuation and 'parenchymatization' of the apical meristem, with concomitant disappearance of vascular tissue, has been observed in other ferns (Wardlaw 1945).

It has been shown that the shape of experimentally induced solenosteles, as seen in transverse section, follows the contour of the incisions. Further evidence of this fact has been obtained during the present investigations. Figure 58, plate 27, shows the vascular system differentiated below an apical meristem which had been isolated on a rectangular panel of tissue; the solenostele is approximately rectangular in cross-section.

Solenostelic buds were common in the experimental materials under consideration. In the classical works on stelar morphology in ferns, the view has been advanced that the solenostele is a stage in the progressive elaboration of the protostele (Bower 1923). That this is so in the normal development is not in doubt. It is also held that the solenostele marks an evolutionary advance on the prior, more primitive, protostelic condition, this being borne out by the facts of ontogeny in solenostelic and dictyostelic ferns. A point of some interest which has emerged from the present investigation is that in some of the large, rapidly growing, induced buds, there is no protostelic stage, the vascular system being solenostelic from the outset.

6. Effect of puncturing the apical cell

As long as the fern shoot apex is in an actively growing condition, lateral buds are not formed, or, if they are already present, they do not develop further. But if the apex is injured, or if its growth is arrested, lateral buds soon appear at specific positions on the shoot, these positions being occupied by detached meristems or bud rudiments (Wardlaw 1943*a*, *b*). If, now, an apical cell is punctured so that a minimal amount of necrosis of the adjacent meristem ensues, it should be possible to observe (i) if the existing leaf primordia undergo further development, (ii) if new leaf primordia are formed, (iii) if buds develop on the apical meristem itself, and, if so, in what positions, i.e. close to, or remote from, the damaged apical cell.

In order to puncture the apical cell a very sharp finely tapering needle was used, the apex being observed under a binocular microscope at a high magnification. The perforation was made with the lightest touch of the needle point so that there was only a slow exudation of liquid. But even in those specimens where the apical cell was punctured with minimal disturbance, a considerable area of the meristem became necrosed (figures 62, 63, plate 27). It may accordingly be inferred that the system of stresses at the extreme apex is such that a release of pressure in the apical cell has a marked effect on the neighbouring cells. The superficial cells adjacent to the apical cell are, in fact, very susceptible to injury. In numerous operations the writer has not found it possible to destroy the apical cell alone. On being perforated, some apical cells freely exuded a liquid which dried to a brown scab.

The perforations neither became extended nor compressed. In some specimens bubbles of gas were observed to escape. Where, by accident, the apical cell was very slightly injured, e.g. on being brushed with a shred of blotting paper, the cell contents did not immediately become discoloured nor did they exude; but after some time—24 hr. or more —the pattern of the apical cell and its neighbours could easily be observed because of the development of a necrotic brown colour in the cell walls. In the writer's experience even slightly injured apical cells eventually become necrotic. The apical cells of young leaf primordia are also particularly delicate and easily damaged. Figures 62, 63, plate 27, give some indication of the extent of the disruption and necrosis of tissue that ensue when an apical cell has been lightly punctured.

Earlier investigations have shown that when the apex is damaged, buds will develop freely in the subapical region. In order to exclude the possible effect of these buds on the development of new ones close to the damaged apical cell, the apical meristem was isolated by vertical incisions. In these specimens, accordingly, such buds and new leaf primordia as appeared would be formed in the narrow ring of meristematic tissue situated between the damaged apical cell and the base of the apical cone. In other shoots six to nine leaf primordia were left round the apex, but the latter was not isolated by vertical incisions. The data of a considerable number of experiments in which the apical cell was punctured are available, but only a few can be selected for consideration here. For convenience, the major findings will be briefly summarized (see figures 64 to 67, 71, plate 28).

Leaf growth. Leaf primordia already present continue to grow in isolated meristems as well as in those not isolated by vertical incisions.

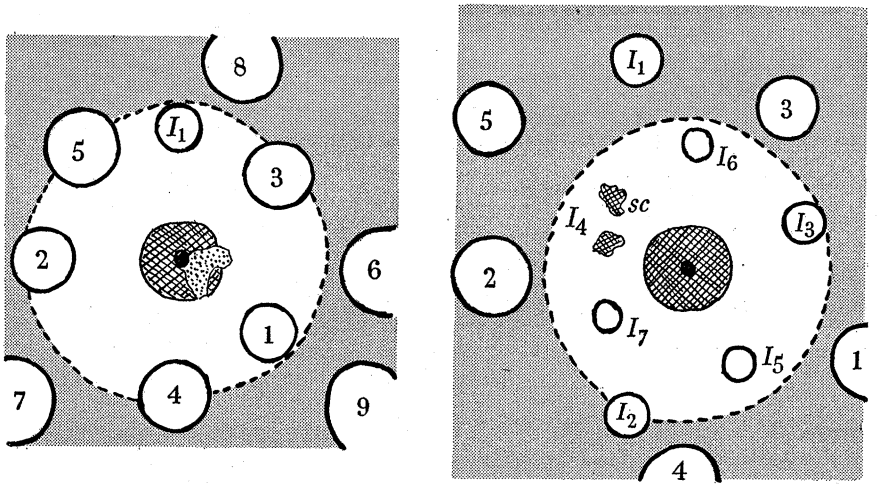
Leaf formation. Three to seven new leaf primordia may be formed. They appear in normal or approximately normal positions, i.e. in continuity with the existing spiral sequence; in fact, they continue to be formed until all the available space on the meristem has been utilized. The last-formed primordium may thus be situated close to the damaged apical region (see also § 8).

Bud formation. Buds are formed on the apical meristem in positions which may be described as axillary or interfoliar. They typically occur close to the basiscopic margin of the apical meristem, i.e. remote from the apical cell; their formation lags behind that of leaf primordia.

Scales. These may be formed on the apical meristem in proximity to necrotic tissue. (In the normal development they are formed along the base of the apical cone.)

Some typical specimens may now be illustrated and briefly described. The potentiality for leaf formation at the apical meristem is illustrated in figures 18 and 19. Only a small necrotic region was present round the punctured apical cell. After 10 days one new leaf primordium (I_1) had appeared in the normal position for that primordium (figure 18), and in the course of the next 5 weeks other primordia occupying approximately normal positions had been formed (figure 19). No primordium was formed in position I_4, probably because of the presence of small accidental scars (*sc*). The only bud in proximity to the apex developed at the base of P_8. Leaf formation thus appears to be the primary morphogenetic expression of the apical meristem. As they arise on the sides of the apical cone, they are subject to tangential stresses and to differences in the supply of nutrients on the abaxial and adaxial sides. In attempts to account for the shape and dorsiventral symmetry of the

leaf, these factors should be considered. In other experiments of this series as many as eight new leaf primordia have been observed on apical meristems in which the apical cell had been punctured. The phyllotactic sequence was less regular than that illustrated in figure 19 in some instances (see Wardlaw 1948).



18 19

FIGURES 18, 19. Apical cell punctured; formation of leaf primordium at meristem.

FIGURE 18. Necrosed tissue (cross-hatched) round punctured apical cell; scab of coagulated exudate. 1, 2, 3, etc., leaf primordia present at beginning of experiment; I_1 new primordium formed 10 days later. The broken circular line indicates approximately the base of the apical cone.

FIGURE 19. The same apex, 5 weeks later. In all, six new primordia have been formed (I_1–I_7), a scar *sc* preventing leaf formation at I_4. (Ultro Pak tracing, semi-diagrammatic, × 25.)

In the specimen illustrated in figure 65, plate 28, the apical cell was punctured and the apical meristem isolated by vertical incisions. Of the leaf primordia present on the meristem, three (P_1, P_2 and P_3) developed subsequently to the puncturing of the apical cell. This specimen shows that leaf primordia may be formed close to the necrosed apical cell, i.e. P_1 and P_2. A bud was also present, in a position near the base of the cone in the axil of P_9 or between P_4 and P_6. Scales (not shown in the illustration) were present round the base of the cone, and also along the margin of the necrosed distal tissue; scales are never formed in the latter position in a normal apex. A comparable specimen is illustrated in figure 66, plate 28; in this specimen it was difficult to determine whether the largest leaves of buds 7 and 8 really belonged to these buds or to the original shoot apex; they are, in fact, in phyllotactic continuity with P_3, P_2 and P_1.

In the specimen illustrated in figure 64, plate 28, the apical cell was punctured with minimal necrosis of the adjacent cells. Two new leaf primordia (P_2 and P_1) and two buds have been formed since the beginning of the experiment. These now abut, or almost abut, on the necrotic distal region. Figure 67, plate 28, shows a bud formed in close proximity to the injured apical cell.

Evidence has thus been obtained that leaf primordia continue to be formed on the apical meristem in approximately normal phyllotactic sequence and at approximately the normal rate after the apical cell has been destroyed; this process continues until all the available

space on the meristem has been used up. It thus appears that *leaf formation is independent of the apical cell*, being neither activated nor inhibited by it. The formation of leaf primordia slightly precedes that of buds. The latter, however, grow rapidly and soon outstrip the developing leaf primordia of the original shoot. As buds are formed when the apical cell and its immediate segments alone have been destroyed, it appears that the physiological dominance, formerly known to be exercised by the growing point as a whole, is an attribute of the apical cell, or of the small group of cells at the summit of the apical cone. Even when the greater part of the apical meristem is left undamaged, buds develop freely in the subapical region and eventually in the apical meristem itself. The apical cell is therefore not only responsible for the continued growth of the shoot but is also the seat of important physiological processes.

Whereas in a normal, actively growing shoot, buds are never formed at the apical meristem, leaf primordia are typically formed there. The growth-regulating substance (or substances) proceeding from the apical cell which inhibits bud formation and development does not inhibit leaf formation. Yet both leaf and bud primordia originate by the outgrowth of a group of meristematic cells, the chief difference between them *being in the positions which they occupy at the time of their formation*. The data also show that leaf primordia do not inhibit bud formation or development. Indeed, as we have seen, induced buds grow considerably more rapidly than the leaf primordia of the original shoot (see also § 8 (*c*)).

7. Organ formation in isolated apical panels

When the apical meristem is isolated by vertical incisions from the adjacent lateral organs, buds are formed in the lateral subapical region, but not in the isolated terminal region (Wardlaw 1947*a*). In an attempt to extend these observations the writer considered the following problem: If a small panel of the apical meristem (as close to the apical cell as possible) were isolated by incisions, what development would take place in it? A cataract knife was used to make two transverse and two longitudinal incisions as shown in figure 20. In a number of specimens the apical cell and those cells adjacent to it became necrotic as a result of the treatment; in others the isolated panel became necrosed. However, a few apices remained viable and yielded results of a rather interesting and perhaps unexpected kind. In each instance a leaf primordium and not a bud was formed in the panel.

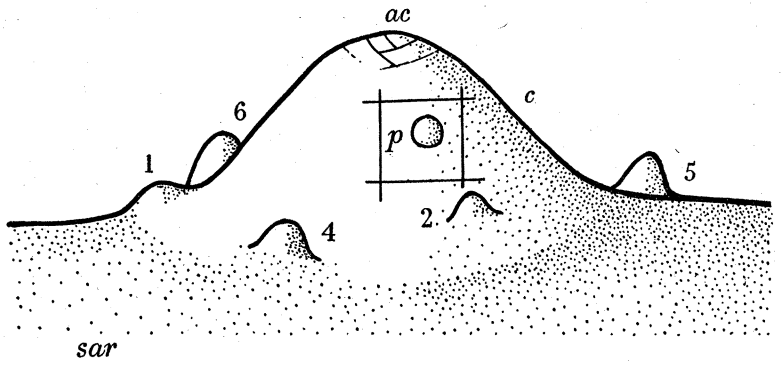


Figure 20. Panel of meristematic tissue isolated by shallow incisions on the apical meristem: a leaf primordium *p* has been formed on the panel; *ac*, apical cell; *c*, apical cone; *sar*, subapical region; 1, 2, 3, etc., leaf primordia in order of increasing age. (Diagrammatic.)

8. Further observations and experiments on leaf formation and development

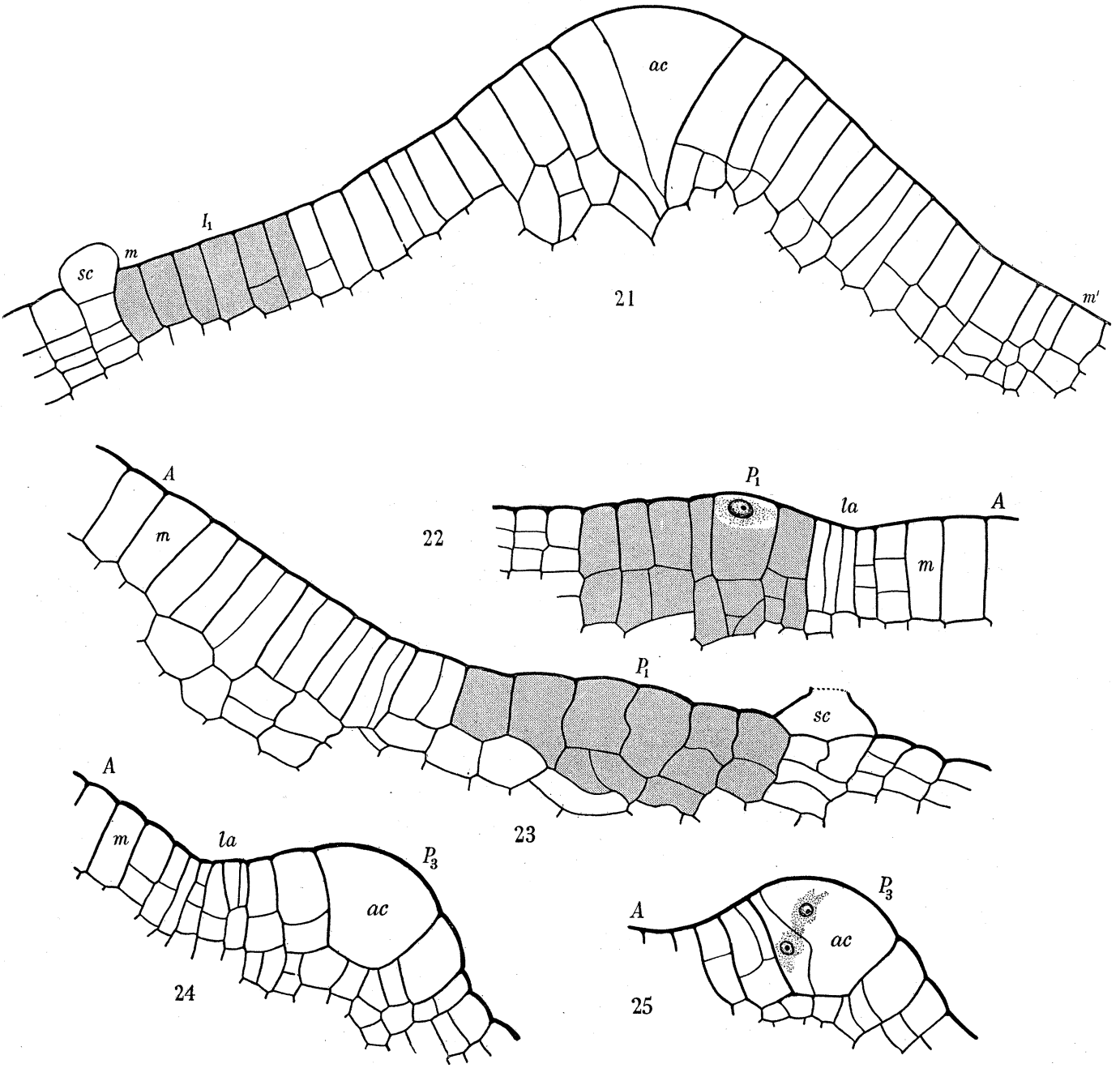
(*a*) *The initial phase of leaf formation*

In standard works on the morphology of leptosporangiate ferns (de Bary 1884; Bower 1923; Campbell 1940; Sifton 1944), the leaf is described as originating from a single enlarged cell of the apical meristem and, indeed, unless a close examination of the apex is made, this appears to be the case. Accordingly, it has been held that in its origin the fern leaf differs notably from the leaves of flowering plants. In them, the leaf primordium consists of a mound-like outgrowth of the shoot, a considerable number of cells being involved. In dicotyledons, the first evidence of leaf formation consists of periclinal divisions in the sub-epidermal layer; in monocotyledons, the first divisions are periclinal divisions in the outer layer. If it is true that the leaf primordium in leptosporangiate ferns is entirely the product of a single cell then the process of leaf formation in ferns and of flowering plants is quite different from the outset. Nevertheless, the evident fact is that the leaves in the two groups have much in common. Considerations such as these have led the writer to undertake a reinvestigation of the earliest stages of leaf formation in *D. aristata*.

The method adopted was as follows: Apices were laid bare as already described and the positions of I_1, P_1, P_2, P_3, P_4 and P_5 observed. Each apex was then dissected longitudinally so that a thin slice of tissue, including the apical cell and either the position I_1 or primordium P_1 or P_2, was obtained (figure 1). These materials were fixed, embedded in wax and sectioned longitudinally. The results are illustrated in figures 21 to 29 and figures 73 to 78, plate 29.

A median longitudinal section through the apical cell and position I_1 is illustrated semi-diagrammatically in figure 21. The prism-shaped meristematic cells (m–m') extend well down the flank of the apical cone on which primordium I_1 will be formed. At the stage illustrated, there is no evidence of enlargement in any one of the prism-shaped cells. The group of cells from which the primordium may originate is indicated by stippling; these cells lie just within the basiscopic margin of the meristem. A longitudinal section of an apex in which the very first evidence of leaf formation (P_1) could be observed under the binocular microscope is illustrated in figures 1 and 23; a slight swelling or outgrowth can be seen near the basiscopic margin of the apical meristem. This is due to the enlargement of a group of the prism-shaped meristematic cells, four or five of which can be seen in the longitudinal section, and to the division of the underlying cells (figures 73, 74, 75, plate 29).

It is evident that a considerable group of cells has been stimulated to growth. At this early stage, then, the primordium consists of a group of the prism-shaped, superficial cells. This stage is soon followed by the enlargement of one of these cells (figure 22); it is situated slightly on the apical side of the centre of the protuberance. Figures 26 to 28 show serial longitudinal sections from the margin to the median plane of a single primordium at this stage of development. The enlarged cell is conspicuous; the underlying cells are in an active state of division. On the adaxial side of the primordium some narrow cells, which subsequently grow slowly, can be distinguished as the cells of the leaf axil. From this stage onwards, the enlarging cell becomes conspicuous and is unmistakable as the apical cell of the primordium (figure 24). It now begins to divide by anticlinal walls (figure 25), and the



FIGURES 21 to 25. Details of leaf formation, as seen in longitudinal median sections of young primordia.

FIGURE 21. An apex, showing the group of meristematic cells (stippled and labelled I_1) which will give rise to a leaf primordium. *ac*, apical cell; *m–m'*, prism-shaped cells of apical meristem; *sc*, scale.

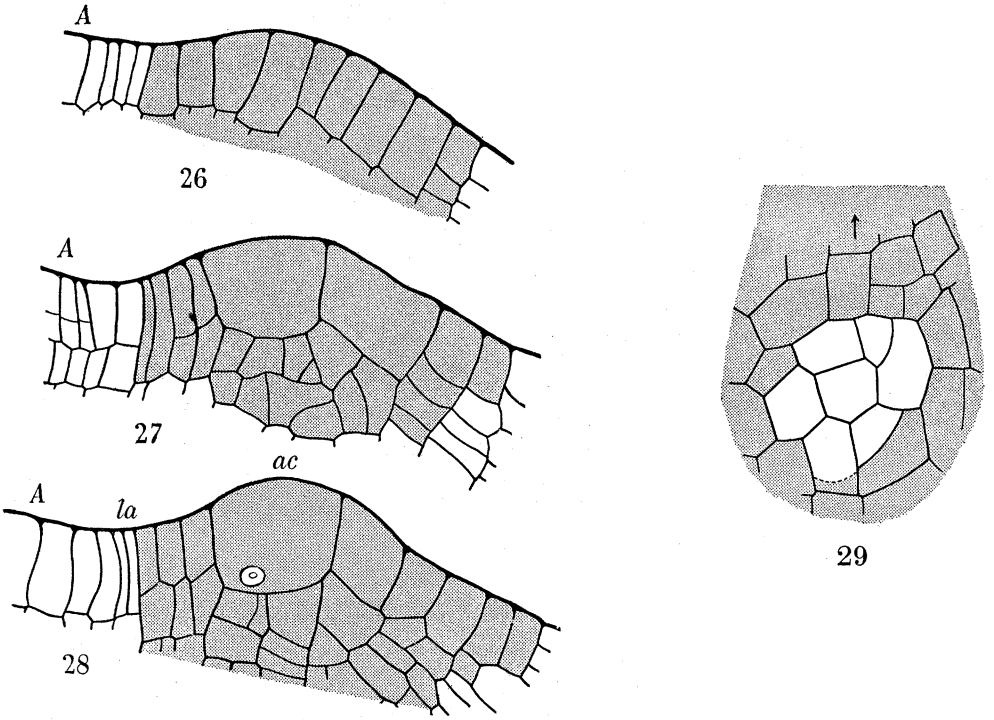
FIGURE 22. An older primordium P_1 in which one of the prism-shaped cells has begun to enlarge. *A*, direction of apical cell of shoot; *la*, narrow cells of leaf axil.

FIGURE 23. The first evidence of the outgrowth of a leaf primordium P_1, several prism-shaped meristematic cells being involved. *A*, indicates direction of apical cell of the shoot.

FIGURE 24. An older primordium P_3 with a large apical cell *ac*.

FIGURE 25. The apical cell of a primordium dividing. (All ×170.)

further developments are as described in the standard works, i.e. the growth of the primordium is thereafter due to the growth and division of this apical cell and its products. Above the leaf axil the prism-shaped meristematic cells grow, divide, and become differentiated as epidermis and parenchyma. From an early stage the cells underlying the apical cell of the primordium undergo many divisions and can be recognized by their distinctive staining as pertaining to the incipient vascular tissue.



FIGURES 26 to 28. Longitudinal serial sections from the margin to the centre of a young leaf primordium (stippled), showing the prism-shaped cells of which the primordium is composed: one of these cells *ac* near the centre is enlarging and will become the apical cell of the primordium. The underlying cells are in a state of active division. *A*, direction of apical cell of shoot; *la*, leaf axil. (× 170.)

FIGURE 29. Young leaf primordium in transverse section. The clear cells are the outgrowing cells of the primordium which have been cut through; the stippled region consists of the outer walls of the adjacent meristematic cells. At this stage a single apical cell is not in evidence. The arrow indicates the direction of the shoot apex. (× 170.)

From an examination of comparable leaf primordia in transverse sections, confirmation was obtained of the fact that the very young primordium consists, not of a single enlarged cell, but of a group of prism-shaped cells (figure 29). These cells rise above the general level of the adjacent cells of the meristem and in thin, basipetal, serial sections are the first to be cut. In figure 29 the stippling indicates the *outer walls* of cells of the apical meristem, the clear cells being those of the outgrowing primordium which, at the same level, have been cut through. This point is illustrated by the photomicrographs in figures 76, 77, plate 29. A later stage, as seen in transverse section, is illustrated in figure 78, plate 29.

There are thus no essential differences between the early stages of leaf formation in ferns and flowering plants. In each, the young primordium arises as an outgrowth of the

apical meristem, a group of cells being involved. But in the ferns there is this difference: at an early stage one of the more centrally placed superficial cells begins to enlarge rapidly and becomes *the* apical cell of the growing primordium.

(*b*) *Effect of transverse incisions above and below presumptive foliar positions*

In several experiments factors affecting the *formation* of leaf primordia at the shoot apex have been investigated. Elsewhere (Wardlaw 1948*c*), some account of factors in leaf formation in leptosporangiate ferns has been given in relation to the problem of phyllotaxis.

In one experiment, transverse incisions were made near the base of the apical cone *below* the positions to be occupied by the new primordia, I_1, I_2 and I_3; in another, punctures were made on the apical cone *above* the positions to be occupied by these primordia. It was anticipated that the former would give some indication of the effect of factors working acropetally from below, e.g. the effect of roots or of older leaf primordia on the formation or development of younger ones, while the latter might show whether the apical cell determined or controlled leaf formation. In both experiments the direct passage of substances to the developing primordia was interrupted. The first of these experiments is in all essentials a repetition of that originally carried out by M. & R. Snow (1931, 1935, 1947) on flowering plants.

Consistent results have accrued from these experiments; new primordia appeared in the positions I_1, I_2 and I_3 as in the normal development (figures 68, 69, plate 28). Only where the wound damage was too severe did a primordium fail to appear. In these experiments metabolites proceeding from the apex or from older regions of the shoot are not completely prevented from reaching the presumptive positions of leaf primordia; but their direct path is interrupted. Together with the data set out in § 6, the conclusion may be drawn that the formation of new leaf primordia is not directly affected either by the apical cell or by the older leaf primordia but is determined by factors at work in the apical meristem. These findings do not exclude the possibility that the *further development* of primordia may be affected by factors proceeding from the apical cell or from older regions of the shoot.

(*c*) *Development of isolated leaf primordia*

Some preliminary observations on the effect of isolating young leaf primordia by vertical incisions, with concomitant severing of the incipient vascular tissue, have already been published (Wardlaw 1947*a*). In these experiments care must be taken to avoid desiccating and damaging the apices. Very young primordia make little growth and in many instances become moribund, but an actively growing bud usually develops in the leaf axil (figures 70, 72, plate 28). Various abnormal vascular and other developments have been observed (figure 57, plate 26).

Uninjured, isolated leaf primordia continue to grow, particularly if they are on a considerable plug of tissue. Figure 30 shows how an apex has been incised so that a number of leaf primordia have been isolated. But the amount of growth, even in larger primordia, is never great. The development of pinnae, for example, has only occasionally been observed (figure 79, plate 30). Curved, awl-shaped, protophyll-like structures have been of common occurrence among the experimental materials. Figure 70, plate 28, shows two primordia on the same plug; the smaller primordium was incipient at the beginning of the experiment.

Bud formation on the plugs of tissue has been general. These buds may originate in the axil of the primordium, or laterally to it. In some instances the bud may be carried upwards on the elongating primordium (figures 79 to 81, plate 30). Not infrequently the original primordium has the appearance of being the oldest leaf of the new bud; in other words, the first bud leaf arises in an approximately normal phyllotactic relation to it (figures 31 to 34). In many instances the bud soon outgrows the isolated leaf primordium (figure 84, plate 30); this is the reverse of what we find in the normal development. Whether the slow and limited development of isolated leaf primordia is directly related to the size of the plug on which it is situated, or whether other factors are involved, has not been ascertained. Primordia isolated on large plugs do grow to greater size than those on small plugs, but as the former tend to be situated in the subapical region, where growth is more rapid, the size of the plug may not be the only or the principal factor at work.

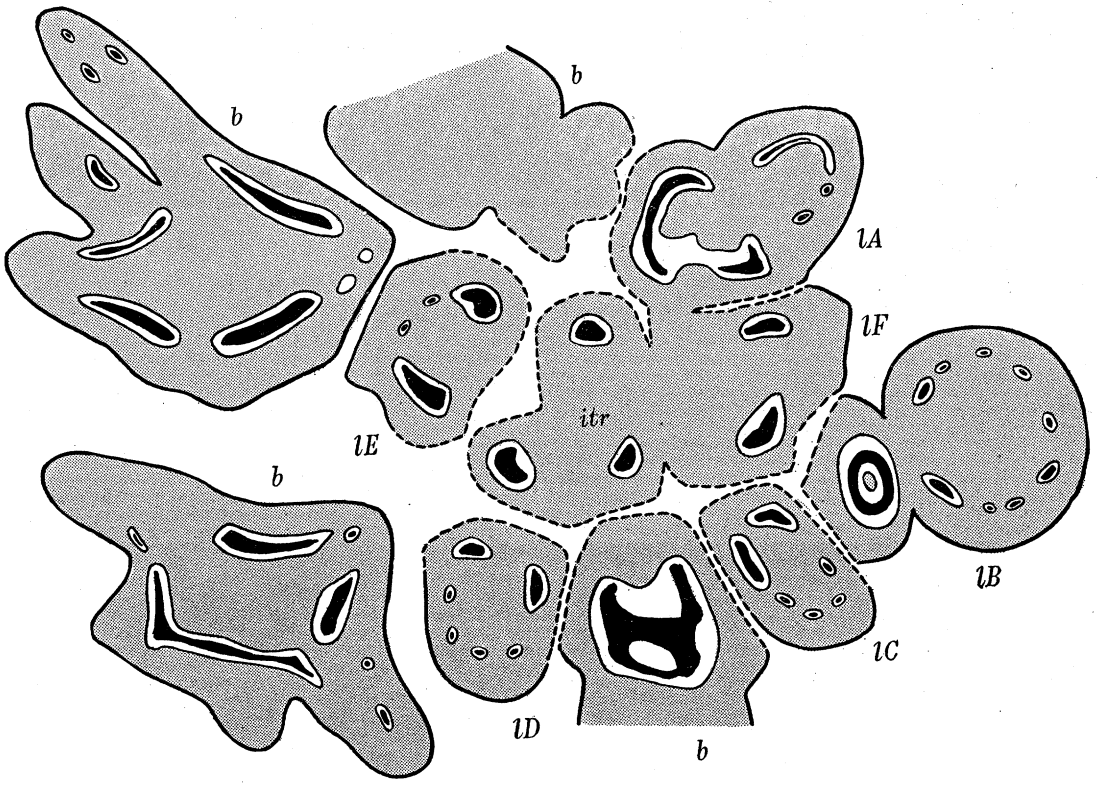
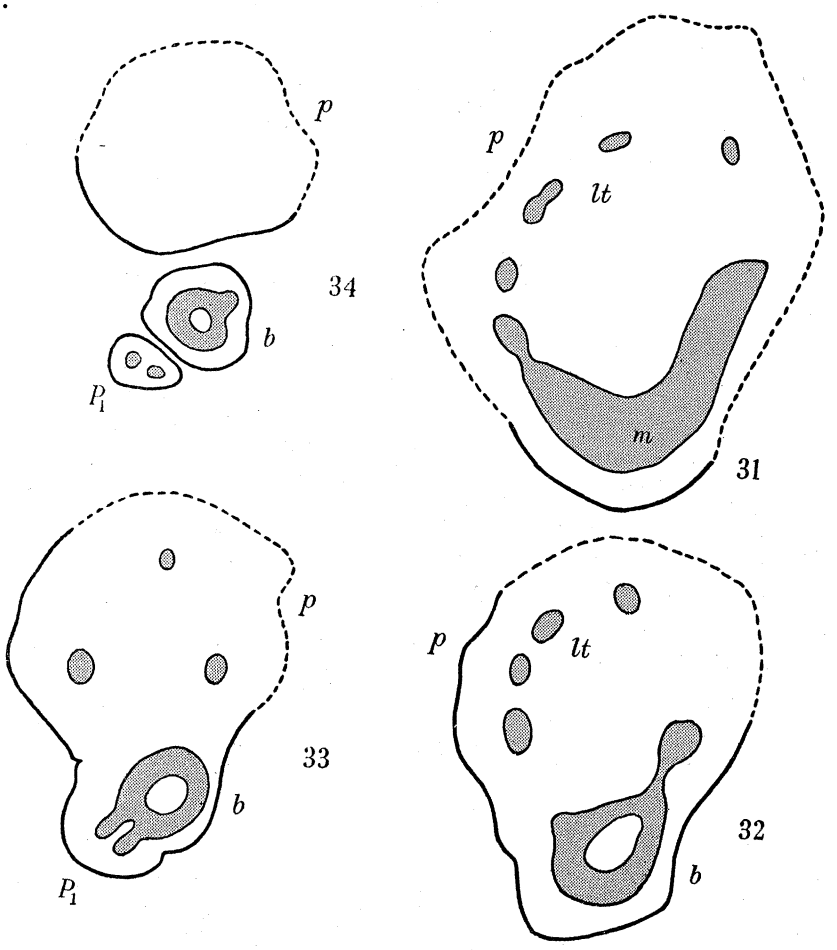


FIGURE 30. Transverse section of an incised apex near the base of the incisions; the latter are indicated by broken lines. *lA*, *lB*, etc., leaf primordia which have been isolated by vertical incisions; *itr*, isolated terminal region; *b*, buds: a good example of a solenostelic axillary bud is shown at *lB*. (Semi-diagrammatic, × 15.)

A typical experiment is illustrated in figure 30, several leaf primordia being isolated radially and laterally and the shoot allowed to grow on. The position of the several incisions and some of the buds which developed are shown. On further growth the several leaf primordia were much alike in their external morphology and internal structure; the details of leaf primordium *lD* are therefore described by way of illustration (figures 35 to 39). The two large meristeles (*m*) in figure 35 are part of the vascular system of the shoot; they are converging in the upward direction as the gap or leaf *lD* 'closes'. In this primor-

dium, as in the others, there was evidence of limited growth; there was no laminate development, the distal region developing as a curved awl-like structure. In the axil of the leaf a solenostelic bud developed (figure 36). The first bud leaf (P_1) occupied a position approximately at right angles to the radial plane of leaf *lD*. Large solenostelic buds were also formed in the axils of the other isolated primordia, e.g. *lB* in figure 30, and figures 82 to 85, plate 30.



FIGURES 31 to 34. Transverse sections of an older, isolated leaf primordium *p* after growth had taken place. Broken lines indicate incised tissue.

FIGURE 31. Near the base of the isolated primordium; *m*, meristele of parental shoot; *lt*, leaf-trace consisting of several meristeles; the vascular tissue is not yet differentiated into phloem and xylem.

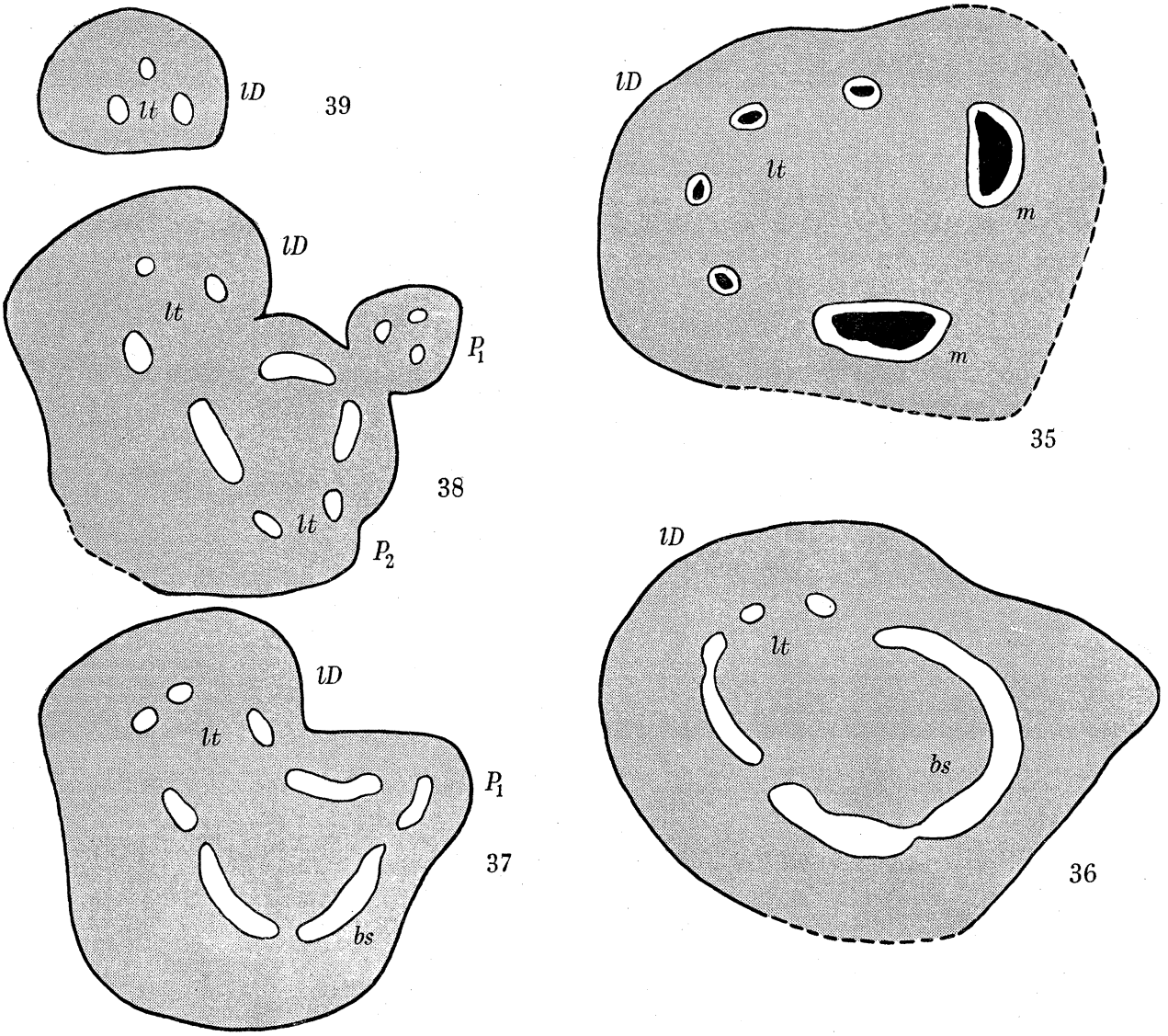
FIGURE 32. Higher up, showing the solenostele of the axillary bud *b*.

FIGURE 33. Higher up than figure 32; the base of the first leaf P_1 of the bud can be seen.

FIGURE 34. Just below the apex of the bud; the vascular tissue of the parental leaf *p* has faded out. P_1 stands in an approximately normal phyllotactic relation to *p*. (Semi-diagrammatic, × 40.)

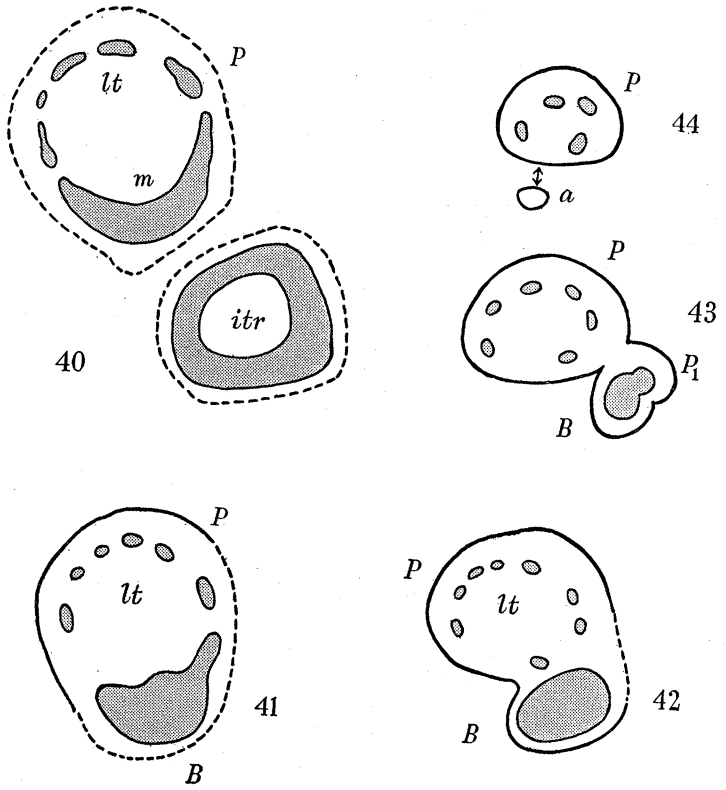
Although isolated leaf primordia are capable of further growth, this is usually inextensive; active growth, in fact, becomes localized in the axillary bud. This bud growth is in marked contrast to that in normal plants; there the bud has only a small protostelic strand passing through the cortex of the parent plant, whereas in the materials under consideration the bud may be solenostelic from the outset, and may soon become dictyostelic, e.g. figures 35 to 37.

In another experiment six leaves, i.e. P_4 to P_9, were isolated as already described. In each instance an axillary, or approximately axillary, bud developed, the parental leaf showing limited growth only. In one instance, the first leaf of the bud was formed approximately opposite to the parental leaf, and the second leaf at right angles to it. In another instance the angle of divergence between the parent leaf and the first leaf of the bud was approximately 138°. Other comparable instances have also been observed.



FIGURES 35 to 39. Transverse sections from below upwards of isolated leaf primordium *lD* of figure 30. *lD*, isolated primordium, with its leaf-trace *lt* at different levels; *m*, meristele of parental shoot (xylem in black); *bs*, bud stele, not yet fully differentiated, of axillary bud; P_1, P_2, first and second leaf primordia of the axillary bud; broken lines indicate incised tissue. The bud which is solenostelic from the outset soon becomes dictyostelic. (Semi-diagrammatic, × 40.)

Occasionally, isolated primordia showed more considerable growth and developed the characteristic circinnate curvature. In these materials the bud grew rather slowly and eventually occupied a position some distance up from the base of the petiole (figures 40 to 44 and figures 79 to 81, 83, plate 30).



FIGURES 40 to 44. Transverse sections from below upwards of an isolated small leaf primordium *P*, with its leaf-trace *lt*. *itr*, the isolated terminal region, with its solenostele which as yet is not fully differentiated; *m*, meristele of parental shoot; *B*, protostelic bud in leaf axil; P_1, first leaf of bud (*B*), *a*, apex of leaf *P*, which shows circinnate curvature. This bud has been carried some distance up the petiole by the growth of the parental leaf primordium. (Semi-diagrammatic, × 25.)

9. THE FORMATION OF SCALES

In the normal shoot scales are formed at the base of the apical cone, i.e. in the region of transition between the meristematic cells of the apex and the developing parenchymatous tissue of the subapical region. This characteristic position of scales is clearly seen in detached meristems; the scales arise along the line of junction between the meristematic cells and the normal epidermal cells. They originate from superficial cells which are of a meristematic or semi-meristematic character. Scales are never present on the normal growing apex, but if the apex is incised or otherwise wounded, scales soon appear round the perimeter of the wound. In these instances parenchymatous cells are first formed in proximity to the wound, and the scales subsequently arise in a position between this parenchyma and the adjacent meristematic cells of the apex.

10. DISCUSSION

(*a*) *Distribution of growth at the apex*

From the configuration of the distal region of the fern shoot it may be inferred that there is a sharp increase in the rate of growth in the region of transition between the apical cone and the subapical region. The densely protoplasmic apical meristem may be specified as the region of organ formation and initial differentiation of tissues; the growth of the shoot, the orderly formation of leaf primordia, and of detached meristems or bud rudiments, are all referable to the apical meristem. The subapical region is the region of development

of organs and further differentiation and maturation of tissues; a conspicuous feature is that much of the meristematic tissue is transformed into large-celled, highly vacuolated parenchyma. But there is also growth and meristematic activity in this region; for example, there may be rapid formation of buds when the apical meristem of the shoot is destroyed. In the apical meristem protein metabolism appears to be of paramount importance; but as cells and tissues pass into the subapical region during growth, they become increasingly affected by substances moving upwards from the older regions, in particular by carbohydrate metabolites. The results are seen in cell vacuolation and enlargement, the formation of complex cell walls, and the deposition of starch. Therefore, while there is undoubtedly active protein metabolism in the subapical region, the more conspicuous developments are those which we associate with carbohydrate metabolism. The region of transition between the apical meristem and the subapical region is thus one of profound physiological interest.

Although more detailed studies are required, some direct observations on the normal distribution of growth at the apex have been made. By a simple technique it has been shown that growth is much more rapid in the subapical region than at the apex; and whereas leaf primordia grow actively, the tissue in their axils and in certain interfoliar positions grows slowly. These observations are of interest in relation to the induction of stresses in the meristem. There is support for the view that new leaf primordia and buds arise from groups of meristematic cells occupying positions of minimal tensile stress (Wardlaw, 1948*a*, *c*). In relation to this distribution of growth at the apex, some of the developments which follow on the isolation of the apical meristem by vertical incisions can be explained. If the incisions are made very close to the meristem, the growth of the isolated terminal region is very slow; but if the meristem is isolated on a larger panel of tissue, the growth of the isolated terminal region is more rapid. Buds situated immediately outside the isolated terminal region grow more rapidly than the latter, and buds further from the centre of the shoot enlarge more rapidly still. An investigation of the factors which determine the distribution of growth at the apex is therefore essential to a fuller understanding of the morphological developments which take place there.

In shoots from which all leaf primordia, leaves and roots have been removed, the apical meristem continues to grow and new leaf primordia are formed at approximately the normal rate; but the shoot usually shows some attenuation. When an apical meristem is isolated laterally by deep vertical incisions from the adjacent primordia and tissues, with concomitant severing of the vascular connexion with the older regions of the shoot, it continues to grow and form leaf primordia. Morphogenetic processes at the apex thus appear to be independent of the older leaf primordia and roots; they also take place in the absence of vascular connexion with the older tissues below. The substances required for the growth of new leaf primordia in an isolated meristem must be obtained from, or through, the plug of pith parenchyma on which the meristem is seated; but ultimately, the substances stored in the pith are referable in origin to the leaves and roots.

(*b*) *The effect of puncturing the apical cell*

Even the most careful puncturing of the apical cell is attended by some disruption of the adjacent cells. An investigation of the distribution of stresses at the extreme tip is therefore likely to prove of interest; the indications are that the apical cell and those ad-

jacent to it are under compressive stress. This is inferred from: (*a*) a consideration of cell growth and division at the apex; (*b*) the fact that when the apex is punctured the hole does not become distended; and (*c*) the considerable exudation of sap on puncturing and the collapse of adjacent cells. It has, however, been found possible to puncture the apical cell so that the subsequent necrosis was slight, i.e. involving the apical cell and its immediate segments only.

When the apical cell is destroyed, lateral buds are formed, first in the subapical region and then in the apical meristem itself; buds are also formed on meristems which have, in addition, been isolated from the subapical region. Hence it may be inferred that the substance responsible for the inhibition of buds in the normal development is formed in the apical cell, and probably its immediate segments, and not necessarily in the apical meristem as a whole. The apical cell in *Dryopteris*, and presumably in other leptosporangiate ferns also, is thus seen to have a particular physiological importance. This finding is very different to that of Sachs (1887), who concluded that the apical cell represented 'merely a break in the constructive system of the growing point; i.e. the apical cell is a spot in the embryonic tissue in which neither anticlines nor periclines nor radial longitudinal walls have yet been formed'. The apical cell in Sachs's view does not dominate the apex; indeed, as Bower (1923) remarks, 'its characters are negative'. This view may now be dismissed.

When the apical cell was lightly punctured and the meristem isolated, leaf primordia continued to be formed, usually in a normal phyllotactic sequence, until all the available space had been used. So far as the writer is aware this observation is quite new. The formation of leaf primordia is thus independent of the presence of the apical cell, although the latter is necessary for the continued growth of the meristem from which primordia develop. Nor does the formation of leaf primordia depend directly on the presence of older primordia or leaves; but the possibility that the latter may exercise an indirect effect, or an effect on the subsequent development of primordia, is not excluded.

(*c*) *Stelar morphology*

It has been shown (Wardlaw 1947*a*) that when an apical meristem, isolated by vertical incisions, grows on, it forms a short vasculated shoot. The vascular system is at first solenostelic, but as new leaves are formed it becomes dictyostelic. Thus, by the experimental procedure adopted, the large dictyostelic shoot of *Dryopteris* can be reduced at will to a small solenostele. A protostelic shoot has now been obtained by making incisions very close to the apical cell. The small shoot later became attenuated, the apical meristem became parenchymatous, and the vascular system faded out. Facts of this kind, as also abundant data relating to the vascular tissue in isolated apical meristems and induced buds, support the writer's hypothesis that the initial differentiation of the vascular system is due to the downward movement of a substance or substances from an actively growing apical meristem (Wardlaw 1944*a*). To what extent the apical cell in particular is responsible for this process is a matter for further investigation.

The only vascular tissue present in an isolated terminal region lies immediately below the active apical meristem, or in the new shoot which results from its growth. In the large number of specimens which have now been examined, there has been no evidence whatsoever of new accessory vascular strands making connexion with the vascular system in

older regions. Evidence in support of this statement is afforded by the photomicrographs which accompany this text. This result may be found to differ from the data of somewhat similar experiments with flowering plants. The formation of a new and completely separate vascular system in the isolated terminal region, and its differentiation into the usual complement of tissues (phloem, xylem, parenchyma, etc.), afford the strongest evidence that, in the ferns at least, these histogenic processes are not determined by 'influences' proceeding from the older, preformed vascular tissue. While the initial differentiation of vascular tissue (by which it becomes distinguishable from the adjacent cortical and pith parenchyma) can be attributed to the activity of the apical meristem, the factors at work in the *subsequent differentiation* of this tissue into phloem, xylem, etc., still await exploration.

In the development of the individual fern plant from a zygote or bud rudiment, a protostelic stage normally precedes a solenostelic stage and the latter a dictyostelic stage. Hence there was a tendency to conclude that the more highly elaborate stage, e.g. a solenostele, was necessarily preceded by a protostele. The same idea underlies the theory of recapitulation, in which it is held that the progressive elaboration of form during the development of an individual plant is a necessary consequence of its ancestry and hereditary constitution, i.e. it recapitulates the history of the race. Although the genetic constitution of an organism must be involved in every aspect of its morphological development, it now seems improbable that the facts of stelar elaboration can be explained in terms of the theory of recapitulation. In these studies, some of the large, rapidly growing buds, which have been induced to develop in the subapical region, may be solenostelic from the outset, with no antecedent protostelic stage. The size and configuration of the stele, in fact, are directly related to the size of the apical meristem and the latter to the nutritional status of the tissues with which it is in organic continuity (Wardlaw 1945).

A comprehensive study of pteridophytes has shown that the progressive elaboration of the stele accompanies its increase in size. That there is such a size-structure correlation may now be accepted as a fact (Bower 1921, 1930; Wardlaw 1947*d*). The *initial differentiation* of vascular tissue immediately below the apical meristem is a process which apparently takes place uniformly throughout development, from the young sporophyte to the adult state; it is during the *subsequent phase of development and differentiation* that the various elaborations of the stele take place. These can be directly correlated with the size and nutritional status of the apical and subapical regions. In a study of the apex of a tree fern, *Cyathea Manniana*, the writer (Wardlaw 1948*b*) has shown that during growth from the small sporophyte a few mm. in length to the adult plant 3 to 4 m. in height, the apical growing point undergoes a considerable enlargement—about seven- to tenfold. But in appearance, outline, growth, differentiation, formative activity and arrangement of parts, the large apex of an adult shoot is closely comparable with the small apex of a young sporophyte. The former is simply an enlarged replica of the latter. In morphogenetic activity, as in structural organization, the fern apex thus remains singularly unchanged throughout development, notwithstanding the vast increase in size of the plant and the number of its component parts.

(*d*) *The shoot type of organization*

When an apical meristem is isolated on a plug of pith parenchyma, it develops into a vasculated shoot bearing leaves. What may be described as the shoot type of organization

is found in all classes of vascular plants. The similarity of the leafy shoot in ferns and in flowering plants may be attributed either to community of descent (homology of origin) or to parallel evolution (homoplastic development). Among contemporary botanists, confronted with the difficulty of establishing the natural relationships of the major groups of vascular plants, the latter view is probably that which is most generally held. Now, on analysis, this is a somewhat surprising state of affairs; for it is as much as to say either (*a*) that many of the major and seemingly distinctive features of vascular plants are to be related, not to intrinsic or genetic factors, but to extrinsic factors, or (*b*) that closely comparable genes have evolved in quite distinct groups.

During the past two decades several investigators (Arber 1925; Priestley 1928; Grégoire 1931; Hamshaw Thomas 1932; McLean Thompson 1934; Foster 1939; Thoday 1939; Watson 1943; Sifton 1944) have made the plea that there should be a breakaway from the old morphological traditions and an approach made to the problems of form and structure from a dynamic point of view. In the following tentative suggestions the writer has attempted to indicate some of the factors which may determine the shoot type of organization during the growth of the individual plant.

In the embryonic development, as also in the development of a bud rudiment, the axis possesses polarity from the outset (Bower 1922) and various physiological gradients are established. The position of the embryonic or meristematic tissue in relation to supplies of nutrients, the quantity and quality of such nutrients, and the path of translocation, may be indicated as factors which are important in the subsequent growth and morphological development of the meristematic region. For the growth and development of a shoot or axial structure there must be: (i) a stimulus to growth; (ii) cells in a distal position capable of sustained meristematic activity; (iii) a source of nutrition in close proximity; or (iv) the differentiation of a conducting system by which nutrients can be quickly conveyed from more distant sources to the active meristem; and (v) the progressive development of rigid structure in the submeristematic region. The shoot, in fact, develops on an accretionary principle. If the rate of supply of nutrients to the growing region were uniform then, other things being equal, the resulting shoot would be of cylindrical form. If the rate of supply of nutrients diminished, for example, as the distance of the apex from the source of supply increased, the shoot would have an attenuated or tapering form. But if the rate of supply of nutrients to the meristem were accelerated, it would undergo a progressive increase in size and the shoot would become an enlarging obconical structure. This obconical development is, in fact, a characteristic feature not only of vascular plants, but of plants in general (Bower 1922). Qualitative as well as quantitative variations in the supply of metabolites may also be expected to induce changes in the morphological development.

The general inference to be drawn from the experimental studies described here is that the shoot type of organization can in the main be referred to non-hereditary factors, i.e. its occurrence in different phyletic lines is the result of development under the influence of the same or similar physical and physiological factors.

(*e*) *The lateral organs*

The lateral organs in ferns (leaves, scales and buds) are normally formed in specific positions (except the scales) and at different levels on the growing point. Leaf primordia

are formed *within* the apical meristem some distance below the apical cell; scales are formed at the *base* of the apical cone, i.e. *in the region of transition* between the densely protoplasmic cells of the apical meristem and the vacuolating parenchymatous cells of the subapical region; buds, which may grow into lateral shoots, arise on the subapical region, i.e. *below* the apical cone, but they originate from cells which formed part of the apical meristem.

Both leaf and bud primordia arise from equivalent groups of superficial meristematic cells. In fact, the initial stages of bud and leaf formation are difficult to distinguish; each begins as an outgrowth or mound of tissue in which, at an early stage, a centrally placed apical cell is formed, all subsequent growth of the primordium being referable to this apical cell. The chief differences between leaf and bud primordia are these: (i) leaf primordia are formed *on an organized apical meristem* and nowhere else, whereas bud primordia arise from a group of isolated meristematic cells lower down on the shoot, i.e. there need not be a pre-existing organized meristem; (ii) leaves have dorsiventral symmetry whereas buds have radial symmetry; and (iii) leaf formation and development are not inhibited by an active apical cell whereas bud formation and development are. These are among the facts of organ formation which await explanation.

Leaf primordia are formed on *the sides or flanks of the conical apical meristem.* As a consequence of the distribution of growth at the apex (§§ 5 (*a*) and 10 (*a*)), the lower or abaxial side of a primordium grows more rapidly than the upper or adaxial side, both in the transverse and longitudinal planes. From the outset, therefore, there is a distinction of upper and lower sides, i.e. dorsiventral symmetry is established. It is suggested that, as a consequence of the different rates of growth on the upper and lower sides, a system of stresses is set up in the young, plastic primordium, which determines the characteristic shape and orientation of its apical cell. This apical initial is lens-shaped—the so-called 'two-sided' apical cell —and is disposed with one edge facing obliquely towards the apical cell of the shoot. In this view, the shape and symmetry of the leaf primordium, and the character and disposition of its apical cell on which the subsequent development depends, are determined by the *position in which the primordium* arises on the growing point. So far, all attempts by the writer to transform a young leaf primordium into a shoot have failed.

When a bud primordium is being formed, the outgrowth of meristematic cells takes place in a region of the shoot which has already attained to approximately its adult girth, i.e. there is not a marked difference in the rates of growth in the transverse planes above and below the primordium, and an organ of radial symmetry, with a 'three-sided' apical cell, results. (It is pertinent to note that leptosporangiate ferns with radially symmetrical shoots, but with 'two-sided' apical cells, are also known, e.g. *Matteuccia struthiopteris*. The formation of the first leaf and of the shoot in the fern embryo also raises special problems.)

If we inquire what differences, if any, there are between *very young* leaf and bud primordia, the answer is that they occupy different positions on the growing point at the time of their inception and that leaf formation precedes bud formation. This matter of position has momentous consequences; they are seen in the morphological differences that distinguish leaf and shoot. In *Dryopteris* the formation of leaf primordia has never been observed in the subapical region or in a detached meristem until a shoot apex has become organized in the course of growth. What we can say of leaf formation is that a group of superficial cells

of an actively growing apical meristem, in a position determined by a system of stresses (Wardlaw 1948*a*, *c*), and presumably in a certain physiological condition, grow out and give rise to a lateral member. For the reasons given above, this member develops with the symmetry and shape which we recognize in leaves. As leaf primordia, with their enlarging bases, develop in sequence, the lower region of the apical meristem becomes largely transformed during growth into epidermis and cortical parenchyma. But in certain positions, which can best be specified as interfoliar, small areas of the superficial meristematic cells persist in an unaltered state. These persistent areas gradually move away from the apex during growth and are recognized farther down the shoot as detached meristems or bud rudiments. They show little tendency to grow; in fact, they are in an inhibited condition and remain so until the apical cell of the shoot is destroyed or becomes quiescent when they give rise to lateral buds.

In the present view, lateral organs are an expression of the growth of the shoot apex; they are not specifically predetermined either by the hereditary constitution of the plant—although the potentiality for their development must be there—or by the pre-existence in the race of organs of different fundamental categories. The category to which a lateral organ belongs, be it leaf, bud, or scale, depends on the position at the apex in which it is formed; and it is to the mechanics of growth in the plastic distal region of the shoot that we must look for explanations of the characteristic symmetry, form and structure of the leaf primordium as compared with the corresponding features of a bud primordium. In this analysis the conclusions regarding the nature of the leaf are in some respects similar to those of Arber (1941, p. 100) though the approach to the problem in the two investigations was very different indeed.

(*f*) *Physiological aspects of leaf and bud formation*

Leaf and bud primordia not only differ morphologically (i.e. after the initial stage), they also differ in their physiological reactions. If, as the evidence suggests, the apical cell and its immediate segments are characterized by a distinctive metabolism, physiological gradients, some basipetal, will be established. Cells within but near the lower margin of the apical meristem will be in a somewhat different physiological state to those close to the apical cell; they will be at a lower point on the concentration gradient of substances proceeding from the apical cell and will be closer to the source of substances moving upwards from below. They may also be affected in various ways by their proximity to the actively growing subapical region. These observations are relevant to the problem of leaf formation.

Each leaf primordium in *Dryopteris* may be regarded as a centre or locus of growth occupying a specific position; collectively these leaf positions constitute a phyllotactic system usually of a high order of regularity. According to the caline theory of Went (1938) leaf formation is due to a growth-regulating substance—phyllocaline. But why should phyllocaline accumulate or exercise its effect in a particular position in the meristem? Went has not attempted to answer this question. In the normal development meristematic cells close to the apex do not give rise to leaf primordia; only those considerably lower down on the apex do so, and, as we have seen, there is reason for the view that physiologically the meristematic cells in the two positions may differ in certain respects.

Certain observations by Thimann (1938) indicate that roots, buds and shoots react to auxin in essentially the same way; they simply differ in the quantities required to produce the effects. Each type of organ is stimulated by a relatively low concentration of auxin and inhibited by a high concentration. This generalization may perhaps be extended to leaves also, along the following lines. In the ferns there is evidence that growth-regulating substances are formed in the apical cell and move downwards in the shoot. From the fact that leaf primordia arise on the apical meristem whereas buds are formed lower down or, more usually, remain inhibited, it may perhaps be inferred that leaf formation is stimulated by concentrations of growth-regulating substances that inhibit bud formation. But in close proximity to the apical cell, where the concentration of growth-regulating substances is greatest, leaf formation will be inhibited. If, however, the apical cell is destroyed, leaf primordia may eventually be formed in close proximity to the extreme tip.

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Description of Plates 24 to 30

(All figures are from untouched photographs)

Plate 24

Figure 45. Downward view of a large shoot apex of *Dryopteris aristata* (with the scales removed) illustrating the kind of material used in these experimental investigations. *p*, leaf primordia. (× 40.)

Figure 46. Downward view of a large shoot apex of *Dryopteris aristata* treated with a suspension of lamp-black in an aqueous solution of gum-arabic. The positions of the extreme apex (centre) and of some of the leaf primordia *p* can be seen. (× 40.)

Figure 47. An apex treated with lamp-black, photographed after some growth has taken place. The apex (centre) is still covered with lamp-black, but there has been a considerable dispersal of it round its base and in the subapical region. Scales *sc* and leaf primordia *p* can be seen; lamp-black still persists in the axils of the latter. (× 40.)

Figure 48. A later stage than that illustrated in figure 47. Only a peppering of lamp-black remains on the apical meristem. *p*, primordium. (× 40.)

Plate 25

Figure 49. Apex in longitudinal median section: deep incisions *i* have been made close to the apical meristem. The isolated terminal region *itr* has grown slowly and has been outstripped by the lateral buds *b*; *P*, pith. (× 20.)

FIGURE 50. Like figure 49, but the superficial tissue surrounding the isolated terminal region has been removed. The isolated terminal region has grown, produced leaf primordia, and become somewhat distended radially. (× 20.)

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FIGURE 52. Like figure 51, but the incisions have been made very close to the apical meristem. The isolated terminal region *itr* has made little growth but its meristem is normal and intact. A bud *b* is developing in an approximately axillary position to leaf *l*. (× 20).

PLATE 26

FIGURES 53, 54. Continued growth of apical meristems, with formation of leaf primordia and differentiation of vascular tissue, after an undercutting operation by which vascular connexion with the older tissues below was severed. (× 20.)

FIGURES 55, 56. Two views of an isolated terminal region in which the apical cell *a* was damaged: the leaf primordia *p* have continued to grow. The growth of the terminal region has been carried on by means of a lateral bud (not shown in these sections). (× 20.)

FIGURE 57. Transverse section, after growth, of an isolated leaf primordium. *a*, apex of primordium; *P*, pith; *vt*, vascular tissue; *c*, direction of centre of shoot. For description, see text. (× 70.)

PLATE 27

FIGURE 58. A rectangular solenostele, in an early stage of differentiation, formed below an apical meristem isolated on a rectangular panel of tissue. (× 50.)

FIGURES 59 to 61. Transverse sections, at different levels, of a protostele which developed in an isolated terminal region.

FIGURE 59. Near the base of the vascular column, the stele is a medullated protostele. *X*, xylem; *P*, pith. (× 150.)

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FIGURE 61. At this level, higher up than figure 59 and below figure 60, the stele consists of a solid core of xylem, surrounded by thin-walled elements. (× 50.)

FIGURES 62, 63. Two transverse sections, at different levels, of an apical meristem in which the apical cell had been very lightly punctured. The illustrations show the extent of disturbance of tissues which has ensued. *m*, superficial prism-shaped cells of meristem. (× 180.)

PLATE 28

FIGURES 64 to 72. These are camera lucida drawings made by means of the Ultro Pak microscope. For detailed description, see text. (All × 25.)

FIGURES 64 to 67. Formation of leaf primordia (1, 2, 3 etc.) and buds on the meristem after the apical cell (dark shading) had been punctured; the meristem had also been isolated by vertical incisions.

FIGURES 68, 69. The formation of leaf primordia I_1, I_2, I_3, when punctures had been made above these presumptive leaf positions; 1, 2, 3, etc., leaf primordia, in order of increasing age, present at beginning of experiment. The dark shading indicates the punctures.

FIGURE 70. Apical meristem and leaf primordia isolated on plugs of tissue by vertical incisions. 1, 2, 3, etc., leaf primordia in order of increasing age; buds have developed on the lateral plugs.

FIGURE 71. Apical meristem which was subjected to diametrically opposite radial longitudinal incisions: these have spread upwards and destroyed the apical cell. The older leaf primordia have continued to grow, small new ones have been formed, and two buds have arisen on the meristem.

FIGURE 72. An isolated leaf primordium with an induced bud in its axil; *a*, panel on which apex of parental shoot is situated.

PLATE 29

FIGURE 73. Longitudinal median section of a shoot apex, passing through a very young leaf primordium *p*, which consists of a group of prism-shaped meristematic cells; *m*, meristematic cells; *a*, direction of apical cell of shoot. (× 180.)

FIGURE 74. Longitudinal median section of apex, passing through a very young leaf primordium *p*; *a*, direction of apical cell; *x*, subepidermal cells which have just divided. (× 350.)

FIGURE 75. Another study of a very young leaf primordium *p*; *a*, direction of apical cell; *nc*, narrow cells of leaf axil. (× 350.)

FIGURES 76, 77. Two consecutive serial transverse sections of a very young leaf primordium *p*, showing that several prism-shaped, meristematic cells are involved. The clear cells *p* are those of the outgrowing primordium which have been cut through; the darker cells have not been cut through; *a*, direction of apical cell of shoot. (× 350.)

FIGURE 78. An older primordium *p* cut in transverse section, showing the 'two-sided' apical cell; *a*, direction of apical cell of shoot. (× 350.)

PLATE 30

FIGURES 79 to 85. Formation of buds *b* on isolated leaf primordia *l*, as seen in transverse section.

FIGURE 79. Shows a small bud, in a lateral position, high up on an older, rolled, leaf primordium. (× 50.)

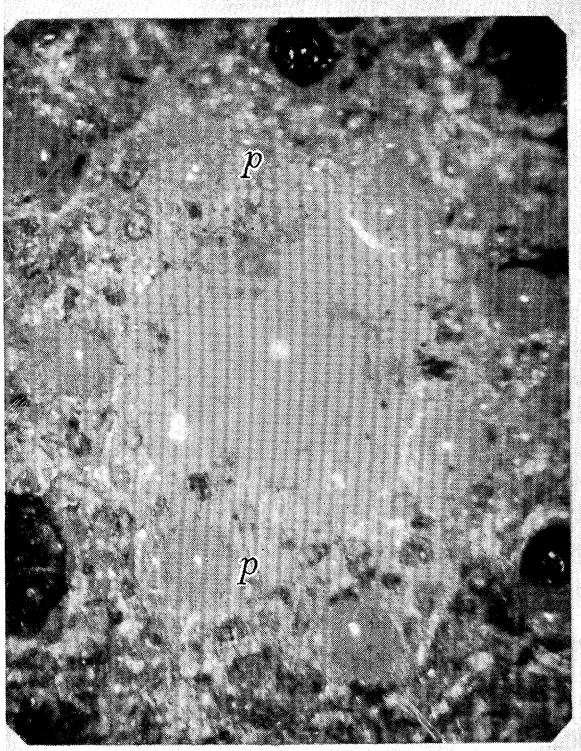
FIGURES 80, 81. Like figure 79, the bud has developed well up on the petiole of the primordium; l_1, leaf primordium of bud. (× 70.)

FIGURE 82. An axillary bud, showing a characteristic arrangement of vascular tissue at the level where the bud stele becomes conjoined with the two meristeles of the shoot (at the 'closing' of the leaf-gap). (× 20.)

FIGURE 83. A protostelic axillary bud. (× 30.)

FIGURES 84, 85. Two examples of large solenostelic axillary buds. (× 30.)

FIGURE 84. Illustrates, in transverse section, the typical awl-shaped leaf primordium, of limited growth, in which the vascular tissue gradually fades out; *c*, centre of shoot.



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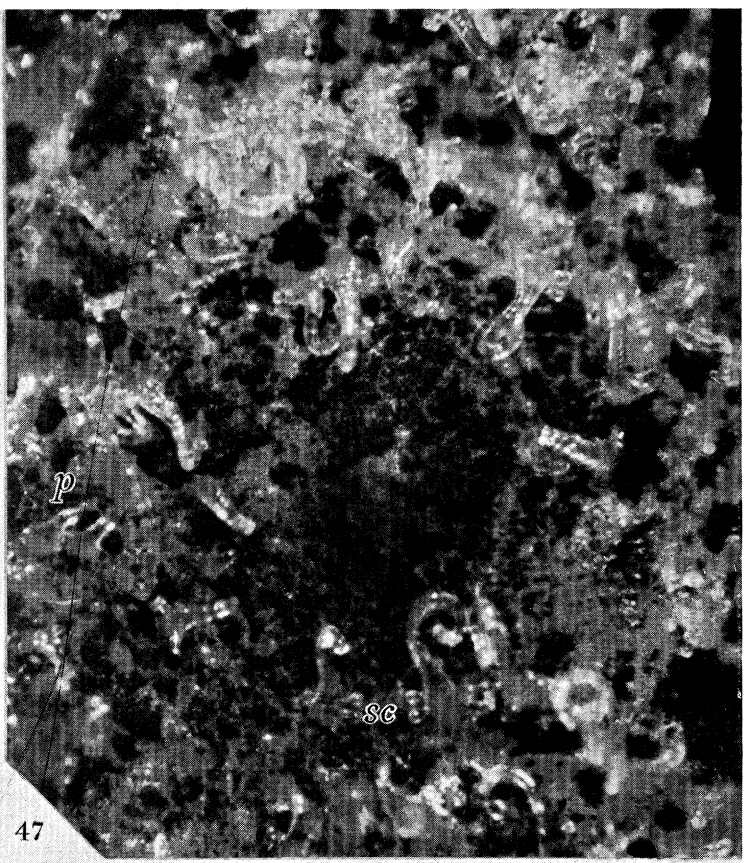

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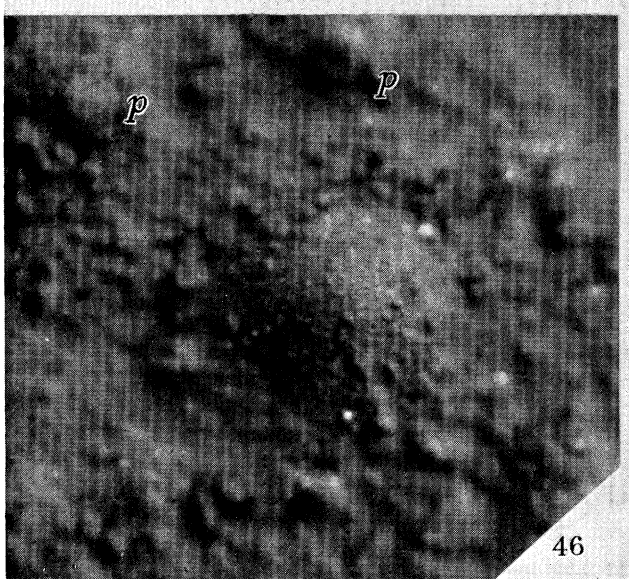

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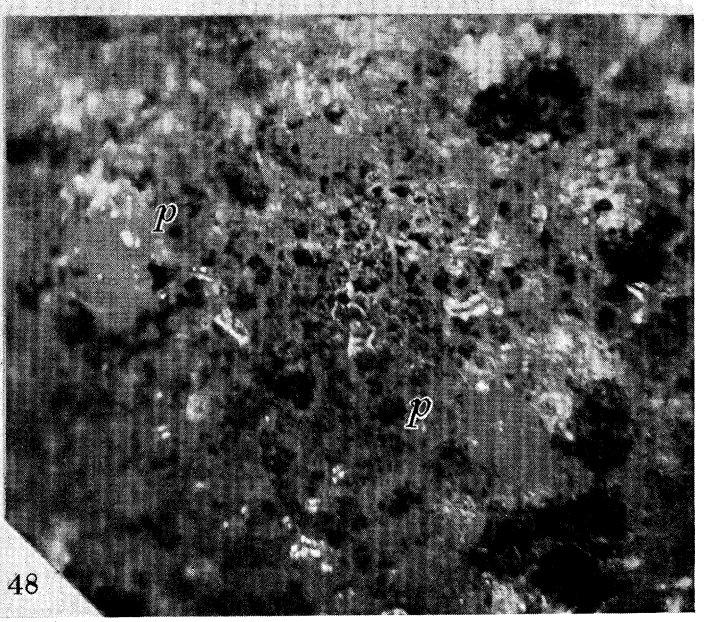

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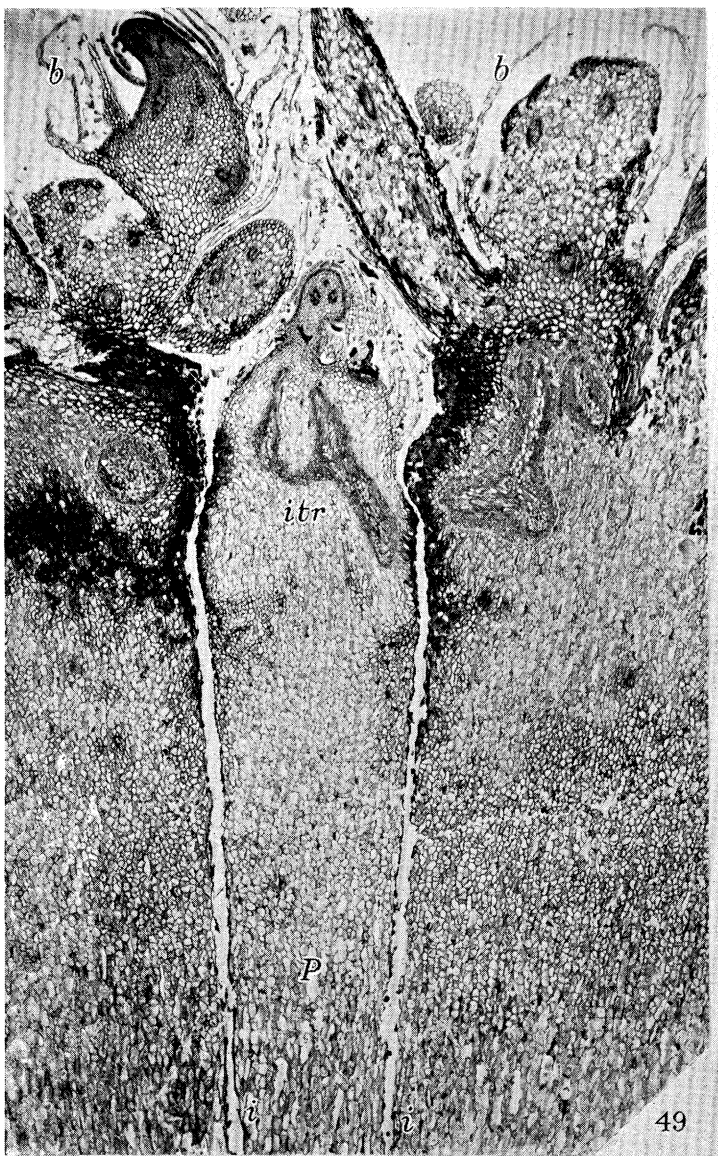

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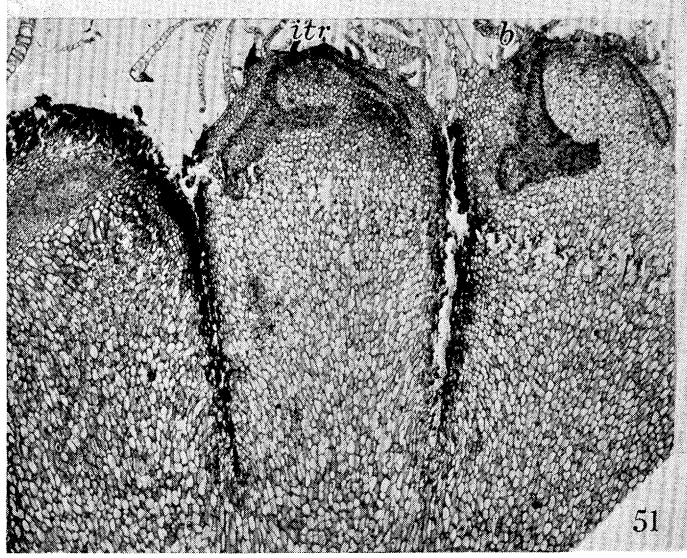

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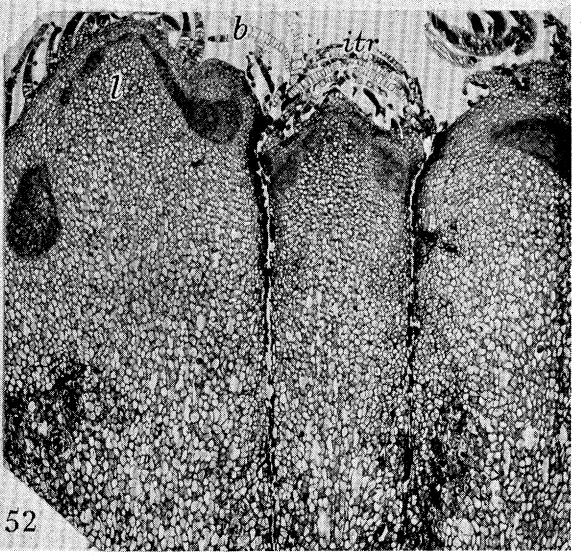

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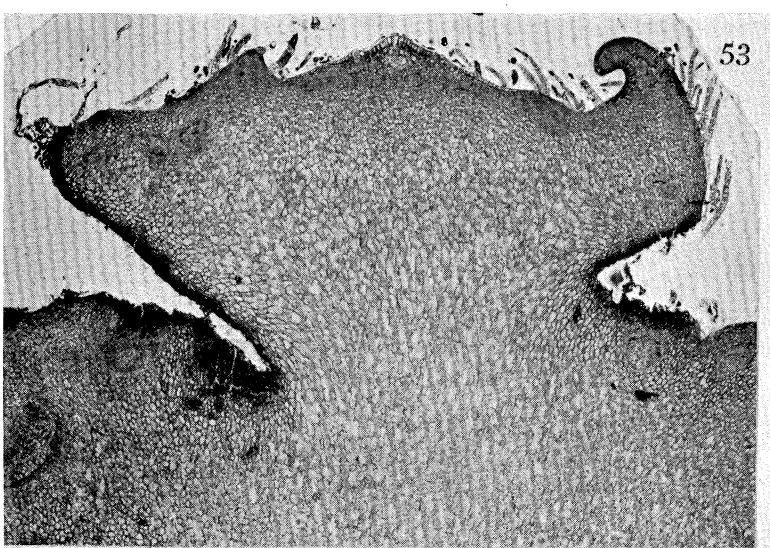
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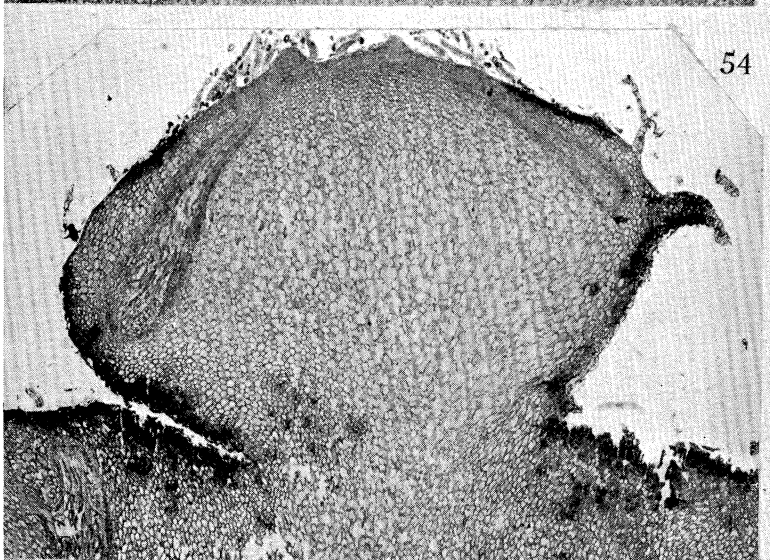
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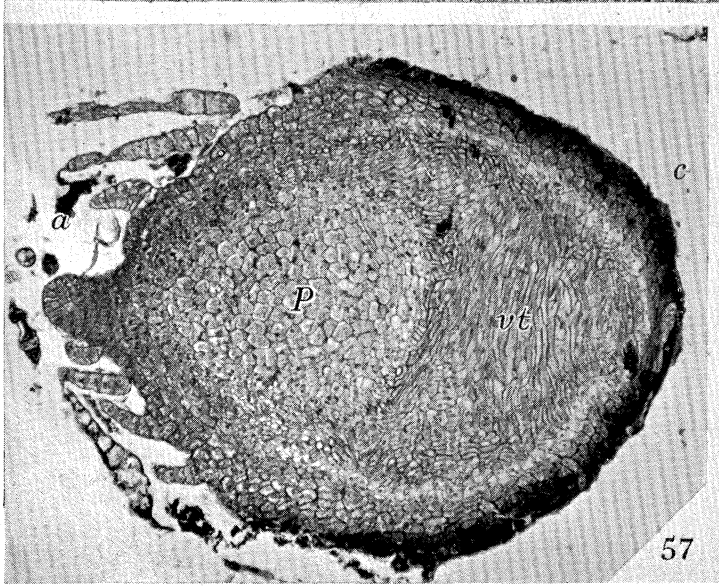

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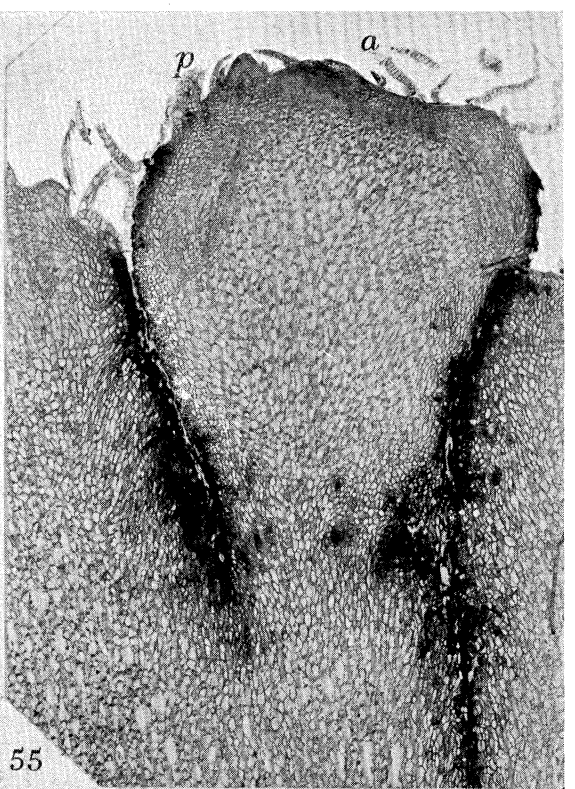

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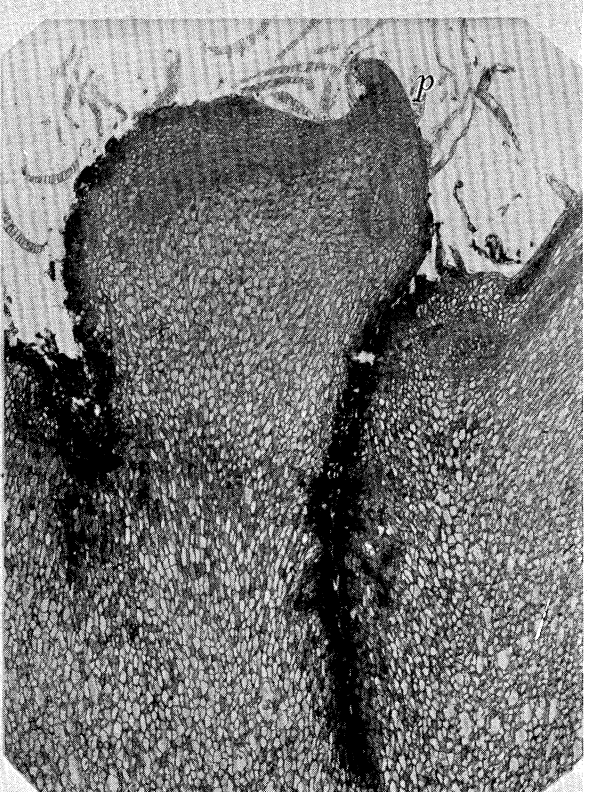

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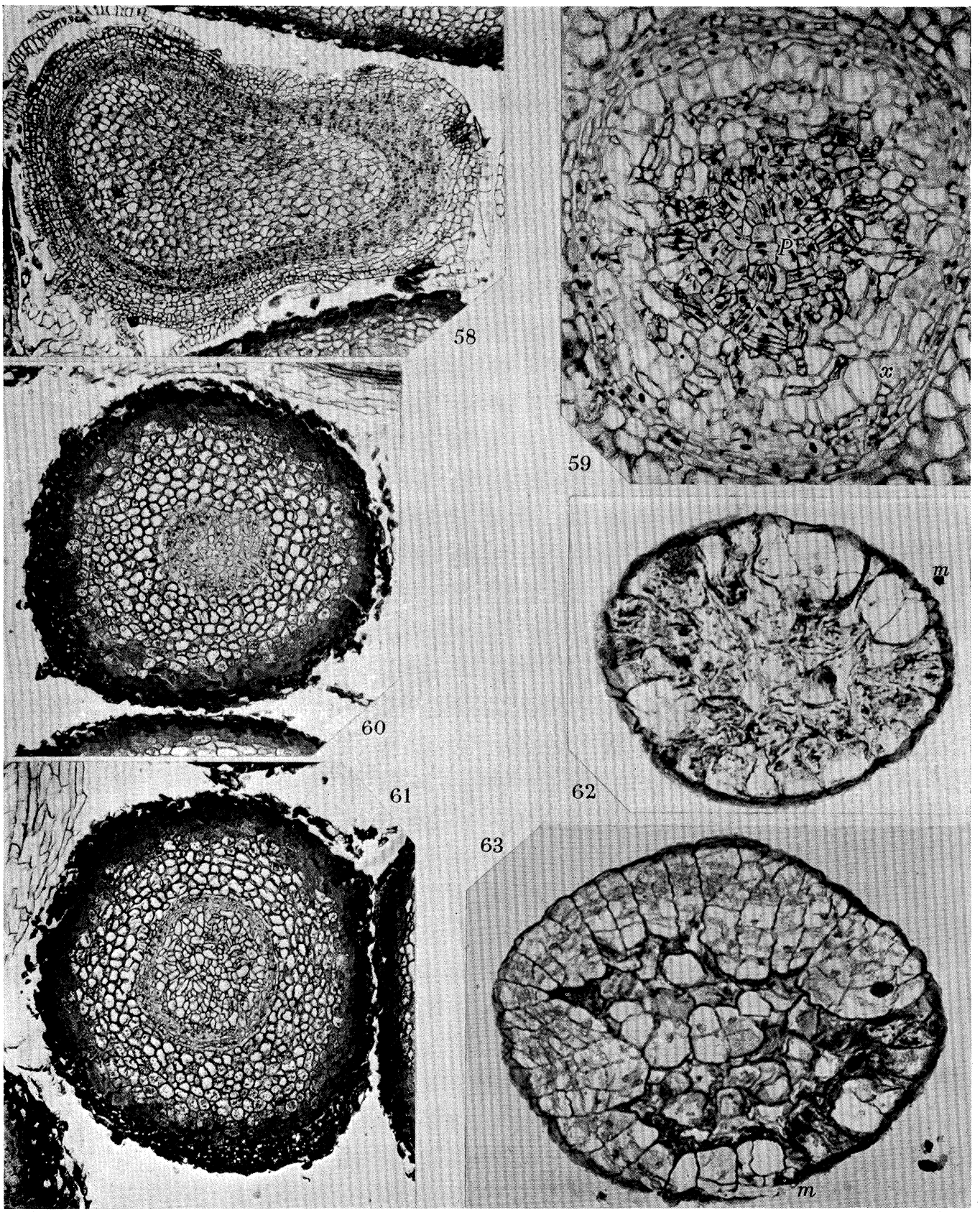
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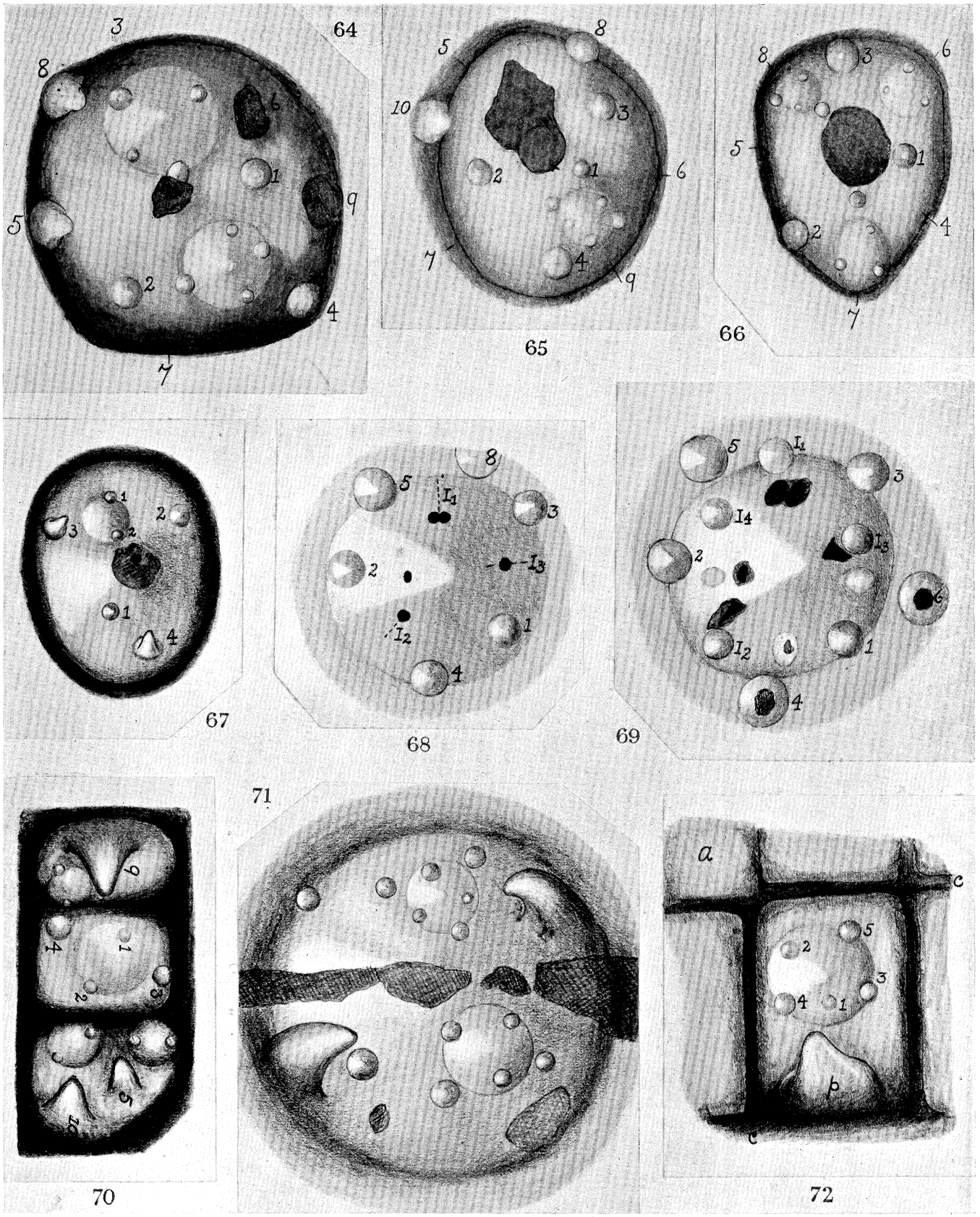
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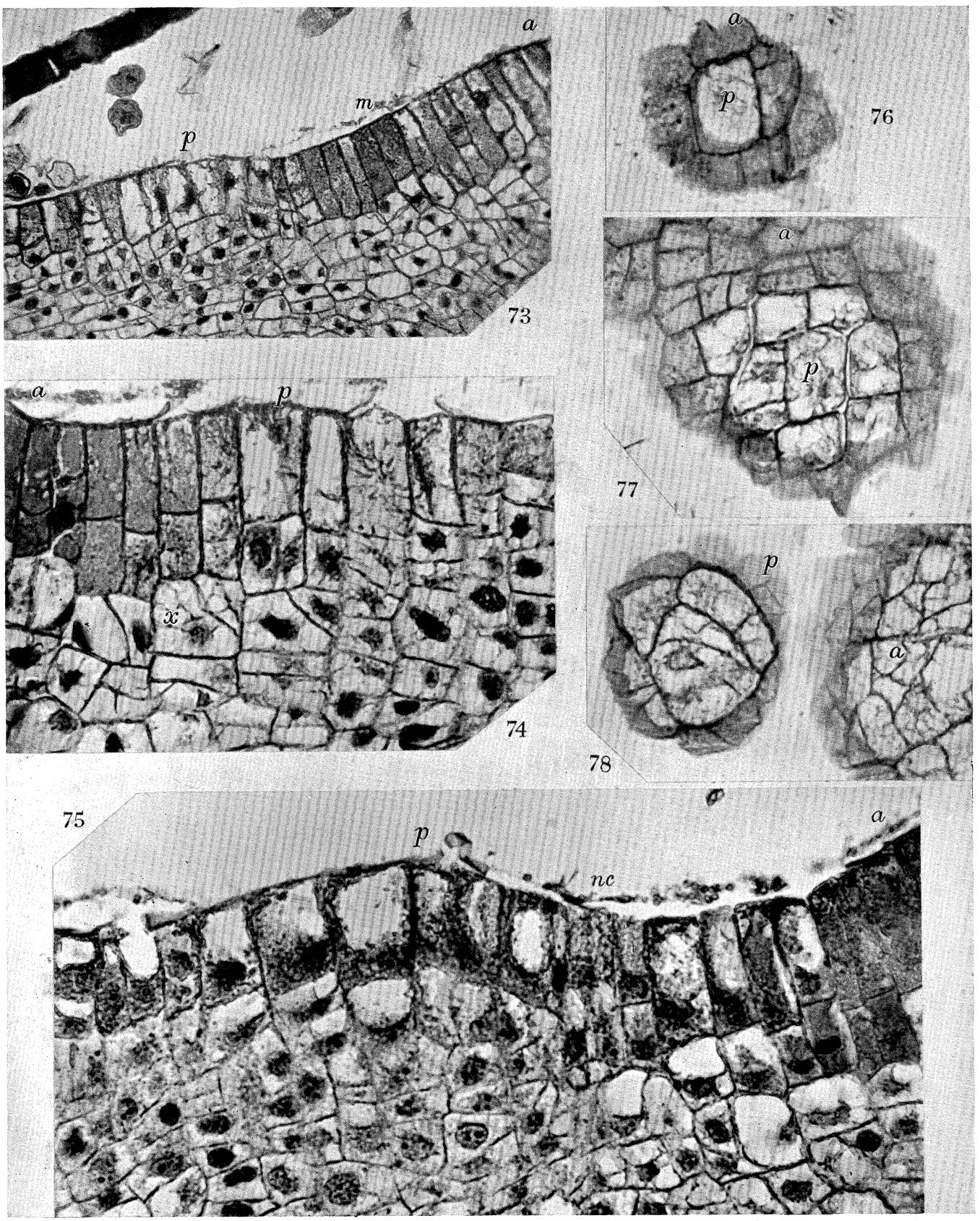
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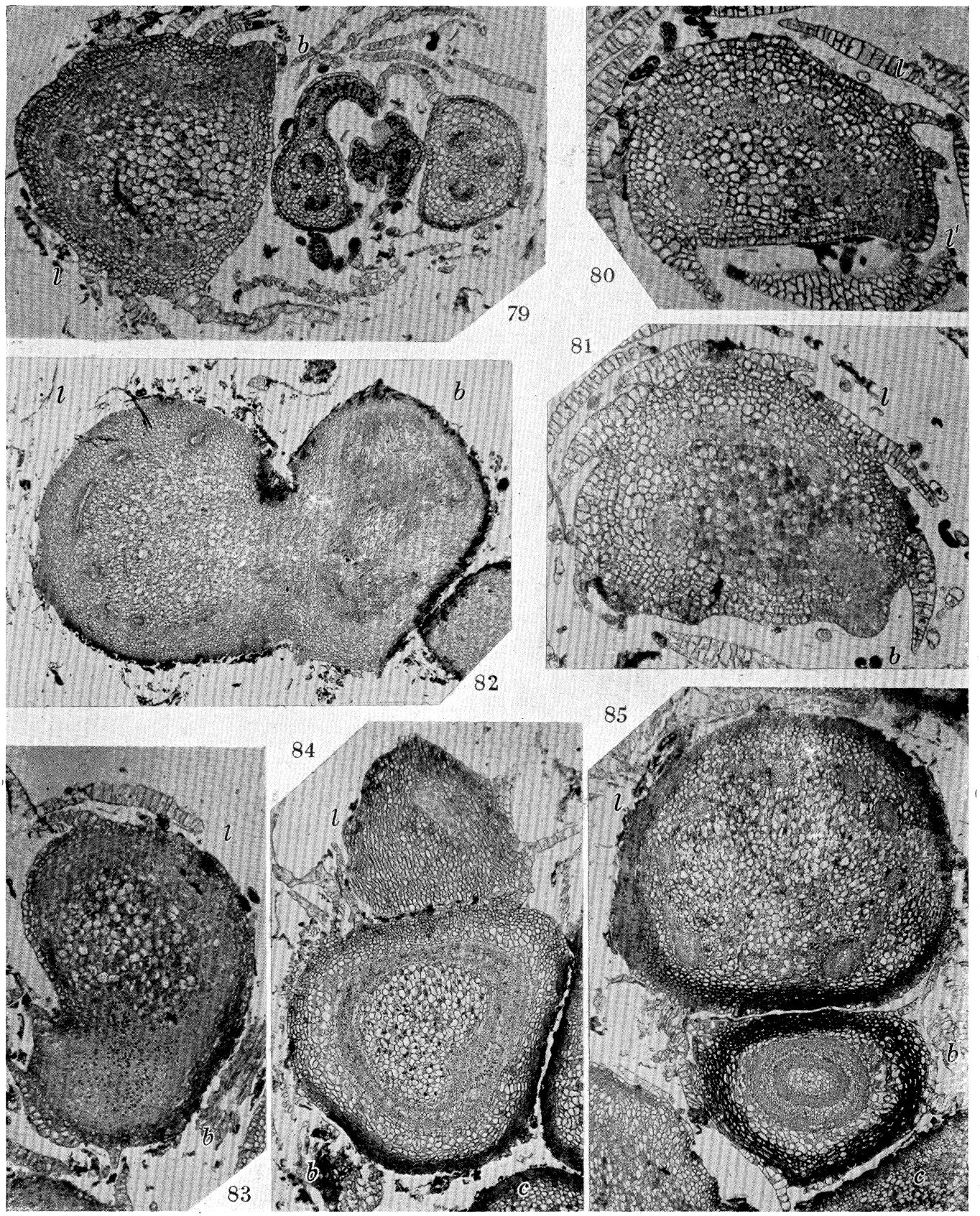
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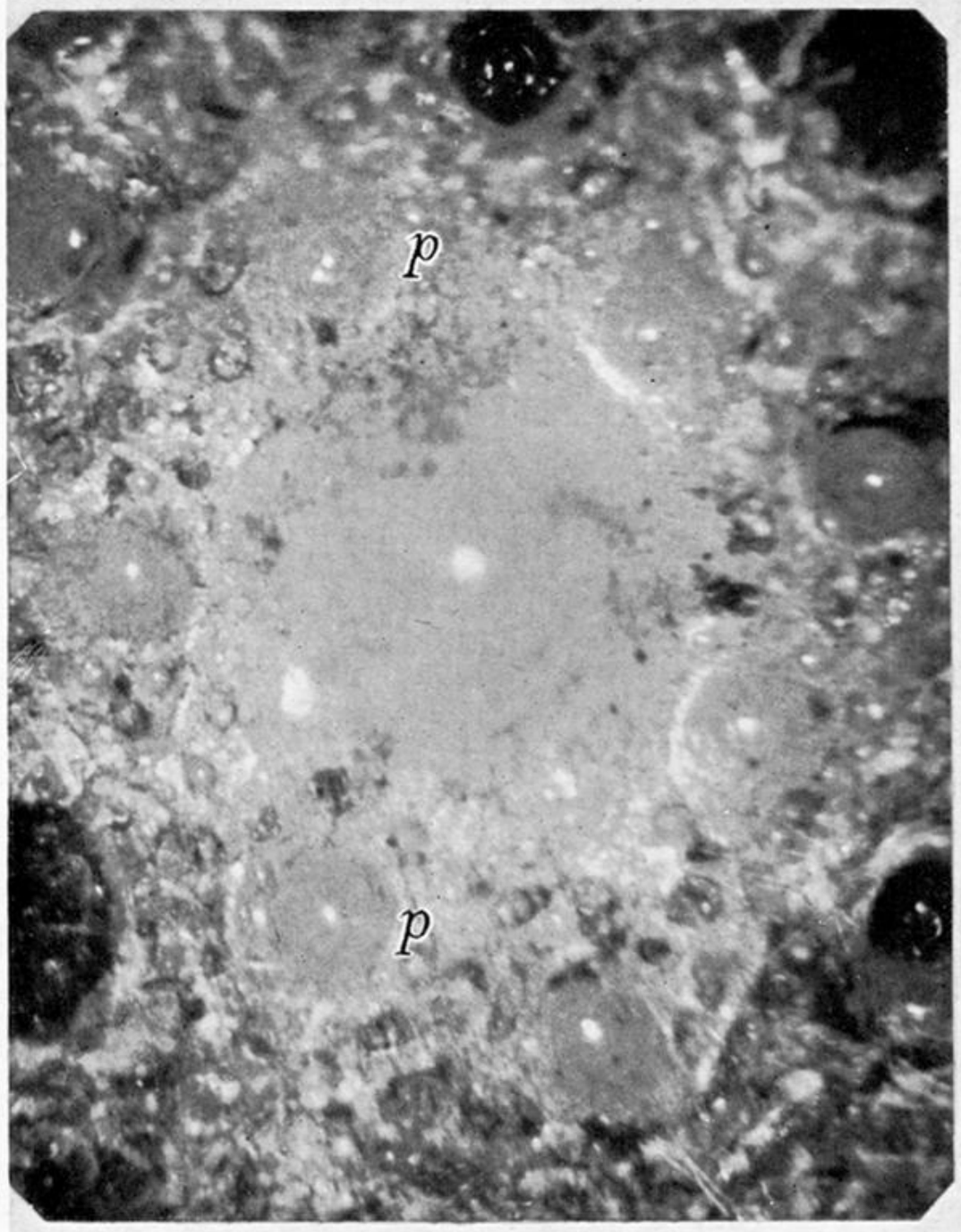

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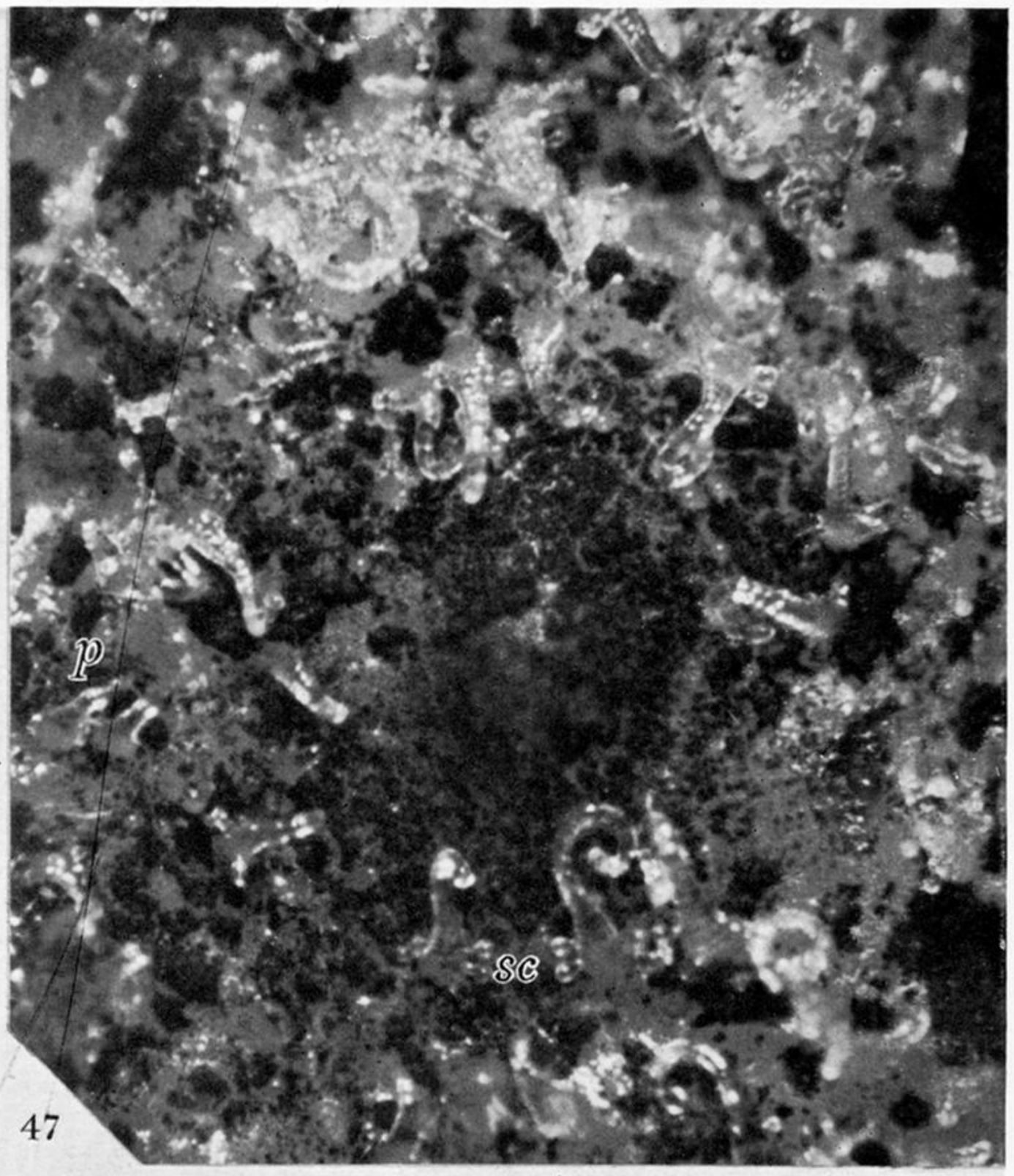

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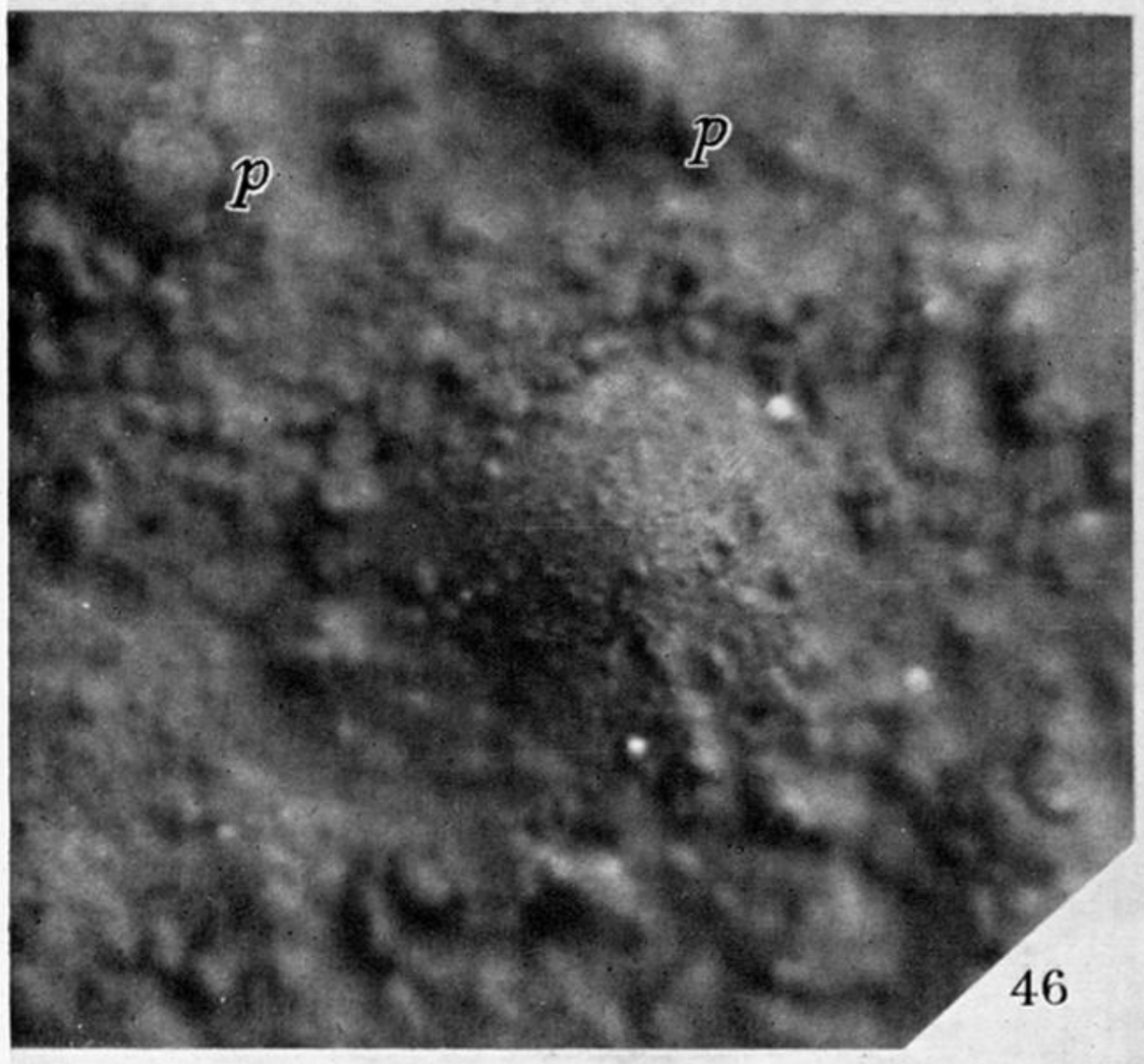

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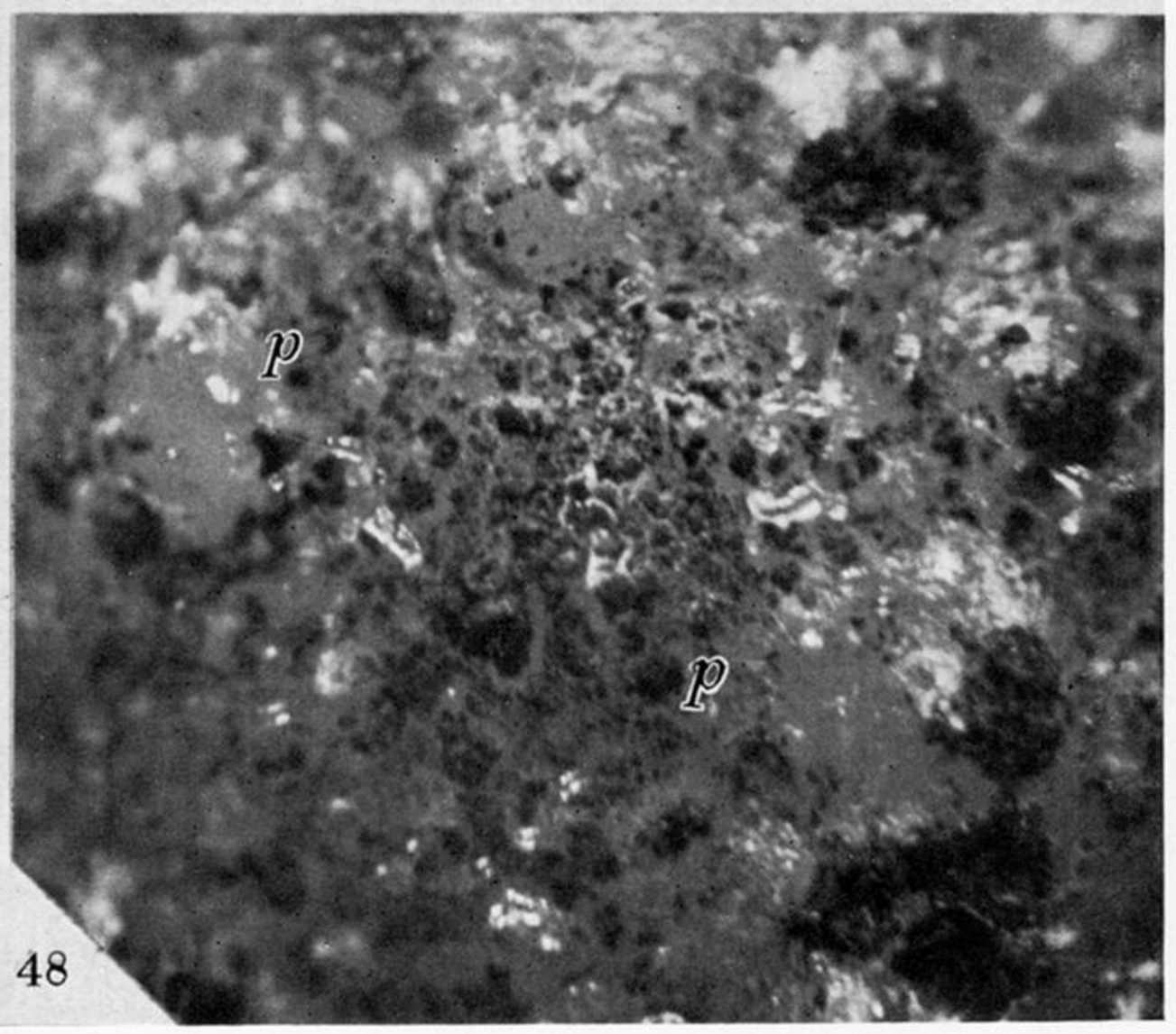

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PLATE 24

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FIGURE 46. Downward view of a large shoot apex of *Dryopteris aristata* treated with a suspension of lamp-black in an aqueous solution of gum-arabic. The positions of the extreme apex (centre) and of some of the leaf primordia *p* can be seen. (× 40.)

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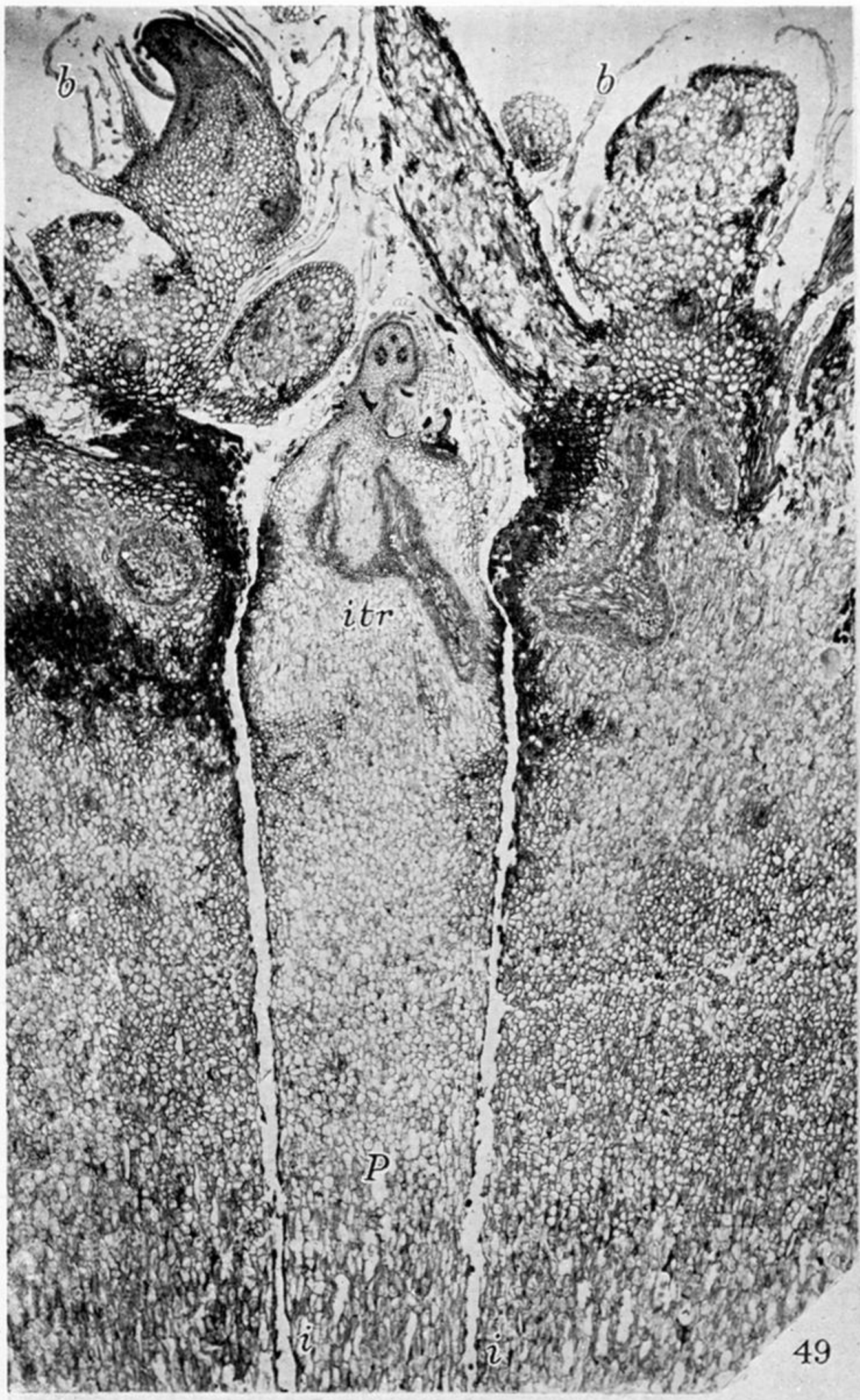

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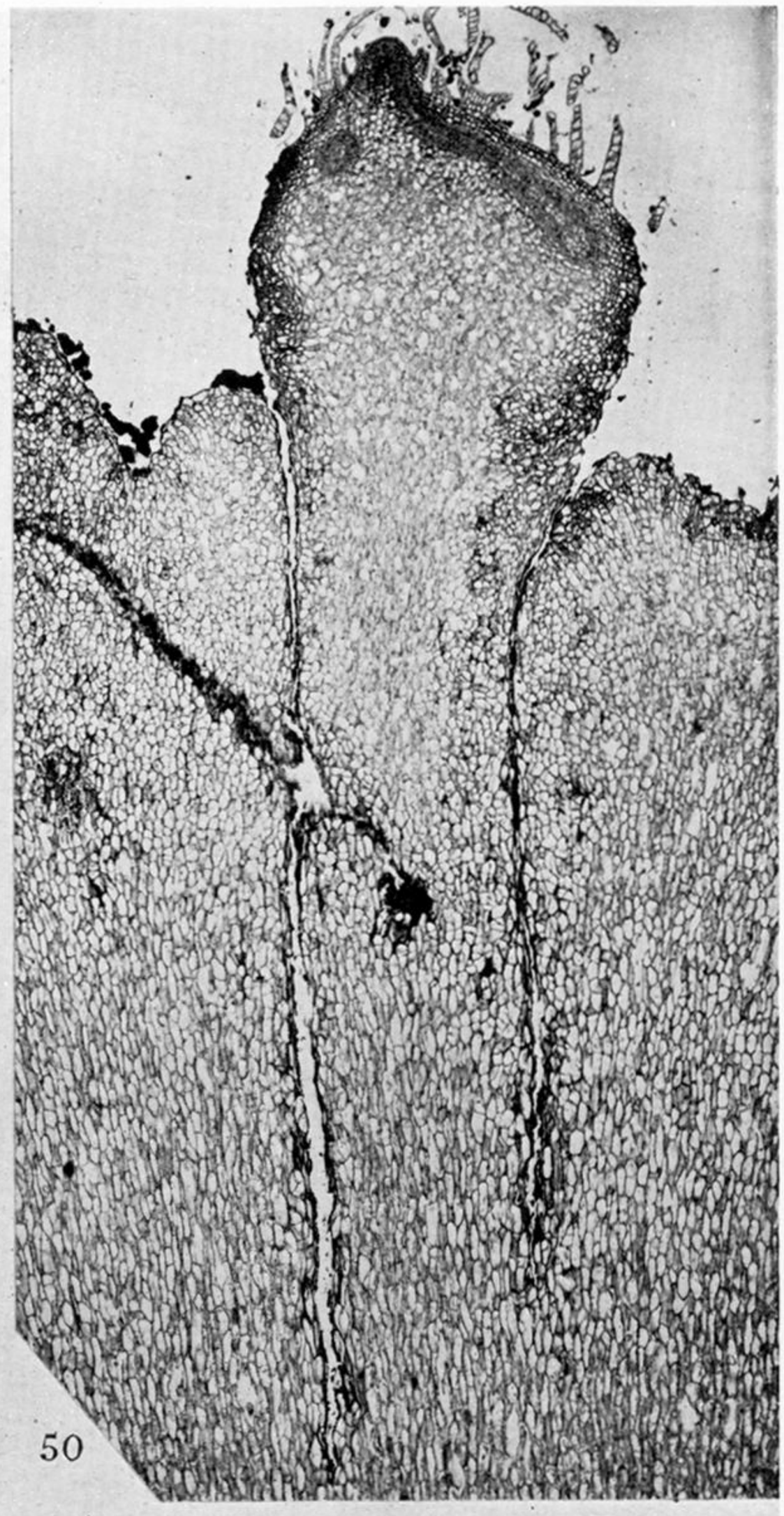

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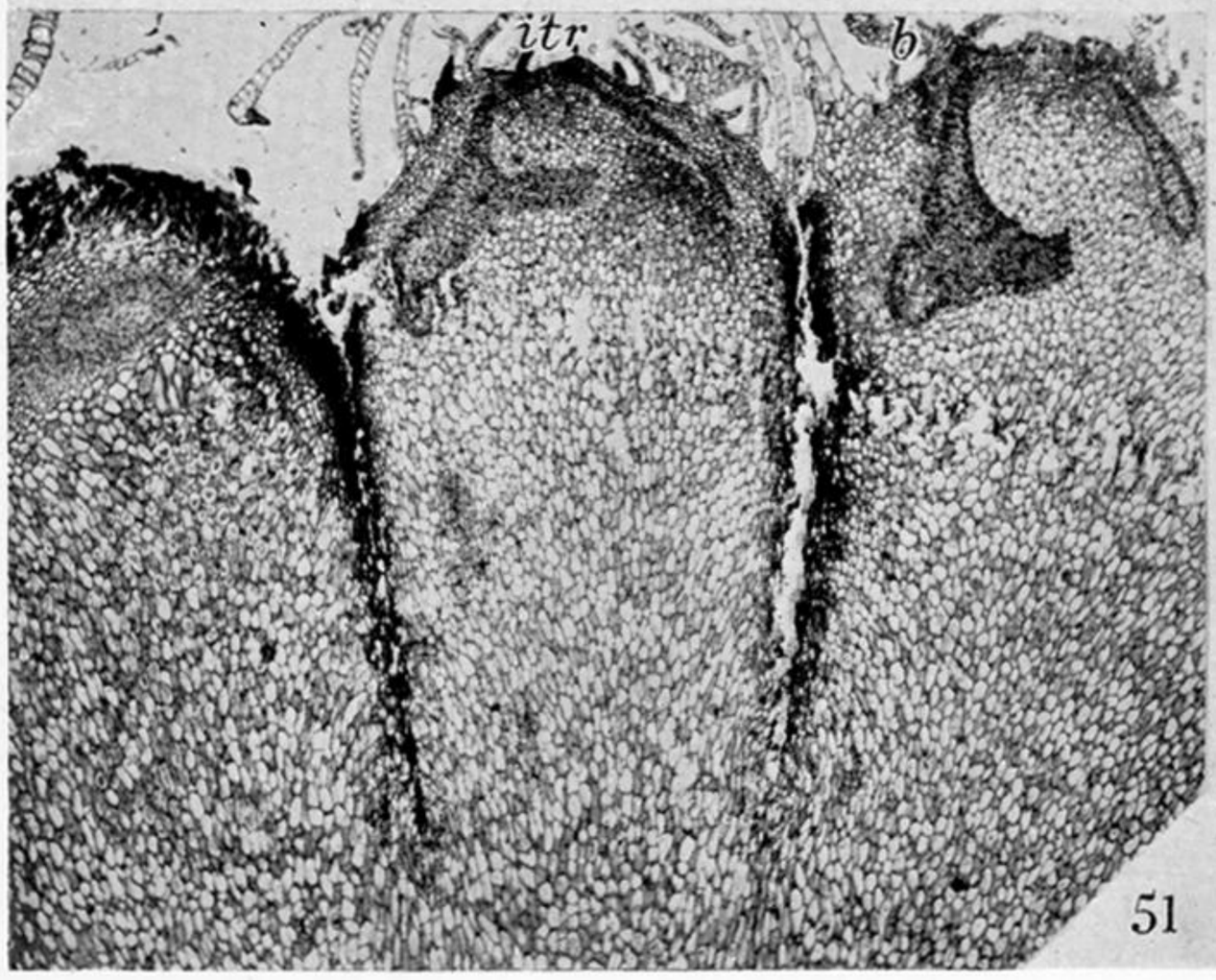

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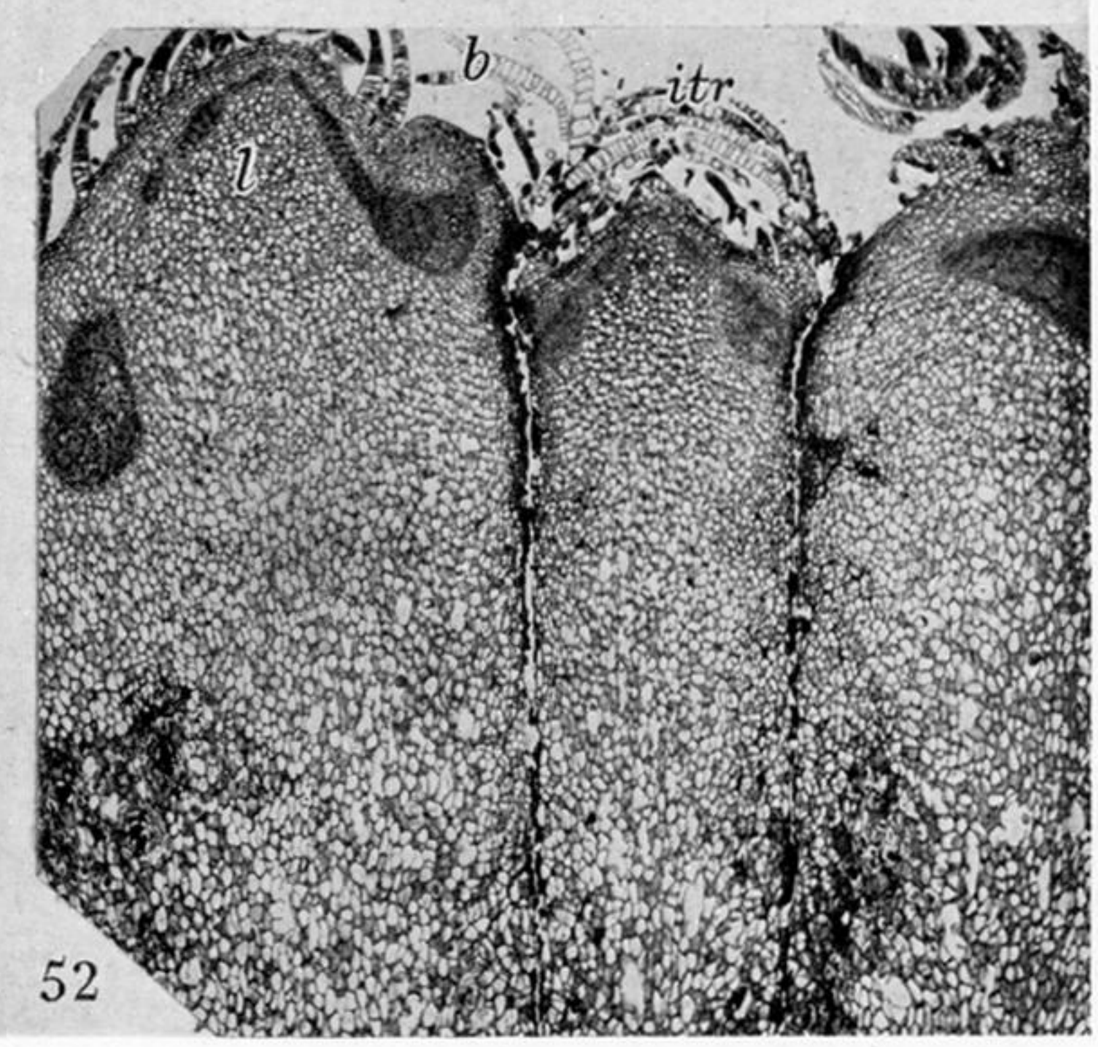

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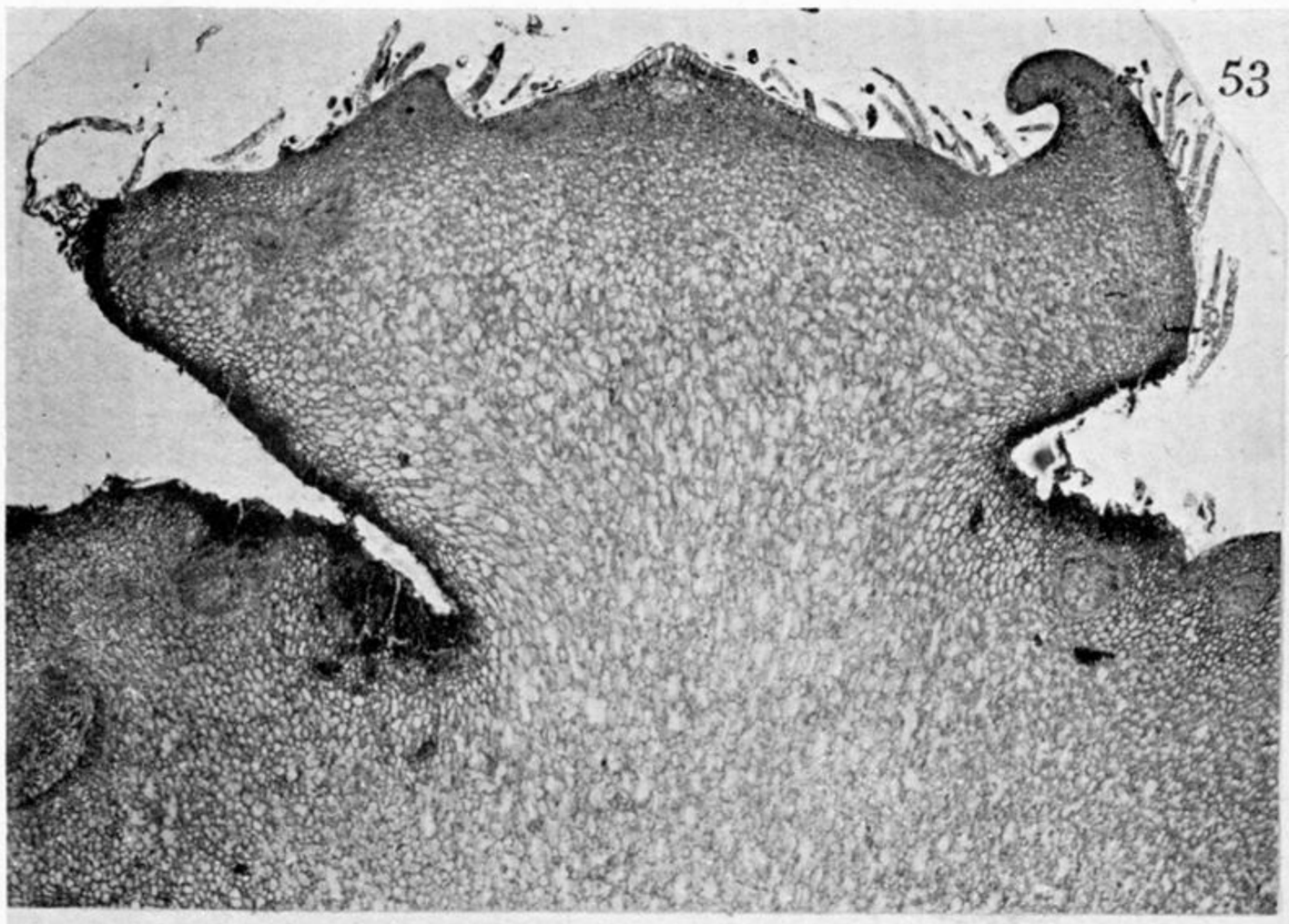

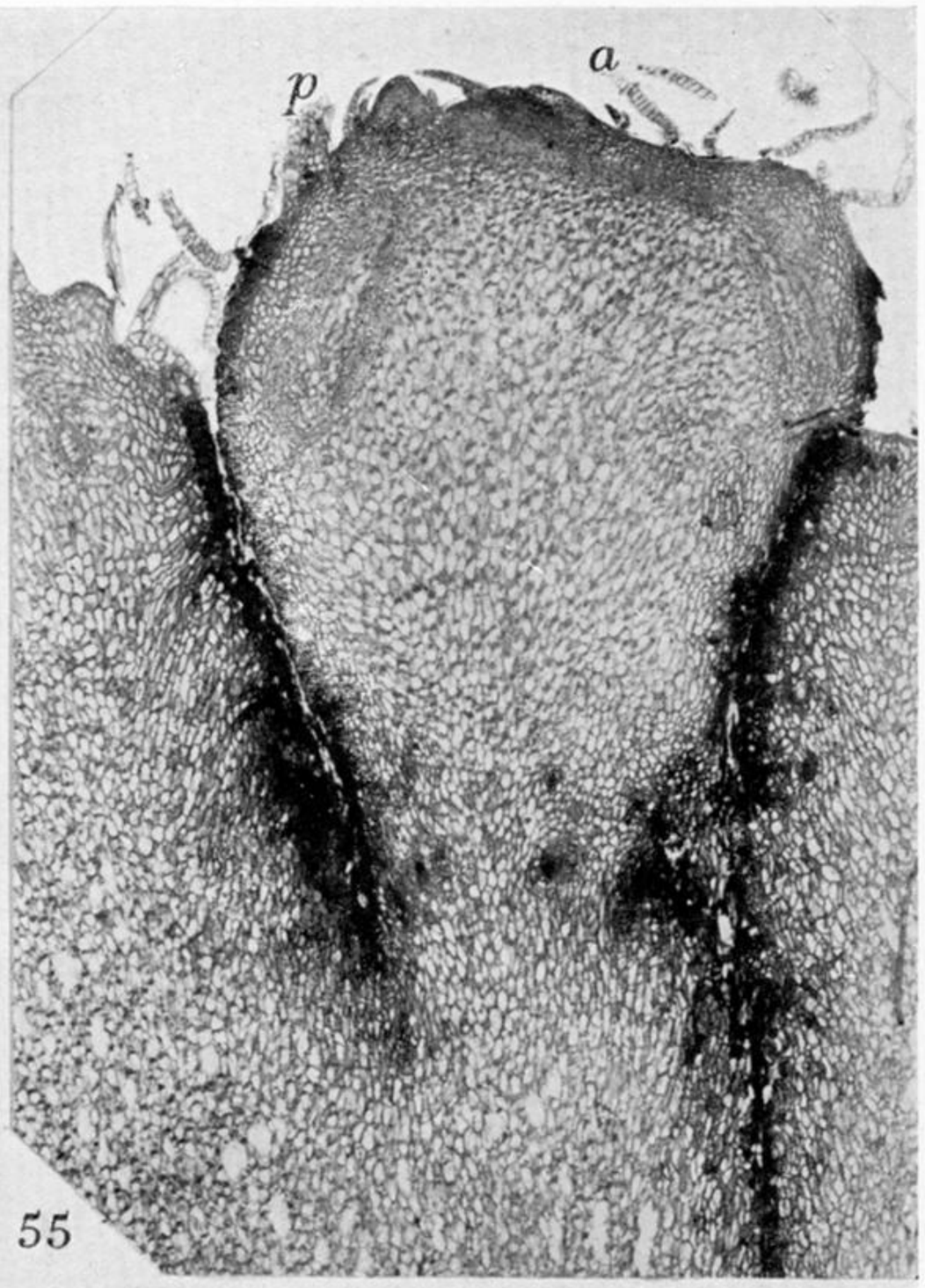

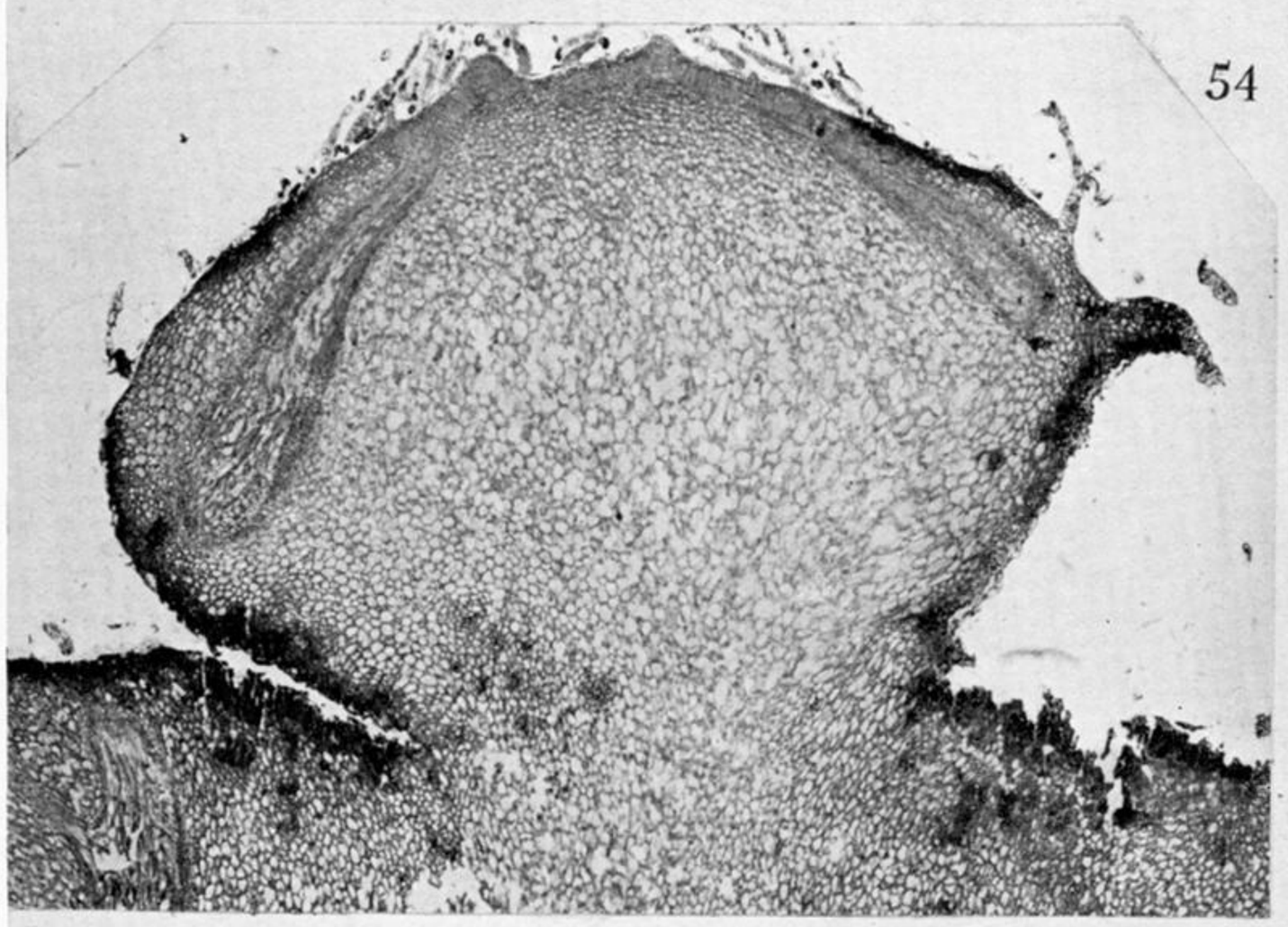

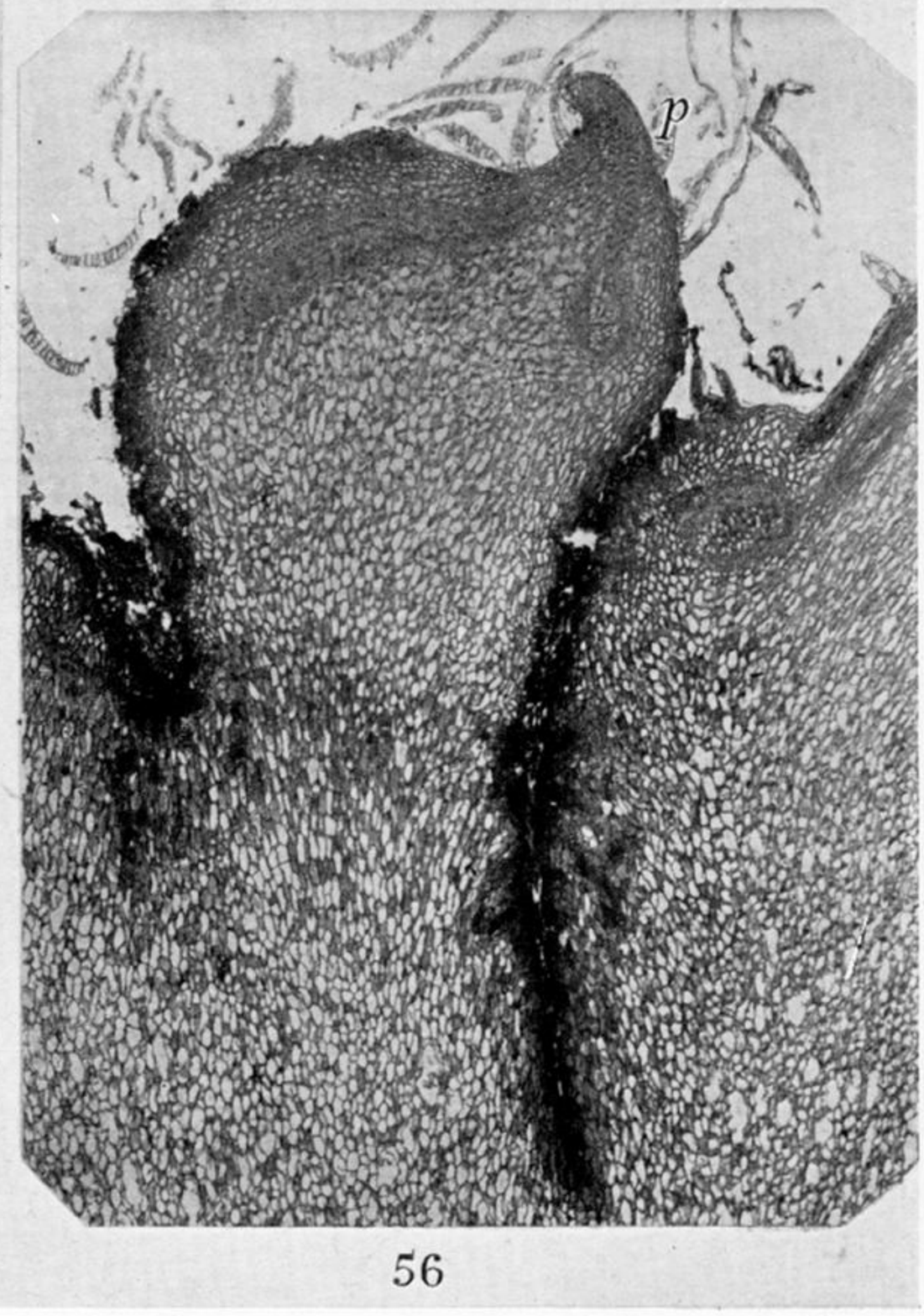

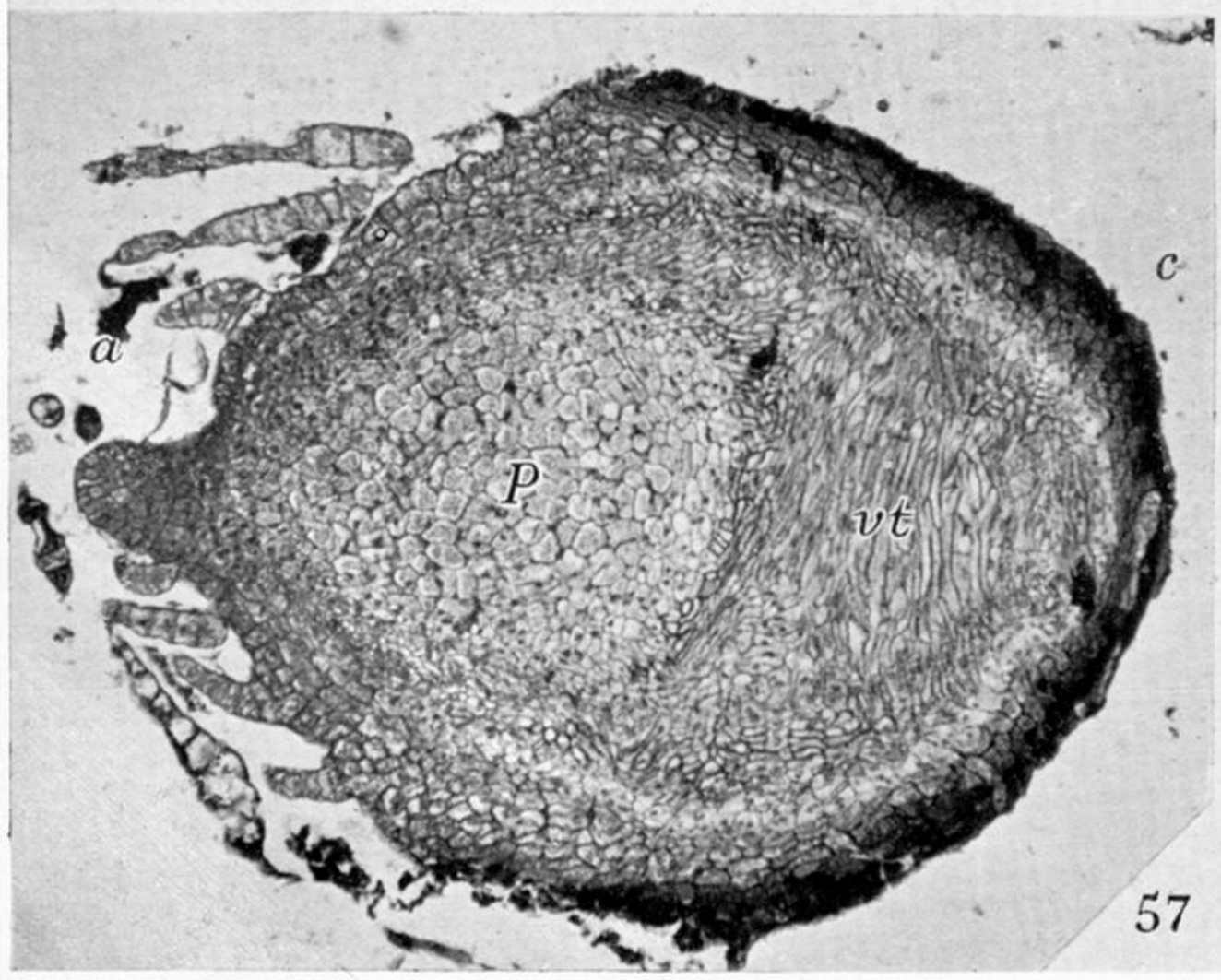

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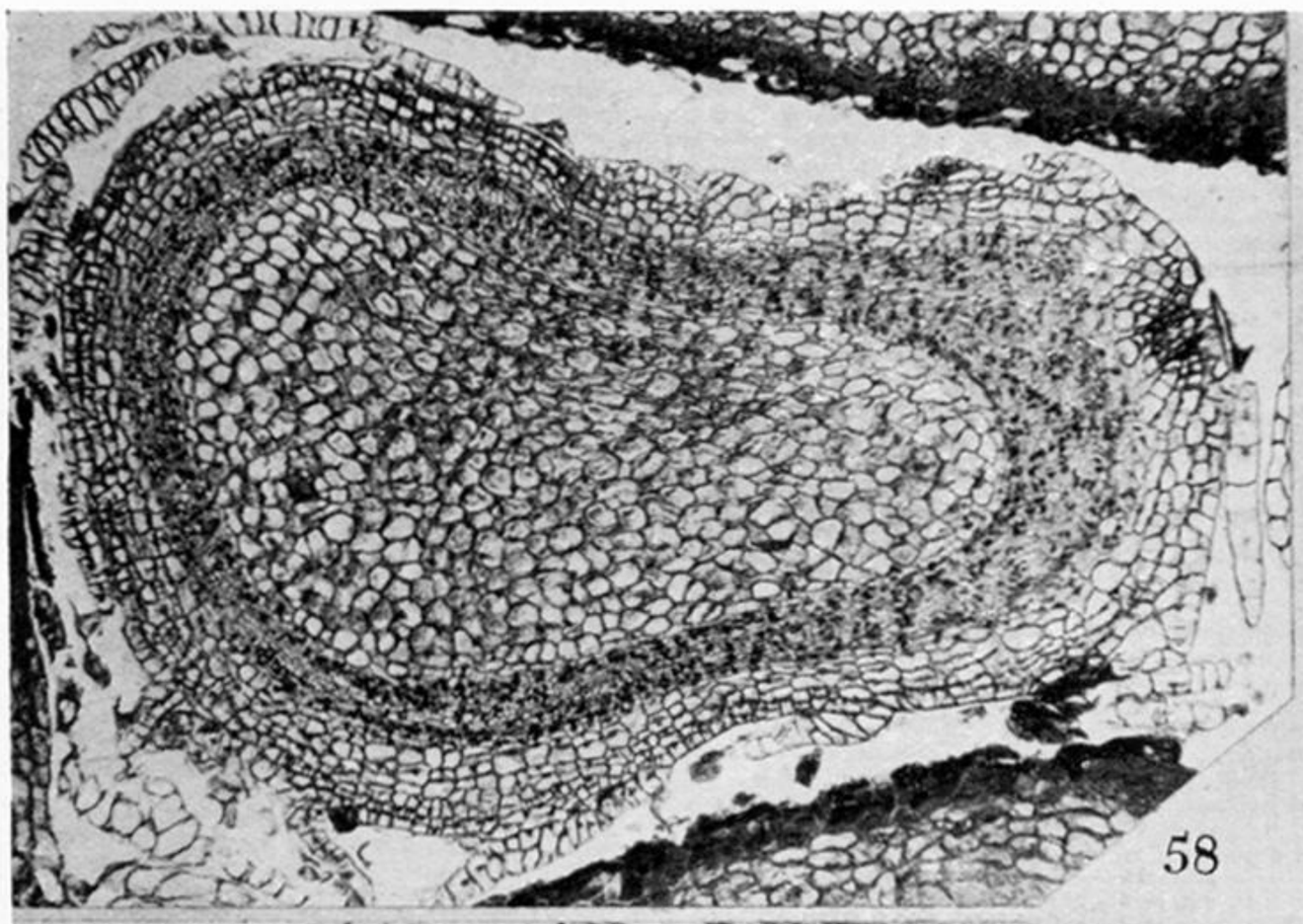

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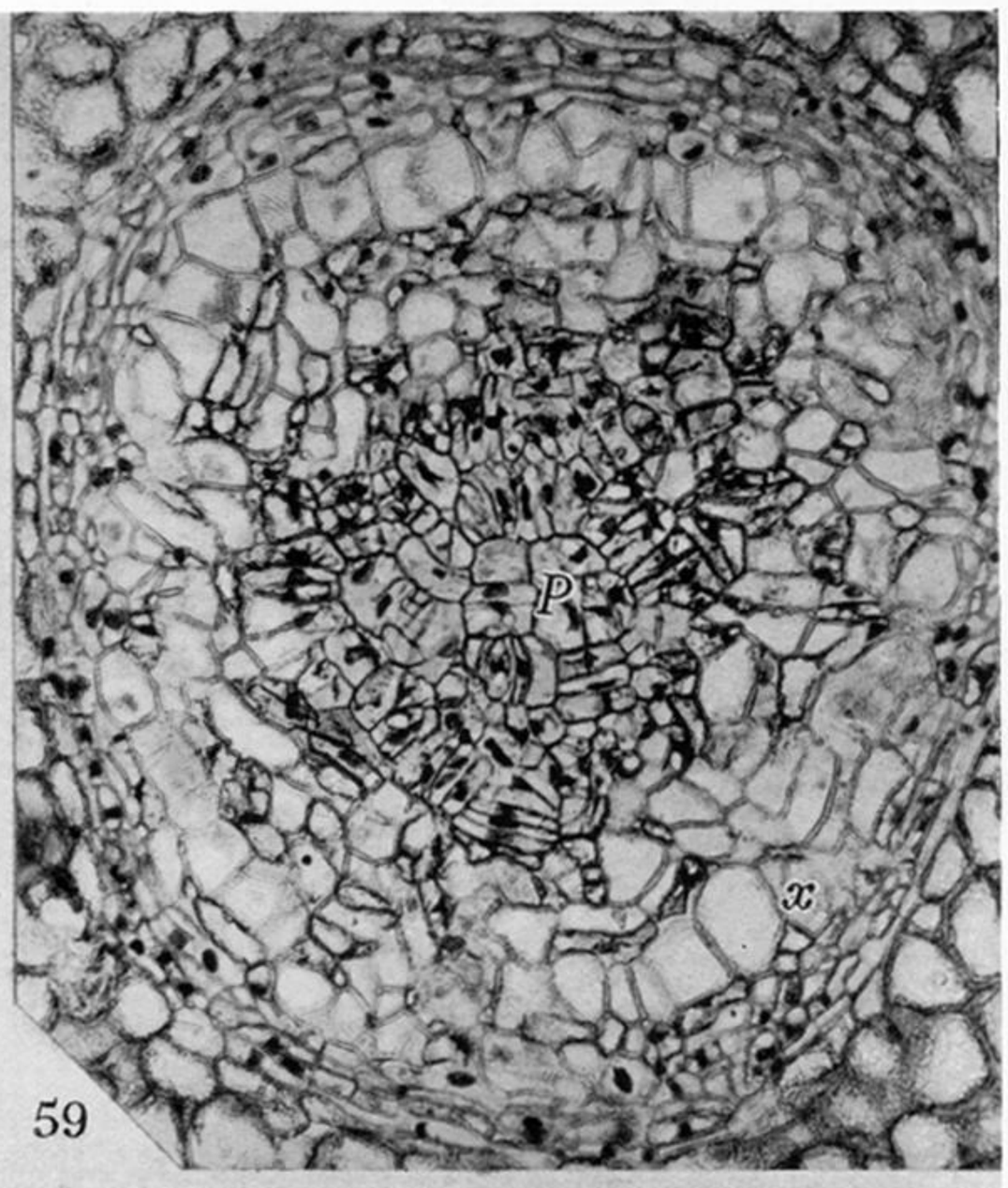

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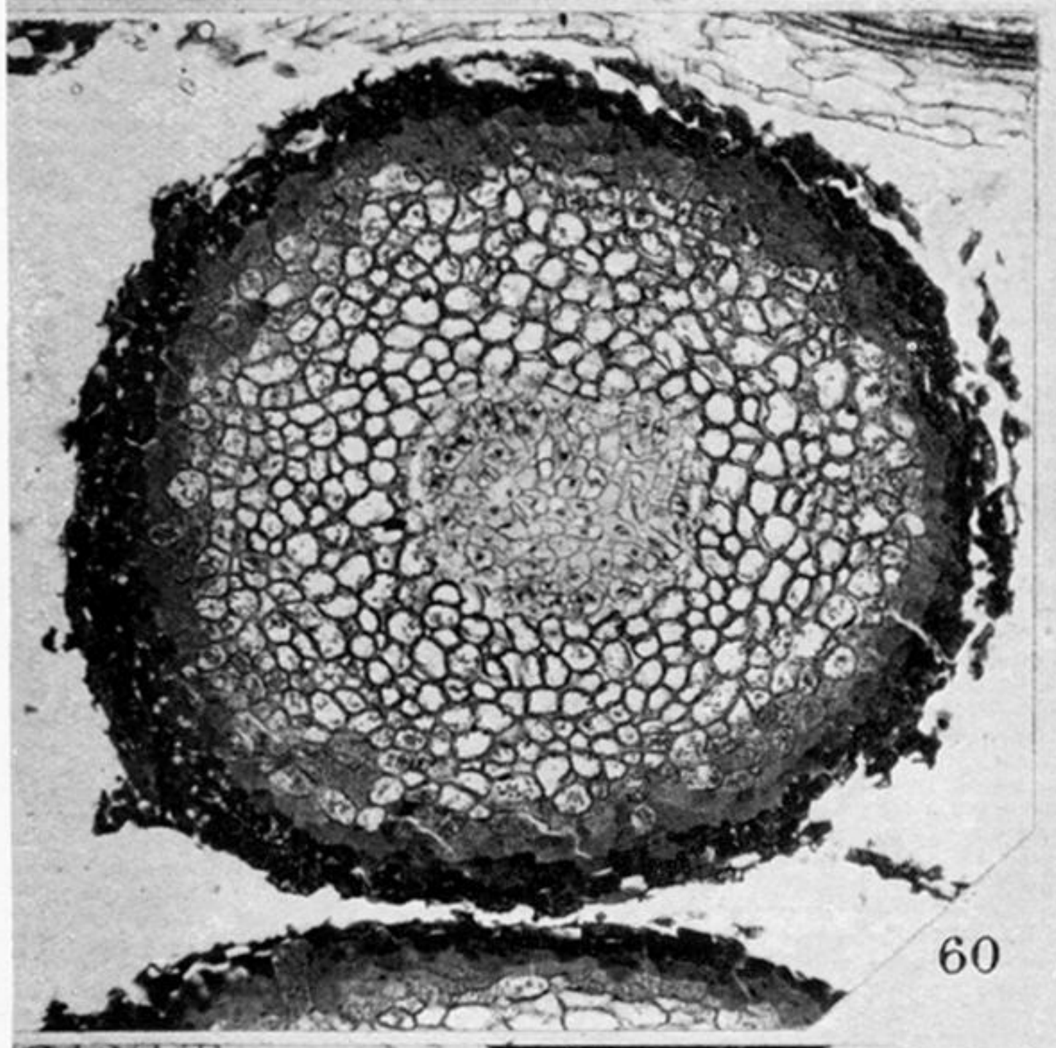

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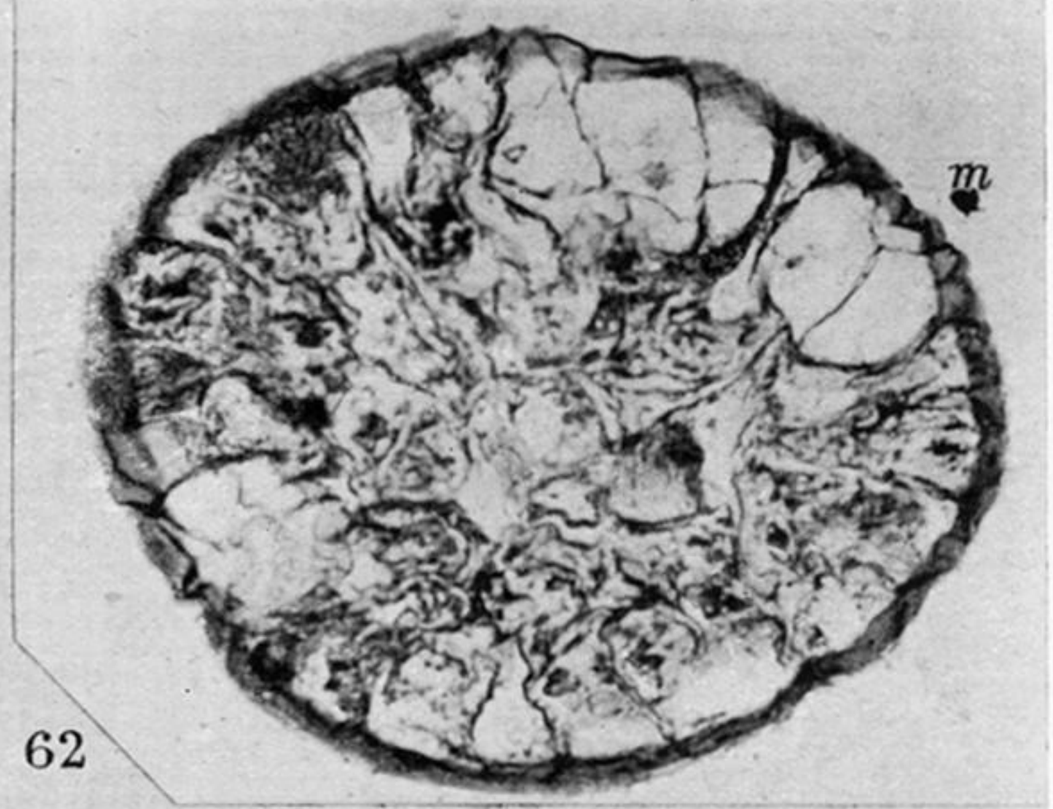

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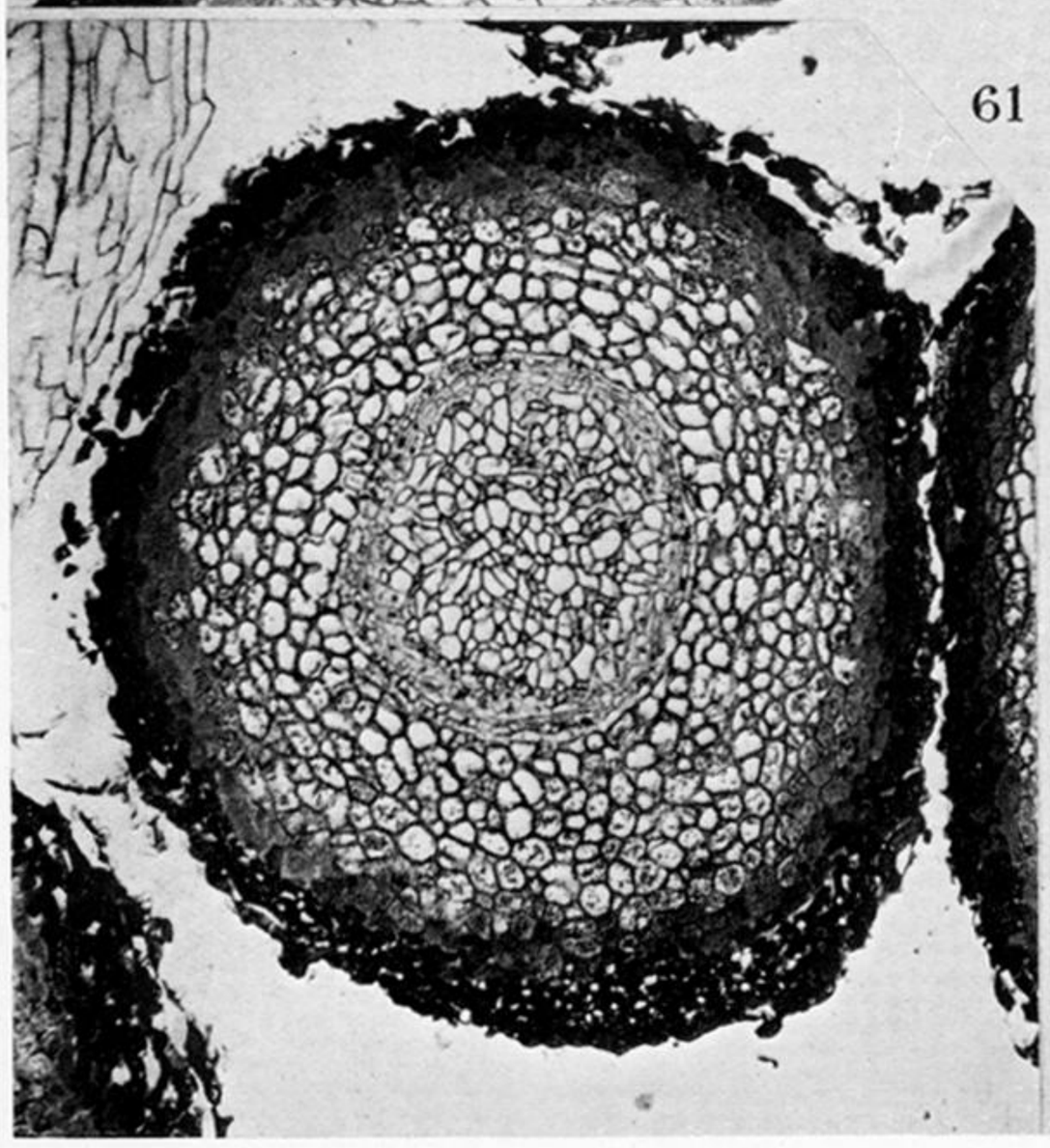

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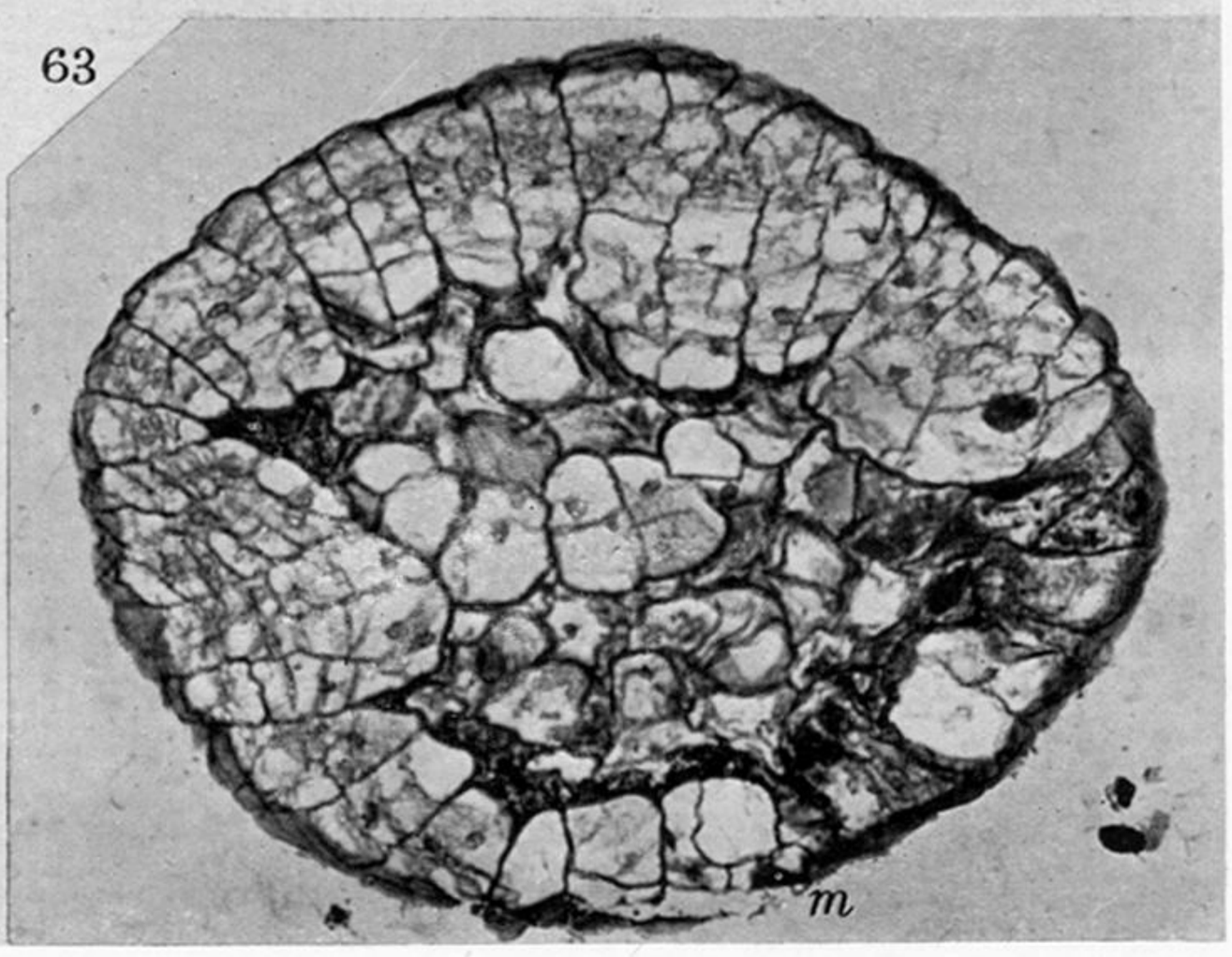

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PLATE 27

FIGURE 58. A rectangular solenostele, in an early stage of differentiation, formed below an apical meristem isolated on a rectangular panel of tissue. (× 50.)

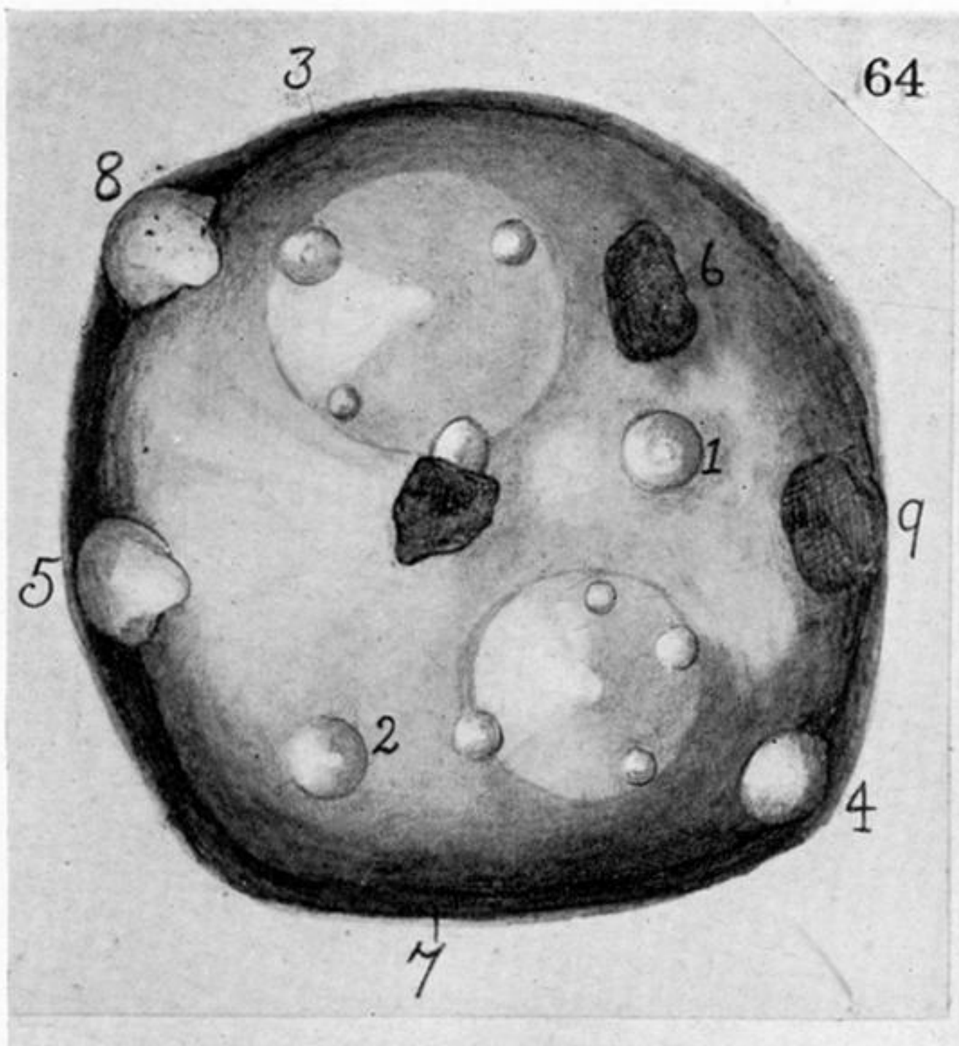
FIGURES 59 to 61. Transverse sections, at different levels, of a protostele which developed in an isolated terminal region.

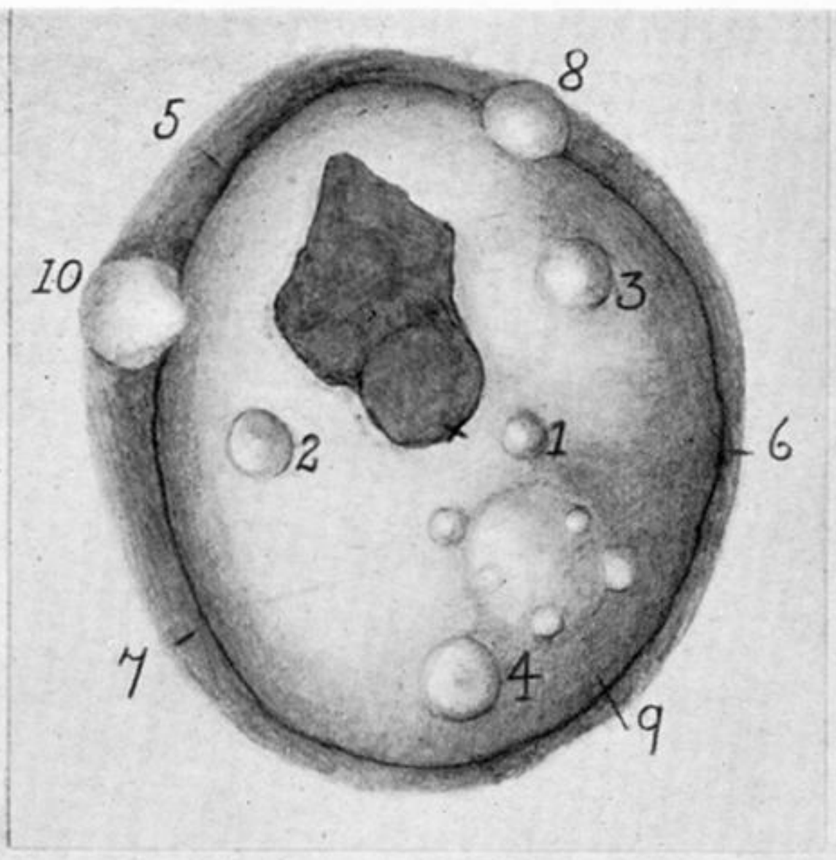
FIGURE 59. Near the base of the vascular column, the stele is a medullated protostele. *X*, xylem; *P*, pith. (× 150.)

FIGURE 60. Taken below the distal end of the shoot; the stele, which consists of small, thin-walled elements, is fading out. (× 50.)

FIGURE 61. At this level, higher up than figure 59 and below figure 60, the stele consists of a solid core of xylem, surrounded by thin-walled elements. (× 50.)

FIGURES 62, 63. Two transverse sections, at different levels, of an apical meristem in which the apical cell had been very lightly punctured. The illustrations show the extent of disturbance of tissues which has ensued. *m*, superficial prism-shaped cells of meristem. (× 180.)





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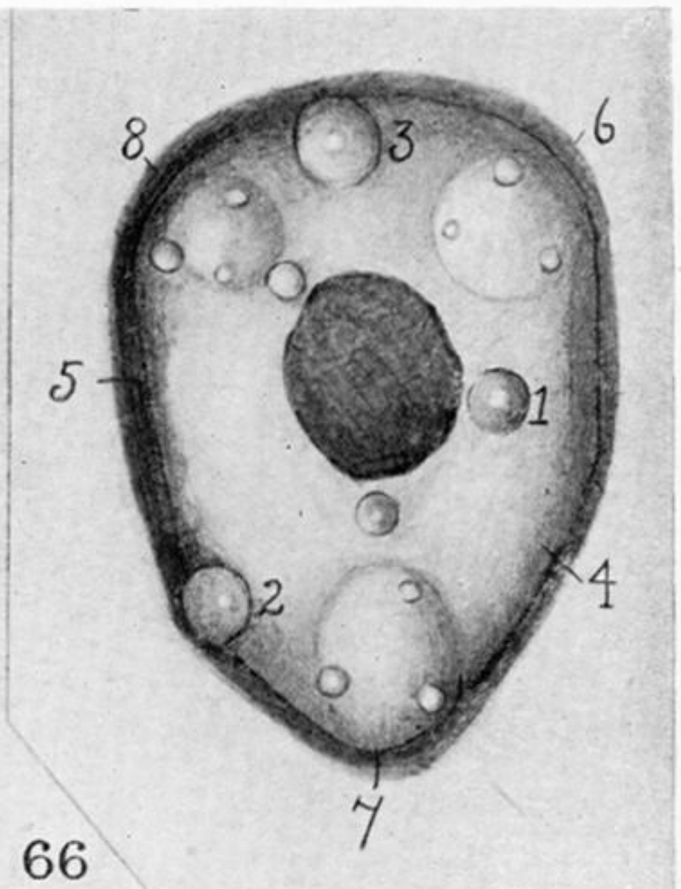

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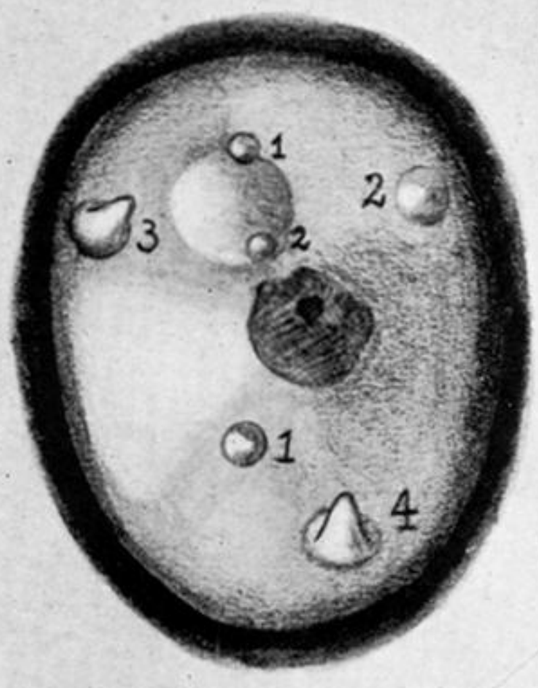

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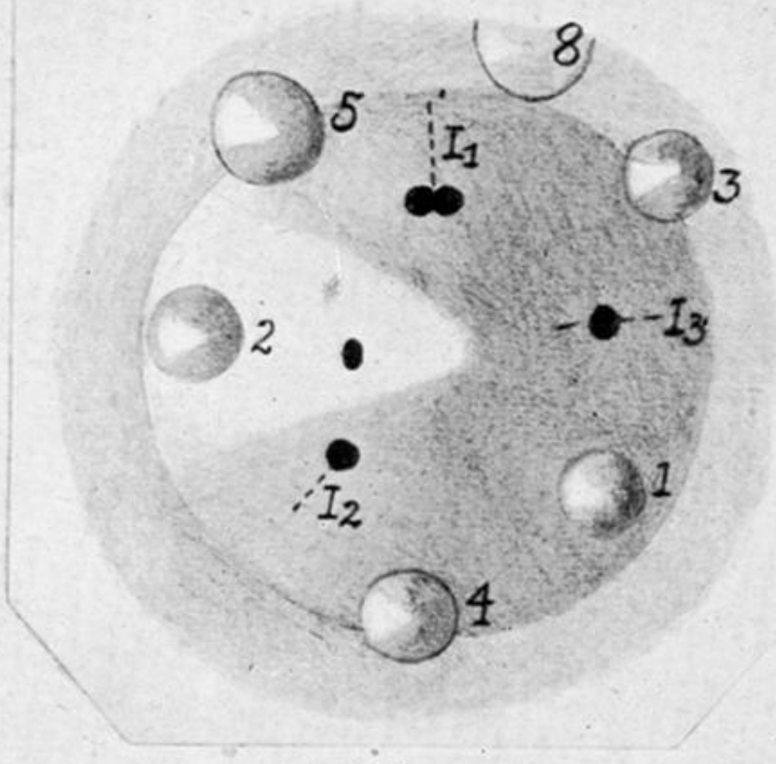

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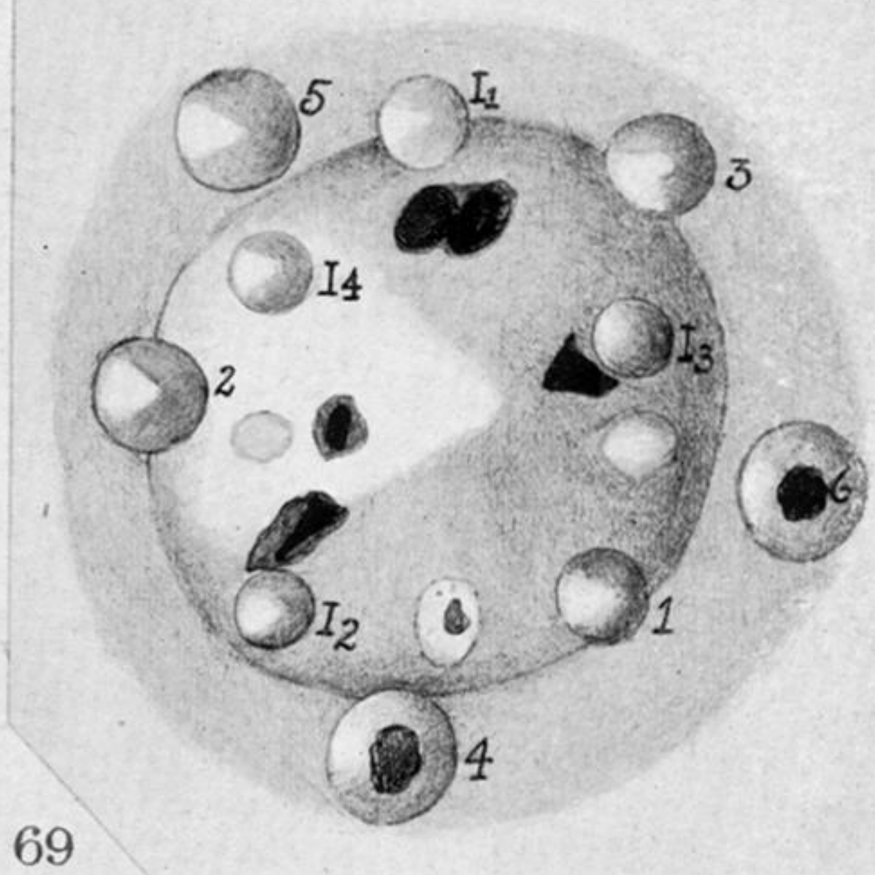

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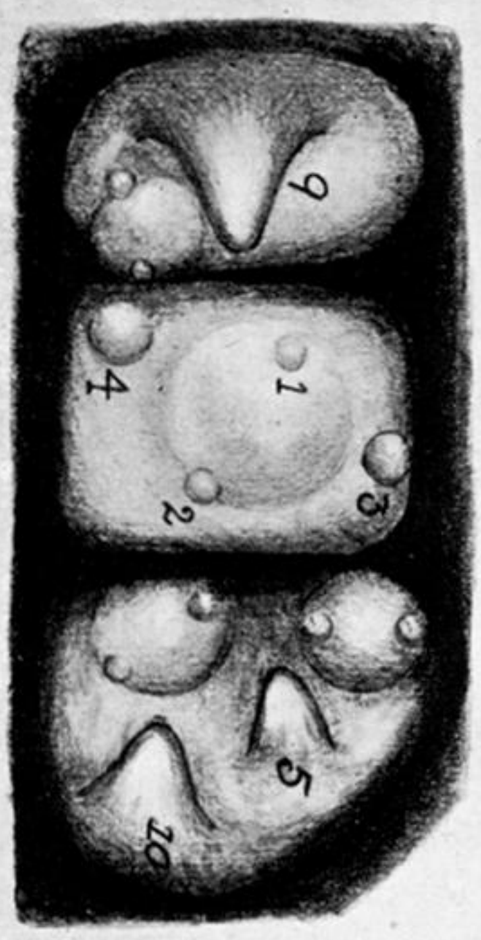

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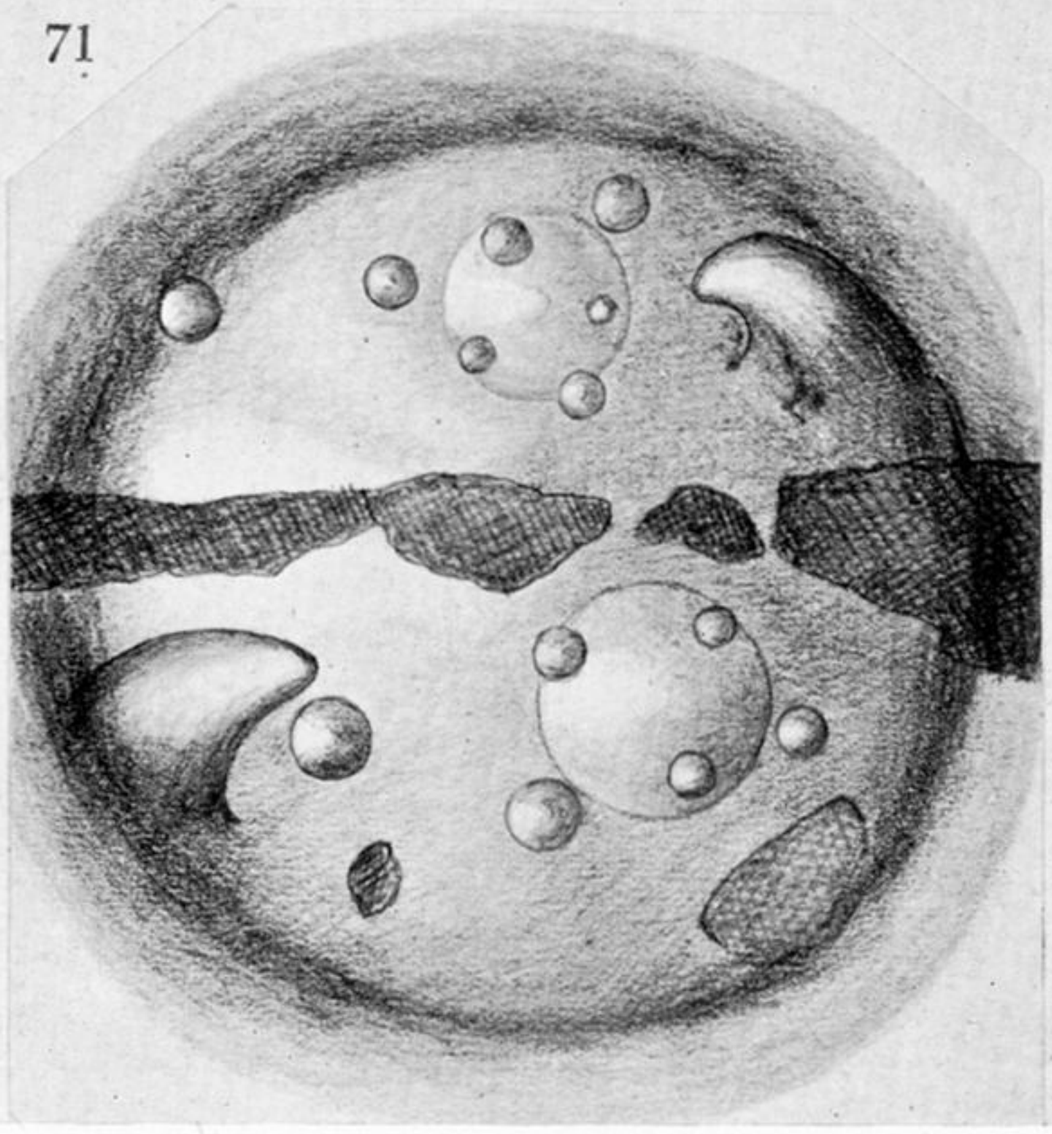

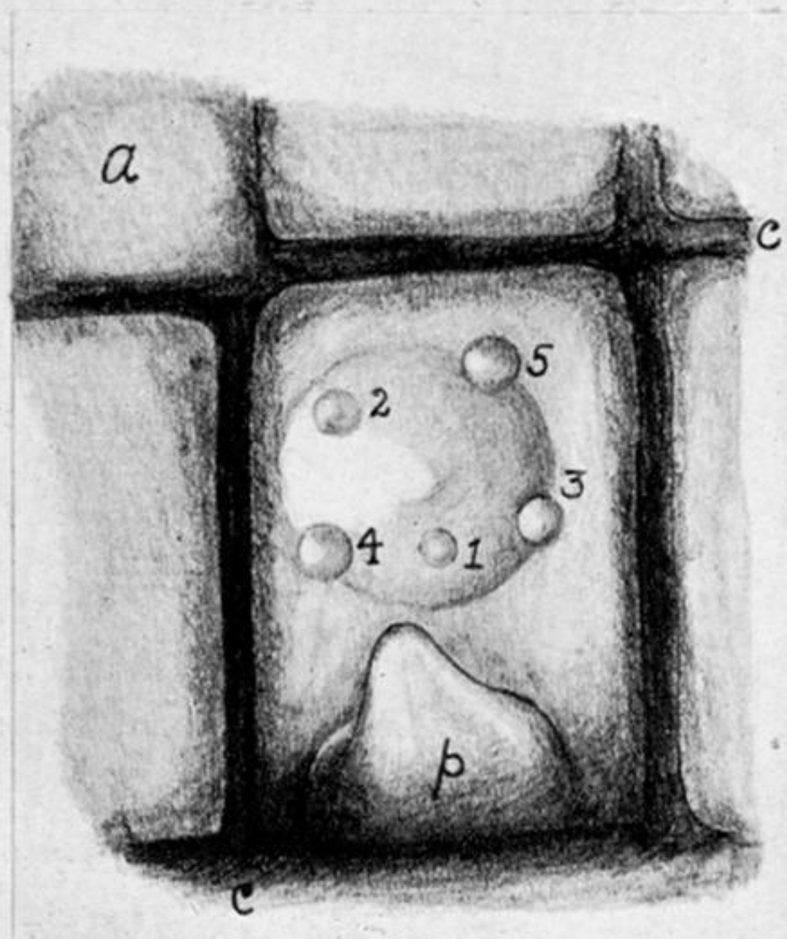

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PLATE 28

FIGURES 64 to 72. These are camera lucida drawings made by means of the Ultro Pak microscope. For detailed description, see text. (All × 25.)

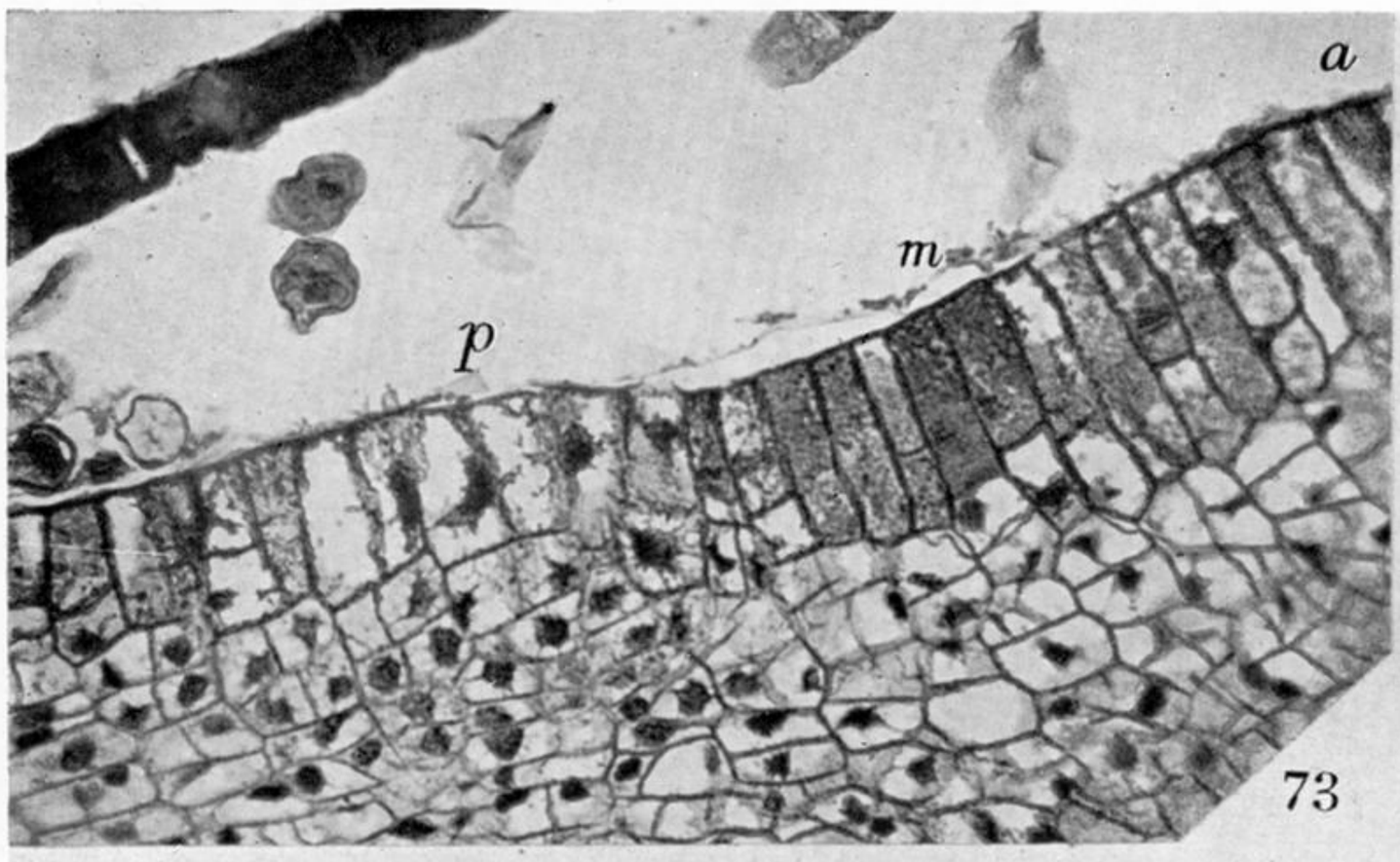
FIGURES 64 to 67. Formation of leaf primordia (1, 2, 3 etc.) and buds on the meristem after the apical cell (dark shading) had been punctured; the meristem had also been isolated by vertical incisions.

FIGURES 68, 69. The formation of leaf primordia I_1, I_2, I_3, when punctures had been made above these presumptive leaf positions; 1, 2, 3, etc., leaf primordia, in order of increasing age, present at beginning of experiment. The dark shading indicates the punctures.

FIGURE 70. Apical meristem and leaf primordia isolated on plugs of tissue by vertical incisions. 1, 2, 3, etc., leaf primordia in order of increasing age; buds have developed on the lateral plugs.

FIGURE 71. Apical meristem which was subjected to diametrically opposite radial longitudinal incisions: these have spread upwards and destroyed the apical cell. The older leaf primordia have continued to grow, small new ones have been formed, and two buds have arisen on the meristem.

FIGURE 72. An isolated leaf primordium with an induced bud in its axil; *a*, panel on which apex of parental shoot is situated.



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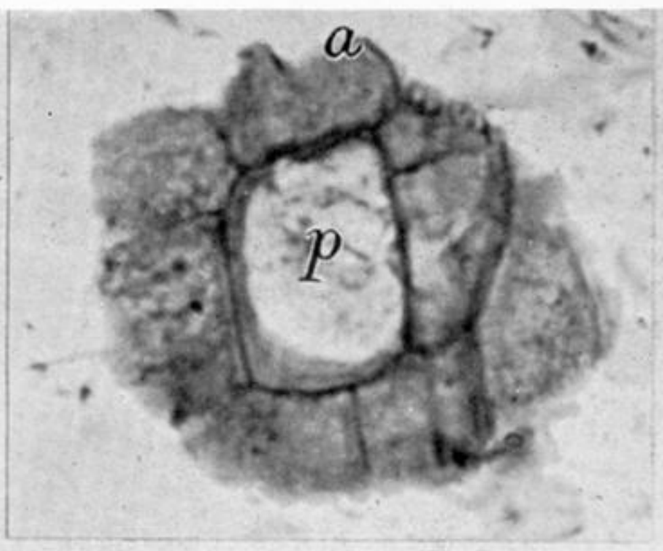

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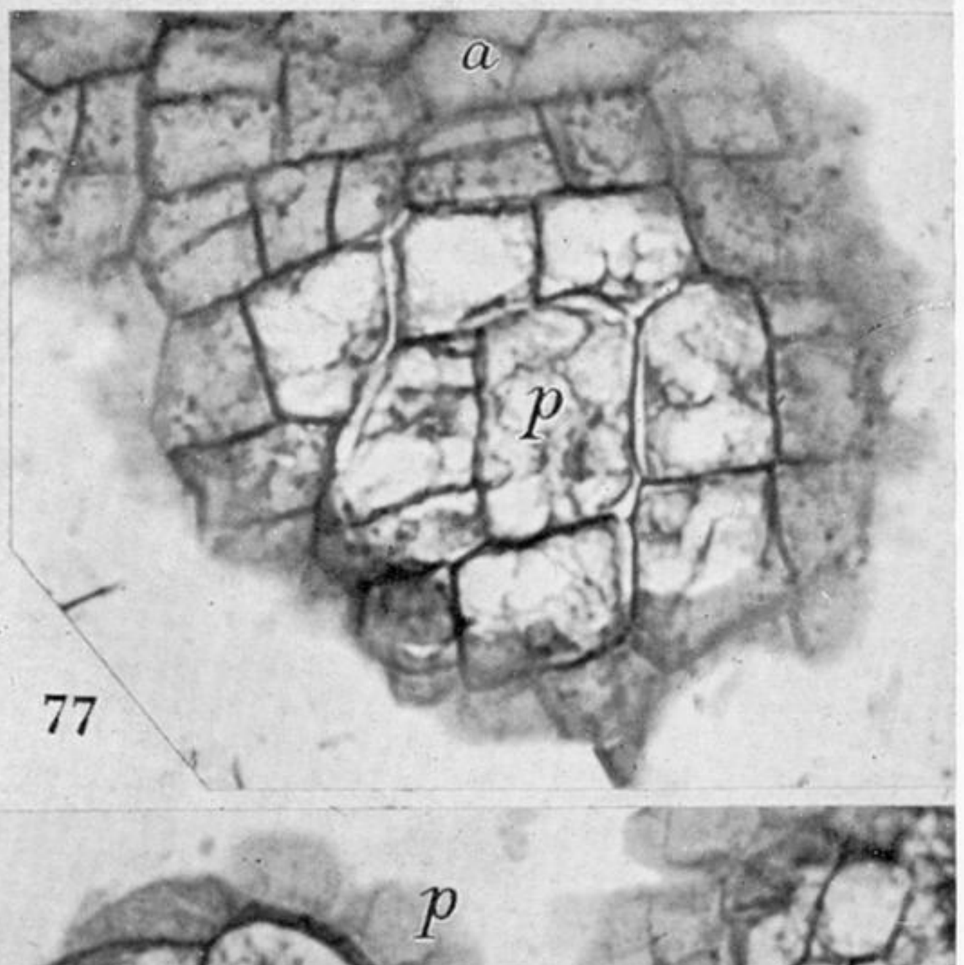

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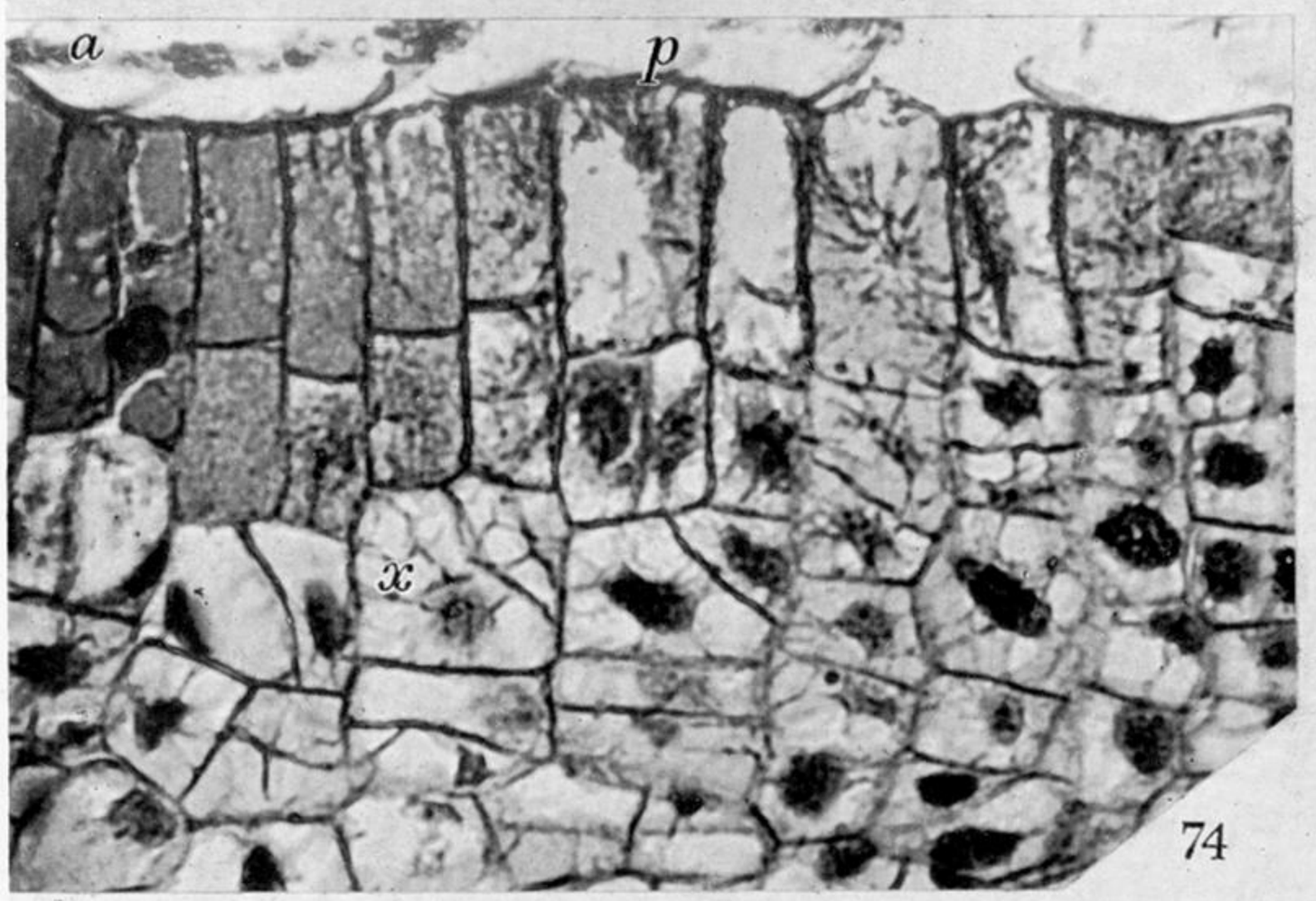

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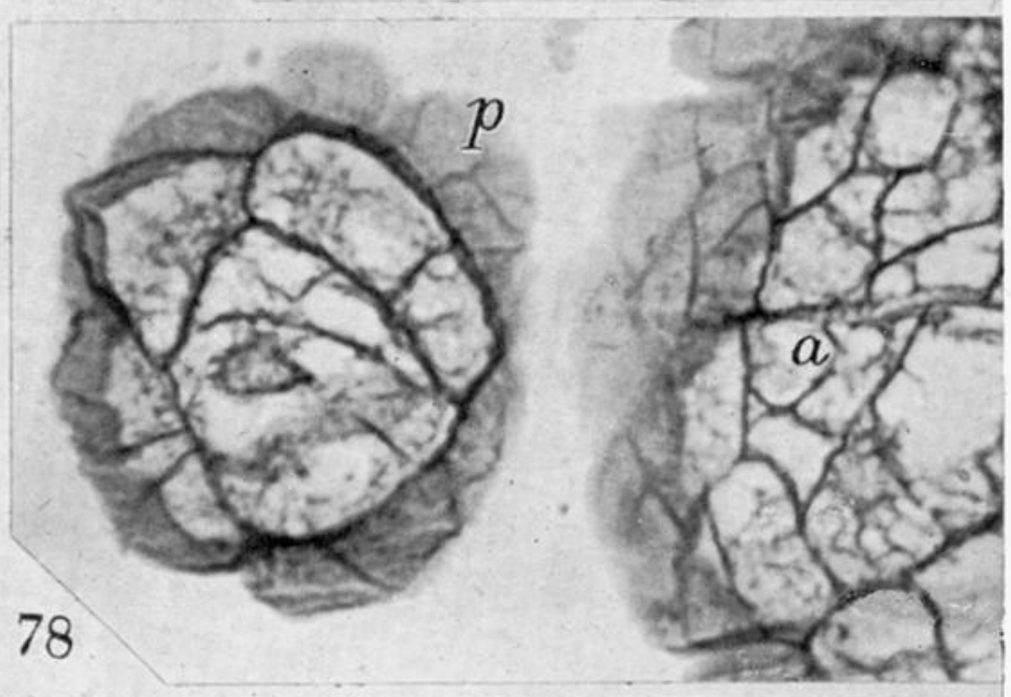

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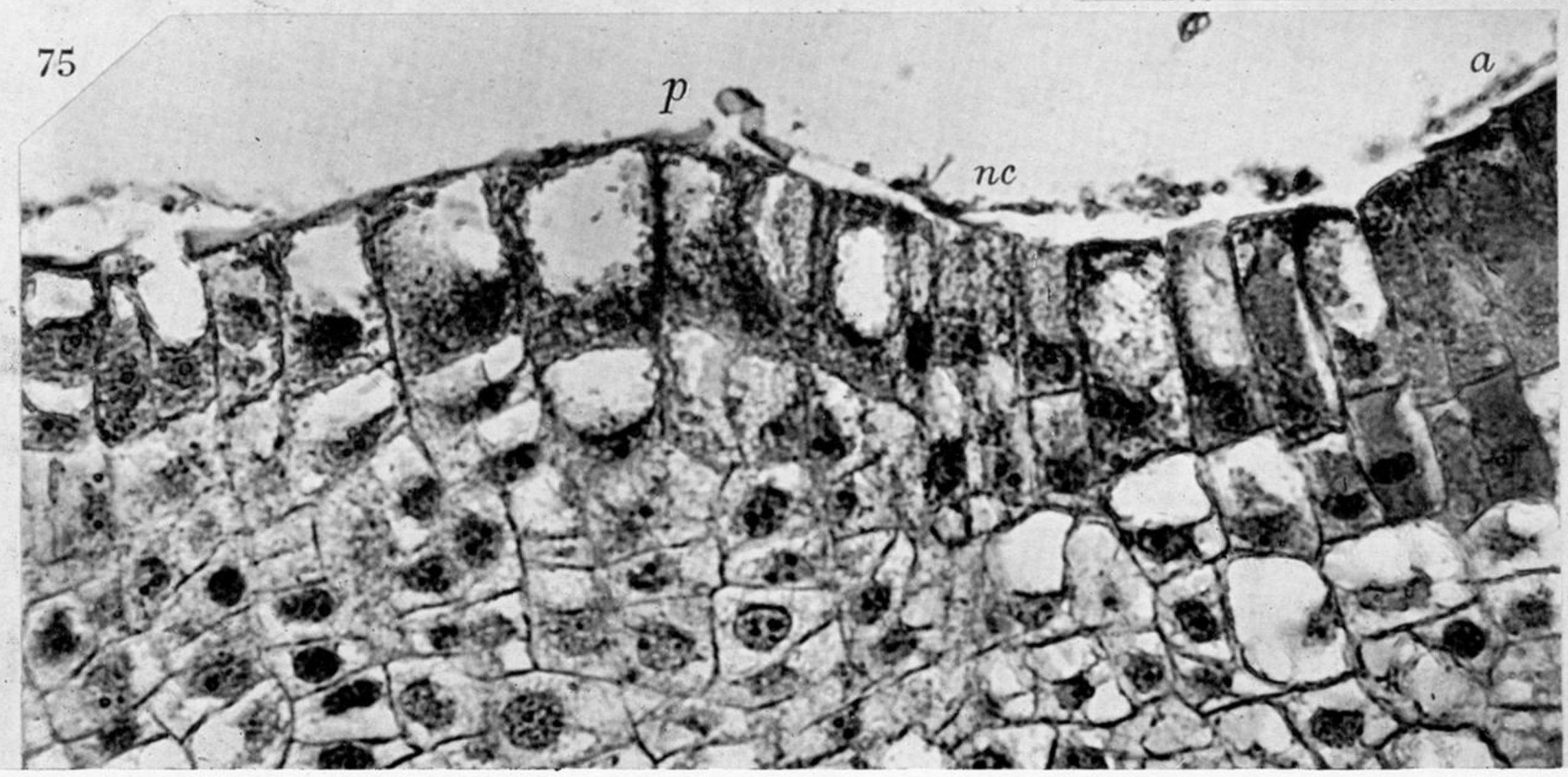

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PLATE 29

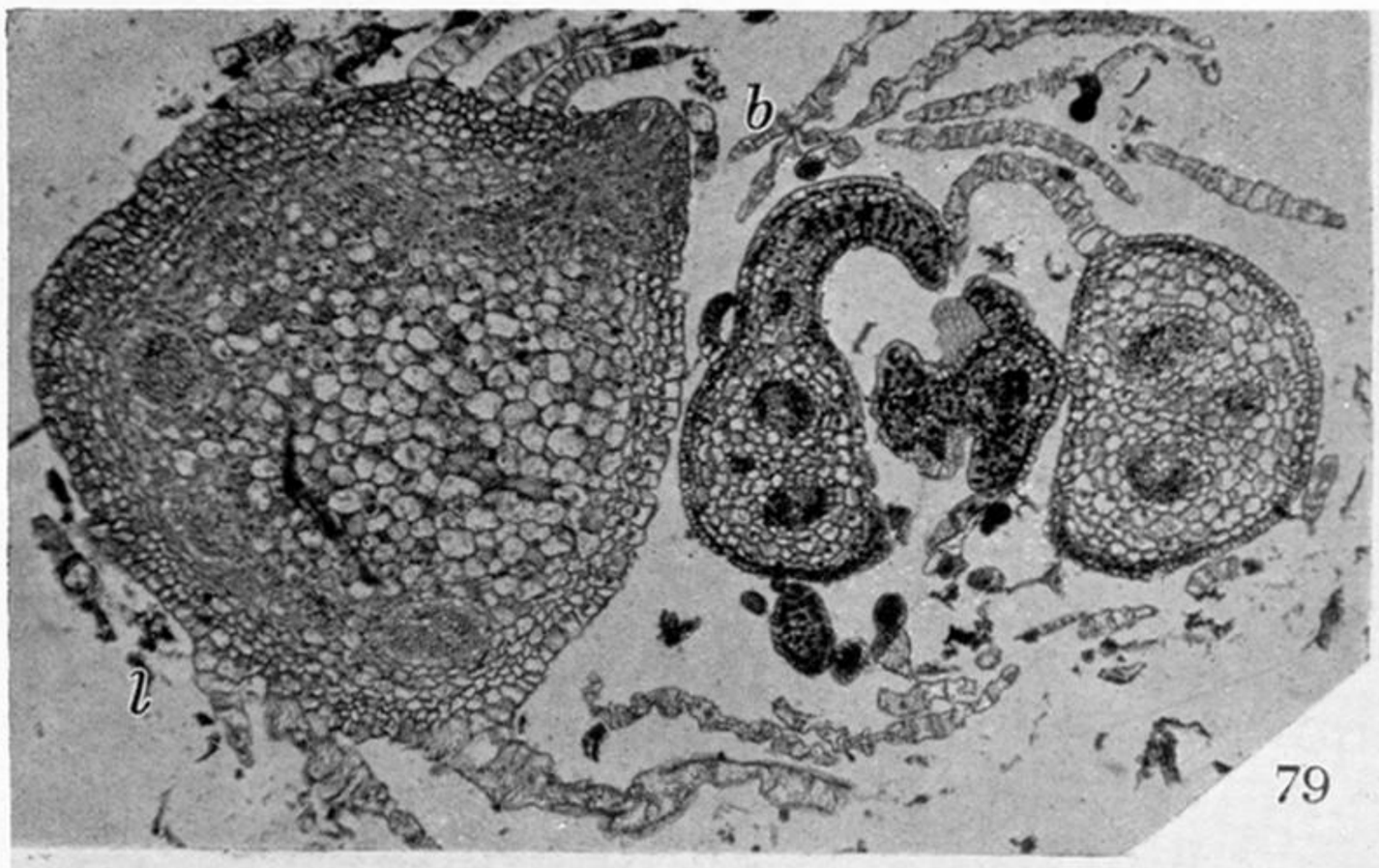
FIGURE 73. Longitudinal median section of a shoot apex, passing through a very young leaf primordium *p*, which consists of a group of prism-shaped meristematic cells; *m*, meristematic cells; *a*, direction of apical cell of shoot. (× 180.)

FIGURE 74. Longitudinal median section of apex, passing through a very young leaf primordium *p*; *a*, direction of apical cell; *x*, subepidermal cells which have just divided. (× 350.)

FIGURE 75. Another study of a very young leaf primordium *p*; *a*, direction of apical cell; *nc*, narrow cells of leaf axil. (× 350.)

FIGURES 76, 77. Two consecutive serial transverse sections of a very young leaf primordium *p*, showing that several prism-shaped, meristematic cells are involved. The clear cells *p* are those of the outgrowing primordium which have been cut through; the darker cells have not been cut through; *a*, direction of apical cell of shoot. (× 350.)

FIGURE 78. An older primordium *p* cut in transverse section, showing the 'two-sided' apical cell; *a*, direction of apical cell of shoot. (× 350.)



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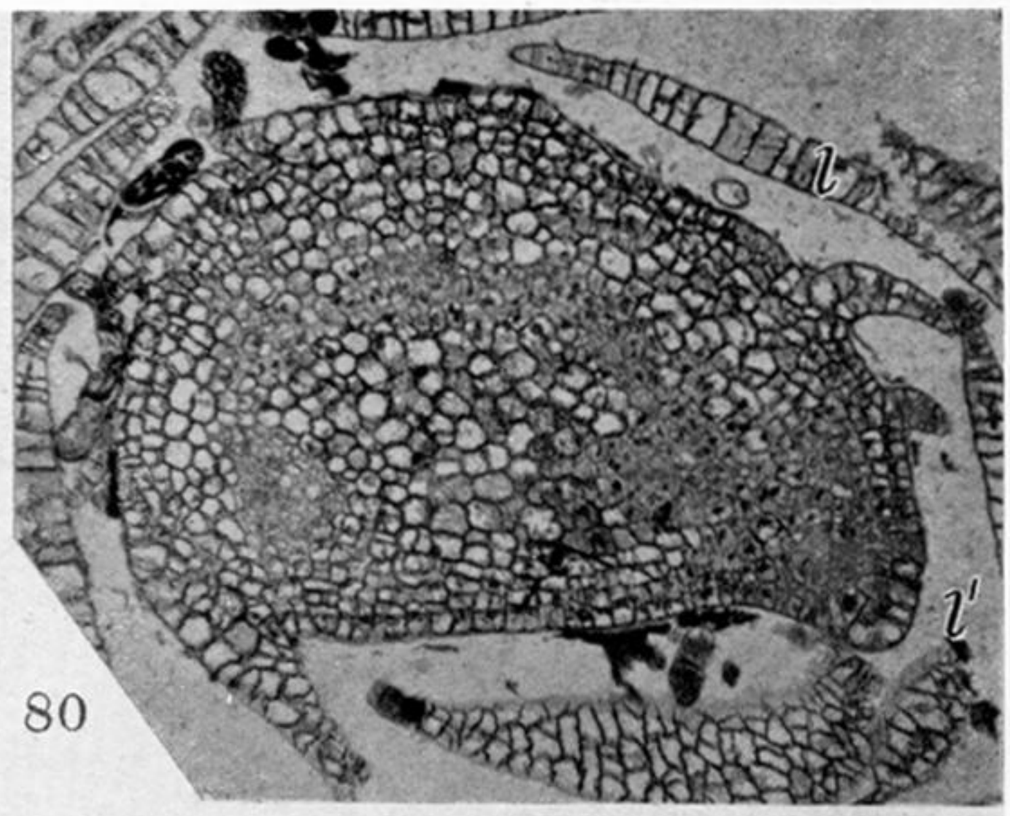

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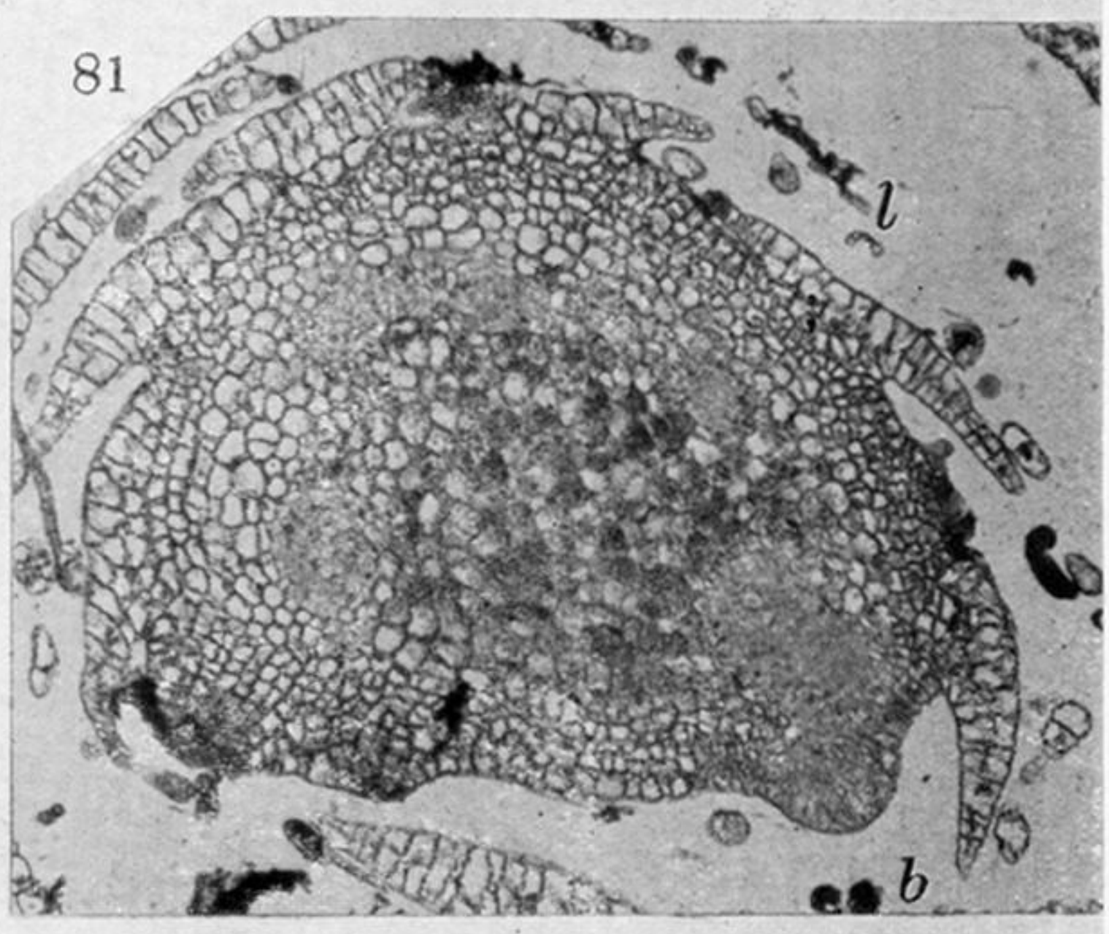

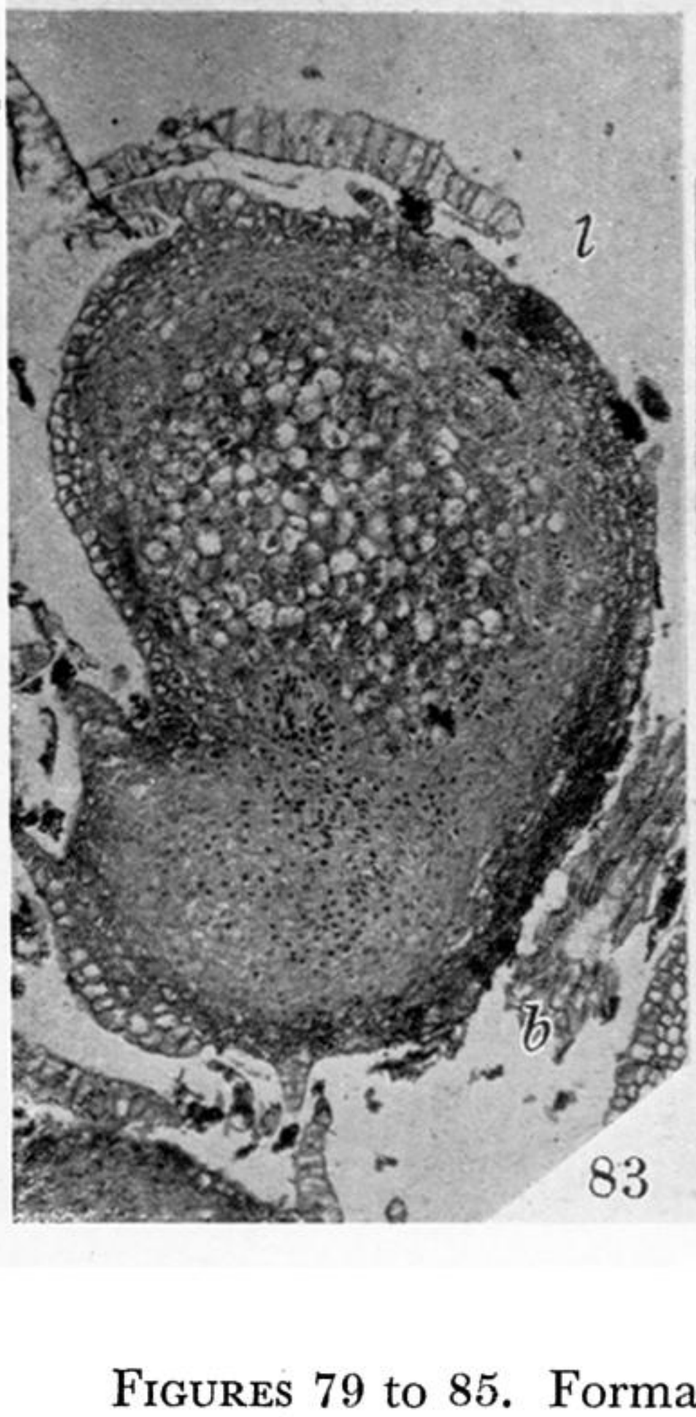

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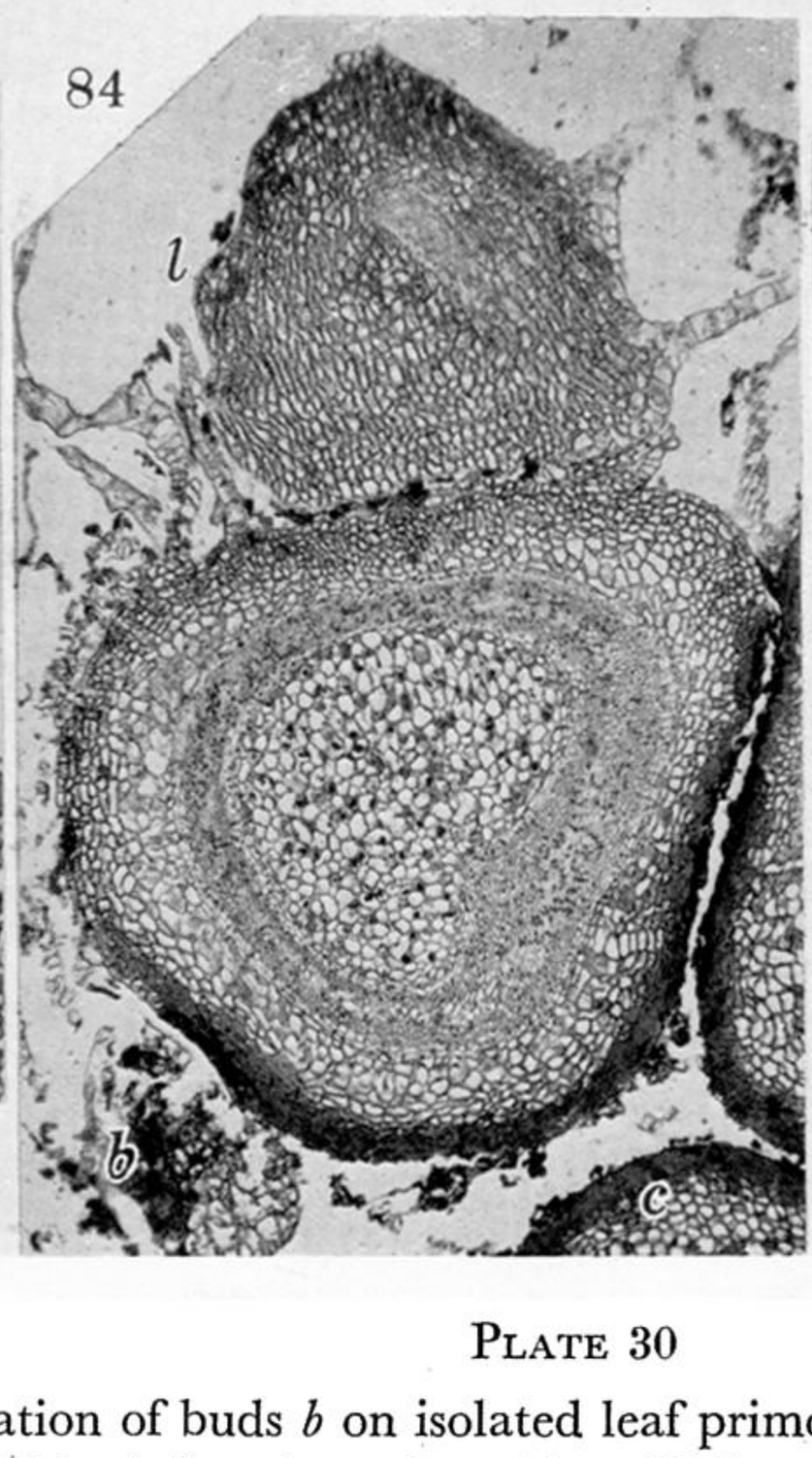

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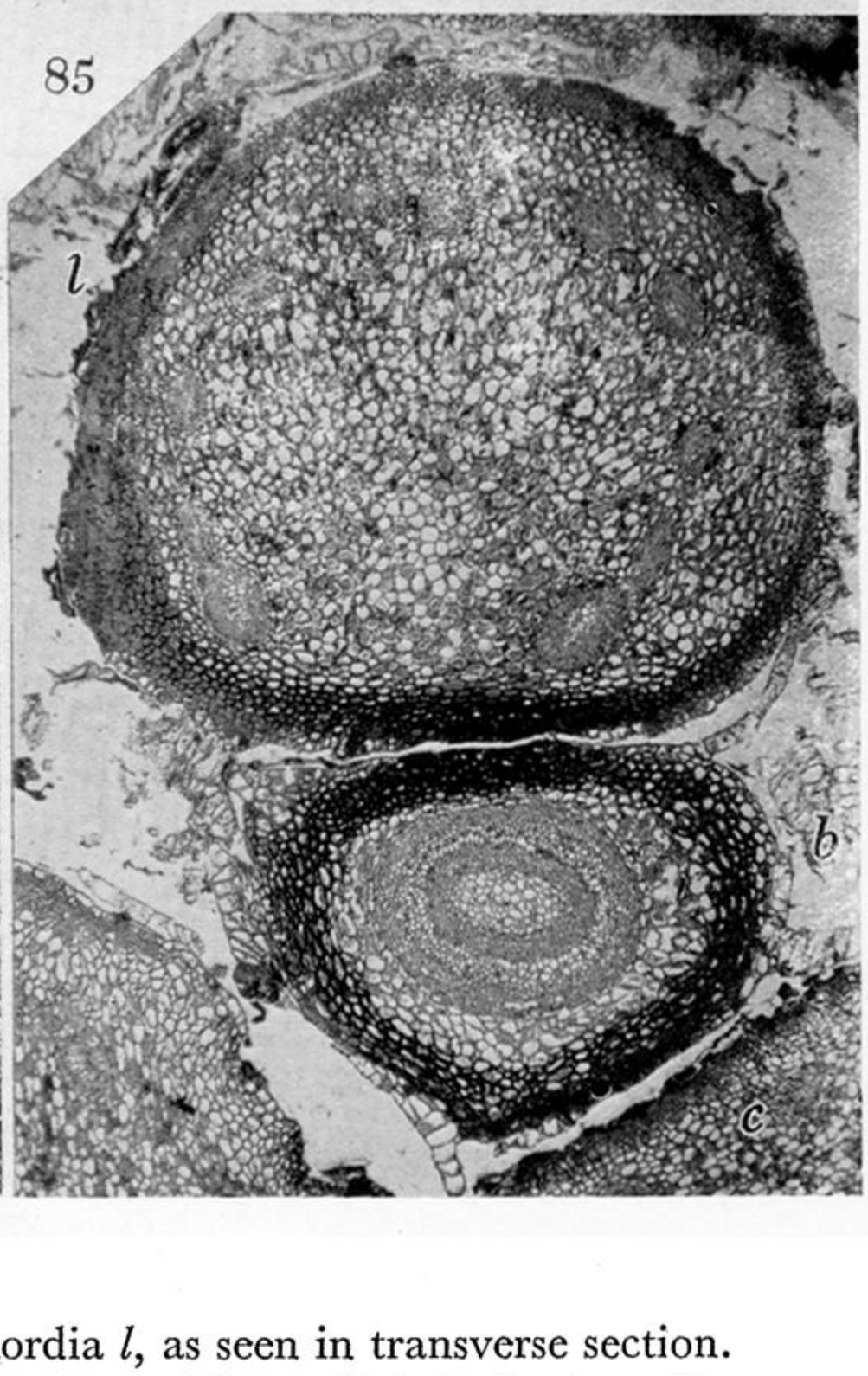

Plate 30

Figures 79 to 85. Formation of buds *b* on isolated leaf primordia *l*, as seen in transverse section.

Figure 79. Shows a small bud, in a lateral position, high up on an older, rolled, leaf primordium. (× 50.)

Figures 80, 81. Like figure 79, the bud has developed well up on the petiole of the primordium; l_1, leaf primordium of bud. (× 70.)

Figure 82. An axillary bud, showing a characteristic arrangement of vascular tissue at the level where the bud stele becomes conjoined with the two meristeles of the shoot (at the 'closing' of the leaf-gap). (× 20.)

Figure 83. A protostelic axillary bud. (× 30.)

Figures 84, 85. Two examples of large solenostelic axillary buds. (× 30.)

Figure 84. Illustrates, in transverse section, the typical awl-shaped leaf primordium, of limited growth, in which the vascular tissue gradually fades out; *c*, centre of shoot.