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Xylem Formation: A Paradigm of Cytodifferentiation in Higher Plants

Plant cells divide and differentiate under the control of changing hormone levels. Xylem offers a model tissue for the study of these cellular events

One of the exciting and elusive quests in biology today is the search for understanding of cytodifferentiation and its control within the organism. Biologists want to comprehend the events which direct similar cells into quite different pathways leading to strikingly different cell types, both biochemically and morphologically. It is clear that the answers lie in the nature of the controls of genetic expression—that somehow cells with the same genetic constitution diverge by virtue of differential activity and expression of their genetic potential. The subtle first events of cytodifferentiation are followed by subsequent slight cellular differences which become progressively more dramatic until morphological or biochemical dissimilarities allow a distinction to be made between cell types.

Before dispersing to opposite ends of the United States, the authors collaborated during a three-year period in Cambridge on aspects of xylem differentiation, each bringing a different perspective to the problem of cytodifferentiation. John G. Torrey is Professor of Botany at Harvard University, where he took his Ph.D. degree in 1950. His research has focused on cell division in relation to differentiation, especially in roots. He is the author of Development in Flowering Plants *and co-author of the plant physiology text* Plants in Action. *Since 1967 he has been Director of the Maria Moors Cabot Foundation for Botanical Research of Harvard University. Donald E. Fosket, Assistant Professor at the University of California at Irvine, received his Ph.D. at the University of Idaho, where his first interest in xylogenesis was aroused. Peter K. Hepler received his Ph.D. in 1964 at the University of Wisconsin. He is now Assistant Professor of Biology at Stanford University, where he continues his interests in plant ecll ultrastructure and in cell walls.* *The authors express their collective thanks to the several agencies which have provided research support: the National Institutes of Health of the U. S. Public Health Service, the National Science Foundation, the Maria Moors Cabot Foundation for Botanical Research, and the Bullard Committee of Harvard University. They also express appreciation to colleagues for frequent discussions and advice, and especially to Professors R. H. Wetmore and M. H. Zimmermann for reading the manuscript.* *Addresses: Dr. Torrey, Biological Laboratories, Harvard University, Cambridge, MA 02138; Dr. Fosket, Department of Developmental and Cell Biology, University of California, Irvine, CA 92664; Dr. Hepler, Department of Biological Sciences, Stanford University, Stanford CA 94305.*

In higher plants, one of the most dramatic cases of cytodifferentiation is the formation of the cell type encompassed by the general term *tracheary element*, a cellular constituent of the xylem. Vascular plants are characterized by usually associated specialized conducting tissues: xylem, the principal water-conducting tissue, and phloem, the food-conducting tissue. The distinctive cells of the xylem are tracheary elements—thick-walled cylindrical cells which at maturity are water-filled and aligned end to end, forming an elaborate, hollow, and continuous tubular system throughout the plant. Tracheary elements are associated with other cells of the xylem specialized for support and storage, including xylem fibers, fiber tracheids, and xylem parenchyma.

Primary xylem develops in the embryo and seedling plant from cells derived from the apical meristems of the root and shoot. During normal development, linear rows of tracheary elements develop proximal to the apex

Table 1. Ontogenetic stages of tracheary element formation (modified from Torrey 1953)

Stage of Differentiation	Cytological and Biochemical Events	Possible Hormonal or Chemical Controls
Cell origination	Critical cell division Gene activation	Cytokinins, auxin, and gibberellins in the regulation of cell division Hormonal activation of genes?
Cell enlargement	DNA synthesis (endomitotic reduplication) Protein synthesis Synthesis of primary cell wall materials Determination of future secondary wall pattern	Auxin-cytokinin regulation? Cytokinin regulation of protein synthesis Auxin regulation of cell enlargement Gibberellins may influence the wall pattern-determining mechanism
Secondary wall deposition and lignification	Synthesis and deposition of cellulose and hemicellulose Synthesis and deposition of lignin	Sucrose may affect the synthesis of sugar nucleotides Auxin may regulate hemicellulose synthesis Both auxin and cytokinin may regulate lignification
Wall lysis and cell autolysis	Rupture of lysosomes? Lysis of portions of the primary wall, cytoplasm, and nucleus	Autocatalytic (Feedback stimulation from autolytic products?)

in characteristic radial and/or collateral patterns, forming a continuous axial conducting system (Fig. 1). If a vascular strand is severed, vascular continuity is restored by the formation of new vascular strands during wound healing. Secondary xylem is derived from cells produced by the vascular cambium, a meristem responsible for diameter growth in plants. Secondary xylem is characteristic of woody species and comprises the wood of commerce. As water-conducting tissues, primary and secondary xylem together form a continuous integrated system. Tracheary elements also tend to occur in tissues grown in sterile culture, but here they serve no obvious conducting function.

Whether they be formed from procambium, from vascular cambium, or from derivatives of a wound meristem, differentiating tracheary elements have a number of cellular features in common. Tracheary elements are characterized by the formation of a secondary cell wall with any one of a variety of patterned wall thickenings. Under the microscope the secondary wall is brightly birefringent in polarized light due to aligned cellulosic microfibrils; it is characteristically impregnated with lignin and forms a rigid and resistant structural element of the plant.

Although primary xylem is characterized by elongate tracheary elements with annular, spiral, or reticulate secondary wall patterns, and secondary xylem by shorter elements with pitted walls, there exists a continuum of these types within the plant that is not categorized easily. Vessel elements are tracheary elements specialized by the dissolution of end walls to form perforation plates.

During cytodifferentiation, the patterned secondary wall is laid down rapidly and becomes lignified, the perforation plates and other wall sculpturings develop by dissolution of portions of the primary cell wall, and then the cell contents autolyze and the protoplast disappears leaving a hollow tubular element, the mature tracheary element. It is this process with its many variations and permutations which serves as a particularly interesting and useful system for the examination of cytodifferentiation in vascular plants. Xylem formation is a paradigm of cytodifferentiation in higher plants.

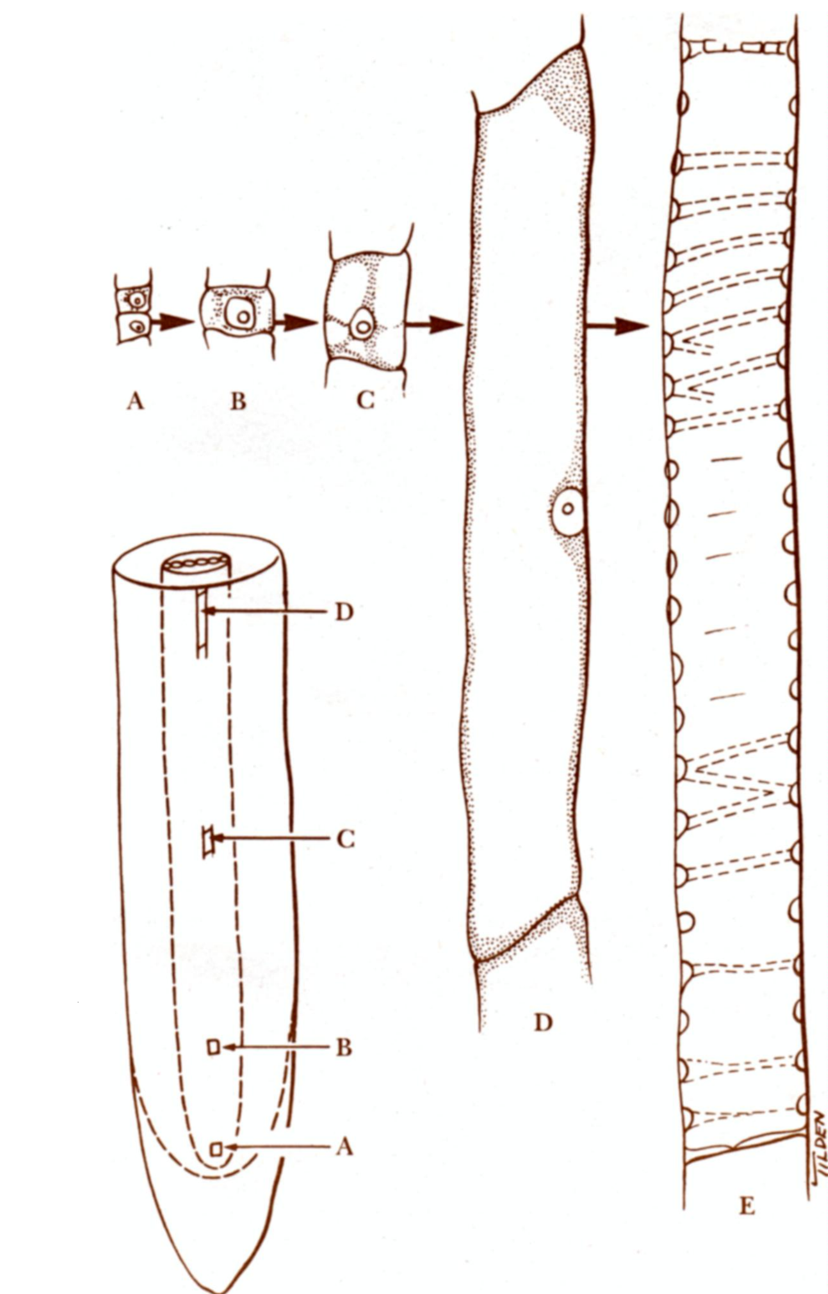


Fig. 1. Diagrammatic representation of the sequential cellular changes observed in the differentiation of primary xylem elements in the tip of the root of the onion *Allium cepa* L. *A* to *E* represent longitudinal views of cells from the apical initials (*A*) through the stages of radial enlargement (*B*) and elongation (*C, D*) to the mature tracheary element (*E*). In the median longitudinal view of the root in the lower left are shown the location of each stage of this cytodifferentiation. The mature tracheary element *E* is formed many millimeters behind the root tip, a distance which depends upon how rapidly the root is growing. Differentiation of primary xylem in the root is continuous and acropetal.

Our intention here is to focus on the problem of cytodifferentiation of this most distinctive vascular cell type, the tracheary element. We will focus on the origin of the tracheary element from its immediate cellular precursors, the nature of the stimuli controlling its origin, and the events which set off the element as a distinctive cell type. In this review of the cytodifferentiative events, we draw upon observations and data which tend to be treated as separate and unrelated phenomena in the literature. By comparing primary xylem formation in intact plants with the formation of xylem around wounds, with tissue cultures grown in vitro, and with secondary xylem formation in intact and experimental systems, we hope to make evident some common features which may help us to define the process of cytodifferentiation more clearly.

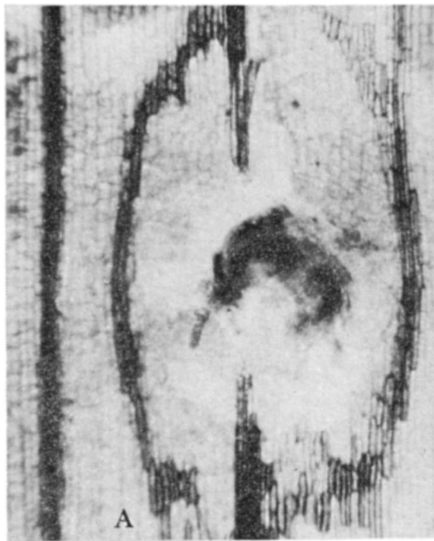
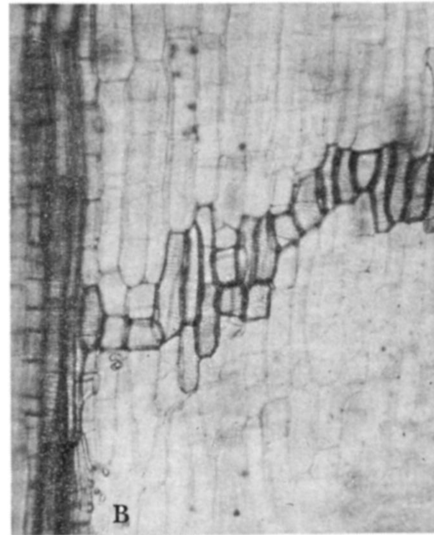


Fig. 2. *A*. Face view of a cleared *Coleus* stem in whole mount, showing the pattern of wound vessel member formation around a wound. A puncture wound was made between two of the main vascular bundles in the second internode that ruptured one of the smaller vascular bundles between them.



Within 7 days after wounding, tracheary elements were formed around the wound restoring the continuity of the vascular tissue. 28×. *B*. An enlargement of the differentiated wound vessel members showing the reticulate secondary walls as seen in cleared whole mounts. Compare with Fig. 9. 70×

In Table 1 are listed the stages in the ontogeny of a tracheary element. From this summary it is immediately clear that we are faced at the onset with the fundamental problem in all cases of cytodifferentiation: At what point can we say tracheary element formation begins? In other words, when is a cell determined to be a tracheary element? This difficult question is not easily answered, and we are forced to explore carefully the evidence concerning the origin of the cells which differentiate as tracheary elements and the control of such origin as well as the subsequent events of cytodifferentiation. Although patterned secondary wall formation is the most certain diagnostic morphological character of tracheary elements, one can see from Table 1 that this event is well along during the ontogeny of the cell.

The ontogenetic events are numerous and complex and we do not expect to cover them comprehensively. We propose to limit consideration here to recent ideas and evidence about the hormonal and nutritional factors necessary for tracheary element differentiation during primary xylem formation and to discuss in particular the hypothesis that cells differentiate as tracheary elements only after they have divided in a cellular milieu containing critical levels of certain hormones. We will then explore the same ideas in relation to the activity of the vascular cambium and the formation of secondary xylem. And finally we will review and assess cellular events and structures concerned with the formation of the distinctive cytological features of this cell type.

Differentiation of primary xylem

The role of auxin. It has been known since the turn of the century that wounding higher plant tissues by severing a vascular strand initiates a sequence of events which leads to the formation of new tracheary elements (Simon 1908; Freundlich 1909). The developmental events which occur after wounding *Coleus* stems were carefully documented by Sinnott and Bloch (1945). They reported that wounding first brought about cell division activity in the parenchyma tissue surrounding the incision. They observed that the plane of division of these cells was parallel to the future course of the wound xylem strands, which arched around the wound to restore vascular continuity. An example of the wound response in *Coleus* is shown in Figure 2.

It was through the analysis of this system that Jacobs (1952, 1954) first demonstrated the limiting role of auxin in xylogenesis. This work showed that the plant hormone, auxin, was produced by the expanding leaves in *Coleus;* if the leaves above an internode were removed at the time the internode was wounded, xylem differentiation did not take place around the wound. Wound xylem formation could be restored, however, if auxin was applied to the petiolar stump after removing the leaves.

Later, Jacobs and Morrow (1957) provided indirect evidence that auxin acted as the principal limiting factor in normal primary xylem differentiation as well as wound xylem formation. They found good correlation between the amount of diffusible auxin produced by a given leaf pair and the relative rate of primary xylem differentiation in the internode below that leaf pair in *Coleus.* Wangermann (1967) supported these observations with the direct demonstration that applied auxin could replace the leaf blade completely in the maintenance of normal xylogenesis in established primary vascular strands in *Coleus* stem tissue.

The work of Young (1954) suggested, however, that auxin was not the only factor regulating the appearance of the primary xylem. In *Lupinus* seedling plants Young found that when the second youngest leaf primordium was surgically removed from the shoot apex neither the procambial tissues nor primary tracheary elements were formed. In contrast to the results of Wangermann (1967) where much older leaves were studied, Young found that auxin would not replace the leaf primordium in the maintenance of normal xylem differentiation below that primordium. Young's work should not be taken to mean that auxin is not necessary for tracheary element formation but rather that other factors are also required.

The role of cytokinin. There are many suggestions that cytokinins represent another major plant hormone which is involved in the regulation of tracheary element formation. Sorokin et al. (1962) reported that kinetin applied to pea stem segments increased mitotic activity and stimulated greater xylem formation, including secondary xylem. Bergmann (1964) reported that kinetin increased tracheary element formation and lignin production in cultured tobacco callus tissues. Torrey (1968) demonstrated that the numbers of tracheary elements formed in several different cultured callus

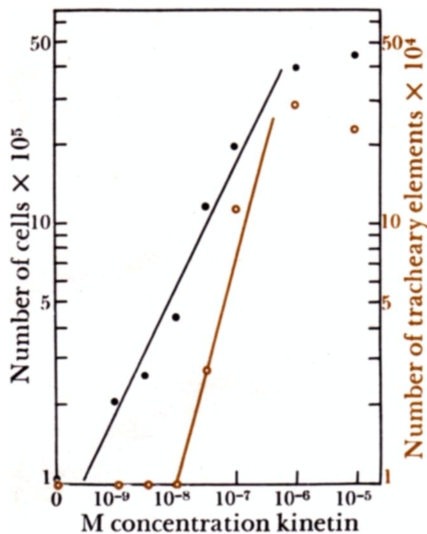


Fig. 3. Growth and tracheary element formation in Biloxi soybean callus tissue grown on agar media containing different concentrations of kinetin. The tissue was grown on media containing 10^{-5}M α-naphthaleneacetic acid and no cytokinin, or with concentrations of kinetin varying from 10^{-9}M to 10^{-5}M. The number of cells (dots) and the number of tracheary elements (open circles) were determined for each callus mass after 3 weeks' growth on the respective media. After subtracting the average cell or tracheid numbers present in the initial inoculum and computing the mean number of cells or tracheids produced during the culture period, these values were plotted against kinetin concentration on a semi-log scale (data from Fosket and Torrey 1969).

tissues was increased in response to addition of kinetin.

Nevertheless, it is difficult to demonstrate in vivo that the formation of tracheary elements is dependent upon an effective level of cytokinin because most growing tissues and organs appear to be capable of producing their own cytokinins. A conclusive demonstration that cytokinins are involved in the regulation of tracheary element formation was obtained from the study of cultured soybean callus, an unusual tissue that does not appear to be able to synthesize this hormone. Callus tissue derived from the cotyledons of soybean will continue to grow indefinitely when periodically subcultured to fresh medium. However, cell proliferation is completely dependent upon the presence of cytokinin in the medium. Growth stops completely within a few days if this tissue is transferred to a medium lacking cytokinin (Miller 1961).

When soybean callus tissue is grown in the dark with 5×10^{-7}M kinetin, 8 to 12% of the cells differentiate as tracheary elements. Since these tracheary elements usually form perforation plates, most of them would appear to be modified vessel elements (Fosket and Torrey 1969). In the absence of cytokinin, no cell division and no cytodifferentiation occurred. Increasing levels of cytokinin resulted in progressively increased cell proliferation and additional xylem differentiation, as is shown in Figure 3. Cell proliferation was stimulated over a much broader range of cytokinin concentrations than was tracheary element formation. Low levels of cytokinin (10^{-8}M or below) did not elicit xylem differentiation, although cell proliferation was stimulated. At kinetin concentration above 5×10^{-8}M, increasing levels of cytokinin actually led to the differentiation as tracheary elements of a progressively larger percentage of the total cell population.

Because of this differential sensitivity to cytokinin, a tracheary element-free population of cells was produced when the callus was subcultured repeatedly on a medium containing 10^{-8}M kinetin. Xylem differentiation resumed when the tracheid-free tissue was placed on a medium containing a higher level of cytokinin (Fosket and Torrey 1969).

Additional evidence that a cytokinin is necessary for xylem differentiation has come from the study of the behavior in culture of isolated pea root segments (Torrey and Fosket 1970). The diploid cells of the pericycle were stimulated to divide when the culture medium contained auxin and lacked a cytokinin, but the cortical cells did not divide. Xylem elements were not formed from the pericycle derivatives or from other tissues of the pea root segment under these conditions. When the root segments were cultured on a medium containing a cytokinin as well as auxin, the cortical cells were also stimulated to divide. From previous work it was known that cytokinin brought into division a population of cells that was polyploid at the time of division (Torrey 1961; Matthysse and Torrey 1967a and b).

Torrey and Fosket (1970) demonstrated that these polyploid cells were cortical cells and that, after one or two divisions, many of the cortical derivatives differentiated as tracheary elements (Fig. 4). After

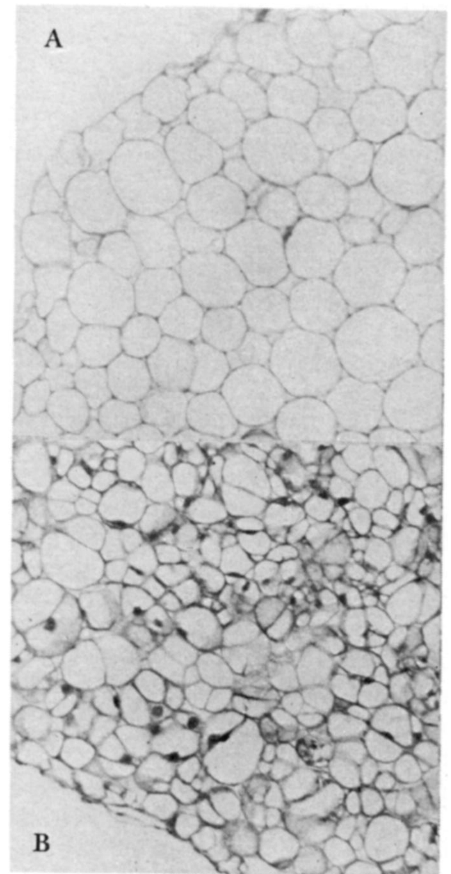


Fig. 4. *A*. Transection of pea root segment cut 10 mm behind root apex, showing cortex and epidermis at beginning of culture. *B*. Transection of pea root cortex as in *A* but after 5 days of culture on synthetic medium containing 0.5 ppm kinetin plus 10^{-6}M 2,4-dichlorophenoxyacetic acid and 10^{-6}M indoleacetic acid. Note the numerous subdivided cortical cells, many of which have differentiated as tracheary elements (from Torrey and Fosket 1970). 220×

10 days in culture, over 16% of the total population of new cells had differentiated as tracheary elements. As was found to be the case with the soybean callus, increasing levels of cytokinin stimulated both cortical cell proliferation and tracheid differentiation. The formation of xylem elements, however, was much more strongly promoted. Increasing the kinetin level from 0.1 to 1.0 ppm brought about a 5-fold increase in cell production and a 50-fold increase in tracheary element formation. The effectiveness of cytokinin in these systems is dependent on an adequate supply of auxin.

Other factors influencing xylem formation. A number of additional substances have been shown to cause quite striking effects on primary xylem formation in various experimental systems. These substances include other hormones, such as gibberellic

acid (Gautheret 1961), and organic substrates, such as sucrose (Wetmore and Sorokin 1955) or L-proline (Roberts and Baba 1968b). Considerable additional work will be necessary before we know the basis for these effects. At the present time it has not been shown that these factors, with the exception of sucrose, are actually required for the initiation of differentiation, and there is some reason to believe that their effects on xylogenesis may be indirect. For example, as will be discussed later, gibberellic acid has been shown to stimulate cambial activity in woody species and thereby to increase the number of cells that can be induced to form tracheary elements. Similarly, in cultured *Coleus* stem segments, gibberellic acid interacts with auxin to increase wound vessel member formation, but it appears to do so by bringing about precocious interfascicular cambial activity (Roberts and Fosket 1966).

The effects of sucrose on xylogenesis are particularly interesting because of the dramatic nature of the response and also because of the complexity of the possible interpretations of the response. As is now well known, Wetmore and his colleagues demonstrated that sucrose is necessary for tracheary element formation in a variety of callus tissues (Wetmore and Sorokin 1955; Wetmore and Rier 1963). In this work V-shaped cuts were made in the upper surface of callus tissue growing on a maintenance medium. The cut was filled with agar containing sucrose and auxin. Over the course of several weeks, meristematic nests of dividing cells appeared in the callus at some distance below the point of auxin application. Tracheary elements were formed in these regions of more recent mitotic activity. While neither the mitotic activity nor tracheary element differentiation occurred in the absence of an effective level of auxin, xylem differentiation also occurred only when sucrose was applied to the tissues along with the auxin. Rier and Beslow (1967) subsequently found that the number of tracheary elements formed in *Parthenocissus* callus varied with the sucrose concentration, with the maximum number of tracheary elements formed in the presence of 8% sucrose. The possible interaction of cytokinins with auxin and sucrose in this system has not yet been explored.

While it is fairly certain that sucrose is necessary for tracheary element formation, it is not at all clear why a substrate such as sucrose should have a regulatory role in cytodifferentiation. Although we cannot answer this question at present, some possible or partial answers have been discussed by workers in this field.

Recent work by Doley and Leyton (1970) suggests that at least part of the sucrose effect can be explained by the osmotic properties of sucrose solutions. They observed that the amount of xylem tissue formed in the wound callus of *Fraxinus* could be increased by adding sucrose to the culture medium, a finding consistent with the observations of Rier and Beslow (1967). However, they found that the tissue exhibited the same behavior when sucrose was replaced with polyethylene glycol, an inert substance which would reduce the availability of water to the tissues. Doley and Leyton (1970) interpreted their findings as signifying that pressure plays a significant role in the control of xylem differentiation.

While this interpretation would be consistent with those of Brown and Sax (1962), the work of Sussex and Clutter (1968) suggests that the increase in xylem differentiation arising from reduced water availability may be more directly related to qualitative changes in the kind of callus growth occurring under such conditions. Sussex and Clutter noted that tracheary elements appeared in a compact callus of *Eucalyptus* when it was grown on agar but that this same tissue lacked tracheary elements when it was grown in a liquid medium, where the tissue became friable and grew as a suspension of cells. Reaggregation and compression of the cells from the suspension cultures in dialysis bags did not lead to xylogenesis.

Doley and Leyton (1970) reported that a similar change in the growth habit of *Fraxinus* callus was associated with decreasing water potential due to elevated levels of sucrose or polyethylene glycol, i.e. the callus changed from a friable, loosely packed mass of cells with few tracheary elements to a harder mass of closely adhering cells with less intercellular space and abundant tracheary elements as the osmotic concentration of the medium increased.

Regardless of how these effects should be explained, it is apparent that the osmotic properties of sucrose solutions have not been properly evaluated in many of the studies where the role of sucrose in xylogenesis has been investigated. This is particularly true where workers have attempted to show a quantitative relationship between sucrose concentration and tracheary element formation.

Another partial explanation for the role of sucrose in tracheary element formation is the obvious one that sucrose acts as a metabolizable substrate. Torrey (1953) demonstrated that a number of metabolic inhibitors, such as dinitrophenol, blocked xylem differentiation in isolated pea roots. Since a cell must expend energy to form a tracheary element, it must also have available to it a substrate that can be metabolized to provide this energy. However, the work of Jeffs and Northcote (1967) indicated that the role of sucrose in xylogenesis may be more specific than this. They tested a number of mono-, di-, and trisaccharides for their ability to support tracheary element differentiation when supplied with auxin to bean callus tissue by the method of Wetmore and Rier (1963). Only the disaccharides trehalose and maltose stimulated as much differentiation as sucrose. Most monosaccharides were ineffective in inducing xylem differentiation, although some such as glucose supported callus growth.

The work of Jeffs and Northcote (1967) suggests that sucrose may have an almost hormone-like function in the induction of xylogenesis. Nevertheless, it must be remembered that sucrose is a natural plant product and as such it could be contaminated by low levels of natural plant hormones, especially compounds such as cytokinins, which are active at very low concentrations. Since sucrose is known to contain impurities which absorb ultraviolet light, it would be advisable to wait until this possibility has been satisfactorily evaluated before we definitely conclude that this substance plays a regulatory role in xylogenesis.

Cell division in relation to cytodifferentiation. Primary xylem differentiation tends to occur in regions of prolonged, persistent, or recurrent cell division activity during normal plant development. For example, the procambium,

a meristematic tissue which characteristically precedes the differentiation of the first tracheary elements in the shoot or root apex (Esau 1965a), usually exhibits continued mitotic activity after cell division has largely stopped in adjacent tissues. Also, as pointed out above, cell division activity is associated intimately with tracheary element formation in cultured pea root segments (Torrey and Fosket 1970), and in soybean callus tissue (Fosket and Torrey 1969).

Careful studies made of the physiological control of cell division in plant tissues have demonstrated the involvement of the same hormonal components that are clearly involved in tracheary element formation, viz. auxin, cytokinins, sugars, and perhaps other limiting factors such as gibberellins, myo-inositol, calcium, or other ions. In their study of tobacco tissue explants, Patau, Das, and Skoog (1957) demonstrated that both auxin and cytokinin were essential for the activation of the cell cycle which ends in cell division. DNA synthesis, mitosis, and cytokinesis are all events dependent upon a critical balance of hormones. Setterfield and the Adamsons (Setterfield 1963) also have demonstrated this dependence in cultured explants from Jerusalem artichoke tuber tissue (*Helianthus tuberosus* L.).

The question then arises whether in effecting tracheary element formation these hormones act on the differentiation process per se or on the preceding cell division. In the case of wound xylem differentiation it is clear that cell division and cytodifferentiation are not just temporally related, but the initiation of tracheary element formation occurs only during some phase of the mitotic cycle. These conclusions (Fosket 1970) come from study of tracheary element formation in cultured 2 mm-thick segments of the second internode of *Coleus*, using the technique of Fosket and Roberts (1964). On an agar medium containing sucrose and auxin, wound vessel members formed in these segments according to the time course shown in Figure 5. No wound vessel members were observed until the third day of culture, and almost 90 percent of the total wound vessel members appeared on or after the fourth day of culture. Figure 5 also shows the time course

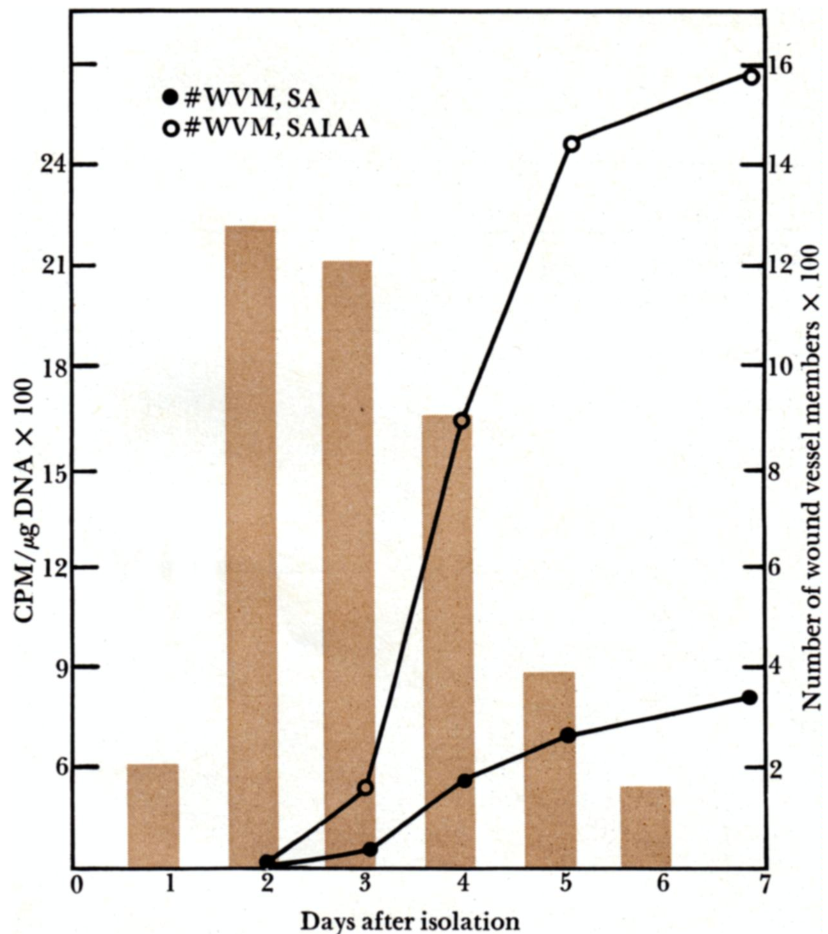


Fig. 5. The time course of DNA synthesis and xylem differentiation in *Coleus* stem segments cultured in vitro. The numbers of differentiated wound vessel members (WVM) were determined at daily intervals after culturing stem segments on a medium containing either 2% sucrose and 1% agar (SA) or SA medium supplemented with 0.05 ppm auxin (SAIAA). The mean number of wound vessel members per stem segment is given for each daily sample. Daily rates of DNA synthesis were estimated from the amount of ^{3}H-thymidine incorporated into DNA within a 12-hr labeling period. The observed incorporation of ^{3}H-thymidine into DNA of SAIAA-grown stem segments is indicated by the vertical bars, the width of which represents the duration of the labeling period (from Fosket 1970).

of DNA synthesis in the cultured stem segments, determined by measuring the daily rate of H^3-thymidine incorporation. DNA synthesis was found to reach a maximum on the second day of culture, two days prior to the peak of xylem differentiation. An examination of Feulgen-stained squashes for mitotic activity showed that the daily rate of H^3-thymidine incorporation into DNA reflected cell division activity.

If cell division is necessary for tracheary element formation, one might expect that agents or conditions which would prevent DNA synthesis and cell division would also block wound vessel member differentiation in these cultured *Coleus* stem segments. This proved to be the case. Inhibitors of DNA synthesis, such as 5-fluorodeoxyuridine and mitomycin-c, and inhibitors of mitosis such as the alkaloid colchicine were found to block wound vessel member differentiation as well as mitotic activity (Fosket 1968). Furthermore, these inhibitors were largely without effect in inhibiting xylogenesis if they were given after the third day of culture, i.e. at a time before most of the tracheary elements have begun secondary wall deposition, but after the peak period of mitotic activity.

There are, however, reliable reports of tracheary element formation in the absence of concurrent cell division activity. Foard and Haber described the development of "gamma-plantlets," which are wheat seedlings that germinate without mitotic activity after previous massive irradiation of dry seed with gamma rays (Foard and Haber 1961; Haber and Foard 1964). Although gamma-plantlets do form normal tracheary

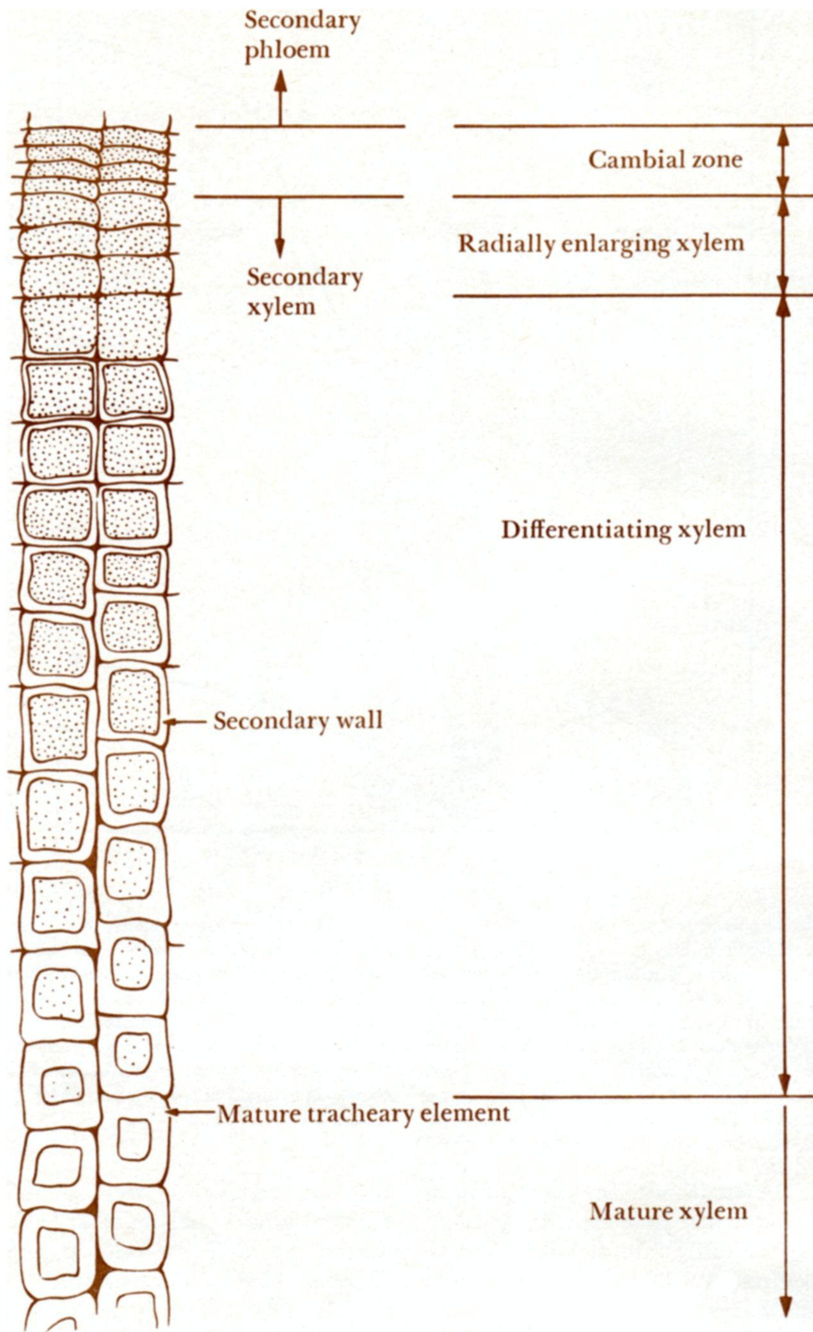


Fig. 6. Diagrammatic representation of the vascular cambium and secondary xylem formation and maturation seen in transection of the woody stem of *Pinus* (modified from the ideas of Wilson et al. 1966).

elements, it is not clear what this means. The tracheary elements produced by the gamma-plantlets are formed from procambial tissue that was already well developed in the dry seed before irradiation.

Does tracheary element formation in the gamma-plantlets represent the maturation of cells that were committed to this course of development before the irradiation, or was tracheary element differentiation initiated from previously uncommitted cells after planting the irradiated seed? If the former possibility is true then even in this case the initiation of xylem differentiation would have been associated with the mitotic activity that occurred during the development of the embryo. We simply do not know enough about the formation of tracheary elements in the intact plant to be able to interpret these observations more precisely at the present time.

Formation of secondary xylem

Primary xylem elements are derived from the differentiation of procambial elements which are formed in turn from the apical meristems. In contrast, secondary xylem elements are derivatives of a lateral meristem, the vascular cambium. Meristematic cells of the cambium are characteristically arranged in the form of a cylinder of longitudinal files of elongate cells. Two types of cambial initials occur within this cylinder: the elongate fusiform initials that form the vessels and tracheary elements and the more isodiametric ray initials that form ray parenchyma. Cell divisions occur within the cambial zone, adding to the cylindrical layers which increase the diameter of the stem axis. In almost abrupt and diagrammatic fashion, especially as viewed in transection (Fig. 6), the cellular derivatives on the inner side of the cambium undergo the characteristic changes of vessel or tracheary element differentiation, viz. radial cell enlargement, secondary wall formation with distinctive patterning of pits, wall lignification, dissolution of the end-wall perforation plates, autolysis of the cytoplasm, and complete maturation of the elements. The sequential events of cytodifferentiation are diagrammatically displayed within a few radially aligned cell rows.

The relationship between cell division in the vascular cambium and the differentiation of the cellular derivatives—secondary xylem on the inner face and secondary phloem on the outer face—is one of the most striking sequences of cytodifferentiation and tissue formation to be observed in higher plants (Fig. 7). What are the control mechanisms that initiate cell divisions in the cambium? What determines the differentiation of xylem internally (and phloem externally) in such a precise fashion? What subtle variations in control allow for the observed variation in cell type within the xylem tissue: the enlarged and distinctive vessel element, the narrow thick-walled xylem fiber, the thick-walled fiber-tracheid, the living, thin-walled xylem parenchyma cell or ray cell, or the other cell types derived from the same cylindrical layer of meristematic cells?

In tropical regions, woody species with

cambial activity may show continuous meristematic activity of the vascular cambium, unless cell division is brought to a halt by drought, other adverse conditions of the environment, or internal regulation. In temperate regions, the vascular cambium is periodic in its cell-division activity starting from a dormant state in the spring of the year with the onset of bud burst, sustaining rapid cell-division activity for several weeks or months, slowing again in mid-summer and returning to a quiescent state in late summer or autumn. In general, the cambium is inactive when trees are leafless and resumes when new leaves develop. This cyclical activity results in a pattern of annual rings of secondary xylem, frequently showing enlarged xylem elements in the spring wood followed in radial sequence by progressively smaller diameter elements with progressively thicker secondary cell walls of the summer wood (see Fig. 7).

This pattern is repeated in concentric rings, each marking a season of cell division and cytodifferentiation. Each period of inactivity of the vascular cambium is marked by an abrupt transition from summer wood to spring wood. It is generally believed that the annual ring pattern reflects the response of the plant to its changing environment. Thus, much interest and a large literature exist with respect to these structural features and their significance. Excellent published accounts are available (Kozlowski 1962; Zimmermann 1964).

The processes controlling vascular cambial activity and the subsequent events of xylem differentiation are subject to experimental manipulation. Because the patterns of differentiation are so diagrammatically presented to the investigator, they should be readily subject to analysis and understanding. Control of the external environment to which the plant is exposed allows one within certain limits to shut down cambial cell divisions, to activate them and to protract or shorten the duration of continued cell divisions of the vascular cambium. In addition, one can work with excised plant parts, roots or stems, and by organ or tissue isolation prevent cambial activity. Restoration of normal vascular cambium activity can be controlled by providing exogenous hormones and nutrients to the isolated plant part. To date, it has proved impossible to produce the distinctive features of vascular cambial activity and the normal pattern of cellular differentiation of secondary xylem and phloem in isolated callus tissue cultures, although some useful observations along this line have been made. What, then, have we learned from such studies that helps us to understand xylem differentiation?

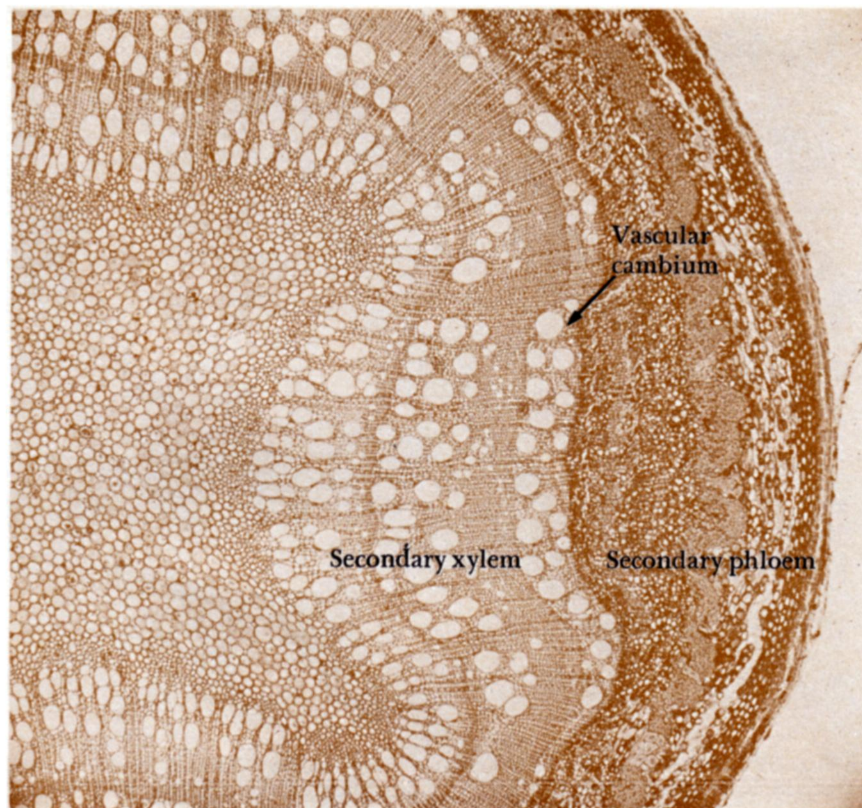


Fig. 7. Transection of the young stem of oak (*Quercus*) showing secondary xylem, vascular cambium, and secondary phloem. Portions of 3 annual rings are evident (photograph courtesy of P. R. Morey).

As is true in considering the differentiation of elements of the primary xylem or of regenerating xylem strands in relation to a wound, so also one finds it difficult to separate experimentally the intimate relationship between cell division and the succeeding events of cytodifferentiation in the formation of secondary xylem from vascular cambium activity. Stimuli which influence and determine the one affect the other also. Cell division is an essential prerequisite for production of the cells that form the wood; yet the cell derivatives may differentiate into a variety of cell types. In attempting to understand the physiological control of xylem differentiation from cell products of the vascular cambium, one is faced again with the problem of separating the events of cell division from the subsequent events of cytodifferentiation. Relatively little attention has been paid to date to this important distinction and few experiments on secondary xylem formation have been reported which allow one to specify controlling influences on cytodifferentiation per se.

Evidence from whole plants. It was early established (T. Hartig 1853, 1857) that in temperate climates activation of cell divisions in the vascular cambium of woody stems occurs annually with the onset of spring and is associated in time with the reactivation of dormant terminal buds. Cell divisions begin in leaf primordia of the developing bud, progress to the procambium of the stem, and thence to the cambium at the base of the bud (Tepper and Hollis 1967). Expanding floral buds also stimulate cell-division activity in the vascular cambium. Cambial cell divisions thus begin below the terminal bud region in the cylinder of dormant vascular cambium, and a wave of cell division moves from apex to base along the stem.

At the onset of cambial activation each spring, a hormonal stimulus ap-

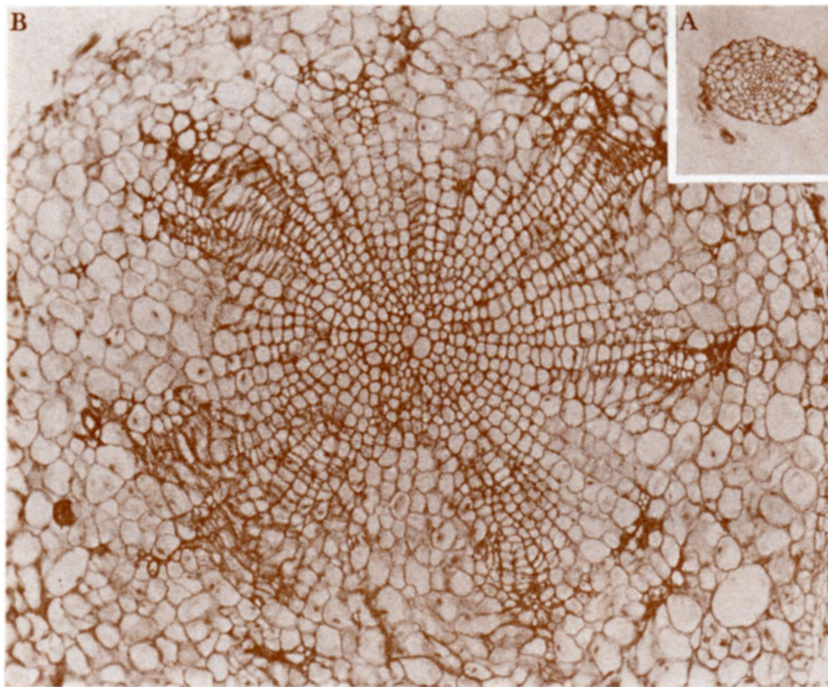


Fig. 8. *A*. Transection of a cultured isolated root of radish (*Raphanus sativus* L.) showing mature primary vascular tissues, but lacking secondary vascular tissues. 100×. *B*. Transection of a cultured isolated root of radish provided via the base with a mixture of sucrose, 10^{-6}M NAA and 10^{-6}M 6-phenylaminopurine. Root sectioned after 33 days in culture. Extensive secondary tissues have formed. (From Torrey and Loomis 1967a.) 100×

pears to move in the dormant cambial zone or in the late phloem or late wood xylem of last year's growth. When the cambium is first formed, the initiating stimulus apparently moves in procambial cells set aside in a preformed pattern by prior differentiation of other cells in the primary vascular tissues of the stem or root. The rate of movement of this stimulus is still a matter of debate. According to some views, this wave of cell division is propagated also into the vascular cambium of the trunk of the tree, but here the evidence is less clear. Under some conditions, activation of cambial divisions in the trunk of ring-porous trees may occur along the vertical length of the whole bole more or less simultaneously.

From those correlations in time, it was concluded that substances stimulatory to cambial activity are formed by the unfolding terminal vegetative buds. This idea was strengthened by the fact that excision of the terminal bud before cambial activation prevents cambial divisions and secondary vascular tissue formation (Gouwentak 1936; Reinders-Gouwentak 1965). The stimulatory substances are believed to be transported in a basipetal fashion. The breaking of bud dormancy is subject to seasonal temperature conditions and to photoperiod. Thus, indirectly at least, these environmental factors also affect cambial activation and wood formation.

In a number of genera, cambial activity leading to secondary thickening of the root occurs in intact plants only when the plant is grown under appropriate conditions of photoperiod. Thus, for example, dahlia plants grown in alternating 12 hr light and 12 hr dark periods produce only fibrous unthickened roots (Zimmerman and Hitchcock 1929). Plants of the same species grown in 8 hr light–16 hr dark conditions produce large underground root tubers from extensive root cambial activity. Apparently substances formed in shoots grown under appropriate day lengths are translocated to the root system where activation occurs and secondary vascular tissue is formed.

Thus, in both roots and shoots there is evidence for the activation of the vascular cambium and the differentiation of secondary xylem and phloem in response to substances translocated to the site of cell-division activity from elsewhere in the plant. The question is what are these stimuli and what controls their movement and effective action? To answer this question one needs good experimental systems for analysis. Intact plants are complex and results difficult to interpret. These difficulties are especially apparent in large woody plants.

Several experimental systems have provided useful information. Excised roots grown in sterile culture can be given nutrients, hormones, vitamins, and other chemical stimuli via the cut basal end, simulating supply from the shoot. In a somewhat analogous system terminal buds can be excised from dormant shoots and chemical stimuli provided to the disbudded stem as replacement for the terminal bud. From each of these experimental systems, some information has been gained bearing on the question of hormonal control of cambial activation and of secondary vascular tissue formation, especially concerning xylem formation.

Secondary thickening in excised cultured roots. Secondary thickening in the root is clearly dependent upon stimuli moving from the shoot into the root. Excised roots grown in nutrient culture develop as primary root structures, completely lacking a vascular cambium or secondary thickening (Fig. 8a). In excised radish roots, it was shown (Loomis and Torrey 1964; Torrey and Loomis 1967a, 1967b) that cambial activation required basal feeding of the following substances: an auxin such as indoleacetic acid, a cytokinin such as kinetin or benzyladenine, and sucrose. In addition, myo-inositol markedly increased the response. Although these substances provided in agar medium in a vial were taken up by the root base across its whole transectional area, vascular cambium activation occurred only at its normal location in the pericycle and in the procambium between primary phloem and primary xylem strands, and normal secondary thickening occurred (Fig. 8b). Differentiation of xylem as xylem parenchyma or as tracheary elements or vessel elements was dependent upon hormonal treatment and other factors not yet completely subject to control. Cambial activation occurred progressively with time, moving from root base toward the root apex.

In the radish experiments, the longitudinal course of activation was limited, perhaps by limited transloca-

tion of the active stimulants. In similar experiments with excised pea roots (Torrey 1963), only sucrose and indoleacetic acid were required, and activation of the cambium occurred over a considerable distance from the root base toward the root tip. Cambial derivatives were mostly vessel elements arranged in usual radial rows typical of secondary thickening in pea roots. Since pea root apices are rich sources of cytokinins, it is presumed that excised pea roots in culture have a nonlimiting supply of cytokinins essential, together with auxins, for cell divisions of the vascular cambium and subsequent cytodifferentiation.

In the intact plant, radish roots grown under optimum conditions as a garden vegetable form secondary tissues that are mostly xylem parenchyma rather than vessels or tracheary elements with secondary cell walls (which would be tough and inedible). Cultured radish roots usually produced many thickened, lignified elements, sometimes in rather inexplicable patterns. To understand the control of such patterns of differentiation is the aim of further research. By analogy to the results with soybean callus tissue, it is possible that subtle differences in cytokinin concentration, in the presence of auxin, could determine whether cambial derivatives differentiate as parenchyma or as vessel elements, depending on the threshold of the response.

Xylem formation in disbudded stems. Wareing and his associates have used the technique of treating disbudded stems in studying vascular cambium initiation and wood formation in woody shoots, a method pioneered by Münch (1938) and Söding (1936, 1940). Removal of the terminal bud just prior to bud break prevents cambial activation in many species. Application to the cut surface of a lanoline paste containing synthetic auxin substitutes in part for the excised bud and causes cambial activation and xylem differentiation to proceed.

Wareing (1958) and Wareing et al. (1964) tested combinations of hormones applied to disbudded shoots of such hardwood species as *Acer*, *Populus*, *Fraxinus*, and *Robinia*. From their various studies they concluded that auxins and gibberellins act together to determine the secondary vascular tissue formation following cambial activation in these tree stems. They showed that gibberellic acid when applied alone caused cambial divisions but not the subsequent differentiation of vessel elements. Auxin alone caused formation of discontinuous groups of xylem elements derived from the cambium. Appropriate concentrations of auxin and gibberellin together produced more or less normal secondary xylem formation, i.e. normal cambial cell divisions and secondary xylem formation. They showed further that the relative amounts of auxin and gibberellin provided affected the type of cells differentiated from the vascular cambium. According to Digby and Wareing (1966), high concentrations of auxin relative to gibberellin enhanced xylem formation while high gibberellin relative to auxin favored phloem formation. Other instances of gibberellin-auxin interactions affecting the pattern of cytodifferentiation in secondary tissues of disbudded plants have been reviewed by Wareing et al. (1964), Reinders-Gouwentak (1965), and Roberts (1969). Here again one can conclude that differences in hormone thresholds determine the cytodifferentiation behavior of cambial derivatives.

Recently Hejnowicz and Tomaszewski (1969) reported similar experiments on the effect of growth regulators on wood formation in the gymnosperm *Pinus sylvestris*. Disbudded pine stems will form normal xylem if the excised bud is replaced by a mixture of lanoline paste containing appropriate concentrations of auxin, gibberellin, and cytokinin. They pointed out that all three of these hormones have been found to occur naturally in growing buds of pine, and they concluded that these substances "control wood formation in intact plants." They believe that while auxin is the principal limiting factor for xylem formation, the other hormones affect auxin distribution within the plant. Their data clearly show that the hormones provided influence tracheid diameters, length, and cell wall thickness, as well as the number of differentiated elements formed. Thus there exists a complex interaction between cell division and the subsequent processes of cytodifferentiation which result in the vascular tissue patterns that are formed.

In the herbaceous species *Xanthium pensylvanicum*, Shininger (1970, 1971) studied the effect of hormone treatment on cambial formation in decapitated shoots. Excision of the terminal bud and leaves allowed continued cambial divisions and vessel formation in the stem but prevented xylem fiber formation. The auxin α-naphthaleneacetic acid (NAA) applied in place of the decapitated shoot resulted in an increase in the number of cambial derivatives and xylem fibers differentiated. Gibberellic acid (GA_3) caused an increase in the number of cambial derivatives formed but did not allow xylem fiber formation. Thus NAA in effect replaced the role of the shoot or of leaves with respect to xylem fiber differentiation. In this case, a specific type of cellular differentiation from cambial derivatives was controlled by application of a single exogenous hormone while other cambial derivatives were unaffected.

Fig. 9. A group of wound vessel members of *Coleus* observed with Nomarski differential interference contrast optics. The cytoplasmic contents of the cells have been cleared away with sodium hydroxide and chloral hydrate, thus making it easy to view the pattern and reticulation of the bands of secondary wall. In several places it is evident that the secondary wall bands of one vessel element are deposited directly across the primary wall from those bands of the adjoining vessel element. Compare with Fig. 2. (From Hepler and Fosket, in press.) 725×

In a unique genetic system, Caruso and Cutter (1970) showed that stem tissue of a leafless mutant of tomato produced no vascular cambium. Grafting a normal shoot tip onto the hypocotyl of the mutant led to the development of a normal vascular cambium and extensive secondary xylem, presumably due to hormonal stimuli produced by the shoot tip. The nature of these stimuli was not determined.

Shelldrake and Northcote (1968) de-

Fig. 10. An electron micrograph of a wound vessel member of *Coleus*. Several bands of secondary wall have been sectioned transversely and appear as ridges or "bumps" evenly spaced along the primary wall. (From Hepler and Fosket, in press.) 9,000×

veloped the hypothesis that hormones involved in cambial activation and the differentiation of secondary xylem and phloem may arise in part at least from the autolysis of the cell contents of xylem and phloem elements themselves. Thus auxin and cytokinin might be products of the breakdown of cytoplasmic components during cellular maturation. They would be formed continuously in a developing system and would tend to act in a self-stimulating inductive manner. Thus, hormonal stimuli could be visualized as derived not only from active meristems and young leaves but also from maturing cell populations in continuity with the differentiating population.

Cellular events during xylem differentiation

Once a cell has been initiated to become a xylem element the most obvious cytological change that occurs is the formation of a secondary wall. This thickened wall serves an important structural role in resisting collapse of the xylem element, thus preventing occlusion of the conducting tube. The composition and formation of the secondary wall in many respects is similar in both primary and secondary xylem elements. However, in primary xylem cells the secondary wall is deposited in discrete ridges or bands, often in an annular, helical, or reticulate pattern (Fig. 9), while in secondary xylem the secondary wall may be deposited in three layers on the primary wall.

The secondary wall is composed of a fibrillar component, cellulose, and encrusting substances, lignin, hemicellulose, pectin, and to a much lesser extent, protein (Frey-Wyssling and Mühlethaler 1965). The cellulose microfibrils within these walls are highly ordered. In primary xylem elements the microfibrils are all oriented parallel to each other and to the banded secondary wall. In secondary xylem elements, where the wall is formed in successive layers, the microfibrils within each layer are oriented parallel to one another in a helical array (Mark 1967). The encrusting materials fill the spaces between the cellulose microfibrils, adding strength and rigidity to the wall.

In the formation of the secondary wall the deposition of the cellulose component is accompanied by the deposition of lignin (Esau et al. 1966a; Hepler et al. 1970). Using $KMnO_4$ as a sensitive stain for lignin, it has been shown in differentiating wound vessel members of *Coleus* that lignification starts in the region of the primary wall and middle lamella beneath the attachment of the developing secondary thickening (Hepler et al. 1970). Although lignification has already begun in the earliest detectable stages of secondary wall formation, it nevertheless appears to lag somewhat behind the deposition of cellulose, as indicated by a more intense $KMnO_4$ staining in the core of the wall and lighter staining toward the periphery. It seems reasonable that the cellulose microfibrils are laid down at the cytoplasm-cell wall interface by apposition and that lignin and perhaps other encrusting substances are deposited by intussusception within the cellulose framework. Lignin biosynthesis involves activation of the enzyme phenylalanine ammonia-lyase, which is correlated in time with xylem differentiation (Rubery and Fosket 1969). The amino acids phenylalanine and tyrosine are enzymatically deaminated and oxidized to the phenolic alcohols that serve as immediate precursors to the lignin polymer (Rubery and Northcote 1968).

How the differentiating cells control the formation and pattern of the wall, especially since the wall is deposited outside of the plasma membrane, is a question of long-standing interest. The high degree of order in the structure of the secondary wall has led several investigators to analyze the structure of the overlying cytoplasm in an attempt to define its interrelation with the wall. As early as 1855 Crüger noted in young plasmolyzed tracheary elements that thickened bands of cytoplasm corresponded in position to that of the secondary wall thickenings.

Ninety years later this same question was brought under much closer scrutiny in a classic study by Sinnott and Bloch (1945) on the cytoplasm-cell wall relationships in differentiating wound vessel elements in *Coleus*. By following the process at time intervals after wounding, Sinnott and Bloch were able to analyze the sequential process of cytodifferentiation. They noted that in cells destined to become tracheary elements the cytoplasm became more granular and eventually banded in a pattern which mirrored that of the subsequently deposited secondary wall. They further noted that adjacent differentiating cells always formed their secondary thickenings directly opposite one another across the primary wall. Or expressed in a different way, primary pit fields tended to occur opposite each other in adjacent cells. The pattern in one differentiating cell thus was related and

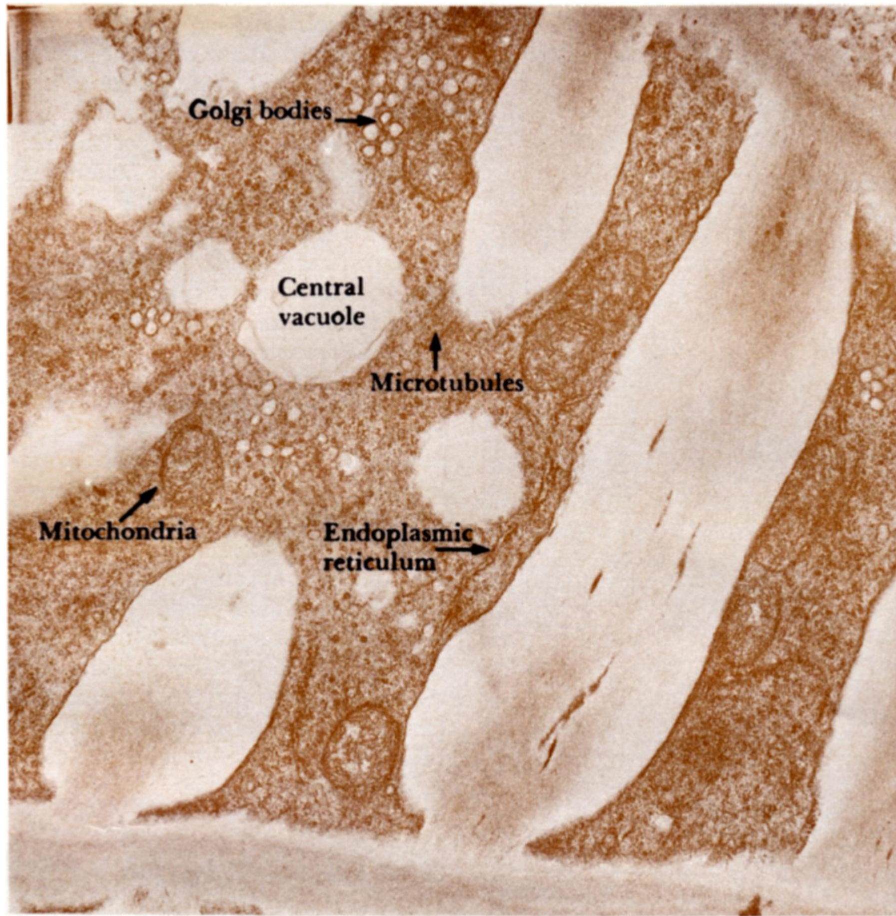


Fig. 11. An electron micrograph of a section made tangential to the cell surface reveals the bands of secondary wall and numerous cytoplasmic organelles. Mitochondria, Golgi bodies with their associated vesicles, elements of the endoplasmic reticulum, ribosomes, and portions of the central vacuole are evident. Of particular interest are the microtubules, which can be observed at the point where the section passes through the wall band into the cytoplasm. These structures are localized specifically over the bands of secondary wall, but are separated from the wall by a plasmalemma. The microtubules are oriented parallel to each other and to the axis of the wall band. 16,000×

integrated with the pattern of the wall in the neighboring differentiating cell. More recent light and electron microscopic studies have confirmed that the cytoplasm of differentiating tracheary elements is more dense than surrounding parenchyma cells, but they have failed to confirm the cytoplasmic prepatterning which Sinnott and Bloch (1945) observed (Cronshaw and Bouck 1965; Esau et al. 1966a, 1966b; Hepler et al. 1970; Wooding and Northcote 1964). The organelles such as mitochondria, plastids, nuclei, dictyosomes, elements of the endoplasmic reticulum, and ribosomes occur throughout the cytoplasm but without any apparent grouping or clustering with respect to the developing wall (Figs. 10, 11).

The role of microtubules. However, when glutaraldehyde was introduced as a fixative for plant cells, one cytoplasmic element, the microtubule, was observed in the cortical cytoplasm clustered specifically over the developing bands of secondary wall (Figs. 11, 12) in primary xylem elements (Hepler and Newcomb 1964). These linear structures, which are 250Å wide and of undetermined length, are oriented in the direction of the wall band and are thus parallel to the cellulose microfibrils of the underlying wall. Numerous reports have confirmed these initial observations and generally support the idea that the microtubules are involved in the formation of the secondary wall, perhaps by controlling the deposition and/or orientation of the cellulose microfibrils (for review see Newcomb 1969).

In secondary xylem, as well as in primary xylem, microtubules are found in the cortical cytoplasm oriented parallel to the cellulose microfibrils of the last-formed layer of secondary wall. For example, in wood fibers of *Acer rubrum*, where the microfibrils of the S2 layer are deposited in a helical array with a steep pitch with respect to the cell axis, the underlying microtubules show a similar orientation (Cronshaw 1965). Furthermore, in the formation of bordered pits, microtubules are localized in the cytoplasm adjacent to the inner rim of the pit aperture, again oriented parallel to the cellulose microfibrils (Robards and Humpherson 1967).

Attempts to unravel the role of microtubules in secondary wall formation have used colchicine, a drug known to have a destructive effect on other microtubules, such as those of the spindle apparatus (Hepler and Jackson 1969). Biochemical studies have shown that colchicine binds to a protein subunit of the microtubule, and may, because of the binding, prevent microtubule assembly (Borisy and Taylor 1967a, 1967b).

Studies in both the light and electron microscope have confirmed that colchicine deforms secondary wall thickenings in differentiating tracheary elements (Pickett-Heaps 1967; Roberts and Baba 1968b; Hepler and Fosket in press). Figures 13 and 14 show the striking effect colchicine has on the wall pattern of wound vessel elements of *Coleus*. The secondary wall is essentially smeared over the surface of the primary wall rather than being deposited in discrete ridges. Perforation plates, however, still occur and possess clear circular profiles (Fig. 13). If some secondary wall had been deposited before the cell was treated with colchicine, the wall still appears banded, but much less so than in the control. New wall is laid down between the older thickenings, tending to obscure the original wall pattern.

A wall thickening is thus formed in the presence of colchicine, and it appears from examination in the electron microscope to have the characteristics of a secondary wall. Microtubules, therefore, appear not to affect the process of deposition, but rather the process of pattern development. On a more careful look at the effect of colchicine on wall formation, using both polarized light microscopy and electron microscopy, Hepler and Fosket (in press) observed that normal microfibril orientation was severely disrupted, with microfibrils occurring in swirls. Thus, it appears that microtubules by some mechanism, as yet unknown, control the orientation of cellulose microfibrils of the secondary wall.

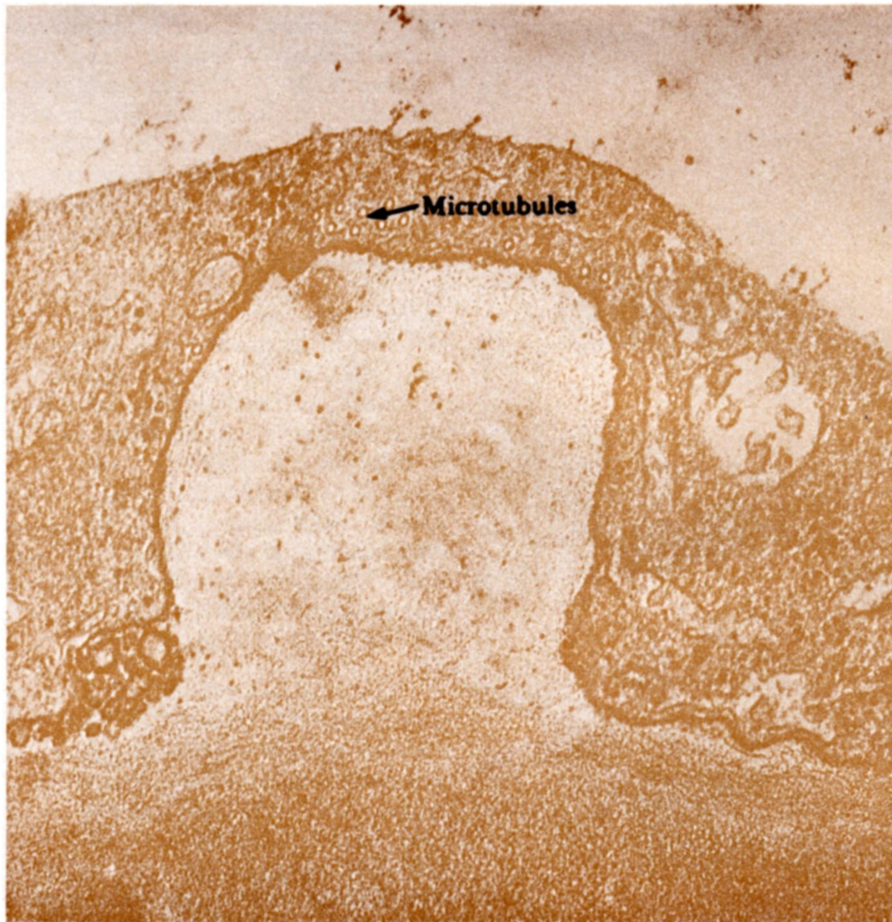


Fig. 12. An electron micrograph of a secondary thickening which has been sectioned transversely reveals the microtubules clustered over the secondary wall but not over adjacent regions of primary wall. Transversely sectioned microtubules are circular in profile, with a densely staining cortex and a lightly staining core. Their overall diameter is approximately 250 Å. (From Hepler and Fosket, in press.) 60,000×

In trying to analyze further the process of control of orientation it is important to bear in mind that the microtubules in the cytoplasm are separated from the wall by the plasmalemma. Microtubules, to the best of our knowledge, do not invade the wall or move through the plasmalemma to deposit a cellulose microfibril, although they may be attached to the plasmalemma by short cross-bridges (Cronshaw 1967). We would support the idea that microtubules mediate control of orientation through the plasmalemma, perhaps by affecting the conformation or structure of the plasmalemma or of the orientation of alignment of important enzymes in or on the plasmalemma, which would in turn affect cellulose microfibril orientation.

The role of other cellular components. In addition to the microtubules, considerable effort has been made in an attempt to understand the role of the Golgi apparatus in secondary wall formation. In other types of cells such as root cap cells, the Golgi apparatus has been implicated in wall deposition (Northcote and Pickett-Heaps 1966). During cytokinesis, vesicles presumably derived from the Golgi apparatus migrate to the mid-region of the cell, where they fuse with other vesicles to produce the new cell plate (Whaley and Mollenhauer 1963). Similarly, vesicles derived from the Golgi in differentiating tracheary elements appear from morphological evidence to participate in secondary wall formation (Wooding and Northcote 1964).

The endoplasmic reticulum (ER) has also been implicated in secondary wall formation. Pickett-Heaps (1966) noted in xylem members of wheat that the elements of the ER were frequently interspersed between the bands of secondary wall. ER-bound polyribosomes are commonly observed in differentiating cells suggesting an increased level in protein synthesis (Hepler and Newcomb 1964; Pickett-Heaps 1967). In the cisternae of the ER fine fibrils have been seen in wound vessel members of *Coleus* (Hepler and Newcomb 1964). Because these fibrils are found only in the ER cisternae of differentiating cells they have been implicated in the formation of the secondary wall. In secondary xylem elements the ER becomes aligned with the cell surface during differentiation and membrane-bound ribosomes become more numerous, again suggesting a role of the ER in wall development (Cronshaw 1965).

Experimental attempts to correlate either the Golgi apparatus or the endoplasmic reticulum with the formation and deposition of the wall components have yielded negative results. Applying the techniques of electron microscopic autoradiography Wooding (1968) and Pickett-Heaps (1968) followed the incorporation of labeled cinnamic acid, phenylalanine, and glucose in primary xylem elements. Cinnamic acid and phenylalanine have been shown to be excellent precursors of lignin, while glucose is a precursor of cellulose and other polysaccharides such as hemicellulose, pectins, etc. With none of these labeled compounds was there any consistent association of the autoradiographic grains with the vesicles or membranes of the Golgi apparatus, or, for that matter, with any other organelle. It would seem from these results that both carbohydrate and lignin precursors move from the cytoplasm to the wall via the soluble matrix and not via a particular organelle.

Recent evidence suggests, however, that the Golgi apparatus may participate in the transport of cell wall-forming enzymes. Ray et al. (1969) have shown in internode segments of etiolated pea seedlings that the polysaccharide synthetase is localized in a fraction composed almost entirely of Golgi membranes and vesicles. In wound vessel members of *Coleus*, cytochemical studies indicate that peroxidase, an enzyme localized in secondary thickenings which may act in the formation of lignin, is present in the Golgi apparatus and its associated vesicles (Hepler and Rice, in prep.). Although the Golgi apparatus may function in the movement of enzymes to the wall, the important question on the development of pattern remains unanswered since it is unknown how these enzymes, once transported to the wall, are localized or, alternatively, why the enzyme-containing

vesicles move only to specific sites on the cell surface.

A final but important stage of xylem differentiation is the maturation phase, when the cell dies and becomes functional in conduction. It is during this stage that certain portions of its primary wall are hydrolyzed, creating openings between adjacent xylem elements, thus leading to the establishment of continuous xylem tubes. The hemicellulose fraction of the primary wall in a maturing xylem element seems to be the most susceptible to attack and may be dissolved away leaving the cellulose microfibrils intact. However, in the region of the perforation plate in vessels even the cellulose is hydrolyzed (O'Brien 1970). The observation that the secondary wall and those portions of the primary wall which are lignified are not attacked has led O'Brien and Thimann (1967) to the conclusion that lignin protects the wall against hydrolysis. Because dissolution of the wall begins when the cytoplasm has already started to degenerate, it is argued that the process of hydrolysis is not under control of the cell but is part of a general autolysis (O'Brien 1970).

The process of xylem differentiation is thus a highly ordered sequence of events from cell origination, through the deposition of a precisely structured and patterned secondary wall, and finally to cell death and the formation of interconnected elements. Our observations suggest that this process is not stepwise but one in which all successive stages occur once initiation has taken place. We can block differentiation entirely, and we can, with inhibitors such as colchicine, cause abnormal xylem elements to form. But in no case do we appear to stop xylogenesis at partial completion. It is extremely difficult, therefore, to relate the successive cytological stages of differentiation to the different hormonal requirements. We are not able to determine whether auxin, for example, acts in the patterning of the cortical microtubules, in the induction of lignin synthesis, or in any other similarly defined step. The answers to many of the important questions on the sequence of structural and biochemical events of xylem differentiation appear to rest with the development of a culture system that can be precisely controlled and monitored.

Should this review have provoked a desire for additional information, the interested reader will find the anatomical aspects of vascular tissues concisely delineated by Esau (1965a) in her text on plant anatomy. Furthermore, the literature on the experimental control of vascular tissue differentiation recently received two very thorough reviews (Roberts 1969; Jacobs 1969), while Esau (1965b) has provided a unique integration of experimental work with the morphological and anatomical manifestation of vascularization.

Fig. 13. The secondary wall of wound vessel members that develops in the presence of colchicine lacks the clear banding and distinctive reticulation observed in untreated cells (compare with Fig. 9). It becomes smeared and covers a great portion of the primary wall surface. A circular perforation plate is evident and appears similar to those observed in normal wound vessel members. (Nomarski differential interference contrast optics.) (From Hepler and Fosket, in press.) 700×

Fig. 14. An electron micrograph of a colchicine-treated wound vessel member in an early stage of differentiation. Patches of secondary wall are thinly spread along the primary wall. The thickenings are no longer in discrete ridges or "bumps" as is commonly noted in untreated cells (compare with Fig. 10). (From Hepler and Fosket, in press.) 6,000×

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