CAMBIAL FORMATION IN ISOLATED PEA ROOTS FOLLOWING DECAPITATION [1]

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THE EXPERIMENTAL modification of developmental patterns has been utilized effectively in studying the factors determining the course of normal differentiation in the tissues of higher plants. In recent years Wardlaw (1944, 1947), Ball (1950 a,b) and others have shown in their experimental studies on shoot apices that problems in plant morphogenesis can be attacked profitably using experimental techniques. Such work is contributing to our understanding of the factors responsible for the normal patterns of development in the shoot of higher plants. Yet surprisingly little critical work has been carried out on the comparable tissue systems in the root.

Some attempts to modify the course of vascular differentiation in roots experimentally have been reported. Jost (1931-1932) showed that in *Zea Mays*, the usually fixed number of xylem strands in seedling roots can be modified by simple experimental techniques such as decapitation or longitudinal splitting of the root tip. In some cases where a new apical meristem was regenerated following decapitation, Jost reported changes in the number of vascular strands formed during subsequent growth. Dormer and Street (1948, 1949) have reported unusual vascular differentiation in excised tomato roots grown in nutrient culture for long periods, which they attributed to the influence of the carbohydrate used in the medium. Others (Levan, 1939; Noirfalise, 1940; Carlton, 1943; Beal, 1944; Duhamet, 1946; D'Amato and Avanzi, 1948) have studied the effects of applied synthetic growth substances on root development and differentiation. In most cases, anatomical modifications involving cortical or pericyclic tissues were noted. Jost (1935) reported increased cell division in cambial tissues of roots treated with auxin. Bond (1948) reported changes in root diameter due to stelar enlargement following treatment of pea roots with growth substances.

Isolated root systems may be cultured in a completely known medium under carefully controlled conditions. Under certain of these conditions roots may be grown which produce many, few or no complicating lateral roots (Bonner, 1940; Torrey, 1950) and which may or may not retain the usual distinctive pattern of vascular differentiation (Dormer and Street, 1948). Cultured roots offer ideal material for studying physiological factors in cellular differentiation in plants. This paper reports the effects of simple root decapitation on vascular differentiation in isolated primary pea roots grown in sterile nutrient culture.

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MATERIALS AND METHODS.—Isolated 3-4 mm. root tips of the garden pea, *Pisum sativum*, varieties Alaska and Pilot, were excised from 48-hr. germinated pea seeds and grown in the dark at 25°C. in nutrient agar medium as previously described (Torrey, 1950). Ten millimeter decapitation of roots was made at the end of 1 week following initial excision when roots were about 50 mm. long. Excised tips were discarded. Decapitated root bases were then maintained in the nutrient medium for periods up to 4 weeks.

Intact and decapitated roots were fixed in formalin-acetic acid-alcohol with aspiration, 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.

OBSERVATIONS.—*The differentiation of vascular tissues in cultured pea roots.*—The sequence of differentiation of primary vascular tissues in isolated pea roots grown in culture, like that in the root of the intact plant, follows the general pattern described by Esau (1941) for intact tobacco roots. The structure of the mature primary root of *Pisum* is described by Hayward (1938). The arrangement of vascular tissues in the root is usually triarch. Whereas the protophloem elements differentiate early in ontogeny, often becoming apparent within 600-700μ of the apical meristem, mature protoxylem elements are first evident several millimeters behind the apical meristem with maturation occurring at unequal levels at the three poles. Centripetal differentiation of the metaxylem is only partially complete at 10 mm. behind the apical meristem, so that the central metaxylem elements may still contain protoplasts and show unthickened cell walls. Three groups of differentiated phloem fibers are evident at this level and clearly mark the phloem regions at alternate radii with the primary xylem poles. Outside the phloem fibers and protoxylem points lies a single-layered pericycle, bordered externally by a poorly defined endodermis. The wide cortex consisting of enlarged vacuolated cells is bounded by the epidermis. Secondary tissues are not usually observed in isolated pea roots grown in culture. Secondary growth due to limited cambial activity has been described in the primary root of the mature pea plant grown in the soil (Hayward, 1938). According to Hayward, a relatively inactive cambium may arise in the zone of interstitial parenchyma between the phloem and the metaxylem regions. A limited number of large xylem elements and phloem elements may be produced by this cambial activity in the root of the mature pea plant. Such secondary growth does not usually occur in cultured pea roots.

The endogenous origin of lateral roots in *Pisum*

has been described by Van Tieghem and Duliot (1888). Lateral root development in the primary root of isolated cultured pea roots usually occurs during the second or third week of continued culture without transfer. Lateral roots appear at this time in acropetal succession, usually 30-50 mm. from the root apex. Each lateral root is initiated by tangential divisions in the pericycle cells immediately outside the outermost protoxylem elements of a xylem arm. By numerous tangential and transverse divisions accompanied by radial elongation of the newly formed cells, the dome-shaped lateral root primordium is formed. The lateral root which projects outward through the cortex is made up chiefly of cellular derivatives of the pericycle. Organization of the primordium into root cap, apical meristem, vascular cylinder, and cortex is evident before the lateral root emerges from the cortex of the primary root.

The first differentiation of vascular tissues of the lateral root occurs just as the lateral projects through the cortex at approximately right angles to the main root axis. At this time the procambial tissue of the lateral root is apparent, extending back from the terminal meristem as progressively more elongated cells. These procambial cells abut upon pericyclic derivatives which lie outside as well as on either side of the protoxylem elements of the main root axis. The first xylem cells of the newly-initiated lateral root differentiate from these pericyclic derivatives in immediate contact with the outer protoxylem elements of the primary root. The numerous shortened xylem elements, which differentiate from the parenchyma-like derivatives of the pericycle into scalariform tracheids and vessel elements, are thus in intimate association with the protoxylem elements of the main axis. Subsequent xylem differentiation occurs centrifugally, so that xyllary continuity is maintained with the main root. The early differentiation of elongate procambial cells and continued acropetal xylem differentiation keep pace with lateral root elongation.

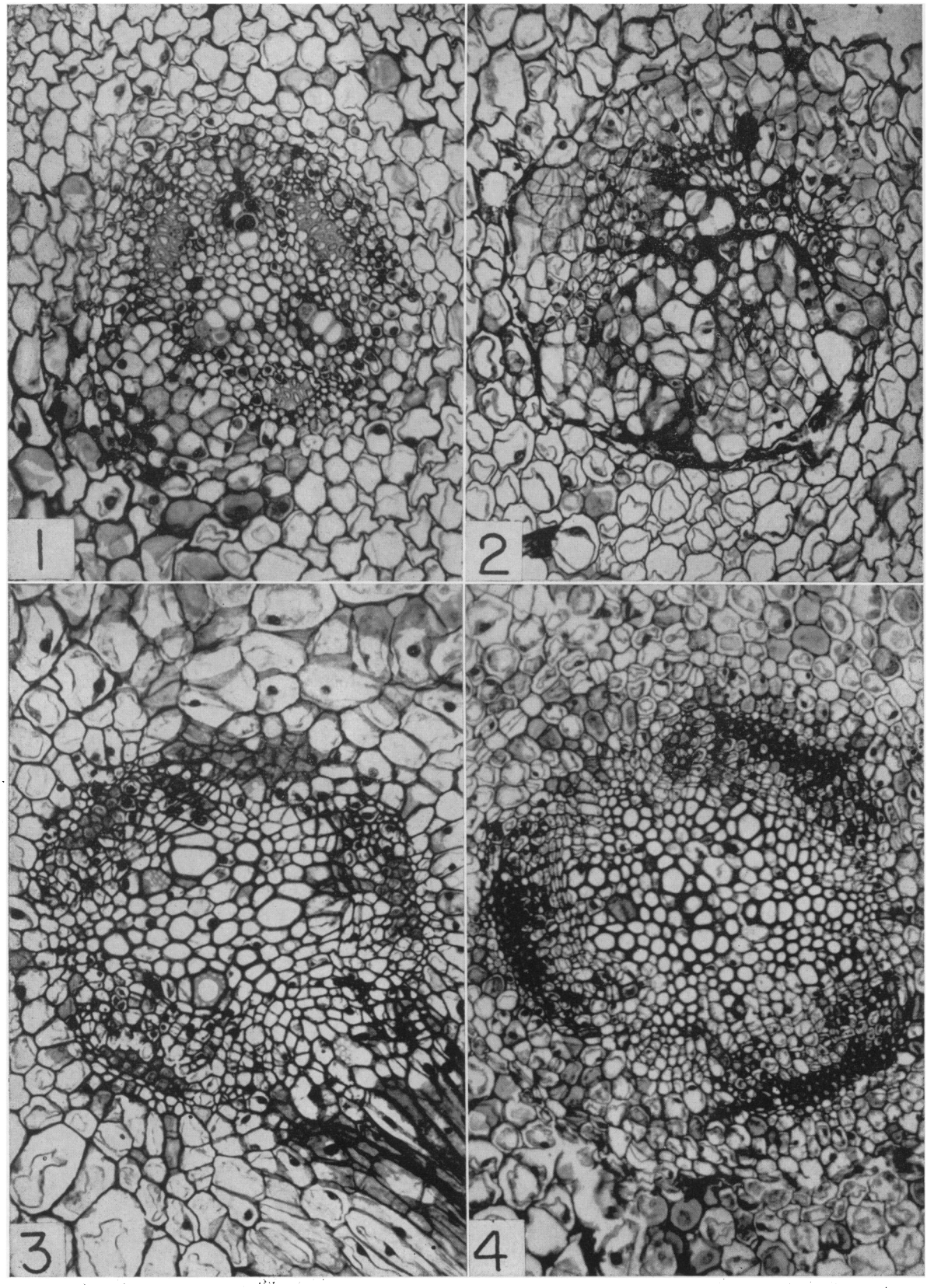
The initiation and development of lateral roots from the primary root usually effect no change in the basic triarch arrangement of vascular tissues in the primary root itself. At the point of lateral origin, the cross-sectional area of the vascular tissues of the primary root is somewhat enlarged due to the differentiation of vascular elements from the vascular tissues of the main axis into the lateral root. A few xylem elements differentiate on either side of the protoxylem arm as well as at the outermost point of the radial xylem arm. These elements form xylem strands which converge laterally, producing the solid diarch or triarch xylem plate of the lateral root itself. Cross-sections cut on either side of the lateral root origin, show the usual triarch pattern in the vascular tissues of the main axis.

Experimental modification of vascular differentiation in cultured pea roots.—The induction of lateral roots by decapitation of isolated initial pea roots grown for 1 week in culture has been described (Torrey, 1950). Usually several lateral roots are formed at the cut end of the decapitated root a few days after 10 mm. decapitation. Whereas the primary root is no longer capable of elongation following the removal of the apical meristem, the lateral roots formed subsequent to decapitation elongate at the usual rate. In such decapitated roots it was noted that at the site of lateral root origin, the primary root showed an increased diameter for a distance of several mm. from the cut surface. Ten millimeter apical sections were taken from decapitated roots at weekly intervals following the decapitation, fixed, and sectioned. The observations made on these roots are reported here.

A cross-section of the primary root cut at the site of decapitation shows the usual arrangement of vascular tissues described above. The triarch pattern of the primary xylem is well defined with large, thin-walled metaxylem elements clearly outlined in the central area of the xylem plate. Centripetal maturation of the metaxylem elements is usually still incomplete. Phloem fibers may be well developed at this level and clearly mark the outermost boundary of the phloem (fig. 1). The vascular cylinder occupies about one-third of the cross-sectional diameter of the root.

One week following decapitation.—One week following decapitation lateral roots are well developed within one to several millimeters of the cut apical end of the decapitated root. When sectioned, remarkable changes in the development of the vascular cylinder are evident. In the region immediately proximal to the cut surface, many irregular radial rows of meristematic cells with large nuclei have been produced by divisions of pericycle cells. These dense-staining cells form concentric layers external to the vascular cylinder. The incompletely lignified metaxylem cells in the central area appear enlarged and may be partially plugged due to the decapitation (fig. 2). Progressively back from the cut surface of the root, the enlarged pericycle decreases in size and the more typical arrangement is usually evident at a distance of about 500 μ from the cut surface. It seems probable that the stimulation of cellular divisions in the pericycle is related to wounding at the decapitation surface. Lateral roots arising within this zone may show early stages of vascular differentiation.

Under these experimental conditions, the distance at which the first lateral root is formed proximal to the locus of decapitation varies greatly. Measurements from the cut surface to the place of origin of the first lateral root varied from 160–1512 μ in the roots studied. In every case, at a given distance proximal to the origin of the first lateral root, an average distance of about 500 μ (table 1, col. 4), initial stages of cambial formation are observ-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3
4

TABLE 1. *Measurements made of isolated roots grown in culture for 1 week following 10 mm. decapitation, showing the relationship between the point of origin of the first lateral root and the occurrence of cambial tissues in the primary root axis. Values in microns.*

1	2	3	4	5
Root number	Distance from cut surface to 1st lateral root	Distance from cut surface to appearance of cambium	Difference between columns 2 and 3	Extent of cambium
1	368	1024	656	1608
2	712	944	232	1848
3	384	760	376	1408
4	160	824	664	1080
5	1512	2064	552	1208
Averages			496	1430

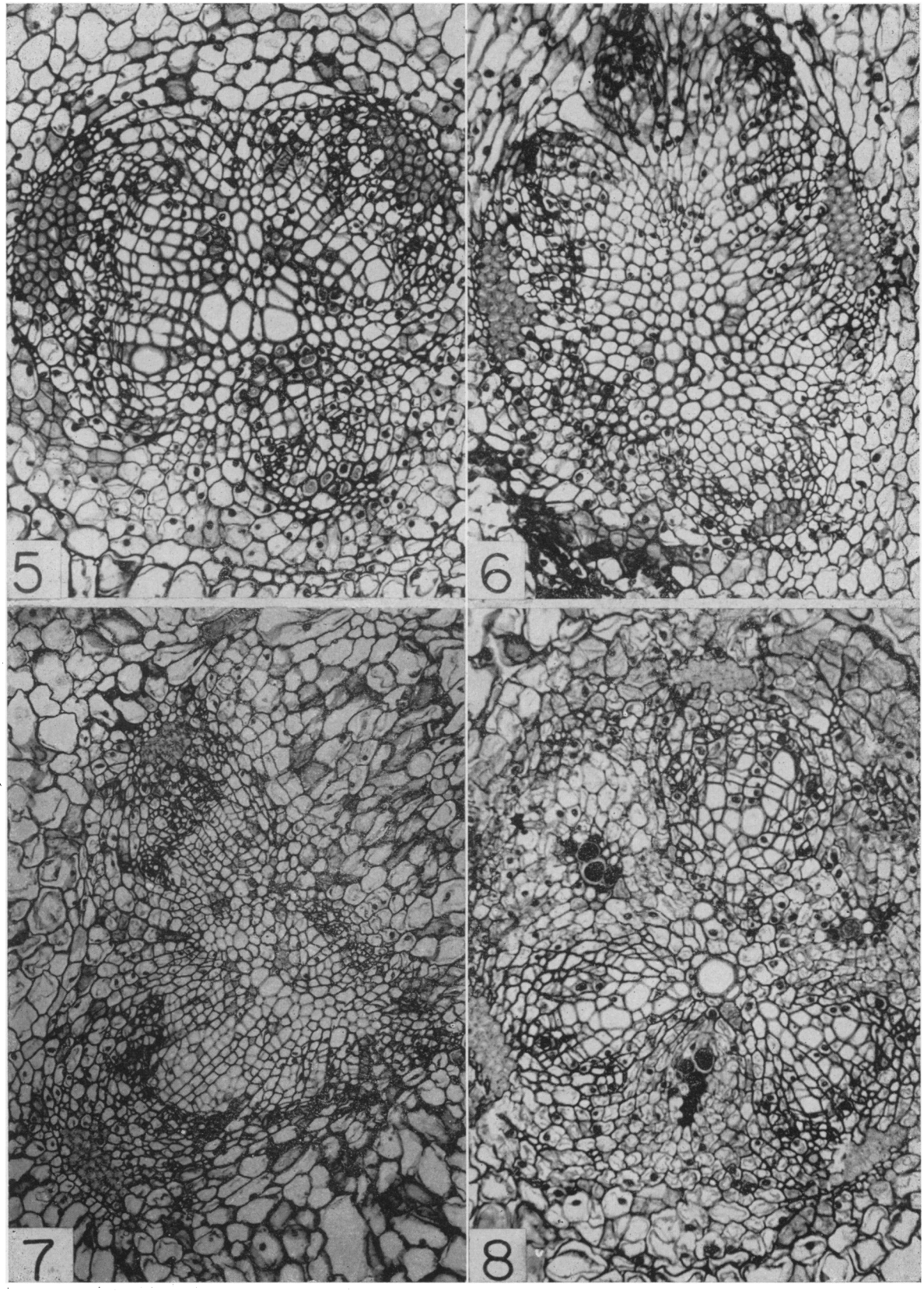
able in the parenchyma cells between the phloem and the metaxylem regions. The alignment of thin-walled cells in the phloem region is evident and in some cases radial rows of xylem derivatives can be seen (fig. 3). The number of vascular derivatives of the cambial layers is greatest in this zone of initiation and lessens basipetally. Finally, the cambium itself disappears completely at an average of about 1400 μ proximal to its initial appearance. Basal to the region showing definite cambial zones is observed the completely differentiated triarch vascular cylinder of the primary root and this arrangement persists to the extreme base of the root. Fig. 3 shows such a root with three discontinuous cambial zones each having given rise to radially arranged rows of cells. Internally, the differentiation of large vessel elements derived from these dividing layers is evident. This secondary xylem is arranged at alternate radii with the original, smaller xylem arms, resulting in strands of secondary xylem which are contiguous with the primary xylem plate. Actual increase in stelar diameter is slight. Differentiation of vascular tissues in the lateral root does not appear unusual.

Additional lateral roots may have been formed in the region of the root showing cambial tissues and also proximal to it. Such branches usually show the typical pattern of vascular differentiation. In table 1 are given figures showing the relationship between lateral root origin and the occurrence of cambial tissues in a representative series of roots.

Later stages showing extended cambial zones were observed in decapitated roots killed at two and three weeks after decapitation. Fig. 4 illustrates a section from the zone of lateral root attachment of a typical root base 10 days after decapitation. The cambial zones in each of the three phloem regions are well defined and the early undifferentiated derivatives of the dividing cambial cells are aligned radially on either side of the cambial zones.

Four weeks following decapitation.—Four weeks after the removal of the 10 mm. tip of the root, the basal portion of the root which is continued on the nutrient medium begins to appear browned. The lateral roots formed at the apical portion of the root continue to elongate and may grow several centimeters in length, forming lateral branches themselves. The swollen portion of the main root may extend 5 mm. or more behind the cut surface. Serial sections made from the cut end toward the root base show remarkable progressive changes in the vascular tissues of the primary root. Immediately proximal to the cut surface, the triarch arrangement of the vascular tissues is evident. The enlargement of the pericycle zone by cell division and enlargement described in week-old decapitated roots may have progressed back as far as the attachment of the first lateral root. Phloem fibers mark the outer areas of phloem between each pair of adjacent xylem arms. Proceeding back from the region of origin of the first lateral root toward the root base, one finds progressive increase in the number of secondary xylem elements produced by divisions of cambial cells. These extensive areas of secondary xylem are formed between the original primary xylem areas. Secondary phloem tissue is also evident. The primary phloem tissues and the cells of the pericycle are pushed outward into the surrounding cortical tissues (fig. 6, 7). Measure-

Fig. 1–4.—Fig. 1. Transverse section of root fixed after 10 mm. decapitation, showing usual arrangement of vascular tissues with incomplete maturation of metaxylem elements. Section made at 1080 μ proximal to the cut surface. 235×. —Fig. 2. Transverse section of root fixed 1 week after 10 mm. decapitation. Section made at 385 μ proximal to the cut surface. The triarch xylem pattern and the radial rows produced by the pericycle can be distinguished. Protoxylem elements are plugged. 210×.—Fig. 3. Transverse section of root fixed one week after 10 mm. decapitation. Section made at 1400 μ proximal to cut surface and 1016 μ proximal to origin of first lateral root. Cambial zones are evident external to the large secondary xylem elements which appear at alternate radii with the original triarch xylem plate. 265×.—Fig. 4. Transverse section of root fixed 10 days after 10 mm. decapitation. Section made at 840 μ proximal to cut surface in region of lateral root origin. Clearly defined cambial zones are evident opposite each phloem region. 200×.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ments within individual roots indicate a twofold or greater increase in the diameter of the vascular cylinder, due chiefly to secondary xylem formation. Maximum stelar diameter is achieved proximal to the level of lateral root origin and is maintained through the entire zone of lateral root attachment. Then a gradual decrease in the number of secondary xylem elements can be traced toward the base of the root until the original stelar diameter and arrangement is reached.

From studies of serial sections of individual roots it is apparent that the formation of cambium, and the formation and differentiation of secondary xylem tissue is always initiated in the region immediately proximal to the first lateral root attachment. Cambial differentiation proceeds basipetally and appears to be causally related to and dependent upon lateral root initiation and growth.

The appearance of cambial divisions following decapitation and the formation of lateral roots does not always occur in exactly the sequence just described, although it was observed in most of the decapitated roots studied. Some differences were observed. In the root illustrated in fig. 7, well defined cambial zones are visible opposite only two of the three phloem regions, although the third area shows some divisions. In some cases libriform fibers were buried between the metaxylem elements and the secondary xylem in the central vascular area (fig. 5), although such fibers were not evident in the region nearer the cut end of the same root (fig. 6). The arrangement of these fibers into three groups, each showing clear relationship to a cambial zone, is evident in fig. 5. The ontogenetic origin of such fibers was not determined.

Cambial differentiation proceeds basipetally following lateral root induction by root decapitation. This fact is clearly shown in the remarkable development of one root in which the primary xylem had become nonfunctional due to plugging of the vessels following decapitation. Near the cut end of the root, the usual triarch arrangement of the xylem was evident (fig. 1 and fig. 9A). Three lateral roots originated within 3 mm. of the cut surface, one opposite each of the three protoxylem points, as illustrated in the drawing of the decapitated root in fig. 9. Centrifugal differentiation of vascular tissues in each of the three lateral roots from pericyclic derivatives in the main root axis was normal. However, instead of establishing the usual continuity with protoxylem elements in the main axis, the xylem of each lateral root was continuous with *secondary* xylem produced by divisions of cambial cells differentiated within the primary root. Cambial differentiation occurred at the level of origin of each lateral root in parenchymatous cells lying on either side of the particular protoxylem point opposite which the lateral root had its origin. Progressive basipetal differentiation of these cambial tissues in the main root axis resulted in the production of a completely new xylem plate proximal to the zone of lateral root formation (fig. 8 and fig. 9C). The original triarch xylem plate is plainly discernible in its former position but is considerably distended by the formation of the secondary xylem. The sequence of development is indicated diagrammatically in fig. 9, which shows drawings of cross-sections cut at three successive levels in the main root axis. Especially notable is the progressive marked increase in diameter, amounting to an increase of two and one-half times the original diameter of the vascular cylinder.

DISCUSSION.—Although isolated roots grown in culture usually do not show cambial formation (White, 1943), Weintraub (1940) suggested that secondary thickening may have occurred in root cultures of the moonflower, *Calonyction*. Dormer and Street (1948, 1949) have shown that secondary growth does occur in isolated tomato roots grown in culture without transfer for 5–6 months. The factors causing cambial differentiation were not completely determined. The advantages of studying cambial formation in cultured tissues grown in a completely known medium under carefully controlled conditions are self-evident.

Under the conditions of these experiments it has been possible to initiate cambial divisions experimentally in young isolated roots grown in sterile nutrient medium. The complicating factor of root elongation as well as the direct influence of the original apical meristem are eliminated by decapitation. In these roots, secondary xylem formation is initiated by 2-week old roots within the differentiated vascular cylinder of the primary root at little more than a centimeter from the root tip.

The specific physiological factors which are responsible for initiating the cambial divisions are not readily determined. In his work on fern shoot apices, Wardlaw (1944) has suggested that the apical meristem plays an important role in the development and differentiation of the vascular tissues of the shoot—that substances moving from the

Fig. 5–8.—Fig. 5. Transverse section of root fixed 4 weeks after 10 mm. decapitation. Section made at 5060 μ proximal to cut surface in region of lateral root origin. Note cambial zones opposite phloem areas and libriform fibers grouped in the center of the vascular cylinder. 210×.—Fig. 6. Transverse section of same root as shown in fig. 5 made at 1550 μ proximal to cut surface, *i.e.*, 3510 μ distal to section in fig. 5. Note greater extent of secondary xylem and lack of libriform fibers. 210×.—Fig. 7. Transverse section of root fixed 4 weeks after 10 mm. decapitation. Section made at 3775 μ proximal to cut surface in region of lateral root origin. Cambial zones are apparent opposite only two phloem areas. 135×.—Fig. 8. Transverse section of root fixed 4 weeks after 10 mm. decapitation. Section made at 2860 μ proximal to cut surface in zone proximal to region of lateral root origin. Primary xylem elements are plugged; secondary xylem elements, differentiated at alternate radii, form a new triarch xylem plate (see fig. 9C). 235×.

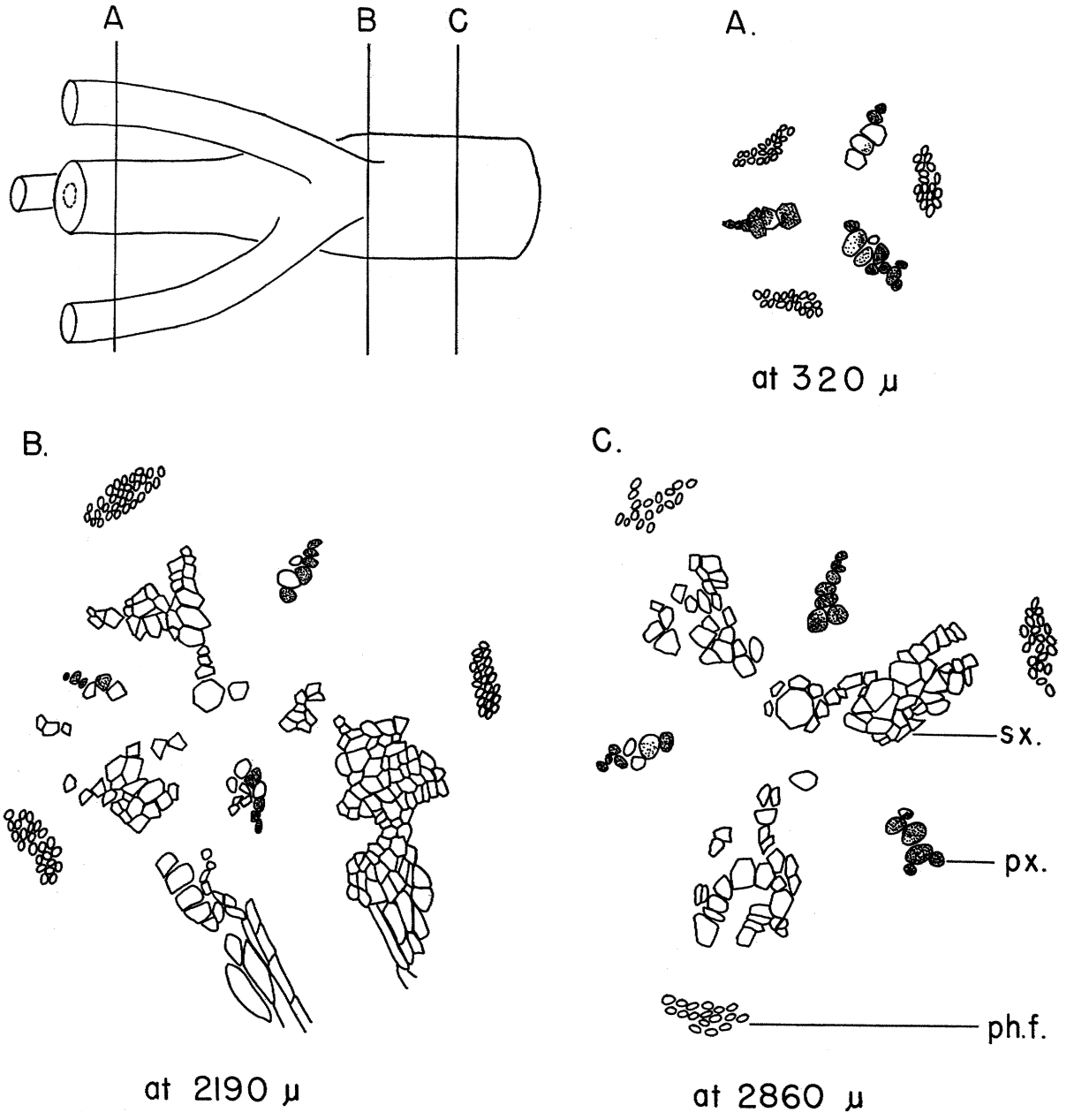

Fig. 9. Diagrammatic representation of decapitated root and its vascular differentiation. Upper left: Three-dimensional sketch of decapitated root fixed 4 weeks after 10 mm. decapitation, showing presence of three lateral roots within 3 mm. of the cut surface. Decapitation surface at the extreme left; proximal end of the root toward the right. Lines A, B, and C indicate the levels at which sections illustrated in drawings A, B, and C were cut. A, B, and C are photographic tracings, made at the same magnification, of the vascular cylinder of the main axis of the decapitated root at different levels proximal to site of decapitation. A. Section cut at 320 μ proximal to decapitation, showing usual arrangement of phloem fibers and plugged primary xylem. B. Section cut at 2190 μ proximal to decapitation, showing same triarch arrangement of primary xylem plus secondary xylem associated with the formation of two lateral roots. Note xylem strands from second lateral root extending to the lower right of the figure. C. Section cut 2860 μ proximal to decapitation, showing original triarch arrangement of plugged primary xylem. Secondary xylem, derived from basipetally differentiated cambial tissue, is arranged at alternate radii. Note increase in stelar diameter compared with A. Section C traced from fig. 8. ph.f.—phloem fibers; px.—primary xylem; sx.—secondary xylem.

meristem probably are causally involved in the developmental process. It is clear from the experimental results presented here that the differentiation of vascular tissues is not dependent solely upon the presence of the original apical meristem. From the abnormal development which occurs in these decapitation experiments, it might be inferred that the apical meristem of the primary root acts as the organizer in the differentiation of the vascular tissues which results in the typical pattern of development. In the absence of the apical meristem of the primary root, tissue differentiation is modified by as yet unidentified factors which affect the mature tissues and change the pattern of development.

In the decapitated roots studied, cambial formation was observed at an apparently definite distance proximal to the origin of the first lateral root. Lateral root primordia, as sites of intense meristematic activity, clearly play an important role in determining the course of vascular differentiation in these roots in the absence of the primary root meristem. The analogy to activation of cambial growth in the stem, shown by Snow (1935) to be related to auxin production by meristematic tissues of the buds and leaves, is evident. Furthermore, considerable evidence exists to show that growth substances have a marked effect on the course of tissue differentiation, including cambial formation, in the roots and shoots of higher plants (Went and Thimann, 1937). It may well be expected that naturally-occurring auxin, produced within the root, plays some role in controlling root tissue differentiation.

SUMMARY

Lateral roots are initiated following 10 mm. decapitation in isolated pea roots grown for 1 week in a sterile synthetic nutrient medium. At the apical portion of the root base continued in culture after decapitation, the primary root shows a considerable increase in diameter through the entire region of lateral root origin. A study of serial sections made of such roots, fixed and killed at weekly intervals following decapitation, indicates that such root enlargement is due chiefly to differentiation of secondary xylem resulting from cambial formation. Cambial divisions occur in the region of interstitial parenchyma between the phloem and the metaxylem regions of the triarch vascular cylinder. Discontinuous cambial layers are evident within 1 week following decapitation in the primary root proximal to the point of origin of the first lateral root. In 1 month the vascular cylinder may show a twofold increase in diameter due to the production of vascular tissue by cambial cells. The differentiation of cambial layers proceeds basipetally from the point of its first appearance proximal to the first lateral root. Abnormalities of vascular tissue development in decapitated cultured root bases are described.

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EVOLUTION OF ACETALDEHYDE BY EXCISED WHEAT ROOTS IN SOLUTIONS OF NITRATE AND NITRITE SALTS [1]

James F. Nance and Leon W. Cunningham

IN STUDIES on nitrate assimilation by excised wheat roots (Nance, 1948, 1950) it was observed that under conditions which favor active nitrate assimilation the roots produce an aroma similar to that of acetaldehyde. In a preliminary experiment a stream of nitrogen was used to carry the volatile products from wheat roots in a solution of KNO_3 to a receiver containing bisulfite. The bisulfite solution, when analyzed by the method of Stotz (1943), was found to contain a compound which reacted like acetaldehyde. This effect is of some interest in view of the studies of Wirth and Nord (1942), who found an accumulation of pyruvate in *Fusarium* cultures supplied with nitrate. These investigators suggest that the accumulation of pyruvate is due to inhibition of carboxylase by nitrite formed in the reduction of nitrate. They suggest, further, that nitrate and nitrite can oxidize reduced coenzyme I. It appeared to us that acetaldehyde evolution by roots assimilating nitrate might be interpreted as evidence for the oxidation of coenzyme I by nitrate, though obviously not evidence of the inhibition of carboxylase by nitrite. The experiments of the present study were designed to determine the relation, if any, between the acetaldehyde production and nitrate assimilation. It was thought that the significance of the acetaldehyde evolution might be inferred from studies of the phenomenon under conditions known to affect nitrate assimilation, and that the effects of manganese, molybdenum, iron, $CaSO_4$, and oxygen would be of particular interest.

Stimulation of nitrate assimilation in wheat roots by manganese and, to a lesser extent, by iron has been observed by Burstrom (1939 a, b). He found that salts of other heavy metals, including Cu, Zn, Al, Mo, Ni, and Co not only fail to stimulate the assimilation but are actually inhibitory. Nance (1948), using the variety of wheat employed in the studies of the present paper, found no enhancement of nitrate assimilation by manganese additions, though in later experiments (unpublished) it was observed that roots grown in dilute $CaSO_4$ solutions did respond to manganese. Mulder (1948) demonstrated an accumulation of nitrate in leaves of molybdenum-deficient tomato and barley plants. When these plants were supplied with molybdenum, the nitrate content of the leaves diminished rapidly.

Inhibitory effects by the salts of various alkali and alkaline earth metals on the assimilation of nitrate were reported by Burstrom (1939 b). Of these $CaSO_4$ is particularly effective, an observation confirmed by Nance (1948, 1950). Inhibition of nitrate utilization in soybeans grown in culture solutions having oxygen concentrations of 8 and 16 p. p. m. is clearly indicated in work by Shive (1941). Oxygen inhibition of the assimilation of previously accumulated nitrate has been reported by Nance (1948).

Acetaldehyde has long been known as a product of higher plant metabolism (Kostytschev *et al.*, 1913). Thomas (1925) investigated the effects of CO_2 and O_2 tensions on acetaldehyde production by apples. He observed acetaldehyde formation not only under anaerobic conditions but in the presence of oxygen at high CO_2 tensions. Boresch (1926) injected acetaldehyde into dormant buds of various trees and caused them to break dormancy. Later he (1928) found that warm baths which are effective in breaking dormancy in the same trees cause increased production of acetaldehyde by the buds. Acetaldehyde was collected from respiring leaves and flowers by Klein and Pirschle (1926), who concluded that this compound is an intermediate of respiration in these tissues. Early in the present work, we found that four plant growth substances, indole-3-acetic acid, indolebutyric acid, napthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid stimulate the formation of acetaldehyde by wheat roots under both aerobic and anaerobic conditions (Nance and Cunningham, 1950). Our experiments seem to indicate that this effect of the growth substances operates through some mechanism other than that by which nitrate and nitrate induce acetaldehyde evolution.

METHODS AND MATERIALS.—All experiments reported in this paper were performed with roots cut from 4-day old wheat seedlings. "White Federation 38", a spring wheat variety grown in California, was used. Grains were soaked overnight in aerated, distilled water, and then an appropriate number from which the coleoptile had emerged was placed on fiberglas gauze stretched tightly over a 2 l. pyrex crystallizing dish. Rubber bands were used to hold the gauze in place. The vessel, equipped with four

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