SEQUENCE OF TISSUE DIFFERENTIATION IN THE ROOT OF *CERATOPTERIS THALICTROIDES*

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Abstract: The external morphology as well as the differentiation and maturation of the vascular tissue of the root and initiation of lateral root in *Ceratopteris thalictroides* (L.) Brongn. are studied. At maturity leaf length is negatively correlated with leaf age. The initial cells of all tissues of the root are present a few cells behind the apical cell. The sequence of the differentiation and maturation of each tissue is strictly constant. Metaxylem appears first but matures last among all of the other vascular tissues. Protophloem is identified next to the metaxlem and matures before the protoxylem appears, then protoxylem matures before the metaxylem matures. The lateral root initial arises in the endodermis at about five cells behind the apical cell. The endodermis matures by the formation of a Casparian strip before the metaxylem matures and after protoxylem maturation. The air spaces arise schizogenously very close to the apical cell usually before the earliest metaxylem appears. The lateral root is derived endogenously from a single enlarged endodermal cell at a definite place. But no other morphologically difference can be seen in this special lateral root initial.

INTRODUCTION

A considerable number of papers concerning the differentiation and development of tissues in the roots of ferns have been published. Most of the studies on vascular differentiation of ferns deal with the protoxylem and protophloem, and frequently only with the first few elements of these tissues. The tissues other than the cap and vascular tissues, *i. e.*, epidermis, cortex, endodermis and pericycle, are delineated at a very early stage, only a few cells away from the apical cell (Bartoo, 1929, 1930; Chiang, 1970; Chiang and Gifford, 1971; Conard, 1908; Nägeli and Leitgeb, 1868; Pal and Pal, 1962). But the information concerning the study on the pattern of the maturatuon of root tissues is not readily available from earlier literature. *Ceratopteris thalictroides* was chosen for this study in order to investigate the sequence of the differentiation and maturation of the root tissues in the later stages of root development. *C. thalictroides* was chosen for the present study because of its rapid growth during the growing season and orderly initiation of the root tissues including the lateral root initials (Chiang and Gifford, 1971).

MATERIALS AND METHODS

All the roots used in this investigation were the roots grown adventitiously from

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the base of the petiole of an aquatic fern, *Ceratopteris thalictroides* (L.) Brongn.. Root tips as well as the roots which were in various states of growth were fixed in Craf III (Sass, 1958) immediately after collection in the greenhouse. Materials were then dehydrated by passing through a tertiary butanol series and embedded in paraffin. Serial sections were made in both transverse and longitudinal planes at a thickness of 8 μ and stained with safranin and fast green (Johansen, 1940).

For study with the electron microscope, roots were fixed in the buffered 1% $KMnO_4$ and dehydrated in an alcohol-propylene oxide series and embedded in Maraglass (Pease, 1964). Sections were cut with a diamond knife and stained with Reynold's lead citrate (1963). Sections were examined with a Hitachi HU-11 electron microscope.

1. External morphology:

The adventitious roots arise from the basal part of the petiole, mainly on the abaxial surface (Fig. 1). As in *C. pteridoides* (Chiang, 1970), the number of roots formed in association with each individual petiole is variable in a sporophyte bearing many leaves whereas in the sporeling, one leaf gives rise to only one root (Chiang & Chiang, 1962). In the present study, ten roots were found arising from the petiole base of a young leaf measuring 14.2 cm in length. The diameter and length of these ten roots were not the same; the diameter measured at their bases varied from 0.2 to 1.2 mm, and the root length varied from 8 to 88 mm (Fig. 2). The root diameter is roughly correlated positively with the length of the root, *i.e.*, a thicker root is generally longer (Fig. 2). The length and number of lateral roots produced on an individual main root are also correlated with the length of the main root; a longer main root usually gives rise to more lateral roots which are long. In another plant, a leaf measuring 27 cm in length had twenty associated adventitious roots varying from 0.2 mm in diameter at their bases, and from several mm to 20 cm or more in length. The length of the longest root formed from an individual leaf at maturity is negatively correlated with the length of the leaf from which it arose. Successively younger leaves are successively longer in size at maturity and the longest roots associated with them are successively shorter (Fig. 3). This probably means that root growth continues for a longer period of time. The lateral roots located at the middle portion of a main root which is still growing are the longest; they are gradually shorter toward the base and the tip of root (Fig. 2). At maturity, however, this relation becomes obscure (Fig. 4). Although the lateral roots on the adult main root are not the same in length, they seldom exceed 4 cm, The lateral (secondary) roots grow obliquely downward, making an average angle of 53.4 with the main root (average of seven measurements) (Fig. 4). Both main and lateral roots are devoid of secondary growth and thus they remain slender, always slightly tapering toward the tip (Table 1). The diameter of a root at its base is nearly twice that of the root at its tip (actually a few mm back from

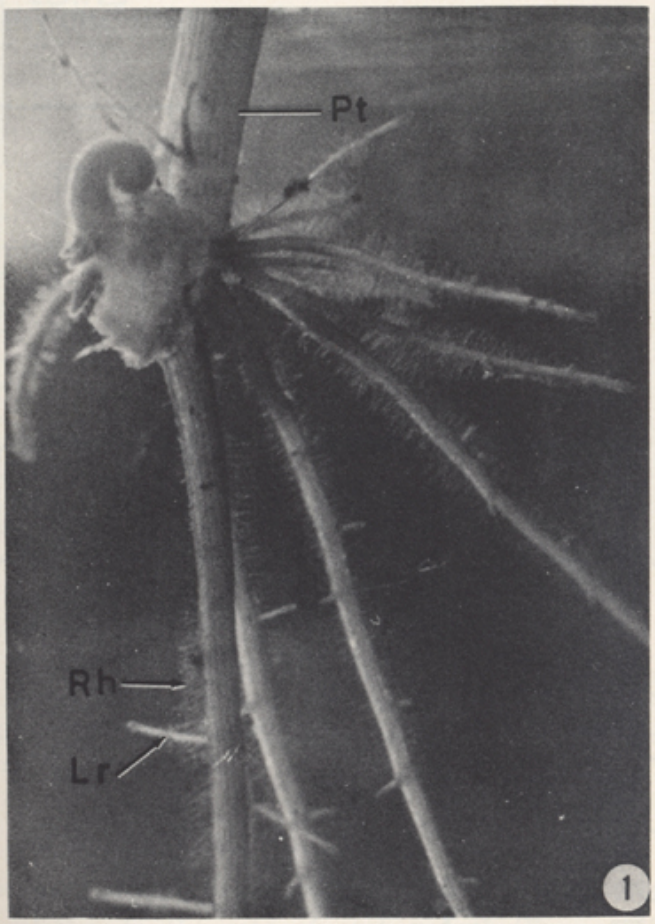


Fig. 1. Photograph showing the attachment of adventitious roots on the base of petiole, ×3. Key for labeling to figures: Ac, apical cell; As, air space; D, dictyosome; En, endodermis; ER, endoplasmic reticulum; Lr, lateral root; Lri, lateral root initial; M, mitochondrion; Mx, metaxylem; N, nucleus; Nu, nucleolus; P, plastid; Pc, pericycle; Pd, plasmodesma; Ph, phloem; Pl, Plasmalemma; Pt, petiole; Px, protoxylem; Rh, root hair; V, vacuole; W, cell wall.

the tip) (Table 1). Tertiary roots are also found occuring on the secondary roots, usually very close to the junction of the secondary root with the main root, but very infrequently. All roots are cylindrical, delicate and easily broken. Root hairs form a dense covering on all surfaces of both main and lateral roots, including the older portion of the root, but excluding the tip. They persist on the roots for a rather long time. In the very old roots, the root hairs still retain their complete

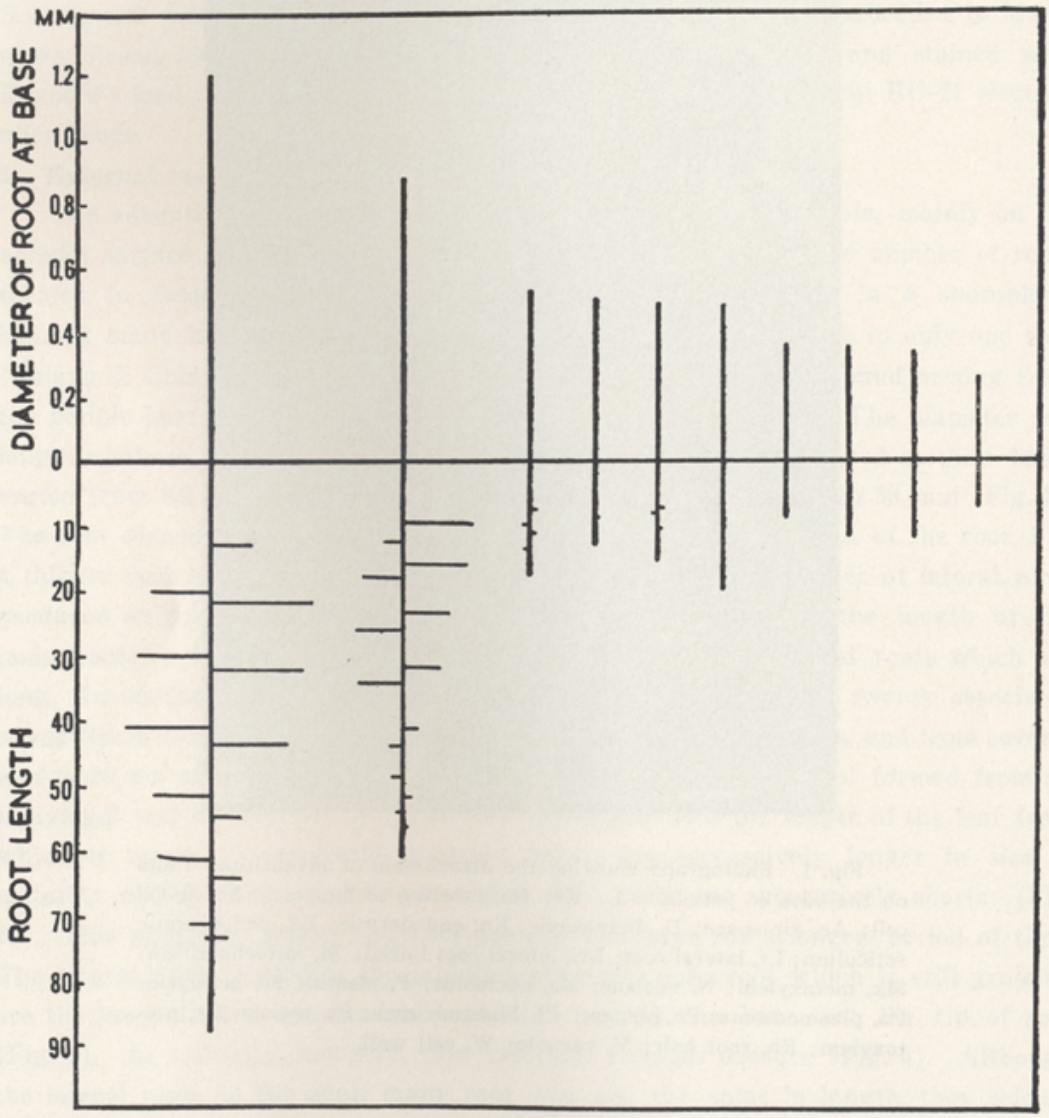


Fig. 2. Relation of root diameter to the length of roots and to lateral root formation. All roots which emerged from the rachis base of the second leaf depicted in Fig. 3 are illustrated in this figure. All roots were still growing at the time of harvest. Root diameter and root length are positively correlated, the thickest being the longest. The longer roots also have the longer lateral roots.

shape. The younger portion of roots growing in water is grass green especially at the tip, but those grown in soil are colorless. The older roots are brown.

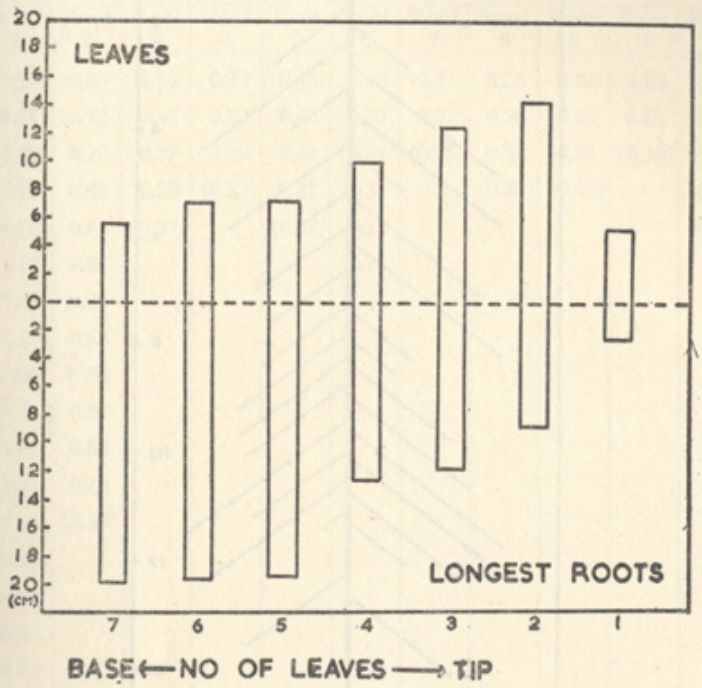


Fig. 3. Relation of the length of the longest roots to the length of leaves from which the longest roots arise, showing that the longer leaves have shorter roots. All leaves are borne on the same sporophyte. The first leaf is a circinate, actively growing leaf.

2. Differentiation and maturation of root tissues.

The distance between the tip of a root and the first mature phloem and xylem elements varies in relation to the rate of root growth (Esau, 1965 a, b). In the present investigation fourteen roots were examined in a study of the initiation, differentiation and maturation of root tissues. These fourteen roots showed a similar sequence of initiation, differentiation and maturation of the tissues. This sequence is illustrated diagramatically in Fig. 5. The immediate precursors of all the tissues of the root proper are present very close to the apical cell, at about 50 μ behind the base of the apical cell from which all the root tissues are derived. The sequence of differentiation and maturation of vascular tissues, indicated by the distance from the apical cell in a particular root, is as follows: metaxylem first appears at 0.14 mm and matures at 10.20 mm; protoxylem is identifiable at 1.46 mm and matures at 2.60 mm; protophloem is identifiable at 0.20 mm and matures at 0.36 mm (Fig. 5). Phloem is the only tissue which matures close to the region of the apical meristem. It is not easy to distinguish metaphloem from protophloem in the root of this plant.

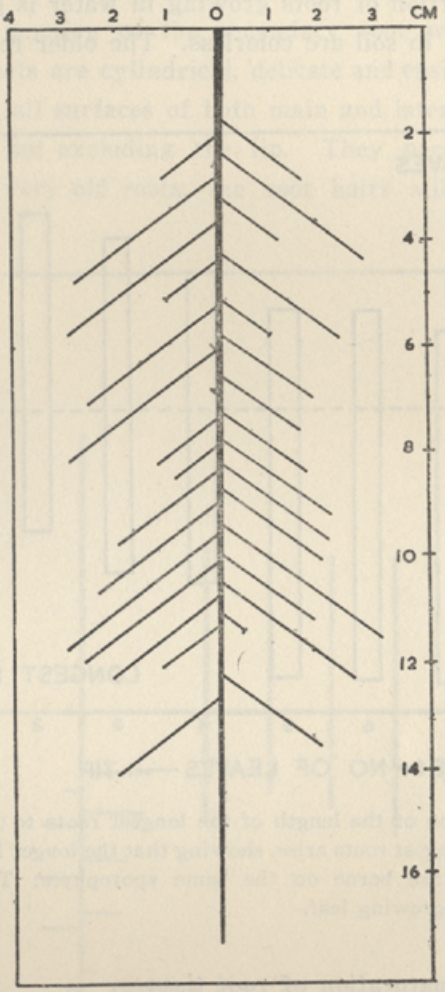


Fig. 4. A diagrammatic illustration of the longest root from the sixth leaf depicted in Fig. 3, showing the attachment of lateral roots to the main root. Four lateral roots were broken and lost at the time of harvesting at the points indicated by short lines in the figure. A lateral root makes an average angle of 53.4 with the main root.

Sieve cells and parenchyma occur at all successive levels between the first phloem and the last phloem to mature, indicating that both protophloem and metaphloem are tissues composed of sieve cells and phloem parenchyma. As seen in transverse section, phloem differentiates and matures centripetally. The first mature phloem element, or protophloem, is on the periphery of the stele (next to the pericycle, Fig. 6e). The root of *C. thalictroides* has two protoxylem poles and two protophloem poles (diarch, Fig. 7), so that the xylem forms a flattened central strand bordered on each side by a strand of phloem.

Table 1. Diameter of main roots and lateral roots at their bases and at various distances from their bases.

Distance from base (mm)	Diameter of main roots* (mm)										Diameter of lateral roots** (mm)		
	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6	Root 7	Root 8	Root 9	Root 10	Root 1	Root 2	Root 3
Base	1.21	0.88	0.53	0.49	0.48	0.48	0.37	0.35	0.34	0.26	0.52	0.18	0.18
5	1.61	0.72	0.46	0.32	0.45	0.39	0.37	0.26	0.52	0.20	0.52	(0.18)	(0.13)
10	1.21	0.70	0.27	0.29	0.44	0.31	(0.25)	0.23	0.22	(0.18)	0.50		
15	1.15	0.68	0.35	(0.23)	0.31	0.31		(0.20)	(0.18)		0.39		
20	1.09	0.67	(0.27)		(0.27)	0.22					(0.38)		
25	1.17	0.64				(0.21)							
30	1.05	0.63											
35	1.12	0.63											
40	1.08	0.59											
45	1.21	0.57											
50	1.05	0.53											
55	1.03	0.48											
60	1.00	(0.35)											
65	0.96												
70	0.91												
75	0.83												
80	0.77												
85	0.65												
	(0.65)												

* These ten main roots are also depicted in Fig. 2.

** Lateral root 1, 2 and 3 are respectively the third lateral root on the main root, the first lateral root on main root 5 and the second lateral root on the main root 5.

() Indicates the diameter at the middle of the rest of the same root.

3. Lateral root initial

In *C. thalictroides*, and also in some other ferns with a single apical cell in its root tip, the lateral roots are always borne regularly according to a definite order. As in most lower vascular plants, the lateral or secondary roots of *C. thalictorides* originate endogenously in the endodermis of the parent root. During the initiation of a lateral root, a specific endodermal cell increases greatly in size to become a large, somewhat cubical lateral root initial with a large nucleus (Fig. 6a). It is identical in size with the apical cell of the parent root (Fig. 9a) and occurs very close to the apical cell of main root (Figs. 5, 9a). No obvious morphological differences (except the size) exist between this lateral root initial and other endodermal cells (Figs. 6a, 8). This enlarged lateral root initial is about four times larger then the ordinary endodermal cells in size in transverse section, containing a conspicuous big nucleus and one to several reticulate nucleolar masses. The morphology of cytoplasmic organelles have no identifiable differences from those in its

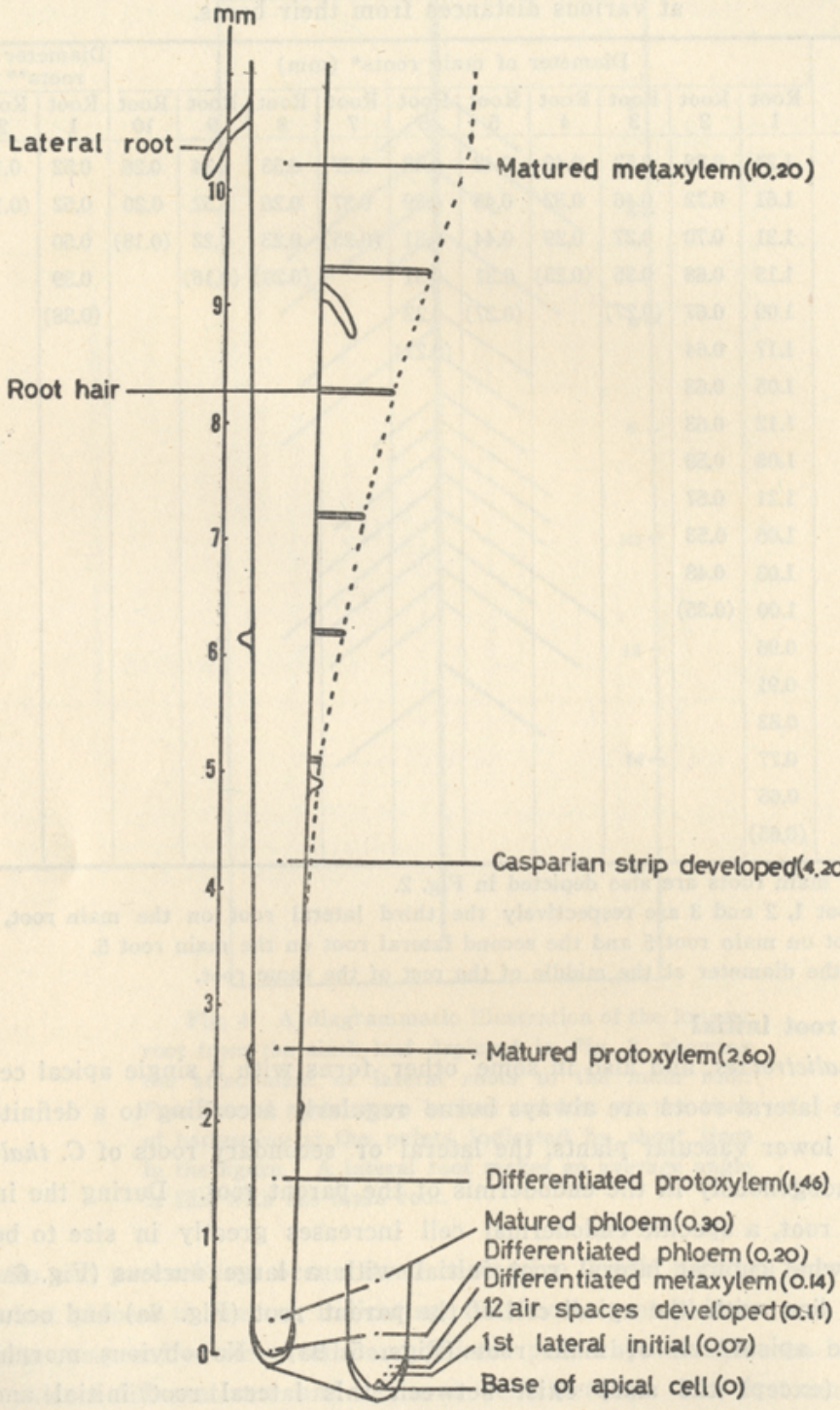


Fig. 5. Diagram illustrating the initiation, differentiation and maturation of various tissues. The numbers in ( ) indicate the distances from the base of the apical cell in mm. Broken line indicates root hair zone.

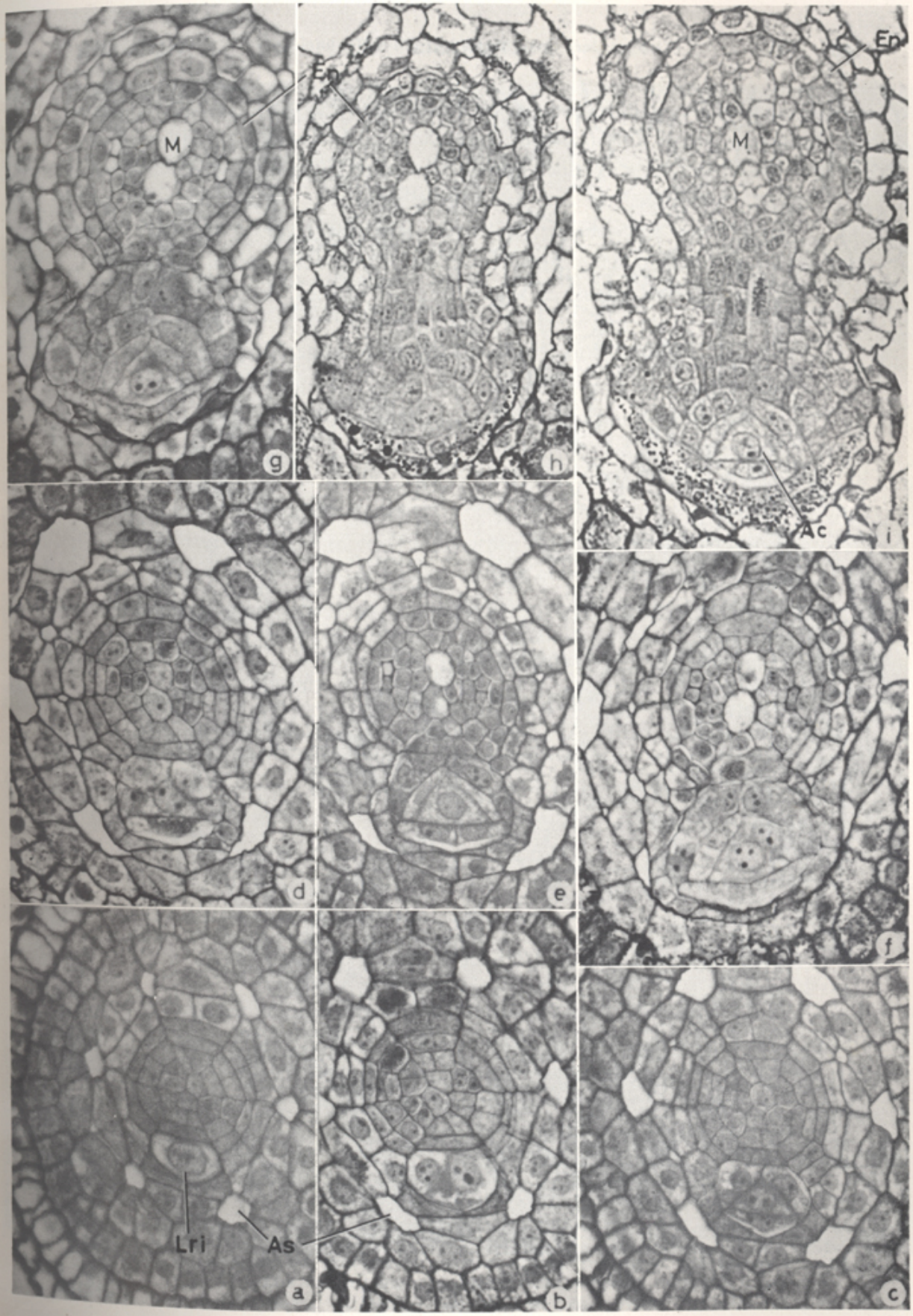
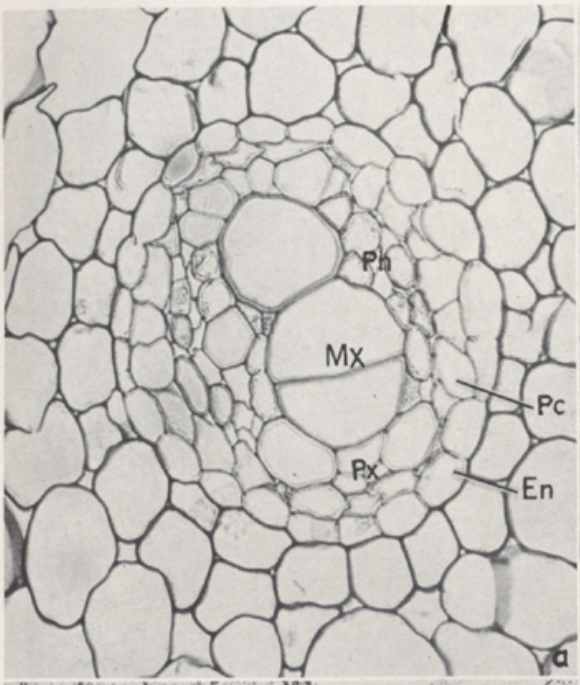
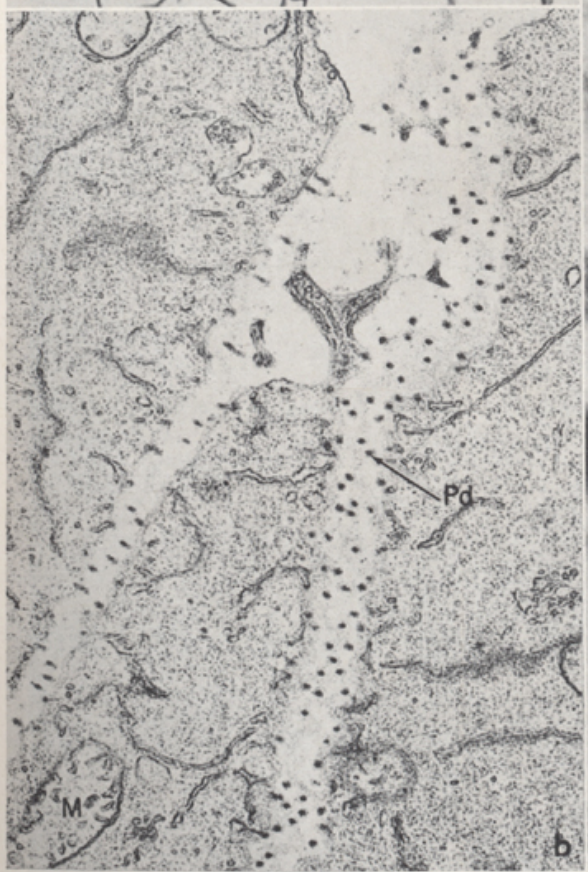


Fig. 6. Lateral root development. Longitudinal sections through lateral roots (*i. e.*, transverse sections through main roots), showing successive stages in the development of a lateral root from a single lateral root initial cell which occurs in the endodermis of the main root, all ×260.





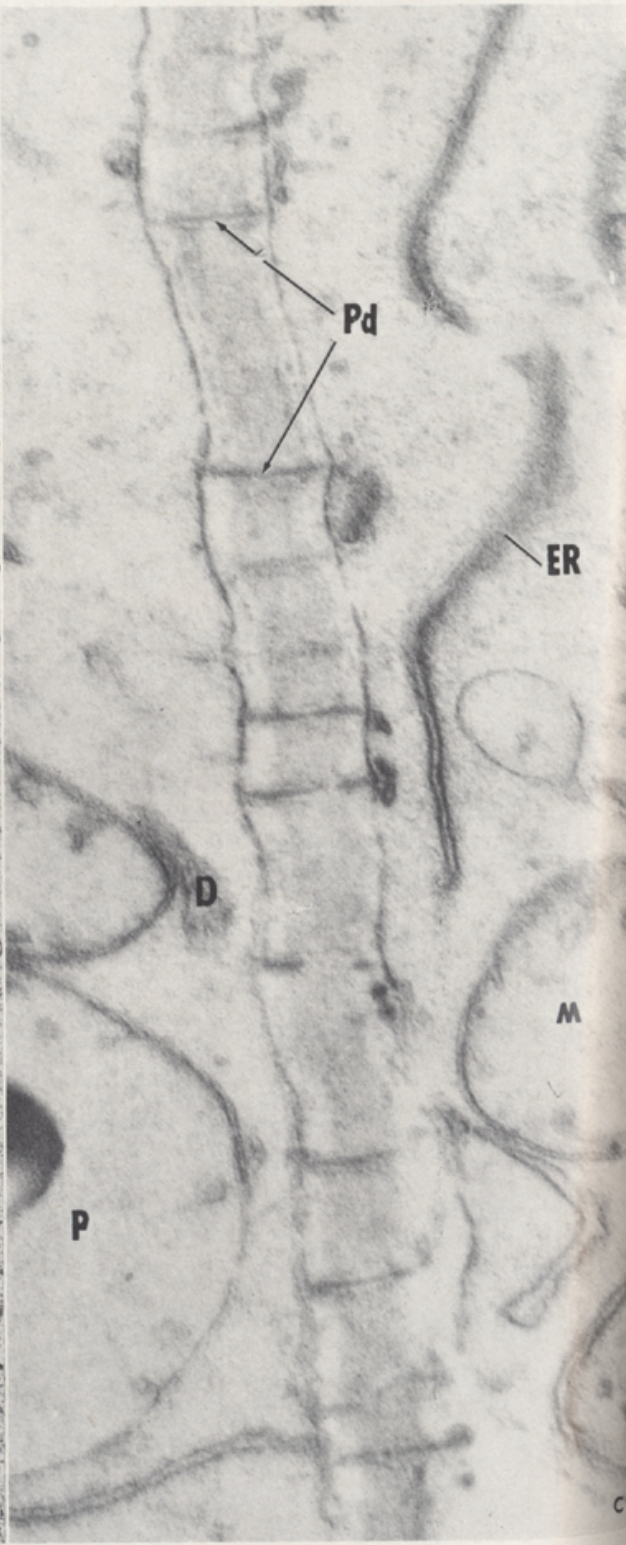


Fig. 7a. Transverse section of central stele in a mature root, ×335.

Fig. 7b. Electron micrograph showing the section in perpendicular view with the plasmodesmata, ×19,700.

Fig. 7c. Electron micrograph showing the parallel view of the plasmodesmata, ×70,000.

neighboring cells. As seen in the section, the numbers of the cytoplasmic organelles in a given area in the lateral root initial are almost about the same as that in other endodermal cells. The cytoloplasmic organelles found in this initial as well as the other endodermal cells in the same level of the root are mitochondria, dictysomes, plastids, endoplasmic reticulum, spherosome-like structure and vacuoles.

Numerous mitochondria are present: elongate, ovoid and circular forms are frequently observed. The mitochondrial matrix is usually slightly lighter than the hyaloplasm but denser than the stroma in the plastids and the inner membrane has many extensions, the cristae, which are numerous and short.

Many plastids are found in these cells but they are less abundant than mitochondria. Most of them are circular to elongate in section while some are more or less polymorphic. Grana are not well-developed, only 1–3 layered lamellae. Intergrana, or frets are short. Starch granules are found in the stroma in some plastids. The stroma appear structurally homogeneous.

Dictyosomes consisting of several cisternae are commonly observed. They are stacks of flattened sacs or cisternae and their profiles are nearly straight being surrounded by vesicles.

Many irregular profiles of endoplasmic reticulum (ER) occur. They often appear in parallel arrays. They have double layered membranes resembling the nuclear envelope and are typically simple, but some are branched.

Spherosome-like structures are present in the cells of endodermis as well as in the enlarged single lateral root initial. They appear very similar to an "unidentified inclusion" described by Diboll and Larson (1966) in the maize egg cell. They are delimited by single layered membranes and are much smaller than mitochondria with denser, finer homogeneous inclusions inside.

Numerous small vacuoles are randomly distributed, but are fewer than in the young cortical cells of the same level of the section.

Numerous plasmodesmata are seen in all the root cells of the same level of the single lateral root initial. The numbers of plasmodesmata on the wall of this specialized lateral initial are of about $36/\mu^2$ as measured in the para-surface view of the cell wall. The plasmodesmata of these embryonic cells consist of three electron dense layers separated by two electron light regions. The middle layer of these three electron dense layers are wider than the other two layers which are located parallel with the middle one (Fig. 7c). Thus many five-layered structures, three dense and two lighter are distributed on the cell wall of enlarged lateral root initials in all directions (Fig. 8). The pattern of the initiation and development of lateral roots from a single endodermal cell to a well-organized lateral root of *C. thalictroides* is the same as that which occurs in the root of *C. pteridoides* (Chiang, 1970).

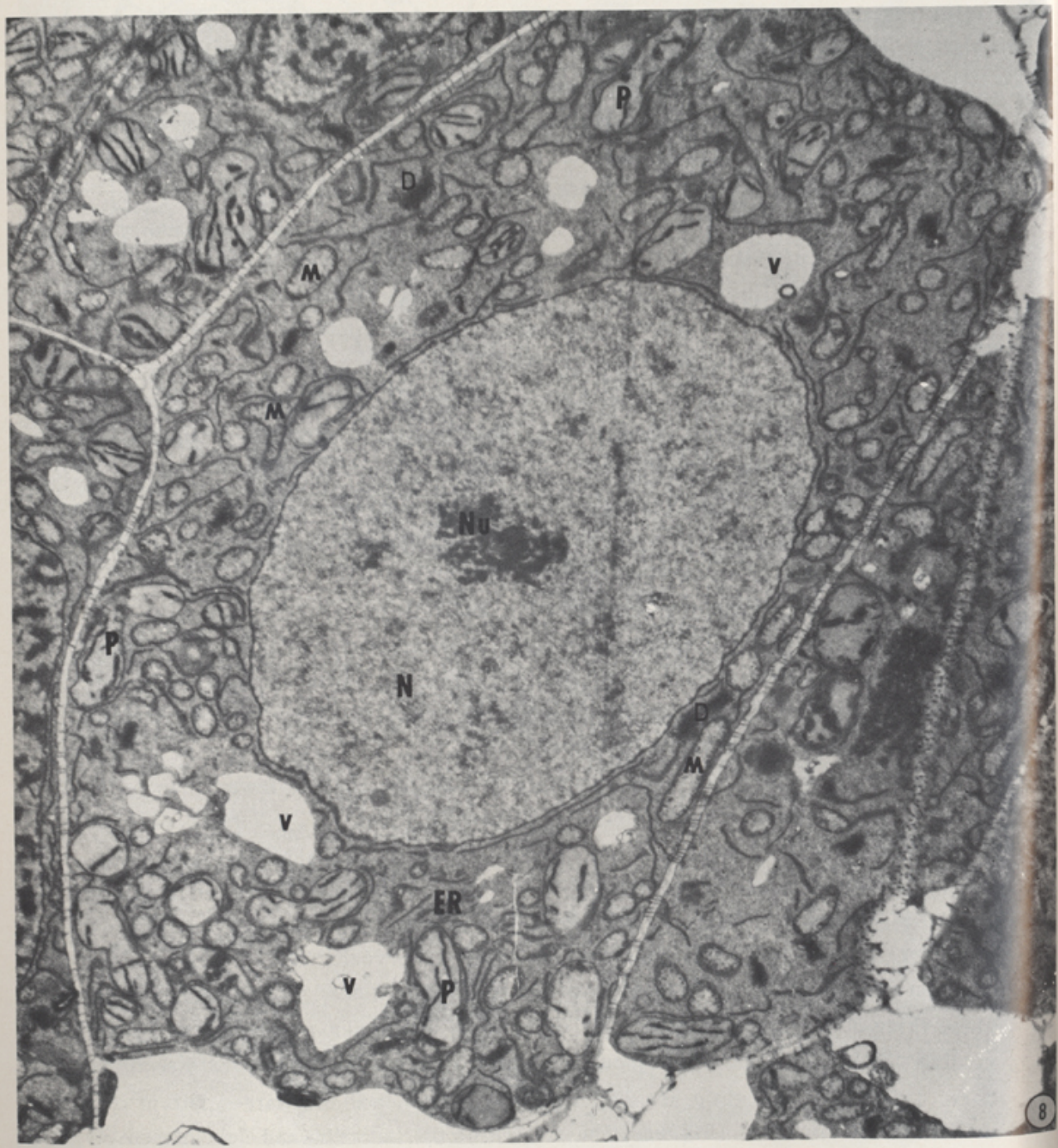


Fig. 8. Electron micrograph of transverse section of lateral root initial, ×730.

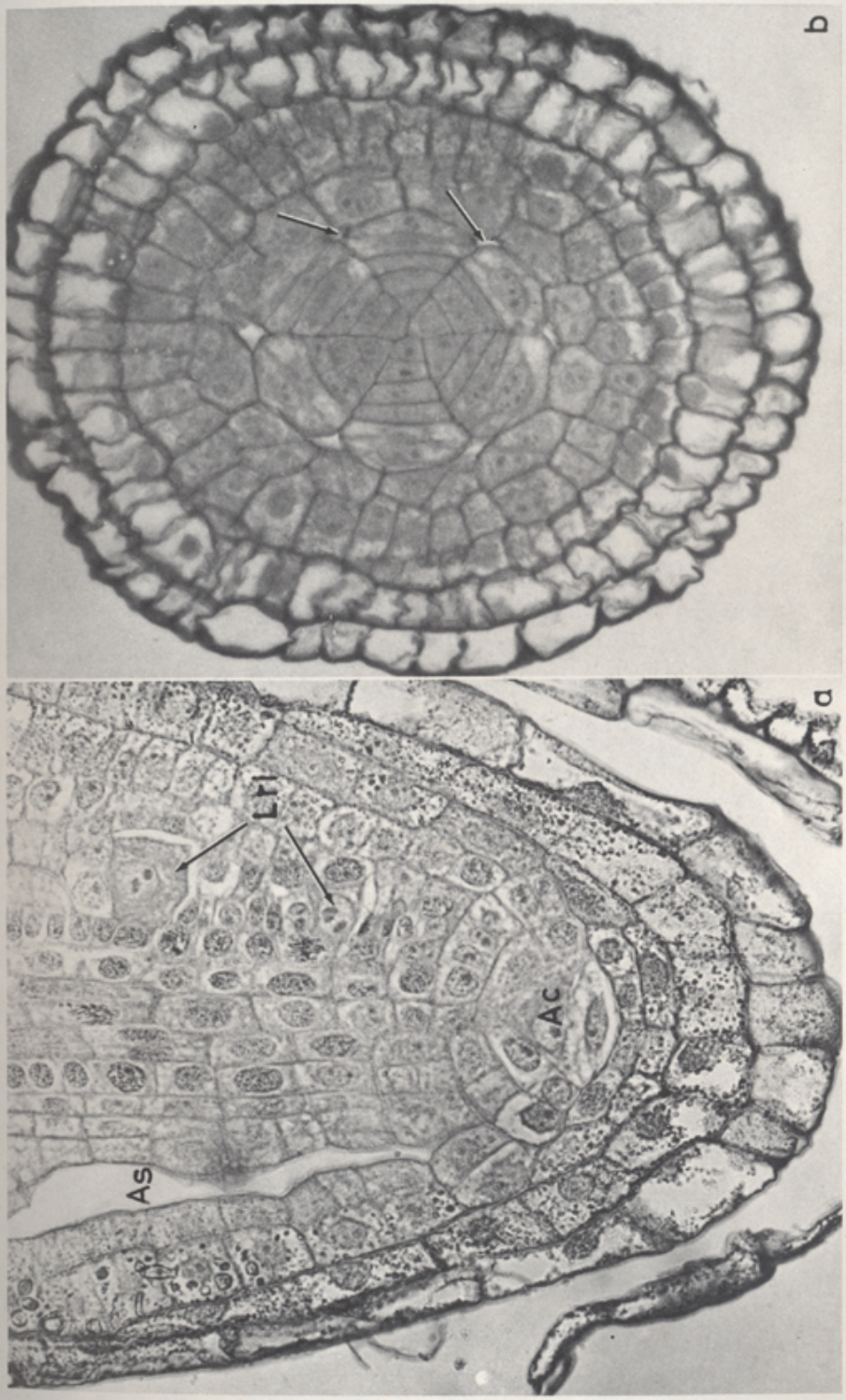


Fig. 9. Transverse and longitudinal sections of apical meristem showing the initiation of the air spaces (arrows in Fig. b), Fig. a, ×240; Fig. b, ×250.

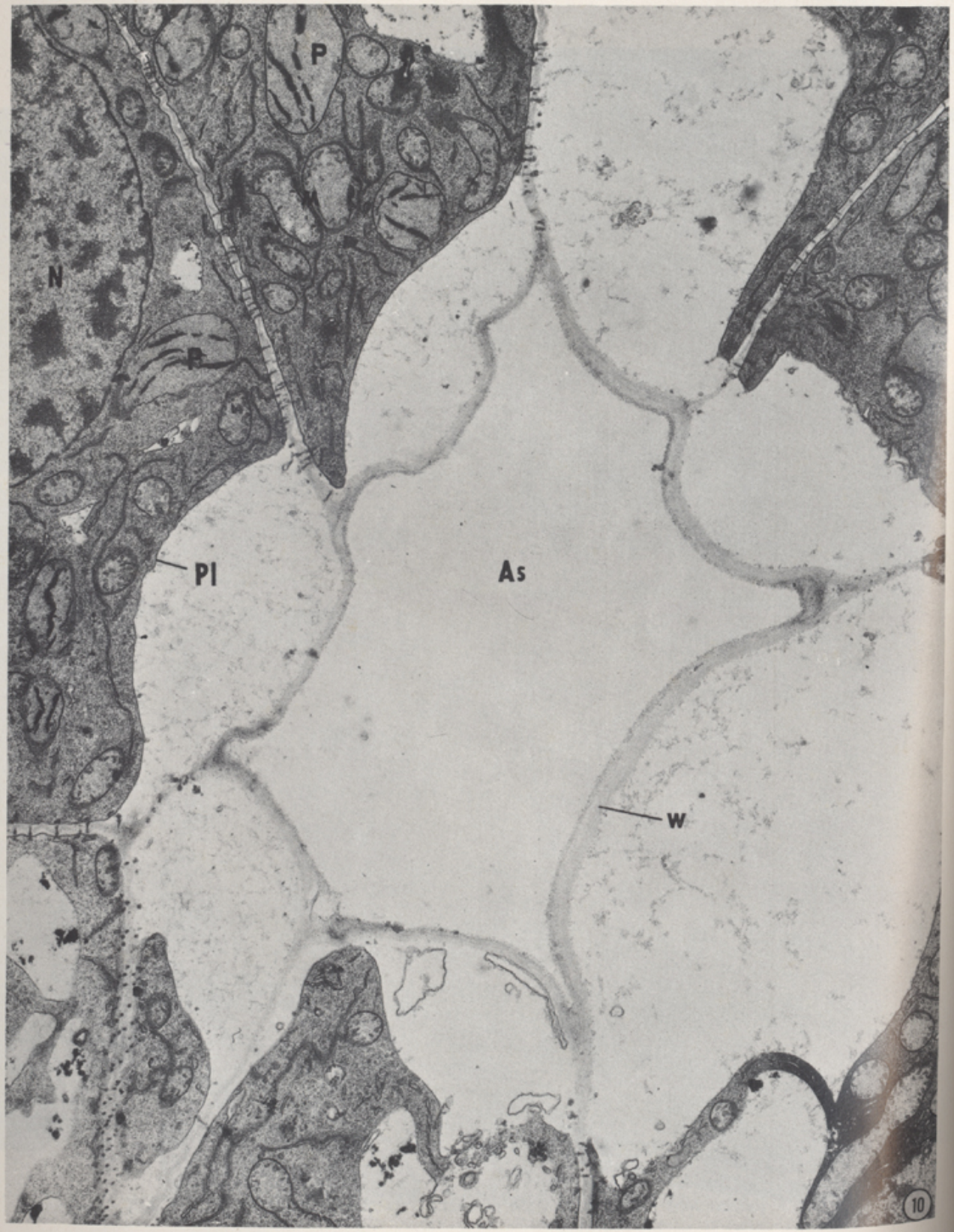


Fig. 10. Electron micrograph of transverse section of cells adjacent to an air space found about 0.06 mm away from the apical cell, ×11,700.

4. Air spaces and their initiation

The air spaces (intercellular spaces, air passages, air channels, air ducts) found in the root of *C. thalictroides* are restricted to the cortex. They arise schizogenously very close to the apical cell, about 50 μ back from the basal wall of the apical cell (Fig. 9). As seen in longitudinal section, they are ducts, increasing in diameter gradually in a basipetal direction (Fig. 9a). The transverse section of the duct in its early stage is roughly circular as seen under the light microscope (Figs. 6a, 9b). Six air spaces are usually initiated at about the same level in the root (Figs. 6a, 6b, 9b). Under the electron microscope, the air space is stellate (Fig. 10). Each air space is surrounded by several cortical cells. The cortical cell walls which are in contact with the air space are thicker than the walls in association with other cortical cells. There is no plasmodesma on the walls between the air spaces and the cortical cells, but numerous plasmodesmata are seen on the walls which are in contact with the neighboring cortical cells (Fig. 10). The cortical cell walls which are in contact with other cortical cells are thinner and homogeneous in electron density. The cortical cell walls which face the air space appear heterogeneous under electron microscope. Rather deeply stained rough granular structures are distributed along the outer part of cell wall (*i. e.*, the face immediately next to the air space) whereas on the inner part (*i. e.*, the face in contact with the cytoplasm of the cortical cell) are deposited finer granular structures (Fig. 10). As the growth of the root progresses, the number of air spaces increase from six to twelve and then they become many more (Figs. 6a–6i). Twelve air spaces are found occuring approximately 112 μ behind the apical cell in the root which is depicted in Fig. 5. In the mature region the cortical cells grow in size and become isodiametric, resulting in irregular air space or air spaces which are reticulately connected with each other. When the root is lightly pressed with a blunt tip of a needle, the sap of the internal tissues is easily pressed out into the air duct to interrupt the gas column in it, indicating that the duct is filled with gases rather than liquids.

DISCUSSION

It is well known to botanists that in most plants, the older leaves are always smaller than the younger ones at least in their early stages of development. This is more conspicuous in seedlings and this fact can be seen in *C. thalictroides* as well. Thus, it is not difficult to understand, or explain that the younger leaves always bear more adventitious roots on their petiole bases than the older leaves. Apparently the leaves affect the associated roots and the lateral roots on them. The detailed relationship between them has not been studied in the present work, but it was mentioned in *C. pteridoides* (Chiang, 1970).

All the roots (fourteen) examined in the present work showed a similar sequence of initiation, differentiation and maturation of the tissues. Phloem matures the

earliest among all the tissues in the central stele whereas metaxylem initiates first but matures last among them (Fig. 5). This sequence of differentiation and maturation of tissues agrees with that of some dicotyledonous roots (Esau, 1938, 1943; Peterson, 1967; Popham, 1955), and the root of *Dendrobium kwashotense* (orchid) (Chiang, 1970), and also with roots of *Dennstaedtia punctilobula* (fern) (Conard, 1908). The distance between the meristem of a root and the first formed phloem and xylem members varies in relation to the rate of root growth (Esau, 1965a, b). The influence of root growth, environmental conditions and age of the root were not examined in the present investigation. Therefore the time relationship between this plant and other groups of plants cannot be discussed here. But it is clear that it is possible to recognized the early initials of each tissue merely by their topographical position at the region where cellular differentiation cannot yet be seen in any other tissues. As reported in the previous paper (Chiang and Gifford, 1971), all root tissues are delineated at the region only a few cells away from the apical cell from which all of the root tissues are derived. Further detailed studies of other ferns should assist in the formulation of a general picture of differentiation and maturation of tissues in fern root tips.

The great volume of both the cell and the nucleus in the single lateral root initial is the most conspicuous morphological difference between it and the ordinary endodermal cells in the same level of root development. But an increase of cellular organelles in the unit area of this initial cell cannot be recognized. This specialization of a single initial cell seems to be physiological or biochemical rather than morphological. As mentioned in the previous paper (Chiang and Gifford, 1971), it occurs only at the definite endodermal cell in the definite tissue layer which was orderly derived from the apical cell of the main root. However, the topographical condition may be one of the most important factors involved in the initiation of lateral roots.

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