

INITIATION OF RADIAL POLARITY IN THE INTERFASCICULAR CAMBIUM OF *RICINUS COMMUNIS* L.

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SUMMARY

The initiation of the cambium in the ray parenchyma of a shoot has been explained by two contradictory hypotheses: (i) the initiation process is dependent on inductive influences arising from the vascular bundles, or (ii) the process is an autonomic one in the sense that the initiation is determined at a very young stage of the shoot and proceeds without influence from other tissues.

Experiments in which small pieces of interfascicular tissue have been isolated and replaced in reverse between the vascular bundles in the hypocotyl have shown that the initiation site and the radial polarity must have been determined many days before the first demonstrable mitotic activity had actually occurred. The experiments provided no evidence pointing to the existence of an inductive effect arising from the vascular bundle or an influence from a surface-dependent gradient on the initiation of the cambium. It is suggested here that the entire initiation process, including fixation of radial polarity, is determined at the primary meristem ring stage.

I. INTRODUCTION

The vascular cambium generally gives rise to two entirely different types of tissues, *viz.* xylem toward the inside and phloem toward the outside. The cambial layer therefore seems to have or to receive information about the position of the centre and the periphery of the part of the plant concerned. The question of how and when this information is transferred to the cambial cells is still unanswered. For the fascicular cambium this problem may have a simple solution. The pre-existing, specifically localized xylem and phloem may induce a radial polarity within the cambial cells. Another possibility is that the radial polarity is already determined and can be traced back to the polarity of the original procambial tissue in the embryonic shoot. As to the interfascicular cambium, which is formed in the parenchymatous tissue of the medullary ray, the problem is complicated by the supposedly undifferentiated and unpolarized state of this tissue.

Several authors have therefore presumed that some inductive influence on the intermediate tissue arises from the vascular bundles (PRIESTLEY 1928; PRATT 1948) or from their fascicular cambia (BÜNNING 1956). This influence may result in determination of the site at which the interfascicular cambium will be formed and in fixation of the radial polarity in this tissue.

For the regenerating cambium, which is formed as a result of injury of the vascular cylinder, J. & P. M. WARREN WILSON (1961) have put forward a hypothesis based on the assumed existence of a gradient of some unknown factor from an

exposed surface inward. Such a gradient may have some relation to the determination of polarity in the interfascicular cambium.

The theories mentioned so far have one feature in common, namely, that the initiation of the interfascicular cambium is in some way dependent on factors originating from outside the interfascicular tissue.

RZIMANN (1932) and LANG (1965) thought that the site at which the interfascicular cambium will arise is determined by the localization of the primary meristem ring already present there at a very early stage in the development of the shoot. Extension of this theory to the aspect of polarity would mean that the initiation process as a whole is determined at the primary meristem ring stage and that this process can proceed without any influence from the surrounding tissues.

One way to interfere with normal development in order to obtain information about polarity is by the inversion of tissue. We therefore attempted to determine whether the polarity of interfascicular tissue could be altered by an inversion over 180 degrees. If this were so, and, for example, xylem still formed on the topographically inner side, it could be concluded that the polarity was induced either by the adjacent vascular bundle or by some radial gradient. If, on the other hand, the inverted tissue blocks did not react to the new situation, it would have to be assumed that the polarity had already been established before the moment of inversion.

2. MATERIAL AND METHODS

Experiments were carried out with 6 to 11 days old Castor bean seedlings (*Ricinus communis* L. received under the name *R. sanguineus* from Vilmourin – Andrieux, Paris) from seeds germinated in plastic beakers filled with gravel and given about 100 ml Hoagland solution every 12 hours. The seedlings were grown with a 16-hour light period (Philips TLF40W, 10 tubes per metre, placed 1.50 m above the seedlings; temperature 25°C) alternated with an 8-hour dark period (temperature 20°C). Strips of interfascicular tissue were isolated from a hypocotyl by means of a rectangular punch (0.8 mm by 2.0 mm) made of small razor-blade fragments inserted in a drop of sealing wax attached to a small glass holder. Tissue in which the cambium was or would be formed was cut out and inverted, except in the control experiments, and then re-inserted into the hypocotyl together with the other parts of the excised block (*fig. 1*). The injured area was covered with a thin coat of vaseline and wrapped with cotton tape. After 9 to 20 days the anatomical reaction of the hypocotyl was analysed. Routinely, transverse sections were stained according to MAÁ CZ & VÁ GÁS (1963).

3. RESULTS

3.1. Radial inversion experiments

Of the total of 54 inversion experiments about half failed due to necrosis of the inverted tissue. Of the remaining inversions about half were discarded because

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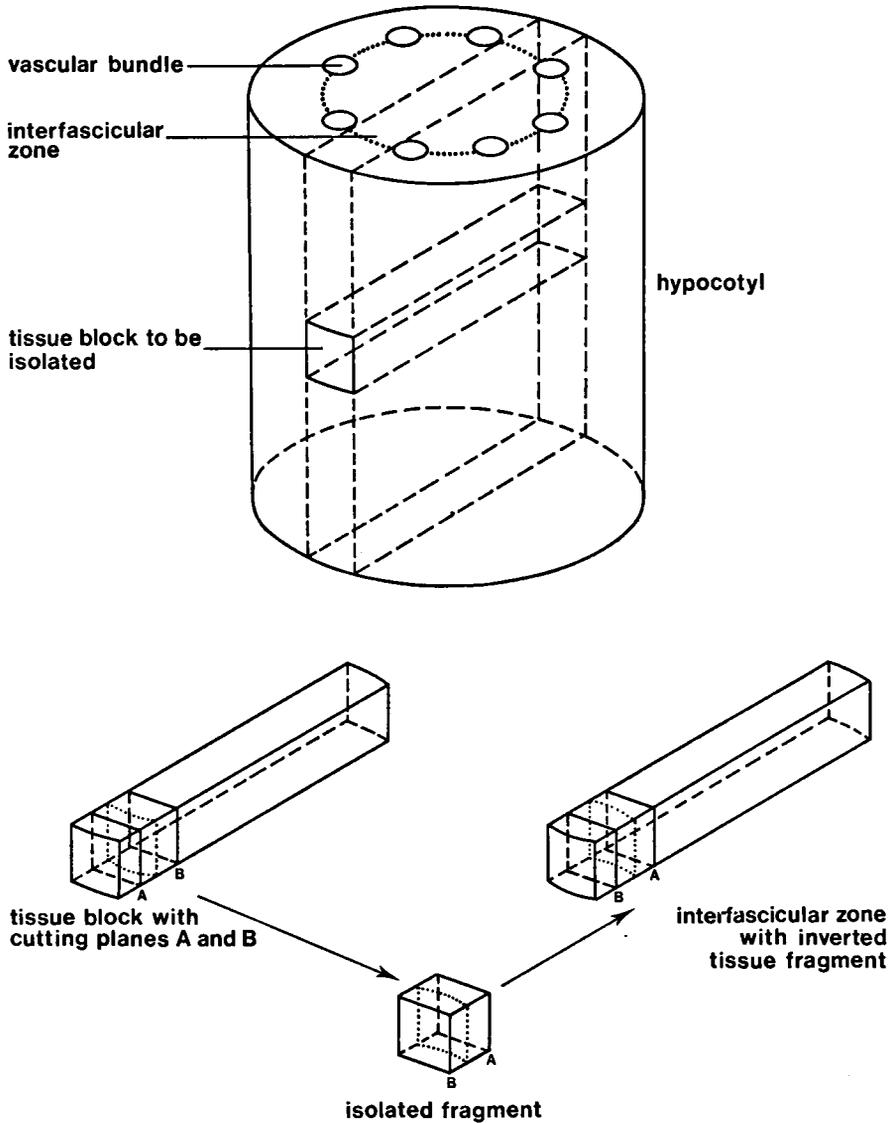


Fig. 1. Schematic representation of experimental inversion of interfascicular tissue.

of various histological complications. Some of the inverted tissue blocks did not unite with the adjacent tissue or had obviously been placed in an incorrect position, others were not exclusively interfascicular and contained parts of a vascular bundle.

The results of the seven successful inversion experiments were uniform. In all

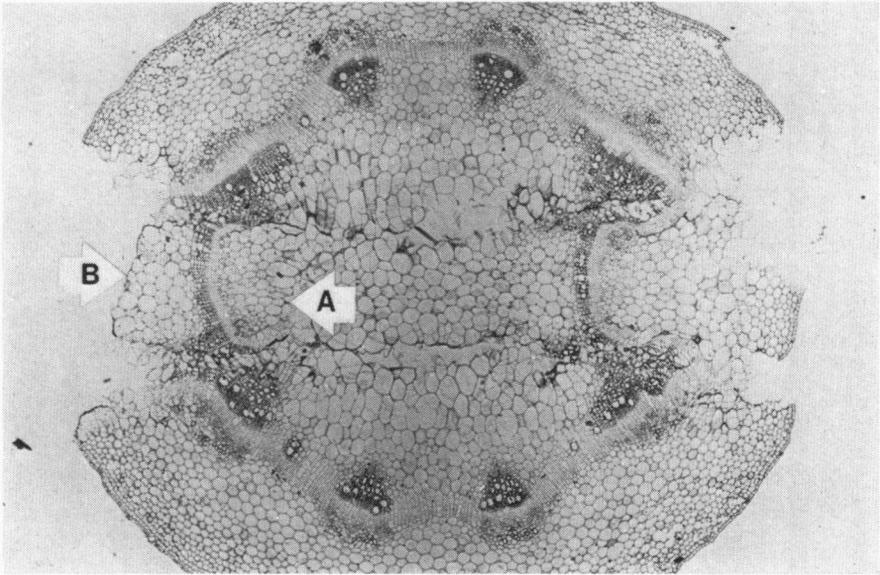


Fig. 2. Hypocotyl with inverted interfascicular tissue (BA), 10 days after inversion. At the moment of inversion the seedling was 7 days old and exhibited no sign of secondary growth. $\times 13$.

cases cambium had formed at the original site in the inverted tissue block. The xylem and phloem were formed at the topographically outer and inner side of the tissue block, respectively (*figs. 2 and 3*). There was no sign of adaptation of the inverted interfascicular tissue to the new position within the hypocotyl.

Under our conditions of cultivation the formation of the first tangential walls in the interfascicular zone takes place on the 11th day. This process starts at the base of the hypocotyl and extends to the apical region. In the successful experiments the age of the seedlings at the moment of inversion was 6, 7, or 8 days. It may therefore be concluded that at least several days before the first demonstrable sign of cambium initiation the radial polarity is already determined in the cells concerned. Plants less than six days old could not be used because of difficulties in manipulating the very small samples of interfascicular tissue.

None of our anatomical observations suggest the existence of an inductive effect exerted by an initiated cambium on the adjacent parenchyma cells. This holds particularly for the experiments in which the replaced interfascicular tissue was found to lie in an incorrect position (*fig. 4*).

3.2. Pith parenchyma grafts

The present experimental results do not exclude an inductive effect from the vascular bundle on radially unpolarized tissue. Central pith parenchyma may be regarded as such a tissue. Preliminary experiments in which interfascicular tissue (AB in *fig. 1*) was replaced by a block of pith parenchyma of the same size failed

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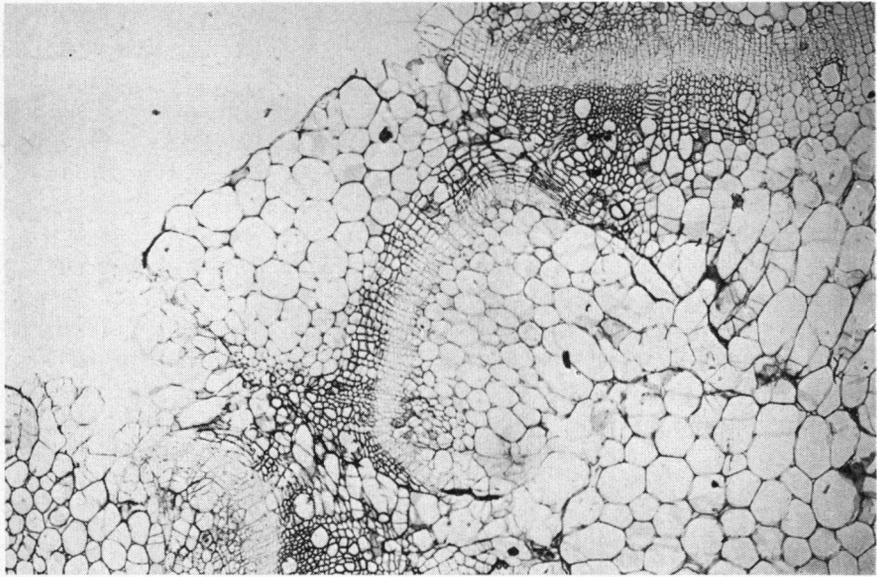


Fig. 3. Detail of fig. 2, showing inverted tissue block and surrounding tissue. Note curvature of the cut cambial ends and the inverted position of the xylem. $\times 30$.

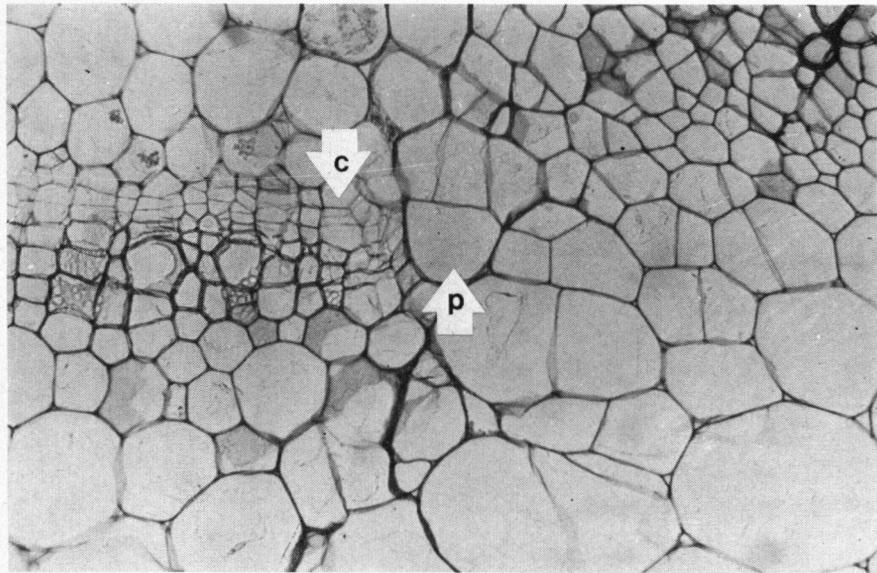


Fig. 4. Interfascicular tissue (left), shifted inward to give it a position contiguous with the pith parenchyma (right), 9 days after the operation. At the moment of the operation the seedling was 7 days old. Note absence of inductive effect from the developing cambium (c) on the pith parenchyma cells (p). $\times 60$.

because of necrosis of the inserted tissue. The injurious effect on the relatively large parenchyma cells was apparently too great. A better method is to replace the entire excised tissue by one piece of pith parenchyma. For this purpose we isolated a longitudinal block of central pith parenchyma by inserting a micropunch perpendicular to the wound surface of a decapitated hypocotyl. In fifteen experiments the tissue block thus obtained was grafted in a radial position in an interfascicular cavity, made with the same micropunch, in the hypocotyl of another plant. None of the seven grafts with complete union between the graft and surrounding tissue showed a tendency of the cambial ring to close through the pith parenchyma cells.

In general, little or no inductive effect from the cambium on the pith parenchyma cells was observed. Only in a very few cases did orientation of the newly formed walls in the inserted pith parenchyma seem to have some relationship with the orientation of the walls in the adjacent cambial tissue (*fig. 5*). This effect is, however, very limited, and did not exceed the one-cell range to any great extent. A beginning of a closure of the cambium through the cells of the graft was observed only in cases where the outermost cells of the graft showed dedifferentiation as a result of a marked wound reaction and the adjacent cambium was situated close to the wound tissue (*fig. 6*).

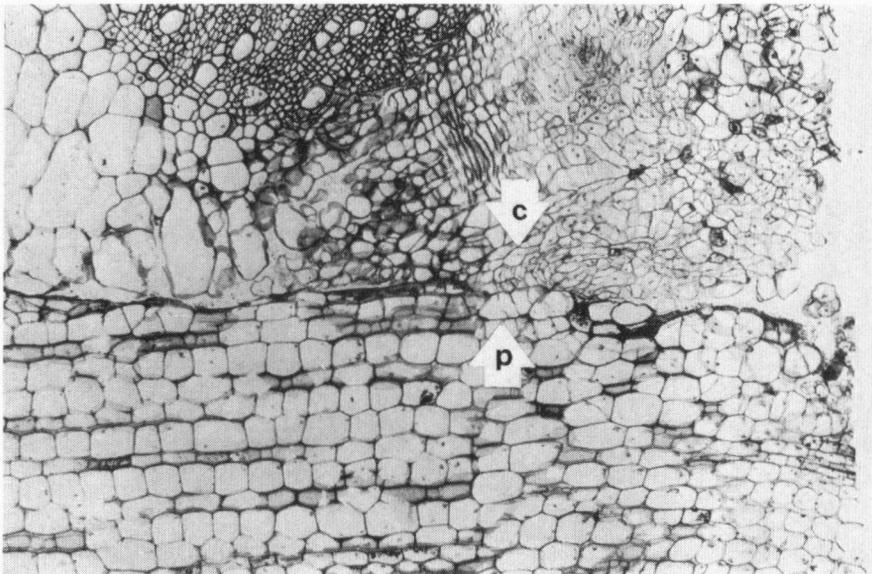


Fig. 5. Limited induction at the margin of a developing cambium (upper region) and a pith parenchyma graft (lower region), 20 days after the operation, performed when both seedling and graft were 9 days old. Note orientation of cell division in the parenchyma cell (p) adjacent to the cambium (c) and absence of cambial ring closure through the graft. $\times 47$.

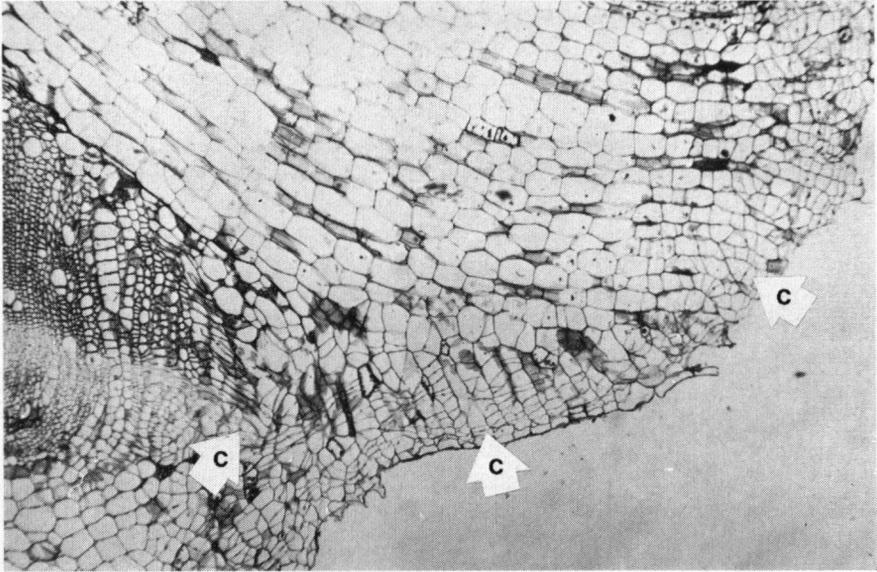


Fig. 6. Experiment similar to the one shown in Fig. 5. Cambial ring (c) beginning to close through dedifferentiated cells of a pith parenchyma graft (right). $\times 37$.

4. DISCUSSION

The vascular cambium is highly suitable for the study of radial polarity because of the very clear bipolar activity of this tissue. But polarity is only one aspect of cambium initiation, others being the special site at which the cambium is initiated and the given time at which the first tangential walls are formed. In considering "induction" of the interfascicular cambium we must keep in mind which of these aspects is in fact meant.

The initiation of the interfascicular cambium has been taken as a good example of homogenetic induction (LANG 1965), i.e., an induction resulting in the development of a tissue identical or very similar in differentiation to the neighbouring tissue (*fig. 7, B*). Here emphasis is placed on the type of tissue formed as a result of the induction and not on the position, polarity, and time sequence of initiation.

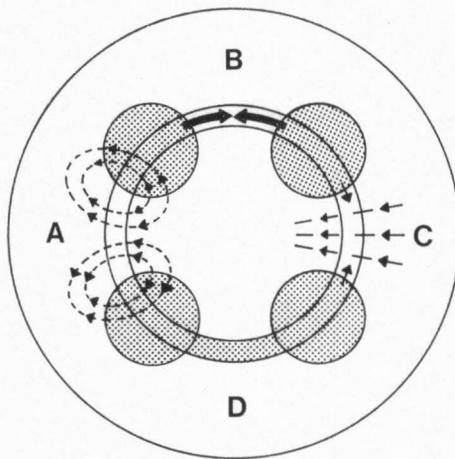
The existence of an inductive effect arising from the fascicular cambium and acting on the intermediate ray parenchyma cells is stated in textbooks by BÜNNING (1953), ESAU (1965), and MOHR (1969). The only evidence available for such an effect, however, is descriptive, being deduced from a developmental pattern in which cambial activity begins in the vascular bundles and then extends to the space between them. To the best of our knowledge this has only been described for *Aristolochia* (BÜNNING 1956). Nevertheless, the consecutive occurrence of two similar, juxtaposed structures does not necessarily mean that the second structure has a causal relationship with the first.

The vascular bundle has also been assumed to exert an influence on the events in the interfascicular region by the induction of a hydrogen ion gradient. According to PRATT (1948) pH is one of the main factors governing the development of cells, and PRIESTLEY (1928) supposed the relatively acid xylem and alkaline phloem to be responsible for the disposition of an ionic gradient within and between the vascular bundles (*fig. 7, A*), this gradient determining the place of initiation (in the zone of intermediate pH) and inducing the polarity (by the direction of the gradient) of both fascicular and interfascicular cambium.

For the regenerating cambium J. & P. M. WARREN WILSON (1961) put forward a hypothesis based on the assumption of a gradient from an exposed surface inward. The cambium can arise in only one position on this gradient (where an unknown factor is at an appropriate level), the orientation of the cambium being determined by the direction of the gradient. Once established in differentiated tissues (xylem, cambium, phloem), the levels of the unknown factor tend to induce similar levels in contiguous undifferentiated tissue. Applied to the problem of the initiation of the interfascicular cambium, this hypothesis implies that the vascular bundle can determine the location and polarity of the interfascicular cambium, albeit over a limited distance. In wide interfascicular regions such as occur in the Castor bean hypocotyl, a direct influence from a radial, surface-dependent gradient may be involved (*fig. 7, C*).

The cited literature is in agreement on one point, namely that the initiation of the interfascicular cambium is in some way dependent on its position between the vascular bundles or, in terms of the gradient induction theory, on its position with respect to the outer surface.

A rather different approach to the problem of the initiation of the cambium can be derived from the fact that in the very early development of every shoot



initiation process dependent on inductive influence:

A. hydrogen ion gradient originating from the vascular bundle (Priestley, 1928; Pratt, 1948).

B. inductive effect from the fascicular cambium (Bünning, 1953; Esau, 1965; Mohr, 1969).

C. surface-dependent gradient (J. & P. M. Warren Wilson, 1961; modified).

initiation process independent of surrounding tissues:

D. determined at the procambial stage (Lang, 1965).

Fig. 7. Schematic representation of hypotheses concerning the initiation of the interfascicular cambium.

there is a stage in which a closed primary meristem ring is present (KAUSSMANN 1963). The meristem ring is ontogenetically related not only to the later vascular bundle tissue but also to the cell layer(s) in which the interfascicular cambium will subsequently be formed. It seems therefore possible that the initiation process has already been determined when this young stage is reached. There is no reason to restrict this assumption to plants with a closed procambial ring – the type mentioned by LANG (1965) – or exclusively to the position of the cambium and ignoring the polarity as argued by RZIMANN (1932). This leads to another possibility, namely that the whole initiation process, including the fixation of the radial polarity, is determined in the primary meristem ring and that further differentiation can proceed without any inductive influence from the vascular bundles or the outer surface (*fig. 7, D*). That the Castor bean has a primary meristem ring was demonstrated by SARKANY (1936).

Our results provided no evidence indicating the existence of an inductive influence. In the inverted tissue blocks the cambium was always formed at the same position, i.e. through the band of relatively small cells just inside the innermost cortical layer. Stellar or cortical parenchyma cells inadvertently placed adjacent to a developing cambium showed no tendency for cell division.

The radial polarity in the inverted tissue always represented the situation before the operation. In other words, there was no sign of adaptation to the new situation. Cambial ends with the same polar orientation tend to unite, whereas inverted cambial ends exhibit a strong tendency to bend off. JANSE (1921) has described a single case in which a junction between inverted cambial ends was observed. RZIMANN did not observe any similar case in her material, nor have we seen any development of this kind.

On the basis of our results we conclude that there is a direct ontogenetical continuity between the primary meristem ring as defined by KAUSSMANN (1963) or the residual meristem as defined by ESAU (1965) and the layer in which the interfascicular cambium is formed. During the development of the hypocotyl it is indeed possible to identify a more or less characteristic layer of relatively small parenchyma cells with its origin in the primary meristem ring and in which the cambial walls can later be seen. Because of its determined state and the possible identification we propose that this layer be called “precambial layer”.

If we accept the theory that the initiation process is determined as early as the primary meristem ring stage and that the vascular bundle exerts no influence at all on the development of the interfascicular cambium, we would expect an isolated piece of interfascicular tissue, taken from a very young hypocotyl and cultured *in vitro*, to show an almost normal initiation of the cambium. The results of our experiments performed to investigate this point will be reported elsewhere (SIEBERS 1971).

The present results clearly show that the interfascicular cambium does not offer appropriate material for the study of an induction process. From a theoretical point of view the regenerating cambium would be suitable material. The position of this newly formed cambium and its radial polarity must inevitably be related in some way to the exposed wound surface. Experiments in which wound

tissue was inverted after various periods of exposure failed, because the cells exhibited no mitotic activity after the operation. The development of a regenerating cambium appears to proceed only under the continuous influence of an exposed wound surface.

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