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Author(s): John G. Torrey

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ON THE DETERMINATION OF VASCULAR PATTERNS DURING TISSUE DIFFERENTIATION IN EXCISED PEA ROOTS¹

John G. Torrey

ONE OF THE most characteristic and diagrammatic patterns established in the seedling structure during the ontogeny of the individual plant is the alternate and radial arrangement of the primary vascular tissues within the primary root. The determination of a precise radial arrangement of alternating strands of phloem and xylem tissue by longitudinal differentiation occurs during early embryogeny. The vascular pattern is evident in the seedling radicle. In many species, this radial pattern with its characteristic number of vascular strands may persist for the life of the primary root.

Prior investigations of the origin of the vascular pattern in roots present two general approaches, each of which may have significance in understanding the basis of the pattern. One approach involves detailed anatomical studies of the root structure in all parts of the root system—primary axis as well as branch roots—of different sizes and ages. The other approach has been one of experimental manipulation, usually by surgical operation, in an attempt to influence the tissue differentiation and pattern formation.

Descriptive studies reported extensively in the classical anatomical literature (van Tieghem, 1870-71; DeBary, 1884) have been interpreted in such works as Bower (1930) and are summarized by Esau (1953). It has been shown that the number of alternate and radially arranged xylem and phloem strands may be constant in any given plant and may be quite characteristic of a species, genus, or even family. In some plant groups the number of vascular strands in the primary root varies among members of the group (Wardlaw, 1928) or varies within the root of the same individual (e.g., Heimsch, 1949; Meyer, 1930). Lateral branches from the primary root axis often show a reduced number of vascular strands. Recent anatomical analyses of root ontogeny (e.g., Esau, Heimsch, and

Bünning) have clearly demonstrated the continuous early establishment of the radial vascular pattern in the root proximal to the apical initials and the uninterrupted acropetal maturation of vascular tissues.

The experimental studies, limited in number, have been reviewed recently by Esau (1954). Two major aspects concerning the determination of the pattern of vascular differentiation have been analyzed using experimental methods. Jost (1931-32) from his studies on vascular differentiation in regenerating decapitated roots concluded that the mature vascular tissues of the root induce differentiation of the newly formed cells derived from the apical initials into the established pattern of the mature region. This concept of longitudinal induction arises from Pfeffer (1904) and Haberlandt (1913) who reported the control of cell division and differentiation of meristematic cells by mature differentiated vascular tissues. Support for the idea of vascular tissue determination by induction was given by some early root-culture work (e.g., Bonner and Addicott, 1937). Further indirect support for this view of differentiation has been given by the consistent observation in anatomical studies of the acropetal sequence of vascular tissue maturation in roots.

The opposed view holds that the determination of the vascular pattern in roots is controlled by the apical meristem which governs the differentiation of the primary tissues of the root. Thoday (1939) has emphasized the self-determining power of the root apex and has cited the evidence derived from culturing isolated root tips in nutrient medium. As early as 1931 Scheitler concluded from experiments with very small excised root tips which grew and differentiated in nutrient culture that the root meristem controls primary tissue differentiation in the root. Recently, Bünning (1952) has reported that if a 2-mm. root tip of *Vicia faba* is excised and replaced on the shortened stump of the same root, it would form a union and grow, but the xylem elements of the two parts are not in line and only later become connected by differentiating elements.

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He concluded that the vascular pattern was already fixed in the 2-mm. root tip and that diffusible substances moved from the mature tissues toward the tip which made possible the maturation of the vessel elements.

The second major aspect of vascular tissue differentiation in roots concerns the question of the determination of the radial and alternate arrangement of xylem and phloem tissues in the primary root. According to the description of differentiation in the diarch roots of *Sinapis* by Bünning (1951), the early enlargement of the central future-metaxylem elements immediately behind the apical initials exercises a determining influence on the subsequent differentiation of all the tissues of the central cylinder. This influence results in the induction of differentiation of the radially-aligned vessel elements of the xylem and the suppression of phloem tissue except at the radial points most distant from the induced xylem tissue. According to Bünning, no phloem can arise in the immediate neighborhood of a xylem strand nor in the vicinity of existing phloem tissue. Thus a radial and alternate pattern of vascular tissue must result. Bünning (1951, 1952) has used staining reactions, measurements of nucleolar size, and the results of wounding experiments to support his speculations.

In studying the determination of vascular-tissue patterns in the root, it is essential to analyze the problem not in terms of two-dimensional transverse or longitudinal root sections, but in the light of the three-dimensional root structure as a whole, considering longitudinal as well as radial forces acting upon the system. The problem then resolves itself into two fundamental questions. First: is the determination of the acropetal differentiation of primary vascular tissues in the root induced by the older and mature vascular tissues or is such determination inherent in the activity of a self-determining apical meristem? Second: what is the nature of the forces that produce the radial and alternate arrangement of the primary vascular tissues of the root?

Both the observational and the experimental approaches have been used in an attempt to find answers to these questions. The first of these problems is the subject of the present investigation. A detailed anatomical analysis of the course of primary vascular tissue differentiation in untreated control roots grown in nutrient culture has been used as the background for the experiments. From anatomical studies of cultured roots, it was possible to determine the proper length for excision of root tips in which no mature vascular tissues occurred and in which incipient vascular pattern formation had just begun. Such root tips were successfully subcultured in a sterile synthetic nutrient medium and the course of vascular tissue differentiation was studied.

MATERIALS AND METHODS.—Procedures for growing 0.5-mm. pea-root tips in a sterile synthetic nutrient medium have been described in detail

elsewhere (Torrey, 1954). The procedure can be summarized as follows: Alaska pea root tips 5.0 mm. in length were excised from sterilized seed germinated for 48 hr. and were grown in a synthetic nutrient medium containing mineral salts, 4 per cent sucrose and 0.5 per cent agar, plus FeCl_3 , lacking added vitamins and trace elements. After growth for 1 week to an average root length of about 50–60 mm., the terminal 0.5-mm. tips (including root cap) were excised from the control roots under a dissecting microscope equipped with an ocular micrometer and were transferred to fresh medium in small dishes. Growth of these experimental tips was dependent upon the addition of the vitamins, thiamin and nicotinic acid, and trace elements. Such 0.5-mm. root tips were grown for various periods from one to several weeks and were then killed in formalin-acetic acid-alcohol (FAA) with aspiration. Root tips were dehydrated in an ethyl-butyl alcohol series, embedded in "Tissue-Mat" and sectioned on a rotary microtome at 8μ . Serial sections were stained with Heidenhain's hematoxylin and safranin. Histological study of large numbers of roots in any one experiment was accomplished with roots killed in FAA and sectioned at 40μ using a carbon-dioxide freezing microtome. Sections were mounted in water and examined under the microscope immediately without staining.

The root diagrams used as illustrations were made by drawing cellular outlines directly on photographic prints, removing the emulsion and completing the line drawings in ink. Both the original photomicrograph and its line tracing are presented to facilitate interpretation.

Structure and vascular tissue differentiation in excised control pea roots in culture.—The organization of the meristematic region of the root apex in the cultured pea roots studied appeared quite uniform. Figure 1 shows, in median longitudinal view, the typical organization of the apical region of a pea root fixed at the start of the culture period. This organization is maintained in elongating roots in culture and represents the arrangement of tissues of the root apex in week-old roots before 0.5-mm. tip excision.

A group of apical initials gives rise to the cells of the central cylinder by cell divisions which early delimit the procambial cone proximal to the apical initials. The same initials give rise to cells distally which undergo transverse divisions, forming the columella of the root cap. The cortical initials appear to lie peripheral to the apical initials where the cortex and epidermis have a common origin with the cells of the root cap lateral to the columella. Thus, although the central procambial cylinder has a clear-cut initial group, the other primary meristematic tissues show no such precise site of origin (fig. 1a). The relatively massive subterminal apical meristem in *Pisum* thus includes the poorly individualized apical initials, which give rise more or less directly to the primary meristematic tissues of the

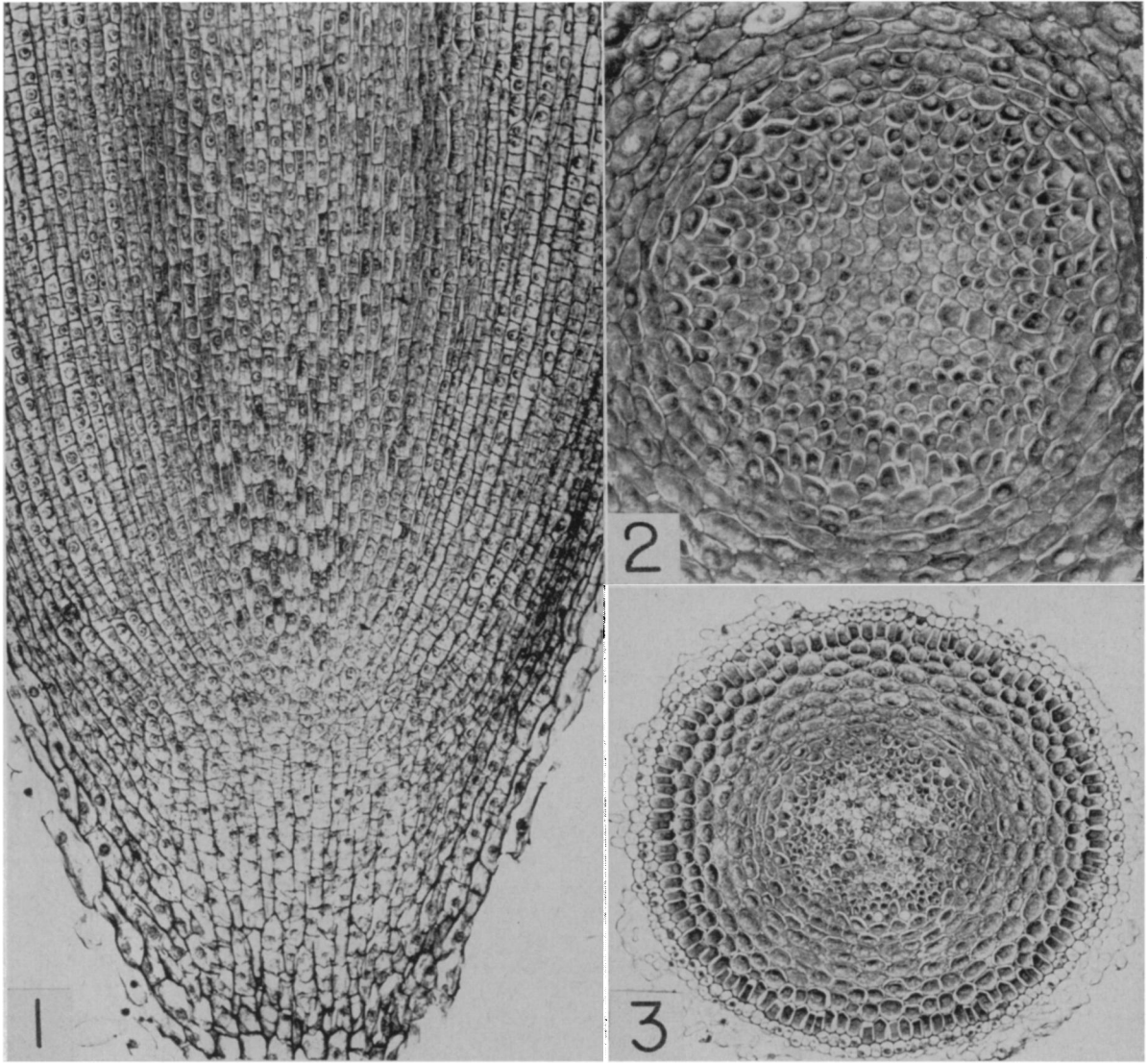
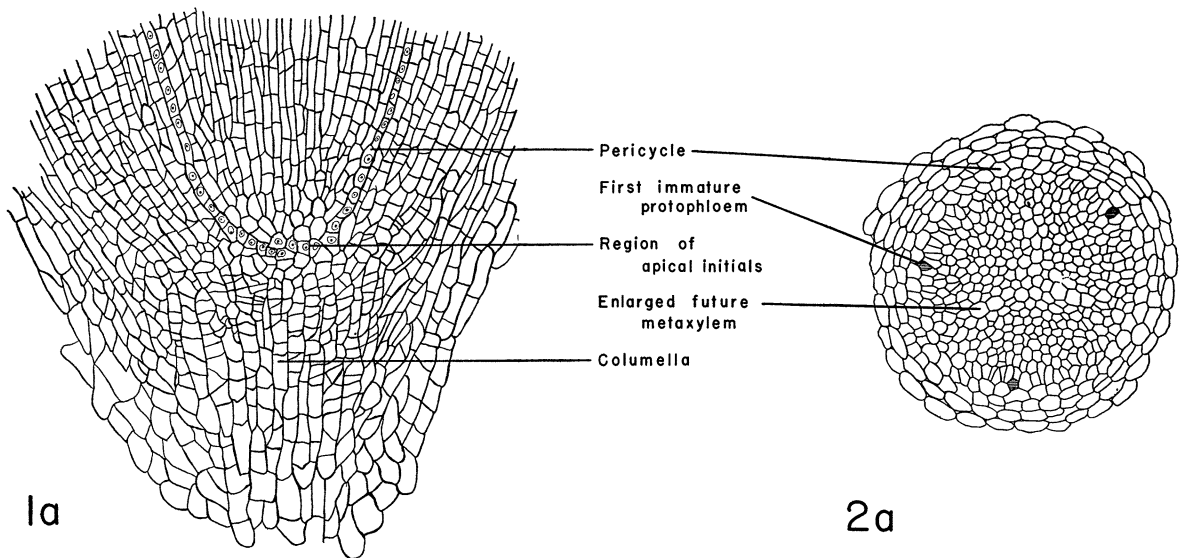


Fig. 1-3.—Fig. 1. Median longitudinal section of root tip of *Pisum sativum* variety 'Alaska,' excised from seed 48 hr. after germination. See photographic tracing in fig. 1a for cellular detail. $\times 175$.—Fig. 2. Transverse section of one-week-old cultured root, showing procambial cylinder at 300μ proximal to the apical initials or 500μ from the extreme tip. See photographic tracing in fig. 2a for cellular details. $\times 350$.—Fig. 3. Transverse section of week-old root grown from 0.5-mm. pea root tip. Section was cut at 288μ proximal to the apical initials, showing the triarch arrangement of the enlarged metaxylem elements and the first mature protoxylem elements. $\times 175$.

root, and the actively dividing cells intimately associated with them. The organization of the root apex is apparently intermediate between the classic open or "*Pisum* type" described by Janczewski (1874) and evident in such roots as *Daucus* (Esau, 1940) and the clearly delimited tiered arrangement, as seen in the roots of tobacco (Esau, 1941) and most monocot roots. One might interpret the initial region in the pea root as an inverted cup not unlike that described for *Fagus* by Clowes (1950). Tiegs (1913) has already pointed out the erroneous nature of Janczewski's interpretation of the "*Pisum*

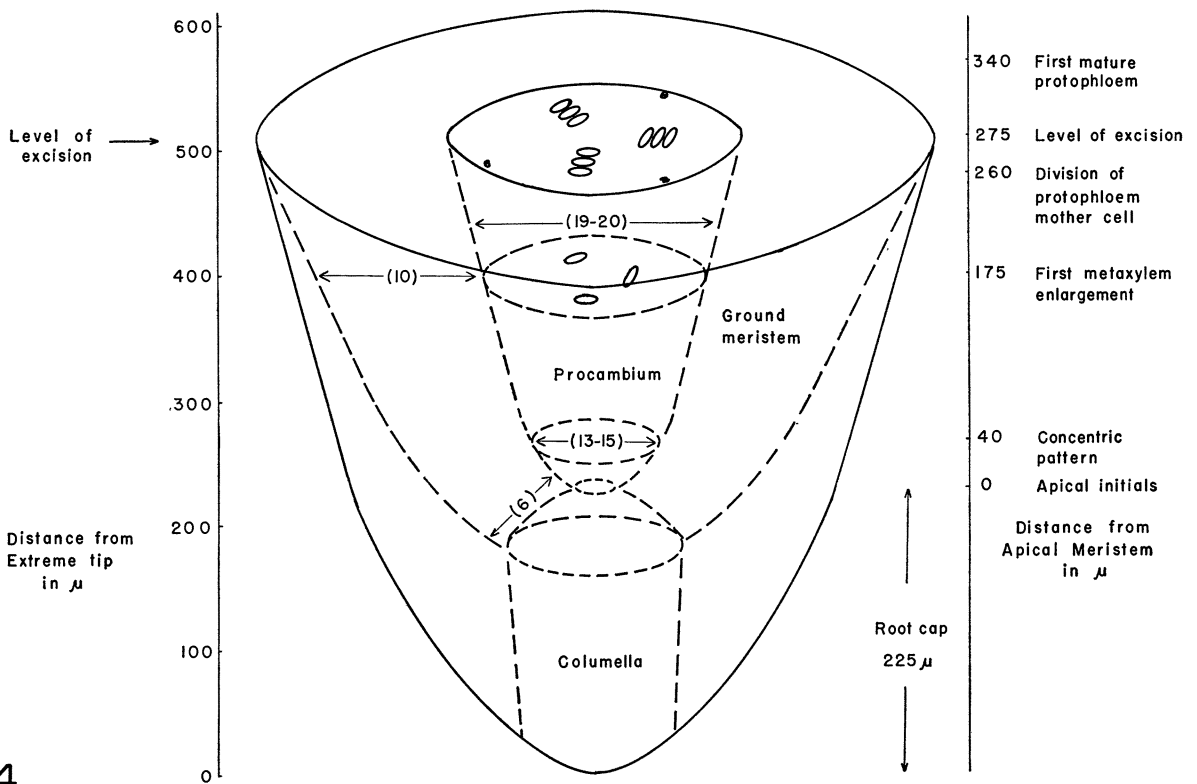
type" which has since been similarly criticized by Reeve (1948) for the pea-embryo radicle.

The ground meristem originates by divisions of the cortical initials peripheral to the apical initial zone. The innermost layer of cells of this tissue continues cell divisions. A precise ring of cells is produced, marking clearly the boundary between the central cylinder and the peripheral tissues of the root. The ground meristem increases in cell number radially from about 6 cells to an average width of 10-11 (fig. 4). This radial increase occurs through successive periclinal divisions of the inner layer,



1a

2a



4

Fig. 1a, 2a, 4.—Fig. 1a. Photographic tracing of fig. 1, showing cellular detail. $\times 110$.—Fig. 2a. Photographic tracing of fig. 2, showing cellular detail. $\times 175$.—Fig. 4. Diagrammatic representation of tissue arrangement in 0.5-mm. pea root tip from which experimental material was grown, showing the spatial relationships in tissue differentiation. See text for details. Approximately $\times 175$.

which finally becomes identifiable as the single-cell-layered pericycle. The pattern of concentric cell layers is evident in transverse section within 30–40 μ of the region of the apical initials and is the first visible tissue pattern proximal to the initial region. Establishment of the origin of the endodermis at this level was not possible as no distinguishing features were observed. Even in the mature root, the endodermis shows no evident characteristic structures. In longitudinal sections, cortical thickening is seen also to occur through T-divisions (Clowes, 1950) in intermediate and outer layers of the ground meristem (fig. 1a).

While the organization of the apical region in isolated pea roots grown in culture is quite consistent, the actual diameter of the region tends to diminish slightly during early growth in nutrient medium. The diameter decrease is attributable chiefly to a reduction in the number of cell layers in the ground meristem which becomes stabilized at a width of approximately 10–11 cell layers. All average dimensions and cell counts of control roots presented in the text or in tabular form represent measurements made on roots fixed after one week's growth in nutrient medium.

In fig. 4 are represented diagrammatically the spatial relationships observed in the root apex of a typical week-old control root grown in nutrient medium. In table 1 are presented detailed measurements of a series of roots, from which the average dimensions shown in fig. 4 were taken.

The central cylinder thus originates from the apical initials and is set off from the future cortical zone by a cylinder of cells forming the inner layer of the ground meristem. At about 30–40 μ behind the apical initials, the diameter of the central cylinder averages 13–15 procambial cells which have been produced by longitudinal and transverse divisions of the cells derived from the apical initials. The central cylinder is comprised of elongate cells, 3–4 times greater in length than width. These cells are of uniform transverse diameter, have large nuclei and their densely staining protoplasts show little vacuolation. The cells are typically procambial in appearance and the entire cylinder,

having a terminal conical shape proximal to the initial region, may properly be termed procambium.

The first evident change in these procambial cells as seen in serial transverse sections is the enlargement of metaxylem elements—one in each of the three future xylem strands. The first manifest enlargement of these cells is observed an average distance of about 175 μ proximal to the initial region (fig. 4). At this level the procambial cylinder has already increased in diameter through the formation of new procambial cells by longitudinal divisions to an average of 19–20 cells. In each radial xylem arm, the metaxylem cells which first enlarge are seen to lie inside the midway point on the radius between the outermost layer of the central cylinder and the root center. Usually two or three small procambial cells lie between them and the root center. Subsequent enlargement of individual metaxylem elements occurs along a radial path outward from the first enlarged cells toward the future xylem pole. At about 275 μ from the initial region three metaxylem mother cells in each arm have enlarged in diameter and the future triarch xylem pattern is clearly indicated. The increase in diameter of these cells usually represents only a two-fold increase over adjacent procambial cells and although clearly evident in transverse section (fig. 2, 2a), one finds difficulty in picking out precisely such cells in longitudinal section close to the apical initials. It has not been possible in these roots to trace such early enlarged metaxylem elements in single file into the initial region.

The central procambial cells remain small in diameter and parenchymatous in appearance for a considerable distance proximal to the apical initials (usually greater than 10 mm. in the control roots). These cells apparently play no role in the determination of the radial vascular pattern. At some stage after the enlarged metaxylem elements have completed their maturation, these central cells undergo changes leading to their maturation, forming a solid central core of primary xylem (Torrey, 1951). The delayed maturation of these central elements is a matter of considerable interest and worthy of further investigation. As referred to in

TABLE 1. Longitudinal dimensions in the apical region of isolated pea roots grown for one week in a synthetic nutrient medium, showing the spatial relationships in the primary vascular tissues during early differentiation. Values in μ .

Root Number	Root Cap μ from tip	First Metaxylem Enlargement	Sieve Tube Element Division	First Mature Phloem	First Mature Xylem
				μ from Apical Initials	
1	0–288	160	208	288	5180
2	0–280	152	208	288	7260
3	0–192	216	272	352	5770
4	0–168	208	224	288
5	0–304	112	408
6	0–160	152	440
7	0–168	224	336
Averages	0–225	175	260	340	>5000

this paper, protoxylem elements are the first matured primary xylem elements which usually occupy a characteristic locus at the periphery of the central cylinder. The metaxylem is that portion of the primary xylem maturing after the protoxylem. Early and late metaxylem are not distinguished, although, strictly speaking, the enlarged metaxylem elements which form the characteristic radial pattern could be interpreted as "early metaxylem" and the later maturing central elements as "late metaxylem."

The early blocking out of the xylem pattern by cell enlargement of the radially-arranged metaxylem mother cells is characteristic of most of the dicotyledonous and monocotyledonous roots reported in detail. It is clear however that in *Pisum*, unlike most monocot roots, e.g., *Allium* (Mann, 1952), *Hordeum* (Heimsch, 1951), etc., and the diarch dicot roots studied, e.g., *Nicotiana* (Esau, 1941), *Sinapis* (Bünning, 1951), etc., metaxylem enlargement does not occur immediately adjacent to the apical initials, but is delayed until some distance behind the initial region. As has been pointed out by Esau (1940, 1941) the early enlargement reflects sluggish cell division of these cells accompanied by relatively rapid increase in cell volume. The surrounding procambium maintains a more uniform rate of cell division with no evident increase in cell diameter. At this level, no marked differential in cell length is evident among the cells, and, in *Pisum*, vacuolation of the enlarged metaxylem elements is delayed for some distance (averaging 400–500 μ) until elongation of the immature metaxylem elements begins. Then small isolated vacuoles form adjacent to the two end walls of the element and eventually coalesce within the elongate vacuolated elements.

Midway between the radial arms of the future xylem and immediately within the cylindrical pericycle cell layer lie the future phloem areas. At an average distance of 340 μ from the apical initials the first mature protophloem elements are evident in transverse section. In each of the three distinct phloem areas, single sieve tube elements are seen, each lying immediately adjacent to cells of the pericycle (fig. 2a, 4). Each mature element is readily discernible by the lack of cell contents, the characteristic pentagonal or four-sided shape and the dark staining cell wall. The first three elements, one at each pole, usually, mature at slightly different levels, but within 30–40 μ of each other. In tracing the origin of these first protophloem elements toward the apical initials, it was found that the file of cells which finally produces the mature sieve tube elements originates regularly by a longitudinal cell division of a protophloem mother cell. This division occurs 60–80 μ distal to the first mature phloem elements or at an average level of 260 μ behind the apical meristem. The longitudinal divisions occur in radial, periclinal or oblique direction without any apparent regularity. Follow-

ing this final longitudinal division, the outermost cell adjoining the pericycle enlarges slightly, becomes vacuolated, and undergoes the characteristic changes to a mature sieve tube element (Esau, 1950) within a distance of about 60–80 μ . The sister cell which is approximately the same size as the sieve tube element in diameter as well as length, remains as a flanking parenchymatous cell usually on the inner side of the sieve tube element. Several additional sieve tube elements form and mature in similar fashion in rapid succession on either side of the first mature elements, adjacent to the pericycle. The origin of sieve tube elements by longitudinal division of protophloem mother cells, after increase in the diameter of the central cylinder by cell division and cell enlargement has occurred, has been described in a number of dicotyledonous roots (e.g., *Nicotiana*, Esau, 1941) as well as in monocot roots (e.g., *Hordeum*, Heimsch, 1951). In *Daucus* no such division of protophloem mother cells has been observed (Esau, 1940).

At the level of these final cell divisions that give rise to the first mature sieve tube elements, enlargement of metaxylem mother cells has progressed to the point that two enlarged cells are clearly evident in each xylem arm. Thus, when the cells finally comprising the mature protophloem pattern have just been formed by cell divisions, the xylem pattern is already clearly established (fig. 2, 2a).

As has been described in the root of *Pisum* (Torrey, 1951, 1953), the maturation of protoxylem tissue proceeds much more slowly than does protophloem maturation. In the material studied here, mature protoxylem elements identifiable by lack of nucleus and the presence of lignified secondary walls, were first evident between 5000–6000 μ (5–6 mm.) behind the apical initials. Xylem maturation occurs centripetally and typical exarch xylem is found in the primary body. In cultured pea roots, completion of primary xylem maturation usually occurs at distances greater than 10 mm. from the root tip.

The week-old cultured roots grown under controlled conditions showed remarkably constant tissue differentiation in terms of pattern and of actual positional relationships (table 1). In the hundreds of control roots examined in sectioned material, the triarch arrangement of vascular tissues was always observed. In the experiments described below, the starting experimental plant materials were 0.5-mm. root tips, including root cap, excised from these one-week-old roots. Figure 4 therefore represents diagrammatically the excised 0.5-mm. tip used as the starting experimental material. At this level of excision the triarch pattern of vascular tissues is well established. Enlargement of three metaxylem mother cells in each xylem arm has occurred and the site of the future sieve tube elements has been fixed. Figures 2 and 2a show the stage of differentiation of the procambial cylinder at the excision surface. Usually no mature proto-

phloem was included in the excised tips, although considering the possibility of variation due to the excision procedure, certain tips may have included the earliest mature protophloem elements. No mature xylem tissue was present in the excised tips.

Vascular tissue differentiation in roots developing from 0.5-mm. root tips in culture.—Half-mm. pea root tips excised from week-old roots grown in culture will increase their initial length about 15–20 times in a week. The roots are white and healthy, but somewhat smaller in diameter than the roots from which they were excised. During extensive experiments on the nutritional requirements of 0.5-mm. tips, (Torrey, 1954), numerous samples of roots of different lengths grown in different nutrient media were fixed in FAA, usually after only one week in culture, and were embedded in paraffin for detailed histological examination or were sectioned with a freezing microtome. Nearly 200 roots were examined histologically. During these studies, in which a number of anatomical abnormalities were discovered, no evidence suggested any correlation between the anatomical changes observed and changes in the nutrient media in which the roots grew. Anatomical modifications appeared in roots grown on many of the different nutrient media, including the medium that produced a maximum rate of root elongation. In describing the structural differences here, the nutrient medium differences have therefore been disregarded since their effect concerned elongation rate chiefly. As will become apparent in the subsequent discussion, the observed abnormalities in tissue patterns appear to be attributable to the excision procedure and to the size of the excised tip.

A large proportion of the 0.5-mm. root tips grew as normal roots with the usual triarch arrangement of the vascular tissue. In these roots the sequence of vascular tissue differentiation at the meristem was quite comparable to that described above for the control roots from which the tips were derived. The root diameter as well as the diameter of the mature vascular cylinder was slightly reduced, due to a reduced number of cells in the total root diameter (compare fig. 2 and 3). Approximately 20 per cent of the roots studied in detail, however, showed abnormalities. The most striking abnormality observed in elongating roots derived from 0.5-mm. tips was the change in vascular pattern. This fundamental change involved a reduction in the number of vascular strands accompanied by a complete readjustment of the tissues of the central cylinder. Although the 0.5-mm. tips were always excised from triarch roots, many of these tips grew as diarch roots and a number were observed which showed a typically monarch arrangement of the vascular tissues. Table 2 lists the number of roots studied histologically and the nature of the vascular patterns observed.

The change in vascular pattern was evident at the base of the root very close to the level of the

TABLE 2. *Summary of root materials studied histologically, showing the observed incidence of transitions in vascular tissue patterns.*

<i>Roots grown from 0.5-mm. root tips</i>	
No. of roots	
Studied in serial paraffin sections:	
Triarch throughout	24
Diarch throughout	5
Monarch throughout	2
Diarch to triarch	3
Monarch to diarch	2
Monarch to diarch to triarch....	1
	—
	37
Studied in freezing microtome sections:	
Sectioned at base:	
Triarch	66
Diarch	11
	—
	77
	Total 114
% of roots from 0.5-mm. tips showing pattern transition 21%	
Sectioned at tip only:	
Triarch	32
<i>Roots grown from one-mm. root tips</i>	
Studied in freezing microtome sections:	
Triarch throughout	16
	—
Total number of roots studied	162
	—
	—

excision. At the point of excision, many cells were crushed or torn open by the cutting operation and these were manifest in sectioned material for a distance of as much as one mm. distal to the cut. In fig. 5, 5a is illustrated in transverse section the central cylinder from the basal portion of a root, showing the distortion and tearing caused by the excision. An immature, slightly asymmetric diarch xylem pattern is evident with phloem tissue irregularly arranged in three separate areas. The central cylinder was flattened laterally by the excision and the original triarch vascular arrangement was destroyed. In fig. 11, 11a is illustrated the mature symmetrical diarch condition seen in another root. In fig. 7, 7a is illustrated in transverse section the central cylinder of a root in which the arrangement of vascular tissue is typically monarch. The central strand of xylem elements is surrounded by phloem elements arranged in the shape of a horseshoe.

The diarch arrangement of the vascular tissue, either completely normal and symmetrical or slightly asymmetric, was found at the bases of 19

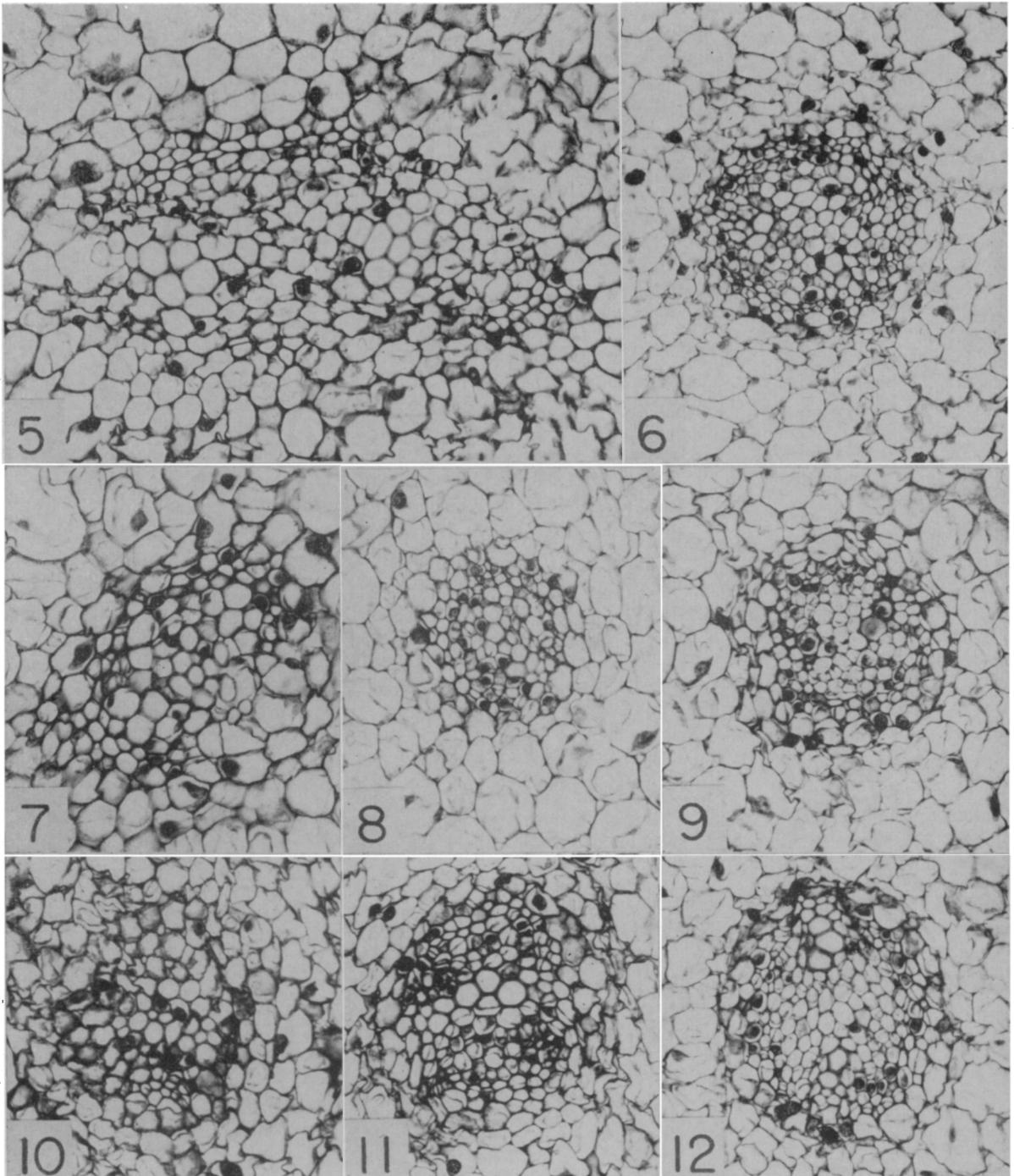


Fig. 5-12.—Photomicrographs of transverse sections of roots grown in nutrient medium from excised 0.5-mm. pea root tips, cut at various levels to show differences in vascular arrangement. Root numbers refer to table 3. Reference should be made to photographic tracings of these figures in fig. 5a-12a. All $\times 350$.—Fig. 5. Asymmetrical diarch arrangement in root 14 cut at 1200μ from extreme base, showing lateral compression and tearing at the protoxylem points due to excision.—Fig. 6. Triarch vascular arrangement in root 14 cut at 2400μ distal to the level shown in fig. 5.—Fig. 7. Monarch vascular arrangement in root 17 cut at 940μ from the extreme root base, showing the horseshoe arrangement of phloem around the single xylem strand.—Fig. 8. Same root 17 cut at 1415μ distal to fig. 7, showing essentially diarch arrangement with one xylem element in the third strand.—Fig. 9. Same root 17 cut at 4640μ distal to fig. 8 and 920μ from the apical meristem, showing the symmetrical triarch arrangement with enlarged metaxylem elements containing nuclei.—Fig. 10. Monarch vascular arrangement in root 11 cut at 2510μ from extreme root base, showing the opposite arrangement of

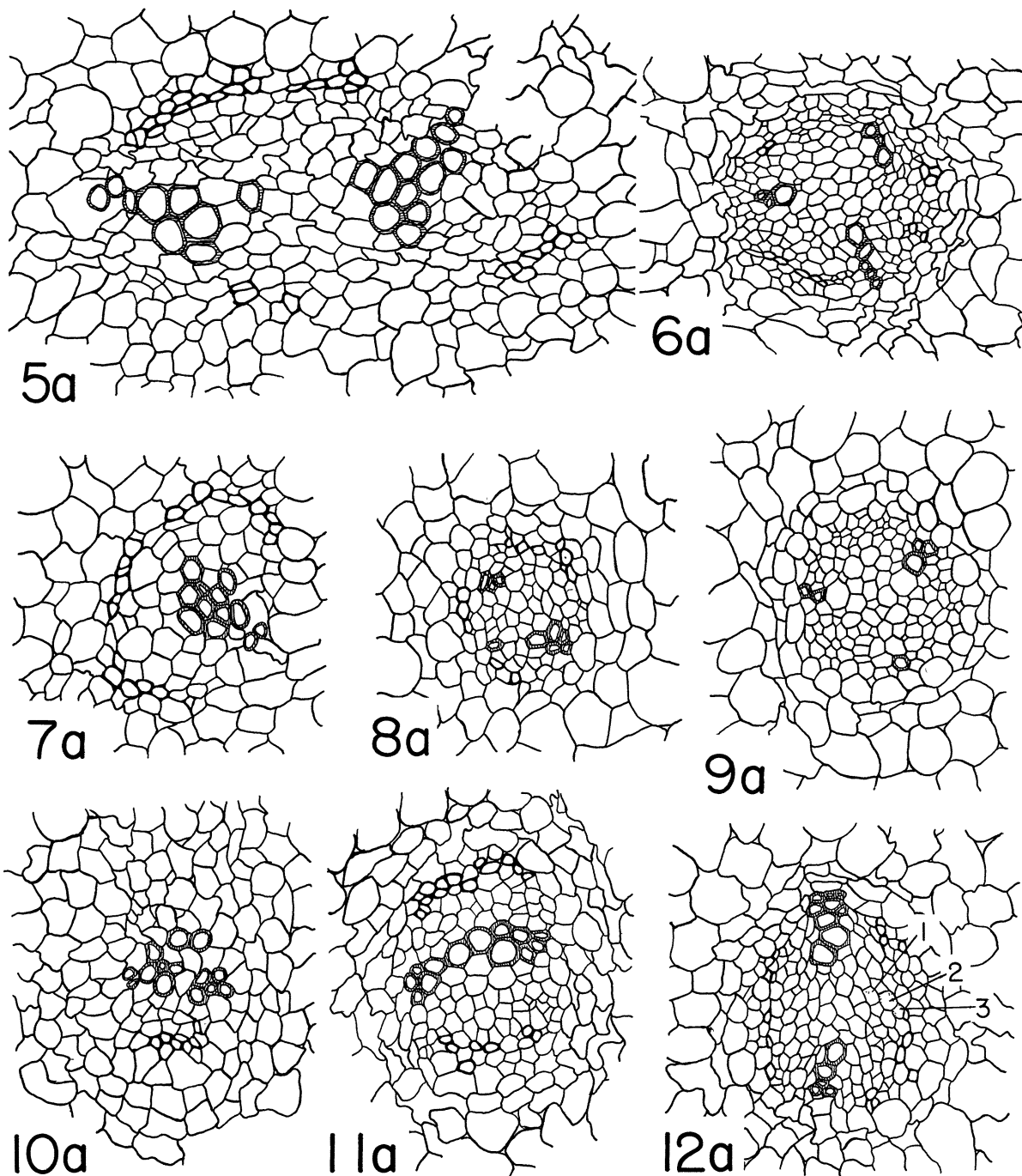


Fig. 5a-12a.—Photographic tracings of sections shown in fig. 5-12. Mature xylem elements are designated by shading of the thick secondary walls; mature sieve tube elements of the phloem are indicated by thickened black walls. Reference should be made to corresponding photomicrographs in fig. 5-12 for detailed descriptions and cellular detail. All $\times 350$.

xylem and phloem.—Fig. 11. Same root 11 cut at 1400μ distal to fig. 10, showing symmetrical diarch vascular arrangement.—Fig. 12. Diarch vascular arrangement in root 12 cut at 2900μ proximal to the apical initials, showing the symmetrical diarch arrangement just proximal to the level of transition to the triarch pattern. Future xylem elements of the third xylem strand can be identified and are indicated in the photographic tracing, fig. 12a, numbered to show the centrifugal sequence of their maturation.

roots. Five roots were observed in which the pattern of vascular tissues at the base could be termed monarch, containing a single strand of xylem tissue with a localized area of phloem tissue. The tissue arrangement illustrated in fig. 7 is typical of the monarch condition such as is found in the root of *Ophioglossum* (Bower, 1930) and in roots of certain other lower plant forms, and is an arrangement very rarely observed under natural conditions in the roots of dicotyledonous plants (Guttenberg, 1940).

At the time of excision of 0.5-mm. root tips, the triarch pattern of vascular tissues is well established in the root tip. If the tissues in which this pattern is determined are not destroyed during excision, the root grows as a triarch root. If, however, the excision damage is sufficiently extensive, the triarch pattern is destroyed or partially destroyed and new patterns are produced within the potentialities of the residual procambial tissues. Thus diarch roots develop or in some roots, even the very simple monarch arrangement is produced. It is of some significance that under these conditions the new patterns always represent a reduction in the number of vascular strands within the root. No matter what vascular pattern is differentiated, the tissues of the entire central cylinder finally adjust by the formation of a symmetrical structure. In the case of the diarch roots formed, one xylem arm may be related in origin to a strand blocked out in the pre-existing triarch pattern of the excised tip. This relationship is difficult to establish with certainty. The opposite xylem arm of the symmetrical diarch arrangement cannot be related, however, to either of the two other strands of a pre-existing triarch pattern, as the new strand lies in a position midway between them. Thus the pattern formed in the apical meristem has changed, as is clearly evident by a study of the meristematic region in a number of such roots.

Transitions in vascular patterns.—Diarch and monarch roots do not continue to grow in culture with a fixed vascular pattern as in cultured triarch pea roots. All roots in which a reduction had occurred in the number of vascular strands to the

diarch or monarch condition, if allowed to grow in culture, ultimately reverted to the triarch vascular arrangement characteristic of the pea root! The transitions from one arrangement to another occurred at various root lengths. Table 3 summarizes data from ten roots and shows the position along the length of the root at which the transition occurred. The transition per se from one pattern to the other was completed usually in about 1 mm. of root length. In one root that was sectioned (root 17, table 3), the complete transition from monarch through diarch to triarch occurred within a distance of 5.3 mm.

In fig. 5, 5a and 6, 6a are illustrated transverse sections taken from root 14 of table 3, showing the diarch and triarch arrangement at the two different levels of pattern. In fig. 7, 7a, 8, 8a, and 9, 9a the sequence from monarch to triarch is illustrated in sections cut from root 17 of table 3. In fig. 8 the transition from diarch to triarch has occurred, although the diarch arrangement is still clearly evident. In several cases diarch or monarch roots were killed and fixed before the transition had occurred (table 2). These roots were all quite short. In several diarch roots, it was possible to study the early delimitation of the vascular pattern behind the apical initials. Such differentiation occurred in the manner described for the control triarch roots except that only two xylem strands were initiated. It is certain from these observations that the meristem itself passed through an active period producing diarch root structure before the transition to the triarch condition was established in the meristem. In every root in which sufficient elongation had occurred, the transition to the "genetically fixed" triarch arrangement of the primary vascular tissues was accomplished.

The anatomy of the vascular transition region.—Anatomical studies were made of roots in which the transition from the diarch to the triarch arrangement had been completed shortly before the root was killed. In such roots, the formation of the third xylem strand could be studied during the early stages of maturation of the newly formed xylem. Since all the cells comprising the central area of

TABLE 3. *The occurrence and position of transitions in vascular tissue patterns in roots grown from 0.5-mm. tips.*

Root Number	Vascular pattern	Root length in μ	Length of Transition in μ	Distance of new pattern from apical meristem in μ
8	Monarch throughout	3050	-----	-----
9	Diarch throughout	4225	-----	-----
10	Diarch throughout	5760	-----	-----
11	Monarch to diarch	7040	1065	4515
12	Diarch to triarch	6690	370	1970
13	Diarch to triarch	7225	840	3985
14	Diarch to triarch	7400	1185	4975
15	Diarch to triarch	>8880	520	-----
16	Diarch to triarch	7380	-----	6430
17	Monarch to diarch to triarch	7970	232, 960	6270, 920

the central cylinder, including the diarch xylem elements themselves, are enlarged and vacuolated, it was not possible to distinguish the initiation of the third xylem strand by differential cell enlargement as is possible in the apical region of the root. Even so, the sequence of xylem maturation of the newly formed xylem strand was readily discerned by a study of serial transverse sections. The first indication of the maturation of the additional xylem strand was the appearance of lignified secondary walls in one of the enlarged vacuolated cells. This cell, located on a radius extending from about the mid-point of the diarch xylem plate, corresponds in position approximately to that of the earliest enlarged metaxylem elements in the already established xylem strands. Thus the inner largest xylem element of the new strand was the first to mature. Subsequently, distal to the first element a second enlarged cell external to the first maturing element began to show secondary wall thickening and lignification. In a similar manner, additional smaller elements in a radial row outward from these first maturing elements also differentiated as xylem elements, forming a new mature primary xylem strand. The position of the radial arm is such as to form an approximately equal tripartite division of the central cylinder with the ultimate establishment of a symmetrical triarch vascular arrangement. The appearance of the third xylem strand results in little or no change in the diameter of the central cylinder. The direction of maturation of the xylem elements in the new xylem arm was exactly opposite that normally observed, that is, maturation was centrifugal instead of the usual centripetal maturation. In fig. 12 and fig. 12a is represented in transverse section a diarch root immediately proximal to the site of initiation of the third xylem strand. In the diagram of the root, fig. 12a, the future xylem elements are marked by stippling and their centrifugal sequence of maturation is indicated. The element numbered 1 matured first and elements 2 and 3 matured subsequently and in that sequence, forming a strand of three mature elements. The xylem elements became differentiated in the midst of many enlarged and vacuolated procambial cells which initially were indistinguishable from the adjacent tissues. In this root the maturation of the smaller central metaxylem elements was not yet complete. No peculiar arrangement of the phloem tissue was evident prior to the initiation of the additional xylem strand. Sieve tube elements were uniformly distributed along the periphery of the central cylinder between the diarch xylem poles. The division of the cylinder into three sections by the differentiation of the new xylem strand resulted in a division of phloem tissues on either side of the strand. In four different roots (table 3, Root 12-15) in which the transition from the diarch to the triarch vascular arrangement could be studied in detail, the centrifugal direction of

maturation of the newly formed xylem strand was observed.

The change in vascular pattern from the single strand to the diarch arrangement was somewhat less precise and thus more difficult to determine critically. In table 3, roots 11 and 17 were studied in detail over the region of transition. In root 11, illustrated in fig. 10, 10a, the monarch condition is represented by a one-sided distribution of the phloem tissue with a central strand of xylem elements. The transition to the diarch condition involved the gradual appearance of a diametrically opposed phloem tissue and the centrifugal extension of the xylem band by differentiation of enlarged vacuolated cells to form the symmetrical diarch condition (fig. 11, 11a). In root 17, illustrated in fig. 7, 7a, in which the monarch condition is manifest in a horse-shoe distribution of phloem tissue surrounding a strand of xylem elements, there occurred first a reduction in the number of xylem elements to three elements in transverse section (distal to the level illustrated in fig. 7). This condition was followed by the centrifugal differentiation of a second strand opposite the first xylem elements with the ultimate establishment of two xylem strands at 180° from each other, separated by enlarged and vacuolated cells of the central cylinder. Subsequently a third xylem area appeared as described (fig. 8) and the full transition from monarch to triarch was complete (fig. 9).

The relation of root size to vascular pattern.—As early as 1877 DeBary stated, as a general rule, that as the thickness of a root diminishes, the number of its radial vascular strands also diminishes. Bower (1930) more specifically stressed the relationship between the diameter of the mature central cylinder and the number of vascular strands in the root, citing this relationship as evidence favoring his "Size-Factor" hypothesis. According to Bower (1930, p. ix), the "Size-Factor" is "that influence which tends to secure by modification of Form a due levelling up of the proportion of surface to bulk as the Size increases." In reference to the root, he quoted Wardlaw (1928) who had shown that in the roots of a wide selection of ferns and monocotyledonous and dicotyledonous plants studied the number of vascular strands in the root increased in almost direct proportion to the increase in stelar diameter. Similar observations on the roots of many plants have been recorded and the correlation has been noted by many investigators. Such observations have been made usually on different roots of different sizes chosen at random from one plant or from different plants. Several reports of this correlation have been based on studies of a single root axis of changing diameter. In the swollen storage root of *Maranta*, Meyer (1930) found that the number of vascular strands increased with increasing total root diameter and then diminished again to the original number past the swollen zone. He also reported a reduction in the number

of vascular strands accompanying reduced root diameter in roots of *Hyacinthus* and *Asphodelus*. Jost (1931-1932) observed in seedling roots of *Zea* striking reductions in vascular strand numbers which were related to decreased diameter of the central cylinder of the root. Similar observations have been made by other authors (e.g., Bouillenne, 1928; Aldrich-Blake, 1930; Hatch and Doak, 1933; Guttenberg, 1940; Preston, 1943; etc.). Dodel (1872) described in detail the reduction of vascular strand number in lateral roots as compared to the primary root, in several species of *Phaseolus*.

Although both Wardlaw (1928) and Bower (1930) attempted to explain the observed correlation between central cylinder diameter and vascular complexity of the mature root in terms of "size and structure" relationships, such an attempt was at best a description and is of little direct assistance in analyzing the physiological basis of the pattern. More recently Thoday (1939) has pointed out that the diameter of the root meristem at the level of pattern formation is the significant dimension in an analysis of the causation and not the final diameter of the mature organ.

It is interesting to note the observed changes in vascular pattern in the present experiments from the point of view of the size-structure relationship. According to Bower's "Size-Factor" hypothesis, one would expect to find that a change in the number of vascular strands in the root is correlated with a change in total root diameter. Table 4 presents measurements of total root diameter and

central cylinder diameter of a number of the experimental roots described earlier, arranged according to increasing vascular complexity. Each measurement is representative of the root at the level of mature primary tissues of a given pattern. Measurements of the central cylinder include the pericycle. Roots which showed transitions are represented by measurements of the mature tissues at each level of vascular pattern. It is readily apparent from these data that no correlation exists between total root diameter and the degree of complexity of the mature vascular pattern. Although in his analysis of the effect of size on vascular complexity in the shoot, Bower correlates increasing vascularization with total diameter, it is interesting to note that, in reference to the root, the only valid correlation established was an increase in the number of vascular strands paralleling an increase in diameter of the vascular cylinder but not total root diameter. Although table 4 shows a general trend of increasing vascular complexity, i.e., monarch to triarch, associated with an increase in the diameter of the mature vascular cylinder, even this relationship is not significant since considerable overlap in size of each of the different types of vascular arrangement occurs. Furthermore, there is no consistent relationship between size and vascular arrangement within a given root. In root 11, the mature diarch arrangement of the vascular cylinder is larger (94μ) than the mature monarch condition (57μ). Similarly in root 12, the diameter increases from 83μ (diarch) to 117μ (triarch). On the other hand, in root 17, the reverse situation is evident with a continuous reduction in the diameter of the mature vascular cylinder accompanied by an increase in the number of vascular strands: monarch— 103μ ; diarch— 69μ ; triarch— 62μ .

Following the suggestion of Thoday (1939), analysis of the size-structure relationship was made in roots at the level of the pattern formation, immediately proximal to the apical meristem. Table 5 presents representative measurements of the diameter of the procambial cylinder of roots at the level of the earliest maturation of protophloem elements, approximately the level at which the vascular pattern first becomes established. Fewer roots were available for measurement which showed the reduced vascular pattern at the meristem at the time of killing. It is evident from this table that a much clearer trend relates the increased procambial diameters to increased vascular complexity. Two types of triarch roots are included—(1) roots derived from 0.5-mm. excised root tips which developed normally and (2) control roots, initially derived from 5-mm. tips from germinating seed and grown for one week without excision. Roots from 0.5-mm. tips show consistently smaller vascular cylinder diameters—both in actual width and in number of cells, compared to the control roots, which are remarkably uniform among themselves. It is apparent that the 0.5-mm. roots were consist-

TABLE 4. Measurements of roots derived from 0.5-mm. tips and control root tips, showing the transverse dimensions of the mature primary tissues. Roots are listed according to increasing vascular pattern complexity. Individual roots with pattern changes are listed at each pattern level.

Root Number	Vascular Pattern	Diameter of Central cylinder		Total Root Diameter in μ
		No. of cells	Width in μ	
Roots grown from half-mm. tips.				
11	Monarch	10	57	507
8	Monarch	9	78	585
17	Monarch	12	103	780
10	Diarch	9	63	187
17	Diarch	11	69	390
12	Diarch	15	83	296
11	Diarch	12	94	296
14	Diarch	14	114	840
9	Diarch	14	125	605
17	Triarch	12	62	304
14	Triarch	15	89	324
18	Triarch	15	132	390
12	Triarch	14	117	351
Control roots grown one week in culture				
3	Triarch	19	172	390
1	Triarch	20	176	449
4	Triarch	20	187	488
2	Triarch	23	195	525

TABLE 5. *Measurements of roots derived from 0.5-mm. tips and from control root tips, showing the dimensions of the procambial cylinder at the level of pattern inception. Roots are arranged according to increasing vascular complexity and increasing diameter.*

Root Number	Incipient Vascular Pattern	Diameter of procambial cylinder	
		No. of cells	Width in μ
Roots grown from 0.5-mm. tips			
8	Monarch	9	109
10	Diarch	9	47
11	Diarch	10	78
9	Diarch	14	82
14	Triarch	14	94
16	Triarch	14	101
19	Triarch	14	105
20	Triarch	15	105
12	Triarch	15	117
17	Triarch	16	117
21	Triarch	14	140
18	Triarch	17	146
Control roots grown one week in culture			
3	Triarch	20	171
4	Triarch	20	172
1	Triarch	21	183
2	Triarch	22	195
5	Triarch	22	195
6	Triarch	23	195

ently smaller, incident to the excision. Even so, many such roots were triarch (fig. 3).

In comparing the procambial diameters in table 5 with the diameters of the mature vascular cylinder of the same roots given in table 4 it is interesting to note that no consistent relationship exists between these two dimensions in this particular experimental material. Thus one must not assume, as has been done in the past, that, in either experimental or in untreated material used for anatomical studies, a constant relationship does exist between these dimensions that makes possible an analysis of the differentiation process based on study of the mature vascular system alone.

The data in table 5 tend to confirm Thoday's contention (1939) that the number of vascular strands in a root is related to the diameter (or circumference) of the central cylinder of the root at the time of initiation—i.e., in the region of the apical meristem, and not to the ultimate size of the mature root. Recently, Wardlaw (1947a) has admitted the improbability of any causal relationship between the final mature functional condition and the initial processes of differentiation, and, in relation to the shoot at least, has concluded that the mature structure is related directly to the activity of the apical meristem.

DISCUSSION.—From the experiments described above evidence suggests that the primary vascular tissue pattern in the excised pea roots arises as a product of the activity of the apical meristem and does not result from the inductive influences of the

older mature vascular tissues, as maintained by Jost. Half-mm. pea-root tips, in which the pre-existent triarch pattern of the primary vascular tissues was destroyed by experimental manipulation, produced new and different vascular patterns during subsequent root growth. These new patterns, typically diarch or monarch, were maintained for varying distances during root elongation, and then the triarch pattern was restored. In these experiments there was no induction of the monarch or diarch pattern in the newly formed cellular derivatives of the meristem as would be expected from Jost's inductive forces. The presence of mature vascular tissues in organic attachment is not essential for the continued differentiation of procambial tissues in these roots, nor does there appear to exist any direct controlling influence of the mature tissues over differentiation of the meristematic tissues. As suggested earlier (Torrey, 1954), the mature differentiated tissues of the normal seedling root may be thought of as providing and/or transporting essential nutritional requirements that regulate the activity of the apical meristem. When isolated and provided with a complete synthetic nutrient medium, the apical meristem with its immediate cellular derivatives appears to be able to develop independently. Thus, as has been suggested for the apical meristem of the shoot by the work of Wardlaw (1947b, 1950) and Ball (1948, 1950), the isolated apical meristem of the pea root grown in nutrient medium appears to be self-determining, capable of producing its own pattern and structure within the genetic capacities of the species. One must look, not to the mature tissues, but to the apical meristem for the physiological control of the vascular tissue pattern in such roots.²

It is evident from these experiments with 0.5-mm. tips that the preformed pattern of vascular tissues at the apical region was destroyed by the excision itself in those roots which produced abnormal arrangements. In these roots, damage and destruction of individual cells of the future vascular tissue interrupted the course of their differentiation and resulted in the differentiation of a reduced vascular system. In the roots showing horizontal compression at the base of the root due to the excision, the suppression of differentiation of one or more arms of vascular tissue was evident (fig. 5a). Thus either diarch or monarch roots were formed where extensive destruction of the preformed pattern had occurred. In all cases, these new patterns resulted from excision and internal adjustment or rearrange-

² In an article published since the above was written, E. Reinhard (*Zeitschr. f. Bot.* 42: 353-376. 1954) states this same general conclusion, based upon observations on the differentiation of tiny fragments of pea root tips cultured in sterile nutrient medium. He concludes (p. 375), "Die normale Ausgestaltung der Wurzelstruktur ist also wohl nicht abhängig von determinierenden Einflüssen älterer Teile der Wurzel, sondern von der Organisation des Vegetationspunktes selbst."

ment ending in an essentially symmetrical arrangement. Boodle (1899) suggested that the monarch arrangement in the root of *Ophioglossum* was derived from the diarch condition in which one xylem group had aborted, a view held earlier by van Tieghem (1870-71). No evidence concerning the cause for the natural suppression of the xylem group was offered. In the experiments reported here, the excision procedure effectively reduced the diameter of the procambial cylinder at the level at which pattern formation occurred and in many roots the reduced vascular pattern resulted. It is significant that excision of root tips at 1 mm. from the extreme tip, at a level where the vascular pattern is well established, produced no abnormalities of vascular differentiation (see table 2).

Reduction of the complexity of tissue arrangements in the vascular cylinder in roots of monocots and dicots by experimental means has been described by several authors, using a variety of experimental manipulations. Flaskämper (1910) found that removal of the cotyledons from seedlings of *Vicia faba* resulted in retarded root growth and a reduction in the number of vascular strands in the root from 6 to 4. Jost (1931-32), in his decapitation experiments on roots of *Zea*, found that regenerated tips produced fewer vascular strands, with reductions from 16 or 17 strands to 10. Similarly, in regenerating root tips of *Vicia* seedlings, he observed reduction of vascular strands from 6 to 5, or from 5 to 4. Fourcroy (1938) found that a transverse puncture of the root tip of *Faba vulgaris* and *Lupinus albus* prevented normal differentiation of vascular strands on the injured side of the root, with a progressive return to the normal condition distal to the wound. In similar experiments in which he cut wedges in the region of the apical meristem of *Vicia faba* roots, Clowes (1953) reported a reduction in the number of protoxylem arms after the excisions. He attributed these changes to a decrease in the size of the meristem produced by the incision. In all these cases, as in the experiments reported here, the general rule applies that the reduction in the diameter of the central cylinder of the root tip at the level of pattern formation is accompanied by a reduction in the number of vascular strands. In roots of monocots especially, the change in vascular pattern may involve striking reduction of vascular strands both peripheral and central, and may not, in fact, be a situation exactly comparable to that in typical dicot roots.

In his analysis of root structure, Thoday (1939) has suggested that the unit pattern in root vascular tissue differentiation, i.e., the unitary vascular strand consisting of xylem and phloem, may vary within relatively narrow limits at the site of pattern inception and that the number of repetitions of the unit pattern may therefore increase as the dimensions of the procambial cylinder increase. Thus, increasing vascular complexity would accom-

pany increasing size of the apical meristem and its immediate procambial derivatives. Such an explanation, while clearly contributing to our understanding of the size-structure relationship, in effect restates the problem of pattern formation in roots in terms of unit pattern determination through cellular differentiation. In studying normal differentiation at the apical meristem and the appearance of the vascular tissue pattern in roots of *Pisum*, it is clear that pattern formation becomes manifest due to differential rates of cell division and enlargement. That is, more rapid enlargement of the future primary xylem elements, especially the metaxylem elements, occurs as compared to the rest of the procambial elements, which maintain a relatively more rapid rate of longitudinal cell division. Pattern formation then appears to be initially dependent upon forces which control, either directly or indirectly, cell division and cell enlargement in the apical meristem. The fundamental problem which the present work raises concerns the nature of these forces and the means whereby their influence is modified according to the dimensions of the apical meristem and its derivatives. For the ultimate understanding of the physical-biochemical basis of vascular pattern in growing roots, one must study and analyze the activities of the individual cellular derivatives of the apical meristem and the inter-relationships existing among them which determine the final structure.

SUMMARY

Isolated pea-root tips cultured one week in sterile nutrient medium show a uniform pattern of vascular tissue differentiation. The earliest visible pattern of primary vascular tissue differentiation following the delimitation of the cylindrical procambium is apparent in the enlargement of future metaxylem elements, beginning about 175μ proximal to the apical initials and resulting in the blocking out of the triarch primary xylem pattern. The divisions of the protophloem mother cells situated on alternate radii occur at about 260μ proximal to the apical initials and mature sieve tube elements are apparent within 340μ of the apical meristem. Half-mm. root tips including root cap, which contain no mature vascular tissues, were excised, grown in sterile nutrient medium and analyzed histologically. Approximately 20 per cent of the tips studied showed a reduction from the normal triarch radial pattern to a symmetrical diarch or monarch arrangement of the vascular tissues. The reduced vascular pattern was evident at the root base and persisted during root elongation for varying lengths. The abnormal pattern resulted apparently from a partial or complete destruction of the pre-existing triarch pattern in the tissues at the level of excision. After continued elongation, roots with a symmetrical monarch or diarch vascular pattern underwent a transition, returning to the original triarch arrangement. This transition usually occurred within about

one mm. of root length and involved the appearance of an additional xylem strand by centrifugal differentiation to form the new symmetrical vascular tissue arrangement. Roots showing transitions from monarch to diarch, from diarch to triarch, and, in one case, from monarch through diarch to triarch, were studied. No correlation was found between vascular complexity and either total root diameter or the diameter of the mature vascular cylinder. A relationship between the diameter of the procambium at the level of pattern inception and the complexity of the vascular pattern was suggested. Two major conclusions were reached from these observations. First, the primary vascular tissue pattern in 0.5-mm. excised pea root tips does

not result from inductive influences of the older mature vascular tissues upon the newly formed cells produced by the apical meristem as was maintained by Jost (1931-32) but arises as a product of the activity of the apical meristem. Second, the determination of the radial arrangement of the primary vascular tissues appears to be related to the dimensions of the apical meristem at the time of pattern inception. It is suggested that pattern determination is under the control of unknown factors which influence cellular division and enlargement in the immediate derivatives of the apical meristem.

DEPARTMENT OF BOTANY,
UNIVERSITY OF CALIFORNIA,
BERKELEY 4, CALIFORNIA

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PHYSIOLOGY AND ANATOMICAL DEVELOPMENT OF TOMATO FRUIT TUMOR¹

Michael Treshow²

TOMATO FRUIT TUMOR, a disorder of green tomato fruits observed in California tomato fields and packing houses, results from the rubbing encountered during picking, handling, and processing of the green fruits.

Although tomato fruit tumor, sometimes referred to as waxy blister or oedema, was reported as early as 1896 by Selby (1896), no detailed study has been made concerning the anatomy, physiology, or development of the disorder.

Gardner (1925) made a study of the disorder and showed that the tumors resulted from cushions of hyperplastic tissue which pushed up beneath the necrotic epidermis. The direct stimulus was thought to emanate from the necrotic tissue and initiate hypertrophy and hyperplasia in the underlying cells.

The first sign of tomato fruit tumor is an inconspicuous wrinkling of the affected area which will later form the tumor. Tumors are initiated only on green fruits and become less conspicuous as the fruit ripens. They are formed only from the affected area, or part of it, and do not spread laterally, although they may attain a height of 1–2 mm. Individual circular or irregularly shaped tumors are generally less than 1 cm. in diameter on fruits in the field and affect only a small portion of the fruit surface. Tumors which result from picking or processing are frequently 1–3 cm. in diameter. A large portion of the pericarp may be affected by such tumors.

ANATOMICAL DEVELOPMENT.—Histological studies were made of the development of tomato fruit tumor. Green Pearson tomato fruits, free of tumors, were rubbed with a rough slat from a packing crate to stimulate tumor formation. All anatomical and physiological work was conducted with fruits detached from the plants. Sections of pericarp tissue were removed from the shoulder and blossom ends

of the fruits at the time of rubbing, and 6, 12, 18, 24, 36, 48, 72, and 96 hr. later. The tissue was placed immediately in formalin-aceto-alcohol or a chromo-acetic fixing and killing solution. Paraffin embedded tissue was sectioned to a thickness of 12 μ and stained with safranin 0 and fast green as described by Johansen (1940).

There was no morphological damage to the epidermis or any of the underlying tissue immediately after rubbing (fig. 1). The cuticle was usually intact, although occasionally the outer portion of cutin was lacerated.

The first sign of any abnormality of the fruit surface was the deposit of tannin in the sub-epidermal collenchyma, and sometimes in the epidermis, as early as 12 hr. after rubbing (fig. 2). Six to 12 hr. later, the collenchyma collapsed and tannins were more abundant. Within 24 hr. after rubbing, the outer layers of parenchyma or inner collenchyma cells started to enlarge under the rubbed area (fig. 2). These cells enlarged by intrusive growth and by crushing the tannin-filled collenchyma tissue. Intrusive growth was most prevalent in the outer three or four layers of parenchyma tissue. Some intrusive growth was evident several cell layers deeper in normal tissue, but it was not so extensive as in the tumorous tissue.

Radial cell division may start in some of the parenchyma cells within 48 hr. after rubbing (fig. 3). Transverse divisions generally followed (fig. 4), but were never so extensive as the radial divisions. This early hyperplasia could occur in the outermost layer of parenchyma tissue, or the innermost collenchyma tissue. In the latter case, only the collenchyma cells next to the epidermis were crushed and filled with tannins.

Nuclei of healthy and tumorous tissue did not differ consistently. However, nuclear divisions of tumor cells were occasionally abnormal and occurred without cell wall formation so that a binucleate cell was formed. Other tumor cell nuclei were observed to be in a tetraploid condition as determined by chromosome counts.

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