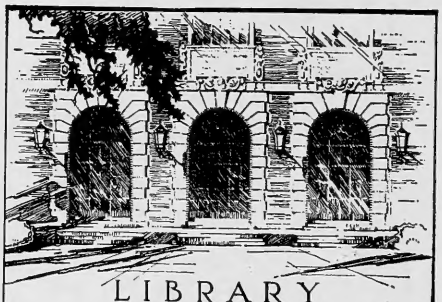

THE DEVELOPMENTAL ANATOMY OF *ISOETES*

DOMINICK J. PAOLILLO, JR.

ILLINOIS BIOLOGICAL MONOGRAPHS
31

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CONTENTS

INTRODUCTION 1

MATERIALS AND METHODS 2

THE GENERAL ORGANIZATION OF THE SPOROPHYTE 3

TERMINOLOGY 7

THE SHOOT 8

REVIEW OF THE LITERATURE 8

OBSERVATIONS AND DISCUSSION 15

SUMMARY AND CONCLUSIONS 51

THE ROOT-PRODUCING MERISTEM 52

REVIEW OF THE LITERATURE 52

OBSERVATIONS AND DISCUSSION 57

SUMMARY AND CONCLUSIONS 75

THE APICAL MERISTEM OF THE ROOT 76

REVIEW OF THE LITERATURE 76

OBSERVATIONS AND DISCUSSION 78

SUMMARY AND CONCLUSIONS 82

GENERAL SUMMARY AND CONCLUSIONS 82

LITERATURE CITED 84

PLATES 89

INDEX 129

INTRODUCTION

In *Isoetes,* the relationships of some of the plant parts are unique, and the structure of the sporophyte is difficult to interpret and explain. One of the best ways to secure an understanding of this structure is to compare plants of different ages to determine the relationships of the component parts throughout ontogeny. Sporophytes from sporeling to adult stages have been examined in this study. Observations are recorded on the growth of the shoot tip, lateral meristem, and root-producing meristem, and on procambial differentiation, root initiation, and the growth of the apical meristem of the root. To enhance the continuity of the report, the review of the literature has been divided into three parts. The pertinent original observations follow each section of the literature review in sequence.

This study was completed at the University of California at Davis during the tenure of a National Science Foundation Predoctoral Fellowship. I wish to express my sincere thanks to Dr. E. M. Gifford, Jr., for guidance throughout the course of this investigation. Thanks are also due to Dr. K. Esau and Dr. L. K. Mann for critical review of the original manuscript and suggested improvements on the original draft.

Mr. W. Russell assisted me in locating populations of *Isoetes howellii* and *I. nuttallii.* Dr. S. C. Tucker supplied me with several specimens of *I. braunii.* Dr. E. M. Gifford, Jr., placed the departmental collection of slides of *I. howellii* at my disposal. I am indebted to Dr. H. B. Currier for the use of his Ortholux microscope for fluorescence microscopy and to Mr. H. B. Tepper for assistance with staining procedures.

MATERIALS AND METHODS

Isoetes howellii Engelm., *I. nuttallii* A. Br., and *I. braunii* Dur. were examined in this investigation. *I. howellii* and *I. nuttallii* were obtained from vernal pools in Lake Co., Calif. *I. nuttallii* was also collected from a moist sod over granite rock in El Dorado Co., Calif. Several specimens of *I. braunii,* collected under one foot of water at Deming Lake, Minn., were given to me by Dr. S. C. Tucker. *I. howellii* and *I. nuttallii* were collected near the beginning of the growing season (Feb. and March). Young and old plants were obtained in the field. All of the plants collected showed signs of new vegetative growth. Some plants were fixed directly after collection, whereas others were fixed after they were transplanted and grown for one month in the greenhouse in sand cultures inundated with half-strength Hoagland's solution. Sporelings of *I. howellii* were obtained from spores that were sown in distilled water, tap water, and half-strength Hoagland's solution in Syracuse watch glasses.

Specimens were fixed in Craf III (Sass, 1958, p. 18), in Regaud's formaldehyde-dichromate mixture (Conn, Darrow, and Emmel, 1960, p. 14) with eight days of postchroming, and in Formalin-Aceto-Alcohol (Conn *et al.,* 1960, p. 7) prepared with 50 per cent ethanol. Fixation times and washing were according to the recommendations given in the references consulted for formulae. Materials were dehydrated with a normal butyl-ethanol series, infiltrated with Fisher tissuemat, and sectioned serially at 7-12 μ. The principal combinations of stains used for morphological studies were hematoxylin-safranine-fast green and chlorazol black-acid fuchsin-malachite green-martius yellow. (Details of these schedules are contained in the dissertation on which this report is based. This dissertation is filed in the University of California Library, Davis.)

Other staining schedules used were as follows: Regaud's hematoxylin (Conn *et al.,* 1960, p. 213); Heidenhain's hematoxylin (Conn *et al.,* 1960, p. 182, using acidified iron-alum as the mordant, and using Johansen's, 1940, p. 50, mixture of the dye); tannic acid-iron chloride-safranine (Foster, 1934, with the addition of fast green in clove oil); tannic acid-iron chloride-lacmoid (Cheadle, Gifford, and Esau, 1953); aniline blue fluorescence (Currier and Strugger, 1956); mercuric bromphenol blue (Mazia, Brewer, and Alfert, 1953); periodic acid-Schiff's reagent (Glick, 1949, p. 44); aqueous pyronine Y (Tepper and Gifford, 1962); and the Fuelgen reaction (Johansen, 1940, pp. 95-97).

Most of the specimens examined were *I. howellii.* All of the developmental investigations were limited to this species. Examination of the

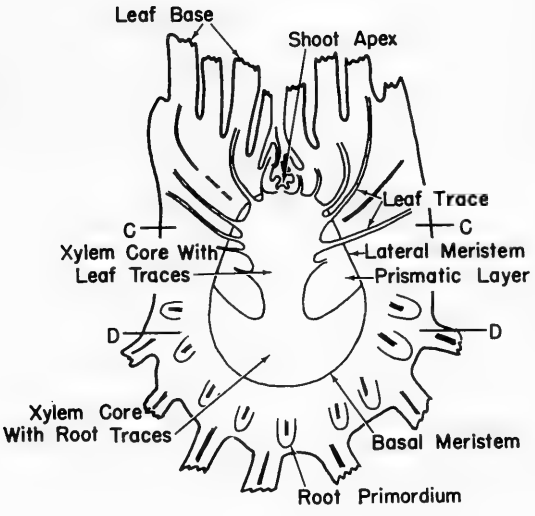
other two species served to support and modify some of the concepts developed. Both *I. howellii* and *I. braunii* are typically two-lobed. *I. nuttallii* is typically three-lobed. For two-lobed specimens, longitudinal sections were made parallel and perpendicular to the basal groove, i.e., between and across the two lobes, respectively.

THE GENERAL ORGANIZATION OF THE SPOROPHYTE

The principal planes of sectioning for two-lobed specimens are illustrated in figure 1. This figure indicates the locations of the meristematic tissues in relation to the whole plant. Figure 1A represents the sporophyte as it is seen in a median longitudinal section in the plane passing through the basal furrow. In the text, this plane of sectioning is referred to as the *plane of the furrow* or the *furrow plane.* The shoot apex is at the bottom of a conical depression. Vertical expansion of the cortex raises the bases of the leaves above the level of the shoot apex. The portion of the xylem core that bears the leaf traces is obconical, whereas the portion that bears the root traces is convex on the lower perimeter and forms a "horn" at each side. The outline of the xylem core of the whole stele has been compared to that of a garden edging-tool, an anchor, and a vegetable chopper (Foster and Gifford, 1959, p. 172). The cambium is composed of two parts: the *lateral meristem* and the *basal meristem.* The lateral meristem produces the so-called *prismatic layer* toward the inside. This layer obtains its name from the prismatic form of its cells and is also called the *secondary vascular tissue.* The basal meristem augments the root-bearing portion of the stele and produces the surrounding ground tissue, in which the root primordia are organized. A median section in the furrow plane does not show any root primordium attached to the basal meristem.

Figure 1B shows the appearance of a median longitudinal section at right angles to the furrow plane. Both lobes of the plant are represented in the figure. Again the shoot apex appears at the base of the conical depression. The shape of the portion of the xylem core that bears leaf traces is the same as in figure 1A, but the shape of the portion that bears root traces is different. This difference indicates that the root-bearing portion of the stele is flattened in the plane of the basal furrow. The root traces are arranged in an *orthostichy* and obscure the relationship between the basal and lateral meristems.

Figure 1C is a transverse section taken at a level through the broadest



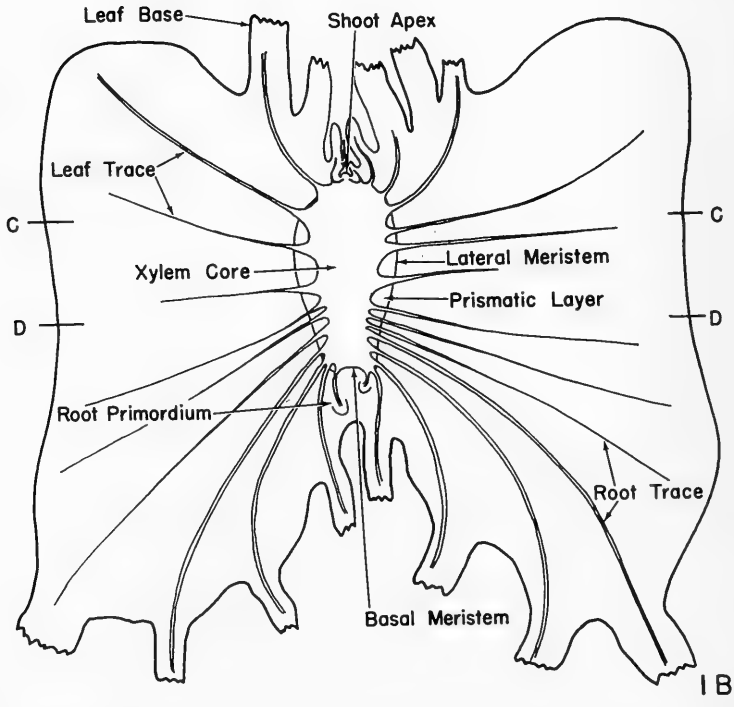
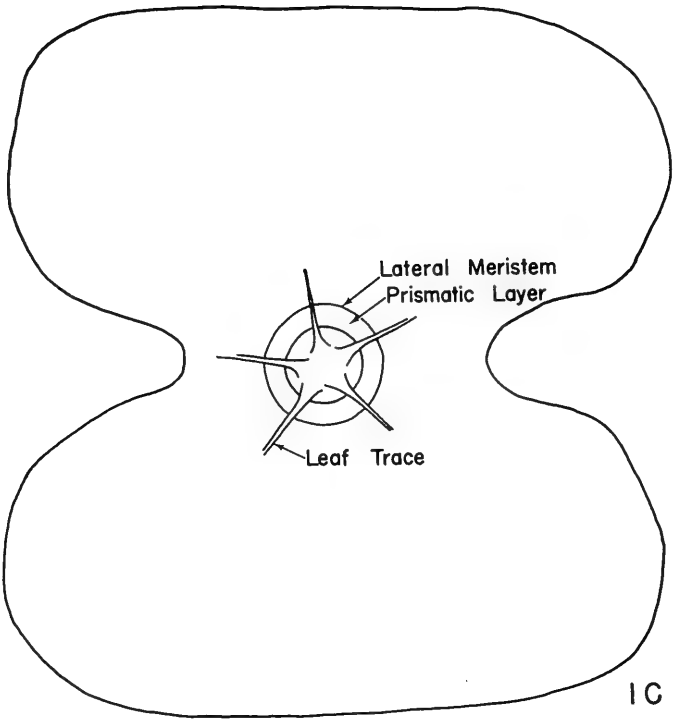
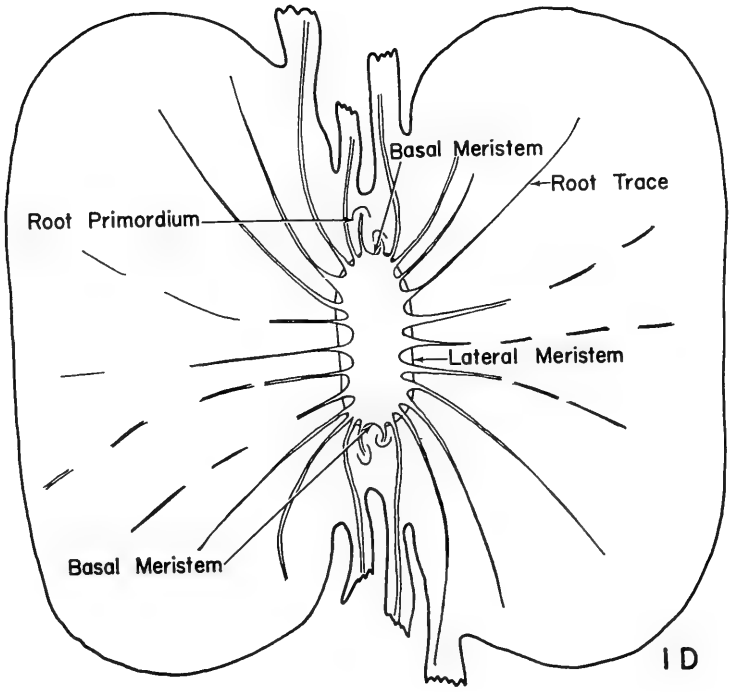


FIG. 1. The principal planes of sectioning for two-lobed plants. A. Furrow plane. B. Plane perpendicular to the furrow. C. Transverse at high level (C-C, in A and B). D. Transverse at low level (D-D, in A and B). Explanation is in text. Schematic.


Lateral Meristem
Prismatic Layer
Leaf Trace

1C


Basal Meristem
Root Trace
Root Primordium
Lateral Meristem
Basal Meristem

1D

part of the leaf-bearing portion of the stele (level C-C, in A and B). The figure is arranged with the basal furrow running from left to right for comparison with figure 1A. At all levels of sectioning, the stem is divided into lobes. At the level of sectioning in figure 1C, the lateral meristem appears circular. Figure 1D shows the appearance of a transverse section taken at a low level in the plant (level D-D, in A and B) and is arranged with the furrow running from top to bottom for comparison with figure 1B. The arrangement of root traces near the basal meristem in 1D is similar to that in 1B, and the root traces again obscure the relationship between the basal and lateral meristems. The basal meristem is arranged in the form of a ribbon on the convex underside of the root-bearing portion of the stele and therefore appears at two locations in figure 1D (cf. level D-D in A).

Orthostichies of root traces are inserted on the root-bearing portion of the stele in several to many places. Figure 1B shows a vertical orthostichy because a longitudinal section at right angles to the furrow plane is represented. Figure 1D is taken at a level where two nearly horizontal orthostichies are represented. The remainder of the orthostichies of root traces are inserted obliquely on the root-bearing portion of the stele. It may be argued that the various insertions of the orthostichies contradict the meaning of the word because an orthostichy should be vertical in the plant. However, with respect to the basal meristem, the arrangements of root traces at different locations are similar. It is convenient to designate the arrangement of root traces one above the other in median longitudinal section as an orthostichy and to apply the same designation to similar arrangements elsewhere on the root-bearing portion of the stele.

For additional orientation, three diagrams representing sporophytes in the first three plastochrons are given as figure 2. All my figures of sporophytes in the first three plastochrons are drawn and photographed from sections in the plane containing the first leaf, foot, and first root. This plane corresponds to the longitudinal plane of sectioning at right angles to the furrow plane in plants where the basal furrow can be recognized. While the plant has a distichous phyllotaxy, the insertions of all the leaves are contained in this plane. The second root also appears in this plane. The first leaf and root form the beginnings of one lobe of the plant; the second leaf and root form the beginnings of the other. The basal furrow forms between these two lobes (fig. 2C). The shoot apex is directly above the basal furrow. Additional figures illustrating the transition from sporophytes with one leaf to sporophytes with many leaves are contained in Baldwin's (1933) account of the early development of the sporophyte.

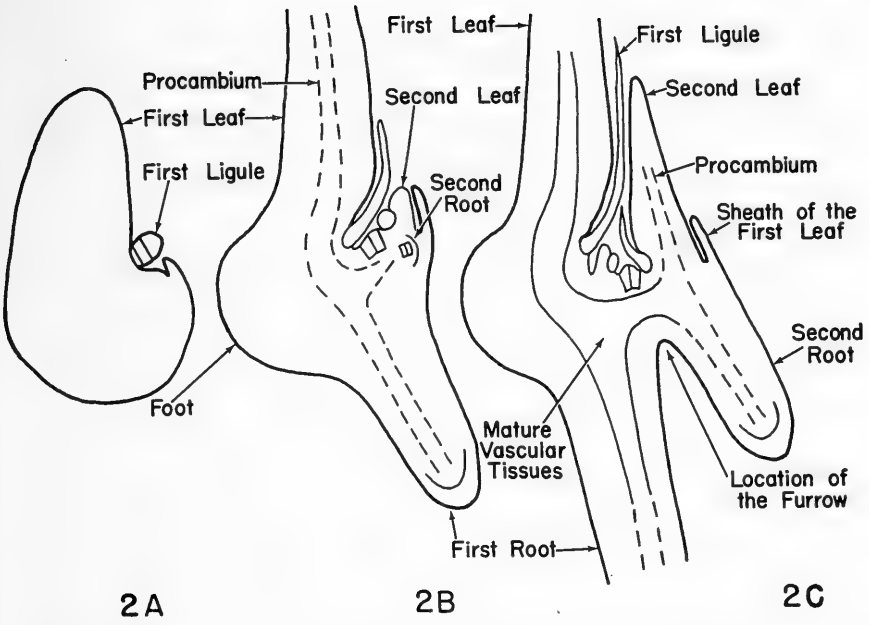


FIG. 2. Three stages in the development of a young sporophyte. A. One leaf. B. Two leaves. C. Three leaves. The plane of sectioning in A, B, C is the same plane that contains all the leaf traces while the plant is in a ½ phyllotaxy. The basal furrow forms between the first and second roots in a plane at right angles of the plane of sectioning of the figures. Schematic.

TERMINOLOGY

Parke (1959) had discussed the problem of terminology in studies of the apical meristem of the shoot. He defined the *shoot apex* as that portion of the shoot tip above the youngest leaf primordium. The *shoot tip* consists of the shoot apex and varying portions of the surrounding tissues, including leaf primordia and young leaves. Parke's definition of the shoot apex is convenient for conical apexes when the primordia arise on the flank of the apex. However, a median longitudinal section does not necessarily include the youngest primordium, and in such a circumstance it is difficult to mark the limits of the apex.

Some longitudinal sections of *Isoetes* show a flattened surface to the left and right of the apical mound (e.g., pl. 2). I designate this flattened region part of the *region of leaf formation.* It is best to exclude all this

region from the shoot apex because young primordia may be located in the region of leaf formation outside of the median plane. Under these conditions, the shoot apex of *Isoetes* may be less than that portion of the shoot tip above the youngest leaf primordium seen in a median section.

Because *Isoetes* is protostelic, the term *plerome* designates the procambium above the stele. The term *stele,* as it has been applied to *Isoetes* by Lang (1915b), indicates the vascular tissues and associated parenchyma formed from the procambium. Lang (1915b) believed that the lateral meristem and its derivatives are extrastelar in origin. If the lateral meristem arises in the procambium (West and Takeda, 1915), the concept of the stele may be extended to include the prismatic layer.

In plants with few leaves, my figures are labeled according to the succession of leaves on the plant, with L_1, L_2, L_3 . . . ; L_1 designates the oldest leaf. For plants with several to many leaves, the leaves are numbered from the youngest to the oldest, as P_1, P_2, P_3 . . . .

THE SHOOT

REVIEW OF THE LITERATURE

Form of the shoot apex. Hofmeister's (1862) report of an apical cell in the shoot apex of *Isoetes* was challenged by Bruchmann (1874) and Hegelmaier (1874). Hegelmaier related the formation of cells to the activity of the surface layer of the entire apex, whereas Bruchmann identified a small group of superficial initials within the apex. Farmer (1890) maintained that there was never any substantial evidence for an apical cell in his preparations of *I. lacustris,* the same species studied by Hofmeister and Bruchmann. Scott and Hill (1900) introduced evidence for the existence of an apical cell in at least some specimens of *I. hystrix,* and Lang (1915b) did not exclude the possibility that an apical cell exists in some plants of *I. lacustris.* Other workers (Stokey, 1909; West and Takeda, 1915; Weber, 1922; Liebig, 1931; Bhambie, 1957; Sharma, 1961) have rejected the idea of an apical cell in the shoot apexes of various species of *Isoetes.* Bruchmann (1874), Hegelmaier (1874), and Rauh and Falk (1959b) have allowed the possibility that the configurations found in the apex may vary among plants of different ages, but Bruchmann (1874) found no apical cell in young or old plants of *I. lacustris.*

Hofmeister (1862), Bruchmann (1874), and West and Takeda (1915)

illustrated the shoot apex of *Isoetes* as a conical or dome-shaped mass, but Farmer (1890) asserted that the apex is flat. Scott and Hill (1900) came to the same conclusion and reported that they could determine the location of the median section of the shoot only by counting serial sections between opposing leaf primordia and choosing the median section of this sequence. One may question the validity of such a procedure in a plant with a spiral phyllotaxy because opposing primordia are of different ages and at different distances from the center of the apex. More important, however, is the possibility that Scott and Hill may have erroneously identified the shoot apex as a leaf primordium. They remarked that Bruchmann's (1874) illustrations of conical apexes in *I. lacustris* bear a "suspicious resemblance" to leaf primordia. But Bruchmann's figures agree with those given by Hofmeister (1862) and Lang (1915b) for *I. lacustris*. One may conclude either that Scott and Hill did not recognize the apexes of their plants, or that the apex of *I. hystrix* is so flat that these authors remained unconvinced by Bruchmann's illustrations of conical apexes in *I. lacustris*. West and Takeda (1915) examined slides of *I. hystrix* which had been prepared and studied by Farmer. They reported that the shoot apex of *I. hystrix* is conical. It may be suggested, therefore, that Scott and Hill (1900) had an erroneous concept of the topography of the shoot tip. One may also ask why Scott and Hill (1900) maintained that their evidence explained Hofmeister's findings on the apical cell, when Hofmeister reported that the apical cell is at the summit of a conical apex and Scott and Hill maintained that the apex is flat. The situation is complicated by Lang's (1915b) report that the apex of *I. lacustris* usually forms a slight conical projection but is sometimes flat. However, Lang has also commented that Bruchmann's (1874) figures are accurate. It may be concluded that the shape of the apex can vary among plants of the same species. Interspecific variations also exist. For example, the figures given for *I. coromandeliana* by Bhambie (1957) illustrate apexes which are more elongate than those illustrated for other species of *Isoetes*.

La Motte (1937) reported that the shoot apex and probably all of the permanent tissues of the sporophyte originate from a single quadrant of the embryo. Mitotic activity is delayed in this quadrant until late in embryogeny. Thus, the apex appears to arise laterally on the embryo. The poles of the embryo are occupied by the tip of the first leaf and the tip of the first root. Baldwin (1933) wrote that the "characteristic leaf growing region of the adult sporophyte" is well defined at the time of the origin of the seventh leaf. Baldwin paid little attention to cellular details in the shoot tip of the young plant. He used outline drawings to indicate the several stages recognized in the development of the young

sporophyte. These are not detailed enough to determine the character of the shoot apex during the first few plastochrons. In the figures of Bruchmann (1874) and Campbell (1891), the shoot apex may be distinguished from the youngest leaf primordium during the second and third plastochrons. Although this distinction cannot be made on the outline drawings given for comparable stages of development by Baldwin (1933) and La Motte (1937), it can be made in the photomicrograph published with La Motte's (1937) account.

Bruchmann (1874), Bhambie (1957), and Sharma (1961) recognized that the form of the apex changes during ontogeny. Bruchmann (1874) reported that the topography of the relatively flat apex of the sporeling is subjected to plastochronic changes and that leaf primordia are formed in the tissues surrounding the conical apex of the adult plant.

Function of the shoot apex in the shoot tip. Hofmeister (1862) attributed the origin of the cells of the shoot of *Isoetes* to the activity of an apical cell. In his scheme of growth, the shoot apex is responsible for the production of cells which later enter into organogenesis and histogenesis in other parts of the growing tip of the plant. Likewise, Bruchmann (1874) and Hegelmaier (1874) forwarded the concept that all the cells of the shoot have their ultimate origin in the shoot apex. Hegelmaier felt that the orientation of cell walls and cell files leaves no doubt that the superficial layer of the apex contributes cells inwardly, and that these multiply to give a plerome directly below the apex and cortical cells lateral to the apex. Bruchmann gave the same general scheme of growth from the apex, although his concept of apical initials restricts the location of the initiating group of cells more than the concept of a meristematic surface layer formulated by Hegelmaier.

The account rendered by Bruchmann offers the opportunity to raise an important point. Bruchmann (1874) wrote that the summital cells of the shoot "sind die Meristem-Initialgruppe" of all of the surrounding cells and "haben als solche auch die Aufgabe, den in Folge der Blattbildung verbrauchten Scheiteltheil bei Erweiterung desselben wieder zu ersetzen. Die Blattbildung ist hier zwar sehr langsam und daher auch die Thätigheit der Stammscheitel-Initialen träge, immerhin aber lässt sie sich verfolgen." If attention is drawn to the word *träge*, it becomes clear that Bruchmann did not necessarily regard the apical initials as mitotically highly active. His concept of initials must have been related to the ultimate source of cells rather than to relative rates of mitotic activity. Furthermore, in the scheme of growth proposed by Hofmeister (1862), emphasis is placed on the division of the derivatives of the apical cell. Whereas the apical initial or initials are the source of all the cells in the shoot in the schemes of Bruchmann, Hegelmaier, and

Hofmeister, the initials are not regarded as sites of organogenesis and do not enter *directly* into the production of the plant body. The apical initials are regarded as histologically undifferentiated cells. The multiplication and differentiation of their derivatives furnishes the materials for the growth of the shoot.

Both Bruchmann (1874) and Hegelmaier (1874) recognized anticlinal and periclinal divisions in the superficial cells of the apex. The scheme of cell division given by Bhambie (1957) for the shoot tip of *I. coromandeliana* closely resembles that given by Popham (1951) for the shoot tip of his typical angiosperm. Bhambie (1957) did not apply either dermatogen or tunica to the superficial layer of the shoot tip, because periclinal divisions occur in the superficial layer during leaf formation. Bhambie did not state clearly the exact location of the sites of leaf formation in relation to the apical cone. From his diagram, it appears that leaf primordia arise in the tissues surrounding the base of the cone. If this is the case, no periclinal divisions occur within the superficial layer of the apex of the species he investigated. Sharma (1961) has reported that the shoot apex of *I. sampathkumarani* has an outer "epidermal" layer in which the cells divide mostly anticlinally, and an inner mass of cells that divide in all planes.

Differentiation of tissues in the primary plant body. Most authors have recognized a plerome above the centrally located vascular tissues of the stem (Hofmeister, 1862; Bruchmann, 1874; Hegelmaier, 1874; Scott and Hill, 1900; West and Takeda, 1915; Lang, 1915b). Because *Isoetes* is protostelic, the plerome is composed entirely of procambium. Although it has been admitted (West and Takeda, 1915) that this plerome is rather poorly defined, Lang's (1915b) photographs offer substantial evidence for its existence. Stokey (1909) has reported that there is no indication of a procambial strand above the mature stele in the stems of the several species she investigated. Farmer (1890) and Stokey (1909) argued that the vascular tissues of the stem represent a sympodium of leaf traces and that there is no cauline portion to the stele. Farmer (1890) pointed out that the distinction between cauline and foliar portions in the stele of *Isoetes* is not easily made and that the distinction may exist more in the mind of the investigator than in the plant. He emphasized, however, that there is clearly no cauline bundle in the young plant. But this point was freely admitted by most workers. West and Takeda (1915), for example, stated that there is general agreement about the absence of a cauline portion in the stele of the young plant. With Bruchmann (1874), they maintained that a cauline portion does exist in later stages of development. No statement has been made as to when in ontogeny the transition is accomplished.

Scott and Hill (1900) and Stokey (1909) reported that Hofmeister (1862) regarded the stele of the mature sporophyte as a composite of leaf traces. Weber (1922) commented that these reports were in conflict with his knowledge of Hofmeister's work, but the Ray Society Translation that served as a reference for Scott and Hill and for Stokey was not available to Weber. West and Takeda (1915) used the reference that was quoted by Scott and Hill and Stokey (i.e., Hofmeister, 1862) and correctly reported that Hofmeister recognized cauline tracheids in the mature plant.

Lang (1915b) recognized a peripheral and central portion in the xylem cylinder of the stems of several species of *Isoetes*. Leaf traces are attached throughout the peripheral portion of the xylem but do not affect the arrangement of tracheids in the central portion, which is regarded as wholly cauline. Lang (1915b) suggested that the cauline procambium gives rise to both xylem and phloem. Hegelmaier (1874), however, stated that the xylem core is "das Umwandlungsproduct des ganzen Pleroms." Bruchmann (1874) reported that there is no distinct boundary at the periphery of the core of xylary procambium. Lang (1915b) emphasized the absence of a definite boundary between the procambium and the surrounding tissues. He stated that radial seriation of cells may be traced from the peripheral portion of the xylem core, through the primary phloem, into the cortex.

Whereas Lang (1915b) and West and Takeda (1915) concluded that primary phloem occurs in the stem, Scott and Hill (1900) reported that cauline primary phloem is absent, except under certain circumstances (see later). They believed that the first tangential divisions around the procambial xylem core are cambial divisions. Hegelmaier's (1874) ideas on the question are close to those of Scott and Hill, and Stokey (1909) offered a somewhat similar concept. For these authors, the primary vascular tissues found within the stem are confined to the xylem core at the center of the plant and the vascular tissues of the leaf and root traces.

Differentiation of procambium to the leaves. The accounts that are available in the literature suggest that differentiation of a leaf trace takes place in the primary cortex and that the trace is initially inclined outward. Hegelmaier (1874) offered that:

An den Stellen, welche in ihrer Lage den Anfängen von Blättern entsprechen, ändert sich jene nach einwärts geneigte Richtung der tangentialen Scheidewände in eine auswärts ansteigende, und es enstehen hier zarte, steil von innen und unten nach aussen und oben gerichtete Zellenbündel, die ersten Anlagen der Blattstränge, deren Anfänge somit in nächster Nähe des Scheitels sich differenziren und deren später hinzuwachsende Theile, entsprechend der Richtung, welche die centripetal sich vermehrenden Radialreihen der Rinden-

zellen annehmen, einen mehr und mehr dem wagrechten sich nähernden Verlauf bekommen, in welchem endlich ein Theil der Zellen bekanntlich schrauben- und ringförmige Verdickungen erfährt.

Hegelmaier's account is rendered without the use of illustrations. According to Farmer (1890), "The leaf trace originates in the division of a row of cells, in an upward and outward direction, which more or less irregularly connect the base of the leaf rudiment with the central part of the stem, at the apex of the woody portion of the bundle. Thence the divisions proceed upward into the leaf and downward into the stem." Farmer's account is also rendered without the use of illustrations. West and Takeda (1915) offered the following description of the initiation of a leaf trace:

> That part of the procambial strand of the foliar bundle which traverses the primary cortex is differentiated at a very early stage in the development of the leaf. It originates by the division of certain cells in the primary cortex, which retain their meristematic character for a considerable period. A strand of small cells, easily distinguishable by their relatively large nuclei, is produced in an upward and outward direction. The upper extremity of the strand extends to the base of the young leaf. Connection of the primary xylem and primary phloem of the stem-stele is established by the downward prolongation of the procambial strand, the tissues of which are differentiated from the 'parenchymatous mantle.'

The figure cited by West and Takeda in support of this description shows a trace in an advanced stage of development.

Rauh and Falk (1959b) reported that the differentiation of the procambial traces of *Stylites* (a member of the Isoetaceae that was recently described by Amstutz, 1957) is basipetal, and that the traces differentiate in the primary cortex and reach the axial procambium before any cytohistological differentiation can be detected in the latter at the place of attachment of the trace. To evaluate these ideas and the reports on procambial differentiation in the leaf traces of *Isoetes,* one must determine if the differentiation of the axial procambium is strictly comparable to the differentiation of the procambium of the leaf traces. One must also determine if the designation *primary cortex* is appropriate for the tissue which gives rise to the leaf traces. These problems are discussed in a later section of this report.

The cambium and secondary growth. The cambium is composed of two parts: the lateral meristem and the basal meristem. All authors have treated the lateral meristem as part of the cambium. The basal meristem, which has been treated in several ways, is discussed later in this report. In the present section of the literature review, the term *cambium* will be used to designate the lateral meristem only. This usage allows facility in treating the literature, because most of the discussions of the cambium relate only to the lateral meristem.

There is some difference of opinion on the place of origin of the cambium in the mature plant. Scott and Hill (1900) reported that the cambium originates in the cells immediately outside the axial xylary procambium. Stokey (1909) stated that the cambium begins its activity in the parenchyma that surrounds the central core of xylem. West and Takeda (1915) indicated that the cambium originates in the outer portion of the *parenchymatous mantle*, which builds the parenchyma sheath, the axial primary phloem, and the cambium as concentric layers around the core of xylem. In their view, the cambium arises from a part of the procambium, for the parenchymatous mantle is considered a part of the plerome. West and Takeda (1915) and Lang (1915b) believed that the cambium begins its activity at the level of the youngest mature phloem. Although Lang (1915b) agreed that in large plants the cambium originates in the layer of cells adjacent and external to the cauline primary phloem, he chose to designate the primary phloem as the outer limit of the stele. In his opinion, therefore, the cambium is extrastelar in origin because it originates in the primary cortex. A logical consequence of this opinion is the exclusion of the prismatic layer from the stele.

For *Stylites*, Rauh and Falk (1959b) reported that the cambium takes its origin from the innermost layer of the primary cortex, which borders on the primary phloem. They designated the cambium as a *secondary meristem* and maintained a distinction between the cell layer that gives rise to the cambium and the so-called *primary meristem*, which gives rise to both the primary phloem and a parenchyma sheath that surrounds the xylem core. A discontinuity of cell files is shown at this location in their diagram of the origin of the cambium, but such a discontinuity is lacking in photographs and drawings distributed throughout the rest of the text.

For the sporeling, Hofmeister (1862) reported that a mantle of cambium is formed during the first growing season and that the cambium arises from the layer of parenchyma adjacent and external to the axial tracheids. Stokey (1909) reported that cambial activity begins early in the ontogeny of the sporophyte. Her figure 3, cited in support of the early origin of the cambium, shows a plant that is probably in its first season of growth.

All authors have agreed that the outer derivatives of the cambium differentiate as parenchyma of the secondary cortex, but Lang (1915b) held that the activity of the cambium toward the outer side has been greatly overrated. He attributed a large portion of the cortical tissues to the extension of the primary cortex. Most authors have agreed that the corky layers of the surface of the plant are formed by the suberization

and death of the outermost cells of the cortex, but West and Takeda (1915) have reported a cork cambium in the parts of the plant where the basal furrow forms.

The inner derivatives of the lateral meristem have been the subject of much discussion. Weber (1922), Rauh and Falk (1959b), and Lamoureux (1961) have given detailed reviews of the literature on the morphological nature of the prismatic layer. This layer has been described as undifferentiated parenchyma (von Mohl, 1845), as secondary xylem (Smith, 1900; Stokey, 1909), and as a mixture of sieve elements, tracheary elements, and parenchyma (Russow, 1872; Scott and Hill, 1900; West and Takeda, 1915). Arguments advanced in favor of the presence of sieve elements in the prismatic layer depend on the presence of callose in the putative sieve elements (Esau, Cheadle, and Gifford, 1953; Lamoureux, 1961), the continuity of the innermost derivatives of the prismatic layer with the functional sieve elements of leaf traces (Russow, 1872; Scott and Hill, 1900), and cytological details of the putative sieve elements (Lamoureux, 1961). Various arguments have been advanced against the presence of sieve elements in the prismatic layer. These are based on the obscurity of the physiological function of the prismatic layer (Smith, 1900), the similarity of the sieve areas to pits on the walls of parenchyma cells and the impurity of the callose deposits (Weber, 1922), or the assertion that the putative sieve elements are actually immature tracheids (Stokey, 1909).

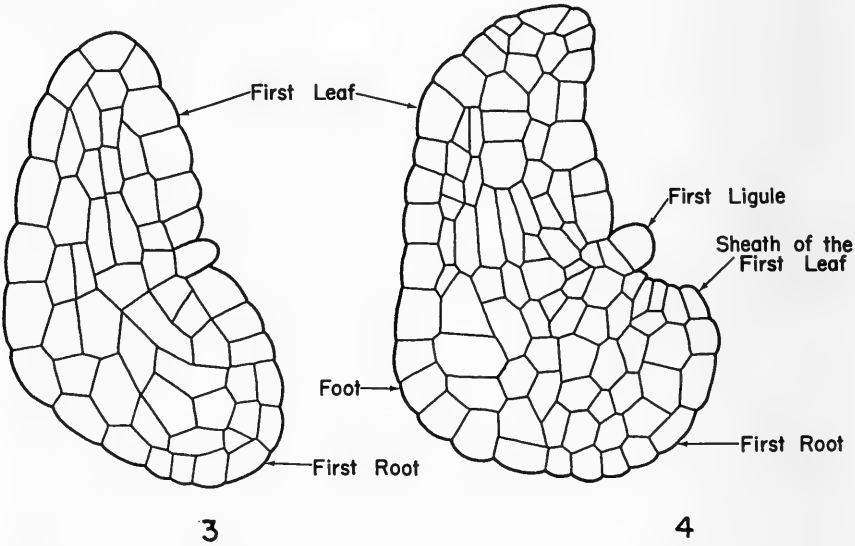
The question of whether the prismatic layer should be called xylem or phloem is unimportant. It has been reported that both sieve elements and tracheary elements differentiate in the prismatic layer (Russow, 1872). Recently, *secondary vascular tissue* has been used to designate the prismatic layer (Foster and Gifford, 1959; Lamoureux, 1961). This designation may be especially apt because the composition of the prismatic layer varies, tracheary elements being absent in some species (West and Takeda, 1915).

OBSERVATIONS AND DISCUSSION

Form of the shoot apex. The shoot apex is organized in the young sporophyte. A careful study of the figures available in the literature (Hofmeister, 1862; Bruchmann, 1874; Keinitz-Gerloff, 1881; Campbell, 1891; La Motte, 1937) and of a number of original preparations suggests that the shoot apex cannot be distinguished from the surrounding cells on morphological (figs. 3, 4) or cytological grounds until the advent of the second plastochron. A median sagittal section of a sporophyte with only one leaf may show a group of superficial cells between the ligule

of the leaf and the portion of the sheathing leaf base that appears in the section (fig. 4). No distinction can be made between the cells that will give rise to the shoot apex and those that will furnish the second leaf primordium.

La Motte (1937) has speculated that the axis of the rudimentary shoot is parallel to the axis of the first leaf because the second leaf emerges parallel to the first leaf. I have redrawn one of his figures as figure 3.



FIGS. 3 AND 4. Sagittal sections of sporophytes in the first plastochron. FIG. 3. The sheath of the first leaf has not yet developed. Taken from La Motte (1937, fig. 13). Magnification not known. FIG. 4. *I. howellii* at a stage older than the sporophyte in figure 3. The sheath of the first leaf is partially developed. The ligule mother cell has divided transversely, initiating the development of the multicellular ligule of the first leaf. × 390.

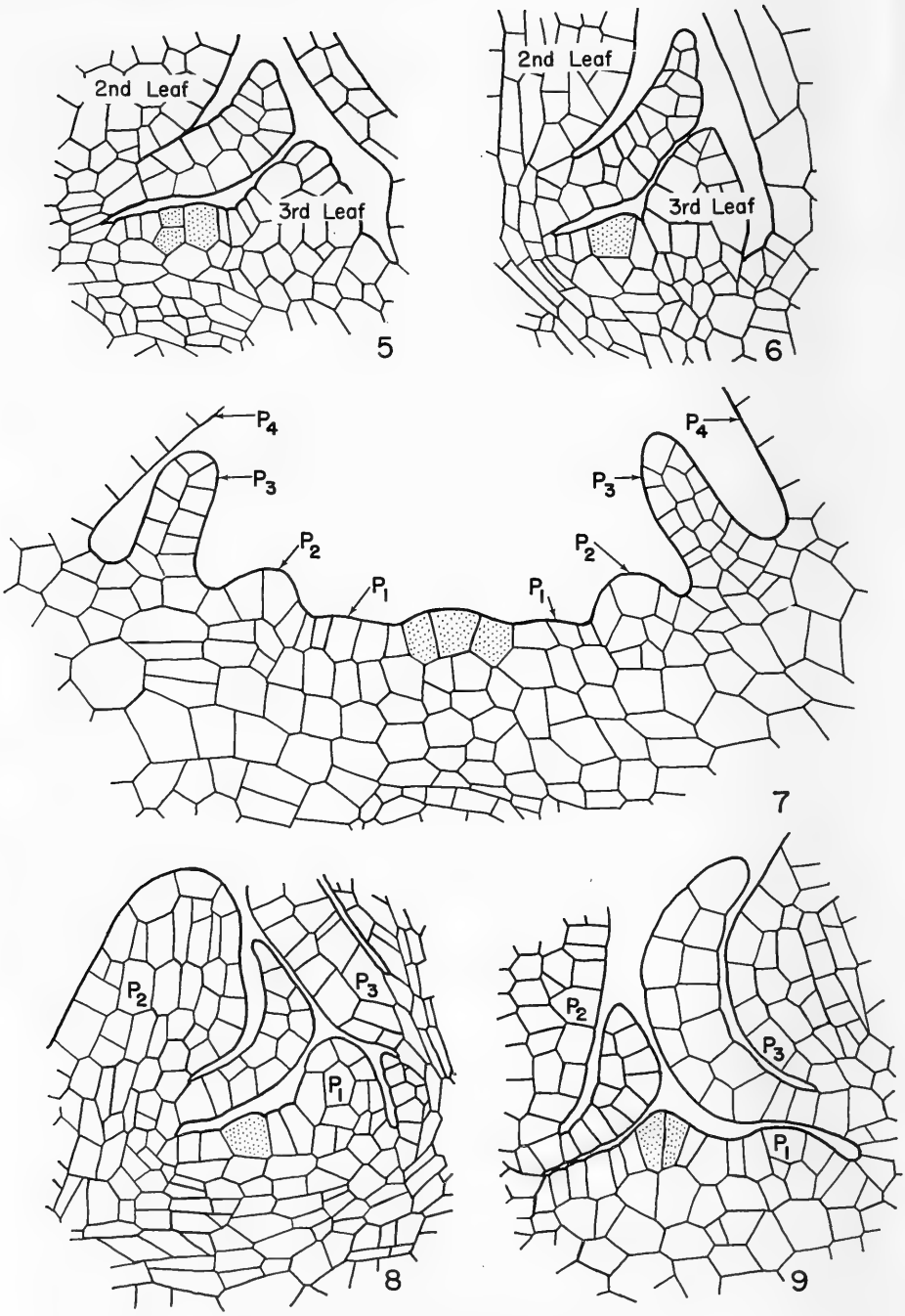
Although the axis of the shoot is difficult to locate when the sporeling has only one leaf, it is not likely that this axis is parallel to that of the first leaf in the early stages of development (fig. 3). However, as the second plastochron is approached, growth in the region opposite the insertion of the first leaf tends to reorient the superficial cells so that the axis of the shoot and the first leaf are more closely aligned than they were initially (fig. 4). With the advent of the second primordium, certain cells of the shoot tip assume characteristics that set them apart from the rest of the meristematic tissue. These cells mark the center of the shoot apex and are the apical initials of the shoot. These initials enlarge and stain less deeply or with a slightly different hue after the sections have been stained with progressive hematoxylin-safranine-fast

green (pl. 1, A, B, C, at arrows), but they are no more vacuolated than the adjacent cells.

During the initiation of the second primordium, tilting of the apical surfaces continues so that an acute angle is formed between the surface of the first leaf and the combined surfaces of the shoot apex and the second leaf (pl. 1, A, B, C). As the second primordium grows, it becomes closely appressed to the first leaf (pl. 1, D, E, at L_2). The angle between the surfaces of the first leaf and the shoot apex becomes very acute, and there is no pronounced topographic distinction between the shoot apex and the second primordium, even after the ligule mother cell of the second leaf is differentiated (pl. 1, E, at Li_2). The broadening of the apex by the multiplication and expansion of cells is accompanied by a shift in the surface of the apex toward a plane more nearly perpendicular to the adaxial surfaces of the first two leaves. As the development of the third leaf proceeds, the topographic boundaries of the apex become more distinct (figs. 5, 6). The apex assumes the form of a small dome. The dome is very low, and the apical initials are deeper than the apex. The apex may be so narrow that the tops of the apical initials account for all of the apex.

The arrangement of leaves in a two-lobed specimen is distichous for the first 10-15 plastochrons, and the shoot tip is not radially symmetrical through these early stages of development. In a longitudinal section at right angles to the basal furrow (figs. 5, 6, 8, 9) the leaves are to the left and right of the apex. The apex may measure from 15-50 μ in width by 3-15 μ in height between periods of leaf initiation. The form of the shoot apex of the young plant is subject to plastochronic variations. The apical initials are usually raised in a small dome (fig. 9). Leaf primordia arise in the tissues *next* to the base of this dome, at least as early as the third plastochron and for all subsequent periods of initiation. In the young plant, the initiation of a leaf primordium may so alter the topography of the shoot tip that the apex appears to have no height above the axil of the primordium (fig. 8). At some stages of the plastochron, the boundaries of the apex are indistinct, so that it is difficult to obtain exactly comparable measurements from one apex to another.

As the phyllotaxy changes from distichous to spiral, the apex enlarges, and the topography of the apex becomes more stabilized. The apex forms a cone in the shoot tip. Plastochronic changes in the apex become less distinct or nonexistent. Rauh and Falk (1959b) have reported that in *Stylites* plastochronic changes in the form of the apex may be detected in young plants but not in old plants. Bruchmann (1874) has reported plastochronic changes in the shoot apex of young plants of *I. lacustris.*



FIGS. 5, 6, 7, 8 AND 9. Legend on facing page.

Shortly after the spiral phyllotaxy becomes well established, the apical cone is about twice as broad as high, and the apical initials occupy the distal region of the cone (pl. 2, A). At this stage, the apex may measure 50-75 μ in width by 20-30 μ in height. As the plant increases in size, the apex may also enlarge. At the same time the apical initials become less distinct, and the apex becomes covered with a uniform layer of cells that are similar to the cells covering the tissues flanking the apex (pl. 2, B, C). The uniform character of the superficial cells of the mature apex makes it impossible to single out a particular group of cells as apical initials on any grounds other than position.

While the apical initials are still distinguishable on the basis of their staining reaction, they comprise a group of cells of similar size. However, several of the specimens examined offer clear indication that a single cell dominated the apical group at the time of fixation (pl. 3, A, B, C, arrows). Whether or not such a cell is regarded as an apical cell depends on one's concept of an apical cell and on one's emphasis on

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FIGS. 5, 6, 7, 8, AND 9. Median longitudinal sections of young sporophytes of *I. howellii*. × 500. FIGS. 5 AND 6. Plants in the third plastochron. Figure 6 represents a more advanced stage of the plastochron than figure 5. In both figures, the surface of the shoot apex is more or less at right angles to the adaxial surfaces of the second and third leaves. FIG. 7. Plant with 8-9 leaves. Sectioned parallel to the developing basal furrow. The sheathing base of each leaf appears to the left and right of the shoot apex. FIG. 8. Plant with 8-9 leaves. Sectioned at right angles to the plane of the furrow (sectioned in the sagittal plane). The plant was still in a ½ phyllotaxy. The shoot apex has no height above the axil of P_1 because of the stage of the plastochron. FIG. 9. Plant cut at right angles to the basal furrow. Fixed at the time the phyllotaxy was changing from ½ to spiral. P_1 represents the edge of the youngest primordium and not the median portion. The apex has some height above the axil of the youngest primordium. In all figures, the stippled cells are members of their respective groups of apical initials. Other cells in these groups appear in sections adjacent to those illustrated. P_1-P_4, youngest to oldest leaves, counting from the shoot apex.

short-term versus long-term cell configurations. In the first dozen or more plastochrons, such a cell would not be easily maintained at the summit of the apex. Plastochronic variations in topography would alter the spatial relations of the cells of the apex so that no one cell would remain continuously elevated above the others. As plastochronic changes become less important, an increased stability of the configurations in the apex may be expected to result. However, the configurations that show a single large cell at the summit of the apex are not stable throughout ontogeny, for no such configuration is found in old plants. The apparent apical cell has been found only in plants that were near the change from distichous to spiral leaf arrangement. The low frequency of occurrence of the apparent apical cell (about one plant in 15-20 of the appropriate size) indicates that such a cell may dominate the apex for only a short period in ontogeny or that it may never occur at all in some plants.

The evidence available does not support Hofmeister's (1862) report of extreme regularity of division from two or three cutting faces of an apical cell that is supposed to exist throughout the life of every sporophyte. The evidence offered by Scott and Hill (1900) for the existence of an apical cell in at least some plants of *I. hystrix* could be considered at this time. It is possible, however, that these authors failed to identify apexes properly in their materials, and their conclusions cannot be accepted without reservations. On the other hand, the assertion by Bhambie (1957) that the configurations shown in his cell net drawings exclude the *possibility* of an apical cell is clearly unfounded. In most of his figures the summit of the apex is dominated by a single cell. The pattern of the cell net below this cell would not reflect the presence or absence of an apical cell so much as it would the number of cutting faces possessed by an apical cell and any symplastic growth adjustments among the cells in and behind the apex. As a matter of fact, certain of Bhambie's drawings and my plate 3, A, B, C, may be interpreted as evidence for the existence of an apical cell which divides mostly anticlinally (and alternately, with respect to the single plane of sectioning) and rarely periclinally. In my opinion, an undue emphasis on configurations like those shown in plate 3, rather than poor microtechnique as suggested by Bhambie (1957), led to the formulation of the concept that every plant of every species of *Isoetes* has an apical cell (Hofmeister, 1862).

Bhambie (1957), in arguing against the existence of an apical cell, has emphasized the lack of an extensively regular cell net which an apical cell might be expected to generate (cf. Hegelmaier, 1874; Rauh and Falk, 1959b). But it may also be argued that the summital cell in some small plants of *Isoetes*, like the apical cell of *Equisetum*, is in position to displace other cells from the center of the apex by its own

growth and division. In this sense, the summital cell is at least the analog of the apical cell of *Equisetum.* Regardless of the interpretation, one observes that the form of the apex just before and after the transition from distichous to spiral phyllotaxy lends itself to the domination of the summital region by a single cell. As the plant matures, the cells of the apex become more equivalent, even at the summital region, for this region of the apex flattens as the plant ages (pl. 2, C).

Popham (1960) described *Isoetes* as an example of a plant "in which the apex of the very young sporophyte exhibits a single apical cell . . . whereas an unstratified layer of cells dividing in many planes may be observed in apices of older and adult sporophytes." In an earlier paper (Popham, 1951), a similar opinion is implied but not stated. In *I. howellii,* the presence of a large summital cell in some small plants offers no guarantee that a similar condition occurs in the ontogeny of all plants. During the second plastochron, a definite *group* of equivalent apical initials is present (pl. 1, B, C, arrows; cf. Bruchmann, 1874). The occurrence of a single summital cell depends on the elevation of the apex into a dome or cone, and I assume that the occurrence of a large summital cell is the result of growth adjustments within the group of apical initials in their topographic setting in the apex.

The changes that occur in the shape of the apex of *I. howellii* during the ontogeny of the sporophyte parallel those reported for *I. coromandeliana* by Bhambie (1957). In the latter species, the form of the apex changes from a flattened dome (plants with 6-15 leaves) to an elongate cone (plants with about 20 leaves), with a tendency toward flattening as the plant approaches old age. In *I. howellii,* the apex of a plant with a ½ phyllotaxy is only slightly raised above the surrounding tissue (figs. 5-7, 9). With the establishment of the spiral phyllotaxy, the apex is further elevated, and becomes a sharp cone which is about twice as broad as it is high (pl. 2, A, B). In the largest plants available, the apexes were clearly elevated above the surrounding tissue, with their widths about three to five times as great as their heights.

Several circumstances detract from any generalization which might be made. First of all, there is no objective way of determining the age of the plants I collected in the field. The plants may attain a certain maximum size, but there is no guarantee that the largest plant is the oldest. The external dimensions of a specimen are determined by the balance between growth and deterioration of its tissues. New increments of tissue are added to the plant by its meristems, and older tissues are lost from the outside by decay. After sufficient time has elapsed, two plants of different ages can attain the same size. But this size need not be stable, because the plant may decrease in size if the equilibrium be-

tween growth and decay is disturbed. One might anticipate that the internal structure of a plant would be a better indication of its age. However, the increments of secondary vascular tissue are not layered in the early stages of growth in *I. howellii.* Likewise, the number of series of roots (see later section) produced by a plant in a season of growth could vary from plant to plant or from year to year. Two plants of similar external size and form may have apexes of rather different form, even though the plants are approximately equal in the number of series of roots they have possessed (pl. 4, A, B). To make comparisons valid, it is also necessary to eliminate variations caused by differences in the stage of the plastochron. It has already been mentioned that plastochronic changes in the apexes of adult plants are not observed. Comparisons of the apexes of different adult plants on the basis of the external and internal form of the plants are valid, but the difficulty with these comparisons is that there is no objective way to relate them to chronological age.

Bhambie (1957) has reported that the "form of the apex is apparently correlated with the mode of growth of the axis. In very young plants, as also in mature ones, radial growth is more pronounced than vertical growth, while in the prime youth of its life the plant elongates most rapidly and possesses, though for a short time, a conical apex." No data on the rates of elongation support this statement.

In *Isoetes,* the internodes do not elongate after they are formed (Hofmeister, 1862). The elongation of a stem during a growing season depends directly on the number of leaves that are added along the axis of the stem during a single season. This number may be estimated by the number of leaves in a rosette, because the rosette is renewed each year so that the number of leaves on the plant is not cumulative. In *I. howellii* the height between successive traces on the stele is the same in plants with conical apexes as it is in plants that are much older and that have broader apexes than their younger counterparts. The number of leaves in the rosette of large plants is greater than the number of leaves in the rosettes of small plants. Thus, the increment of length added to the axis each season is larger in large plants than it is in small plants. It follows that the conical form of the shoot apex is not correlated with the highest rate of elongation. I cannot suggest what factors are responsible for the variations in form of the apex through ontogeny. Apparently, however, the change is not related in any simple fashion to the rate of elongation of the plant.

Cytological details of the shoot apex and the adjacent tissues. The use of Regaud's fixative has facilitated the study of vacuoles, mitochondria, and plastids in the cells of the shoot tip. The discussion of

these cell components is based on the examination of specimens prepared after Regaud's fixation and begins with a description of the shoot tips of mature plants (pl. 4, C, D).

Bhambie (1957) has reported that the cells of the superficial layer of the apex of *I. coromandeliana* are less vacuolate than the subjacent cells. In *I. howellii* the vacuoles of the superficial layer are smaller than those of the subjacent cells. The transition from small to large vacuoles may occur very abruptly along the axis of the plant so that large vacuoles are found in the second layer of cells (pl. 4, C). More often, the transition occurs gradually, so that several cell layers are traversed before cells with large vacuoles are encountered (pl. 4, D). The mitochondria of cells of the superficial layer are granular and short, rodlike forms; they are seldom elongated. In the cells with larger vacuoles, the mitochondria are long and threadlike, attaining a length of several microns (pl. 5, A, at M, and opposite pl. 5), and granular mitochondria are inconspicuous or absent.

Because a separate publication has been devoted to the plastids of *I. howellii* (Paolillo, 1962), they are mentioned only briefly here. During the interphase period in a meristematic cell, typically only one plastid is present (Stewart, 1948). This plastid may be called a proplastid because it is immature. With Regaud's fixation, the proplastid in its least differentiated state is revealed to be a flattened object, often circular. It stains very deeply at the rim and lightly in the interior after treatment with hematoxylin and other protein stains. This staining reaction is obtained for proplastids in the superficial cells of the shoot apex, in young leaf primordia, in the intercalary meristematic tissues of growing leaves, and in the apical meristem of the root. For convenience in description, I call the proplastid in this condition the *undifferentiated plastid*. The plastids of the cells of the superficial layer of the apex are undifferentiated plastids (pl. 5, A, at U Pl). Below the superficial layer, the plastids are less condensed in form than the undifferentiated plastids (pl. 5, A, B, at Pl). Most of the plastids of the internal tissue of the apex have a deeply staining reticulum and a lightly staining ground substance (see opposite pl. 5).

The cytological characteristics of the superficial layer of the apex and of the region of leaf formation surrounding the base of the apex are essentially the same. The cytological characteristics of young leaf primordia also resemble those of the superficial layer of the apex. With regard to this similarity of cytological characteristics in cells of the shoot apex and sites of leaf formation, *Isoetes* resembles such gymnosperms as *Ephedra* (Dayes-Dujeu, 1957) and *Cryptomeria* (Tribot, 1961) and

contrasts with some angiosperms (Buvat, 1952) in which the leaf primordia differ cytologically from cells of the apex.

All of the comments on the vacuoles, mitochondria, and plastids of the superficial layer of the apex of the mature plant apply to the apexes of young plants, with a few minor differences. In small plants, the transition to cells with large vacuoles occurs in the second layer. In young plants with conical apexes, the transition extends over whatever number of cell layers are present in the apex. Two plants among twenty young specimens examined after Regaud's fixation showed exceptionally large cells at the summits of sharply conical apexes, and the vacuoles of these cells were intermediate in size between those typical for the superficial layer and those of the underlying tissue.

When acid fixation and a staining schedule containing safranine are used, the most marked difference between the apical initials and the adjacent cells in small plants is the lack of safranine in the apical initials. This difference in staining with safranine may persist until after the spiral phyllotaxy is established (pl. 2, A). In small plants, fixation with Regaud's fluid and staining with Regaud's hematoxylin revealed no special cytological properties for the apical initials, except that the two plants mentioned in the previous paragraph showed some difference in vacuolation between the summital cells and the other superficial cells of the shoot tip. In small plants, the apical initials do not stain distinctively with acid fuchsin or with mercuric bromphenol blue. In large plants, the superficial layer of the apex stains uniformly in all of the staining schedules I have used, and the apical initials have no distinctive staining properties. The most consistent feature in plants of all ages is the deeper staining of the superficial layer compared to the underlying tissues.

In the staining schedules used, safranine is regressed with acidified alcohol and acid fuchsin is regressed with basified alcohol. From the observation that these two dyes produce different results in delimiting the apical initials of young plants, it may be suggested that the apical initials of these plants differ from the adjacent cells in some, but not all, of their stainable constituents. To determine if the differential staining properties of the apical initials might result from a lower content of stainable RNA, ten small plants, of the size that might contain distinct apical initials, and one large plant were stained with pyronine Y. This stain may be used as an indicator of RNA in plant cells (Tepper and Gifford, 1962) and is more or less specific for this compound under certain conditions. The materials were fixed in Craf III and FAA, and the stain had to be applied for several hours or overnight in order to obtain an intensity of staining equivalent to that obtained in six minutes in

higher plants in the same 2 per cent aqueous solution. It was also necessary to reduce the washing of the stained materials (in n-butyl alcohol) to the minimum required for dehydration in order to prevent complete loss of stain. No tests were made to determine what constituents other than RNA contributed to the staining, although it is obvious that pyronine Y stains more than RNA in plant tissues (Tepper and Gifford, 1962). In *I. howellii*, starch grains, secondary walls of tracheary elements, and callose deposits stain with pyronine Y. Large starch grains are absent from the apex (see later), and secondary walls and masses of callose are absent from the shoot tip, so there may be a close relationship between pyronine staining and stainable RNA content within the shoot tip. Even if this relationship exists, the morphological significance of the phenomenon is an open question. With these reservations in mind, the results of staining with pyronine Y will be discussed.

In the small plants, there was some indication that the cells which corresponded to the apical initials seen after safranine staining were more lightly stained than the surrounding cells. In the large plant, no differences in staining could be detected within the superficial layer of the apex. In both the large and small plants, the superficial layer of the apex and of the region of leaf formation was more deeply stained than the underlying tissues. The adaxial cells of the youngest leaf primordium of a given shoot tip stained more deeply than any of the cells of the apex, but differences in staining were much less pronounced between the apex and surrounding cells where no leaf primordium was present.

The most striking differences in staining are not among cells within the apex, but between the cells of the apex and those of the young leaf primordia. These differences are demonstrated to varying degrees with pyronine Y, safranine, Regaud's hematoxylin, and other hematoxylins. Safranine, used regressively after progressive hematoxylin, brings out the differences most dramatically. The staining differences which do exist separate the shoot tip into the same two regions that one would designate on other grounds: the region of the shoot apex and the region of leaf formation. In the shoot apex, the superficial layer stains more deeply than the underlying layers. In the region of leaf formation, the superficial cells closely resemble their counterparts in the shoot apex, except where they are directly concerned with leaf formation. In the latter case they stain more deeply than any of the cells of the shoot apex.

The distribution of starch can be studied by the use of IKI solution or by the application of periodic acid followed by Schiff's reagent (PAS reaction). Both of these staining techniques are appropriate for the detection of large starch grains, but the PAS reaction may be used for the coloration of very minute particles. One may assume that small particles

that are limited to plastids and have the same color as the large starch grains are also starch granules. If this assumption is correct, the PAS reaction enables one to detect smaller starch granules than may be easily detected with the IKI reaction. The PAS reaction, therefore, provides a useful tool for the study of the distribution of starch in the shoot tip.

Starch may or may not be detected by the PAS reaction in the summital region of the apex (pl. 5, C, D, E, F). Likewise, starch may be present or absent in young leaf primordia (pl. 5, C, E, F). The starch grains found at the summit of the apex are always minute (pl. 5, D), but large grains of starch may occur at the base and tip of a young leaf primordium (pl. 5, F). Bhambie (1957) has reported that starch is absent from the shoot apex and young leaf primordia of *I. coromandeliana.* In *I. howellii,* all of the four possible combinations of the presence and absence of starch in the shoot apex and the leaf primordia occur. Too few specimens have been treated with the PAS reaction to allow any definite statement on the conditions that determine the distribution of starch in the shoot tip.

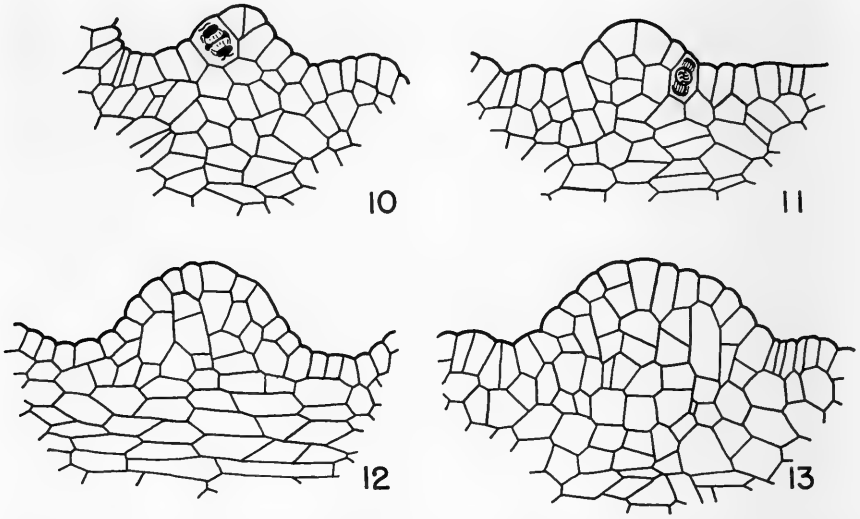
Function of the shoot apex in the shoot tip. The function of the shoot apex must be considered in relation to the function of the entire shoot tip. Plants with the spiral phyllotaxy well established (pl. 2, A, B, C, and older) will be considered first. It may be freely admitted that mitoses are infrequently encountered within the limits of the shoot apex. Bruchmann (1874) has suggested that the frequency of mitoses in the apical initials may be low, and Stewart (1948) reported that the cells of the apex are seldom found in division. I have located mitoses in median or near median longitudinal sections of nine out of eighty apexes from plants with a spiral phyllotaxy. The search for mitotic figures was not designed to obtain data on the relative frequencies of mitoses in the apex, but rather to determine whether or not the pattern of the cell net is a reliable indicator of the location and orientation of mitoses within the apex. The arrangement of cell walls was used by Bruchmann (1874) and Hegelmaier (1874) in the formulation of their concepts of the growth of the shoot tip. The concept that apical growth may be interpreted by the cell net pattern is supported by my observations on the location and orientation of mitotic figures within the apex. The following discussion, therefore, is based primarily on the pattern of the cell net, and information on the location and orientation of mitotic figures is introduced where it is pertinent.

One of the striking features of the cell net of *Isoetes* is the presence of radial files of cells beneath the surface of the shoot tip. As Lang (1915b) and others have pointed out, these radial files are a manifesta-

tion of the primary thickening of the plant body. Near the shoot apex, the files do not extend indefinitely into the cortex. Instead they bend upward and terminate at the bases of the leaves (pl. 6, A, B). The uppermost files are the shortest. New leaves are added to the rosette from the inside and are displaced outward. It may be assumed, therefore, that the short files found below the distal part of the shoot tip are extended to the length of the longer files found below them. At the same time, it is obvious that new files are added on top of the older files. If this were not the case, the files near the shoot apex would become indefinite in length, and it is a matter of observation that this does not happen. One must, therefore, determine the origin of new radial files.

The uppermost radial file in a section may be found to lie parallel to the surface of the region of leaf formation where the latter has not been raised in connection with the formation of a leaf (pls. 2, B, C; 6, A, B, at F). The cell file and the surface layer separate where the latter rises over the contour of the apex. The wedge of cells at this location in the section is in position to form a new radial file, which would be inserted above the older cell file as both are extended outward (pls. 2, B, C; 6, A, at W). This wedge could originate either from a transverse division, with reference to the shoot axis, of a cell near the inner end of the older file, or from a cell which has been contributed downward from within the shoot apex. Cell net patterns and division figures which indicate both of these origins have been observed. Growth of the wedge into a new file results in the transfer of cells at the base of the apex to the region of leaf initiation. That such a transfer of cells does occur is supported by the observation that a young leaf primordium may be found to abut directly on the base of the apex (pl. 2, B). The region of leaf formation may then be regenerated from the apex at that particular site. The spiral phyllotaxy of the adult plant allows for the uniform transfer of cells from the apex to the region of leaf formation along all radii of the plant.

Because some of the cells within the apex are in a position to form new radiating files, it is important to know the source of the internal cells of the apex. The observation of mitoses and the cell net pattern indicate that the superficial layer along the flanks of the apex contributes cells inwardly (figs. 10, 11; and pls. 4, B; 5, F; 6, A, at PF). These inward derivatives are then in a position to produce the radial files of cells mentioned above. Periclinal divisions also occur in and near the summital cells of the apex, so that derivatives of the superficial layer are contributed inwardly along the axis of the plant (pls. 4, C; 6, A; at PS). These derivatives are not necessarily in a position to contribute to the radial files, and need never do so in large apexes. In small apexes,



FIGS. 10, 11, 12, AND 13. Apexes of mature plants of *I. howellii* as seen in longitudinal sections. FIGS. 10 AND 11. Apexes showing periclinal divisions in the superficial layer of the apex. × 400. FIGS. 12 AND 13. Apexes of larger plants than those of figs. 10 and 11, showing an increase in number or in width of cell files toward the summit as an expression of lateral expansion in the summit region. × 350.

such derivatives might produce new cells both vertically and horizontally in the shoot tip. Mitoses and cell net patterns which indicate a contribution from the summit to the flanks of the apex have also been observed. Anticlinal divisions occur at the summit of the apex (pl. 6, B, at AS). The cell net pattern of an increasing number of files toward the summit in some apexes also leads one to believe that these apexes were growing in the summital region before the time of fixation (figs. 12, 13).

The apexes of young plants consist of relatively few cells. The mitotic activity within the apexes of small plants cannot be accounted for by growth of the apex itself, because the size of the apex does not increase markedly in the first dozen plastochrons. The contribution from the apex to other regions of the shoot tip is more direct in small plants than in large ones. This contribution is indicated by the cell net pattern and by mitotic figures.

The above analysis agrees in essentials with the accounts of Bruchmann (1874) and Hegelmaier (1874), but in particulars supports Bruchmann's more restricted concept of a group of apical initials rather than Hegelmaier's concept of an apical cell surface. The group of apical initials present within the apex is distinguishable with certain staining

techniques until after the spiral phyllotaxy is well established. Subsequently, the summital cells are not visibly different from the adjacent cells of the superficial layer. Popham (1951) has registered some objection to the use of the term apical initials when the distal axial cells are not distinguished on the basis of size, shape, and plane of cell division from the adjacent cells. He argued "The term 'apical initials,' however, would seem to imply (1) a group of cells showing a distinctive 'fixed or regular scheme of segmentation' and (2) a group of cells ultimately responsible for the initiation of all cells of the shoot apex." For the definition of initials, the second of these two criteria may be accepted without hesitation, but the first need not be accepted. Popham (1951) apparently regarded both criteria as necessary for the definition of initials. Yet the second criterion does not require that the summital cells be distinguishable from the adjacent cells on the basis of size, shape, and plane of cell division. The term apical initials may be used in a functional sense, i.e., to indicate a group of cells responsible for the initiation of the other cells of the shoot apex. It is precisely *because of their position* that these cells can furnish derivatives to regenerate the rest of the shoot apex. Position and function are the intrinsic properties of initials. The distinctive size and staining properties of the apical initials of young plants are only incidental to the concept that these cells are the initials of the shoot.

If the concept of initials is related to the ultimate origin of cells, rather than to the relative rates of cell division of different regions of the shoot tip, the concept of the *anneau initial* (Buvat, 1952; Gifford, 1954) should be rejected for *Isoetes*. The region of leaf formation in the shoot tip of *Isoetes* may be considered a more active region of growth than the shoot apex, but the shoot apex furnishes cells to replenish the region of leaf formation as the latter is used up in organogenesis. The region of leaf formation is not an independent region of the shoot tip, although the exact nature and frequency of contributions to this region from the apex cannot be estimated. Cell divisions can be found in the shoot apex, and because cells are contributed from the apex to the portions of the shoot tip involved in histo- and organogenesis, the shoot apex contains the initials of the shoot. The initials are a group of cells in the distal region of the apex. No claim is made that the apex itself is a a region of organogenesis. My interpretation of the concept of apical initials in *Isoetes* is in agreement with Bruchmann's (1874). The designation *initials* does not have to be applied only to cells which are mitotically highly active (Paolillo and Gifford, 1961).

Differentiation of tissues in the primary plant body. The purpose of the following discussion is to explain the structure of the primary plant

body of *Isoetes* in relation to the function of the shoot tip. To facilitate this discussion, a brief account of the tissues present in the primary plant body is given. Only large, well-grown plants are considered at this point. The differentiation of tissues in the primary body of young plants is described together with the differentiation of procambium to the leaf primordia.

As Lang (1915b) found for other species, the primary xylem of the protostele of *I. howellii* can be divided into a peripheral and a central part (pl. 7, A, and D at PX and CX). The boundary between the two portions of the xylem cannot be drawn with certainty, and the proportions of the two vary with the specimen. The number of tracheids in the peripheral xylem may be low (pl. 7, B) or high (pl. 7, C). The distinction between central and peripheral xylem in *I. howellii* and *I. nuttallii* is usually less marked than that in the species studied by Lang (1915b), and much less marked than in *Stylites* (Rauh and Falk, 1959b). Most of the cells of the central xylem are procumbent, whereas the cells of the peripheral xylem may be erect and may be arranged in radial files (pl. 7, A, B, C). Directly outside of the peripheral xylem, one to several cell layers differentiate as a parenchyma sheath (pl. 7, A, at Par S), and in contact with the parenchyma sheath is a layer of primary phloem (pl. 7, A, B, at PSE). When the primary sieve elements first mature, they are bounded by residual procambium and primary cortex, but directly below this level they are separated from the primary cortex by the cambium and cambial derivatives. During secondary growth, the primary phloem is usually obliterated.

An understanding of the differentiation of the tissues of the primary plant body may be obtained by relating these tissues to the growth of the shoot tip. The portion of the shoot tip under consideration has been divided into the shoot apex and the region of leaf formation. The apical initials of the shoot are located within the superficial layer of the shoot apex and contribute cells laterally to the superficial layer of the region of leaf formation and basipetally to the interior of the plant. The internal cells of the shoot apex enter into vertical and lateral growth of the shoot. The cells away from the axis of the plant grow to form the radial cell files which underlie the region of leaf formation and the bases of leaves. On and near the axis of the plant, the cells contributed by the shoot apex eventually add to the centrally located procambium of the stem. The procambium is seated upon the primary vascular tissues and has the form of a tapering mound or a blunt solid cone because there is considerable broadening of the procambium before its elements finally mature.

According to Lang (1915b) the primary phloem is the outer limit of

the stele. This arbitrary boundary need not be accepted because the cells inside and outside of the primary phloem may occur in the same radial files as the phloem elements (pl. 7, A, B). Radial seriation in the peripheral region of the xylem indicates that the presence of radial files of cells cannot be used to delimit the procambium if the peripheral xylem is considered a part of the primary plant body. Lang (1915b) himself has pointed out that cell files may be continuous from the peripheral portion of the xylem into the cortex. Bruchmann (1874) also reported that there is no sharp boundary to the procambium.

West and Takeda (1915) designated the outer portion of the procambium as the *parenchymatous mantle.* This mantle surrounds the xylary procambium and gives rise to the parenchyma sheath, the primary phloem, and the cambium. Rauh and Falk (1959b) recognized a similar layer, said to give rise to the parenchyma sheath and the primary phloem of *Stylites,* and called it the *primary meristem.* Although these authors maintained that this meristematic sheath arises in the cortex, such a distinction cannot be consistently made in their figures. In *Isoetes,* the meristematic layer comparable to that which gives rise to the primary phloem of *Stylites* is inseparable from the rest of the procambium. It is but a small step to change the terms parenchymatous mantle (West and Takeda, 1915) and the primary meristem (Rauh and Falk, 1959b) to *procambium.* The use of the term procambium is justified because (1) in ultimate origin there is no clear-cut discontinuity between the xylary core and the surrounding cell layers; (2) the leaf traces are attached to this tissue from the time they are formed; (3) the cells near the outer limit of this tissue differentiate as phloem.

Where leaf traces do not intervene between mature and immature tissues in a longitudinal section (pl. 7, A, at PSE), one may project upward the approximate limits of the procambium. The trajectory followed in this projection leads upwards and inwards from the location of the primary phloem. As best as can be determined by the size, shape, orientation, and staining properties of cells, the approximate boundary of the procambium can be followed to a level two or three cell layers below the surface of the region of leaf formation, and then it tapers abruptly toward the axis of the plant. In designating the primary meristematic tissues, one might call the tissue adjacent to the procambium the ground meristem. The procambium and ground meristem are not readily separable from one another because a common primary meristematic activity of the tissue below the shoot apex and region of leaf formation is responsible for additions to both the procambium and the ground meristem.

In a longitudinal section, the region of leaf formation is located

above the peripheral region of the procambial cylinder (pls. 6, A, B; 7, A, D). Therefore the designation of the tissue underlying the region of leaf formation as primary cortex (Hegelmaier, 1874; West and Takeda, 1915; Rauh and Falk, 1959b) is subject to criticism. The designation of this tissue as ground meristem is also inaccurate because at the same level in the plant the tissue near the axis of the shoot cannot be readily identified as procambium. The tissue intervening between the procambium and the surface of the shoot tip is histologically undifferentiated. Bruchmann (1874) reported that the upper limits of the procambium fade into an undifferentiated tissue which he called the "Urmeristem." This histologically undifferentiated region has special cytological features. In median longitudinal sections of specimens fixed in Regaud's fixative and stained with Regaud's hematoxylin, cells containing conspicuous, long mitochondria are found beneath and within the shoot apex (pl. 5, A, at M; opposite pl. 5). Unfortunately, the chondriome is not photogenic (pl. 4, C, D). The region of cells containing the conspicuous chondriome varies in depth and grades off below into a region where the chondriome is inconspicuous. This latter region coincides with the centrally located portion of the procambial cylinder. The lateral limits of the region containing the conspicuous chondriome underlie the region of leaf formation. As is the case for the procambium, there are no sharp boundaries for the region containing the conspicuous chondriome.

Centrally this undifferentiated tissue yields to the acropetal differentiation of procambium, whereas laterally it differentiates into the ground meristem, except where leaf traces are formed. At these locations, procambium is formed in the lateral position also (see later).

The advantage of using the above concepts is not so much in recording new or different observations as in allowing a coherent terminological treatment which clarifies the processes of differentiation. If the tissue underlying the region of leaf formation is not regarded as cortex, the cortex does not give rise to leaf traces and varying portions of what may rightfully be called the procambial cylinder. The problem is not one of mere semantics, because the designation of tissue within the shoot tip as primary cortex is not justified on the basis of the degree of morphological differentiation of this tissue. Moreover, the leaf traces arise in the tissue underlying the region of leaf formation, and are only subsequently carried away from the shoot apex. The tissue in which the leaf traces arise is the periphery of the histologically undifferentiated tissue below the surface of the shoot tip. Avoidance of the use of primary cortex to designate this histologically undifferentiated tissue can assist in a better understanding of the process of the differentiation of procambial leaf traces.

Differentiation of procambium to the leaves. A number of circumstances make it difficult to observe the differentiation of procambium to the youngest leaves of mature plants of *Isoetes*. First, the youngest leaves are crowded at the base of the apex, and the differentiation of procambium from the level of one leaf to that of the next occurs over a very small vertical height. This height may be no more than two cell layers. Second, a layer of parenchyma differentiates completely around the xylem of the leaf trace. At the insertion of the trace on the stele, the parenchyma cells of the xylem sheath are oriented with the axis of the leaf trace rather than with the vertical axis of the plant. In a section passing adjacent to a leaf trace, continuity of the axial vascular elements above and below the trace appears to be lacking. The leaf traces are numerous and closely placed on the stele. Consequently, only fortuitous longitudinal sections show the continuity of the primary phloem with the region of procambial differentiation to the youngest leaves (pl. 8, A).

A further difficulty arises out of the artifacts of sectioning resulting from the orientation of certain cells in the vicinity of the shoot apex. At the level of the mature stele, the cells of the prismatic layer are oriented so that their long axes are nearly vertical in the plant. A transverse section, therefore, gives an accurate idea of the radial dimensions of these cells (see opposite pl. 8, at 1). In the region of procambial differentiation, however, the cells are inclined inward so that a transverse section gives an exaggerated radial dimension for these cells (opposite pl. 8, at 2). The situation is further aggravated by the increase in the tangential dimensions of the cells on the periphery of the xylem core as the stele matures. At the periphery of the mature stele the tangential and vertical dimensions of cells are approximately equal, whereas in the region of procambial differentiation the tangential dimensions of cells are about one-half the vertical dimensions (opposite pl. 8, at 1 and 2). With their small tangential dimensions and their exaggerated radial dimensions, the cells in the region of procambial differentiation to the youngest leaves do not resemble the cells at the periphery of the mature stele in transverse sections, and their continuity with the latter may be easily overlooked.

Most of the accounts of procambial leaf traces in *Isoetes* give the impression that the initial course of the trace is upward and outward in the primary cortex. However, Liebig (1931) has mentioned that the traces are initially vertical in their orientation and assume an upward and outward course during later growth. The observations which are reported below support Liebig's opinion, and it may be suggested that other investigators have reported on advanced stages in the development of a trace rather than on the initial stages of its formation. The

reports of suspended leaf traces are, then, even more remarkable but are referable to a fourth difficulty which is encountered in the study of procambial differentiation.

The cells of the leaf traces undergo the changes typical of procambial differentiation. They become elongate and densely cytoplasmic before they begin to mature as xylem and phloem elements (pl. 7, D, at LT). The cells outside of the xylem core, which mature as the primary phloem of the stem and are in continuity with the first formed phloem of the leaf trace, do not elongate and do not become densely cytoplasmic before they mature as phloem (pl. 7, A, D, at PSE). If their phloic nature is accepted (see later) it may be argued that they are procambial at some stage in their development. However, it is not difficult to acknowledge that the contrast between the procambium of the leaf trace and the procambium of the axial phloem of the stem can give the impression of a suspended leaf trace, even when the two are actually observed in contact with one another, especially if the plane containing the leaf trace does not exactly coincide with the plane of sectioning (pl. 7, D, left side). The report of basipetal differentiation of leaf trace procambium in *Stylites* (Rauh and Falk, 1959b) may be explained in this way, for the figures offered in support of basipetal differentiation show only traces in advanced stages of development.

As Hegelmaier (1874) has reported, the tangential walls of the cells beneath the region of leaf formation slant inward (pls. 6, A, B; 7, A, D; 8, A). As seen in longitudinal sections, the cells of this region form a bridge for the differentiation of procambium from the mound of procambium below to the base of the forming leaf. The exact time, in relation to the ontogeny of the leaf, that these cells differentiate as procambium may be somewhat variable or even a matter for debate. Throughout the following discussion, the term procambium will be applied to those cells which may be distinguished by their position and degree of morphological differentiation in terms of form, staining properties, or both as the precursors of vascular tissue.

Procambium is differentiated to the base of an emerging leaf when the leaf is still very close to the apex. The procambium differentiates across the two or three cell layers intervening between the axial procambium and the base of the growing leaf primordium. The procambial strand is not clearly defined in longitudinal sections, even at the time the ligule mother cell is visible (pl. 8, B), but in transverse sections indications of traces may be detected to leaf primordia that do not show any sign of a ligule mother cell. The leaf trace follows a path determined by the tangential walls of the cells that compose it so that the trace is initially straight and stands nearly vertical or slants inward

depending on the specimen (pl. 8, B, at LT). A new trace lies on a tangent from the approximate edge of the pre-existing axial procambium. Differentiation of the axial procambium is acropetal. Whether or not differentiation of the trace is also acropetal is difficult to establish, because this differentiation takes place over two or three cell layers. No conclusive evidence has been obtained on this point.

Leaf traces are subjected to the radial displacement which occurs along all radii of the plant. Thirteen plastochrons elapse before a trace is formed directly inside an older trace. As radial displacement proceeds, the tissue that adjoins the inner side of a trace differentiates as ground meristem and then as cortical parenchyma. Meanwhile, the axial procambium expands laterally throughout, and the cells at its periphery become more erect. During this expansion, the base of a trace assumes an upward and outward course, but the upper part maintains nearly its original direction (pl. 8, C, D, E). As the radial files of cells of the ground tissue continue to grow, the upper part of the trace is moved further and further from the axis. The lower part of the trace assumes the direction of the radial files and keeps pace with the radial expansion of the shoot by intercalary growth as the base of the leaf is carried away from the axis of the plant. The trace becomes sharply bent below the base of its leaf (pl. 8, F).

When the trace is first established, the continuity of its tissues with those of the axial procambium is not strongly expressed, but changes in the orientation of cells are consummated before the tissues of the trace and stele mature. As the base of a trace assumes a more and more nearly horizontal position in the plant, its cells become more nearly aligned with other procumbent cells in the procambial cylinder (pl. 6, A, at CXLT). These latter cells form the connection between the xylary procambium of the trace and that of the procambial core, and their arrangement allows for the attachment of the trace throughout the peripheral region of the primary xylem (pl. 7, B, C, at CXLT). At the same time, meristematic activity continues on the abaxial side of the trace. The radially oriented files of cells which result from this activity contact the procumbent elements of the base of the leaf trace. These files are a manifestation of the general radial growth of the plant, but because of their location their component cells differentiate in a particular fashion. Some of these cells become the axial primary phloem, differentiating as sieve elements in contact with the first-matured sieve elements of the leaf trace (pl. 7, B, at PSE).

These phenomena may also be followed in cross sections, but a particular limitation of cross sections must be kept in mind. The radial files of cells near the shoot tip bend upward. A transverse section,

therefore, is an artificial plane of sectioning with respect to the growth of the plant. Instead of extended cell files, short portions of many files appear in transverse sections. Along a single radius in one section, portions of successively older files are encountered away from the axis of the plant. The arrangement observed is not as orderly as that found in longitudinal sections.

The photographs taken to illustrate the characteristic appearance of leaf traces of various ages in transverse sections (pls. 9-12) are from a single large plant. This particular series of sections, cut at 10 μ, is slightly oblique, but the majority of transverse series are somewhat oblique in their orientation. Because of this obliquity some leaf traces appear more distinct than others. The first level of sectioning illustrated (pl. 9, A) is 10 μ below the summit of the apex. All the visible transections of leaf traces and leaf primordia are numbered. The lowest number is given to the youngest primordium in the section. In this particular plant, P_4 is approximately 10 μ high and shows no indication of a ligule mother cell. All of the older primordia and leaves have multicellular ligules (pl. 9, A). The smallest of these is the two-celled ligule of P_5. In the next section (not illustrated), three younger leaf sites can be identified, and the youngest of these is called P_1 in this account. Whether this leaf site should be called P_1 or I_1 (first incipient primordium) is arbitrarily decided, because there is no sharp distinction between P_1 and I_1. A slight uplifting of the surface occurs coincident with anticlinal divisions in the superficial layer of the region of leaf formation and an increase in the stainability of the resulting cells. Thus, the initial indications of the formation of a leaf site are not readily separable from the changes in the contour of the shoot tip which accompany leaf initiation. It is reasonable, as well as convenient, to designate the youngest identifiable leaf site in transverse sections as P_1.

Ten μ lower, or 30 μ below the summit of the apex (pl. 9, B), the obliquity of sectioning is manifest. The level of sectioning in the lower part of the photo is higher in the plant than that in the upper part of the photo. The cell net below P_1 (pl. 9, B, at 1) is not very distinctive, but below P_2 (pl. 9, B, enclosed in hexagon) a distinctive group of cells may be identified. These cells are a part of the trace to P_2 (abbreviated TP_2). The traces along the arc from TP_4 to TP_5 (clockwise) are somewhat obscure because of the obliquity of sectioning. In this arc, the bases of cells of the superficial layer of the region of the leaf formation are probably represented, while the complementary arc (4-5 counter clockwise) contains cells of the histologically undifferentiated tissue underlying the region of leaf formation as well as leaf traces and ground meristem. In a section 10 μ deeper (pl. 10, A) TP_5 may be distinguished,

but TP_2 cannot be seen. The cells underlying TP_2 are indicated (pl. 10, A, at 2). These cells are on the perimeter of the procambial cylinder, and it can be seen that this perimeter passes inside TP_7, comes adjacent to TP_4, underlies P_1, adjoins TP_6, underlies TP_3, passes inside of TP_8, and adjoins TP_5. Its relationship to the traces in the arc 4-5 (clockwise) is somewhat obscured by the obliquity of sectioning. Ten μ deeper (pl. 10, B), TP_4 has disappeared in the perimeter of the procambial core, and TP_5 is on the perimeter of the procambial core. The shape of the cells of TP_5 in this section (pl. 10, B, at 5) resembles that of the cells of TP_2 at a level 20 μ higher (pl. 9, B, at 2). In plate 10, B, TP_6 still adjoins the perimeter of the procambial core. Twenty μ lower (pl. 11, A), TP_5 has disappeared in the perimeter of the procambial core. TP_6 and TP_7 are attached to this perimeter (pl. 11, A, at 6 and 7), and the cells at these locations resemble those at the attachment of TP_5 at the level 20 μ higher (pl. 10, B, at 5). Abaxial to TP_6, a radial file of cells (pl. 11, A, at arrow) of the ground tissue may be seen. The cells of this file differ in tangential dimensions from the cells of TP_6.

Thirty μ lower (pl. 11, B), the cells attaching TP_9 to the procambial cylinder (pl. 11, B, at 9) may be compared with their counterparts for TP_6 and TP_7 (pl. 11, A, at 6 and 7). Radial files of cells appear at the attachment of TP_{12} (pl. 11, B, at 12), and the base of TP_{12} adjoins TP_{17}, whereas the base of TP_9 adjoins TP_{14}. Ten μ lower (pl. 11, C), radial files of cells appear under the attachment of TP_{12} (pl. 11, C, at 12), whereas at a level an additional 20 μ deeper, the cells in this position (pl. 11, D, at 12) are confluent with similar radial files at the base of TP_{17} (pl. 11, D, at 17). Twenty μ deeper (pl. 12, A), TP_{20} is attached to the procambial cylinder, and radial files of cells are seen at this location (pl. 12, A, at 20) and under the attachment of TP_{17} (pl. 12, A, at 17). Sections 20 μ deeper (pl. 12, B), an additional 10 μ deeper (pl. 12, C), and again 20 μ deeper (pl. 12, D) show the attachment of TP_{28} and some younger traces. The field of view has been changed between plate 12, C, and 12, D, to show TP_{21} and TP_{26} which do not appear in plate 12, A, B, or C. Adjacent to the attachments of TP_{20} to TP_{28} (pl. 12, A, B, C, D), radial files of cells fill the bays between leaf traces. The inner members of these files mature as part of the peripheral xylem and often as tracheids (pl. 12, D, at 21). The cells outside of the xylem core mature as a sheath of parenchyma, and those outside of the latter become sieve elements. The over-all picture obtained is the same as that seen in longitudinal sections (pl. 7, A, B, C, D), except that the length of the radial files of cells cannot be fully appreciated in transverse sections because the files are tilted with respect to the horizontal plane (pl. 7, A, B, C, D).

Directly above the level of maturation of the conducting elements of the xylem and phloem, radial files of cells are distributed all around the procambial cylinder. At higher levels, the radial files are most pronounced at the bases of leaf traces (pl. 11, B, C, D, at 12). The highest mature phloem in the stele is also associated with the attachment of a leaf trace (pl. 12, A, B, at Ph, near 19). It may be suggested that the original sequence of initiation of leaf traces is followed acropetally by tangential divisions on the periphery of the axial procambium. These divisions occur while the bases of the leaf traces are assuming a horizontal position, so that the base of a trace is ultimately embedded in radial files of cells (pl. 8, F). Stokey (1909) interpreted parts of these files as the inner derivatives of the cambium and described the situation by reporting that there is an overgrowth of the leaf traces by the secondary tissues. In the present account these files are regarded as entirely primary to the level where the first axial sieve elements are matured (see later).

In plants that have a distichous leaf arrangement, the procambium to the youngest leaf primordium differentiates directly above the procambium to the second older primordium. The traces of the primordia are bent sharply and the cells of a given trace seem to be continuous with the cells of traces to primordia on the opposite side of the stem (pl. 13, A). Median longitudinal sections in the plane of the basal groove give the impression that the stele and procambial cylinder of the young plant is a miniature of that of the older plants (pl. 13, B). However, all of the mature and immature vascular elements, except those in the prismatic layer, are oriented with respect to the leaves and roots. In other words, practically all vascular elements participate in the formation of leaf and root traces (pl. 13, A). The process of differentiation of the procambium of the leaf trace could not be followed in the materials available. In all of the plants with distichous arrangement of the leaves, primordia which showed ligule mother cells also showed a clearly defined trace in longitudinal section. No suspended traces were found in any of these plants.

In longitudinal sections in the plane of the basal furrow, sieve elements are located to the left and right of the tracheids of the leaf traces (pl. 13, B, at PSE). It is noteworthy that sieve elements form in lateral positions in association with trace tissues. Away from the axis of the plant, the phloem of the trace is on the abaxial or underside of the trace. Near the axis, the phloem is found in two lateral groups. Only the tracheary elements reach the axis, and thus the integrity of the xylem core is preserved. The phloem of a trace in a plant with spiral phyllotaxy is abaxial throughout its extent and connects directly with

the axial sieve elements beneath the attachment of the trace to the stele.

When the phyllotaxy changes to spiral, the leaf primordia begin to form over regions that do not contain traces to older primordia. Eventually, median sections of leaf primordia may be seen in longitudinal sections taken in the plane of the basal furrow. From this time onward, the adult pattern of procambial differentiation is established, but the stele of the plant is still too small to show the differentiation of central and peripheral xylem. Because of the spiral insertion of leaves the newly formed portions of the stele assume a circular or polygonal cross-sectional outline. Tangential divisions at the periphery of the stele are more pronounced than in the younger plants. The stem has primary sieve elements which are not directly a part of the leaf traces, although all of the tracheids which differentiate are members of leaf traces. The obconical growth of the stele increases the cross-sectional area of the xylem core so that leaf traces no longer account for all of the tracheids that are formed in the stem. For the specimen in which the transition to this condition was found, the xylem core measured 270 μ across at the level of the highest mature tracheids and 540 μ in height from the level of the tracheids of the original leaves of the plant to the level of the highest mature tracheids. In contrast, the width of the combined traces of the first few leaves is about 35 μ, and that of the xylem core of a large plant may be 1,400 μ or more. The height of the tallest leaf-bearing stele observed exceeded 7,000 μ (7 mm.).

Esau (1954) has interpreted the *foliar* portion of the vascular tissue of the shoot to be that portion which differentiates with a definite positional relationship to the leaves. *Cauline* vascular tissues do not show a definite positional relationship to leaves. The terms cauline and foliar have been applied to the stele of *Isoetes* in this sense throughout the literature. In *I. howellii,* all of the procambial tissues and all of the mature primary vascular tissues of the shoot show a positional relationship to leaves as long as the plant is in a distichous phyllotaxy. After the spiral phyllotaxy is established, the leaf traces become separated enough so that some primary cauline phloem is formed. The formation of cauline tracheids is not accomplished until later in ontogeny, when the primary xylem core has increased in diameter much beyond that of the sporeling.

It has been suggested (Lance-Nougarède and Loiseau, 1960) that the pith of higher plants is a cauline tissue region. Rauh and Falk (1959b) considered the central region of the primary xylem core of *Stylites* as the homolog of the pith in higher plants. Lang (1915b) maintained both that the central xylem of *Isoetes* is wholly cauline, and that part of the peripheral xylem among the attachments of the leaf traces

can be considered cauline. Wetmore (1943) has reported that in certain protostelic lycopods a central column of cauline procambium may be identified below the shoot apex and that this column may even ascend above the level of the youngest leaf trace. In all plants of *I. howellii* that have a spiral phyllotaxy, there is a core of procambium that initially shows little relationship to the leaves. As differentiation and maturation of the leaf traces proceed, portions of the procambial core assume a positional relationship to the leaves. In large plants, this positional relationship does not extend to the center of the xylem core. In smaller plants, the leaf traces may account for all the tracheids of the shoot, even when indications of an axial core of procambium are present (pl. 2, A, at Pr).

Scott and Hill (1900) reported centripetal maturation of the xylem core of *I. hystrix.* West and Takeda (1915) reported that xylem maturation begins at the center of the stem in *I. japonica,* but Ogura (1938) recognized a parenchymatous pith in the same species. Lang (1915b) located the protoxylem of the shoot at the attachments of the leaf traces, i.e., at the inner limit of the peripheral xylem. In *Stylites* (Rauh and Falk, 1959b), the maturation of the first tracheids at the inner limit of the peripheral portion of the xylem is easily seen because of the marked distinction between central and peripheral xylem. In *I. howellii,* the highest tracheids of the xylem core are associated with the bases of leaf traces (pls. 7, A and D, at T; 12, C, at T at 22). These locations indicate the inner extent of the peripheral xylem. The location of the first mature xylem elements is a reflection of the degree of development of the central portion of the xylem. In other words, centrifugal maturation of the xylem would be expected when no central portion of the xylem core is recognizable. It may be suggested, therefore, that the apparent direction of maturation varies with the age and vigor of the specimen investigated. This type of variation might account for the reports of centrifugal (West and Takeda, 1915) and centripetal (Scott and Hill, 1900) maturation of xylem in *Isoetes.* Any designation of the direction of maturation that can be made is subject to the reservation that maturation throughout the xylem core of *Isoetes* takes place over very few cell layers (pl. 7, A, D). In *Stylites,* where the shoot is generally more elongate than in *Isoetes,* the maturation of primary xylem is completed over many cell layers (Rauh and Falk, 1959b).

The cambium and secondary growth. The cambium is composed of two parts: the lateral meristem and the basal meristem (fig. 1A). In this section of the report, the term *cambium* designates the lateral meristem only.

There is some difference of opinion on the place of origin of the

cambium in mature plants. In one view, the first tangential divisions around the core of xylary procambium are recognized as cambial (Scott and Hill, 1900); in another, the cambium differentiates outside the primary phloem at a level where the latter is mature (Lang, 1915b; West and Takeda, 1915). The second view has been adopted in this study. The early divisions around the core of xylary procambium are regarded as part of the growth of the procambium. If one adopts this interpretation and recognizes that there is no clear-cut boundary between the procambium and the adjacent ground meristem, then it is possible to designate the layer of cells outside of the primary phloem as part of the procambium. According to this view, the cambium would arise from part of the procambium (cf. West and Takeda, 1915). Primary growth passes over to secondary growth in a continuous process of radial expansion. Cambial activity represents a sustained meristematic activity in a particular part of the procambium. Esau (1953, p. 381) has remarked that "The procambium and the cambium may be looked upon as two developmental stages of the same meristem." This general statement can be applied to *Isoetes*. However, the cambium of *Isoetes* arises outside of the primary phloem, so that it is anomalous in its place of origin, as well as in its subsequent activity. Nonetheless, the primary plant body of *Isoetes* is directly comparable to that of other protostelic species (cf. Lang, 1915b). A core of xylem is surrounded by a sheath of parenchyma that is in turn surrounded by the primary phloem.

In the higher plants, tangential divisions that produce radial files of cells may occur high in the procambium with respect to the level of mature tissue (Esau, 1953, p. 381). Radial files may exist in bundles where no secondary growth occurs, as in *Zea* (Esau, 1943). Thus, radial seriation alone is not a reliable criterion for determining the presence of a cambium. In *Isoetes*, the first tangential divisions around the core of xylary procambium occur at a level higher than the level of mature vascular elements, and Scott and Hill (1900) regarded these divisions as cambial. It is more fruitful and convenient to recognize these divisions as part of the primary thickening of the plant (Lang, 1915b; Rauh and Falk, 1959b) rather than to designate them as a manifestation of cambial activity.

The cambium may be regarded as arising at the level at which the primary phloem matures. This is a natural definition, because the sieve elements which appear in the primary axial phloem differentiate centripetally (cf. Rauh and Falk, 1959b). Then, by definition, the sieve elements which are added abaxial to the first formed sieve elements (centrifugally) are secondary, but there is no other morphological boundary between primary and secondary phloem.

To understand the origin of the cambium during the ontogeny of the sporeling one must have a clear understanding of the arrangement of the vascular tissues while the plant has only a few leaves (see fig. 2). The xylem and the phloem of the first leaf is continuous with the xylem and the phloem of the first root. The vascular bundles of both of these organs are collateral, and the phloem is on the abaxial side of the bundles. Tracheids differentiate across the axis of the plant to the second leaf and the second root. The phloem of the second leaf and root is also abaxial. The phloem of the first leaf and root stands in relation to that of the second leaf and root as one side of the letter *H* stands in relation to the other, with the connection between the two sides being formed by sieve elements which are located out of the median sagittal plane of sectioning, for median longitudinal sections in the plane of the basal groove show that there are phloem elements to the left and right of the axial tracheids in the young plant (pl. 13, B, at PSE). Phloem differentiates in these locations when the trace of the second leaf matures. Additional sieve elements differentiate next to the abaxial face of the first-formed sieve elements in this position (pl. 13, B, at SSE). By the time the plant has several leaves, radial seriation of cells is noted in sections in the furrow plane (pl. 13, B). Such seriation is not pronounced in the plane containing the leaf traces because the traces are so closely placed that they adjoin one another with little intervening tissue (pl. 13, A).

One has two alternatives in the treatment of the first sieve elements formed in the lateral positions in sections in the plane of the basal furrow: they may be designated as primary or as secondary phloem. If the cambium arises in the layer of parenchyma surrounding the first tracheids (Hofmeister, 1862; Stokey, 1909) the lateral sieve elements are secondary. As a consequence, the cambium would be active in the plant before two leaf traces have matured and it would contribute cells to the leaf traces. On the other hand, if the lateral elements which are first formed are considered primary, then a parallel between the youngest and the oldest plants is immediately evident. The innermost and most acropetal axial sieve elements may be regarded as primary (pl. 13, B, at PSE). The choice between these two alternatives is of no import to the phenomena discussed, but designating the first lateral sieve elements as primary allows a consistent treatment of the description of the primary plant body throughout ontogeny. From this perspective the cambium is interpreted as arising in the procambium outside of the primary phloem. This interpretation is also consistent for sporophytes of all ages. Stokey (1909) reported that the cambium arises in the layer of parenchyma surrounding the xylary core, and this description, too,

could be consistent for plants of all ages. The matter once again resolves itself into a choice of a point of view. The perspective that has been adopted in this report allows a direct comparison between the primary plant body of *Isoetes* and other protostelic forms (cf. Lang, 1915b) and has been chosen for that reason.

When the plant is young, the inward derivatives of the cambium differentiate as sieve elements only (pl. 13, B, C, at SSE). In larger plants of *I. howellii*, the inner derivatives of the cambium may differentiate as layers of sieve elements alternating with layers of parenchyma (pl. 13, D), and some or many of the cells in the layers of parenchyma may differentiate as tracheary elements (pl. 13, E, at ST). In *I. nuttallii*, the prismatic layer consists almost entirely of secondary sieve elements, with few parenchyma cells.

The tracheids of the secondary xylem of *I. howellii* occur both in small groups and as extensive layers of cells, depending on the specimen examined. Secondary xylem has not been found in very small plants, and is absent in even some very large plants. The tracheids which occur in the layers of parenchyma may replace the parenchyma entirely in some locations. The presence of secondary tracheids is dependent on the presence of alternating layers of secondary sieve elements and parenchyma, but in some plants where layering occurs, no secondary tracheids are found. The pattern of formation of tracheids does not appear to be fixed from layer to layer, and one cannot use the position of tracheids in one layer to predict the position of tracheids in another layer.

Rauh and Falk (1959b) have reported that there are at present no diagnostic studies on the origin of secondary tracheids in *Isoetes*, and no comparable tissue occurs in *Stylites*. They have suggested that the explanation of the occurrence of layers of secondary tracheids in *Isoetes* might be the same as for alternation of xylem and phloem in *Beta*, i.e., multiple cambia. Investigations on *I. howellii* indicate that this is not the case. Maturing tracheids may be found nearly in contact with the inside of the cambial region (pl. 13, F). More often, the cambium surrounds a layer of parenchyma located outside of a layer of sieve elements, or it surrounds a layer of sieve elements located outside a layer of parenchyma that contains tracheids. Because of the unpredictability of the pattern of maturation of the secondary xylem elements, it is impossible to relate these observations directly in a sequence of maturation. However, because there are no indications that phloem maturation occurs outside of the cambium, no similarity to the multiple cambia of *Beta* is apparent.

Based on present evidence, the following sequence is proposed. The

cambium produces a layer of parenchyma. Some of these cells begin to mature as tracheids while the cambium produces a layer of sieve elements. As the latter elements mature, maturation of the tracheids continues. Whether or not the cells differentiating as tracheids differentiate first as parenchyma cells has not been determined. It appears that at least some of the cells which differentiate as tracheids are distinct from parenchyma cells at their formation. As far as the order of maturation within a parenchyma band is concerned, immature tracheids have been found (1) inside, (2) outside, (3) both inside and outside of mature tracheids within a single file of cambial derivatives. All of these variations can be found in one plant.

Scott and Hill (1900) reported multiple cambia in *I. hystrix.* They wrote that sometimes a normal cambium differentiates around the xylem core, and that this cambium contributes secondary xylem inwardly. This first-formed cambium is said to be supplanted by another cambium, which is anomalous in its activity and which arises outside of the first-formed axial phloem. They interpreted the presence of the normal cambium inside of the first-formed axial sieve elements to mean that these sieve elements are primary, whereas if the cambium is anomalous from the start, these first sieve elements are secondary. If the perspective used in this study is applied, the situation can be explained more simply. Scott and Hill interpreted the first tangential divisions around the procambial xylem core as cambial divisions, but I have interpreted them as part of the growth of the procambium (cf. Lang, 1915b; West and Takeda, 1915). According to my interpretation, the "secondary xylem" formed by the "normal cambium" comprises late-maturing primary tracheids differentiating in the part of the procambium which normally matures as cells of the xylem parenchyma sheath (pl. 13, G, at LMT). Lang (1915b) has already pointed out this possibility in comparing his observations on *I. lacustris* with those of Scott and Hill on *I. hystrix.* If it is held that the cambium always arises outside of the primary (first-formed) axial sieve elements, all of the cells from the cambium inward originate from procambium, regardless of the presence or absence of late-maturing primary tracheids.

Similar late-maturing primary tracheids occur in *Stylites.* Rauh and Falk (1959b) have described these tracheids as secondary xylem arising from some of the derivatives of the primary meristem. The other derivatives of this meristem differentiate as primary sieve elements and parenchyma cells. The so-called secondary xylem arises centripetal to the primary phloem, and is appressed to the peripheral primary xylem in the location where the parenchyma sheath might otherwise differentiate. The use of the term secondary for these tracheids is a misnomer,

because they correspond to the late-maturing primary tracheids that may be formed in *I. howellii* and in other species of *Isoetes* (Lang, 1915b). Scott and Hill (1900) and Rauh and Falk (1959b) have arrived at the same name, secondary xylem, for these late-maturing primary tracheids, although their interpretations of the origin of these cells are different.

Scott and Hill (1900) also reported that in two specimens they found evidence that a second anomalous cambium had arisen inside of the first. Both of the cambia in each plant produced sieve elements, parenchyma, and tracheids on their inner faces. Therefore, these multiple cambia in *I. hystrix* cannot be compared directly with the multiple cambia of *Beta*, because in *Beta* each cambium produces xylem toward the inside and phloem toward the outside.

As Lang (1915b) has noted, secondary xylem may be in contact with the abaxial face of the primary phloem. A layer of tracheids embedded in parenchyma may be found external to the first-formed sieve elements (pl. 14, A). Lang (1915b) suggested that such an occurrence provides a convenient way of showing the transition from primary to secondary growth, because at this location the first secondary elements are tracheids, whereas the primary phloem consists of sieve elements. When the first secondary vascular elements are sieve elements, there is no visible boundary between mature primary and secondary elements (pl. 14, A, at PSE + SSE).

Mature secondary tracheids are easily identified. They are empty and their secondary wall thickenings are in the form of rings and helices. They resemble the tracheids of the primary xylem and, except for size and form, also the tracheids of the leaf and root traces. The recognition of secondary sieve elements in the cambial derivatives seems to have caused considerable difficulty for some workers. The secondary sieve elements resemble the primary axial sieve elements in all details and, except for shape and size, also the sieve elements of the leaf and root traces. The cytological characteristics which are discussed below pertain to all of the sieve elements of the plant. The sieve elements of the prismatic layer, which are cambial derivatives, are singled out for discussion because it is the phloic nature of these elements that has been denied.

Russow (1872) recognized sieve elements in the prismatic layer. Esau *et al.* (1953) and Lamoureux (1961) have demonstrated the presence of callose in this tissue by using modern techniques (Cheadle *et al.*, 1953). Stokey (1909) reported that all of the elements of the prismatic layer which do not mature as parenchyma or as tracheids are actually immature tracheary elements. She based her conclusions mainly on wall

characteristics, and she failed to detect callose in any cells of the prismatic layer with the use of aniline blue. Lamoureux (1961) investigated both *I. howellii* and *I. nuttallii* and demonstrated callose deposits in the sieve elements of the prismatic layer. The latter of these two species was investigated by Stokey (1909). Both of these species have been used in the present report. My observations support those of Lamoureux (1961), which indicate the presence of sieve elements in the prismatic layer.

The most cogent argument which may be advanced in support of the phloic nature of some of the elements of the prismatic layer is based upon several lines of evidence taken in unison. First, callose is deposited on thin areas in the walls. These deposits can be detected with the aniline blue fluorescence techniques (pl. 14, B) described by Currier and Strugger (1956), and with resorcin blue (pl. 14, C) by the method of Cheadle *et al.* (1953). The callose deposits may become definitive and fill the lumen of a cell (pl. 14, D). Second, the cytoplasm of the sieve elements appears clearer, and the nuclei are smaller and more chromatic than those of the parenchyma cells (pl. 14, E). Third, there is continuity between the prismatic layer, the primary axial phloem, and the phloem of leaf traces (pl. 7, B, at PSE). All of these observations have been reported in the literature, and the evidence offered in this report is only confirmation of earlier accounts (cf. Lamoureux, 1961).

Weber (1922) waged the most comprehensive attack on the arguments given above. He reported that the thin areas on the walls of the sieve elements are not different from the pits of parenchyma cells. He also applied a battery of chemical tests to the definitive callose deposits and concluded that callose, cellulose, pectic substances, and proteins are present in these deposits. He argued that the presence of protein disqualified the cells containing the deposits as phloem. He further pointed out that callose is not unique to sieve elements. The last observation is supported by recent studies (Eschrich, 1956; Currier, 1957). In *I. howellii*, I have found callose in the ligule (pl. 14, F) and in parenchyma cells elsewhere in the plant (pl. 14, G, H).

The conclusion offered by Weber (1922) is that the putative sieve elements are only a specialized type of parenchyma cell. This alternative designation of the sieve elements may be only a matter of terminology. But Weber's arguments are subject to criticism on other grounds. The callose deposits in the parenchyma of the leaf and root and in the parenchyma of the primary xylem core of the stem are found in cells affected by disruptive growth. Large air cavities form in the leaf and in the root. The cells of the ligule separate as the ligule grows. The xylem core is stretched laterally by the continued expansion of the

primary meristematic tissues above the mature stele so that large intercellular spaces form in the xylem core. It is possible that injury caused during disruptive growth could induce the formation of callose in the affected cells. The sieve elements accumulate large quantities of callose, even though they are *not* subjected to disruptive growth of the type found in the ligule and in the other places where I have found callose in *I. howellii*. The callose deposits found in sieve elements begin as small accumulations on the thin areas in the walls and appear when no such deposits are present in the adjacent parenchyma cells of the prismatic layer. Together with the accumulation of callose, the sieve elements show a clear cytoplasm, chromatic nuclei, and thicker walls than parenchyma cells (cf. Rauh and Falk, 1959b). This combination of characters distinguishes the sieve elements of *Isoetes* from parenchyma cells and identifies them with the sieve elements of other plants. In his arguments, Weber (1922) has pointed out that *individually* the characteristics of the sieve elements do not offer conclusive evidence for their phloic nature. However, it is the combination of characteristics which makes the recognition of sieve elements possible.

Weber regarded his observations on the impurity of the callose masses as evidence against the presence of sieve elements. The concept of sieve elements, however, does not require that the callose accumulated must be pure. Weber's (1922) observations are instructive for the understanding of the composition of the definitive callose masses, and several new observations may be added to them. The following discussion applies only to large masses of callose. The fluorescence reaction and resorcin blue staining are the only reactions which yield positive results with both large and small deposits of callose in *I. howellii*.

When stained in the PAS reaction, large masses of callose are colored slightly more than by Schiff's reagent applied after no hydrolysis treatment. Callose is supposed to be a β-1, 3 glucan (Kessler, 1958), and periodic acid should form aldehydes only on the end groups of the callose chains. A weak staining reaction with Schiff's reagent would then be expected. If cellulose and/or pectic substances are present as impurities in the callose masses, they may add to the staining reaction. My attempts to identify pectic substances with ruthenium red and with the Prussian blue precipitate (Rawlins, 1933, pp. 43-44) have yielded no conclusive results.

Mercuric bromphenol blue (Mazia *et al.*, 1953) has been used for a comparison with the impressive list of reactions for protein used by Weber (1922). This compound stains the definitive callose masses of *Isoetes* a deep blue or purple. To determine whether or not the callose deposits of other plants also stain with mercuric bromphenol blue,

trumpet hyphae of *Nereocystis* were stained. The callose plugs in these hyphae stained deeply with both mercuric bromphenol blue and resorcin blue. Parker and Philpott (1961) have reported, without comment, that mercuric bromphenol blue stains the callose plugs in trumpet hyphae of *Macrocystis*. E. M. Engleman (personal communication) has stained the callose deposits of the sieve elements of *Impatiens sultani* with mercuric bromphenol blue. If this dye colors only protein in the callose deposits of plant cells, protein may be mixed with callose in a variety of plants. On the other hand, mercuric bromphenol blue may stain callose deposits because it actually stains callose as well as proteins in plant cells. The extent to which callose gives a positive reaction with mercuric bromphenol blue and with the tests for protein used by Weber (1922) remains an open question. At present, Weber's tests stand as substantial evidence for the presence of protein in the callose deposits of *Isoetes*.

The definitive callose of *Isoetes* stains with an aqueous solution of pyronine Y. The walls of the sieve elements, starch grains, and secondary walls of the tracheids give a similar staining reaction. It is not suggested that these staining reactions indicate the presence of RNA, because Tepper and Gifford (1962) found that removal of RNA failed to inhibit the affinity for pyronine Y in secondary walls and in the hila of starch grains of *Chenopodium album*. Further tests are required to explain the affinity of various substances for pyronine Y. Other dyes which stain the definitive callose of *Isoetes* are chlorazol black, crystal violet, hematoxylin, and safranine (cf. Foster and Gifford, 1959, p. 175). The staining reactions carried out to date do not contradict Weber's idea that callose, pectic substances, cellulose, and proteins are present in the definitive callose masses. However, the strong reaction of definitive callose with such reagents as mercuric bromphenol blue and pyronine Y point out the difficulty of interpreting microchemical tests. Careful and extensive investigations with enzyme and chemical hydrolyses are required before a comprehensive appraisal of the composition of the definitive callose of *Isoetes* can be made.

Attention will now be given to the matters of the external derivatives of the cambium and the proportion of secondary and primary cortex in a plant at any time. If one adopts the view that the first tangential divisions around the core of xylary procambium are cambial (Scott and Hill, 1900), then radial files of cells very close to the apex would contain secondary elements and the multiplication of these elements would produce a large quantity of secondary cortex, even above the level of maturation of the xylem and phloem. When the cambium is said to arise outside of the mature primary axial phloem (Lang, 1915b), a wide

primary cortex surrounds the cambium where it originates. It is in this context that the question of the relative amounts of secondary and primary cortex is real.

Lang (1915b) has postulated that most of the cortex found on a plant at a given time is formed by the extension of the primary cortex. His hypothesis is based on the discovery of plants in which little or no secondary vascular tissues had been formed by the cambium. He believed that the sparcity of secondary vascular tissues indicated an inactivity of the cambium. Because the cortex was no smaller in these plants than in plants where a large prismatic layer was present, he concluded that the cambium does not produce much of the cortex. An alternative explanation would be that the cambium was unifacial in the specimens showing little or no secondary vascular tissues, so that derivative tissues were formed on only one side of the cambium.

West and Takeda (1915) have suggested that all of the primary cortex is soon replaced at the level of secondary growth. It has also been suggested that secondary cortex replaces the cortical tissue which is sloughed off at the outer extremities of the plant at seasonal intervals (von Mohl, 1845). It cannot be denied that there is a rapid loss of cortical cells by sloughing in many species of *Isoetes* (von Mohl, 1845; Eames, 1936, p. 55). However, at the cellular level there is no clear distinction between the primary and secondary cortex of *Isoetes,* and, as Lang (1915b) has reported, the leaf and root traces are stretched throughout their lengths and not only at the level of the cambium. Were the latter the case it might indicate that growth were restricted to the region of the cambium.

Figure 41 given by Eames (1936, p. 55) is drawn as if the traces to leaves and roots were broken in the region of the cambium during the season in which their respective leaves and roots become nonfunctional. To this extent, the figure is incorrectly drawn. Traces to old leaves and roots may be detected throughout the cortex after the leaves and roots have been lost from the surface of the plant (pl. 15, A), and these traces may remain functional because their sieve elements do not become crushed or blocked during at least the first season after a leaf or root separates from the plant (pl. 15, B; cf. Hofmeister, 1862). Eventually, a trace is stretched so thin that all remnants of it are lost at the level of the cambium. The cambium grows over the stump of the trace (cf. Hofmeister, 1862) and covers it with secondary vascular tissues. From the time the trace is broken at the level of the cambium, the newly produced secondary cortex includes no remnant of the trace. Thus, if the cortex can be shown to have no remnant of leaf traces at a given level, it is entirely secondary at that level. Near the bottom of a leaf-bearing

portion of the stele of one old plant no remnant of leaf traces was found in the cortex. The height of the leaf-bearing portion of the stele of this plant exceeded 7,000 μ. At a level between 2,000 and 2,500 μ below the uppermost mature tracheids, the leaf traces were stretched so thin that they were hardly perceptible. At the lowest level of the leaf-bearing portion of the stele, the cortex of this large plant was all secondary, beyond a doubt.

Among the many plants examined, few were tall enough to give any indication that the oldest leaf traces had been stretched beyond recognition. However, another line of evidence can be used to judge the production of a secondary cortex. Farmer (1890) has pointed out that the effect of radial or near radial divisions in the cambium can be traced in the derivatives of the cambium. But cell files may be followed away from the cambium for only a fraction of the total width of the cortex, because the formation of intercellular spaces with the rounding-off of cells near the central region of the cortex destroys the continuity of cell files. In one plant of *I. howellii* (pl. 15, C, D) I observed a transition from one cell to two cells separated by a radial wall, on the inside of the cambium (pl. 15, D, from Par to SSE). The number of cambial cells matched the number of sieve elements (pl. 15, D, at LM), and three boundaries could be followed outward from the radial walls of these cambial cells (pl. 15, C, D, at a, b, and c). The middle boundary could be followed for many cell layers into the cortex (pl. 15, C, at b) but only two cell layers into the secondary vascular tissue. The conclusion is that the quantity of secondary cortex produced during a given time is greater than the quantity of secondary vascular tissue produced during the same time.

The peripheral cells of the cortex become emptied of reserve materials. In some specimens, a broad, lightly staining region of cells occurs on the periphery of the plant (pl. 15, A, at NEC). At the very edge of the plant, a corky layer is formed by the compaction of the surface cells. No cork cambium can be identified, but indications are that the cells undergo divisions before they die, and the increase in cell number assists in making the peripheral tissues compact (pl. 15, E). The effect is much like that obtained with the so-called storied cork of some monocotyledons (Esau, 1960, pp. 151-152). West and Takeda (1915) reported a cork cambium in the region of the cortex where the basal furrow forms. The cells of this region become quite long in response to the tensions which cause the ultimate tearing of cells in the formation of the furrow. Division figures may be located among these cells (pl. 15, F, arrows). However, no generative layer can be recognized, and the designation of a cork cambium is not justified.

SUMMARY AND CONCLUSIONS

The shoot apex of *I. howellii* originates with the advent of the second plastochron. During the second plastochron the apex remains topographically indistinct from the second leaf. In the third plastochron the form of the apex begins to resemble that of the apexes of older plants, but is subject to plastochronic changes until the spiral phyllotaxy is well established. As the plant matures, plastochronic variations in the form of the apex subside and disappear. The apex continues to enlarge and shows a tendency toward broadening as the plant ages. While the plant is still small, the apical initials may be larger and lighter staining (after treatment with some dyes) than the adjacent cells. In older plants, the superficial layer of the apex stains uniformly, and the apical initials may be designated only on the basis of their position in the apex. The superficial layer of the apex stains more deeply than the underlying tissues in plants of all ages. Cytologically, the superficial layer of the apex resembles the superficial layer of the region of leaf formation and young leaf primordia. Leaf primordia show greater basophilia than the shoot apex.

The apex contains a group of apical initials from the time the apex can be distinguished. At about the time the phyllotaxy of the plant changes from distichous to spiral, the form of the apex lends itself to domination by a single cell of the original apical group of initials. There is no evidence that this configuration persists, and the idea that an apical cell, in the sense of Hofmeister (1862), exists throughout the life of the plant cannot be supported. The superficial layer of the apex contributes cells laterally to the superficial layer of the region of leaf formation and inwardly to the rest of the shoot tip. The internal derivatives grow vertically and laterally to form a region of histologically undifferentiated tissue, which yields to the differentiation of the procambial core from below and to that of leaf trace procambium and ground meristem laterally. Leaf traces differentiate to primordia as young as P_2, and initially are vertical or slant inward to the primordia. The horizontal course of the lower portion of the trace is a consequence of radial expansion of the shoot.

The primary plant body of the shoot consists of a core of primary xylem, a xylem parenchyma sheath, primary phloem, residual procambium, and primary cortex, as well as leaf traces and leaf tissues. The cambium arises in the layer of procambium remaining outside of the primary phloem and gives rise to secondary vascular tissue from its inner face. The secondary vascular tissue (the prismatic layer) consists of sieve elements, parenchyma cells, and tracheids in variable proportions, depending on the species, age, and size of the plant as well as on un-

known conditions. Externally the cambium produces a secondary cortex. The immediate outer derivatives of the cambium are meristematic, and meristematic activity continues for some distance from the cambium. The secondary cortex ultimately replaces all of the primary cortex, but the proportion of primary to secondary cortex in a plant at a given time is not easily determined.

THE ROOT-PRODUCING MERISTEM

REVIEW OF THE LITERATURE

The location of roots and the sequence of root initiation with respect to the root-producing meristem. Von Mohl (1845) pointed out that in a two-lobed specimen the youngest roots arise in two lines that are parallel to the root-producing meristem. One line of the two is displaced toward each lobe of the plant because the root-producing meristem is extended in the plane of the basal furrow. New roots continue to arise from the furrow as the older roots are displaced from the median plane of the plant. Thus, the order of root initiation of successive lines of roots appears to be basipetal with respect to the shoot apex, or acropetal with respect to the root-producing meristem. Von Mohl felt that this sequence of root initiation set *Isoetes* apart from other vascular cryptogams, where the sequence of root initiation is acropetal with respect to the shoot apex. Hofmeister (1862) investigated the sequence of root initiation within each line, or series, of roots and found that for each series, the roots nearest the axis of the plant are the oldest, and those that are nearest the ends of the furrow (nearest the shoot apex) emerge last. This sequence of initiation was said to unite *Isoetes* with the other vascular cryptogams.

Both Hofmeister (1862) and von Mohl (1845) reported on *I. lacustris*, but each had emphasized a different aspect of the initiation of the roots. There are two sequences of root initiation that must be considered: this point is essential to an understanding of the literature. The sequence emphasized by von Mohl (1845) is the sequence of initiation of successive lines, or series, of roots. The sequence emphasized by Hofmeister (1862) is the sequence of initiation of roots within a series.

Scott and Hill (1900) reinvestigated the sequence of root initiation, and they determined that Hofmeister's (1862) report was correct. They wrote that "the relative age of roots could be determined by their state of development, the oldest root-traces having their vascular tissues more

or less obliterated, while the youngest were still wholly meristematic, and all the intermediate stages were represented." No proof was given that the state of development of a root is an indicator of its age. This was assumed to be true.

Stokey (1909) endorsed the idea that the sequence of initiation within a series of roots is acropetal with respect to the shoot apex. Lang (1915a) expressed some hesitancy, but also accepted this point of view. West and Takeda (1915) made a radical departure from Hofmeister's concept when they claimed that all the roots of a given series are initiated simultaneously. To explain the emergence of roots of a series in a sequence that is acropetal with respect to the shoot apex, they argued that "It so happens that the nearer the long axis of the corm a root is differentiated, the narrower the zone of cortical parenchyma it has to traverse before reaching the surface; it follows, therefore, that in any given series, all the roots of which are differentiated simultaneously, that the root which has to travel the shortest distance before reaching the surface . . . will be the first of that series to emerge from the cortex." West and Takeda (1915) carried out their principal investigations on *I. japonica* and applied the results to *I. lacustris,* which had been studied by von Mohl (1845), Hofmeister (1862), and Stokey (1909). The latter two authors had come to the conclusion that the sequence of initiation within a series is acropetal with respect to the shoot apex.

Schoute (1938) asserted that West and Takeda (1915) offered no evidence in support of their statement that all the roots of a series are initiated simultaneously. He also asserted that their explanation that the acropetal sequence of emergence results from the variable thickness of the cortex is contradicted by the drawings of longitudinal sections in the furrow plane, as given by von Mohl (1845) and Lang (1915a). Schoute considered that the concept of acropetal origin of the roots should be reinstated. However, the view of West and Takeda (1915) that all roots of a series are of the same age has become established in recent textbooks on plant morphology (Eames, 1936, pp. 52-53, 56; Foster and Gifford, 1959, p. 174).

Eames (1936, p. 56) has reported that each series of roots is "uniformly placed" with relation to the older series, so that members of successive series line up to form rows. Hence, each root, or each root trace, occurs in two lines. The first of these is the *series* of initiation parallel to the basal furrow. The second is perpendicular to the first and has been called an *orthostichy* by Lang (1915a). Little emphasis has been placed on the orthostichies of roots, although they are amply illustrated in the literature (von Mohl, 1845; Hofmeister, 1862; Scott and Hill, 1900; Stokey, 1909; Lang, 1915a; West and Takeda, 1915; Liebig, 1931).

Schoute (1938) asserted that each new series of roots "forms its members without any relation to the roots of the previous row; there is no alternation or juxtaposition to be observed; such a spatial relation moreover would hardly be expected in view of the different ages of adjacent roots (of the different series)." Schoute (1938) cited figures given by Scott and Hill (1900) in support of his assertion, but these figures (Scott and Hill, 1900, text figs. 11, 12) do not support his opinion, because series and orthostichies are represented in both figures.

The morphological nature of the root-producing meristem. One hundred and twenty years ago, von Mohl (1845) proposed three alternatives for the designation of the root-producing meristem of *Isoetes:* (1) the basal meristem is a short main root, (2) is some type of primary meristem on a par with the apical meristem of the shoot, or (3) is a part of the cambium. Von Mohl placed the technical solution of the problem within the scope of ontogenetic and developmental studies of the sporophyte. Although the appropriate information was not at his disposal, he speculated that there is probably no sustained growth of the primary root of the sporeling of *Isoetes.* He concluded that if this is the case, the root-producing meristem is not the homolog of a primary root. On the other hand, his investigations of the sequence of initiation of successive series of roots and certain similarities between the growth of the leaf- and the root-bearing portions of the plant supported the idea that the basal meristem, or root-producing meristem, is a primary meristem on a par with the shoot apex. He concluded that "der untere Theil der aufsteigenden Achse sich in den Verhältnissen seines Wachsthumes ganz nach Art eines ursprünglichen Caudex descendens verhält, ohne dass man ihn desshalb wirklich als solchen betrachten darf."

For the most part, subsequent discussions of this controversy have been amplifications of one or another of the alternatives proposed by von Mohl (1845). Braun (1847) championed the idea that the root-producing meristem is a short primary root. Hofmeister (1862) regarded the root-producing meristem as a portion of the cambium. Scott and Hill (1900) strongly advanced the same opinion as Hofmeister. Lang (1915a) restated von Mohl's position, discussing both sides of the question. He pointed out that during ontogeny initials of the lateral meristem may be converted into initials of the root-producing meristem. This conversion of initials and the continuity of the cambium with the basal meristem were taken as indications of a close relationship between the lateral and basal meristems. They were not considered proof that the root-producing meristem is part of the cambium. Lang recognized that, for each series, the sequence of appearance of roots along the basal furrow is acropetal with respect to the shoot apex. This sequence makes

a comparison with the acropetal sequence of adventitious roots in other vascular cryptogams attractive. However, Lang (1915a) proposed that the sequence of initiation of roots in a single orthostichy is acropetal with respect to the basal meristem and allows a direct comparison with the formation of lateral roots on the main roots of higher plants. Lang further suggested that the many similarities of the morphology of the leaf-bearing and root-bearing portions of the plant make a direct comparison of their respective "apical meristems" possible. He concluded that although the root-producing meristem may not arise as a primary meristem, or as a main root, its growth produces a structure comparable to the shoot (cf. von Mohl, 1845).

West and Takeda (1915) also regarded the basal meristem as a primary meristem on a rank with the apical meristem of the shoot. They wrote: "This meristematic tissue may possibly be regarded as a part of the cambium; but it is present in the embryonic plant (cf. Hofmeister, 1862, pl. 48, fig. 3), persists throughout the life of the plant, and is quite distinct both in origin and appearance from the true cambium. It therefore seemed advisable to distinguish between the two tissues; the term 'primary meristem' is therefore employed with reference to the former." Their reference to Hofmeister's figure was made in spite of the fact that Hofmeister (1862, p. 357) considered the lateral and the basal meristems to be parts of one cambium. Moreover, the figure cited represents a plant in the third plastochron, and the designation of this plant as "embryonic" is misleading. Their assertions as to the origin and appearance of the basal meristem are left unsupported. Weber (1922) argued that the basal meristem must be considered a part of the cambium because it can never be found in the young plant before the lateral meristem or separated from it. Baldwin (1933) reported that the basal meristem arises in the tissue between the first two roots so that at least these two roots are formed without benefit of the activity of the basal meristem.

Liebig (1931) claimed that all of her median longitudinal sections of *Isoetes* supported the thesis of Braun (1847) that "Die Wurzeln sind Seitenwurzeln einer bloss nicht verlängerten Hauptwurzel." She argued that if all the roots of *Isoetes* were adventitious their vascular attachments would be closely associated with individual leaves, as is the case for the roots of the ferns. Concentrating on longitudinal sections at right angles to the furrow plane, Liebig emphasized the parallel between the acropetal sequence, with respect to the basal meristem, of members of successive series in the same orthostichy and the acropetal sequence of root initiation on the main roots of higher forms (cf. Lang, 1915a). Schoute (1938) vigorously attacked the position maintained by

Liebig (1931). He stated that the sequence of root initiation established by Scott and Hill (1900) is acropetal with respect to the shoot apex, and he considered that no direct comparisons can be made between root initiation in *Isoetes* and root initiation on the main roots of higher forms. Schoute failed to note that the sequence he referred to applies to the order of initiation of roots *within a series,* and not to the sequence of formation of *successive series.* The latter sequence is acropetal with respect to the root-producing meristem, as has been indicated by Scott and Hill (1900) and all other workers who have investigated the matter.

Liebig (1931), like other authors, recognized that the basal meristem is distributed as a narrow ribbon over the base of the centrally located vascular tissues and is not directly comparable to the tap root of higher forms. She assumed that *Isoetes* has undergone many modifications of its original form in migrating to semiaquatic and aquatic habitats. Among the modifications is the degeneration of the taproot, so that in present forms the root-producing meristem now occupies the place and performs the function of the original taproot. She admitted that the absence of intermediate forms prevents the elucidation of the derivation of the present condition. She wrote, however, "Da diese Zone die Funktion der Hauptwurzel versieht, gebe ich ihr auch diesen Namen." If analogy is intended, rather than homology, Liebig's interpretation of the basal meristem of *Isoetes* is much like that of West and Takeda (1915). These authors stated that the tissue intervening between the basal meristem and the surface of the furrow is in many ways analogous to a root cap. This statement justifies the assumption that the basal meristem itself is in some way analogous to the apical meristem of a root.

Liebig (1931) gave little attention to the origin of the first root, but her account and those of Hofmeister (1862), Bruchmann (1874), Kienitz-Gerloff (1881), and La Motte (1937) indicate that the first root is the "main root" of the plant and has no sustained growth. The second root arises at the base of the second leaf (Hofmeister, 1862; Bruchmann, 1874) in a manner which may be termed adventitious in the sense that the term is applied to the roots of the ferns. The basal meristem arises from the tissue located between the first two roots (Baldwin, 1933) and not as the first root meristem of the plant. Technically, it is certain that the basal meristem is not a primary root. The alternatives available are to designate the root-producing meristem as some type of primary meristem on a par with the shoot apex or to designate it as a part of the cambium. The essence of this question is whether or not the basal meristem produces primary or secondary tissues. This subject shall receive further treatment in a later section.

OBSERVATIONS AND DISCUSSION

The location of roots and the sequence of root initiation with respect to the root-producing meristem. The discussions of the location of roots within the plant body of *Isoetes* have been given in the literature without definitions of the spatial arrangements that have been described. To allow precise discussion, the *series* and *orthostichies* of roots may be characterized in terms of their morphological properties. Only two-lobed specimens will be considered. Plate 16, A, is a photograph of a mature sporophyte, in which orthostichies of root traces are demonstrated. This photograph of a whole plant is taken in a plane parallel to that passing through the basal furrow. The root traces appear at the surface as nearly vertical rows because the members of an orthostichy are attached one above the other on the sides of the root-bearing portion of the stele. The uppermost traces appear the smallest because they are the oldest and have been stretched the most during the growth of the cortex in which the traces are embedded. If the surface of a plant is viewed from the underside, with the basal furrow running from left to right, the orthostichies of root attachments will appear in vertical lines (pl. 16, B). An orthostichy is then seen to cross the basal furrow so that it is represented on both lobes of a plant (pl. 16, B, at 0 to 0).

Plate 16, B, shows the presence of series of roots (S and S′). A series is most easily recognized when the live roots of a plant are limited to one row along each side of the basal furrow (fig. 14). If each of the two rows constitutes a series, the properties of a series can be described. The three characteristics of a series recorded in figure 14 are (1) a series is more or less parallel to the basal groove, (2) inside each root of a series a large air cavity forms toward the side away from the furrow and the vascular bundle is placed toward the side facing the furrow, and (3) the members of a series do not occupy the same orthostichies as the members of the series most similar in age on the opposite side of the furrow. The first of these properties has been emphasized in the literature. The second can be deduced from the observation that all of the roots appearing on a single lobe of a two-lobed specimen show the same eccentric organization with respect to the furrow plane of the plant (Hofmeister, 1862; Ogura, 1938). The last of the three characteristics of a series has not been reported previously and deserves further attention because of its bearing on the arrangement of roots in orthostichies. If each root is contained in a series and in an orthostichy, each of the roots represented on the plant in figure 14 must be in a different orthostichy because the members of the same orthostichy should be aligned at the base of the plant (pl. 16, B). It is concluded that members of the same

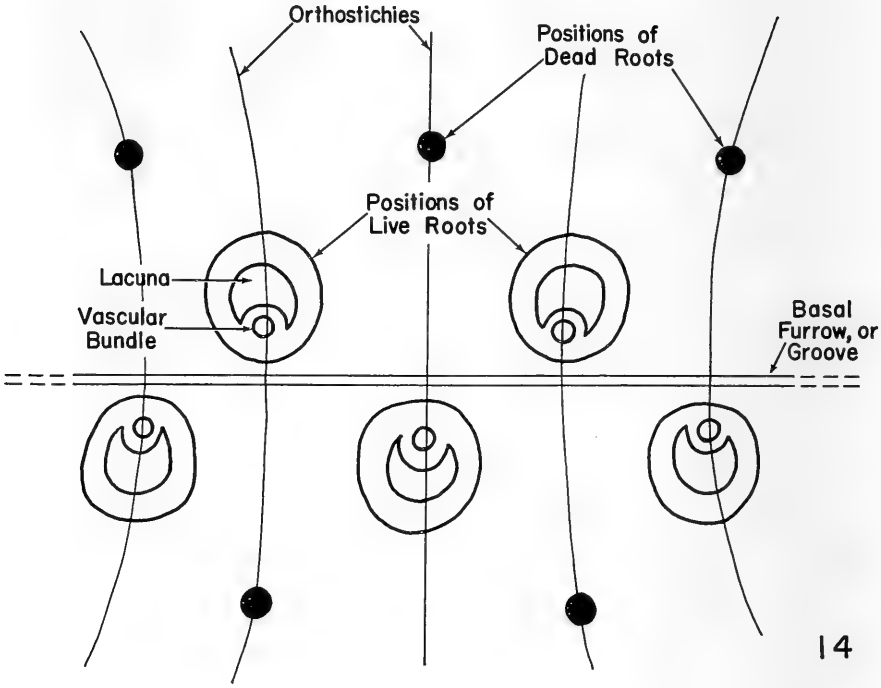


FIG. 14. Sketch of the lower surface of a mature sporophyte in which the living roots were limited to one row along each side of the basal furrow. These two rows each form a series of roots. The members of the two series are on alternate orthostichies. Schematic. The abbreviation "orth." is used for "orthostichy" in figures 15 to 19.

series occupy alternate orthostichies. This alternation can be seen in plate 16, B, by comparing series S and S′.

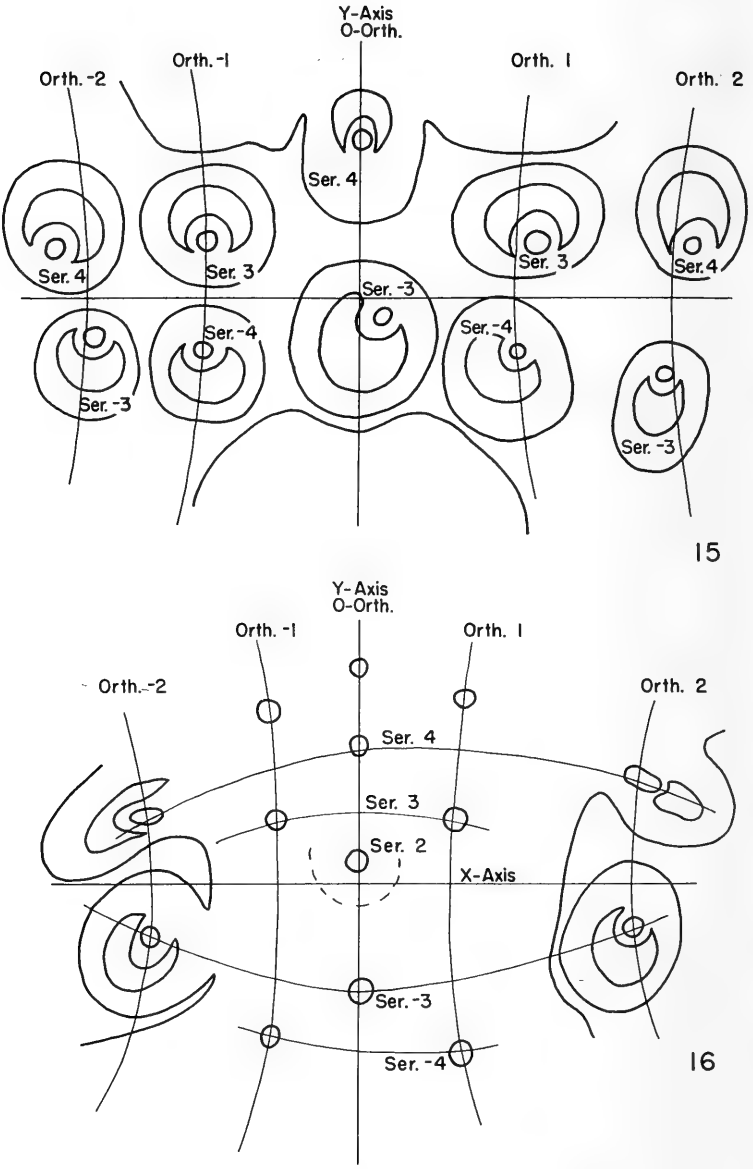
A precise designation of the location of roots or root traces is obtained when the locations are represented graphically. For this purpose, it is best to view transverse sections of two-lobed specimens in a standardized position so that the basal furrow runs from left to right. A drawing of the section may then be related to a set of coordinates by the following procedure. A straight line is drawn through the middle of the furrow and is regarded as the x-axis. Series of roots lie more or less parallel to the x-axis. The perpendicular bisector of the x-axis is drawn and is named the y-axis. Orthostichies appear in cross-sections along lines which are more or less parallel to the y-axis. In some plants the y-axis contains an orthostichy. Such an orthostichy is the zero orthostichy (0-orthostichy), which is the most basal in the plant. Orthostichies located to the right of the y-axis receive values that are consecutive and increasingly posi-

tive. Orthostichies located to the left of the y-axis receive values that are consecutive and increasingly negative. The series above the x-axis are given values that are consecutive and increasingly positive, and the series below the x-axis receive values that are consecutive and increasingly negative.

The properties of a series have been obtained by the inspection of plants with one row of live roots on each lobe, but it may be assumed that these properties hold when more than one series of live roots is present on each lobe. It must be further assumed that, developmentally, some of the members of a series are close enough to each other and distinct enough from the next younger series on the same lobe so that differences in maturation of successive series can be detected at some place on a lobe of a plant and a reference series for the assignment of roots can be obtained. The reference series of one lobe has a counterpart, similar in stage of development or maturation, on the opposite lobe.

The location of a root or a root trace in a section can be designated by naming the orthostichy and the series to which it belongs. The numerical values of the orthostichies and series of roots are determined after data are collected from serial transverse sections, so that the youngest series of root primordia may be recognized. At least one of the two series of root primordia closest to the x-axis receives the value of 1 (+ or —, depending on location). If the youngest series of root primordia on each of the two lobes are matched in their degree of differentiation, one is designated series 1 and the other is designated series —1. If the youngest series has no counterpart, it receives the designation 1 or —1. The younger series yield information on the sequence of root initiation, but it is necessary to study several of the older series on each lobe in order to make a correct determination of the members of each series. The number of series studied may be arbitrarily limited, but the number of orthostichies that can be studied is limited by the insertion of the orthostichies with reference to the plane of sectioning. Whereas orthostichies near the y-axis are sectioned nearly transversely, those far from the y-axis are sectioned very obliquely so that they are ill-defined in the sections.

Figure 15 shows the relative positions of the innermost live roots at the surface of a plant which had more than one series of live roots on each lobe. Four series of roots are represented in figure 15 because members of the same series occupy alternate orthostichies. The roots are crowded because they all emerge from the plant in the basal furrow. In figure 16, which is taken at a higher level in the same plant, absolute displacement from the x-axis justifies the separation of the traces from the ten roots in figure 15 into four series. Figure 16 further demonstrates

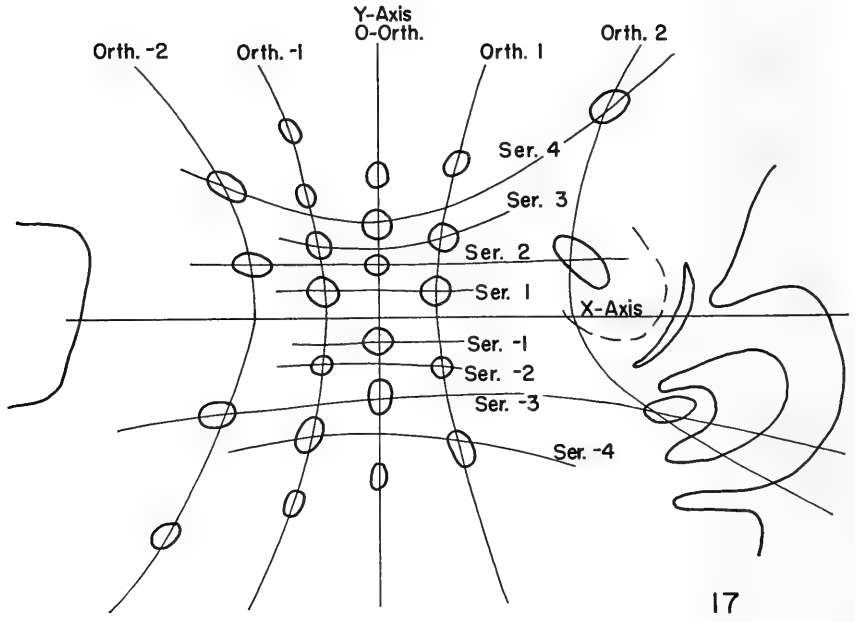


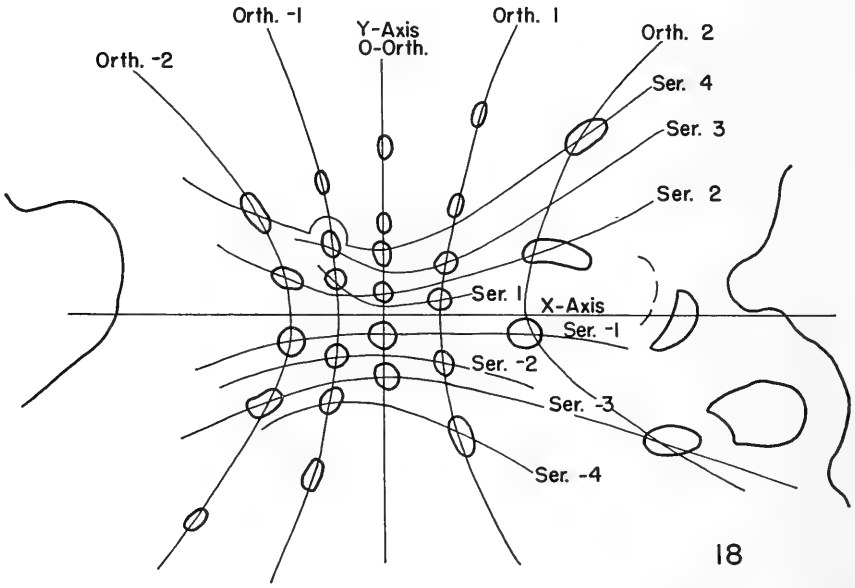
FIGS. 15 AND 16. Outlines of roots and locations of root traces in transverse sections of a plant of moderate size. × 50. Figure 16 is at a higher level in the plant than figure 15. The basal furrow runs from left to right so that the drawings can be viewed in a standardized position on the x- and y-axes. Series of roots are numbered according to information obtained from a study of serial sections. Other sections in the series are drawn as figures 17, 18, and 19. Explanations of the figures are in the text. In figure 15, the series converge with the x-axis. The abbreviation "ser." is used for "series" in figures 15 to 19.

the validity of assigning members of the same series to alternate orthostichies. If the assignment were made otherwise, the regularity of the series would be destroyed. Figure 17, taken from a section near the basal meristem, shows that the traces become crowded as their attachments to the stele are approached. In figure 18 all of the traces and primordia located in series 4 to —4 and in orthostichies 2 to —2 are shown. The irregularities in the location of the sections of traces and primordia are explained later.

In figure 18, series —1 contains primordia in the 0-orthostichy and in orthostichies —2 and 2. Of these three primordia the one on the 0-orthostichy is the most mature. The other two are about equal to one another in maturity. In series 1, which is comparable in degree of maturation to series —1, primordia are present in orthostichies —1 and 1. These two primordia are similar in degree of maturation, are less mature than the centrally located primordium in series —1 (the one on the 0-orthostichy), and are more mature than the laterally placed primordia in series —1 (the ones on orthostichies —2 and 2). A similar situation prevails in the series 2 and —2. For series 2, the primordia that are in orthostichies —2 and 2 are less mature than the primordium that is in the 0-orthostichy. They are also less mature than both the roots of series —2, which are in the orthostichies —1 and 1. Figure 19 represents these relationships. Each zigzag line connects primordia in a pair of series of comparable maturation. Starting in each line with the primordium on the 0-orthostichy, one can follow a single bidirectional sequence of a decreasing degree of maturation because the 0-orthostichy contains the most mature primordium in a pair of comparable series and because the members of the pair of comparable series occupy alternate orthostichies.

West and Takeda (1915) stated that all of the roots of a series are initiated simultaneously. One may infer that the primordia of the same series should be found in the same state of maturation because West and Takeda explained the appearance of roots in an acropetal sequence at the surface of the plant only on the basis of variations in the thickness of the cortex. This inference is contradicted by my observations and by those of Scott and Hill (1900). The latter authors interpreted the maturation differences of root primordia of a series as indications of age differences among the primordia of a series. But differences in the maturation stage of the primordia of a series would not preclude the possibility of simultaneous initiation of a series if the direction of the maturation sequence were acropetal. If a simultaneous initiation of a series is to be rejected, one must show that the extent of the youngest series is less than that of the next older series, at least in some cases.





FIGS. 17 AND 18. Transverse sections in the same series as figures 15 and 16, at higher levels in the plant. × 80. FIG. 17. A level near the basal meristem. The traces are crowded. The series no longer converge with the x-axis. FIG. 18. At the level of the recent derivatives of the basal meristem. All of the primordia within the youngest series are recorded within the limits of orthostichies —2 and 2. The series diverge from the x-axis.

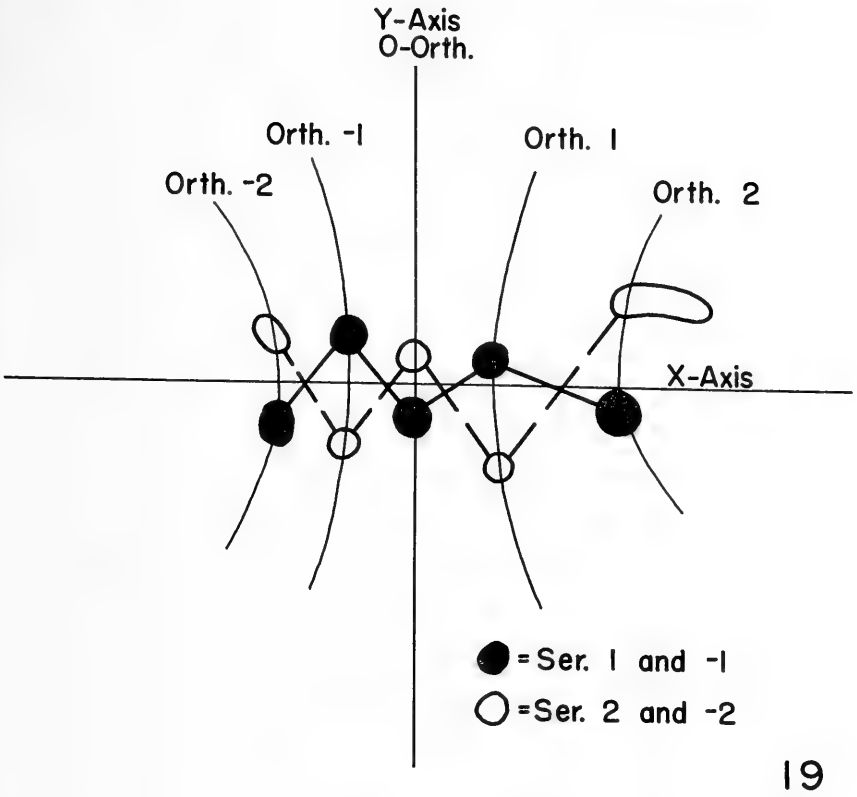
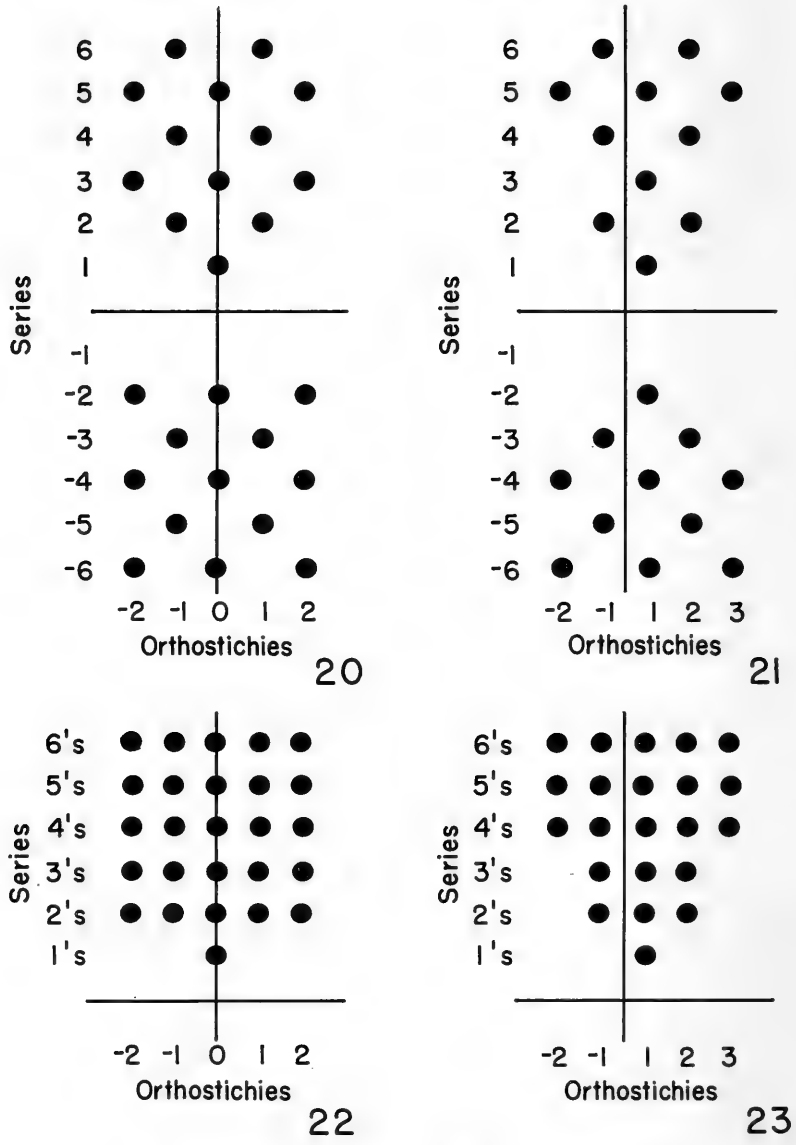


FIG. 19. A portion of figure 18. The two zigzag lines (broken one connecting empty spots; solid one connecting solid spots) trace bidirectional sequences of decreasing maturation. Each sequence starts on the 0-orthostichy and includes members of the pair of series most comparable in maturation.

Scott and Hill (1900) represented roots in series with different lengths in their text figure 11, and their explanation that the youngest series drawn is shortest because it is incompletely initiated is feasible. But their figure offers no conclusive evidence because it represents only an isolated section and does not necessarily show the youngest series of primordia in the plant.

Figure 20 is a graphic condensation of the data obtained from a series of transverse sections of a plant of the same size as that in figures 13 to 16. Orthostichies —2 to 2 are represented over the range of series 6 to —6. The coordinates used in this figure are rectangular abstractions of the actual spatial relationships of the roots in the original sections. All techniques of observation are the same as in the previous example. There is a greater number of series on the +y side of the graph than



FIGS. 20, 21, 22, AND 23. Graphic condensations of data on the location of root traces and root primordia in two plants. FIG. 20. Data from a plant similar in size to that in figures 15 to 19. Series 1 and series —1 are incomplete. FIG. 21. Data from a larger plant than that in figure 20. No 0-orthostichy is present. Series 3, series —2, series 1, and series —1 are incomplete. FIG. 22. Rearrangement of data of figure 20, combining series of comparable maturation in a single row (e.g., series 6 and series —6 combine to give the row marked 6's). This arrangement emphasizes the lateral (acropetal) extent of the combined pairs of series. FIG. 23. Rearrangement of data of figure 21. Similar to figure 22 in arrangement.

on the —y side. All of the available spaces on the graph are taken except in the 1 and —1 series. The 3-2-3-2 alternation at successive y values indicates that initiation of primordia has been regular for all of the roots represented. The most important aspect of the graph is that series 1 is limited in its extent. Figure 21 is a condensation of the data from a larger plant. The range of orthostichies represented is from —2 to 3, and there is no 0-orthostichy. Again there are more series of roots on the +y side of the graph than on the —y side. Series 1 is limited to one primordium. In this plant, however, series —2 and 3 are also limited in extent, though not so much as series 1. Figures 22 and 23 illustrate the data of figures 20 and 21, respectively, in another form, so that the acropetal (lateral on the graph) extent of the combined pairs of series of comparable maturation is emphasized.

The one primordium of series 1 in figure 20 is on the 0-orthostichy. This is the same orthostichy as that occupied by the most mature primordium in the combined pair of series 1 and —1 of figure 19. Similarly, the primordium in series 1 and orthostichy 1 of figure 21 is very near the y-axis, although in this particular plant no orthostichy was actually on the y-axis. Figure 19 may be thought of as a later stage in the initiation of series than figures 20 and 21. Because the most basal orthostichy is the first occupied in a series and also contains the most mature primordium in the more complete series (fig. 17), one may conclude that the sequence of maturation coincides with the sequence of initiation and that differences in maturation are reflections of differences in age.

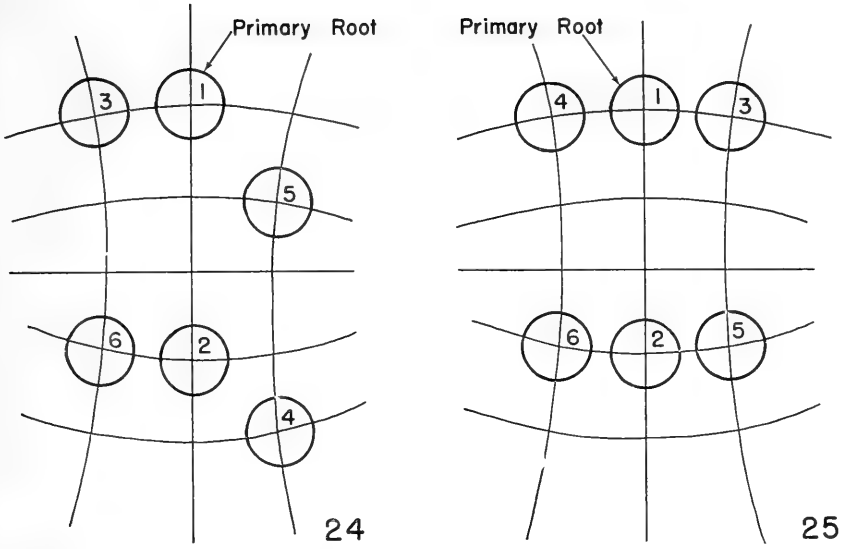
It is now possible to recite a few of the observations and conclusions obtained in this section. Each root is contained in an orthostichy and in a series. The series are roughly parallel to the basal furrow, and the orthostichies cross the basal furrow. The delimitation of series has been described and applied, and the conclusion is reached that members of a series occupy alternate orthostichies. Initiation of the members of a series follows an acropetal bidirectional sequence that begins at the base of the plant and proceeds at both ends toward the shoot apex. The members of two series of comparable age from opposite lobes may be integrated into a single acropetal and bidirectional sequence of initiation because the members of these series occupy alternate orthostichies. No two roots in the same orthostichy are of the same age. Successively younger members of an orthostichy are displaced to opposite sides of the basal meristem. The place where an orthostichy crosses the basal meristem may be designated a site of root initiation.

The observations recorded above may be summarized in two ways. With emphasis on the series, the initiation of a series may be said to begin in the orthostichies at the base of the plant and to continue in a

bidirectional and acropetal sequence by contributions from alternate sites of root initiation. However, the members of comparable series can be integrated into a single sequence of initiation. Emphasizing the orthostichies, one may state that each site of root initiation participates in each *acropetal wave* or *period* of root initiation. The most basal site is the first to produce a root in a new wave of initiation. A bidirectional acropetal sequence follows, and alternate sites of root initiation contribute roots to the same side of the basal furrow. The formation of roots in comparable pairs of series is only incidental to the regular operation of the sites of root initiation.

The formation of primordia at one side and the other of the basal meristem allows the distribution of roots toward both lobes of the plant. Whether the site of root initiation is one site that contributes primordia to alternate sides or two sites that alternate in their activity cannot be decided on morphological grounds. The region between opposing root traces is uniform in its appearance until the advent of a new root primordium. I cannot suggest what it is that provides for the regular distribution of activity during successive waves of root initiation. However, regularity is not complete in all plants. In a few specimens two adjacent orthostichies were found operating in phase in their contributions to the two lobes of the plant. In a few other plants, there were indications that root primordia had formed at the center of the basal meristem rather than only at its sides. In these plants, remnants of root traces were found embedded on the center line of longitudinal sections taken in a plane at right angles to the basal furrow (pl. 16, C, at RRT). Ordinarily, root traces are displaced laterally. The traces along the center lines of the sections were stretched vertically. No permanent impairment of the activity of the sites of root initiation could be detected.

My observations on young plants are incomplete. Available data indicate that where three orthostichies can be studied, two may operate in phase while the third is out of phase. Each site of initiation alternates between lobes in its contribution of new primordia, and the bidirectional sequence of initiation of a series is acropetal with respect to the shoot apex. The pattern suggested is the same as that given for the first six roots of *I. lacustris* by Hofmeister (1862) where the third and fourth roots are displaced toward opposite sides of the basal furrow (fig. 24). The sequence is supposed to be repeated for additional roots (Hofmeister, 1862), and the pattern may persist in the young sporophyte. The transition to the mature pattern requires further study. Baldwin (1933) has reported a sequence of initiation for the first roots of *I. engelmanni* which indicates that adjacent sites of root initiation are all in phase, because the third and fourth roots are displaced to the same side



FIGS. 24 AND 25. Schematic representations of the relative positions of the first six roots of sporophytes of *Isoetes*. The locations are tentatively placed in series and in orthostichies. FIG. 24. Location of the first six roots of *I. lacustris* according to Hofmeister (1862). The sites of root initiation of two orthostichies may be in phase in the production of primordia on alternate sides of the basal meristem. FIG. 25. Location of the first six roots of *I. engelmanni* according to Baldwin (1933). The sites of root initiation for all three orthostichies may be in phase in the production of root primordia on alternate sides of the basal meristem.

of the furrow as the first root (fig. 25). The variability of the patterns of initiation in young plants has not been assayed and the exact relationship of the pattern of initiation in young plants to the pattern in mature plants is not known.

Attention may now be given to the curvature seen in series and orthostichies in cross-sections taken at various levels of the plant (figs. 15-18). One might expect that the lines connecting the series would converge toward the x-axis in transverse sections, because of the bidirectional and acropetal sequence of root initiation. The primordia are displaced from the basal meristem after they are formed, and the oldest member of a series should be displaced the farthest from the median plane. The expected spatial arrangement is found at low levels in the plant, but not at high levels. To explain this variation, one needs to acknowledge that the basal meristem lies in a plane that is convex when viewed from below. The attachments of roots in the 0-orthostichy are encountered first in serial transverse sections taken in an upward sequence in a plant,

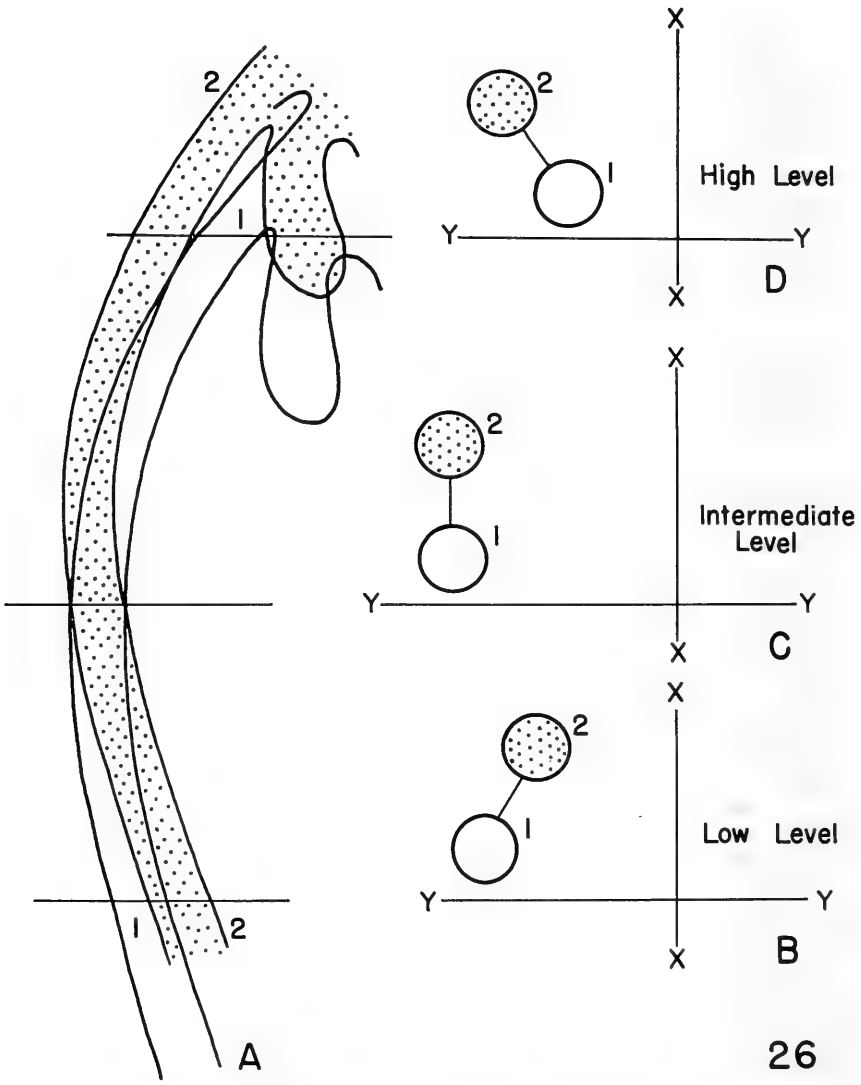


FIG. 26. Schematic representation of the relative positions of successive members of the same series. Trace 1 is in a more basal orthostichy (is closer to the y-axis) than trace 2. A. Superimposition of successive members in longitudinal view at right angles to the furrow plane. B, C, D. Appearance of the series segment 1-2 in transverse sections at various levels represented in A. The inclination of segment 1-2 with respect to the x-axis varies with the level of sectioning. For comparison, see figures 15 to 17.

and the attachments of the traces of other orthostichies are seen in higher sections. Further, the root traces are curved within the plane that contains all of the root traces of a given orthostichy (pl. 16, C). Successive members of the same series are attached at different levels in the plant. A schematic superimposition of the appearance of successive members of a series, as seen in longitudinal sections perpendicular to the furrow plane, is given as figure 26, A. Root 1 is closer to the median plane than root 2. Transverse sections viewed in relation to x- and y-axes would show the series segment 1-2 converging with the x-axis at low levels (fig. 26, B), parallel to the x-axis at intermediate levels (fig. 26, C), and diverging from the x-axis at a level near the attachment of the trace of root 1 (fig. 26, D). The three inclinations of series segments with respect to the x-axis are encountered in the above order in serial sections of the plant taken in an upward sequence (figs. 16-18). The series in figure 16 converge toward the x-axis, but in figures 17 and 18 there is an increasing tendency toward divergence from the x-axis.

In transverse sections observed in the standardized position, all orthostichies except the 0-orthostichy diverge from the y-axis. The intercept of two flat planes is a straight line, and the appearance of the 0-orthostichy as a straight line in transverse sections (figs. 16-18) indicates that the root traces of the 0-orthostichy are contained in a *flat* plane. The more acropetal orthostichies appear as curved lines in transverse sections; their root traces are, therefore, contained in *curved* planes. In other words, the growth vector parallel to the x-axis in a given transverse section is not constant at different values of y. The data obtained for the curvature of orthostichies in cross-sections cannot be easily used in a quantitative description of the growth of the plant. A cross-section represents an artificial plane of reference because the basal meristem is curved. Transverse sections do not reveal different orthostichies at comparable levels. Further, because the planes containing all but the 0-orthostichy are curved, the curvature of the line connecting members of the orthostichies in cross-sections varies according to the obliquity of sectioning for a given orthostichy. Distortion of this kind increases toward the ends of the basal meristem. As a final difficulty, the curved plane containing the root traces of an orthostichy is not the surface of a cone but approaches a portion of the surface of the solid of rotation of a hyperbola or parabola. The rotational axis of the solid that is bounded by the curved plane is oblique to the axis of the plant, and the properties of the curved surface are not easily discovered in transverse sections.

The morphological nature of the root-producing meristem. As von Mohl (1845) has pointed out, if the first root of the sporeling has a

short and definite life span, the root-producing meristem cannot be correctly designated the homolog of a main root. Bruchmann (1874) has reported that the first root of the sporeling is short-lived. My observations support Bruchmann's, and the obvious conclusion is that the basal meristem is not the main root of the plant. The alternative designations available are: (1) the basal meristem is some type of primary meristem other than a main root; (2) the basal meristem is a part of the cambium. Throughout the following discussion, the terms *basal meristem* and *root-producing meristem* will be used as synonyms. The term *lateral meristem* will be used to designate the portion of the cambium which is located above the basal meristem.

Baldwin (1933) has reported that the basal meristem is organized after two roots are present on the sporophyte. For ten or more plastochrons following this event, the plant remains in a ½ phyllotaxy. The median plane of sectioning perpendicular to the furrow contains all of the leaf traces and the 0-orthostichy of root traces and exposes the site of root initiation for the 0-orthostichy (pl. 13, A, at BM). The relationship between the basal meristem and the rest of the plant is obscured by the root traces. However, in the plane of sectioning that coincides with the furrow plane, a different picture is obtained. The basal meristem (pl. 13, B, at BM) is found to be continuous with the lateral meristem (pl. 13, B, at LM). This continuity is established during the origin of the basal and lateral meristems, and is very striking while the root-bearing portion of the stele is small (pl. 17, A, at BM and LM). The basal and lateral meristems originate in the periphery of the procambial strand that forms across the axis of the plant during the formation of the traces to the second leaf and root. Viewed in the furrow plane of sectioning, this strand is circular in outline. Tracheids form at the center of the strand and sieve elements form laterally. It has already been mentioned that the lateral meristem originates outside of the first sieve elements. Divisions in the lower part of the strand produce the basal meristem and complete the origin of the cambium. No direction or sequence in the formation of the cambium has been observed in the materials available. The lateral and basal meristems of a young plant together form a U in sections in the furrow plane (pl. 17, A), and repeated tangential divisions produce derivatives inside and outside of the basal and lateral meristems. Radial seriation of files of cells is evident when the plant has several leaves.

It is reasonable to suggest that the similarity of origin and the continuity of the basal and lateral meristems indicate that these meristems may be regarded as parts of the cambium. Thus, both the basal and the lateral meristems are active in secondary growth. However, from the earliest time of their activity the lateral and basal meristems produce

inner derivatives which mature as different types of cells (pl. 13, B). The lateral meristem at first produces sieve elements on its inner face (pl. 13, B, at SSE), whereas the basal meristem produces tracheary elements (pl. 13, B, at T). As the plant grows, the extent of the basal meristem increases. The production of inner derivatives from the basal meristem adds to the mass of the root-bearing portion of the stele. All of these inner derivatives are regarded as secondary tissues.

As the plant ages, the lateral meristem may produce parenchyma and tracheids on its inner face in addition to sieve elements (pl. 13, E). The basal meristem may produce parenchyma as well as tracheids on its inner face, and a layering of these elements parallel to the basal meristem is often noted (pl. 17, B; cf. Hofmeister, 1862, pl. 52, fig. 6). The lateral layers of parenchyma are confluent with tracheary elements derived from the basal meristem. When secondary xylem is formed inside the lateral meristem, the tracheids differentiate among the cells of the parenchyma layers. In view of the acropetal sequence of maturation of root series, one may postulate that the differentiation of tracheids in the derivatives of the lateral meristem is an expression of the acropetal maturation of tracheary elements along the series of root traces at their attachments to the stele. The continuation of this acropetal maturation would produce tracheary elements in the lateral layers of parenchyma beyond the uppermost root primordia.

As the perimeter of the root-bearing portion of the stele increases and the length of the basal meristem becomes greater, a conversion of initials may occur at the ends of the basal meristem. The evidence for this conversion comes from the interpretation of cell files that were derived from cambial activity. Within radial files of cells that are inside the limits of the basal meristem, the oldest cells of a file may be sieve elements, whereas sieve elements are absent near the initials in the meristem (pl. 17, C, within brackets at FC). The old sieve elements (pl. 17, C, at OSSE) were produced as derivatives of the lateral meristem. The presence of root primordia external to the initials of the cell files (pl. 17, C, at RP) indicates that the initials were in the basal meristem at the time of fixation and suggests that a conversion of initials has occurred. This conversion allows the root-producing meristem to progress toward the shoot apex. The acropetal progression of the root-producing meristem makes it possible to obtain transverse sections in which the attachments of root traces appear inside the limits of the lateral meristem and outside secondary sieve elements (pl. 18, A, B, at BRT). Again, it may be noted that these tracheary elements at the bases of root traces are confluent with a layer of parenchyma inside the lateral meristem (pl. 18, B, at BRT and Par). The appearance of a transverse section such as that in plate 18, B, is very similar to the appearance of a cross-section of

a stem of *Stylites* at a level where the so-called "Wurzelstele" is present (Rauh and Falk, 1959b). Judging from the description given by Rauh and Falk (1959b), I believe that the acropetal formation of the root stele of *Stylites* involves the same processes as the acropetal progression of the root-producing meristem of *Isoetes*. The course of differentiation of the inner derivatives of the cambium is altered from one that can produce sieve elements to one that produces parenchyma cells and tracheary elements but no sieve elements. At the same time, the outer derivatives of the meristem attain the ability to form root primordia. The causes of these changes are not known.

The idea that the basal meristem is a primary meristem (Lang, 1915a; West and Takeda, 1915) may be better understood if attention is turned to the appearance of the root-bearing portion of the stele in a longitudinal section taken at right angles to the basal furrow. Such a section may contain an orthostichy of roots (pl. 16, C), or may pass between orthostichies (pl. 18, C). In the first case, the arrangement of root traces is like the arrangement of leaf traces while the plant has a ½ phyllotaxy. In the second case, the relation of the basal meristem to mature tissues in the lower portion of the plant is similar to the relation of the shoot apex to mature tissues in the upper portion of the plant (cf. pls. 7, A, and 18, C). The comparison between the basal meristem and a primary meristem is attractive if attention is confined to longitudinal sections perpendicular to the furrow plane. But information from transverse sections should be examined before the comparison is accepted as valid. In plate 18, B, near the top, there is no need for hesitation in designating the layers of clear cells as layers of secondary sieve elements (SSE). Toward the right, however, root traces are present, indicating that at that location the initials of the lateral meristem have been converted to initials of the basal meristem. Were it not for these root traces, the outermost layer of phloem would be continuous at the right in the photo. At a higher level in the plant, the phloem layer is continuous around the right side of the stele and at that level would be designated secondary throughout its extent. At the level of sectioning in plate 18, B, the phloem adjacent to the root traces must be designated primary if the root-producing meristem is a primary meristem (cf. West and Takeda, 1915). Because the basal meristem is greatly extended in the form of a ribbon, the transition between primary and secondary phloem takes place along a line parallel to the edge of the basal meristem. Starting with one horn of the root stele, this line descends in an arc, convex from below, and rises to the other horn of the stele, crosses to the other side of the meristem, and descends in another arc to rise again to the first horn of the stele, and crosses the end of the basal meristem back

to the starting point. In the median plane of sectioning at right angles to the basal furrow (pl. 18, C), the transition from secondary to primary phloem offers no conceptual difficulties. At the ends of the ribbon-shaped meristem, however, the conversion of initials leads to difficulties of description. If the concept that the basal meristem produces primary tissues is maintained, the conversion of initials requires that initials producing secondary vascular tissues revert to the production of primary tissues at the ends of the basal meristem. Likewise, the production of secondary cortex must revert to the production of primary cortex.

In choosing the appropriate designation for the root-producing meristem, it is reasonable to assert that one should strive for a concept which allows a unified description of the growth of the entire plant. Lang (1915a) argued that the conversion of initials at the boundary between the lateral and basal meristems does not "prove" that these meristems are both part of the cambium. Certainly this relationship cannot be proved in a rigorous sense. When the basal meristem is regarded as a part of the cambium, however, terminological difficulties are avoided, and a clear description of the growth results. If one adopts this concept, the processes of growth in the basal portion of the plant can be summarized as follows.

Early in the life of the plant, the lateral and basal meristems begin their activity together as parts of the cambium. The differentiation of their respective derivatives is different from the first stages of cambial activity. The basal, or root-producing, part of the cambium does not produce sieve elements on its inner face, but the lateral portion of the cambium does. The first several roots form in close association with the first several leaves (Hofmeister, 1862; Bruchmann, 1874), but soon root primordia begin to form at the bases of older roots (Hofmeister, 1862). Sites of root initiation are established in the basal meristem and orthostichies of roots are produced. The inner derivatives of the basal meristem add to the root-bearing portion of the stele, and these derivatives differentiate as tracheids and parenchyma cells which are attached to the bases of the root traces. Root primordia are initiated in the outer derivatives of the basal meristem (Scott and Hill, 1900) in the secondary cortex. Because the newest roots are located at the edge of the basal meristem, the phloem matures toward the edge of the meristem in conjunction with the formation of sieve elements in the procambial root traces.

The arrangement of leaves changes into a spiral as the plant ages. Hence, the leaf-bearing portion of the stele becomes radially symmetrical. The arrangement of roots at a site of root initiation remains in a ½ "rhizotaxy," and orthostichies are formed. Hence, the root-bearing portion of the stele does not become radially symmetrical. The ends of

the ribbon-shaped basal meristem advance acropetally, so that at these locations the inner derivatives of the cambium cease to differentiate as sieve elements, while the outer derivatives of the cambium attain the ability to differentiate as root primordia.

If the basal meristem is regarded as a part of the cambium, the roots of *Isoetes* may be thought of as adventitious. Liebig (1931) argued that if the roots of *Isoetes* are actually adventitious, they should arise in association with leaves, as do the adventitious roots of other vascular cryptogams. A root primordium in the mature plant is organized at the edge of the basal meristem, at the base of an older root trace, but there is no reason why the appellation adventitious should be any less applicable than if the root had been organized at the base of a leaf trace. The continued formation of root primordia along a particular part of the cambium allows a convenient mechanism for the direct and simple attachment of the collateral traces of the roots. The xylem of the root traces attaches directly to the xylem of the stele, and the phloem, which in all root traces is abaxial with reference to the median plane of the furrow, connects directly to the secondary sieve elements produced toward the inside of the lateral meristem.

For the root-bearing portion of the stele, the designation of a peripheral and central portion is not possible, even in large plants, and it appears that all of the tracheids of the root-bearing portion of the stele are differentiated with positional relationship to root traces. Although the shortness of the tracheids in this part of the stele makes their orientation somewhat obscure, the layering found in the inner derivatives of the basal meristem (pl. 17, B) is an indication of the relationship of the tracheids to the bases of the series of root traces.

The difference between the kinds of derivatives found inside the basal and lateral meristems does not destroy the continuity of the cambium between the lateral and basal locations. Examples from other species may be cited to indicate that a cambium may produce both xylem and phloem from its inner surface, and in varying patterns (Esau, 1960, p. 253). In these species, the derivatives of a single initial in the cambium may differentiate as phloem for a limited time and then as xylem, so that a parallel for the conversion of initials at the ends of the basal meristem is available. It must be recognized, however, that the pattern of root initiation and the acropetal progression of the root-producing meristem of *Isoetes* are quite orderly. For this reason, it is profitable to distinguish between the basal meristem and the lateral meristem, even though they are parts of the same cambium. At any given time, the root-producing meristem may be treated as a bounded portion of the cambium, recognizable on the basis of the presence of root primordia at its

external face. The mere presence of tracheary elements inside the cambium does not identify that portion of the cambium as the basal meristem because the lateral meristem also produces derivatives which differentiate as tracheids, and these are in layers confluent with tracheids in the root-bearing portion of the stele. The presence of elements adjacent to the internal face of the cambium does identify that portion of the cambium as the lateral meristem, because the inner derivatives of the basal meristem do not differentiate as sieve elements.

Rauh and Falk (1959b) compared the structure of *Isoetes* and *Stylites*. They concluded that the root-bearing portions of the steles of the two genera are directly comparable. I agree with their conclusion. However, their suggestion that the basal and lateral meristems are not directly and mutually related at their origin in either genus cannot be accepted, because it makes an unnecessary and artificial distinction between the growth of the upper and lower portions of the plant. Their idea is based on the study of the acropetal progression of the "Wurzelstele" along the axis of the plant. The sporophyte of *Stylites* decays from the base upward as the plant ages, and it is not difficult to acknowledge that the relationship of the "Wurzelstele" to the lower extremity of the leaf-bearing portion of the stele is obscure in a mature plant. Rauh and Falk (1959a) discussed the sporeling of *Stylites*, but did not describe the earliest activity of the root-producing meristem.

The apparent separation of the stele of *Isoetes* into leaf-bearing and root-bearing portions has led to the designation of the lower portion of the plant as a rhizomorph (West and Takeda, 1915) and as a rhizophore (Lang, 1915a; Foster and Gifford, 1959, p. 174). I can offer no objection to either of these terms if they are used in a descriptive sense and if the implication that the basal meristem is a primary meristem is avoided. The whole sporophyte, exclusive of leaves and external roots, has been called a stem, a stock (Lang, 1915a), and a corm (Foster and Gifford, 1959, p. 172). All of these are suitable descriptive terms.

SUMMARY AND CONCLUSIONS

The roots of *Isoetes* are arranged in series and in orthostichies. An orthostichy is produced by the formation of root primordia on a site of root initiation and by the alternate displacement of primordia to one side and the other of the basal meristem. Each wave of root initiation is bidirectional and is acropetal with respect to the shoot apex, because it begins in the most basal orthostichy. During each wave of root initiation, alternate sites of root initiation contribute primordia to the same side of the basal meristem. Two series of comparable age are thus pro-

duced, and these series are displaced to opposite lobes of the plant. The two comparable series do not contain sets of roots of exactly equivalent ages.

The basal, or root-producing, meristem and the lateral meristem are best regarded as parts of a cambium. It is possible to distinguish between the basal and lateral meristems on the basis of the differentiation of their respective derivatives. These meristems originate together, as parts of the same cambium, and during ontogeny portions of the lateral meristem may be added to the basal meristem. An acropetal progression of the ends of the basal meristem results from this conversion of initials. A parallel development occurs in *Stylites* (Rauh and Falk, 1959b), but in *Stylites* the acropetal progression is much more pronounced than in *Isoetes*. The root-bearing portions of the steles of *Isoetes* and *Stylites* are probably homologous (cf. Rauh and Falk, 1959b).

THE APICAL MERISTEM OF THE ROOT

REVIEW OF THE LITERATURE

The process of root initiation. The apical meristem of the root is organized during root initiation. Little has been reported on root initiation in mature plants, but Bruchmann (1874) has given a detailed account of the initiation of the first several roots of the sporeling. He considered the first root to be exogenous and all the later roots endogenous. His decision was based on the locations of the first divisions associated with root formation. Bruchmann recognized histogens in the root apex and asserted that the differentiation of the initials of these histogens proceeds in basipetal order. The first initial recognized is that of the calyptrodermatogen. For the first root this initial is set off by a periclinal division in the superficial layer of the plant, but for subsequent roots the division is in hypodermal tissue. The course of further differentiation of initials of the second and later roots depends on the distance between the initial of the calyptro-dermatogen and the existing vascular tissues of the plant. If sufficient cell layers are present at the origin of the first initial, the other initials differentiate directly from available cells. If only one or two layers are present between the initial of the calyptrodermatogen and the vascular tissues, divisions occur to produce the number of cell layers required for the differentiation of the initials of the remaining histogens.

Farmer (1890) agreed that the first root is exogenous. Campbell (1891) suggested that the first root is a primary organ and also recorded that the second root is recognized as a mass of dividing cells before its characteristic organization is obtained. Scott and Hill (1900) reported that root primordia are differentiated in the external derivatives of the basal meristem but gave no further details. Hofmeister (1862) stated that the original direction of the axis of the root primordium in large plants is oblique to the vertical axis of the plant, and Liebig (1931) reported that the primordia are seldom found in a vertical position. Hofmeister (1862) also reported that the root traces are curved, so that they form an arc which is concave with respect to the median plane of the furrow. West and Takeda (1915) have asserted that the roots penetrate the cortex actively, but Lang (1915a) has stated that the roots are carried to the surface by the growth of the cortex and begin active apical growth after the surface is reached. On this basis, Lang (1915a) suggested a similarity between the roots of *Isoetes* and the leaves, which originate exogenously.

The organization of the apical meristem of the root. Hofmeister (1862) believed that the root of *Isoetes* grows with an apical cell. Naegeli and Leitgeb (1868) supported Hofmeister's opinion, but they could adduce no positive evidence for the existence of the apical cell. Instead, they came to their conclusion by eliminating, to their own satisfaction, the other possibilities they could suggest. However, they also considered that the plerome of the root grows with its own initial. Farmer (1890) severely criticized Naegeli and Leitgeb (1868), charging that their observations were not consistent with their hypothesis. Farmer suggested that the existence of a plerome initial precludes the existence of an apical cell because the latter should act as the initial of all tissues. Naegeli and Leitgeb (1868) had suggested, however, that the apical cell may be rather inactive.

Bruchmann (1874) reported the existence of several histogens in the root apex of *Isoetes:* a calyptro-dermatogen, an outer periblem, an inner periblem, and a plerome. The calyptro-dermatogen and the outer and inner periblem layers were supposed to be supplied by the activity of their respective layers of initials, and the plerome was said to grow with a single initial. Farmer (1890) asserted that the boundary between the outer cortex and the inner cortex is sharp and that this boundary can be traced through the initials of the root. He criticized Bruchmann (1874) for not recognizing the continuity between the outer cortex, epidermis, and root cap. Farmer recognized a single initial for the procambium (plerome), a layer of initials for the inner cortex, and a layer of initials furnishing cells for all other regions of the root. Campbell

(1891) reported that the plerome has several initials and that an additional layer of initials furnishes cells to all other tissues of the root. Mager (1907) adopted Farmer's (1890) concept of the organization of the root apex, whereas Liebig (1931) followed Bruchmann (1874).

The concept of the organization of the root of *Isoetes* outlined by Kienitz-Gerloff (1881) stands apart from all others. He maintained that the root has an undifferentiated meristem. In other words, the initials cannot be designated in terms of the mature tissues of the root. It is only away from the region of the initials that the distinctions among tissues become obvious. He offered many figures in an effort to refute the concept of Bruchmann (1874), and some of these were said to show that the distinction between the plerome and the inner periblem cannot be maintained. The concept of Kienitz-Gerloff also came under the scrutiny of Farmer (1890). Farmer charged that the evidence proposed by Kienitz-Gerloff cannot be accepted because the figures presented show the procambial strand on the axis of the root. Farmer maintained that the longitudinal plane of sectioning at right angles to that in Kienitz-Gerloff's figures (i.e., the plane that would show the procambium eccentrically placed) is the only one that shows the proper relationship of the plerome to the other tissues.

The dichotomy of the root. Bruchmann (1874) asserted that in the development of the dichotomy of the root the plerome initial takes the initiative by dividing longitudinally with reference to the axis of the root. The first dichotomy occurs in the plane which shows the procambium on the axis of the root. After the plerome initial divides, the initials of the other histogens organize themselves with respect to the two newly formed plerome initials. Sister apexes remain in a common root cap for some time. Naegeli and Leitgeb (1868) have reported that fifth-order dichotomies may be found within a single root cap. Because branching is cruciate and sister apexes diverge from each other at a slight angle from the axis of the root, longitudinal sections which show the apexes in proper relationship to mature tissues are difficult to obtain. Naegeli and Leitgeb offered a figure of a section of a root in which dichotomies had occurred and stated that the figure does not disclose the apexes of the root. However, Bruchmann (1874) chose to cite this figure (Naegeli and Leitgeb, 1868, pl. 19, fig. 12) as a correct representation of the organization of the apical meristem of the root.

OBSERVATIONS AND DISCUSSION

The process of root initiation. I have not made sufficient observations on young sporophytes to allow any statement on the origin of the

first root. I have observed the origin of the second root by the enlargement and division of hypodermal cells at the base of the second leaf (pl. 1, D, at R_2), but my observations are too incomplete to reveal the relationship of these first-recognized cells to the final organization of the meristem of the root. Greater attention has been given to the initiation of roots in plants with several to many leaves. A plant of this size has a well-established basal meristem, and root primordia are differentiated in the external derivatives of the basal meristem. The time at which the apical meristem of the root primordium is organized in relation to the length of the primordium varies with the thickness of the cortex. In young plants, where the cortex is thin, the apical meristem is organized before the primordium becomes extended. In older plants, where the cortex outside of the basal meristem is composed of many layers of cells, the primordium may increase in length before any activity of an organized apical meristem is apparent (pl. 18, D). This variation in the time of organization of the meristem is consistent with the observation that the first few roots, which are formed close to the surface of the sporeling, have organized meristems directly after they are first recognizable and before any extension occurs (Bruchmann, 1874).

A primordium first appears as a group of cells with deeply staining cytoplasm and chromatic nuclei. This group of cells is located at the edge of the basal meristem with the axis of the group slightly inclined away from the axis of the plant. The plastids of a newly differentiated primordium have the condensed form of the undifferentiated plastids found in the superficial layer of the shoot apex (pl. 5, A, at UPl). Elsewhere in the basal meristem, the plastids resemble those of the internal cells of the apex (pl. 5, A, B, at Pl). The starch content of a cell in a young primordium is much smaller than that of a cell in the surrounding ground tissue (pl. 18, E).

A procambial strand is differentiated in the growing primordium. This event may occur before the complete organization of the apical meristem (pl. 18, D). The procambial trace is usually curved, so that it is concave with respect to the median furrow plane. The tip of the trace is very close to the median furrow plane of the plant and is displaced practically straight downward along the central plane, but the attachment of the trace is displaced laterally. The curvature of the trace is maintained as the trace is extended. The roots do not penetrate the cortex actively until the tip is close to the surface. In young plants, the penetration may occur soon after the primordium is organized. The tip of a primordium is carried outward along the median plane, so that the root breaks out of the cortex within the basal furrow. After a root

emerges, lateral displacement is very pronounced. Displacement at the surface of the plant exceeds that nearer the attachment of the trace, so that the original curvature of the trace is reversed as the trace ages.

The organization of the apical meristem of the root. I have observed the apical meristems of the following types of roots and root primordia: (1) the first root, unextended, (2) the first root, extended, (3) root primordia in young plants, (4) root primordia in old plants, (5) roots of mature plants after emergence from the cortex but before complete extension, (6) roots of mature plants after complete extension. The information obtained from these various sources agrees very well, so it appears that the organization of root apexes in *I. howellii* does not vary greatly during ontogeny. The apical meristem can be described in terms of the differentiated tissue regions of the root. These regions are the root cap, the epidermis, the outer cortex, the inner cortex, and the procambium (pl. 19, A). The procambium is near the side of the root that faces the furrow. The adaxial side of the procambium differentiates as xylem. The inner cortex is thickest on the abaxial side of the root but breaks down to form a large cavity in this location (cf. fig. 14). The eccentricity of the procambium and inner cortex is manifest to the region of the initials.

In agreement with Farmer (1890), I have found that the most definite boundary among tissues occurs between the inner cortex and the outer cortex. This boundary can be followed through the region of initials (pl. 19, B, C, D, between OC and IC). The outer cortex, epidermis, and root cap have a common origin. In contrast to Naegeli and Leitgeb (1868), Bruchmann (1874), Farmer (1890), Mager (1907), and Liebig (1931), I find that the procambium does not have its own permanent initials. The distinction between the initials of the procambium and those of the inner cortex is lacking in most of the apexes examined. To this extent, my observations agree with those of Kienitz-Gerloff (1881), but the concept of an undifferentiated meristem cannot be accepted because the boundary between the inner cortex and the outer cortex can be traced through the initials. Although Campbell (1891) reported that the procambium has several initials of its own, his figures reveal that he did not distinguish between the procambium and the inner cortex in his preparations. Therefore, the cells he identified as the "initials of the plerome" are actually the initials common to the inner cortex and procambium.

In some apexes, the procambium does not appear continuous with the initials of the inner cortex. This may indicate that the organization of the apex varies among roots or that the procambium may grow for certain periods with its own initials, which are displaced by new con-

tributions from the cells generating the inner cortex. These contributions may be related to the dichotomy of the root apex.

There is no indication of a quiescent center (Clowes, 1959) in the roots of *Isoetes*. Division figures are observed in the initials. Divisions are observed near the apex even in extended roots. Bruchmann (1874) has reported an early curtailment of apical growth in the first root of *I. lacustris*, but my observations on *I. howellii* do not support his findings.

The dichotomy of the root. Several stages of the dichotomy of the apex of the root have been observed. Plate 19, E and F, show two of these stages. The following account is tentative because the early stages of dichotomy were observed in relatively few roots. The first dichotomy occurs near the time the root emerges from the cortex. At this time, a distinction between the procambium and the initials of the inner cortex may be possible. The distal cells of the procambium and of the inner cortex cease to act as initials. The procambium becomes broad and blunt at the tip (pl. 19, E, at Pr), and the distal cells of the inner cortex subdivide (pl. 19, E, below tip of procambium). Two or more cells laterally placed among the initials of the inner cortex maintain their size (pl. 19, E, at arrows).

These cells enlarge and divide so that their derivatives near the axis are contributed to the procambium. These derivatives become the new temporary initials of the procambium. At this stage in the dichotomy, the procambium appears to be continuous with the initials of the inner cortex. The configuration of the apexes in plate 19, F, can account for the configuration of the apex in plate 19, C and D. Therefore, it is reasonable to assume that some of the longitudinal sections which show an apparent continuity of the procambium with the initials of the inner cortex show the apex after the initiation of a dichotomy. Other dichotomies follow soon after the first. The production of new temporary initials for the procambium by the initials of the outer cortex allows multiple dichotomies to occur with the production of relatively few new layers of cells. The production of new apexes within one root cap does not contribute appreciably to the elongation of the root.

Bruchmann (1874) believed that the procambium takes the initiative in a dichotomy. My observations do not contradict this idea, but it is also possible that the initials of the inner cortex and the initials of the procambium act together in the dichotomy. Bruchmann's figure of a dichotomy (1874, fig. 20) represents a stage earlier than that given here in plate 19, E. In his figure, the distal cells of the inner cortex have not yet divided into smaller cells. Bruchmann believed that the procambium is distinct throughout the life of the root. He did not describe

any reorganization of the apex during the dichotomy. Farmer (1890) reported that his observations did not conflict with the ideas forwarded by Bruchmann (1874), but gave no details on his own observations.

SUMMARY AND CONCLUSIONS

Root primordia are differentiated in the external derivatives of the basal meristem. Elongation of a primordium may or may not precede the organization of its apical meristem, depending on the thickness of the cortex outside of the basal meristem. Root primordia do not actively penetrate the cortex until they are very close to the surface of the plant. The apical meristem of the root is composed of a layer of initials which gives rise to the cells of the outer cortex, epidermis, and root cap, and of a group of initials common to the inner cortex and the procambium. The distal cells of the procambium may function as temporary initials, but are replaced during a dichotomy and possibly at other times.

GENERAL SUMMARY AND CONCLUSIONS

Synoptic treatments of the principal observations and conclusions are given at the end of each section of this report, and there is no need to repeat these summaries here. Instead, a few closing remarks will be made. In relation to the previous literature on *Isoetes*, this report is synthetic to the extent that it incorporates the concepts of many workers into one description. The literature on *Isoetes* spans a century and a half, and it is not surprising that many pertinent observations have been made. I have tried to develop the ideas available in the literature and to determine which observations are common to the many descriptions of *Isoetes*.

Principal among the concepts which have been treated with different perspectives are the origin of the cambium and the nature of the basal meristem. I recognize that the choice of perspectives in the treatment of some aspects of these concepts is subjective, and that descriptions can be logically constructed within more than one perspective. The perspectives chosen in this study facilitate description of the whole plant. All of the meristematic tissues of the corm have been considered together, and the consequences of a concept in one part of the plant are applied to the description of other parts of the plant. The perspectives employed in this report also yield a description which facilitates com-

parison of *Isoetes* with other plants. Here, the concepts of primary vascular differentiation and of the primary plant body are most strongly affected.

The use of a particular concept may provide a terminology which avoids many semantic difficulties, but the benefits do not end there. Description is the first stage in obtaining an understanding of growth and form. The benefits of description are enhanced if the perspectives adopted are styled to reveal the most instructive analogies and homologies. From this standpoint, it is best to regard the tissue underlying the shoot apex as a histologically undifferentiated tissue which furnishes cells to the procambium and the ground meristem. It is best to regard the early tangential divisions around the procambial xylem core as a part of the growth of the procambium. Also, there is ample justification for regarding the lateral meristem and the basal meristem as parts of one cambium. Each of these ideas has appeared in the literature. They occur together in this report as the combination of concepts which are supported by my observations.

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PLATES

All plates, except plate 16, B, are of *Isoetes howellii*. Plate 16, B, is of *I. braunnii*.

Plate 1

Sagittal longitudinal sections of sporelings in the second plastochron. Craf fixation; stained with hematoxylin-safranine-fast green.

A. Large portion of section showing the general relationship of the parts of the sporeling. The top of the first leaf is excluded from the photo. Below the ligule of the first leaf, part of the group of apical initials can be seen (arrow). Between this location and the sheathing base of the first leaf is the second leaf primordium. A procambial strand is continuous from the first leaf to the first root but is slightly bent near the level of the differentiating shoot apex. × 250.

B. Apical region of the shoot in A. The arrows indicate cells which are members of the group of apical initials. There is little topographic distinction between the shoot apex and the second leaf (L_2). × 750.

C. Section adjacent to that in B. The arrow indicates a cell which is a member of the group of apical initials. × 750.

D. Later stage of the plastochron than in A, B, C. The primordium of the second leaf (L_2) is appressed to the ligule of the first leaf. The arrow indicates one of the apical initials. The second root (R_2) was forming at the base of the second leaf primordium. × 750.

E. Differentiation of the second ligule mother cell (Li_2). The surface of the second leaf (L_2) and of the shoot apex (arrow) have been inked to separate L_1 from L_2. The arrow indicates the location of the apical initials, which do not show well in the photograph because of the plane of focus. × 750.

L_2, second leaf; Li_1, first ligule; Li_2, second ligule; R_2, second root; SFL, sheath of first leaf; arrows, cells of the groups of apical initials.

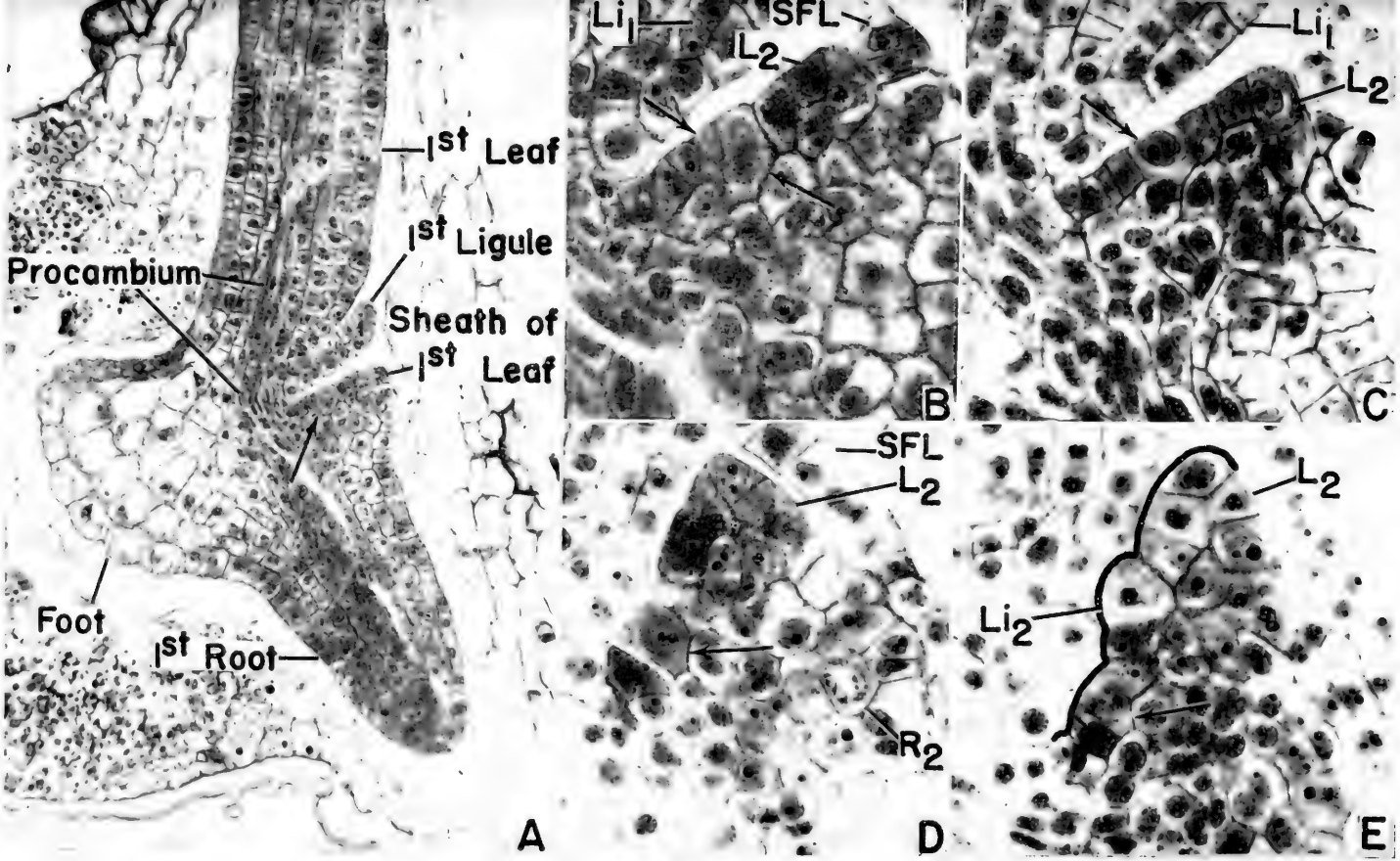

1st Leaf
1st Ligule
Procambium
Sheath of 1st Leaf
Foot
1st Root
A
Li1
SFL
L2
B
Li1
L2
C
SFL
L2
R2
D
L2
Li2
E

Plate 2

Median longitudinal sections of shoot tips. × 580. All three plants had a well-established spiral phyllotaxy. The size of the plant increases from A to C.

A. Lightly staining group of apical initials still visible in the apex. Foster's (1934) stain, plus fast green. Sectioned perpendicular to the furrow plane.

B. Uniformly stained superficial layer. Stained with hematoxylin-safranine-fast green. Sectioned in the furrow plane.

C. No distinct group of apical initials. Stained with hematoxylin-safranine-fast green. The staining reaction of the superficial layer of the shoot apex is much the same as that of the superficial layer of the region of leaf formation to the left and the right of the apex. Sectioned in the furrow plane.

F, files of cells parallel to the superficial layer; Pr, top of procambial cylinder of the stem; W, wedges of cells in position to form new cell files.

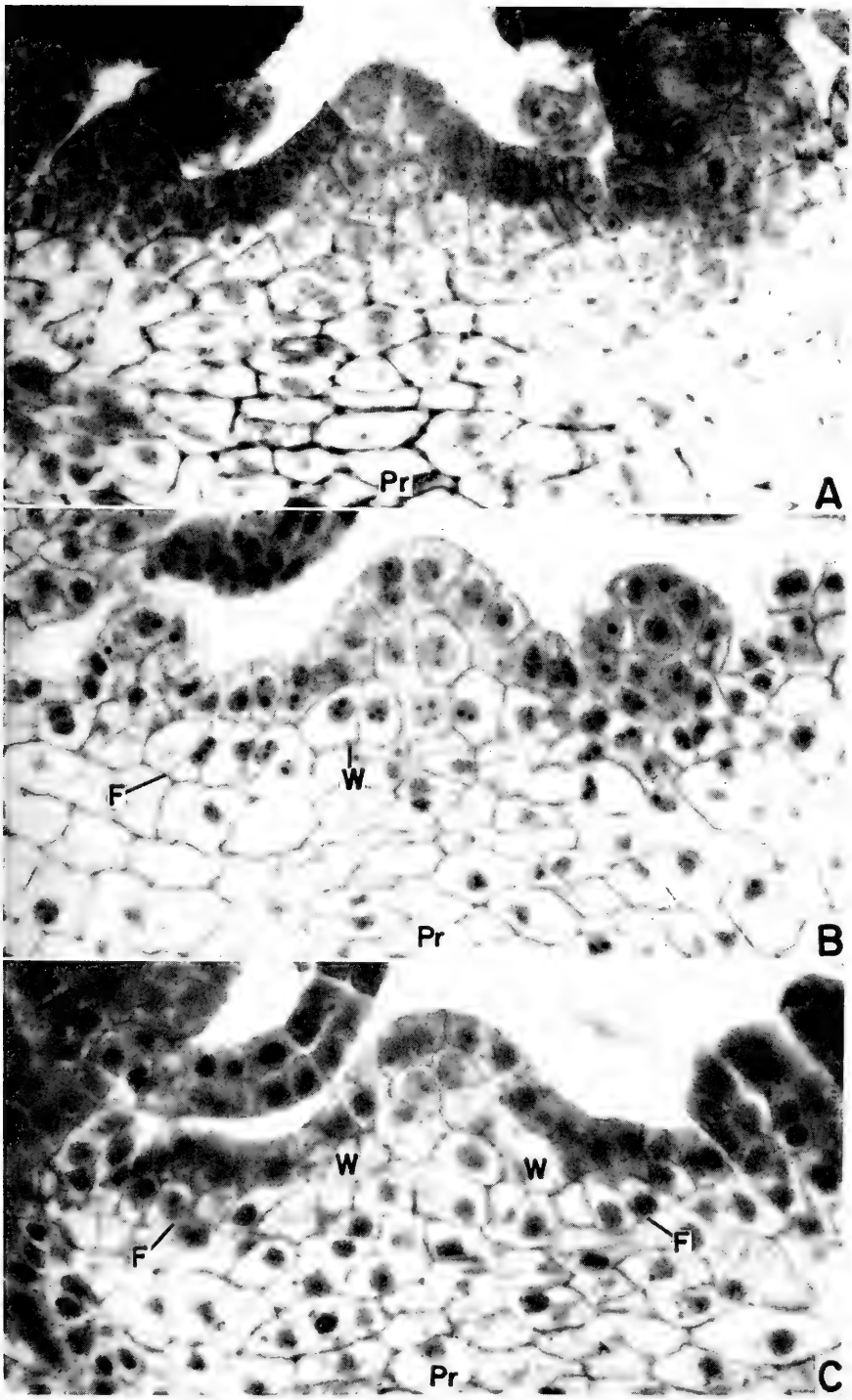

Pr
A
F
W
Pr
B
W
W
F
Fl
Pr
C

Plate 3

Longitudinal sections of apexes dominated by single cells. × 590. The size of the plant increases from A to C. All three plants were near the change from distichous to spiral phyllotaxy. The arrow in each case indicates the cell most highly elevated in the apex. In A and B, this cell is the largest in the apex. In no case was the summital cell unique in staining properties. A and B were stained with hematoxylin-safranine-fast green. C was stained with the chlorazol-acid fuchsin-malachite green combination. A and B are sectioned in the furrow plane; C perpendicular to the furrow plane.

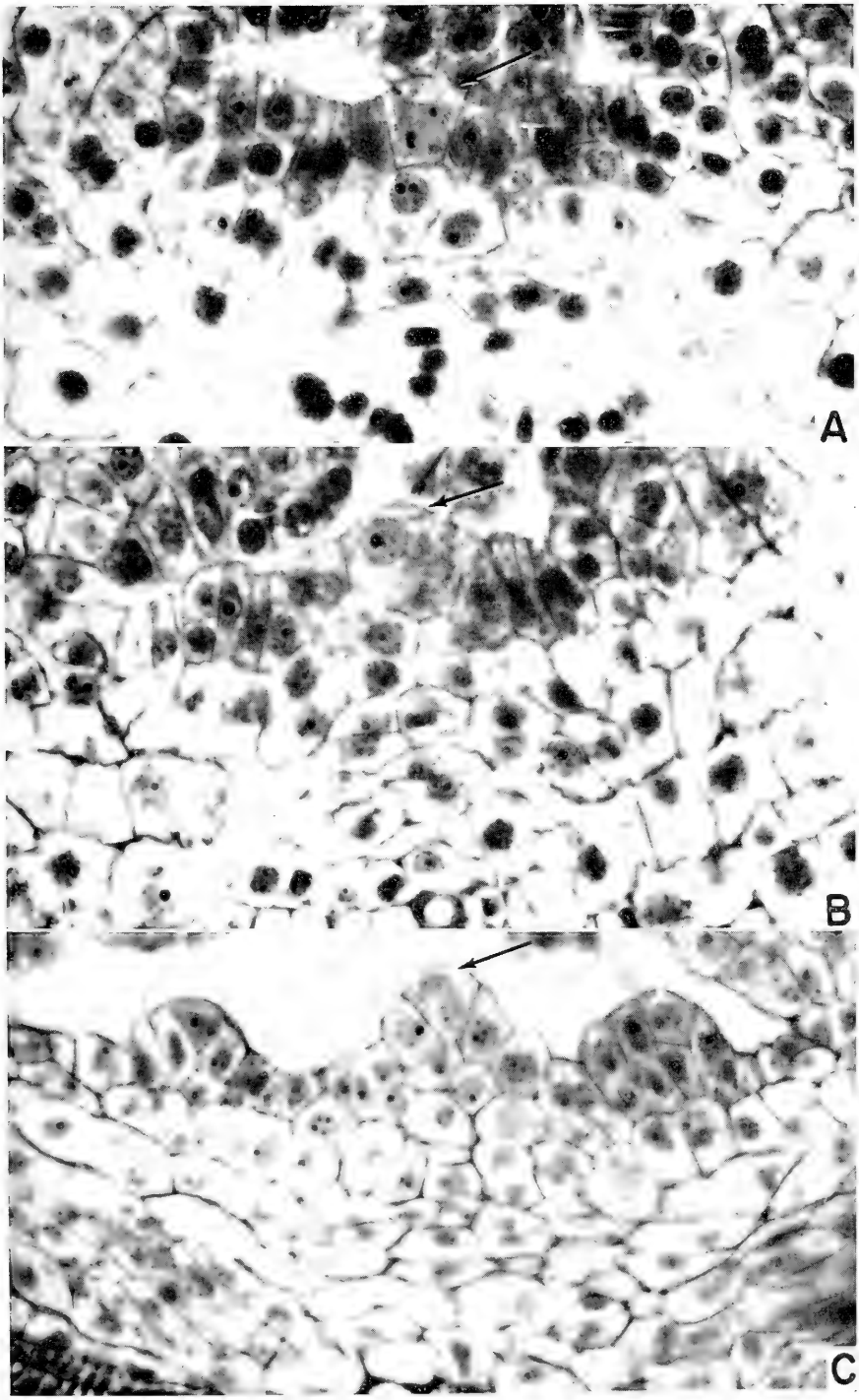

A
B
C

Plate 4

Median longitudinal sections of shoot tips of mature sporophytes.

A and B. Apexes of two plants with nearly identical external dimensions. Each of the plants contained approximately forty series of roots. Both sectioned in the furrow plane. Both × 290.

C and D. Transition in size of vacuoles from the superficial to subjacent layers in the apex. In C the transition to large vacuoles is abrupt. In D it is gradual. Mitochondria and plastids are not conspicuous in these photos. Both Regaud's fixation and Regaud's hematoxylin. Both × 700.

PF, periclinal wall in the superficial layer on the flank of the apex; PS, periclinal wall in the summital region.

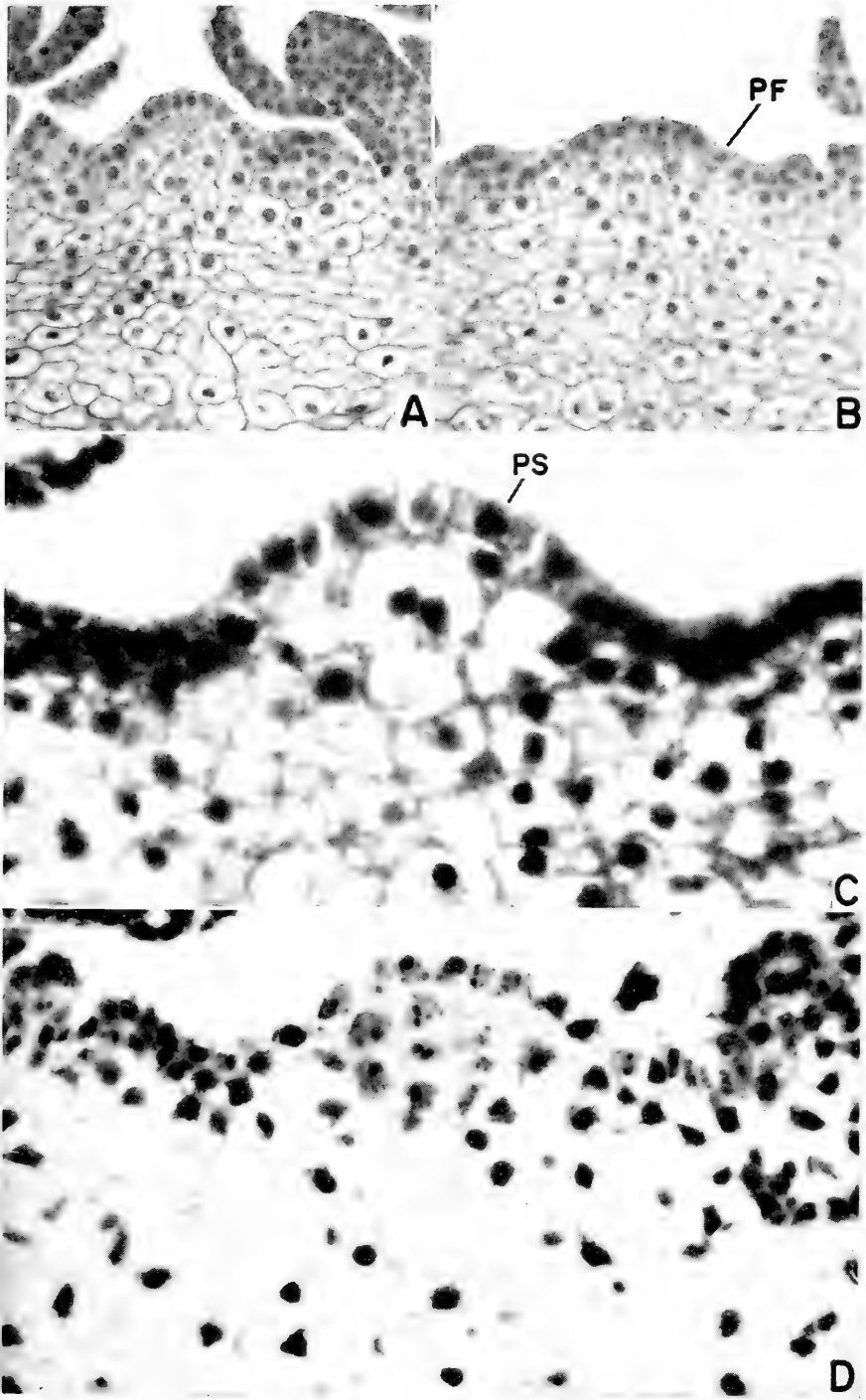

PF
A
B
PS
C
D

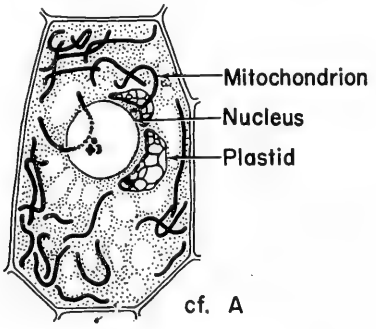
Plate 5

Cytological details in the shoot tip.

A, B, and drawing below. Portions of adjacent longitudinal sections from a large apex. The plastids of the superficial layer (A, at UPl) are undifferentiated and are more condensed in form than those in the cells of the underlying tissues (A and B, at Pl). The cell in A containing the identified mitochondria (M) is drawn below this legend. The mitochondrion identified in the drawing is one of those identified in A. The mitochondria are drawn as they appear in the peripheral cytoplasm, so that vacuoles are not adequately represented. The length of the mitochondria is manifest. The plastids drawn have a darkly staining reticulum and a lightly staining ground substance. Part of one of these plastids is visible in A (Pl). Both A and B, Regaud's fixation and Regaud's hematoxylin. Both × 1,700. Drawing × 1,500.

C, D, E, F. Distribution of starch in the shoot tip is revealed by the PAS reaction. Longitudinal sections. In C and D, starch is absent from the leaf primordia and present in the summital cells of the shoot apex. D is at a higher magnification and shows only the summital cells of C. The starch grains in the summital cells are minute. C, × 500; D, × 880. In E, starch is absent from the leaf primordia and nearly absent from the summital cells of the apex. A few starch grains appear in an adjacent section (not shown) in the summital cells. × 330. In F, starch is present in the leaf primordia but absent from the summital cells of the apex. The starch grains in the leaf primordia are larger than those shown in the apex in D. × 330.

M, mitochondrion; PF, periclinal wall in the superficial layer on the flank of the apex; Pl, plastid; UPl, undifferentiated plastid.



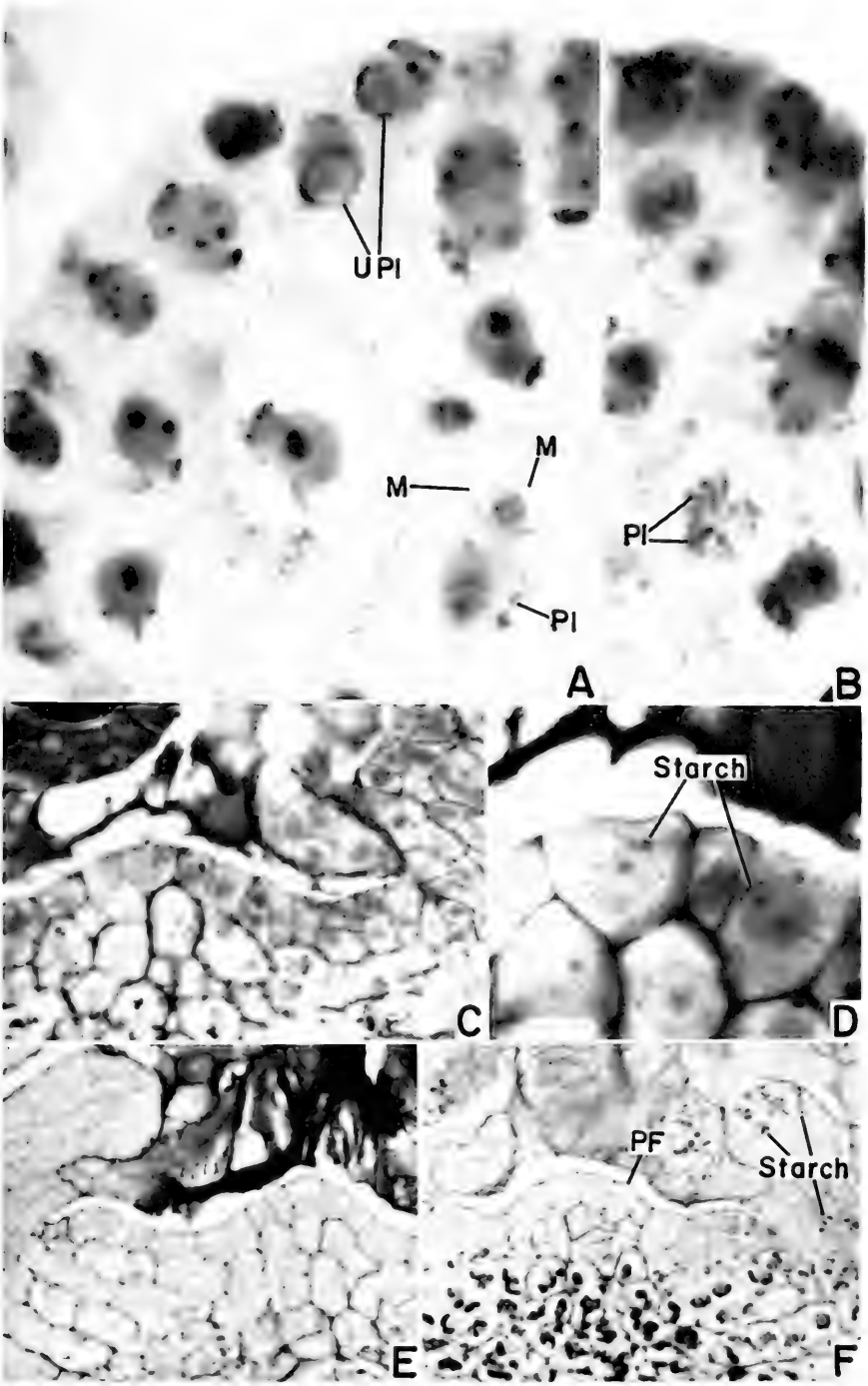

U
Pl
M
M
Pl
Pl
A
B
Starch
C
D
PF
Starch
E
F

Plate 6

Longitudinal sections of shoot tips of mature plants.

A and B. The arrangement of parts is similar in these two figures, but the shoot apex and the subjacent cells appear broader in B than in A. Radial files of cells underlie the region of leaf formation. The tangential walls of cells slant inward at these locations (near F). In A, the cells at CXLT were differentiating with a positional relationship to a leaf trace and were to provide the attachment of the xylem of the trace throughout the peripheral portion of the primary xylem of the stele. Both × 300.

AS, anticlinal metaphase in the summit region; CXLT, cells to form the connection of the xylem of the leaf trace to that of the cauline stele; F, files of cells parallel to the superficial layer; PF, periclinal wall in the superficial layer on the flank of the apex; PS, periclinal wall in the superficial layer in the summital region; W, wedge of cells in position to form new cell file.

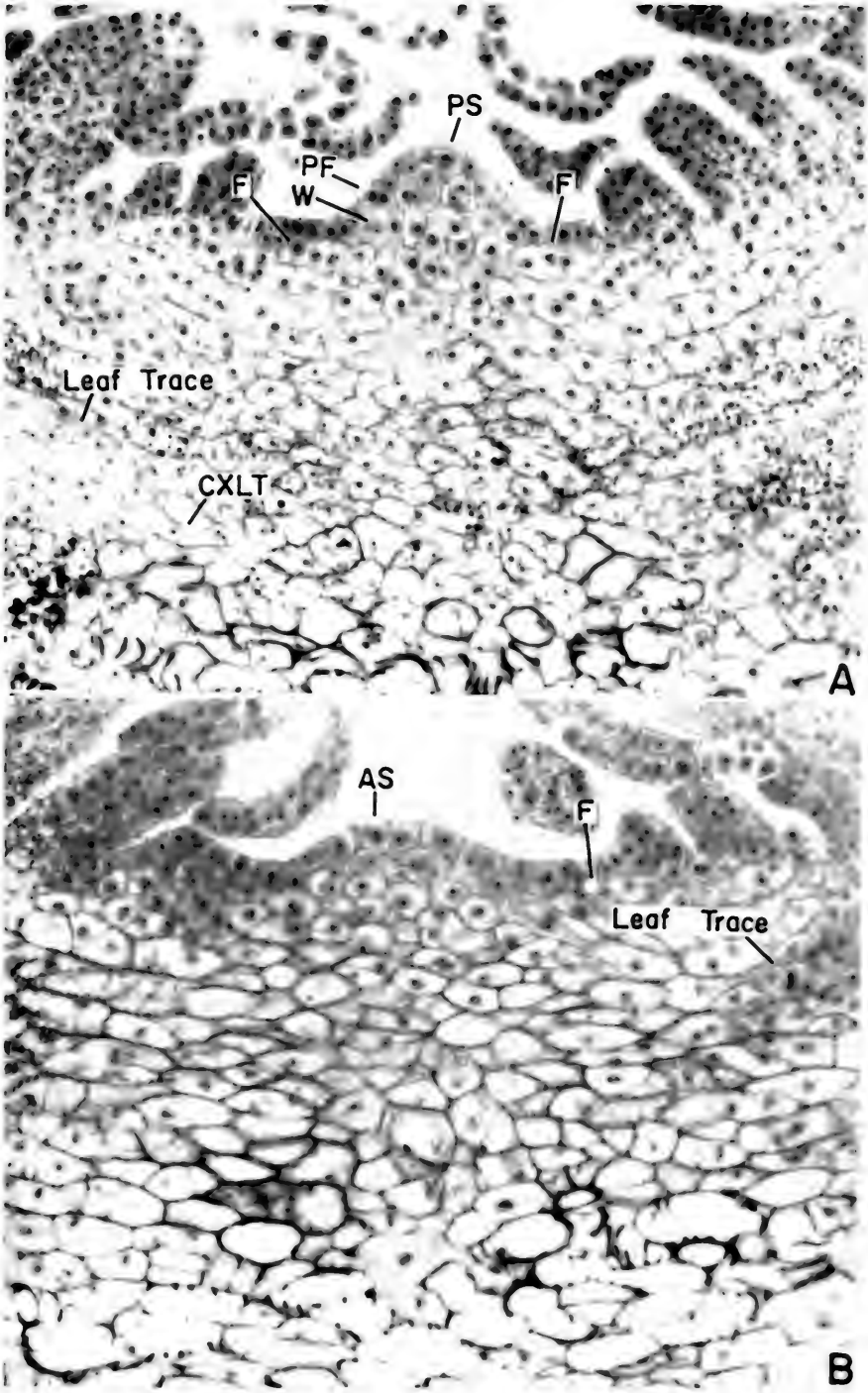

PS
PF
W
F
F
Leaf Trace
CXLT
A
AS
F
Leaf Trace
B

Plate 7

Longitudinal sections showing mature and procambial portions of the stele.

A. Side and upper portions of the stele, plus adjacent tissues. From the primary sieve elements (PSE) the approximate limits of the procambial core can be followed upward and inward. The youngest leaf primordia stand above the periphery of the stele. Beneath the primordia, the tangential walls of the cells slant inward. The xylem is composed of a central and a peripheral portion and is surrounded by a sheath of parenchyma cells (Par S). The tracheids at T are at the base of a leaf trace which is visible in adjacent sections. These tracheids are at the approximate inner limit of the peripheral portion of the xylem core. × 160.

B. Peripheral portion of mature stele. The cells at CXLT have differentiated with a positional relationship to a leaf trace (LT) and form the attachment of the xylem of the trace to the cauline tracheids of the stele. The axial primary sieve elements (PSE) are continuous with the sieve elements of the leaf trace. Scattered tracheids are present in the peripheral xylem (PX). × 160.

C. Similar to B, but with many tracheids at the edge of the peripheral xylem (PX). × 160.

D. Shoot tip and subjacent tissues, showing an arrangement similar to that in A. The tracheids at T are associated with a leaf trace which is visible in adjacent sections. × 190.

CX, central portion of xylem core; CXLT, cells forming the connection of the xylem of the leaf trace to the cauline tracheids; LT, leaf trace; Par S, parenchyma sheath; PSE, primary sieve elements; PX, peripheral portion of the xylem core; T, tracheids, in each case associated with a leaf trace visible in adjacent sections.

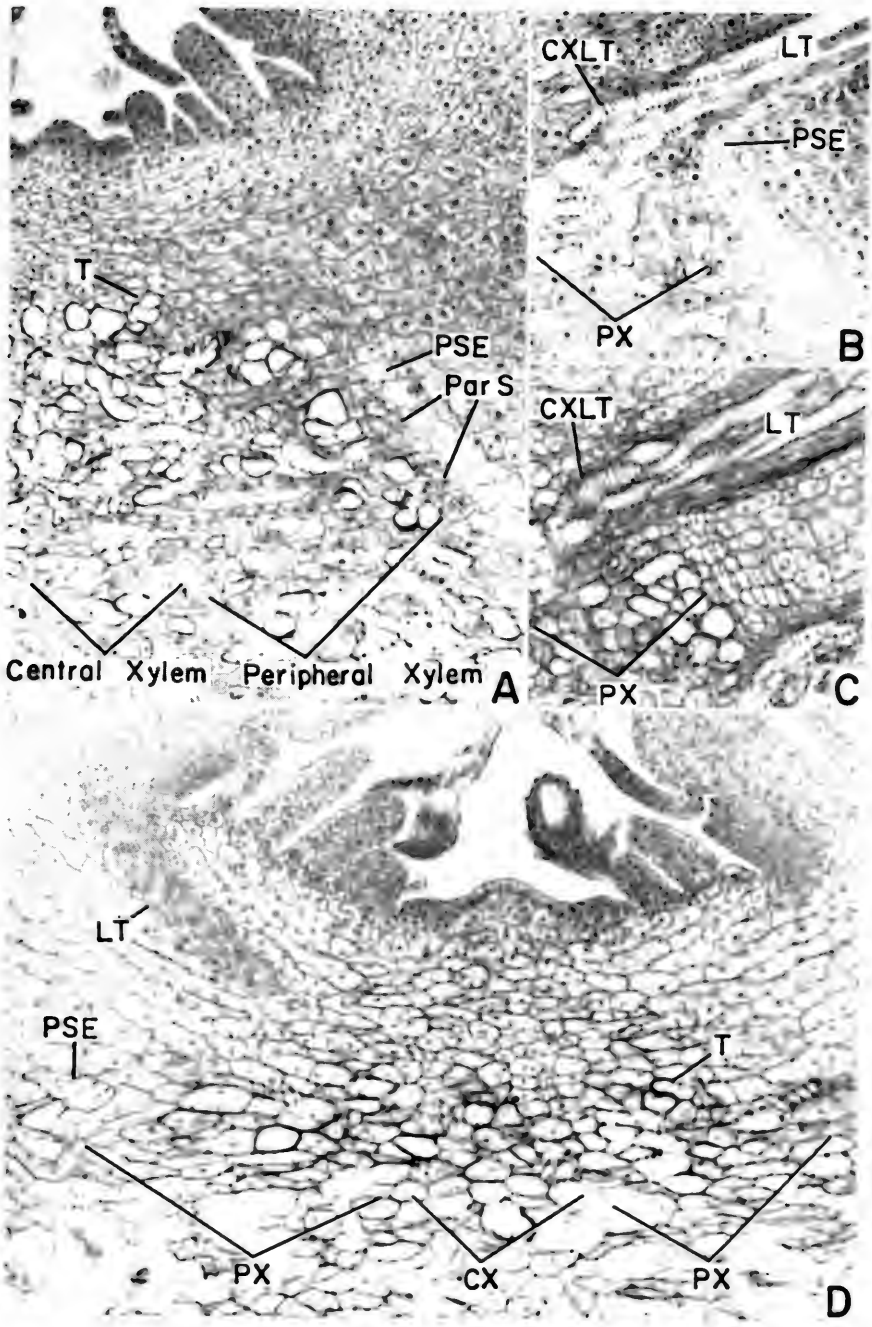

T
PSE
Par S
Central Xylem
Peripheral Xylem
A
CXLT
LT
PSE
PX
B
CXLT
LT
PX
C
LT
PSE
T
PX
CX
PX
D

Plate 8

Stages in the development of leaf traces as seen in longitudinal sections. The axis of the shoot is vertical in all figures.

A and drawing below. Continuity of peripheral region of the stele with the region of formation of procambium to the leaves. The section is median for a young leaf primordium (LP). The shoot apex is to the right, at the margin of the photo. The tangential walls of the cells beneath the leaf primordium are inclined inward. The cells marked 1 and 2 are for reference to the drawing of A that is below this legend. The rectangles above the numbers in the drawing represent the appearance of cells 1 and 2 in transverse sections. For cell 1, the radial dimension is not greatly exaggerated in transverse section, but this is not true for cell 2. The tangential dimensions of the rectangles above the numbers in the drawing are representative of cells found in the appropriate locations in transverse sections. A, × 300, drawing, × 150.

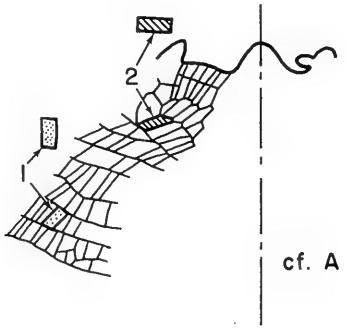
B. Primordium with ligule mother cell. The leaf trace (LT) slants inward. The shoot apex is off to the right. Pr indicates cells of the procambial core. × 370.

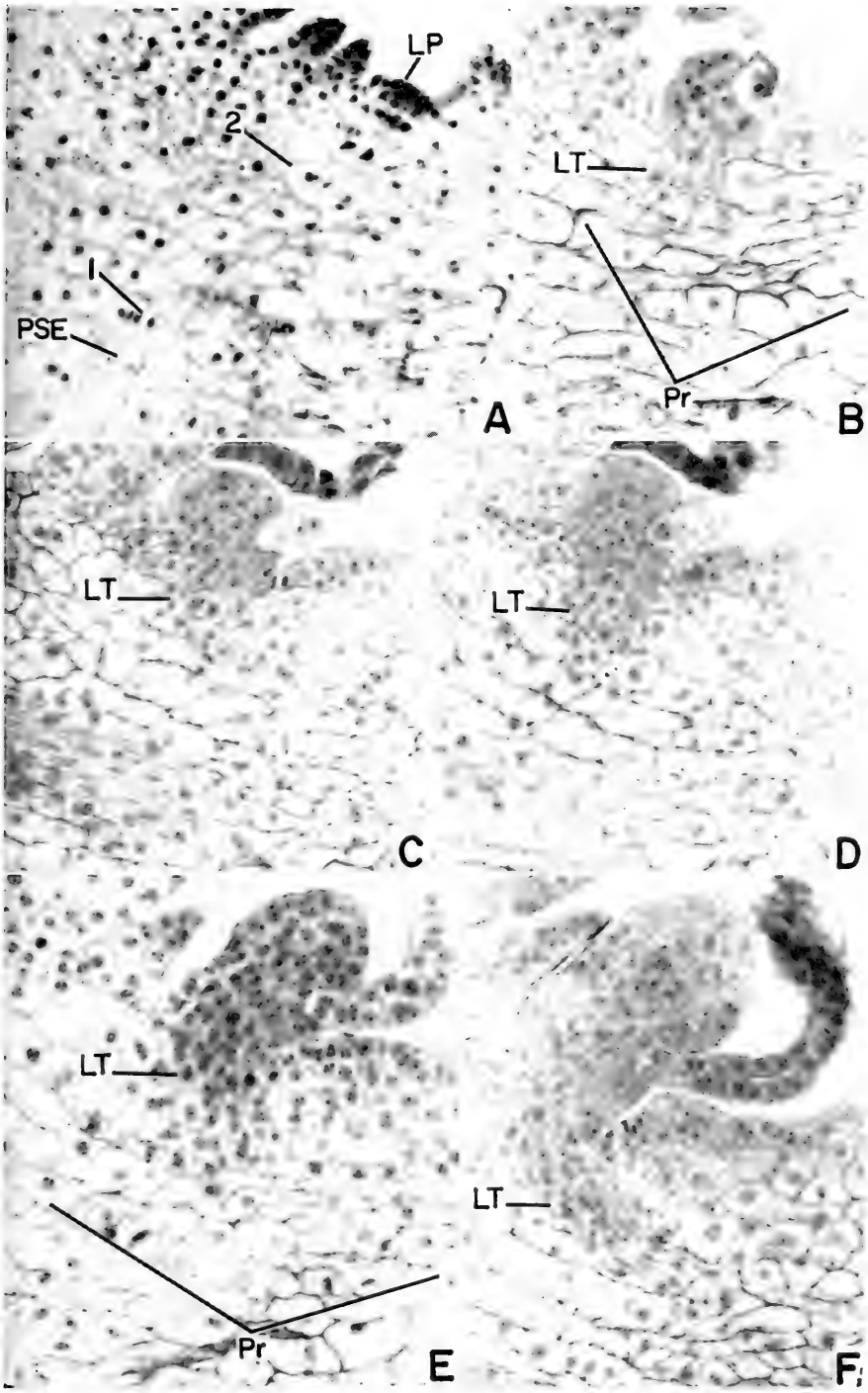
C and D. Serial sections of a primordium (ca. P_7) with a multicellular ligule. The leaf trace (LT) was in the initial stages of bending. × 340.

E. Later stage in the bending of a leaf trace. The procambial leaf trace (LT) stains more deeply than the radial rows of procambial cells at the margin of the procambial core (Pr). × 460.

F. Late stage in the bending of a leaf trace. The attachment of the leaf trace is not perfectly shown, but it can be seen that radial files of narrow cells occur along the base of the trace on its abaxial side. × 325.

LP, leaf primordium; LT, leaf trace; Pr, cells of the procambial core; PSE, primary sieve elements.




LP
2
1
PSE
A
LT
Pr
B
LT
C
LT
D
LT
E
LT
Pr
F

Plates 9 through 12 are serial transverse sections of the upper portion of a large plant, showing leaf traces and their attachments to the vascular core of the stem. Explanations are in the text. The numbers mark the locations of leaves or primordia and their respective traces.

Plate 9

Distances in μ from the summit of the apex are: A, 10; B, 30. $\times$ 340.
SA, shoot apex.

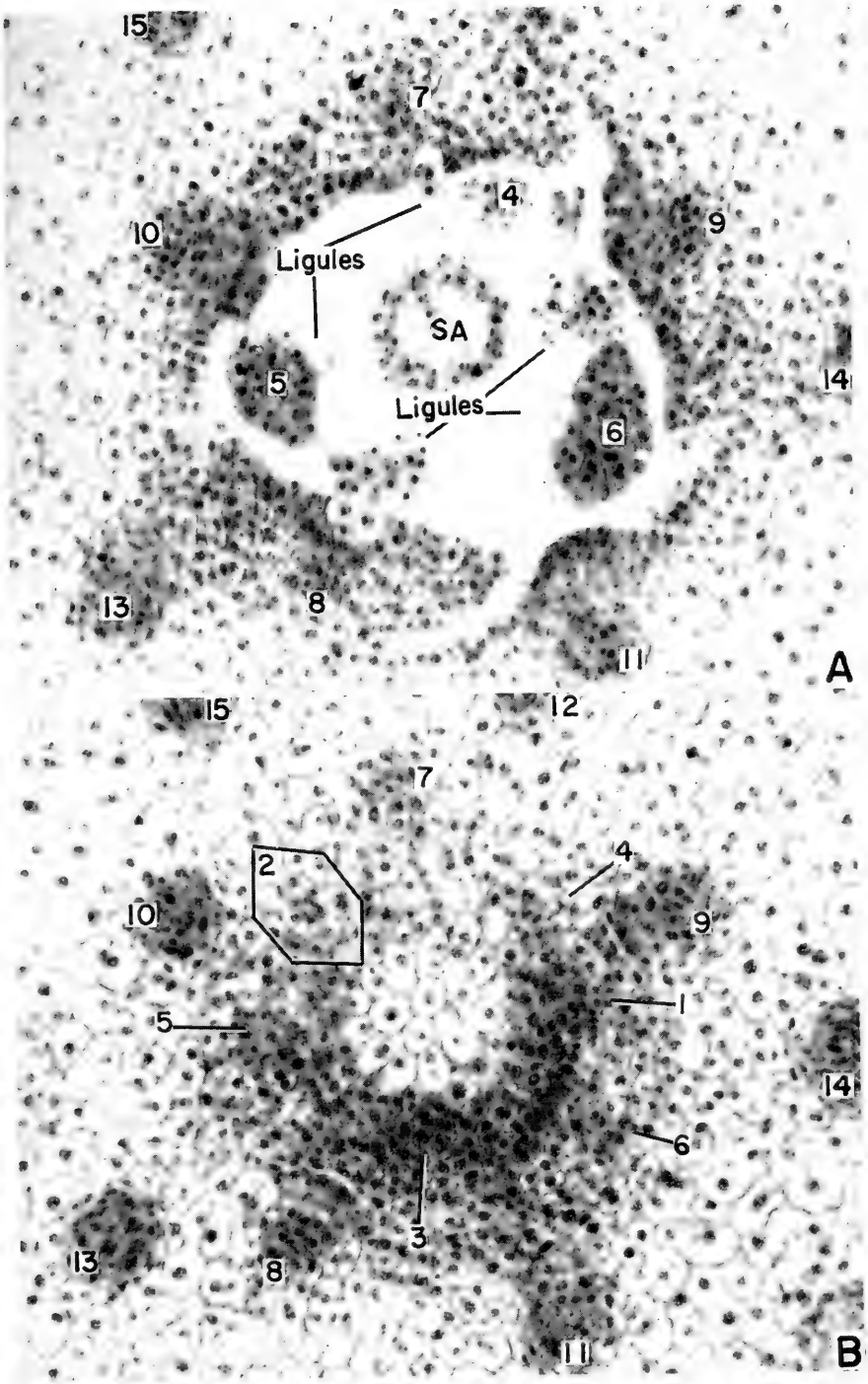

15
7
4
9
10
Ligules
SA
5
Ligules
14
6
13
8
11
A
15
12
7
2
4
10
9
1
5
14
6
3
13
8
11
B

Plate 10

Distances in μ from the summit of the apex are: A, 40; B, 50. $\times$ 340.

The vertical lines on B mark the approximate lateral limits of plate 11, A, B, C, D.

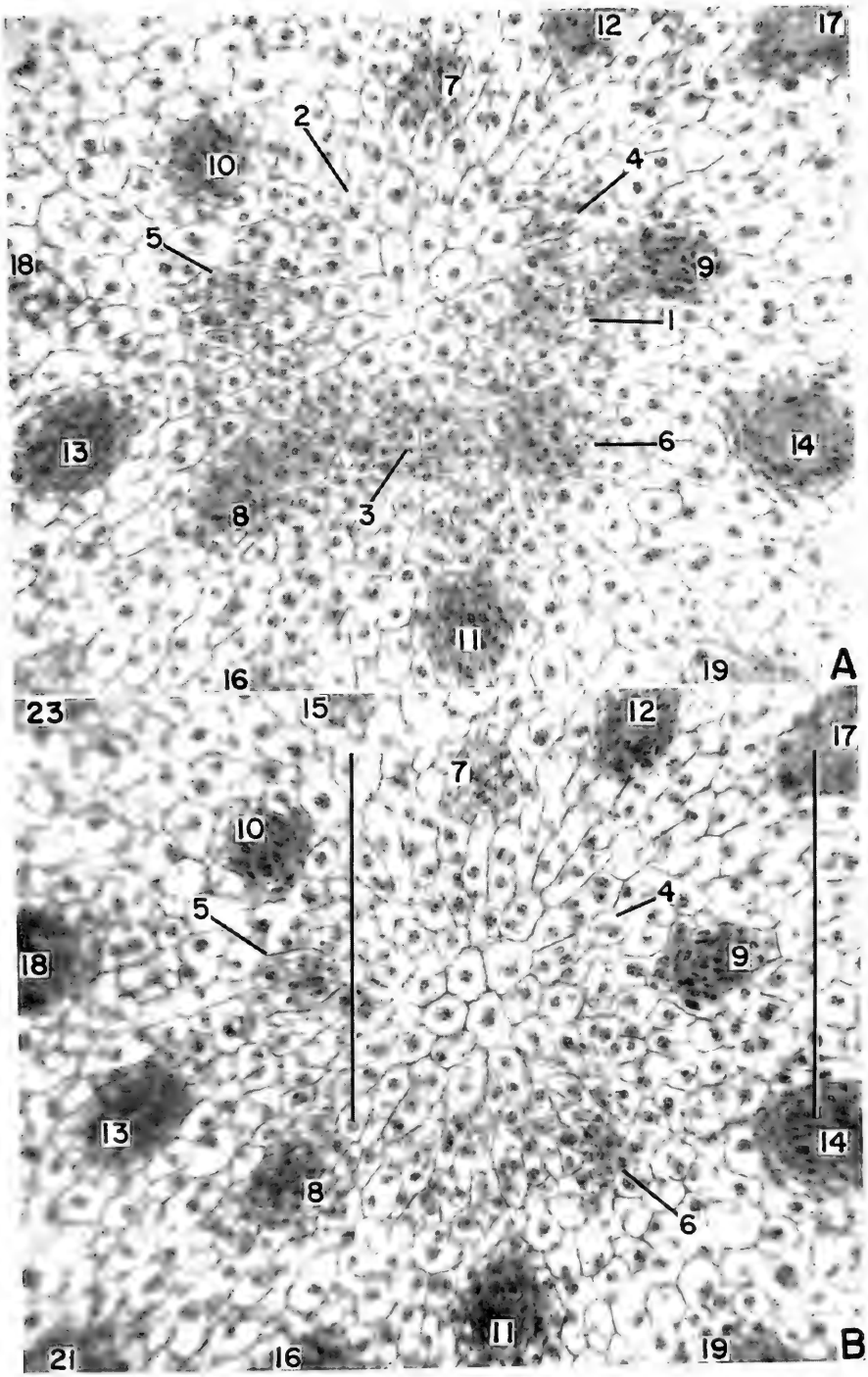

12
17
7
2
10
4
5
18
9
1
13
6
14
3
8
11
16
19
A
23
15
12
17
7
10
5
4
18
9
13
14
8
6
11
21
16
19
B

Plate 11

Distances in μ from the summit of the apex are: A, 70; B, 100; C, 110; D, 130. $\times$ 340.

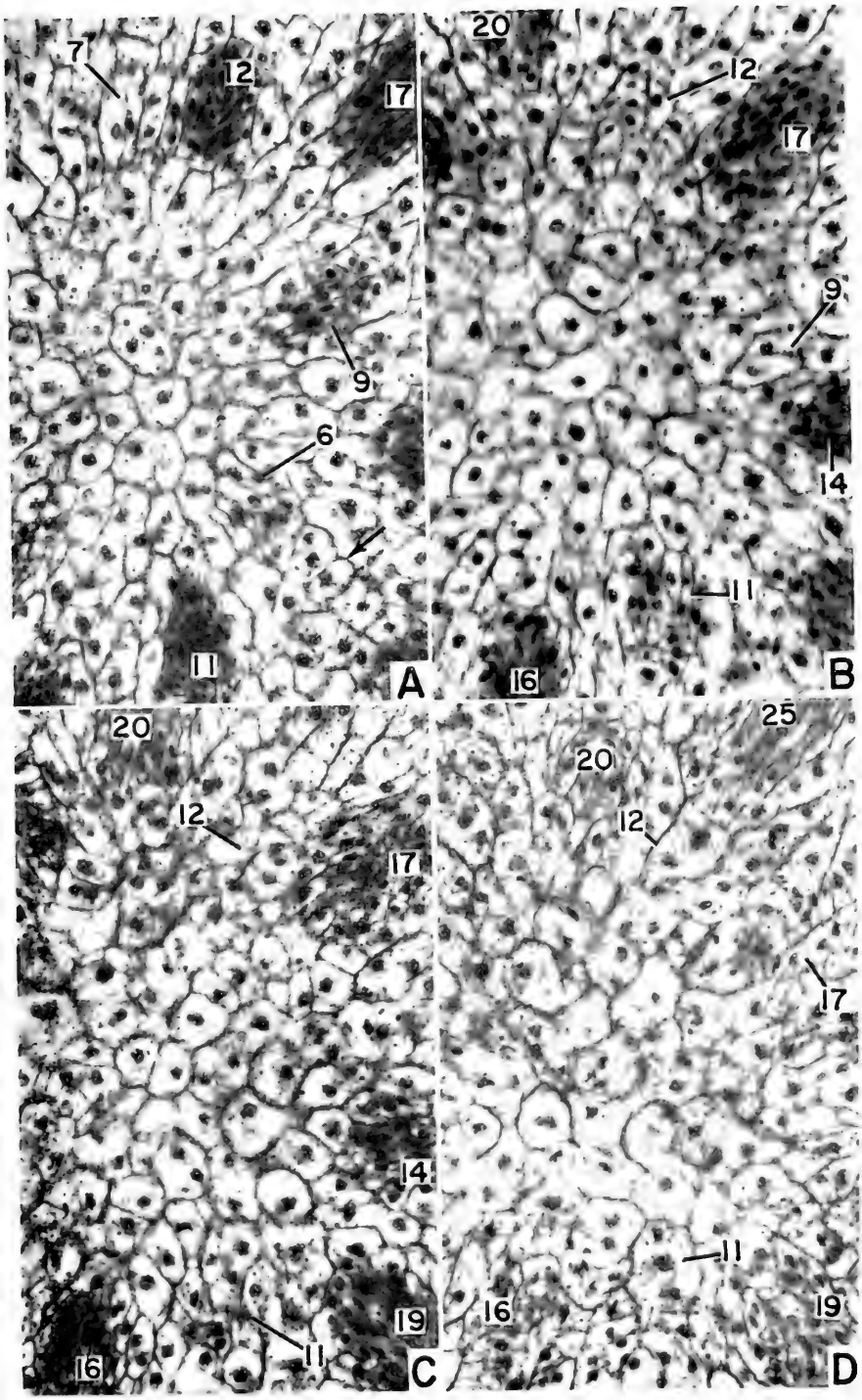

7
12
17
9
6
11
A
20
12
17
9
14
11
16
B
20
12
17
14
19
11
16
C
25
20
12
17
11
16
19
D

Plate 12

The field of view in this plate is broader than that in plate 11, but not as tall. 12, D, is centered on the side of the stele opposite the center of 12, A, B, C. The shift in centers was made to show the arrangement of cells at TP_{21}, which does not appear in A, B, C. Distances in μ from the summit of the apex are: A, 140; B, 160; C, 170; D, 190.

Ph, phloem; T, tracheid. × 230.

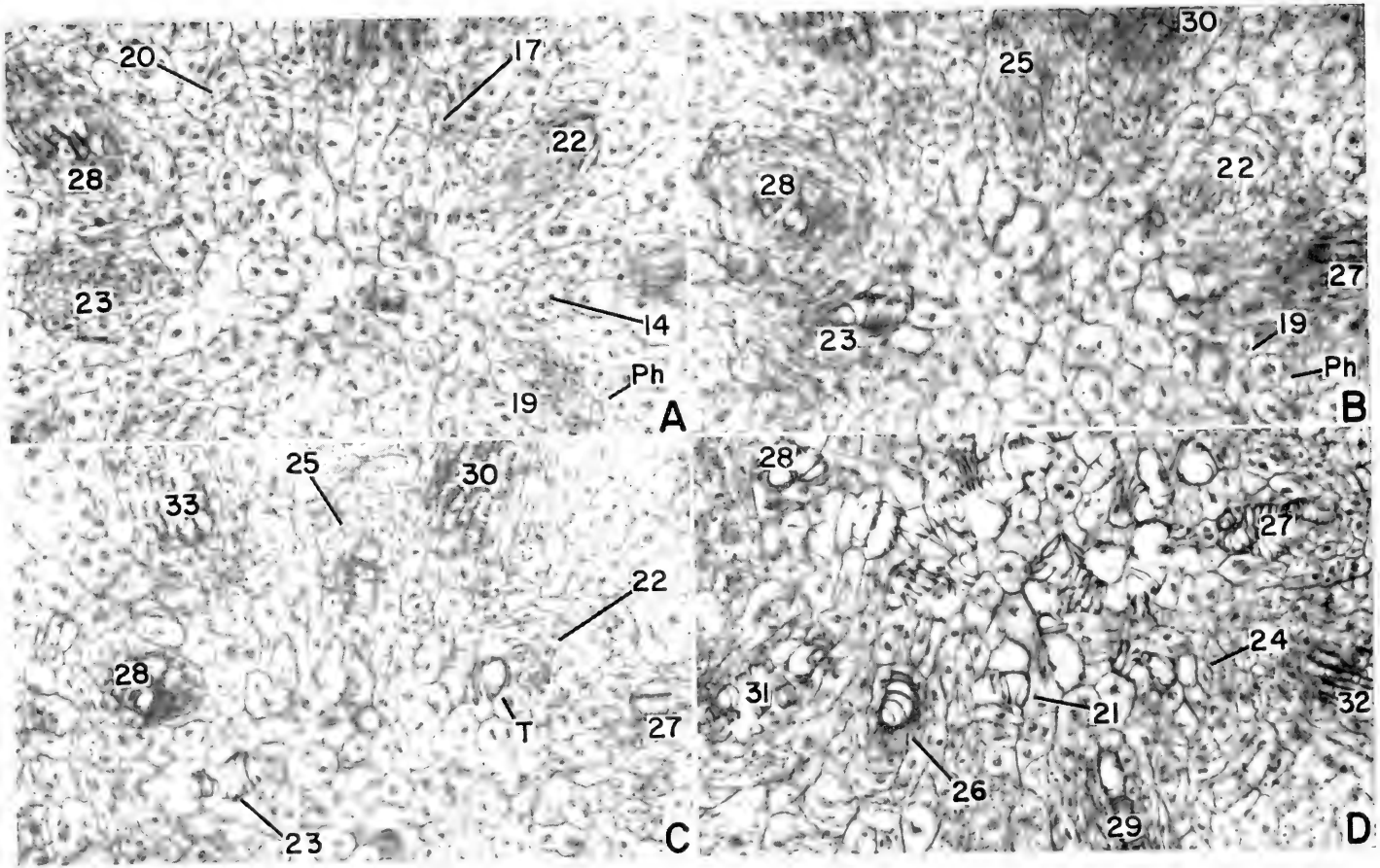

20
17
22
28
23
14
Ph
19
A
30
25
22
28
27
23
19
Ph
B
25
30
33
22
28
T
27
23
C
28
27
24
31
21
32
26
29
D

Plate 13

Relationship of tissues in young and old sporophytes.

A. Sporophyte with several leaves. Longitudinal section in a plane perpendicular to the furrow plane. All of the vascular tissues have a positional relationship to the leaves and the roots. The relationship of the basal meristem (BM) to the rest of the plant is obscured by the presence of root traces (RT). × 370.

B. Sporophyte with several leaves. Longitudinal section in the furrow plane. The basal meristem (BM) is continuous with the lateral meristem (LM). The lateral meristem has produced some secondary sieve elements (SSE). Some primary sieve elements are obliterated (Obl). The basal meristem has produced tracheids (T) on its inner face. × 230.

C, D, E. Longitudinal sections showing the derivatives of the lateral meristem in plants of increasing age. The axis is vertical in all of the photos. In C, only secondary sieve elements (SSE) have differentiated inside of the lateral meristem (LM). × 260. In D, secondary sieve elements and parenchyma (Par) have differentiated in layers inside the lateral meristem. × 260. In E, secondary tracheids (ST) have differentiated among parenchyma cells in alternating layers with secondary sieve elements. × 250.

F. Transverse section showing immature secondary tracheids (ST) nearly in contact with the lateral meristem (LM). × 300.

G. Late maturing tracheids (LMT) in the parenchyma sheath outside of the peripheral xylem (PX). Longitudinal section; axis vertical. × 300.

BM, basal meristem; LM, lateral meristem; LMT, late-maturing tracheids; LT, leaf trace; Obl, obliterated primary sieve elements; P_1, P_2, P_3, youngest, second youngest, and third youngest leaf primordia, respectively; PSE, primary sieve elements; PX, peripheral xylem; RT, root trace; SSE, secondary sieve elements; ST, secondary tracheids inside lateral meristem; T, secondary tracheids inside basal meristem.


P3
P2
P1
LT
LT
RT
BM
A
PSE
SSE
LM
Obl
T
BM
B
SSE
LM
C
LM
SSE
Par
D
SSE
ST
LM
Par
E
LM
ST
F
LMT
PX
G

Plate 14

Morphological and cytological aspects of secondary sieve elements and callose deposits in various cells.

A. Peripheral region of the stele at the level of secondary growth. Longitudinal section with the axis of the shoot vertical. Secondary tracheids (ST) are appressed to primary sieve elements (PSE). Secondary sieve elements (SSE) are confluent with primary sieve elements (at PSE + SSE). Part of the peripheral xylem (PX) also shows in the photograph. × 285.

B, C, D. Callose deposits in sieve elements in the secondary vascular tissue, or prismatic layer. Transverse sections. B and C show the same sieve element. B shows aniline blue fluorescence and C shows the results of staining with resorcin blue. The agreement of the two techniques in the detection of callose is very good. The large white area near the upper end of the sieve element in B is caused by several deposits of callose which are out of focus. B and C are × 1,000. D shows definitive callose (D) fluorescing with aniline blue. Parenchyma cells and tracheids of the prismatic layer are to the right in the figure. × 500.

E. Comparison of nuclei and cytoplasm of sieve elements and parenchyma cells in the prismatic layer, or secondary vascular tissue. Longitudinal sections; stained by the Feulgen reaction and a fast green counterstain. The nuclei of the sieve elements (NSE) are flattened against the walls of the cells and are more deeply stained than the nuclei of the parenchyma cells. × 700.

F, G, H. Fluorescence of callose in parenchyma cells. F shows fluorescence of callose in cells of the ligule. Longitudinal section with the axis of the plant horizontal. × 450. In G, the fluorescence of callose in parenchyma cells (CPC) at the edge of the xylem core may be compared to that of callose in sieve elements (CSE). Transverse section. × 500. H is a transverse section of a root showing a mature vascular bundle. The fluorescence of callose in parenchyma cells (CPC) may be compared with that of callose in sieve elements (CSE). The parenchyma cells containing callose are at the edge of a large air cavity that formed by the breakdown of the inner cortex of the root. × 290.

CPC, callose in parenchyma cells; CSE, callose in sieve elements; D, definitive callose; NPC, nucleus of parenchyma cell; NSE, nucleus of sieve element; PSE, primary sieve elements; PX, peripheral xylem; SSE, secondary sieve elements; ST, secondary tracheids; T, tracheids showing primary fluorescence.

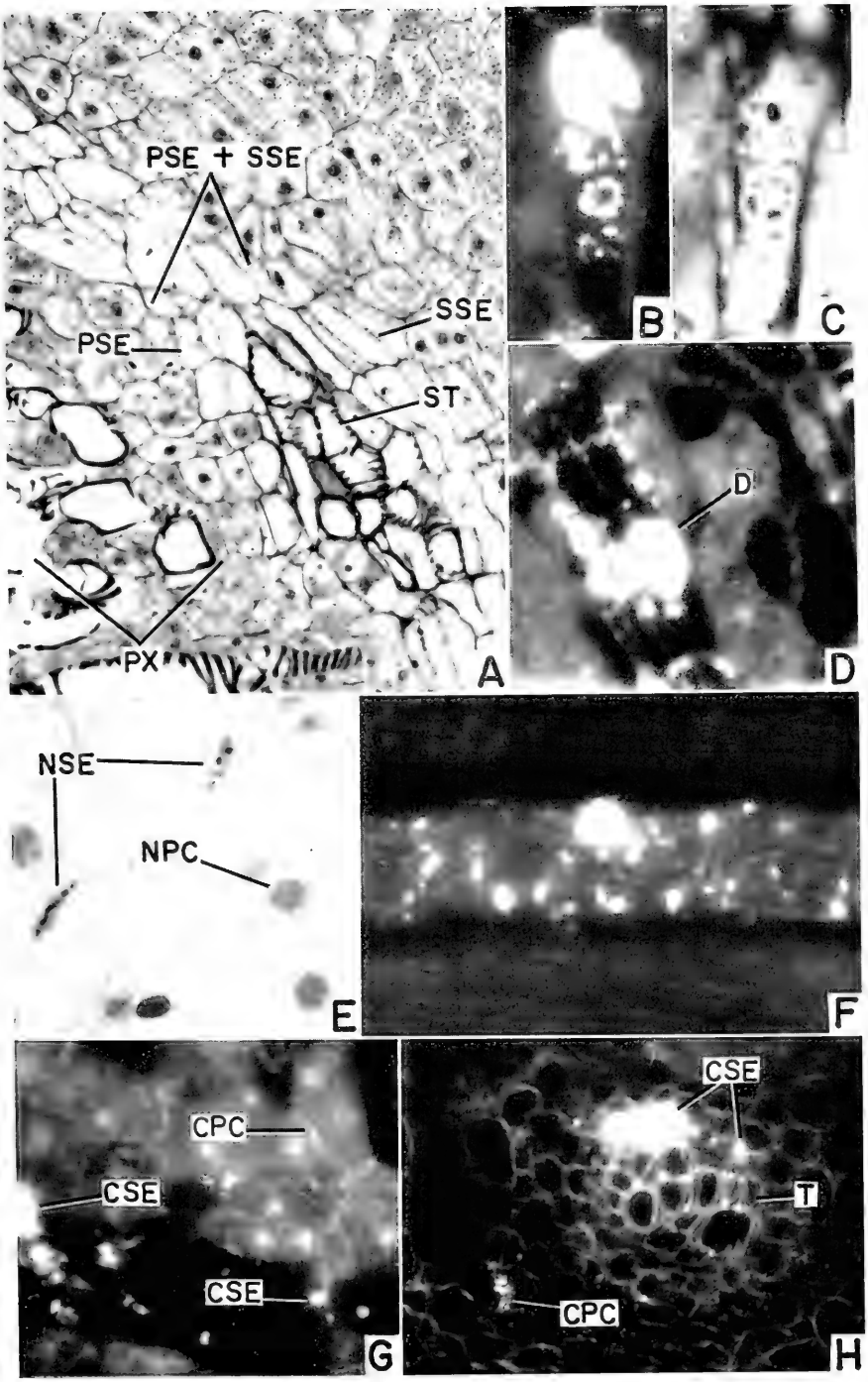

PSE + SSE
SSE
PSE
ST
PX
A
B
C
D
D
NSE
NPC
E
F
CPC
CSE
CSE
G
CSE
T
CPC
H

Plate 15

Various aspects of cortical tissues in mature plants.

A. Upper portion of a longitudinal section of a large plant. Leaf traces (LT) to leaves already lost from the plant may be seen. The peripheral portion of the cortex is composed of nearly empty cells (NEC). × 40.

B. Tissues from the upper (unmarked) trace in A. Functional sieve elements (SE) are present. Crushed tracheary elements appear at CT. Similar tissues were in the other trace (marked LT) in A. × 380.

C, D. The effect of radial division in a cambial initial, as seen in cell files in a transverse section. D is at a higher magnification than C. The boundaries of the cell files at a, b, and c match the radial walls of the initials in the lateral meristem. Explanation is in the text. C, × 270; D, × 500.

E. Periphery of plant, away from basal furrow. Longitudinal section. The cells are compactly arranged. The straight walls (Walls) indicate that the cells of the cortex subdivide before they enter into formation of the corky layer which covers the sporophyte. × 250.

F. Periphery of plant where the furrow was forming. Transverse section. The furrow was forming from the right. The cells indicated by the arrows show division figures. × 220.

CT, crushed tracheary elements; LM, lateral meristem; LT, leaf trace; NEC, nearly empty cells; Par, parenchyma; SE, sieve elements; SSE, secondary sieve elements; ST, secondary tracheids; arrows, cells showing division figures; a, b, c, radial boundaries matching the radial walls of initials in the lateral meristem.

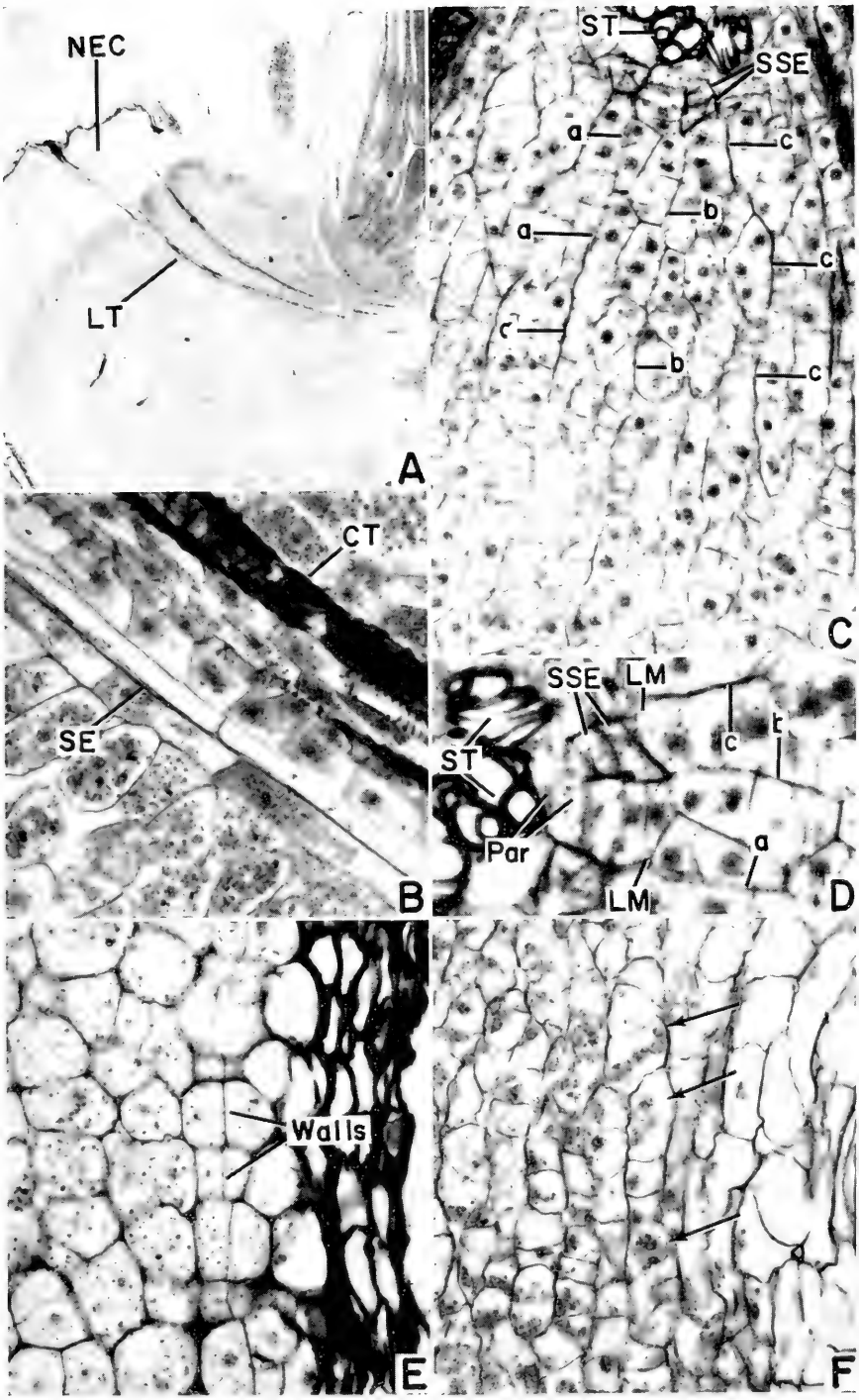

NEC
LT
A
ST
SSE
a
c
b
a
c
c
b
c
C
CT
SE
B
SSE
LM
ST
c
t
Par
a
LM
D
Walls
E
F

Plate 16

Various aspects of the attachments of roots and root traces in mature plants.

A. Photo of a whole plant, showing orthostichies (O) of *root traces* in a view parallel to the furrow plane. The decayed tissue which covered this face of the corm was removed before the photograph was taken. The orthostichies appear as nearly vertical rows of dots. The more lateral orthostichies (those nearest the shoot apex) slant inward toward the top. The oldest root traces of each orthostichy are at the top. × 5.

B. Photo of the underside of a whole plant, showing orthostichies and series of *root attachments*. One orthostichy is marked at both ends (O and O). Two series of comparable age are marked (S and S′). The members of S and S′ are on alternate orthostichies. Compare with figure 14. Basal furrow runs from left to right. In comparing B to A, it must be kept in mind that the total area of attachment of a root (shown in B) is larger than the cross-sectional area of a root trace (shown in A). × 10.

C. Part of an orthostichy of root traces in a large plant, as seen in longitudinal section at right angles to the furrow plane. RRT indicates a remnant of a root trace which may have belonged to a primordium formed at the center of the basal meristem. Stained with the PAS reaction. The portion of the tip of the primordium at the right in the section contains much less starch than the comparable number of cells in the ground tissue. The section is slightly oblique with reference to the orthostichy and the orthostichy does not show at the upper right in the figure. × 250.

O, orthostichy; RRT, remnant of a root trace; S, S′, series of root attachments.

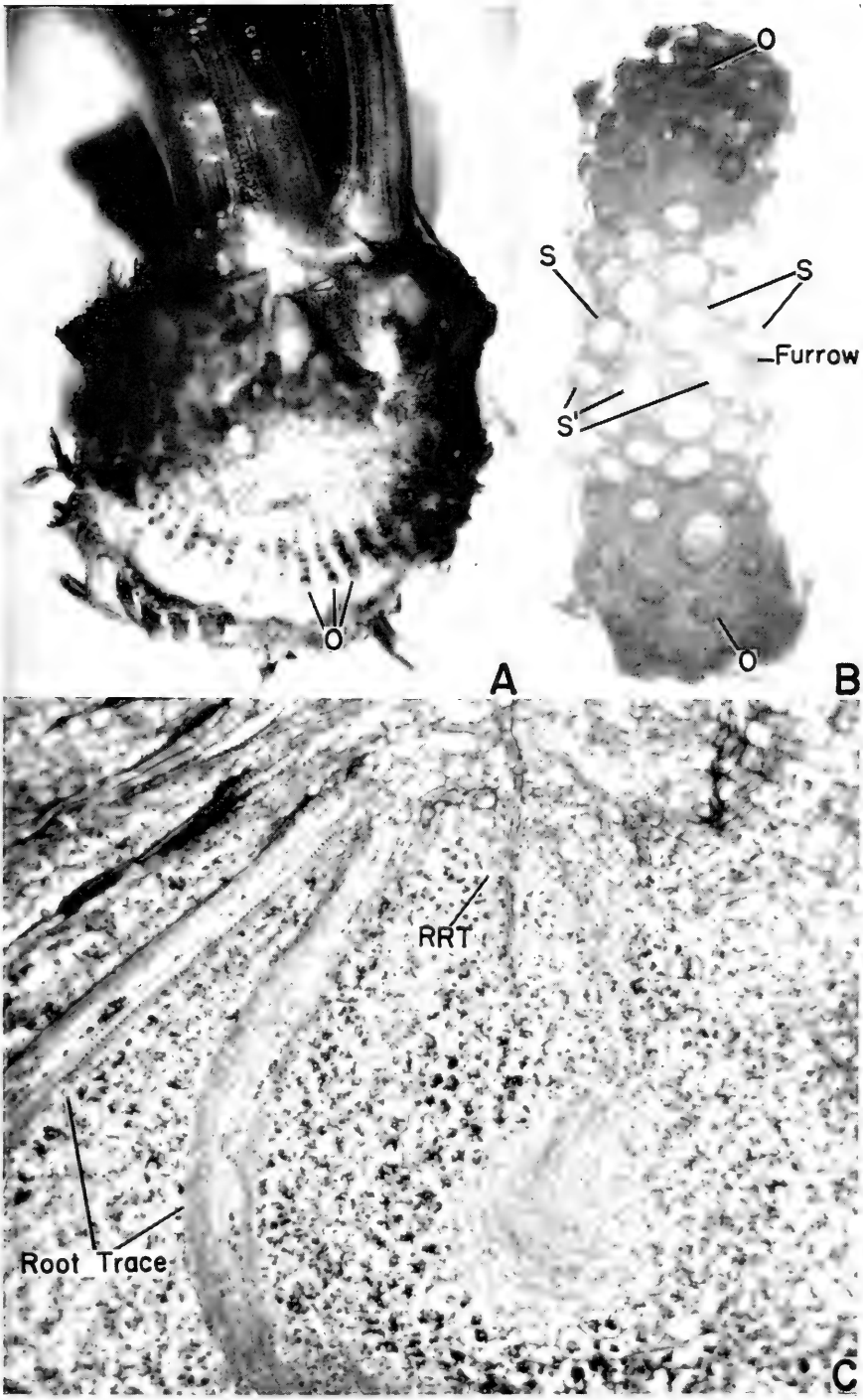

O
S
S
Furrow
S'
O
A
B
RRT
Root Trace
C

Plate 17

The relationship of the cambium to derivative tissues.

A. Longitudinal section in the furrow plane of a young plant. The basal meristem (BM) and lateral meristem (LM) are confluent at the right but not at the left because of obliquity of sectioning. The lateral meristem has produced secondary sieve elements (SSE) in contact with the primary sieve elements (PSE). Primary sieve elements are obliterated in part (Obl). At the upper part of the stele, primary sieve elements (PSE) are still intact. At the left, a root trace (RT) is attached to the stele. × 350.

B. Basal meristem (BM) and derivatives, as seen in longitudinal section in the furrow plane in a large plant. The tracheids (T) and parenchyma (Par) which compose the inner derivatives of the basal meristem are weakly layered in a direction parallel to the basal meristem. × 210.

C. Basal and lateral portions of the stele of a plant of moderate size, as seen in longitudinal section in the furrow plane. The section is off median so that the bases of some root traces may be seen. The uppermost root primordium (RP) marks the upper limit of the basal meristem. Some files of cells below this limit (within brackets at FC) contain old secondary sieve elements (OSSE), and this indicates that the course of differentiation of the derivatives of the initials of these files has changed from one that produces sieve elements to one producing no sieve elements. The initials of these files were in the lateral meristem at one time (while the inner derivatives of the initials matured as sieve elements), although they were in the basal meristem at the time of fixation. Secondary tracheids (ST) inside the basal meristem are confluent with parenchyma (Par) inside the lateral meristem. This plant had not yet produced secondary tracheids inside of the lateral meristem at the time of fixation. × 170.

BM, basal meristem; FC, files of cells containing sieve elements; LM, lateral meristem; Obl, obliterated primary phloem; OSSE, old secondary sieve elements; Par, parenchyma; PSE, primary sieve elements; RP, root primordium; RT, root trace; SSE, secondary sieve element; ST, secondary tracheid; T, tracheids.

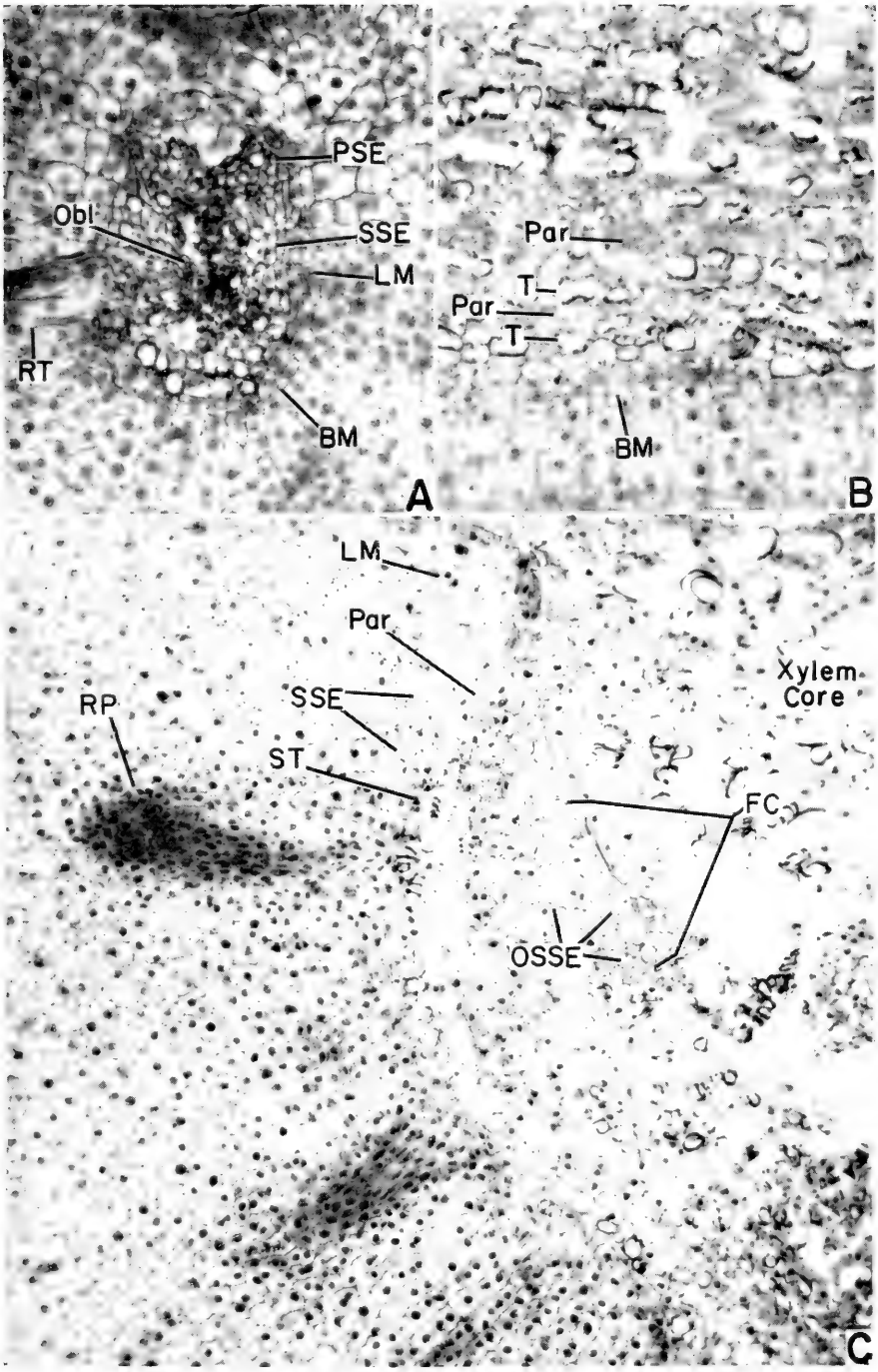

PSE
Obl
SSE
LM
RT
BM
A
Par
T
Par
T
BM
B
LM
Par
Xylem
Core
RP
SSE
ST
FC
OSSE
C

Plate 18

Various aspects of the basal and lateral meristems and of young root primordia.

A. Transverse section of a large plant in which the basal meristem has advanced toward the shoot apex. At the center of the photo is a portion of the stele bearing leaf traces (LT). Secondary sieve elements (SSE), which were produced by the lateral meristem (LM), occur between this location and the bases of root traces (BRT). The lateral meristem was once operative throughout the entire perimeter of the stele at this level in the plant but has been converted, in part, to the basal meristem (left and right extremities of the stele). The lateral meristem has produced several layers of secondary tracheids (ST). × 60.

B. Similar to A, but at a higher magnification. From another large plant. Tracheids at the bases of root traces (BRT) are confluent with parenchyma (Par) produced by the lateral meristem (LM). × 150.

C. Relationship of the basal meristem (BM) to mature vascular tissues. Longitudinal section perpendicular to the plane of the furrow and passing between orthostichies. Secondary sieve elements (SSE) differentiate toward the basal meristem. × 300.

D. Root primordium showing no distinct indications of organized apical activity. A procambial strand (Pr) has differentiated. Longitudinal section perpendicular to the plane of the furrow in a plant of moderate size. × 290.

E. Low starch content in an organizing primordium. Section perpendicular to the furrow plane and from a large plant. The starch content of the root primordium (RP) is lower than that of the ground tissue. Compare with plate 16, C. × 250.

BM, basal meristem; BRT, bases of root traces; LM, lateral meristem; LT, leaf trace; Pr, procambium; RP, root primordium; RT, root trace; SSE, secondary sieve element; ST, secondary tracheids.

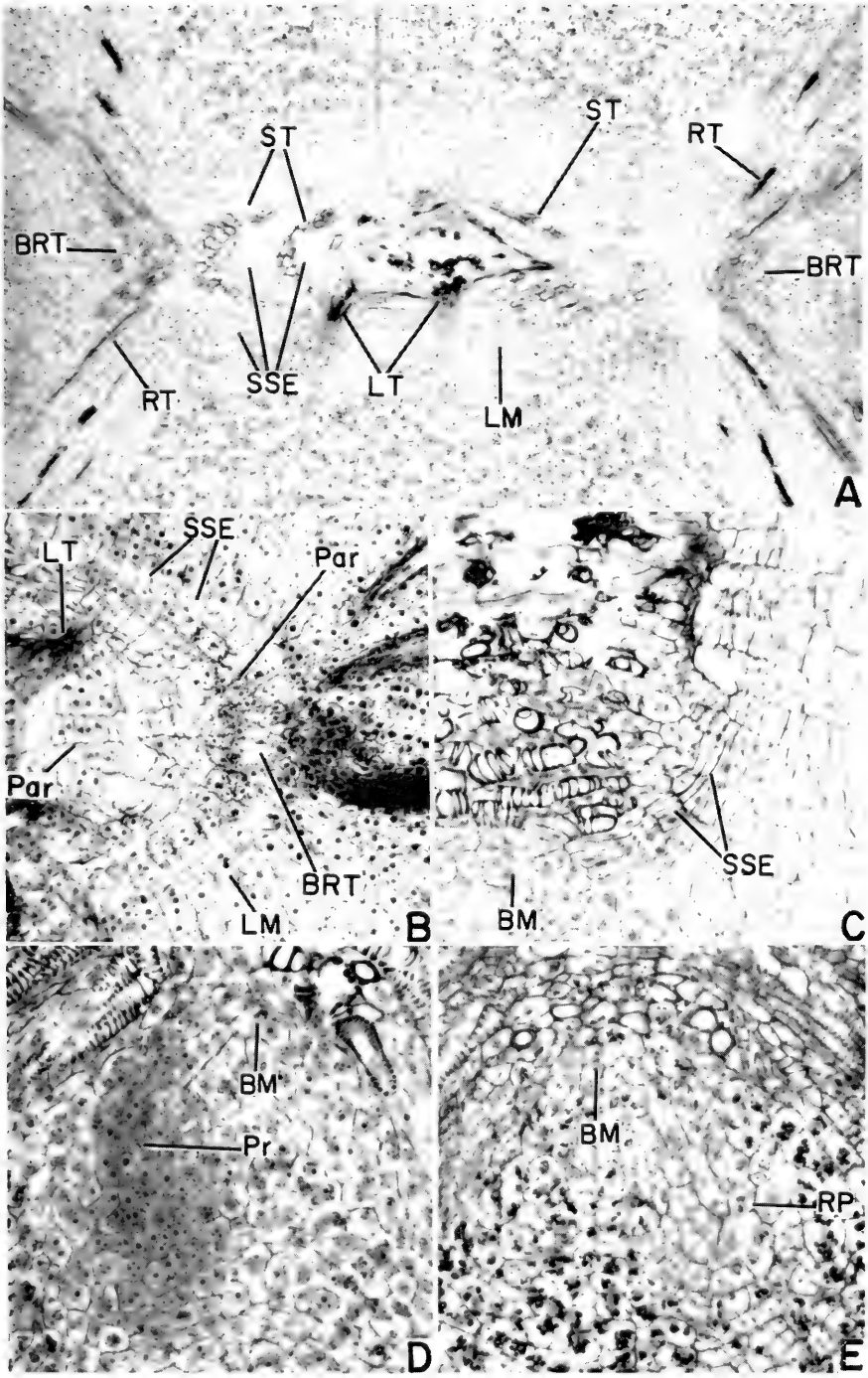

ST
ST
RT
BRT
BRT
RT
SSE
LT
LM
A
LT
SSE
Par
Par
BRT
LM
B
SSE
BM
C
BM
Pr
D
BM
RP
E

Plate 19

Transverse and longitudinal sections of root tips.

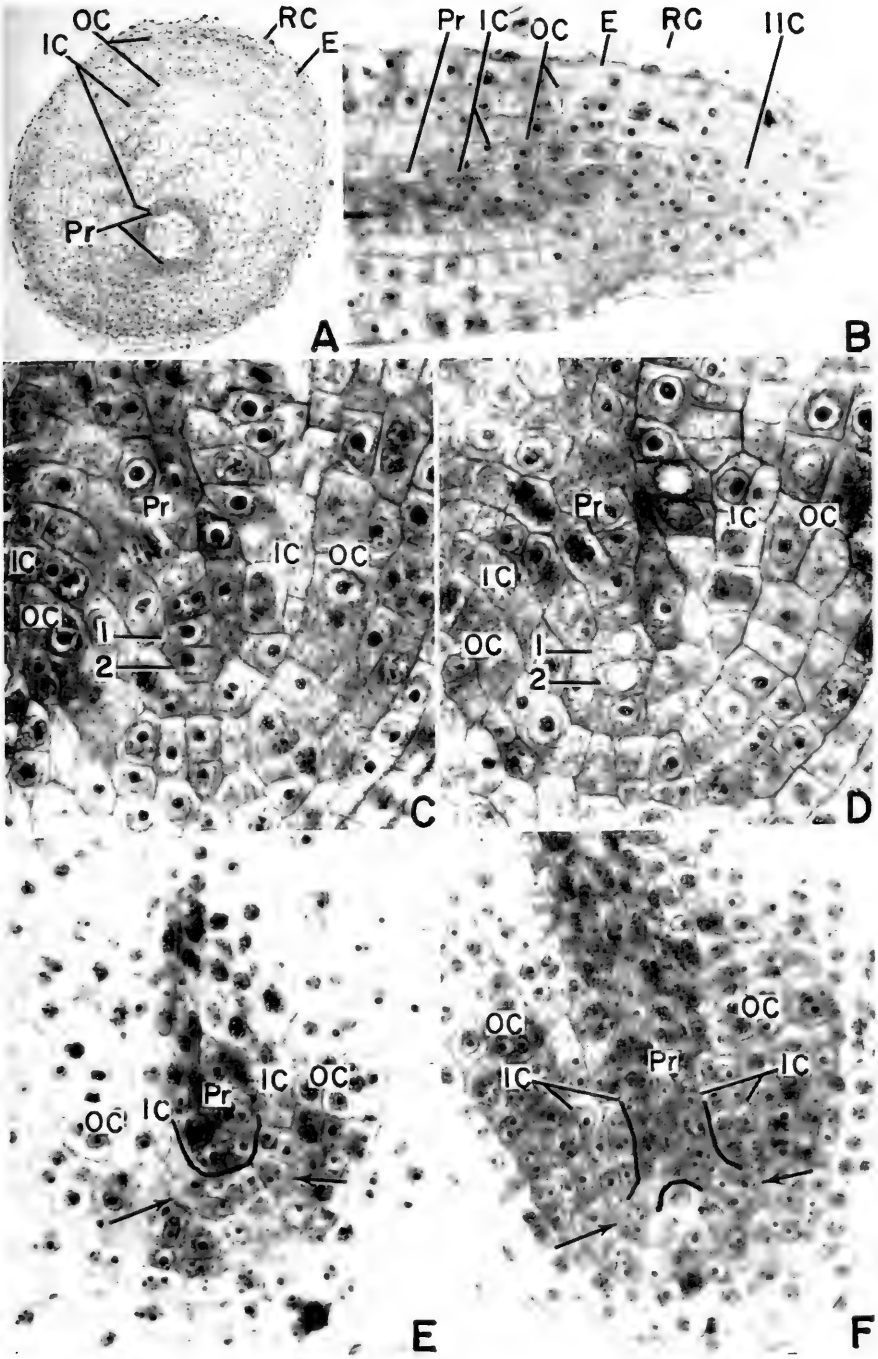
A. Transverse section of a root tip of a partially extended root of a mature plant. The procambium (Pr) has an eccentric position in the section. This eccentricity depends on the development of the inner cortex (IC). × 80.

B. Longitudinal section of the tip of a partly extended primary root of a sporeling. The root cap (RC), epidermis (E), and outer cortex (OC) share a common origin. The cell net indicates continuity between the procambium (Pr) and the initials of the inner cortex (IIC). The inner cortex (IC) does not develop markedly in the primary root. × 430.

C, D. Adjacent longitudinal sections showing continuity of the procambium (Pr) and the initials of the inner cortex. A sharp boundary exists between the outer cortex (OC) and the inner cortex (IC). The apex represented is one among several within the dichotomized tip of a partially extended root of a mature plant. Cells 1 and 2 appear in both sections. × 700.

E, F. Stages in the dichotomy of an apex. F is a later stage than E. Longitudinal sections of roots emerging from the cortex of a small plant. The inked lines follow the tip of the procambium (Pr). In F, the lines are open below because the procambium is continuous with the initials of the inner cortex (at arrows in F). This continuity was caused by a contribution to the procambium from cells among the initials of the inner cortex (arrows in E). E, × 500. F, × 420.

E, epidermis; IC, inner cortex; IIC, initials of inner cortex; OC, outer cortex; Pr, procambium; RC, root cap; 1 and 2, cells appearing in both C and D; arrows, cells among the initials of the inner cortex.


OC
RC
IC
E
Pr
A
Pr
IC
OC
E
RC
IIC
B
Pr
IC
OC
IC
OC
1
2
C
Pr
IC
OC
IC
OC
1
2
D
OC
IC
Pr
IC
OC
E
OC
OC
IC
Pr
IC
F

INDEX

Anneau initial, 29
Apical cell: in root, 77; in shoot, 8, 19-21
Apical initials: differentiation of, in young shoot, 16, 19, 24, 28-29

Basal meristem, 3, 13, 54-56, 69-75

Callose, 15, 45-48
Cambia: multiple, 43, 45
Cambium, 3, 13-15, 40-45; origin of, 14, 40-43
Cauline vascular tissue, 11-12, 39
Cork, 14, 50; storied, 50
Cork cambium, 15, 50
Cortex: primary, 32, 48-49; secondary, 14, 48-50

Dichotomy of root, 78

Foliar vascular tissue, 11-12, 39
Furrow plane of sectioning, 3

Ground meristem, 31-32

Histogens in root, 76-77

Initials: concept of, 10, 29; conversion of, in cambium, 73

Lateral meristem, 3, 13. *See also* Cambium

Mitoses: in root apex, 81; in shoot apex, 26, 28

Orthostichies of roots, 3, 6, 53-54; defined, 57

Parenchyma: secondary, 43, 71
Parenchyma sheath, 30, 37
Parenchymatous mantle, 14, 31
Phloem: primary, 12, 30, 35; secondary, 43, 45, 71
Phyllotaxy, 17
Pith, 39-40
Planes of sectioning, 3
Plastids: in root primordium, 79; in shoot apex, 23
Plastochronic changes, 17, 20
Plerome, 8, 11
Primary meristem of *Stylites*, 14, 31
Primary tissues: differentiation of, 11-12, 29-32
Prismatic layer, 3, 15
Procambium, 11, 30-31, 79; of leaves, 12-13, 33-40; of root primordium, 79

Region of leaf formation, 7
Rhizomorph, 75
Rhizophore, 75
Ribonucleic acid: stain for, in shoot apex, 24-25
Root: origin of tissues in, 80; primary, 54-56
Root apex: dichotomy of, 78; organization of, 77-78, 80-81
Root initiation: location of, 52-54, 57-69; process of, 76-77, 78-80; sequence of, 61-65; site of, 65
Root maturation: sequence of, 61
Root-producing meristem, 54, 69. *See also* Basal meristem
Roots: adventitious, 55, 56, 74; endogenous, 76; exogenous, 76; kinds observed, 80

Secondary meristem: of *Stylites*, 14
Secondary vascular tissue. *See* Prismatic layer
Series of roots, 52-54; defined, 57
Shoot apex: defined, 7; cytology of, 22-26; form of, 8-10, 15-22; function of, 10-11, 26-29; mitoses in, 26, 28; origin of, 9-10, 15-17; size of, 19
Shoot tip, 7
Standardized position: for transverse sections, 58
Starch: in root primordium, 79; in shoot tip, 25-26
Stele, 8

Wurtzelstele: of *Stylites*, 72, 75

X-axis: defined, 58
Xylem: central, 12, 30, 39-40; centrifugal maturation of, 40; centripetal maturation of, 40; peripheral, 12, 30, 37, 39-40; secondary, 43, 71

Y-axis: defined, 58

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