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## AUXIN-CYTOKININ CONTROL OF SECONDARY VASCULAR TISSUE FORMATION IN ISOLATED ROOTS OF RAPHANUS<sup>1</sup>

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### A B S T R A C T

A comparative study was made of the effectiveness of various hormone and metabolite mixtures in inducing vascular cambium initiation and secondary vascular tissue formation in isolated first-transfer roots of the radish, *Raphanus sativus* L. 'White Icicle,' when provided to the cut basal end of the root grown in sterile culture. An auxin, such as indoleacetic acid (IAA) at  $10^{-5}$  M, a cytokinin, such as 6-benzylamino purine at  $5 \times 10^{-6}$  M, a cyclitol, such as *myo*-inositol at  $5 \times 10^{-4}$  M and sucrose at 8% were all required for maximum response. Requirements for auxin and cytokinin were absolute; in their absence no cambium was formed. The addition of cyclitol, while not an absolute requirement for cambium initiation, increased the magnitude of the response markedly. Alternative auxins such as  $\alpha$ -naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid were equally as effective as IAA. Alternative effective cytokinins included 6-furfurylamino-purine, 6-phenylaminopurine and 6-( $\gamma,\gamma$ -dimethylallylamino)purine. Alternative cyclitols equivalent to *myo*-inositol were scyllitol and pinitol. Other related cyclitols tested were much less effective or totally inactive.

ISOLATED EXCISED ROOTS grown in sterile nutrient culture usually do not form a vascular cambium or secondary vascular tissues. Only under unusual culture conditions (Dorner and Street, 1948; Seeliger, 1956; Torrey, 1951) are secondary vascular tissues observed. Using a technique devised by Raggio and Raggio (1956) for culturing excised roots by providing organic nutrients via medium introduced into the cut basal end of the root, Torrey (1963) was able to induce vascular cambium and limited secondary vascular tissue formation in excised roots of *Pisum sativum* L.

Loomis and Torrey (1964) demonstrated with the same techniques that subcultured isolated

roots of the radish, *Raphanus sativus* L., could be induced to form cambium and secondary vascular tissues by introducing appropriate metabolites and hormone mixtures through the cut basal end. They showed that, in addition to sugar in the form of sucrose, the excised radish roots must be provided an auxin such as indoleacetic acid and a cytokinin, such as 6-benzylaminopurine, both at appropriate low concentrations, in order to induce vascular cambium initiation. The addition of *myo*-inositol increased the response markedly.

A further study has been made to determine the optimum conditions in vitro for cambial initiation and for extended vascular cambium activity in the excised radish root. A broad range of concentrations of the essential components, alone and in combination, was tested on first-transfer radish roots in culture, and the anatomical responses were determined in fixed and sectioned material. In the studies presented below a comparison is made of anatomical responses observed

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at the end of a period of approximately 4 weeks of treatment of roots subjected to a wide range of experimental conditions. Conclusions concerning the optimum concentrations of the chemical constituents required for cambial initiation and maintained activity are presented. Detailed considerations of the ontogenetic sequence in cambial initiation and secondary vascular tissue formation are presented elsewhere (Torrey and Loomis, in press).

**MATERIALS AND METHODS**—Roots of radish, *Raphanus sativus* L. 'White Icicle,' were used in the anatomical studies described here. Methods of culturing and treating the excised roots have already been described in detail (Loomis and Torrey, 1964) and may be summarized briefly. Ten-mm root tips from 3-day-old seedlings germinated aseptically in sterile distilled water in the dark at 23 C were excised and cultured in a modified Bonner nutrient agar medium. After 4 days, when the isolated root tips were about 50 mm in length, 15-mm first-transfer tips were excised with a sharp scalpel and transferred to fresh nutrient medium in 11-cm petri dishes. The basal 5-mm portion of each root was inserted into a separate agar medium contained in a 12 × 35 mm glass vial. Usually two roots were cultured in each 11-cm dish. The roots were grown in the dark at 23 C for additional periods up to about 5 weeks. After various periods of culture, whole roots were removed, fixed in formalin-acetic acid-alcohol, dehydrated through an ethyl-butyl alcohol series, embedded in Tissuemat, and sectioned at 10  $\mu$ . Sections were stained for anatomical study using Heidenhain's iron haematoxylin and safranin.

From the studies reported earlier (Loomis and Torrey, 1964), it was known that anatomical differences, especially with respect to the initiation of the vascular cambium and the development of secondary vascular tissues, were dependent upon the presence of hormones and hormone-like materials in the vial medium. Auxins, cytokinins, and cyclitols, as well as the concentration of sugar provided, appeared especially important. A large number of different combinations of nutrients both in the vial and in the plate medium had been tested for physiological response. Macroscopic observation of roots in culture usually showed whether secondary thickening of the roots had occurred, but it was necessary to make careful anatomical investigations in order to assess the extent and nature of the response to the various treatments. In the present study anatomical analyses were made of roots taken from approximately 64 different treatments. In the results described below, emphasis has been placed on the following aspects: the effects of auxin concentration including omission, auxin type; cytokinin concentration including omission, cytokinin type; cyclitol

omission and type of cyclitol. A comparative study was made of structures formed in response to optimum concentration of these factors and to their absence from the vial medium.

In making such an anatomical study large numbers of roots must be sampled and some variation in response is inevitable. Some arbitrary selection must be made of roots to be studied in this way. Usually each treatment involved 8–16 roots, but fewer than half of the roots were fixed and even fewer were selected for sectioning. Among those sectioned were the roots showing the maximum response as measured by root diameter. Despite this selection, the number of different treatments was sufficiently great so that a wide range of responses could be studied. The anatomical responses to similar treatments were remarkably consistent. Photographs of sections are selected as typical of the particular treatment and as showing good physiological response. In most cases any of a number of roots sectioned could have been used as illustrations.

In the presence of optimum concentrations of substances stimulatory to cambial activity, the anatomical response was dramatic and a large root diameter resulted; in the absence of any one critical component, the response was markedly reduced or absent. Photographic comparison among roots from various treatments was made difficult, since useful magnifications varied markedly. In the accompanying photographs some compromise has been made in choice of magnification so that, where possible, cell detail can be seen. Large-diameter roots are reproduced to show overall response rather than cell detail. Some attention must be paid by the reader to the magnification of the photographs in comparing responses.

In most instances, the thickening was macroscopically complete within 14–21 days, but to be sure that all effects were observed the experiments were sometimes continued for longer periods. Some of the cambial derivatives continued to differentiate after cambial activity ceased, but the anatomical patterns in young (14–21 days) and old (28–35 days) roots were generally quite similar. The principal difference was that a cambial region of small, rapidly dividing cells was not as clearly evident in the old roots. The reasons for cessation of cambial activity constitute an important problem (Loomis and Torrey, 1964) which is being investigated further.

**RESULTS**—As has been described briefly by Loomis and Torrey (1964), isolated 'White Icicle' radish roots in culture show maximum secondary vascular-tissue formation under the following cultural conditions.

Plate medium: Modified Bonner medium containing 2% sucrose and vitamins (Bonner and Devirian, 1939).

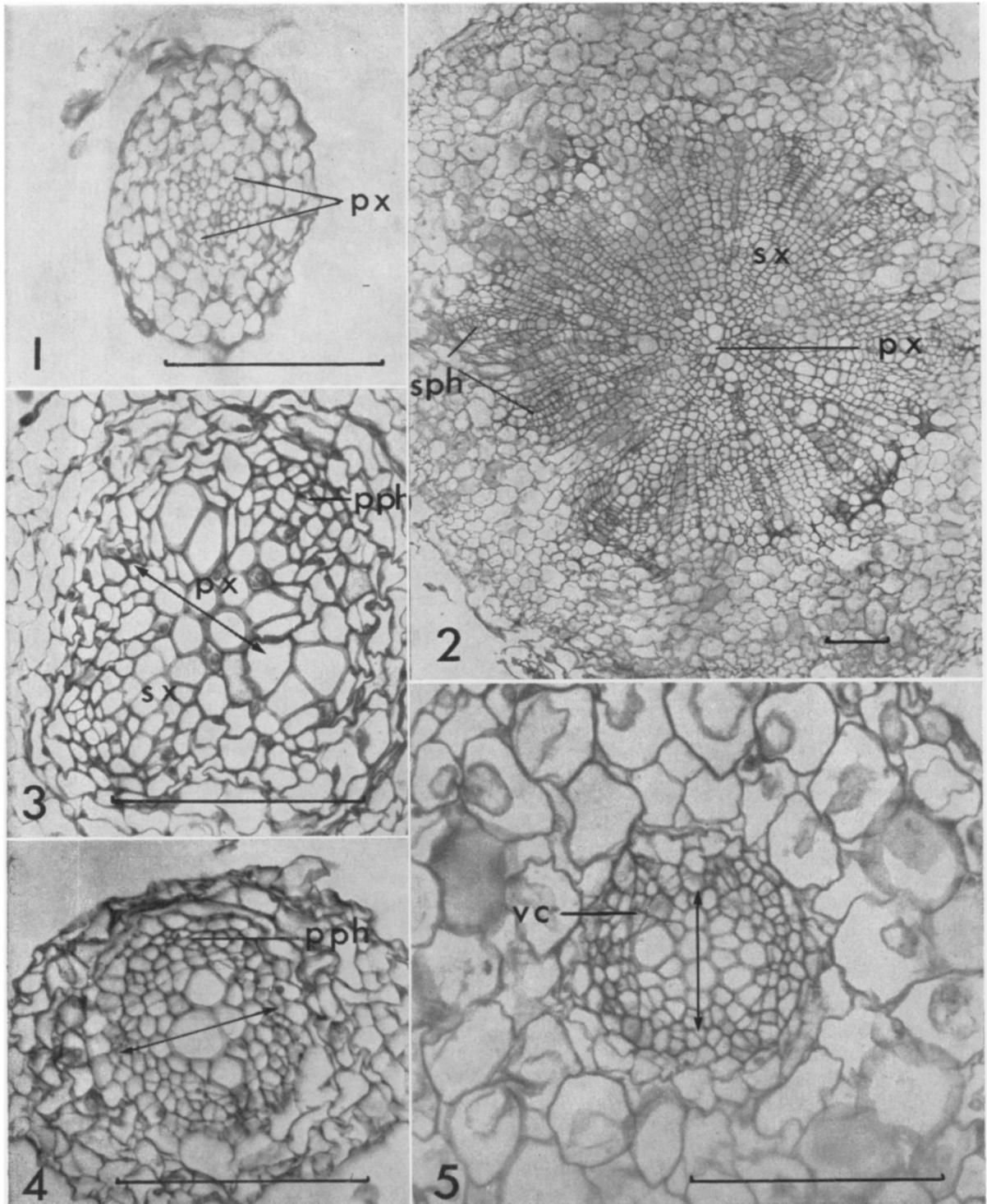


Fig. 1-5. Transsections through mature tissues of vial-fed first-transfer radish roots grown in vitro. Sections were made in the region of maximum root diameter. v.c., vascular cambium; s. ph., secondary phloem; s.x., secondary xylem; p.ph., primary phloem; p.x., primary xylem. The double arrow represents the diarch primary xylem axis. The scale represents 100  $\mu$ .—Fig. 1. Root provided vial medium lacking growth factors, fixed after 31 days in culture. Only primary tissues are evident, including a simple diarch vascular pattern,  $\times 350$ .—Fig. 2. Root provided complete vial medium, fixed after 34 days in culture. Extensive secondary xylem and limited secondary phloem have been formed. The diarch

Complete vial medium: Modified Bonner medium supplemented with

1. Auxin: Indoleacetic acid  $10^{-5}$  M (1.75 ppm).
2. Cytokinin: 6-benzylaminopurine  $5 \times 10^{-6}$  M (1 ppm).
3. Cyclitol: *Myo*-inositol  $5 \times 10^{-4}$  M (100 ppm).
4. Sugar: Sucrose 8%.

A first-transfer root tip provided the Bonner medium without supplement elongated rapidly for a few weeks but produced only primary tissues. No vascular cambium was initiated and no secondary vascular tissues were formed (Fig. 1). The typical diarch arrangement of the primary vascular tissues is evident.

Roots provided the same medium supplemented as shown above with optimum concentrations of auxin, cytokinin, cyclitol and sugar in the vials showed a dramatic increase in root diameter, reflecting extensive vascular cambium activity during the first 2-3 weeks of culture (Fig. 2). As reported earlier this response represents about a 20-fold increase in the diameter of the central cylinder region of the root, resulting largely from formation of many concentric layers of secondary xylem. Note that Fig. 2 is reproduced at about one-fourth the magnification of Fig. 1 and shows only a portion of the whole root diameter. In Fig. 2 a vascular cambium layer is difficult to find because this is an old root which has ceased cambial activity. The radially aligned secondary tissues consist principally of xylem in which enlarged vessels alternate with rays of xylem parenchyma. Large rays are particularly apparent opposite the protoxylem points of the diarch primary xylem plate. Secondary phloem tends also to occur in discrete groups of cells outside the radial bands of vessels. In other roots with similar treatment a more normal cylindrical vascular cylinder was observed (see below). The original cortex of this root had been sloughed off and a secondarily formed cortexlike layer forms the outer tissues of the root, derived perhaps from pericyclic activity and divisions in the secondary phloem. No clear periderm layer is present.

*The effect of variations in auxin treatment*—Omission of auxin from the vial medium essentially eliminates vascular cambium initiation. The cross section in Fig. 3 is from a root grown for 29 days with the complete vial medium, lacking only auxin. The simple diarch xylem pattern seen in Fig. 1 has been distorted by the differentiation of a few secondary xylem elements, especially in two radial sectors normal to the

primary plate. Again, the vascular cambium is not clearly discernible and the response is restricted to a very limited number of cell divisions. The cortex is slightly crushed and has collapsed in fixing.

The root shown in Fig. 4 received 34 days of treatment with the complete medium, but contained IAA at a very low concentration ( $10^{-7}$  M). As in the root seen in Fig. 3 a limited number of cell divisions have occurred, giving rise to a few secondary xylem vessels.

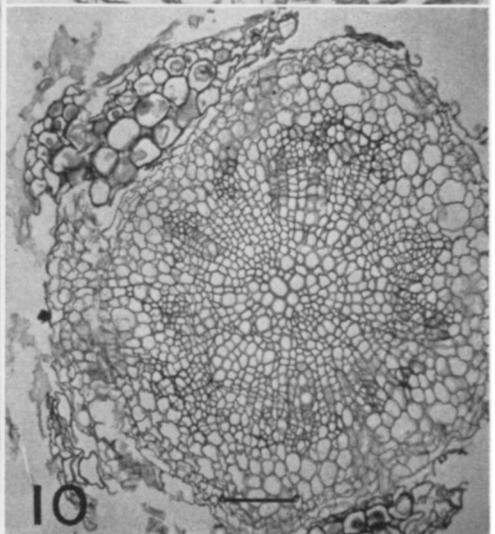
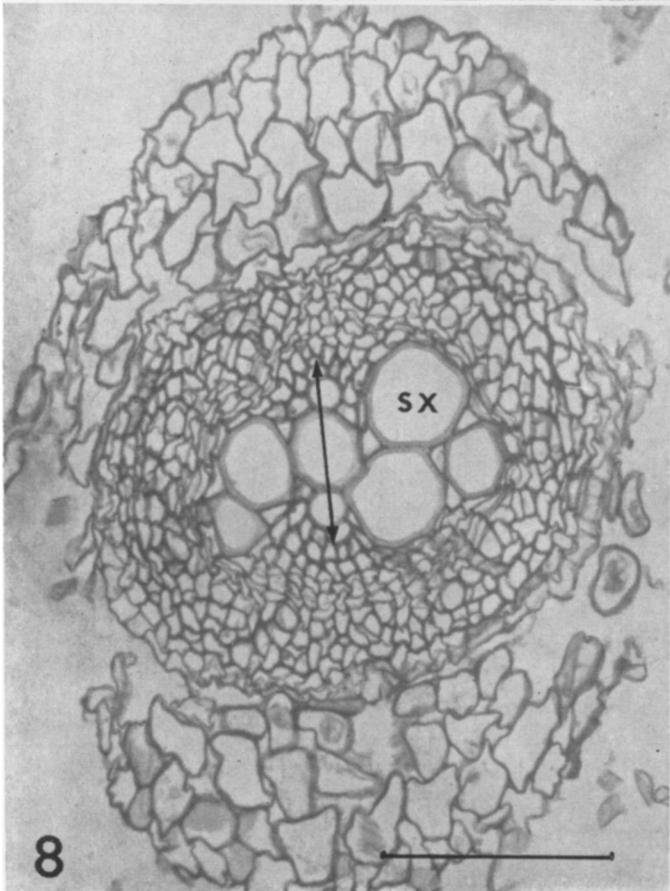
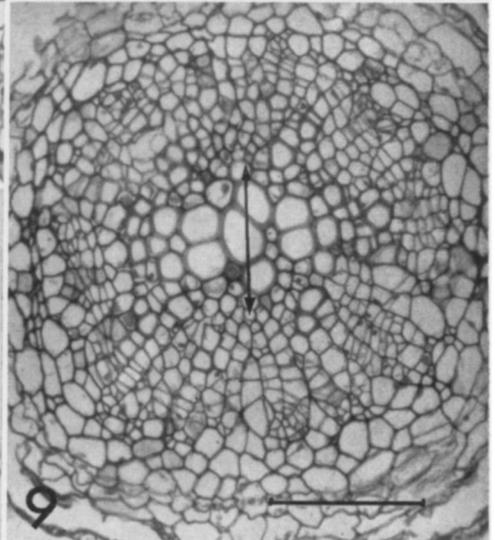
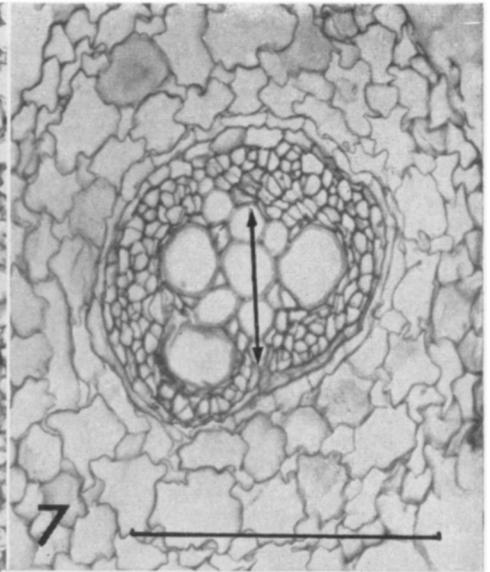
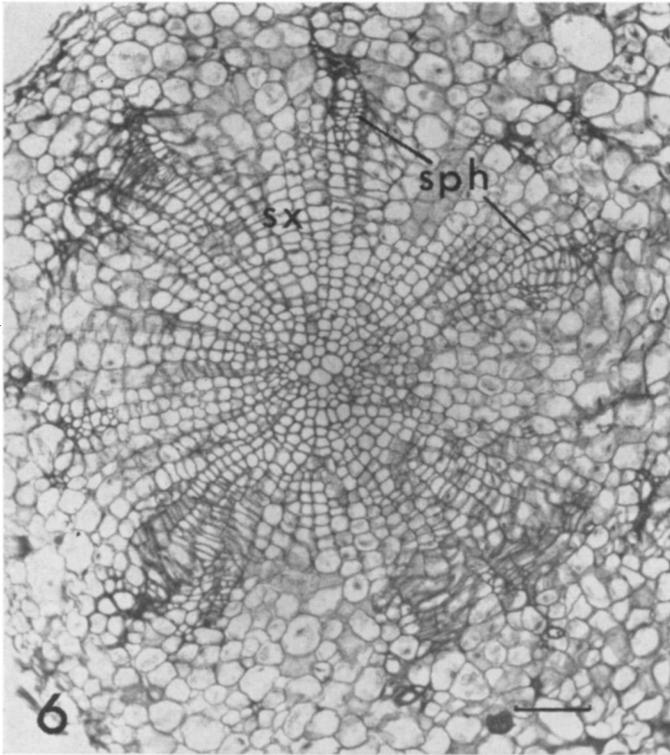
In Fig. 5 is the interesting response shown when the root is provided with the complete vial medium lacking auxin, but with IAA provided at a low concentration ( $5 \times 10^{-8}$  M) in the plate medium surrounding the root. The central cylinder is essentially similar to that seen in Fig. 4—that is, a very limited vascular cambium initiation is apparent. The cortex is more or less intact, but the cortical cells show marked enlargement, apparently in response to the direct exposure of the cells to low auxin in the medium.

Indoleacetic acid was the auxin used in most of these experiments; it is probably the natural auxin in this plant. However, other auxins have been tested in place of IAA and shown to produce a response. The auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and  $\alpha$ -naphthaleneacetic acid (NAA) are effective, in the presence of the other vial factors, in inducing cambial activity. In Fig. 6 is illustrated a root grown for 33 days provided NAA at  $10^{-6}$  M in the vial medium. In this particular treatment the cytokinin was 6-phenylaminopurine at  $10^{-6}$  M. The root which developed showed a radially symmetrical structure with many concentric layers of xylem tissue derived from a cylindrical vascular cambium. Remarkable uniformity in cell size and in arrangement of radial rows resulted from this treatment. Discontinuous areas of secondary phloem are also evident. NAA is equally as effective as IAA in eliciting cambial activity.

*The effect of variations in cytokinin treatment*—Both cytokinin and auxin at near optimum concentrations are needed to induce appreciable vascular cambium activity; omission of a cytokinin from the vial medium in an otherwise complete treatment prevents root thickening. The response to lack of cytokinin is quite striking as is shown by the root in Fig. 7 after 29 days of vial treatment. The central cylinder is small, but several quite enlarged vessels, probably of secondary origin, are evident immediately adjacent to elements of the primary xylem. Note

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primary xylem poles are indicated,  $\times 100$ .—Fig. 3. Root provided complete vial medium lacking auxin, fixed after 29 days in culture. Limited secondary xylem was formed,  $\times 400$ .—Fig. 4. Root provided complete vial medium with IAA at  $10^{-7}$  M, fixed after 34 days in culture,  $\times 400$ .—Fig. 5. Root provided complete vial medium lacking IAA but provided  $5 \times 10^{-8}$  M IAA in the plate medium surrounding the root. Root fixed after 37 days in culture. Note limited secondary xylem and marked enlargement of cortical cells,  $\times 400$ .



in Fig. 7 the alignment of the cells of the vascular cambium between primary xylem and phloem, extending to the pericycle at the protoxylem points of the diarch xylem plate. The enlargement of the vessel elements is quite characteristic of roots provided optimum auxin ( $10^{-5}$  M) but no cytokinin. Note also that in this root the cortex is still intact, although distorted in fixing.

The root shown in Fig. 8 was provided the complete medium in the vial but with adenine sulfate added at 10 ppm ( $2.5 \times 10^{-5}$  M) in place of cytokinin. The root was fixed at 31 days. As was seen in the root in Fig. 7, this root also shows only a few enlarged vessel elements of cambial origin typical of roots treated with high auxin in the absence of cytokinin. The formation of a limited number of radial rows of cells caused sufficient diameter increase to produce splitting of the root cortex. Cytokinins are, for the most part, 6-substituted adenine derivatives, but it is quite clear that adenine itself does not serve as a cytokinin in this system. Similarly, other purine and pyrimidine bases in various forms have shown no effects on the system. Also 1,3-diphenylurea was found to show no activity. The limited cell division activity which occurs in roots provided a cytokinin-free medium may be due to the presence of residual endogenous cytokinin which is rapidly exhausted in the excised root.

The optimum concentration for cytokinin has not been rigorously established, but  $5 \times 10^{-6}$  M 6-benzylaminopurine was clearly more effective than  $5 \times 10^{-7}$  M or  $5 \times 10^{-5}$  M. The cross section in Fig. 9 is from a root fixed after 34 days of culture with a complete vial medium containing 0.1 ppm (ca.  $5 \times 10^{-7}$  M) 6-benzylaminopurine. A considerable amount of secondary vascular tissue was formed, but the response was quantitatively less than at the higher cytokinin concentration. Note the discrete clumps of secondary phloem and the sloughed cortical tissue.

Other 6-substituted adenine derivatives in addition to 6-benzylaminopurine proved to be effective in eliciting the response. Thus, 6-furfurylamino purine (kinetin), 6-phenylaminopurine, 6-( $\gamma,\gamma$ -dimethylallylamino)purine (kindly provided by Prof. F. Skoog, University of Wisconsin) and another adenine derivative, 6-benzylamino-9-(2-tetrahydropyran-9H) purine (SD-8339; kindly provided by Dr. J. van Overbeek of Shell Development Co., Modesto, Calif.) all elicited similar anatomical responses in the concentration

range of  $10^{-6}$  to  $10^{-5}$  M. Kinetin was less effective than 6-benzylaminopurine so that about  $5 \times 10^{-5}$  M was required for maximum response; 6-phenylaminopurine and 6-( $\gamma,\gamma$ -dimethylallylamino)purine seemed active equally at  $5 \times 10^{-6}$  M and  $5 \times 10^{-5}$  M. In Fig. 10 is illustrated a transection of a root, fixed after 30 days, which had been provided a complete vial medium containing 1 ppm (ca.  $5 \times 10^{-6}$  M) 6-phenylaminopurine. This section, like those in Fig. 2 and 6, shows clearly the extensive secondary tissue formed by a cylindrical vascular cambium, the radially arranged sectors of secondary phloem, the outer periderm-like layer, and the sloughed-off tissues of the original cortex.

In Fig. 11 is illustrated a transection of a root provided a complete vial medium containing 1 ppm ( $5 \times 10^{-6}$  M) kinetin for 31 days. Extensive secondary vascular tissues were formed in response to this treatment. Note the scattered and somewhat enlarged vessel elements which are relatively few in number and the rather large rays opposite the protoxylem points, characteristic of many secondarily thickened diarch roots.

*The effect of variations in cyclitols*—In the absence of *myo*-inositol or other added effective cyclitol, isolated radish roots provided sugar, auxin, and cytokinin at optimum concentrations in the vial medium will initiate a vascular cambium and form secondary vascular tissues, but the response is usually much reduced as compared to the complete medium. In Fig. 12 is illustrated a transection of a root sectioned 34 days after treatment with a complete vial medium lacking cyclitol. Cambial activation occurred and secondary vascular tissues were formed, but typically perhaps only half the number of radially aligned cambial derivatives developed compared to the response of roots in the complete medium.

A sugar alcohol, sorbitol, at 100 ppm (ca.  $5 \times 10^{-4}$  M) produced a response markedly reduced as compared to the same concentration of *myo*-inositol, although a well-organized vascular cambium developed (Fig. 13); galactinol was more effective. Various cyclitols related to *myo*-inositol were also tested in this system. Roots provided scyllitol at 100 ppm (ca.  $5 \times 10^{-4}$  M) showed a response equal to that produced by *myo*-inositol. Pinitol was as effective as scyllitol, whereas onanitol and quebrachitol were ineffective (these materials were kindly supplied by E. McComb

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Fig. 6–10. Transections of radish roots grown *in vitro* as described in legend Fig. 1–5.—Fig. 6. Root provided complete vial medium but with  $10^{-6}$  M NAA and 6-phenylaminopurine at  $10^{-6}$  M, fixed after 33 days in culture,  $\times 100$ .—Fig. 7. Root provided complete vial medium lacking cytokinin, fixed after 29 days in culture. Three or four enlarged vessels of secondary origin are evident,  $\times 400$ .—Fig. 8. Root provided complete vial medium lacking a cytokinin and with  $2.5 \times 10^{-5}$  M adenine sulfate, fixed after 31 days in culture,  $\times 300$ .—Fig. 9. Root provided complete medium with 6-benzylaminopurine at  $5 \times 10^{-7}$  M, fixed after 34 days in culture,  $\times 200$ .—Fig. 10. Root provided complete medium with 6-phenylaminopurine at  $5 \times 10^{-6}$  M, fixed after 30 days in culture,  $\times 100$ .

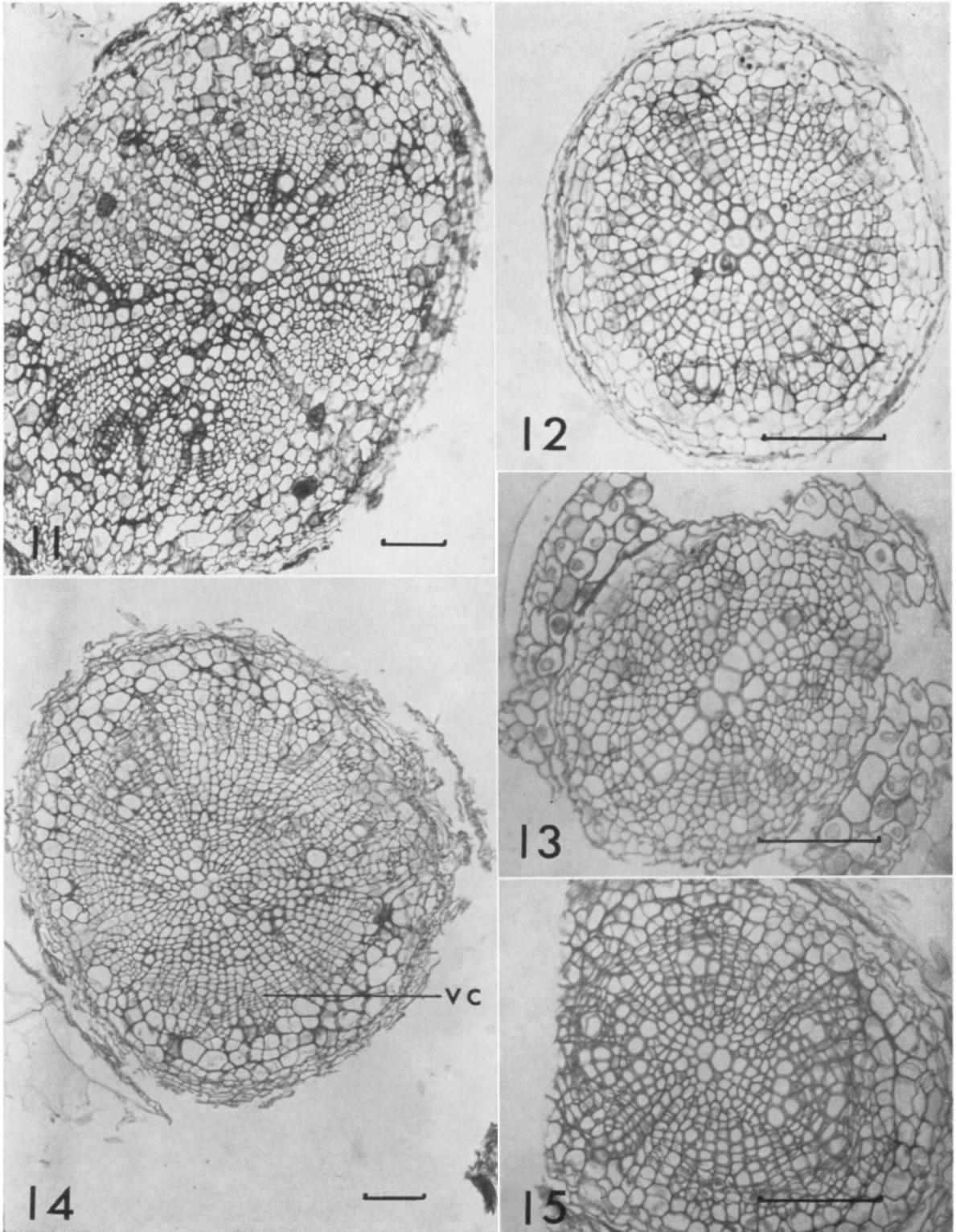


Fig. 11-15. Transsections of radish roots grown in vitro as described in legend Fig. 1-5.—Fig. 11. Root provided complete medium but with 6-furfurylaminopurine at  $5 \times 10^{-6}$  M, fixed after 31 days in culture,  $\times 100$ .—Fig. 12. Root provided complete medium lacking *myo*-inositol, fixed after 34 days in culture. Compare with Fig. 2,  $\times 200$ .—Fig. 13. Root provided with complete medium but with  $5 \times 10^{-4}$  M sorbitol in place of *myo*-inositol. Root was fixed after 30 days

and V. V. Rendig, University of California, Davis). With scyllitol (Fig. 14), a complete cylindrical vascular cambium was active in forming many radial derivatives, especially of secondary xylem. The interesting differential enlargement of isolated vessel elements seen here is unexplained although this is similar to what may occur in intact plants.

*The effect of sugar concentration*—The role of the sugar provided via the vial medium was not explored in detail. Tests of sugar concentrations were made in early experiments without added *myo*-inositol. It was found that best root elongation was obtained with 2% sucrose in the plate medium; the concentration of sucrose in the vial, which was varied from 2% to 12%, seemed to exert little influence on the magnitude of secondary vascular tissue formed. Without sugar in the plate medium, 8 and 12% sucrose in the vial permitted better root elongation than was obtained with higher or lower concentrations. In most experiments described above, 2% sucrose in the plate medium and 8% sucrose in the vial medium were used. In one experiment sucrose was provided at 1 M (34%) as part of the complete vial medium. Roots about equivalent in diameter to minus-*myo*-inositol roots were formed. Much of the secondary xylem tissue was made up of vessels with heavily thickened secondary walls (Fig. 15). Thus, at this high sucrose concentration root diameter was reduced, but the basic hormonal response of cell division was relatively less affected. The high sugar concentration might be expected to have an influence in the differentiation of xylem elements since sugar concentration (although at much lower concentrations) was reported to affect the differentiation of vascular elements in callus tissue (Wetmore and Rier, 1963). No tests of the effectiveness of different sugars as substrates were made. Further study of the role of sugars in the response seems warranted.

**DISCUSSION**—The effective interaction of auxins and cytokinins in eliciting specific morphogenetic responses is well known from the studies of *in vitro* systems initiated by Skoog and Miller (1957) and extended by many others. That the auxin-cytokinin interaction influenced specifically cell division was clearly shown by Das, Patau, and Skoog (1956), and Patau, Das, and Skoog (1957), who offered a model for understanding at the cellular level the nature of the interdependence of these hormones for the initiation of cell division in tissue systems. In ways far from understood, the cell division response in tobacco callus tissue was channeled into root initiation

and bud formation or unorganized callus proliferation, depending upon relative auxin-cytokinin concentrations. Other metabolites such as mixtures of amino acids could influence the magnitude of the tissue response.

The same general type of auxin-cytokinin interaction seems to be involved in the initiation of cell divisions leading to vascular cambium in the isolated roots of radish grown *in vitro*. In this case the cell divisions are initiated within an organized tissue system at a place and in a sequence not dissimilar from that occurring in the intact plant. Since the isolated root does not form a cambium in the absence of treatment but does respond by initiating a fairly typical vascular cambium when provided an appropriately balanced hormone mixture, one is led to conclude that in the intact plant, the same sort of hormones in proper balance are provided the root from the shoot system and act in vascular cambium formation. Proof of this idea depends upon establishing that the hormones are indeed formed in the shoot and that they move into the root in such a manner as to lead to normal secondary thickening. The rate and concentrations at which the hormones are supplied to the root are probably influenced by variations in environment, particularly day length.

Kende (1964) and Weiss and Vaadia (1965) have suggested that cytokinins are normally produced in the root. If their system, in which cytokinin-like activity was obtained from root exudates, and that of the radish are similar, their results might be explained on the basis of previous accumulation within the root of cytokinins translocated from the shoot. Or it may be that the radish roots in these experiments lacked some essential condition (precursor?) necessary for cytokinin synthesis. While this point is unresolved, we know that auxin and cytokinin must be supplied together; separate, sequential applications are ineffective. This result leads one to believe that the roots do not accumulate cytokinins.

In attempting to explain the function of auxin and cytokinin in eliciting this developmental response, it is probably most useful to attribute to the effective hormone balance a stimulatory role in initiating cell divisions. Thus, auxin and cytokinin provided together at optimum concentrations via the root base reach procambial cells of the central cylinder of the root which are sensitive to stimulus and which are activated into division. The subsequent events of cell differentiation, *i.e.*, of secondary xylem and phloem formation, are consequences explicable more in terms of existing root structures and their

influences than in terms of the hormones provided. There is some suggestion in the above anatomical study that cell size, e.g., vessel diameter, may be influenced by auxin concentration when the cytokinin level is sub-optimal (cf. Fig. 2 with Fig. 7, 8), but there is no general relationship between hormone treatment and the amount of xylem versus the amount of phloem tissue formed. Rather, the variations in response point to a fundamental role of the hormone treatment in the stimulation of cell divisions and the determination of root size by an influence on total cell number. In this system, then, the balanced auxin-cytokinin stimulus can be equated to the well-documented response of cell division studied in tobacco callus tissue.

At our present state of knowledge, it is fruitless to speculate about the role of *myo*-inositol in this system. The compound is provided in near-substrate amounts. *Myo*-inositol is presumably incorporated into the glycerophospholipids of cells forming part of the structural components of cell membranes. It is known from the work of Loewus, Kelly, and Neufeld (1962) and others that *myo*-inositol can serve as a precursor for the synthesis of pectic compounds and it is possible that either of these roles is involved in the radish root response. The presence of *myo*-inositol seems to enhance the auxin-cytokinin cell-division response. This enhancement could be compared to a similar effect of *myo*-inositol on tobacco callus (Linsmaier and Skoog, 1965). It is interesting to note also the similarity of *myo*-inositol response in this system to that observed by Raggio, Raggio, and Burris (1959) on nodulation in excised bean roots.

Skoog and Miller (1957) reported that *l*-tyrosine markedly stimulated the growth of tobacco callus in the presence of auxin and cytokinin. While the mechanism of *l*-tyrosine action is unknown, it may be related to the causes of cessation in cambial activity in the radish root system. Tissues induced to cell division by treatment with auxin and cytokinin must have extensive demands for various substrates essential in the synthesis of cell constituents. If the system lacks the capability to synthesize these substrates in adequate amounts, growth would soon be limited in its rate or extent. Oaks (1965) has recently shown an example of this, in that excised corn roots lack the ability to synthesize sufficient amounts of certain amino acids required in growth. As a simple hypothesis, the cessation of cambial activity in the radish system may result from such deficiencies.

A number of organic supplements have been tested as vial additives with varying results. Purine and pyrimidine bases were without effect. Various amino acids were added individually and in combination with some stimulation noted by glutamine and glycine added separately at

$10^{-4}$  M; these trials were not extensive and further tests are warranted.

It may be that the deviations, noted in cultured roots, from normal patterns of differentiated vascular elements in intact radish plants will be traced to such deficiencies. The most apparent deviations relate to the great abundance and small size of xylem elements. Since the lignified thickenings of the walls of such cells appear normal, the deviation seems to occur in the very early stages of cell differentiation.

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