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VASCULAR BUNDLE DIFFERENTIATION AND CAMBIAL DEVELOPMENT IN CULTURED TISSUE BLOCKS EXCISED FROM THE EMBRYO OF RICINUS COMMUNIS L.

A. M. SIEBERS

Botanisch Laboratorium, Leiden

SUMMARY

Small interfascicular and fascicular tissue blocks were excised from the hypocotyl of mature castor bean embryos. When cultured on a liquid medium containing mineral salts, sucrose, and kinetin, many of the interfascicular tissue blocks produced a cambium. Fascicular tissue blocks exhibited remarkably little development of vascular bundle tissue, particularly in the morphologically apical side of the block. Cambial development was frequently observed in this kind of tissue block, however, and was apparently not influenced by the lack of vascular bundle differentiation. Omission of kinetin from the medium prevented cambial development in both interfascicular and fascicular tissue blocks but did not lead to any further reduction of vascular bundle differentiation in the latter.

It is therefore concluded that vascular bundle differentiation and cambial development represent two essentially unrelated developmental processes, both initiated in the mature embryo but each depending for its realization on different specific internal and external conditions.

The precambial layer, i.e. the layer(s) considered to represent the initiated cambium, can be identified in the interfascicular zone as the innermost of two cell layers characterized by the relatively small size of the cells and nuclei. Attempts to obtain enzyme-histochemical evidence corroborating the special character of the precambial layer have been unsuccesful.

1. INTRODUCTION

There are two contradictory hypotheses concerning the initiation of the cambium in the interfascicular region: (i) the initiation process is dependent on inductive influences arising from the vascular bundles (PRIESTLEY 1928; BÜNNING 1963), and (ii) the initiation process is essentially independent of the surrounding tissue and is determined in a very early developmental stage of the shoot (LANG 1965; SIEBERS 1971a). On the basis of descriptive evidence, according to which the interfascicular cambium starts to grow in the vicinity of the fascicular cambium and then proceeds to the space between the vascular bundles, the first hypothesis has gained general acceptance (for a discussion of this point, see SIEBERS 1971b).

However, experiments in which interfascicular tissue blocks were excised and cultured in a simple medium showed that cambial development can proceed normally without the presence of vascular tissue (SIEBERS 1971b). Since the youngest experimental material was taken from hypocotyls of 6-day-old seedlings and cambial development *in situ* starts on the 11th day, it was concluded that in the castor bean hypocotyl cambium initiation had been determined a long time before the first mitotic activity could be observed in the interfascicular

region, and that an inductive influence from the fascicular cambium on the events in the intermediate interfascicular cells could be excluded.

The next step in these studies was to isolate interfascicular tissue from much younger material, e.g., the embryonic hypocotyl lacking differentiated vascular bundle tissue, and to attempt to culture these embryonic tissue fragments. The onset of cambial development in such material would provide evidence that we are concerned here with an autonomous developmental process that can proceed without an inductive influence from the vascular bundle tissue.

Comparison of cambial development in embryonic interfascicular and fascicular tissue blocks (the latter containing procambial bundles) would, on the other hand, show whether there is any stimulatory influence from procambial or vascular bundle tissue on the rate of cambial development.

Morphologically the cells of the interfascicular region are not homogeneous. In all developmental stages there is a certain inequality in cell size in the transverse section: going from the centre to the periphery of the stele, the volume of the cells of the interfascicular region decreases, but from the stelar-cortical boundary to the middle of cortex it increases.

The smallest cells roughly form an uniseriate tangential band of cells connecting the neighbouring procambial or (later) vascular bundles. In the hypocotyl of the mature embryo the band of small cells corresponds with the interfascicular parts of the primary meristem ring (originally described for *Ricinus* by SÁRKÁNY 1936), and in the full-grown hypocotyl mitotic activity leading to cambial development can be seen to take place preferentially in what supposedly is the same band of small cells. If cambium is indeed initiated in the embryonic phase, it would have to be assumed that in spite of its normal parenchymatous appearance in the growing hypocotyl, the band of small cells originally belonging to the primary meristem ring represents specialized cells containing all information needed to develop into a cambium. The use of the term "mother layer" by REINDERS & PRAKKEN (1964, fig. 111) for the layer of cells in which the cambium will be formed in the interfascicular region suggests the same conception of cambium initiation.

The present publication is in part concerned with attempts to identify these cells (for which the name "precambial cells" has been proposed by SIEBERS 1971a) on a basis other than that of cell size. Initially, identification was made on the basis of nuclear volume and DNA content, but the latter parameter had to be discarded because of the poor results obtained with the Feulgen reaction in this kind of material.

The successful application of enzyme-histochemical methods for the demonstration of physiological differences between root hair initials and the other epidermal cells (AVERS & GRIMM 1959a,b; AVERS 1963; CZERNIK & AVERS 1964; MIA & PATHAK 1965) led us to look for similar differences between precambial cells and adjacent parenchymatous cells.

To ensure correct comparison of the same material at different ages a check was made to determine which part of the embryonic axis gives rise to the hypocotyl. Special attention was given to the basal region of the hypocotyl because of

its relatively wide interfascicular zones which, unlike the middle and apical regions, are not disturbed by the formation of additional procambial bundles.

2. METHODS

2.1. Differential growth of the embryonic axis

Castor bean seeds (*Ricinus communis* L., received under the name *R. sanguineus* from Vilmourin-Andrieux, Paris) were soaked in tap water at room temperature for one hour and kept on wet filter paper in petri dishes placed in a growth-chamber conditioned as described earlier (SIEBERS 1971a). After 24 hours the seeds (to be referred to here as 1-day-old seedlings) were opened and half of the endosperm was removed together with one cotyledon.

To ascertain which part of the embryonic axis corresponds with the basal part of the mature hypocotyl, the axis was subdivided into 8 equal parts marked by a row of 8 superficially inserted needles (glochidia) originating from *Cylindropuntia kleiniae* (DC.) Knuth, the uppermost needle marking the position of the cotyledonary node (*fig. 1*). In later experiments the two lowermost needles were omitted because they appeared to give severe damage to the apical meristem of the root, resulting in reduced and abnormal growth of the seedlings. (For convenience in noticing accidental loss of one of these needles they were marked with red or black sealing wax and the colours used alternately.)

The starting material in all experiments consisted of twelve 1-day-old seedlings, each fixed in an upright position with a drop of warm diglycolstearate on the rim of an opening (diameter 7 mm) punched in a plastic disc (12 openings in all) placed in a glass beaker just above the level of a half-strength Hoagland solution. The seedlings thus arranged were placed back in the growth chamber, for the first 5 days in total darkness interrupted only once daily for a short period of light required for measurement of the distance between the needles. Data from seedlings exhibiting reduced or abnormal growth were discarded.

2.2. Isolation and culture of embryonic interfascicular and fascicular tissue

Isolation of interfascicular and fascicular tissue was performed in 1-day-old seedlings with the embryonic axis exposed as described above. The axis was then subdivided aseptically into 8 equal segments and the third segment from the apex was placed horizontally under a dissecting microscope. Under oblique light from above, the procambial bundles could be identified, after a few seconds, as 8 small, slightly concave spots on the surface of the segment. Interfascicular and fascicular tissue blocks measuring $1.3 \times 0.4 \times 0.16$ mm were prepared by making two perpendicular cuts along the diameter of the segments (*fig. 2*) with a corner of a razor blade. The tissue blocks (as a rule 30 per experiment) were transferred to 100 ml erlenmeyer flasks (2 blocks per flask) each containing 10 ml culture medium consisting of a mineral salt solution according to Heller (pH 6.0) fortified with 4 per cent sucrose and phytohormones (cold-sterilized by ultrafiltration). Culture and anatomical observation (after 3 weeks) were performed as described earlier (SIEBERS 1971b).

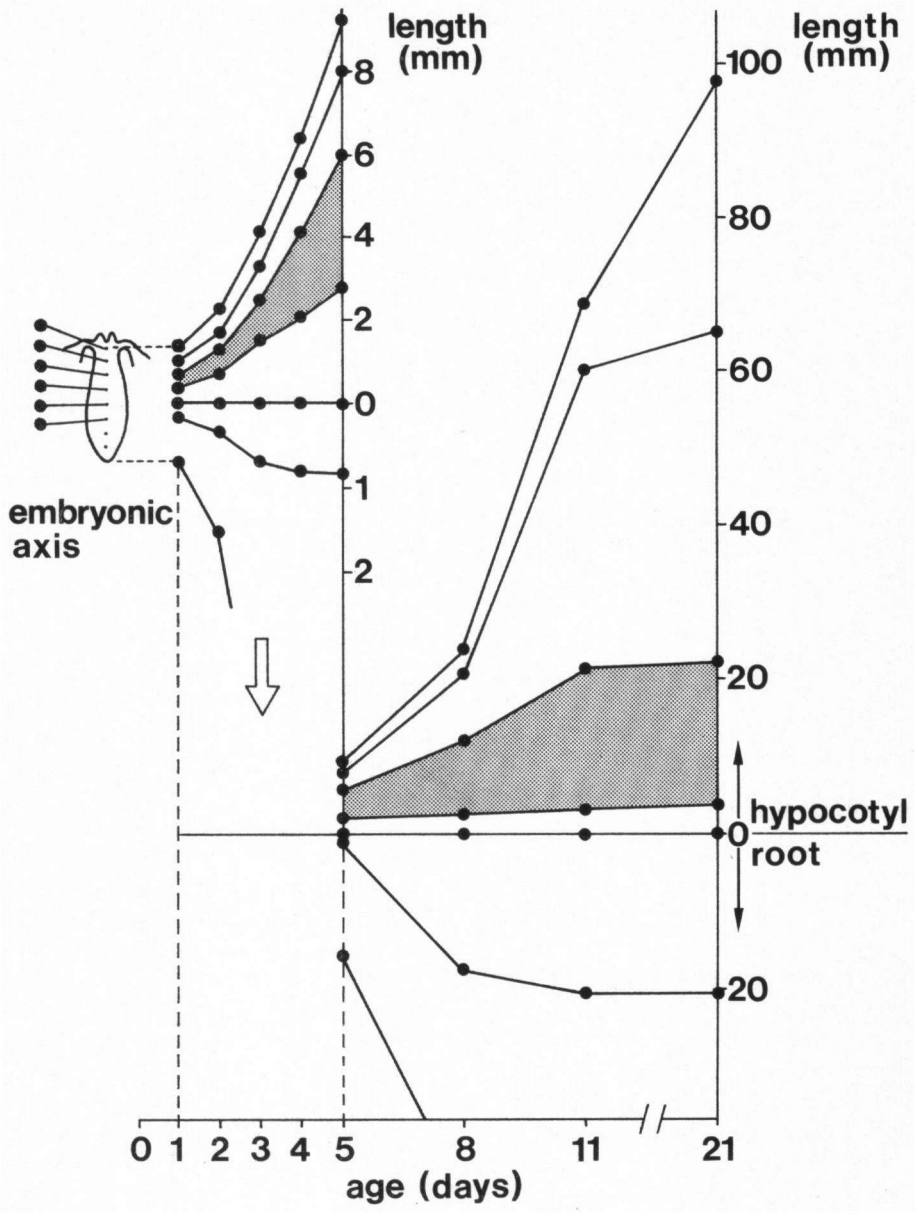


Fig. 1. Differential growth of the embryonic axis. Displacement pattern of 6 small needles inserted at equal distances along the embryonic axis of 1-day-old seedlings. The dotted area represents the part of the hypocotyl from which material was chosen for the culture experiments and the histochemical reactions. (Data based on measurements of 5 seedlings.)

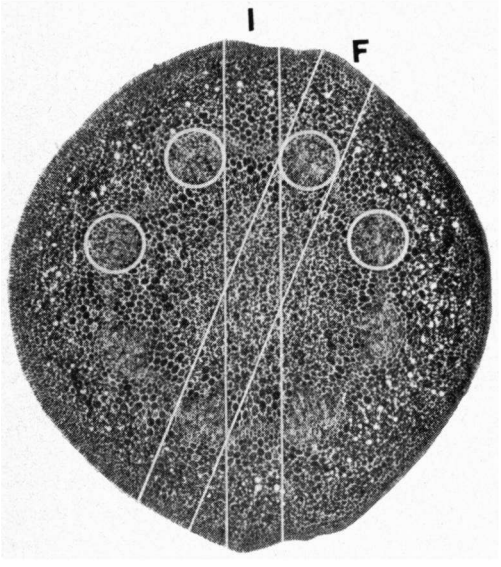


Fig. 2. Transverse section of the embryonic axis of a 1-day-old seedling, taken at the level of the basal part of the future hypocotyl. Four of the eight procambial bundles are indicated by circles. I = interfascicular, and F = fascicular tissue block to be isolated. Hematoxylin – orange G. ×49.

2.3. Histochemical reactions

The 2,4-dinitrofluorobenzene (DNFB) test for proteins, the dihydroxydinaphthyldisulfide (DDD) test for sulfhydril groups, and the Feulgen reaction for DNA (hydrolysis with 0.1 N HCl at 60 °C for 10 to 40 minutes) were performed in transverse sections (12 μ) of FAA-fixed and diglycolstearate-embedded material from hypocotyls aged 1, 2, 3, 4, 5 and 7 days. The diglycolstearate (Pegosperse "100 S", and not "1005" as erroneously indicated in SIEBERS 1971b) was obtained from Glyco Chemicals, New York. Fresh material of the same ages, sectioned by hand-microtome and 60, 60, 80, 100, 125 and 150 μ thick, respectively, was used for the Gomori reaction for acid phosphatase, the amine method for cytochrome oxidase and peroxidase, the benzidine-nitroprusside test for peroxidase (STRAUS 1964), the indigogenic method for esterase, and the method of KOSHUCHOWA & GÖRING (1968) for reducing sugars. All reactions except those for peroxidase and reducing sugars were performed after JENSEN (1962). Reactions of the different tissues were only compared qualitatively.

2.4. Nuclear volume determination

Use was made of transverse and radial sections of material fixed in FAPA (50 ml ethanol 96%, 10 ml 40% formol, 3 ml acetic acid, 3 ml propionic acid, 34 ml distilled water) and embedded in diglycolstearate.

Due to the divergent developmental stages of the material used, nuclear volume dimensions had to be determined in sections subjected to different staining procedures. For the embryonic and young hypocotyls (i.e. from 1- and 3-day-old seedlings, respectively) good results were obtained with 0.2% aqueous solution of basic fuchsin (G.T. Gurr) for 1 minute, which leaves the starch grains (present in abundant quantities and obscuring the nuclei in this material) rela-

tively unstained in the sections (8 μ). For the older material use was made of sections (10 μ) stained with 0.05% acridine orange (Chroma) in 0.2 M acetate buffer (pH 4.8) (LIBBENGA 1970), and examined under ultraviolet light (Leitz ortholux microscope, excitation filter BG 12, transmission at about 400 nm). Under these circumstances the nuclear envelope can be detected as a yellowish-green fluorescing structure. Apical meristem nuclei (from the stem apex of a 1-day-old seedling) were measured in longitudinal sections (12 μ) stained with Heidenhain's iron hematoxylin–orange G (JENSEN 1962), originally prepared for other purposes.

Measurements of nuclear dimensions were performed with a Leitz screw-micrometer ocular on 25 nuclei per type of tissue, deriving from 2 plants from one of which 12 nuclei were taken and from the other 13.

Nuclei of apical meristem and young hypocotyl were spherical, and the values were calculated according to $^4/_3\ \pi\ r^3$. In hypocotyls derived from 5-day-old seedlings the nuclei showed some flattening in the transverse view. Since in transverse sections nuclei lying against the transverse and the longitudinal cell walls appeared to be predominantly round and oval, respectively, and the mean diameter of the former roughly coincides with the mean largest diameter of the latter, it was concluded that on maturation of the tissue the original spherical nuclei change shape by flattening and not by elongation. Investigation of the nuclear shape in radial sections supported this view. Consequently, nuclear volumes were calculated in these cases according to $^4/_3\ \pi\ a^2b$, where a is the largest diameter, and b the smallest diameter, which is measured perpendicular to a. To obtain the required diameters, only nuclei lying against the longitudinal cell walls in the transverse sections were used for measurement (flat nuclei paralleling transverse walls show only one diameter, namely a). Care was taken to avoid measurement of nuclear segments by using only nuclei in which a large nucleolus and a well-developed nuclear envelope were visible.

3. RESULTS

3.1. Differential growth of the embryonic axis

From *fig. 1*, which shows the results of an experiment in which a number of small needles were inserted at equal distances in the embryonic axis of 1-day-old seedlings, it may be concluded that the third segment of the axis (when subdivided into 8 sections and numbered from the shoot apex) represents the region corresponding with the basal part of hypocotyl. Needle number 5 proved to mark the hypocotyl root boundary. Therefore, for all tissue culture experiments material was taken from the third segment. In the selection of material of different ages for the histochemical reactions, use was made only of those parts of the hypocotyls indicated by the dotted area in *fig. 1*.

3.2. Development of isolated embryonic interfascicular tissue blocks

In the simple medium containing only mineral salts and sucrose (4%), no growth or differentiation was observed, in contrast with earlier experimental results obtained in tissue blocks from 8-day-old hypocotyls (SIEBERS 1971b). Addition of caseine hydrolysate (400 ppm) or yeast extract (Difco, 500 ppm), raising of the sucrose concentration (to 6%) or replacement of the nitrate by ammonium as the nitrogen source (RAGHAVAN & TORREY 1964), all failed to give better growth or differentiation. Addition of gibberellic acid (0.05 and 0.5 ppm), which had given almost normal cambial development in the tissue blocks derived from the 8-day-old seedlings, unexpectedly resulted in necrosis of the cultured tissue when used in the medium for the embryonic tissue blocks.

Kinetin gave good results: vigorous growth of the tissue blocks occurred frequently; many brilliant cells containing anthocyanins were present, which, together with a great number of chloroplasts in the cortex and pith parenchyma cells, gave the tissue a very vital appearance. A number of the tissue blocks showed the onset of cambial development, i.e. 2 of the 19 tissue blocks cultured with 0.05 ppm kinetin and 4 of the 15 cultured with 0.5 ppm kinetin. In none of the 23 tissue blocks cultured on the basal medium was cambial development observed.

Growth of the tissue blocks was partially accomplished by a callus-like outgrowth from the wound surface. In the original core of the tissue block, growth seemed to consist predominantly of cell enlargement. Here cambial development started with the production of tangential walls in a place and in a way comparable with the development occurring *in situ (figs. 3, 4)*. In the callus-like region cambial development occurred parallel to the wound surface as is normally the case in cambia regenerating in parenchyma after wounding.

In some of the tissue blocks cambial activity reached a high level and was followed by the differentiation of a number of xylem elements. Cambial activity and xylem differentiation, however, ceased in all tissue blocks after three weeks. In an attempt to prolong cambial activity, tissue blocks kept for one or two weeks in a medium with kinetin were transferred to a medium supplemented with GA_3 (0.5 ppm) or TIBA (2, 3, 5-triiodobenzoic acid, 10 ppm). Although both these substances had been very effective in stimulating cambial development and activity when applied to cultured hypocotyls derived from 9-day-old seedlings (SIEBERS, unpublished results), success was not obtained in this younger material.

The differentiation observed in the presence of 0.05 or 0.5 ppm kinetin was not equally distributed over the tissue block. One side frequently showed two or three small procambial bundles *(fig. 5)*. Since, *in situ*, bundles like these formed in the middle and apical region of the maturating hypocotyl, this side of the tissue block was called the apical side. Here cambial development was always poor or absent. Toward the middle of the tissue block an increase of cambial activity could be observed, accompanied in more basal regions by the formation of secondary xylem. Linked with the xylem formation, tracheary elements of

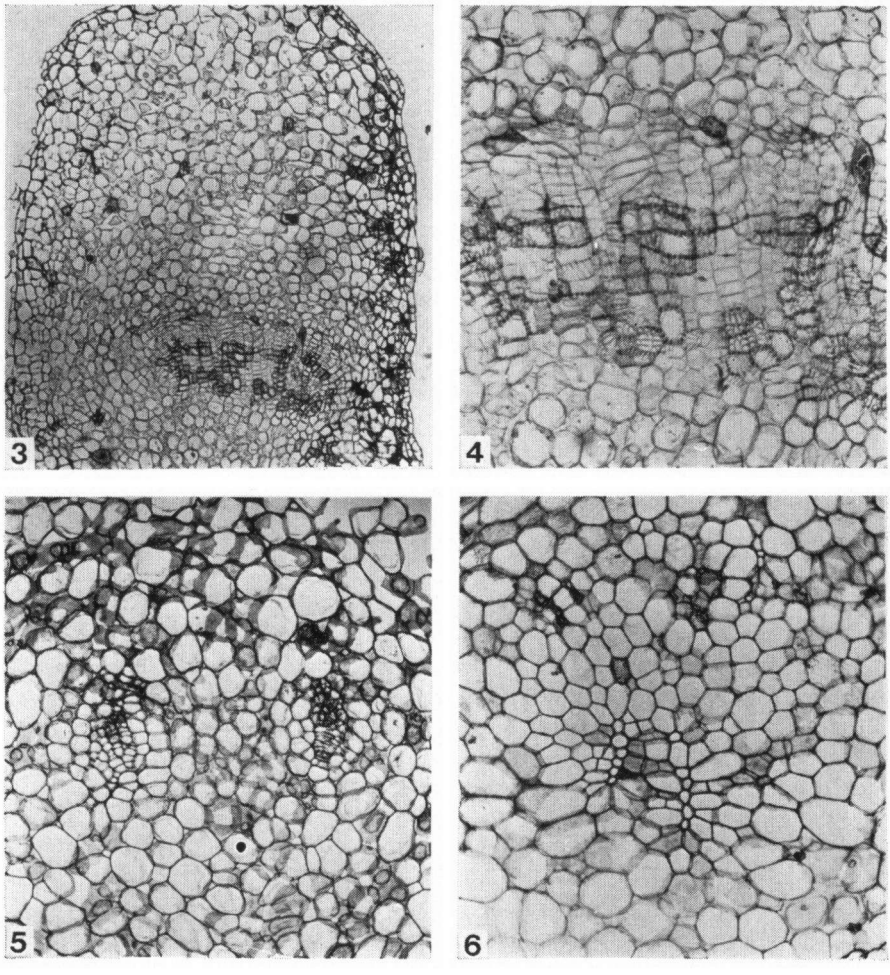


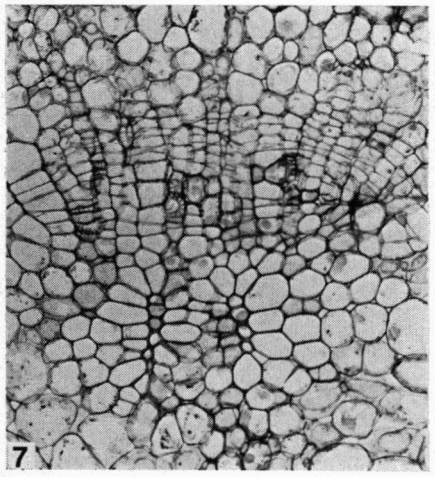
Fig. 3–8. Interfascicular and fascicular tissue blocks taken from 1-day-old seedlings and cultured for 3 weeks in a medium with or without kinetin. Transverse sections taken from the apical and basal side of the tissue blocks. Staining according to Maácz & Vágás.

Fig. 3. *Interfascicular tissue block* cultured in medium with kinetin. Section from the basal side. A normally positioned and actively xylem-producing cambium is visible in the original core of the tissue block. ×41.

Fig. 4. *Interfascicular tissue block*, detail of *fig. 3*, showing cambial development and formation of numerous secondary xylem elements. ×128.

Fig. 5. *Interfascicular tissue block* cultured in medium with kinetin. Section from the extreme apical side showing development of two small procambial bundles. ×77.

Fig. 6. *Fascicular tissue block* cultured in medium without kinetin. Section from the basal side. Only two rows of protoxylem elements present. ×103.



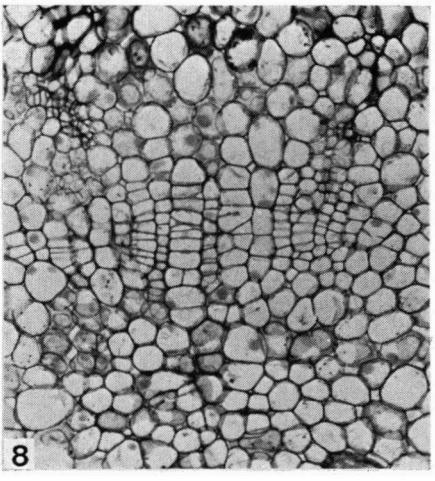


Fig. 7. *Fascicular tissue block* cultured in medium with kinetin. Section from the basal side. Cambium development and secondary xylem formation visible. Primary xylem formation restricted to some protoxylem elements. × 103.

Fig. 8. *Fascicular tissue block*, as in *fig. 7*, but section from the apical side. Note cambial development in absence of vascular bundle differentiation. × 103.

non-cambial origin and derived from stele parenchyma cells were frequently observed.

3.3. Development of isolated embryonic fascicular tissue blocks

Embryonic fascicular tissue blocks were cultured on a basal medium with or without kinetin. In the absence of the phytohormones, 22 of the 28 tissue blocks (distributed over 4 experiments) showed moderate growth and abundant anthocyanin and chlorophyll production in the tissue (representing substantially better results than those obtained with the interfascicular tissue blocks cultured on this medium). The development of vascular bundle tissue appeared to be reduced and was restricted to one side of the blocks, supposedly the morphologically basal side, where between 9 and 14 small protoxylem elements were seen in transverse sections (*fig. 6*). At the opposite side the tissue blocks lacked any sign of vascular differentiation. In 3 blocks some precambial cells showed formation of tangential walls, suggesting the onset of cambial development.

Tissue blocks grown on a basal medium supplemented with kinetin (0.05 ppm) showed a more pronounced growth. In the 7 investigated blocks (distributed over 2 experiments) vascular development was again reduced and was mainly limited to the basal region of the tissue block. Cambial development was, however, abundant and occurred more widely over the tissue block, sometimes reaching as many as 6 to 8 tangential walls per row, frequently followed by differentiation of some secondary xylem. In the basal region this led to a remarka-

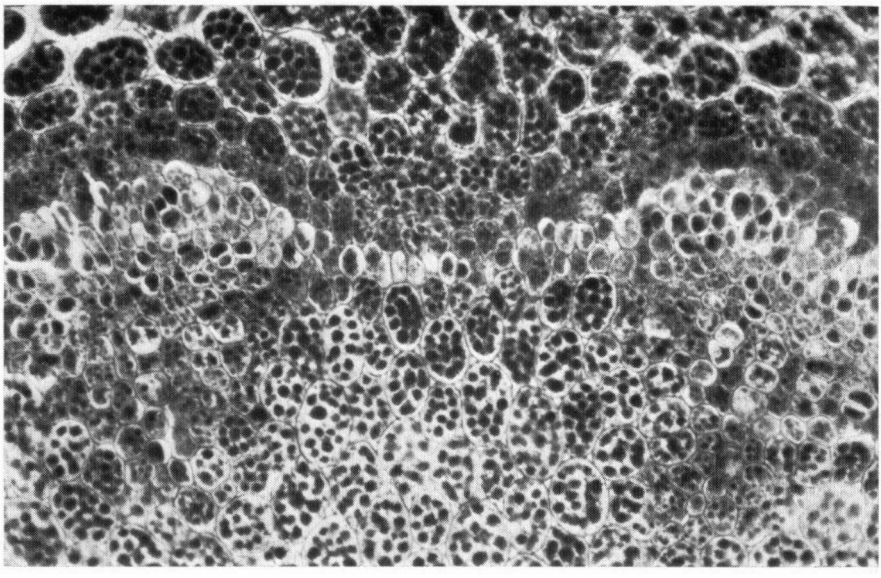


Fig. 9. Precambial layer between two procambial bundles in the embryonic axis of a 1-day-old seedling, taken at the level of the basal part of the embryonic hypocotyl. Transverse section; hematoxylin-orange G. ×380.

ble vascular pattern in which the protoxylem was separated from the secondary xylem by a number of parenchyma cells (*fig. 7*) instead of being connected by metaxylem, as occurs *in situ*. In the apical region the complete failure of primary xylem and phloem differentiation transformed the original fascicular pattern present at the start of the culture (*fig. 9*) into a typical interfascicular picture (*fig. 8*).

3.4. Morphological and histochemical identification of the precambial layer in the interfascicular region

Preliminary observations showed that during all phases of the development of the hypocotyl the assumed precambial layer is actually present, since it can be identified from the relatively small dimensions of the cells in transverse sections (*fig. 9*). In longitudinal sections differences in cell length between the precambial layer and the adjacent cell layers appeared to be very small. To investigate other characteristics that might help to identify the cells of the precambial layer, attention was first given to the nuclear volume. For two ages (3 and 7 days) the nuclear dimensions of all hypocotyl tissues situated along the interfascicular radius were investigated in transverse sections.

In the young hypocotyl, particularly the cells of the precambial and the innermost cortical layers (both identified on the basis of cell size and position) are distinguished by relatively small nuclear volumes (*table 1, column A*).

With aging of the hypocotyl, all tissues exhibited nuclear growth, but es-

Table 1. Nuclear volumes (in μ^3) of the tissues in the interfascicular zone in the hypocotyl of 3- and 7-day-old seedlings. The values in columns A and B represent the mean of 25 determinations (derived from 2 plants); the standard deviation for each value is shown in parentheses.

tissue	A 3 days	B 7 days	
epidermis	133 (± 60)	162 (± 60)	
outer cortex*	120 (± 50)	486 (± 83)	x
middle cortex	143 (± 71)	479 (± 208)	
inner cortex**	125 (± 40)	512 (± 232)	
innermost cortical layer	81 (± 24)	193 (± 75)	x
precambial layer	72 (± 22)	138 (± 46)	
adjacent stele parenchyma	107 (± 29)	236 (± 106)	
pith parenchyma	135 (± 55)	636 (± 365)	x

* the outermost 4 cell layers

** the innermost 5 cell layers, exclusive the innermost cortical layer.

x On basis of the Kruskal-Wallis test (MILLER 1966) the null hypothesis, i.e. all populations of nuclei do not differ, was rejected for the 7-day-old material ($\alpha = 0.05$). The multiple comparison method belonging to the test showed significant divergence in nuclear size at pairs of populations indicated by x in the interline

pecially the cells of the cortex and stele (which is hardly surprising, because cell enlargement often reaches an extraordinarily high level in these cells). The cells of the epidermis, the innermost cortical layer (from the 7th day assuming the form of a starch sheath), and the precambial layer, are relatively competitive in this respect. The cells of the last of these three layers have the smallest nuclei, although the divergence in size from the nuclei of the immediately adjacent cell layers is not statistically significant (*table 1, column B*).

Preliminary investigations, in which nuclear dimensions of the precambial cells were measured during the course of development of the seedling (on the 1st, 3rd, 5th, etc. to the 15th, and finally on the 21st day), revealed that nuclear volume rapidly increases from 70 μ^3 (a value also obtained for all other tissues in the interfascicular sector at the beginning of germination) to about 200 μ^3 at the moment of the onset of cambial activity (i.e. the 11th day). During further cambial development, nuclear volume (measurements limited to the central cells of the cambial zone) decreased to about 120 μ^3 in 21-day-old seedlings. SCOTT (1940) mentioned a value of 38 μ^3 as the typical volume of the castor bean meristem nucleus, and according to her this value also holds for the cambial nuclei. In our material the nuclei of the cambial cells proved to have appreciably higher values, particularly at the onset of cambial activity.

The results of the histochemical reactions for various enzymes (acid phosphatase, cytochrome oxidase, peroxidase, and esterase) and the reaction for reducing sugars, although indicating interesting differences between different cells of

a given tissue and between the different tissues of the hypocotyl, did not provide convincing data contributing to a histochemical identification of the precambial layer. Only in its youngest developmental phase, i.e., directly succeeding the primary meristem ring, can the precambial layer be identified by its reaction to different staining procedures, e.g. hematoxyilin–orange G (*fig. 9*), or to such histochemical procedures as the DNFB and DDD test for proteins and sulfhydril groups, respectively.

4. DISCUSSION

By the term induction is understood the result of a certain influence determining the initiation of a special type of cell, tissue, or organ, i.e. new characteristics are acquired by the cell, tissue, or organ, which under normal conditions no longer has a freedom of choice with respect to other potential courses of development. The inductive influence can arise from neighbouring cells, tissues, or organs, or be exercised by factors originating outside the plant. The term stimulation is reserved for the quantitative aspects of cambial activity. The nature of the stimulus that activates the cambial development will be the subject of a forthcoming paper (SIEBERS & LADAGE 1973).

It is clear from our experiments with both interfascicular and fascicular tissue blocks that to obtain cambial development the medium must contain kinetin. It is known that vascular tissue may contain or produce growth factors that can induce mitotic activity in pith parenchyma (HABERLANDT 1913; JABLONSKI & SKOOG 1954; CARUSO 1971) and that auxins and cytokinins are thought to be produced as a result of autolysis of the contents of xylem and phloem elements (SHELDRAKE & NORTHCOTE 1968a, b). It therefore seems conceivable that *in situ* differentiating vascular elements supply surrounding tissues with kinetin, which is especially active in stimulating cambial development, and that in culture experiments the influence of the vascular tissue is at least partially replaced by an external supply of the phytohormone.

Objections to a specific role of kinetin in cambial development can be derived from the observation that the effect of the applied phytohormone is a very wide one. It stimulated cell division in both cortex and stele parenchyma cells and enhanced chlorophyll and idioblastic anthocyanin production, suggesting that all cells of the embryonic tissue block need kinetin, not just those of the precambial layer. Whether the required kinetin can originate from a vascular tissue that has hardly begun to differentiate is another question. It seems more likely that in this case the source of kinetin must be sought elsewhere, viz. in the thick layer of gelatinous endosperm (a source of kinetin in a number of plants, see SALISBURY & ROSS 1969) enveloping the embryo and remaining in contact with the young seedling until the 5th or 6th day.

An unexpected feature in the cultured fascicular tissue blocks was the greatly reduced vascular bundle differentiation, even in media supplemented with kinetin. This held especially for the more apical parts of the tissue block, where, in contrast to the basal parts, there was no protoxylem and probably also no pro-

tophloem formation (but this was more difficult to distinguish in this kind of material). The lack of xylem differentiation, especially at the apical side, may be attributed to a certain auxin deficit (SHININGER 1971; see also ROBERTS 1969). The auxin produced in limited amounts by the dividing cambial cells (DÖRFFLING 1963) and transported to the basal side as a result of the preferential basipetal transport of this phytohormone (ROBERTS & BABA 1970; see also GOLDSMITH 1969) could account for at least a minimal xylem differentiation at the basal side of the tissue block. Preliminary experiments in which IAA (0.1 ppm) was supplied to the medium in combination with kinetin (0.5 ppm) indeed revealed that primary xylem differentiation can extend longitudinally over the entire tissue block, although the bulk of the xylem elements is still formed at the basal side.

The appearance of a cambium in a quite normal position in the interfascicular tissue blocks strongly suggests that the initiation of the cambium had already been determined in the embryonic phase of the development of the hypocotyl. This finding excludes participation of vascular tissue in the determination of the cells in the interfascicular region. In the fascicular tissue blocks cambial development can also be seen to take place normally, in spite of minimal vascular differentiation, which implies that even in the fascicular region vascular bundle differentiation and cambium initiation must be two essentially unrelated processes.

On the basis of our experimental findings and the observations of the primary meristem ring in the embryonic hypocotyl (uniseriate in the interfascicular region, multiseriate and showing a gradual transition to the procambial tissue in the fascicular region, see also SÁRKÁNY 1936) it may be said that the primary meristem ring represents the predetermined cambium, so that both parts of the cambium, viz. the interfascicular and fascicular, originate from the same meristematic layer. Both these parts must therefore be seen as "primary meristem" in the sense originally given by Nägeli (in KAUSSMANN 1963). The view that according to its origin the interfascicular cambium represents a secondary meristem (FAHN 1963) – which is also implied by the acceptance of homogenetic induction as the causal agent in the initiation of the interfascicular cambium (BÜNNING 1963) – appears to be incorrect.

In relation to the problem of cambium initiation in the epicotyledonary parts of the shoot, we suggest that here, too, the determination will be completed during a very young stage, i.e. at the time when the primary meristem ring is developing in the shoot apex. NEVILLE (1968) has stated that the primary vascular cylinder in a tree like *Gleditsia triacanthos* L. arises directly from the residual meristem (i.e. in our terminology the primary meristem ring), and that the position of this meristem in the shoot apex seems to be the result of a transverse gradient of a kind mentioned by J. & P. M. WARREN WILSON (1961). According to our view there is no essential ontogenetic difference between the cambium of plants with a closed primary vascular cylinder, e.g. *Gleditsia*, and plants with a system of separated vascular bundles, since in both types of plant all parts of the cambium originate from the primary meristem ring. This leads to the hypothesis

that in all dicotyledons and gymnosperms the initiation of the cambium in both hypocotyl and epicotyl is completed during a very early stage, viz. the stage in which the primary meristem ring is developed. The duration of the period between the actual development of fascicular and of interfascicular cambium may, however, vary widely among different plants, as stated by ESAU (1943).

Support for our hypothesis could be sought in certain physiological or cytological properties characterizing the cells of the primary meristem ring and their descendants, the precambial cells, as specialized meristematic cells whose mitotic activity is temporarily repressed. For the detection of such properties the interfascicular region was thought to offer excellent material, because the precambial layer is inserted here in a mass of "unspecialized" parenchymatous cells. One easily observed property is the relatively small size of the precambial cells, which coincides, as measurements have shown, with small nuclear volumes. Marked differences in nucleolar size, like those described by CATESSON (1964) for the interfascicular region of *Acer pseudoplatanus* L., were not observed.

In view of the possibility of a positive correlation between nuclear volume and DNA content (TSCHERMAK-WOESS 1956; TISCHLER & WULFF 1963; BUVAT 1965; NAGL 1968) it would be interesting to know whether the relatively small nuclear volumes reported here for the epidermis, the innermost cortical layer, and the precambial layer are associated with low DNA levels and low degrees of polyploidy. In the root pericycle, for example, TORREY (1965) described both these characteristics as related to a marked tendency to undergo mitosis. Further investigations to elucidate the possible significance of these differences in nuclear volume are planned.

The results obtained with the enzyme-histochemical methods require only brief mention. Our findings do not support the existence of differences in enzyme activity between the tissues under discussion, viz. the precambial layer and the adjacent tissue layers. It may be that the specialized character of the precambial cells is not expressed by special physiological properties at all, or that the methods chosen for this study were inadequate to detect them.

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