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Auxin Transport in Secondary Tissues

A. R. SHELDRAKE
Department of Biochemistry, University of Cambridge

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ABSTRACT

Auxin transport was investigated in excised stem segments of *Nicotiana tabacum* L. by the agar block technique using [1-^{14}C] indol-3yl-acetic acid (IAA). The ability of the stems to transport auxin basipetally increased as secondary development proceeded; by contrast the ability of the pith to transport auxin declined with age. By separation of the stem tissues it was shown that the great majority of auxin transport took place in cells associated with the internal phloem and in cells close to the cambium; in both cases similar velocities of transport were found (*c.* 5·0 mm h^{-1} at 22 °C). The effects of osmotic gradients on auxin transport through the internal phloem were investigated. IAA was found by chromatography to account for practically all the radioactivity in receiver blocks and ether extracts of stem segments. The significance of these results is discussed.

INTRODUCTION

Most investigations of polar auxin transport have been carried out on seedlings or on young plants. Fewer studies have been made of woody stems, but there is convincing evidence that they are also capable of polar auxin transport (van der Weij 1933; Oserkowsky, 1942; Gregory and Hancock, 1955; Balantinecz and Farrar, 1966). A general conclusion which has been established by work on young plants is that basipetal auxin transport declines as a tissue grows older (Jacobs, 1950; Leopold and Guernsey, 1953; Leopold and Lam, 1962). For this reason in woody stems the primary tissues, which are old or may even be dead, are unlikely to be responsible for a very significant amount of the auxin transported. This implies that secondary tissues are capable of polar auxin transport. I have obtained results which confirm this conclusion.

MATERIALS AND METHODS

Tobacco plants (*Nicotiana tabacum* var. Java, a variety with long internodes) were grown from seed in a greenhouse and kindly supplied by Mrs. M. I. Instance of Rothamsted Experimental Station. They were used when they were 60–90 cm high (3–5 months old), before the onset of flowering.

Transport of auxin was measured in excised segments, 7 mm long unless otherwise stated, which were placed horizontally on a glass slide. The segments rested on a strip of filter-paper coated with petroleum jelly to prevent the capillary movement of water between the segment and the slide. Agar (1·5 per cent w/v) donor and receiver blocks were used. Receiver blocks were applied to the segments first. Donor blocks contained [1-^{14}C]-indol-3yl-acetic acid (IAA) obtained from Amersham (as NH_4^+ salt, 57 mCi/mmol) at a concentration of 3·0 μM. In most experiments blocks of constant size were used; but in experiments where stem segments of

widely differing diameters were compared, the size of the agar blocks also differed. This could introduce a source of error if the segments transported auxin not as a function of the concentration of auxin applied but as a function of the absolute amount. However, control experiments with tobacco stem segments showed that the size of the agar donor block had little or no effect on the amount of auxin transported under the experimental conditions used in this work. During the transport period (usually from 2 to 4 h) the segments were kept in the dark in a Petri-dish containing moistened filter-paper. The laboratory temperature was 22 ± 1 °C.

At the end of the transport period the receiver blocks were placed in scintillation vials with 4 ml of scintillation fluid (Bray, 1960) and counted on a Nuclear Chicago scintillation counter for at least 10 min. Blanks containing plain agar blocks were used for background measurements, and background readings (25–30 ct/min) were subtracted from all results.

Auxin was extracted from stem segments with peroxide-free ether at 2 °C for two periods of 2 h each. The extracts were pooled, evaporated to a small volume, transferred quantitatively to scintillation vials, and evaporated to dryness. Scintillation fluid (4 ml) was added and the samples were counted as above. The quenching caused by similar ether extracts of unlabelled tissue was determined and experimental results were corrected appropriately.

For chromatographic investigation of radioactive material, extracts of tissue and of receiver blocks were prepared as above. The samples were applied to the origin of cellulose thin-layer plates which were developed with isopropanol/ammonia/water (10 : 1 : 1 v/v/v). After the plates had dried the cellulose was scraped off in appropriate zones and placed in scintillation vials to which scintillation fluid was added as above. These were shaken several times and allowed to settle before counting.

RESULTS

A very high degree of polarity of auxin transport was found in segments of tobacco stem; in most experiments no significant counts were found in acropetal receiver blocks. The amounts of auxin transported in a given time by successive segments of a single internode were similar except for segments taken from the apical end of the internode, which generally transported larger amounts of auxin. Therefore when comparisons were made between segments or between internodes, only segments from the middle of the internode were used.

A comparison of the ability of successively older internodes to transport auxin showed an initial fall followed by a rise (Fig. 1). The increase first appeared in internodes which had stopped growing longitudinally and in which secondary thickening had begun. Thereafter, in successively older and thicker internodes, more auxin was transported basipetally. Similar results were obtained in three separate experiments.

The bark could be stripped off easily from segments in which secondary thickening was taking place. This process results in a clean separation of tissues in the region of the cambium (Figs. 12 and 16 in Sheldrake and Northcote, 1968). Bark and inner tissues separated in this way are known to form a callus only at the basal end (Sheldrake and Northcote, 1968) suggesting that both are capable of polar auxin transport. This was investigated directly. The amounts of auxin transported basipetally by the bark and inner tissues from successively older internodes are shown in Fig. 1. A striking feature of these results is that the sum of the amounts of auxin transported through the separated bark and inner tissues is considerably less than that transported through complete stem segments from the same internode.

The 'inner tissues' consisted of the xylem, internal phloem, and pith. Pith was excised and tested for its ability to transport auxin. Although polar auxin transport could be detected, the amounts transported were very small and showed that the

pith could account for only a small fraction of the auxin transported by the combined inner tissues. The transport of auxin in pith segments of different ages was examined using pith excised from successively older internodes. There was a marked decline, more steep in the younger internodes, in the amount of auxin transported

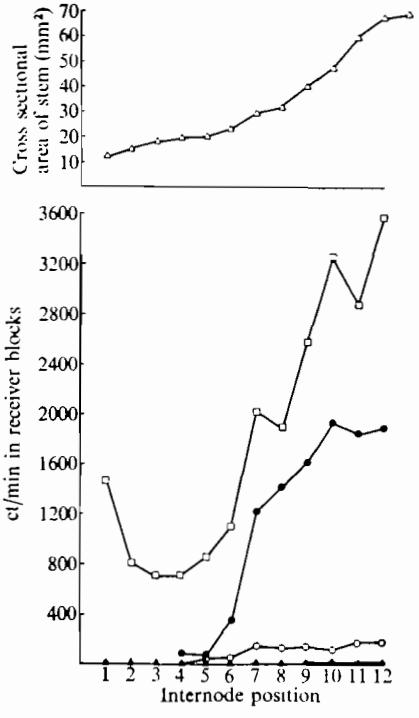

Fig. 1.

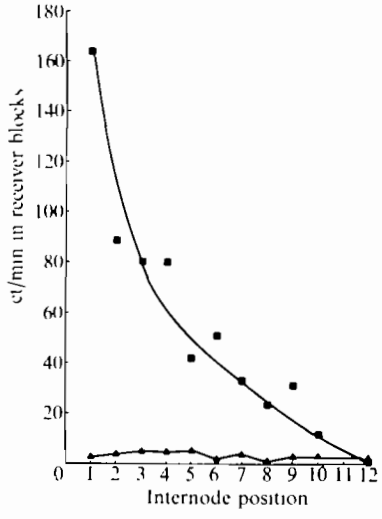

Fig. 2.

Fig. 1. Transport of auxin by segments of tobacco stem and by separated bark and inner tissues. Transport time 3·5 h. —□— stem segments, basipetal transport; —▲— stem segments, acropetal transport; —○— bark, basipetal transport; —●— inner tissues, basipetal transport. Two sections were used for each experimental point.

Fig. 2. Transport of auxin by isolated pith from tobacco stem segments. Transport time 3·5 h. —■— basipetal transport; —▲— acropetal transport. Three segments were used for each experimental point except in the case of internodes 1 and 2 where one and two segments were used respectively and the results were corrected appropriately.

basipetally (Fig. 2). Practically no acropetal movement was found in any samples. The progressive decline of auxin transport in the pith of successively older internodes contrasts strongly with the progressive increase found in whole stem segments and in the isolated inner tissues (Fig. 1).

In segments which had been longitudinally bisected, the xylem tissue could be separated from the internal phloem and the outer layers of the pith by scraping off with a scalpel all tissues interior to the xylem. Microscopic examination showed that this procedure did indeed free the xylem from both pith and internal phloem. Xylem tissue obtained in this way showed no detectable ability to transport auxin (Fig. 3). Therefore the internal phloem, or cells closely associated with it, must account for the great majority of the auxin transported by the inner tissues (Fig. 3).

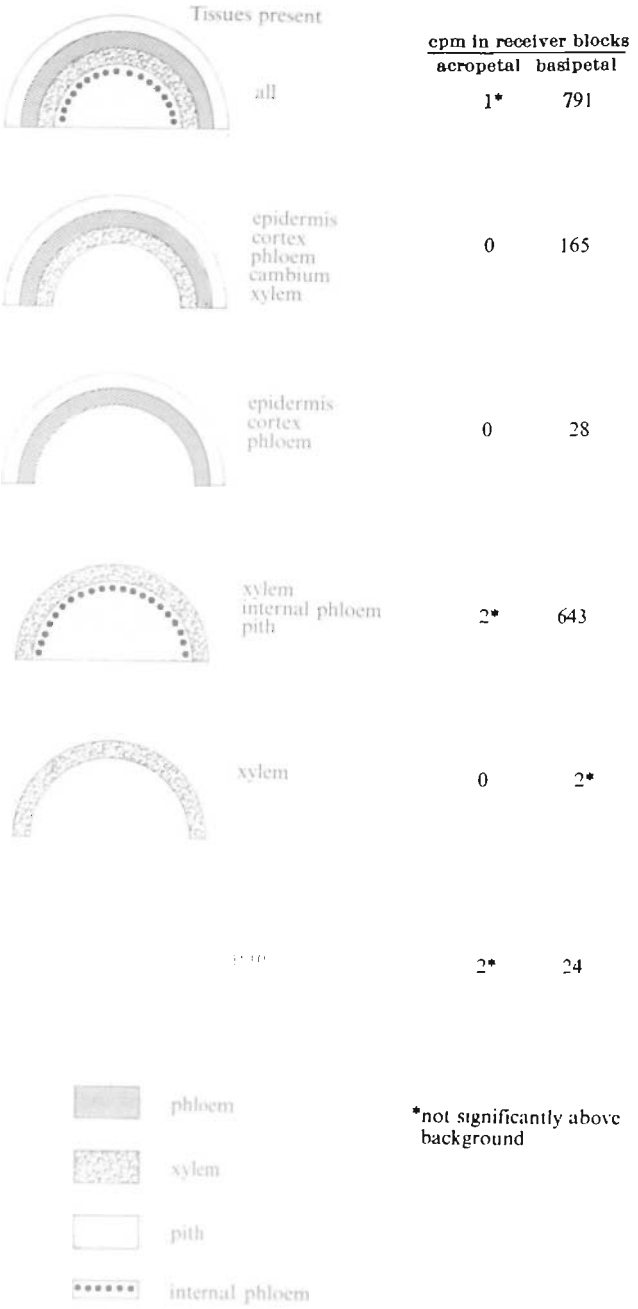

FIG. 3. Auxin transport by tissues of tobacco stem segments. Transport time 3·5 h.

The pith and internal phloem were removed from longitudinally bisected segments of stem from which the bark had not been removed. The amounts of auxin transported by such segments were considerably greater than the amounts transported by the separated bark (Fig. 3). Since the xylem tissues do not transport auxin, this result can only mean that cells with a high transporting capacity for auxin are

damaged or killed when the bark is stripped from the inner tissues. As in Fig. 1, the results in Fig. 3 reveal a considerable discrepancy between the sum of the amounts of auxin transported through the bark and inner tissues compared with complete stem segments. However, the sum of the amounts transported through the inner tissues and through the xylem+cambium+bark segments is in close agreement with the figure for the complete stem segments. This is the result that would be expected if the xylem tissue makes no contribution to the polar transport of auxin.

TABLE 1. *Effects of osmotic gradients on auxin transport by 'inner tissue' segments*

Transport time 3·5 h. Two segments were used for each test

Donor block	Receiver block	ct/min in receiver blocks	
		Acropetal	Basipetal
Water	Water	2*	94
Water	0·6 M sorbitol	0	42
0·6 M sorbitol	Water	3*	191
0·6 M sorbitol	0·6 M sorbitol	0	142

* Not significantly above background.

The internal phloem undergoes progressive development as the internodes grow older. The number of strands seen in transverse segments increases: for example, in the plant used for the experiment shown in Fig. 1, internode 1 had 35 strands, internode 4 had 47, internode 7 had 70, internode 10 had 76. Furthermore new phloem cells are added to existing strands. Esau (1938) has studied the secondary development of the internal phloem of tobacco in detail and has shown that new strands and new phloem cells originate from cells in the outer region of the pith. Since the internal phloem accounts for the great majority of the auxin transport in the 'inner tissues', the increasing ability of the inner tissues of older internodes to transport auxin seems very likely to be associated with the progressive secondary development of the internal phloem. This accounts for part of the increase in auxin transport found in older stem segments (Fig. 1); the remainder of the increase must be associated with the progressive activity of the cambium.

The effects of osmotic gradients on auxin transport through 'inner tissue' segment were examined in experiments using donor and/or receiver blocks containing 0·6 M sorbitol. The results (Table 1) show that a high osmotic pressure in receiver blocks reduced, and in donor blocks stimulated basipetal auxin transport considerably. Similar results were obtained using mannitol as the osmoticum. This phenomenon recalls the results of McReady and Jacobs (1967) who found that the presence of 0·2 M mannitol in donor and receiver blocks increased basipetal auxin transport through segments of young *Phaseolus* petioles.

The velocity of auxin transport in internode segments was determined by the classical method of van der Weij (1932). Older internodes showed a velocity of about 5·0 mm h^{-1}; younger internodes showed a slightly lower velocity. The velocities of transport in separated tissues were also determined. Results of an

experiment of this type are shown in Fig. 4. Lines of closest fit were calculated by the least squares method and the velocities of transport given by the positions of the intersects were: complete segments 5·1 mm h^{-1}, inner tissues 5·1 mm h^{-1}, xylem+cambium+bark 5·0 mm h^{-1}, bark 3·8 mm h^{-1}, pith 3·1 mm h^{-1}. Thus the velocities found for the inner tissues and for the xylem+cambium+bark segments were very similar to each other and to the velocity found in complete stem segments. The lower velocities found in the pith and separated bark are associated with a lower capacity for transport and probably reflect the effects of age: the cells in the separated bark responsible for auxin transport are likely to be ones some distance from the cambium since they survive the process of stripping.

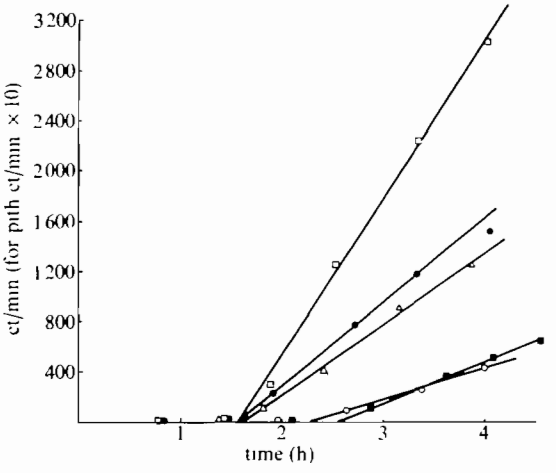

FIG. 4. Rates of basipetal auxin transport by tobacco stem tissues (8-mm segments). —□— complete stem segments; —●— inner tissues; —△— xylem+cambium+bark; —○— bark; —■— pith. The slopes of the lines and positions of intersects were calculated by computer. The last time point for the inner tissues was ignored since inner tissues were found in other experiments to show a decline in rate of transport after about 3·5 h.

The relative abilities of the different tissues to take up auxin from donor blocks and to transport it was also examined by the extraction of auxin. Segments of stem (1 cm) were supplied with donor and receiver blocks and at the end of a 4-h transport period were cut transversely into four 2·5 mm segments. The tissues of these were separated into bark, xylem+internal phloem, and pith. The amounts of radioactivity found in ether extracts of these tissues are shown in Fig. 5. Substantially similar results were obtained in several comparable experiments. In the acropetal system insignificant quantities of auxin were transported and the uptake of auxin was less than that found in the basipetal system. In both cases the highest uptake occurred in the xylem+internal phloem tissues; in the basipetal transport experiment the movement of auxin was greatest in these tissues, less in the bark, and least in the pith. These results confirm those obtained in the experiments on auxin transport through separated tissues.

Experiments involving further separation of tissues from stem segments which had been transporting auxin were carried out in order to obtain a clearer idea of the site of auxin transport in the bark and in the cambial region. The middle 5-mm

portions of 1-cm stem segments were separated as described above and two further samples were obtained by scraping the cambial face of the xylem with a scalpel and by scraping the phloem region from the bark. In the former case the scrapings contained undifferentiated cells of the cambial region and differentiating and recently mature xylem cells; the scraping of the bark caused the removal of the

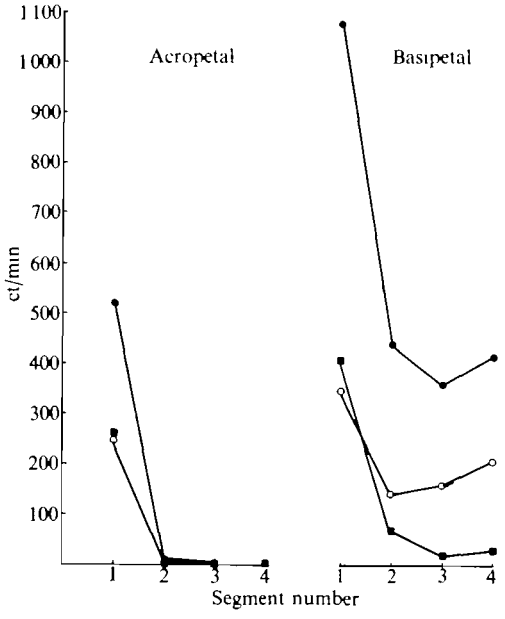

FIG. 5. Ether-extractable radioactivity in tissues of tobacco stem segments. Two 1-cm stem segments were used; they were cut transversely into four 2·5-mm segments at the end of a 4-h transport period and the tissues were separated and extracted with ether. Segments were numbered from 1 to 4; segment 1 was adjacent to the donor block, segment 4 adjacent to the receiver block. —●— xylem + internal phloem; —○— bark; —■— pith.

cambial region and most of the mature phloem tissues as well. Ether extracts of the samples were counted for radioactivity. Results of a typical experiment are shown in Table 2. Although the percentage of total counts in the scrapings from the cambial face of the xylem was low, these samples contained high amounts of radioactivity per unit fresh weight. The expression of results on this basis does not permit a fair comparison with the internal phloem (since the sample containing this

TABLE 2. *Radioactivity in separated tissues of tobacco stem segments*

The middle 5 mm portions of two 1 cm segments were excised at the end of the transport period (3·5 h). Ether extracts of their separated tissues were counted. The results have been corrected for quenching.

	Bark (after scraping)	Phloem	Xylem scrapings (from cambial face)	Xylem + internal phloem	Pith
ct/min	300	358	62	1170	179
% of total	14·5	17·3	3·1	56·4	8·6
ct/min/100 mg f.w.	187	1021	1014	432	169

also contained the xylem tissue and some pith) but the amount of auxin per unit weight found in the cambial scrapings from the xylem can be compared directly with that in the external phloem tissues and is similar. These results suggest that auxin is transported as effectively by young cells on the xylem side of the cambium as by cells on the phloem side.

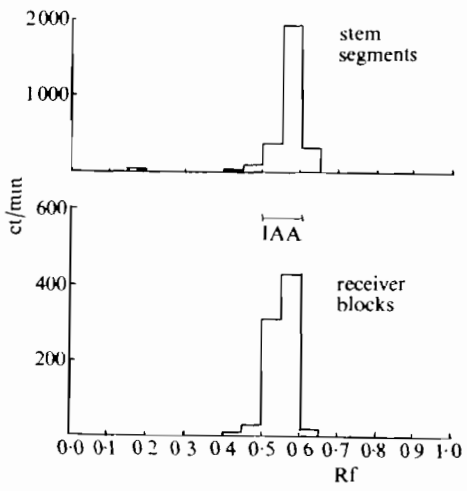

FIG. 6. Distribution of radioactivity on chromatograms of ether extracts of tobacco stem segments and receiver blocks after 3·5 h basipetal transport. All parts of the stem segments were extracted except the region immediately adjacent to the donor block.

Practically all the radioactivity present in ether extracts of tissues and of receiver blocks was found on chromatograms at the Rf of IAA (Fig. 6).

DISCUSSION

The ability of tobacco stems to transport auxin after the bark has been removed depends on the internal phloem and, to a much lesser extent, the pith (Fig. 3). In woody stems which have no internal phloem and in which the pith is old or dead, removal of the bark would therefore be expected to leave no pathway for polar auxin transport. 'Ringing' the branches or trunks of trees does in fact lead to effects which are consistent with an interruption of auxin transport, such as root and callus formation above the ring and shoot formation below (e.g. Vöchting, 1878).

The loss of the majority of the ability to transport auxin in the tissues exterior to the xylem which occurs when the bark is stripped off (Fig. 1, Fig. 3) suggests that the cambium and/or young cells derived from it are the most active in auxin transport, since these are the cells which would be expected to suffer most damage in the stripping process.

A similar conclusion is implicit in the results of Söding (1937, 1940). He found considerable amounts of auxin in the region of the cambium and its young derivatives, but very little in older tissues of the phloem and the xylem. Auxin is probably produced as a consequence of cambial activity (Söding, 1937, 1940; Dörffling, 1963; Sheldrake and Northcote, 1968) and therefore the distribution of auxin in the tissues of the cambial region is likely to reflect the site of its production as well as the site of its transport; but whatever are the relative contributions of these two processes Söding's data make it clear that both must be confined to cells quite near the cambium.

Cells on both sides of the cambium appear to be capable of polar auxin transport (Table 2), although much more occurs on the phloem side. On the xylem side the ability to transport auxin must be confined to the youngest cells: as differentiation proceeds the xylem vessels and tracheids die and cannot retain any potential for auxin transport. The inability of separated xylem tissue to transport auxin (Fig. 3) shows that the only living cells present, the xylem parenchyma, are also unable to

transport auxin. On the phloem side, the ability to transport auxin persists to a limited extent in cells far enough away from the cambium not to be destroyed when the bark is stripped off (Fig. 1; Fig. 3). However, mature sieve tubes are unlikely to be responsible for polar auxin transport (although auxin can be shown to travel in the translocation stream both acropetally and basipetally if applied to leaves in high concentration (Eschrich, 1968)). The rates of movement of translocated substances in the phloem are much higher than the velocity of auxin transport; and phloem transport is non-polar and probably depends on some sort of osmotically directed mass-flow (MacRobbie, 1971). The results in Table 1 show that the application of osmotic gradients to excised segments in a direction which might be expected to inhibit or reverse an osmotically directed mass-flow in fact stimulates auxin transport. Therefore if any polar auxin transport does occur in mature sieve tubes it does so not as a result of their specialized mechanism for translocation, but by virtue of features which they share with undifferentiated or parenchymatous cells. Since sieve tubes have so few features in common with such cells, it is difficult to imagine how any conceivable mechanism for polar auxin transport could function in much the same way in both. I conclude that sieve tubes, like xylem vessels and tracheids, probably lose any ability to transport auxin that they may have possessed by the time that their differentiation is complete. On the other hand, one or more of the other cell types present in phloem tissue (companion cells, parenchyma and fibres) may well retain a declining ability to transport auxin as they mature.

The marked ability of newly formed or differentiating cells of the secondary vascular tissue to transport auxin basipetally agrees well with the results of Sachs (1968, 1969) who has demonstrated indirectly that regenerating or artificially induced vascular strands (or cells closely associated with them) not only form under the influence of auxin and in positions determined by the initial polarity of movement of auxin but also acquire an increased ability to transport auxin.

One of the more mysterious aspects of auxin transport is the question of how the polarity of the cells comes to be established in the first place. Osborne (1968) has suggested that in tissues arising from apical meristems by sequential divisions the difference in age between the apical and basal ends of the cells could account for their polarity. But this hypothesis cannot explain the polarity of secondary tissues, which arise not by transverse but by longitudinal divisions. Went (1941) found that acropetal auxin transport could be detected in inverted *Tagetes* cuttings several weeks after roots had been induced to form at the apical end and shoots at the basal end. The original basipetal polarity persisted as well; Went suggested that the new polarity was established only in cells (presumably cambial derivatives) formed after the inversion of the cuttings. This implies that polarity is determined by chemical or physical gradients to which the newly formed cells are exposed, at least in the case of secondary tissues.

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