Polarity and the Induction of Organized Vascular Tissues

Author(s): T. SACHS

Source: *Annals of Botany*, MARCH 1969, New Series, Vol. 33, No. 130 (MARCH 1969), pp. 263-275

Published by: Oxford University Press

Stable URL: https://www.jstor.org/stable/42908125

Polarity and the Induction of Organized Vascular Tissues

BY

T. SACHS

Department of Botany, The Hebrew University, Jerusalem, Israel

With three Plates

Date accepted: 12 September 1968

Abstract

This work deals with those properties of plant tissues which are responsible for the organization of vascular cells in ordered strands. It is shown that auxin alone is sufficient to cause the differentiation of strands of xylem cells in the parenchyma of pea roots. An artificially induced strand, once it is formed, attracts towards itself newly induced vascular strands, and this attraction results in the union of old and new strands. It is also shown that the application of auxin to natural vascular tissues prevents their being joined by newly induced vascular strands. It is proved that this is dependent on a directional effect and not simply on a local accumulation of auxin.

To understand these results, it must be assumed that the polarity in terms of auxin transport is increased during the process of vascular tissue induction. The same polarity, once established, is maintained by the presence of auxin, so that the differentiation of strands perpendicular to the axis of this polarity is prevented. These characteristics of plant tissues concerning auxin transport explain the basic phenomena of the organization of vascular cells in defined and ordered strands.

Introduction

THE differentiation of the vascular tissues of higher plants is induced by the growing leaves, and there is good evidence that the stimulus involved is auxin (Jacobs, 1956; Jacobs and Morrow, 1957; Wetmore and Rier, 1963; Wangermann, 1967; Sachs, 1968*a*, *b*). The concept of induction, however, does not account for the fact that the vascular tissues differentiate in well-defined and ordered strands. If differentiation were to depend only on the concentration of a diffusing substance, the vascular tissue which would differentiate below a source of inductive influence would have the shape of hemispheres rather than that of longitudinal strands. This work deals with the properties of plant tissues which are responsible for the arrangement of the vascular elements.

The fact that vascular tissues form in longitudinal strands of cells suggests that some influence must pass from one cell to another. It has been shown that vascular differentiation is due to an inducing stimulus coming from only one end of the vascular system, that of the shoot apex, while the root acts as a sink into which this stimulus flows (Sachs, 1968*a*). The inducing substance should thus pass along longitudinal files of cells. It may be suggested, therefore, that a basic concept necessary to account for the pattern of vascular tissue

[Ann. Bot. 33, 263–75, 1969].

differentiation is that the inducing stimulus flows through the differentiating strand.

This suggestion is supported by the fact that when a vascular strand is wounded a bridge of both xylem and phloem elements differentiates around the wound (Simon, 1908; Kaan Albest, 1934; Jost, 1940; Jacobs, 1956). This regeneration around wounds may be understood if it is assumed that the normal flow of an inducing stimulus through the vascular system is interrupted by the wound. The stimulus is, therefore, forced to pass through the parenchyma cells around the wound, and it causes them to change to xylem and phloem elements.

The concept of differentiation occurring along the path of the flow of an inducing stimulus, however, does not in itself account for the formation of defined and well-ordered strands. The experiments presented here are aimed at an understanding of this aspect of vascular differentiation. Advantage is taken of the influence of the presence of a pre-existing strand on the location of a newly induced vascular strand. Such a new strand forms in the direction of union with a pre-existing strand which is poor in auxin and is inhibited from forming in the vicinity of a pre-existing strand which is well supplied with auxin (Sachs, 1968*b*).

Materials and Methods

Pisum sativum, var. *arvense* Poir. was used for the experiments reported in this paper. The seeds were supported on gauze and the roots grew into tap water. The plants were kept at a constant temperature of 24 ± 1 ° C and under continuous fluorescent light. The development of the plants depended on the materials stored in the cotyledons. The details of the procedure have been described earlier (Sachs, 1968*b*).

Experiments with root tissue were performed 2 days, and those with stem tissue 4 days, after soaking the seeds. After treatment the plants were kept under the same conditions as those in which they were germinated.

Indole-3yl-acetic acid (IAA) was applied in a lanolin solution, the concentration of which is given in the text. About 0·1 mg of lanolin was applied in each treatment, but this amount was only estimated approximately.

Results and Discussion

1. *The induction of xylem strand formation by auxin*

The first experiment to be reported here was designed to test whether IAA can alone cause the differentiation of vascular elements arranged in strands. If IAA alone can induce such strands then the properties responsible for the ordered differentiation of the vascular system may be sought in the reaction of plant tissues to this substance.

Auxin applied to wounded plants can cause the formation of new vascular strands, but these strands are generally short, as they immediately join the vascular tissues already present (Jacobs, 1956; Sachs, 1968*a*). When auxin

is applied to tissue cultures, which are devoid of a vascular system, tracheary elements may differentiate, but these cells are not arranged in well-defined new strands (Wetmore and Rier, 1963; Clutter, 1960). It may be, therefore, that some traits of organized tissues are necessary for strand formation. For this reason the application of auxin was tried on tissues taken from intact plants which were devoid of pre-existing vascular systems.

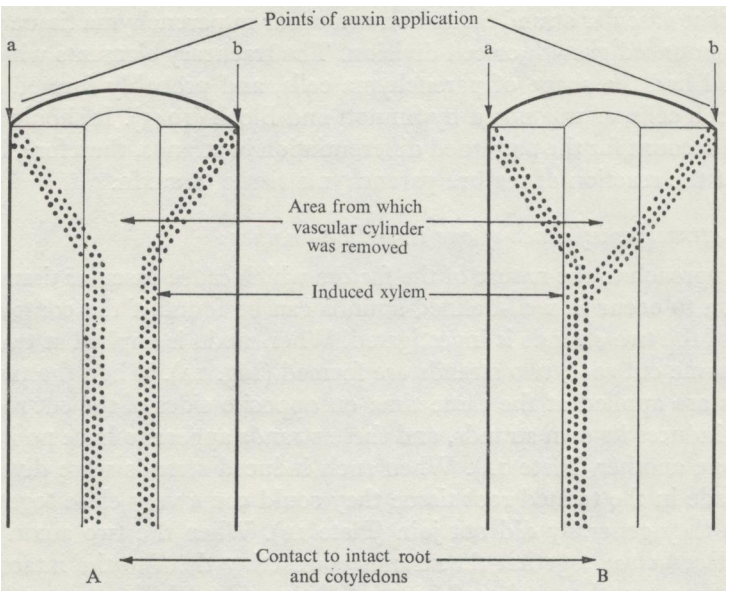


FIG. 1. Induction of xylem in cortical root tissues from which the vascular cylinder was removed. The treated tissues were supplied with water and nutrients by contact, at their base, with the intact part of the root and, through it, with the cotyledons. The course of the xylem strands which were induced by the auxin is marked by dots. In A a 1 per cent auxin solution was applied to points (a) and (b) at the same time. In B the auxin was applied at point (a) one week before it was applied to point (b). Note the change in direction of the strand which was induced by the auxin at (b). Photographs of this type of experiment are shown in Plate 1.

The best material found for this purpose was roots of pea seedlings cut along their longitudinal axis. Since the vascular system of the root is concentrated in its centre, it could be removed relatively easily from half of the root with a pair of fine forceps (Fig. 1A). The half-roots from which the vascular cylinder was removed were left connected to an intact part of the same root, which dipped into water. Through this intact root and the other half of the cut root they were also connected to the cotyledons, and were thus supplied with nutrients. These half-roots have already been described and used for experiments, though without the removal of the vascular cylinder (Sachs, 1968*b*).

When auxin in a lanolin solution was applied to these cut root halves from which the vascular tissue had been removed, it caused the differentiation of one or more strands of xylem (Plate 1A). The tracheary elements of these strands could be observed easily when the half-roots were cleared with lactic

acid 1 week after the auxin was applied. The induced strands always connected the place to which the auxin was applied with the intact root below. Xylem was not formed anywhere else except along these strands. The observed pattern of xylem differentiation, therefore, fits the concept that this differentiation occurs along the path of the flow of auxin from its source to the intact root below (see Introduction).

This influence of auxin on the differentiation of the cortical cells of roots shows that vascular strand formation can occur in parenchyma tissue without any pronounced growth or cell division. The tracheary elements which were observed had the shape of parenchyma cells, and probably formed directly from such cells, as was noted by Sinnott and Bloch (1945). It should be possible to account for the patterned differentiation in strands, therefore, by some traits of the reaction of mature parenchyma tissue to auxin.

2. *The attractive influence of one strand on another*

An approach to the nature of the factors which cause vascular tissue differentiation to occur in well-defined strands can be found if the control of the union of the two strands is investigated. When auxin is applied at two points of the same cut root, two strands are formed (Fig. 1A). When the two auxin sources are applied at the same time on opposite sides of the cut root, each source induces its own strands, and these strands appear to have no influence upon one another (Plate 1A). When such induced strands were diverted by cuts made in the treated root tissue they could come very close together, although they generally did not join (Plate 1B). When the two auxin sources were placed close together the strands connecting them to the intact part of the root ran together and could not be distinguished from one another.

A clear interaction between vascular strands was found when one week elapsed between the time of application of the two auxin sources and the first auxin in lanolin solution was removed at the time the second one was applied on the opposite side of the root. The interaction of the two strands was expressed by the fact that the second, newer strand was formed towards the first strand and united with it (Plate 1C). The exact course of the induced xylem varied greatly for different roots, but cases in which one xylem strand was attracted towards another, as in Plate 1C, were found in every experiment of this type. Such an interaction between strands never occurred in the controls, in which both auxin sources were applied at the same time (Plate 1A).

This experiment, showing the influence of one artificial vascular strand on the formation of another, was suggested by the attraction of the natural vascular cylinder, when it is devoid of auxin, for an auxin-induced vascular strand (Sachs, 1968*b*). The interaction of natural and artificial vascular tissues, however, could be interpreted either as an influence of the vascular system itself or of some pre-determined traits of the cells which surround it. The experiment reported here, in which both strands are artificial, clearly proves that auxin induction changes the cells in such a way that they influence the formation of a newly induced vascular strand.

This conclusion does not depend on the assumption that all the cells remaining in the half-root from which the vascular cylinder was removed are identical. As may be seen from Plate 1B and C, the xylem strands induced by auxin tended to pass through the cells which had been in contact with the vascular cylinder and it may be assumed that these cells have some special traits. Even the cells which were in contact with the vascular cylinder cannot be assumed to be identical among themselves, for in the intact root some of them were in contact with xylem tissue and the others with phloem. The results shown in Plate 1A and C, however, were obtained consistently, and they prove that whatever the original traits of the cells, induction by auxin changed these cells in such a way that they influenced a newly formed xylem strand.

We may now inquire into the meaning of this change induced by auxin. The only interpretation which seems consistent with the results presented in this work is that the cells which attract a newly induced vascular strand have become better transporters of the substance responsible for strand induction. They would then become a sink for the inducing stimulus and, as a result, attract towards themselves any newly induced strand. This must mean that the process of induction is, in a sense, autocatalytic; the passage of the inducing stimulus through the cells makes them better transporters of the very same inducing stimulus.

This property of the process of induction can explain why the differentiation of vascular tissues occurs in definite strands with relatively sharp boundaries. The longitudinal file of cells in which the process of induction starts may be chosen by chance, but once induction starts it continues in the same cells because they have become the preferred channels for the inducing stimulus. Cells which have begun to differentiate to vascular elements, therefore, attract the inducing stimulus and prevent the induction of the neighbouring cells. A similar prevention of vascular differentiation may be observed in mature stems and roots, in which the cortical cells are capable of becoming vascular elements but only do so if the flow of the inducing stimulus through the vascular system is interrupted by wounding (see Introduction).

It would be of interest to know for how long the parenchyma cells of the root have to be induced before their attractive influence for a new strand becomes optimal. When one auxin source is applied to a cut root only 4 or fewer days before another source, there is no clear interaction between the induced strands. The attractive influence on a new strand was found most commonly when there was a time difference of 6 or 7 days between the two auxin applications. It may be significant that at this time, about 1 week after the beginning of induction, fully differentiated sieve and tracheary elements could be observed in the induced strand. It is not clear, however, that it is this differentiation which is necessary for the attractive influence on a new strand to be expressed, since it is also possible that the passage of time is necessary to empty the attracting strand of auxin.

3. *The inhibition of the formation of one strand by another*

The next question to be dealt with here relates to the prevention of union, the opposite of attraction, between a new and a pre-existing vascular strand. It has been shown that the application of auxin to the vascular cylinder of a cut root inhibits the formation of an auxin-induced strand in the root cortex (Sachs, 1968*b*). In those roots in which the artificial strand was formed it did not join the vascular cylinder directly, as was the case when the latter was not supplied with auxin, but ran along the vascular cylinder for some distance and the joining was at a narrow angle.

This experiment could not be repeated simply with the material in which both vascular strands were induced by auxin. It was possible to induce one strand a week ahead of the other, and in contrast to the experiment shown in Plate 1C, to reinforce rather than remove the auxin source of the first strand at the time the induction of the second strand was started (Fig. 1B). This experiment often showed a complete inhibition of the formation of the second strand, as compared with the controls in which the first auxin source was not reinforced. In other cases a relatively weak strand was formed, and it joined the older strand at a narrow angle (Plate 1D). This result is as might be expected from the experiment using the natural vascular cylinder of the root, which was described above. The inhibition of the second strand when both strands were artificially induced, however, was never fully reproducible. The reason for this inconsistency in the results will be discussed below.

The inhibitory effect of auxin supplied to a natural vascular system on the differentiation of new xylem elements is, however, a readily repeatable phenomenon. A convenient system for the analysis of this inhibition of differentiation is shown in Fig. 2 and Plate 2. The lower part of a pea epicotyl, the apex of which was removed, was cut so as to separate some young stem tissues which had no vascular elements. When a 0·1 per cent solution of auxin was applied to the separated flap of tissue, a strand of xylem formed within 6 days connecting the auxin source to the central vascular cylinder (Plate 2A). If auxin was applied to the cut vascular cylinder as well as to the flap of stem tissue, the induced xylem failed to connect to the vascular cylinder though it did form within the separated stem tissue (Fig. 2B and Plate 2B). The differentiation of cells next to the vascular cylinder, therefore, can be induced by auxin coming from a lateral direction and can be prevented by auxin in the main vascular cylinder.

This influence of auxin may be explained in two ways. The first is that xylem differentiation in the vicinity of the vascular cylinder is determined by the concentration of auxin. Inhibition of differentiation would be the result of a supra-optimal concentration of auxin which accumulates when it is supplied both to the flap of tissue and to the vascular cylinder. A second hypothesis for explaining the double effect of auxin on xylem differentiation is that not only the concentration but also the direction from which auxin is coming is important. According to this hypothesis the influence of the application of auxin to

the vascular cylinder may be thought of as keeping the cells polarized in the longitudinal direction. These cells would then be relatively insensitive to induction by the auxin stream coming from the lateral direction of the isolated stem tissue.

The concentration of auxin at the point where the xylem does or does not differentiate cannot be calculated from the amount of auxin which was applied

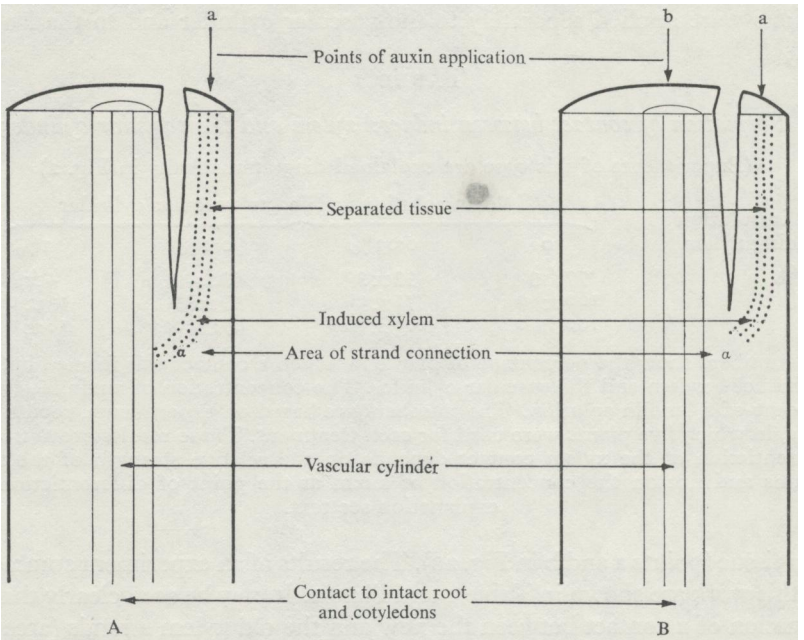


FIG. 2. Diagram of experiment using pea epicotyl tissue to test the relative influence of the direction from which auxin is flowing and of its local concentration on the differentiation of xylem. In A an auxin solution in lanolin was applied to the separated flap of stem tissue. Within 6 days strands of xylem were differentiated, connecting the source of auxin with the vascular cylinder. In B the auxin solution was applied both to the flap of stem tissue and to the vascular cylinder. The differentiation of xylem in the area marked by the letter *a* was prevented by the application of auxin to the vascular cylinder. If the concentration of auxin applied to the flap of stem tissue was raised while the concentration applied to the vascular cylinder was kept constant, the differentiation of xylem at *a* did occur. This experiment proves that the prevention of xylem differentiation at *a* was not due to a supra-optimal concentration of auxin. For further explanation, see text, Table 1 and Plate 2.

to the plant. It is possible, however, to perform qualitative experiments which distinguish between the influence of a local concentration of auxin and the direction from which it is coming. The basis for these experiments may be two opposite predictions for the result of raising the concentration of auxin applied to the isolated tissue (point (a) in Fig. 2). For if the differentiation of a contact between the new and old xylem strands (area *a* in Fig. 2) is prevented by a local supra-optimal concentration of auxin, raising the concentration of the solution will not cause additional differentiation. On the other hand, if differentiation

is controlled by the direction from which auxin comes, the contact between the new and old xylem strands may fail to form because the amount of auxin coming from the lateral direction is not high enough. The two hypotheses, therefore, offer opposite predictions for the result of raising the amount of auxin applied to the isolated stem tissue while the amount of auxin applied to the vascular cylinder is kept constant.

Experiments were therefore carried out in which a variety of auxin concentrations were applied separately to the vascular cylinder and to the isolated

TABLE 1

Formation of contact between induced xylem and the vascular cylinder

(Experiments of this type are explained diagrammatically in Fig. 2)

IAA in solution inducing xylem	IAA in solution applied to vascular cylinder			
	0	0·03%	0·1%	1%
0·03%	77±32	33±30	43±29	4± 6
0·1%	100± 0	71±26	57±15	13±16
1%	100± 0	100± 0	87±16	18±15

The numbers show the percentage of plants in which a contact was formed between the induced xylem and the vascular cylinder. The concentration of auxin is given in per cent of a lanolin solution. The numbers are based on experiments, repeated six times, in which five plants were used for each treatment. These results prove that the differentiation of the xylem contact depends on the relative strength of the auxin streams and not on the concentration of auxin at the point of differentiation; for explanation see text.

stem tissue (points a and b in Fig. 2B). The results of an experiment summarizing this work are shown in Table 1 and Plate 2. It may be seen clearly that the formation of a contact between the new and the old xylem strands (area *a* in Fig. 2B) depends on there being a difference between the concentrations applied to the isolated tissue and to the vascular cylinder. The percentage of plants in which such a contact was formed was directly dependent on the difference in the concentrations of auxin which were used. Raising the concentration of auxin applied to the isolated stem tissue, while keeping constant the treatment of the vascular cylinder, always raised the percentage of plants in which the new xylem was connected to the vascular cylinder. As explained above, this is a proof that the formation of xylem depends on the direction from which the auxin is coming and not only on its absolute concentration at any given point.

It may be concluded from this experiment that auxin moving along the axis of the vascular cylinder in some way reduces the influence of auxin moving at right angles to this axis. This influence may be thought of as an orientating influence of auxin, which in some way polarizes the cells along its axis of flow.

It was mentioned at the beginning of this section that the inhibitory influence of auxin on differentiation is very variable if both strands are artificially induced, as in Fig. 1 and Plate 1D. This difference from the results with natural vascular tissues may be understood if it is remembered that the cells next to the

vascular cylinder are elongated, and were presumably polarized at the time the stem was formed and its vascular tissues were induced. These cells are, therefore, readily influenced by auxin moving along the axis of the vascular cylinder. In the system in which both vascular strands are induced artificially by auxin vascular differentiation occurs after growth has ceased. In this case the cells adjoining the vascular strands are not polarized and are not readily influenced by auxin flowing along the axis of the strand. Auxin flow in this system may be much more confined to the strand itself.

4. *The influence of polarity on the orientation of induced strands*

It is well known that plant tissues transport auxin primarily in the direction of the root (for literature see Bonnett and Torrey, 1965; Goldsmith, 1966; Leopold and Hall, 1966). This is an oriented transport of auxin, as it is not dependent on the presence of a gradient of auxin concentration. The properties of plant tissues concerning auxin transport may be used to define the *polarity* of these tissues. The polarity of a tissue would then be measured by the degree to which it transports auxin in one direction. The pattern of the formation and growth of adventitious buds and roots on plant cuttings has been shown to be determined by this polarity (Warmke and Warmke, 1950).

Since the polarity of auxin transport plays a major role in determining the distribution of auxin, it must also be important for vascular tissue induction (Jacobs, 1952; Fosket and Roberts, 1964; Thompson and Jacobs, 1966; Sachs, 1968*b*). The polar distribution of auxin which affects vascular tissue differentiation is not merely an influence of gravity. This is proved by the induction of strands such as those shown in Plates 1A and 2A in plants which are completely inverted. The performance of these experiments requires special arrangements for the supply of water to the roots, which may be achieved by the use of wet filter-paper.

The transport of auxin and the differentiation of vascular tissues are not dependent on the presence of the root system as a sink for auxin (Sachs, 1968*a*). On the other hand, it may be suggested that vascular tissues act as a sink for auxin, and that they transport this auxin in the direction of the root. The influence of mature vascular tissues on the direction of the differentiation of new strands would then be understood as an influence of a sink for the inducing stimulus (see Plate 1C and Sachs, 1968*a*). An experiment was therefore designed to compare the influence of cell polarity and of the vascular system as a sink for auxin on the direction of xylem differentiation (Fig. 3 and Plate 3).

When auxin is applied to a root or a stem of a pea seedling from which the young shoot was removed, it induces the formation of strands of xylem which always connect the source of auxin with the vascular system (Sachs, 1968*b*). If a cut be made as shown in Fig. 3A, the xylem strands could form in two directions: (*a*) follow the polarity of the cells downwards, but connect to no differentiated sink for auxin, or (*b*) move a short way upwards, against the polarity of the tissue, and then connect to the vascular system of the plant. This

experiment, therefore, compares the effect of the vascular system as a sink for auxin with the influence of the polarity of the tissue.

When a 1 per cent IAA solution was applied to root tissue or a 0·1 per cent solution to the more sensitive stems, new xylem always formed directly downwards and ended blindly in the cut tissue (Plate 3A and C). If the auxin was

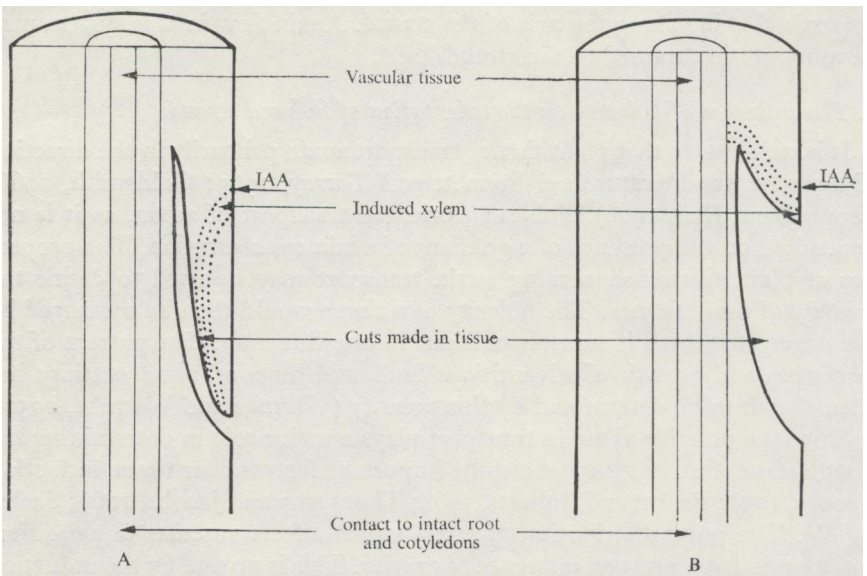


FIG. 3. Diagram of experiment designed to test the relative importance of polarity in determining the direction of induced xylem strands. Both young root and epicotyl tissues were used, and auxin was applied laterally to the area marked by an arrow. The shoot apex was removed in all cases. A cut separated the source of auxin from direct contact with the vascular cylinder, which should form a sink for auxin. If tissue was present below the applied auxin, as in A, the xylem strand formed downwards and ended blindly without joining the vascular cylinder. If the tissue was removed, as in B, the auxin stream overcame the polarity of the parenchyma cells and joined the vascular cylinder. For photographs of this type of experiment see Plate 3.

placed in the same way but the tissue below it was removed (Fig. 3B) xylem was formed which connected the auxin source with the vascular system of the plant (Plate 3B and D). It is, therefore, clear that the need to overcome the polarity of the cells for a short distance presents no serious obstacle to xylem formation. The tissue which is present in experiments of the type shown in Fig. 3A and absent in those of the type shown in Fig. 3B has a definite influence on the direction of xylem differentiation. It can be assumed that this tissue moves the auxin downwards along the polarity of the cells. Whether the applied auxin simply accumulates at the lowest point in the tissue, or whether such an accumulation induces the formation of an auxin-destroying system in this region, is not clear from these results. Regardless of the answer to this question, this experiment demonstrates the effect of the original polarity of the cells as an important additional factor to that of a sink for auxin in the determination of the course of xylem differentiation.

Conclusion

The central conclusion of this work may be that the pattern of vascular tissue differentiation can be understood on the basis of the polar properties of plant tissues concerning auxin transport. In view of this conclusion the results presented above can be understood as follows: (*a*) Auxin alone can cause the formation of defined xylem strands (Plate 1A), but this induction of an organized system can occur only in tissues which have a certain degree of polarity. In tissue cultures there appears to be no pattern to auxin movement, and therefore no patterned differentiation. (*b*) One of the changes which occur as a result of vascular tissue induction is an increase in the polar transport of auxin. As a result of this trait of vascular induction, differentiated vascular tissue which is not supplied with auxin may be thought as acting as an attractive sink for any new stream of auxin. A new xylem strand therefore forms towards a pre-existing strand which is not supplied with auxin (Plate 1C). (*c*) The passage of auxin through a vascular strand keeps the tissue polarized along the axis of this strand. Polarized cells are not labile to the influence of an inductive source of auxin coming from another direction. Auxin applied to a vascular cylinder, therefore, may prevent the expression of the influence of another source of auxin (Plate 2). (*d*) The direction and location of a new strand induced in organized parenchyma is dependent on the polarity determined in the tissue when it was in a meristematic state as well as on the presence of a sink for auxin (Plate 3).

Literature Cited

Bonnett, H. T., and Torrey, J. G., 1965. Auxin transport in *Convolvulus* roots cultured in vitro. *Pl. Physiol., Lancaster* **40**, 813–18.

Clutter, M. E., 1960. Hormonal induction of vascular tissue in tobacco pith in vitro. *Science, N.Y.* **132**, 548–9.

Fosket, D. E., and Roberts, L. W., 1964. Induction of wound vessel differentiation of isolated *Coleus* stem segments in vitro. *Am. J. Bot.* **51**, 19–25.

Goldsmith, M. H. M., 1966. Movement of indoleacetic acid in coleoptiles of *Avena sativa* L. II. Suspension of polarity by total inhibition of basipetal transport. *Pl. Physiol., Lancaster* **41**, 15–27.

Jacobs, W. P., 1952. The role of auxin in the differentiation of xylem round a wound. *Am. J. Bot.* **39**, 301–9.

—— 1956. Internal factors controlling cell differentiation in higher plants. *Am. Nat.* **90**, 163–9.

—— and Morrow, I. B., 1957. A quantitative study of xylem development in the vegetative shoot apex of *Coleus*. *Am. J. Bot.* **44**, 823–42.

Jost, L., 1940. Zur Physiologie der Gefäßbildung. *Z. Bot.* **35**, 114–48.

Kaan Albest, A. von, 1934. Anatomische und physiologische Untersuchungen über die Entstehung von Siebröhrenverbindungen. Ibid. **27**, 1–92.

Leopold, A. C., and Hall, O. F., 1966. Mathematical model of polar auxin transport. *Pl. Physiol., Lancaster* **41**, 1476–80.

Sachs, T., 1968*a*. The role of the root in the induction of xylem differentiation in peas. *Ann. Bot.* **32**, 97–117.

—— 1968*b*. On the determination of the pattern of vascular tissue in peas. Ibid. 781–90.

Simon, S., 1908. Experimentale Untersuchungen über die Entstehung von Gefäßverbindungen. *Ber. dt. Bot. Ges.* **68**, 227–32.

SINNOTT, E. W., and BLOCH, R., 1945. The cytoplasmic basis of intercellular patterns in vascular differentiation. *Am. J. Bot.* **32**, 151–6.

THOMPSON, N. P., and JACOBS, W. P., 1966. Polarity of IAA effect on sieve tube and xylem differentiation in *Coleus* and tomato stems. *Pl. Physiol., Lancaster* **41**, 673–82.

WANGERMANN, E., 1967. The effect of the leaf on the differentiation of primary xylem in the internode of *Coleus blumei* Benth. *New Phytol.* **66**, 747–54.

WARMKE, K. E., and WARMKE, G. L., 1950. The role of auxin in the differentiation of root and shoot primordia from cuttings of *Taraxacum* and *Cichorium*. *Am. J. Bot.* **37**, 272–80.

WETMORE, R. H., and RIER, J. P., 1963. Experimental induction of vascular tissues in callus of angiosperms. Ibid. **50**, 418–30.

EXPLANATION OF PLATES

The plates show the course of xylem strands induced by solutions of auxin in lanolin and their place of joining with other strands. The auxin was applied to definite places, which are marked by arrows. Young pea seedlings were used and they were cleared with lactic acid one week after treatment. All × 14.

PLATE 1

Half roots from which the vascular tissue was removed, of the type shown in Fig. 1. A 1 per cent solution of auxin was applied to the places marked by arrows. Note the xylem strands which differentiated in the parenchyma tissue as the result of this treatment.

A. The auxin solution was applied at the same time to the two sides of the root. Two independent systems of xylem were formed, connecting the sources of auxin with the intact part of the root below.

B. The auxin was applied in two places, as in A. The xylem strands were forced close to one another by cuts (marked 'c') made at the time the auxin was applied. The two strands came very close together but did not join one another.

C. One auxin source (on the left) was applied one week before the other. The first auxin source was removed at the time the second one was applied. Note the influence of the earlier artificial strand on the direction of the second strand. The two strands join at a broad angle. An attractive influence of the first strand on the second, newer strand may be assumed.

D. As in C, but the auxin source on the left, which induced the first strand, was reinforced, rather than removed, at the time the second auxin source was applied. In many plants the second strand did not form; in the example shown in the plate the new strand formed and joined the first strand at a narrow angle. The attractive influence of the first strand when its source of auxin was reinforced was, therefore, weaker than in the case shown in Plate 1C.

PLATE 2

Experiments with cut pea-stem tissues of the type explained diagrammatically in Fig. 2. The results prove that the formation of a contact between an induced xylem strand and a natural vascular cylinder which is supplied with auxin is dependent on the relative strength of the auxin streams. The formation of this contact is not dependent only on the concentration of auxin at the place of differentiation. IX marks the artificially induced xylem; X the vascular cylinder, which is centrally located in the lower part of the pea epicotyl; F marks the strands of fibres, which had no influence on the induced xylem in these experiments.

A. A 0·1 per cent solution of IAA was applied to the cut tissue. An induced xylem strand was formed, and it always joined the vascular cylinder to which no auxin was applied.

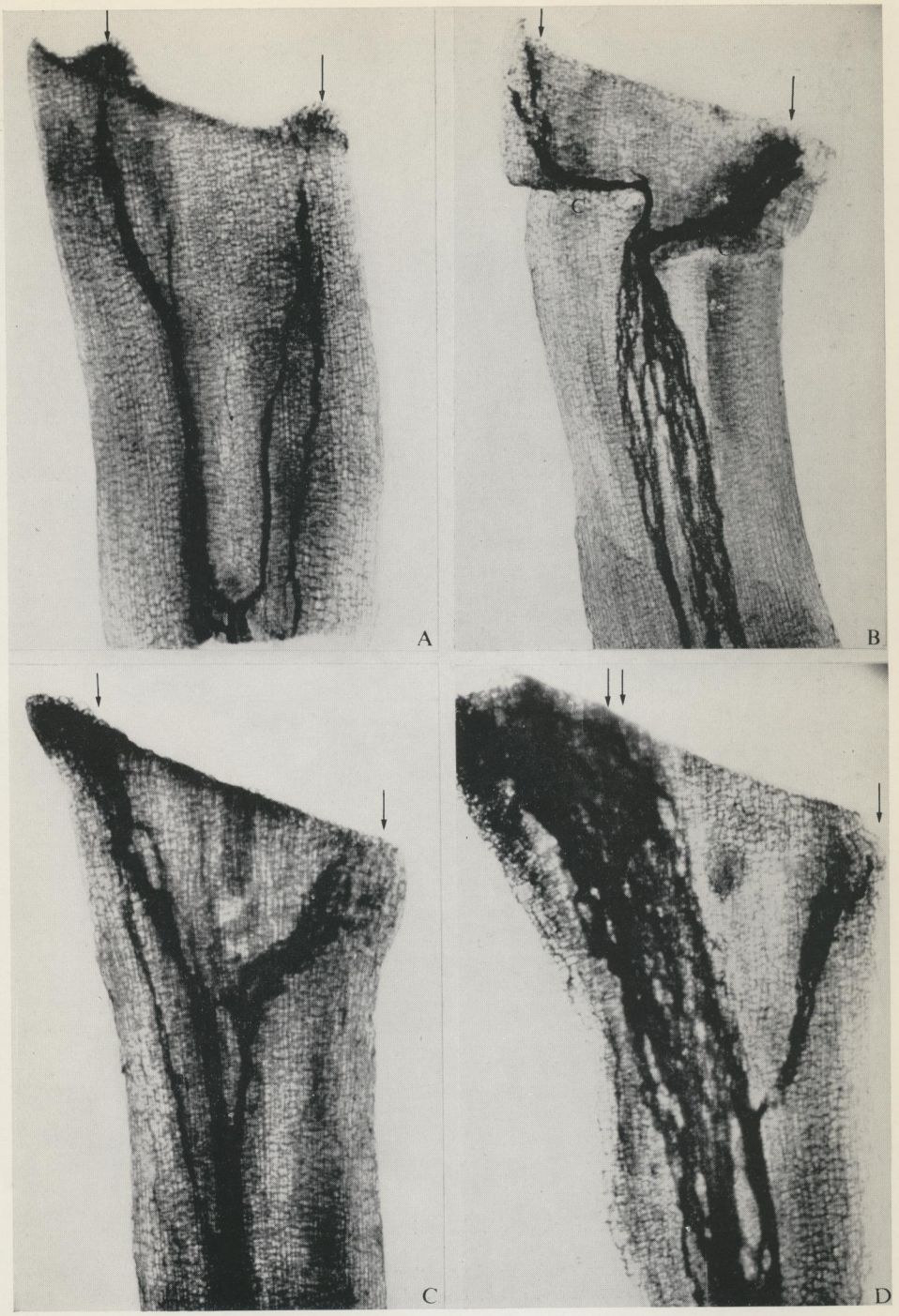
B. The same 0·1 per cent solution was applied to the cut tissue and a 0·03 per cent solution to the vascular cylinder. In many plants, such as the one shown in the plate, the induced xylem did not join the vascular cylinder.

C. The concentration of auxin applied to the cut tissue was raised to 1 per cent. The induced xylem always joined the vascular cylinder to which a 0·03 per cent solution was applied.

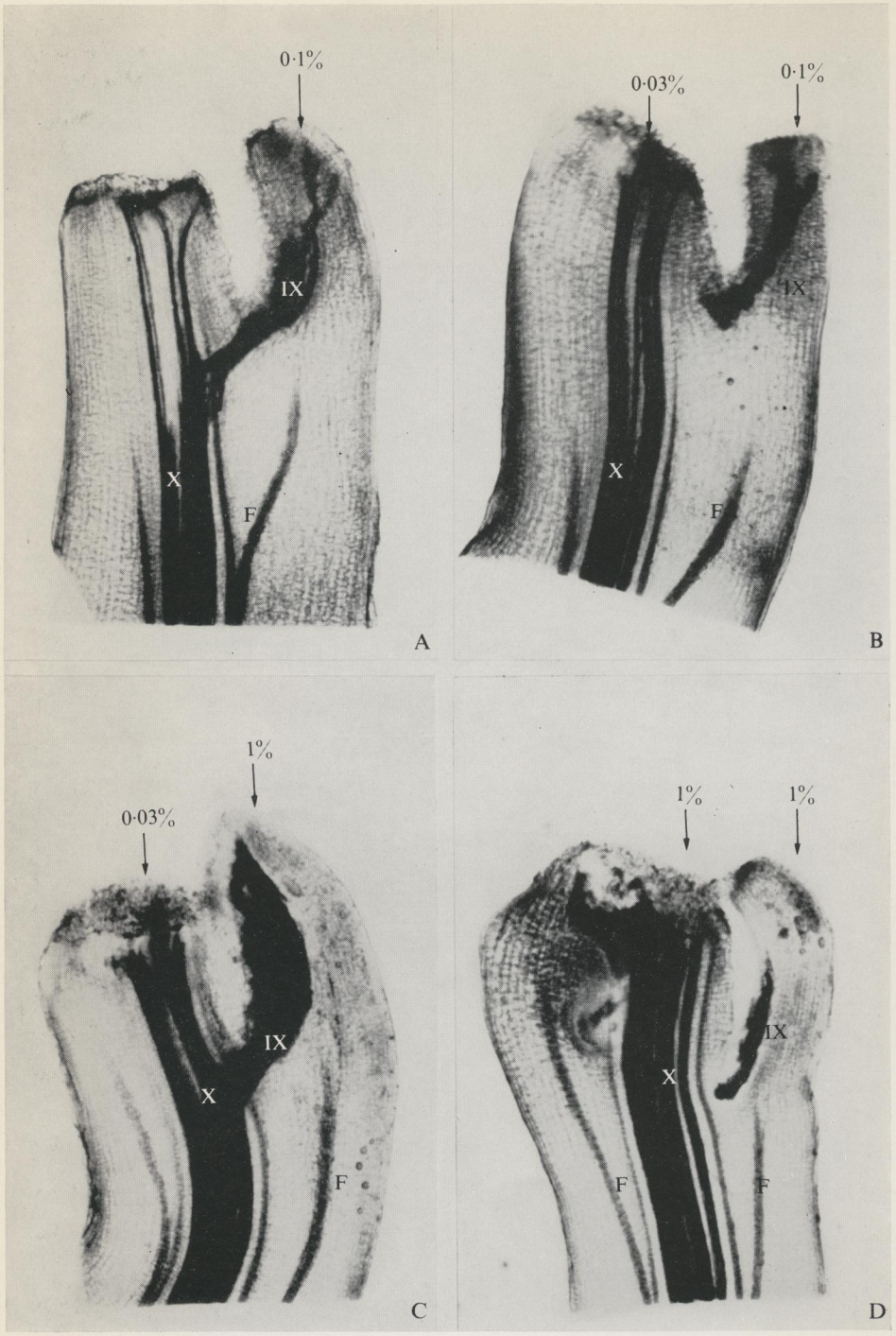
D. A 1 per cent solution was applied to both the cut tissue and the vascular cylinder. The high concentration of auxin in the vascular cylinder prevented its being contacted by the induced xylem in most of the treated plants.

PLATE 3

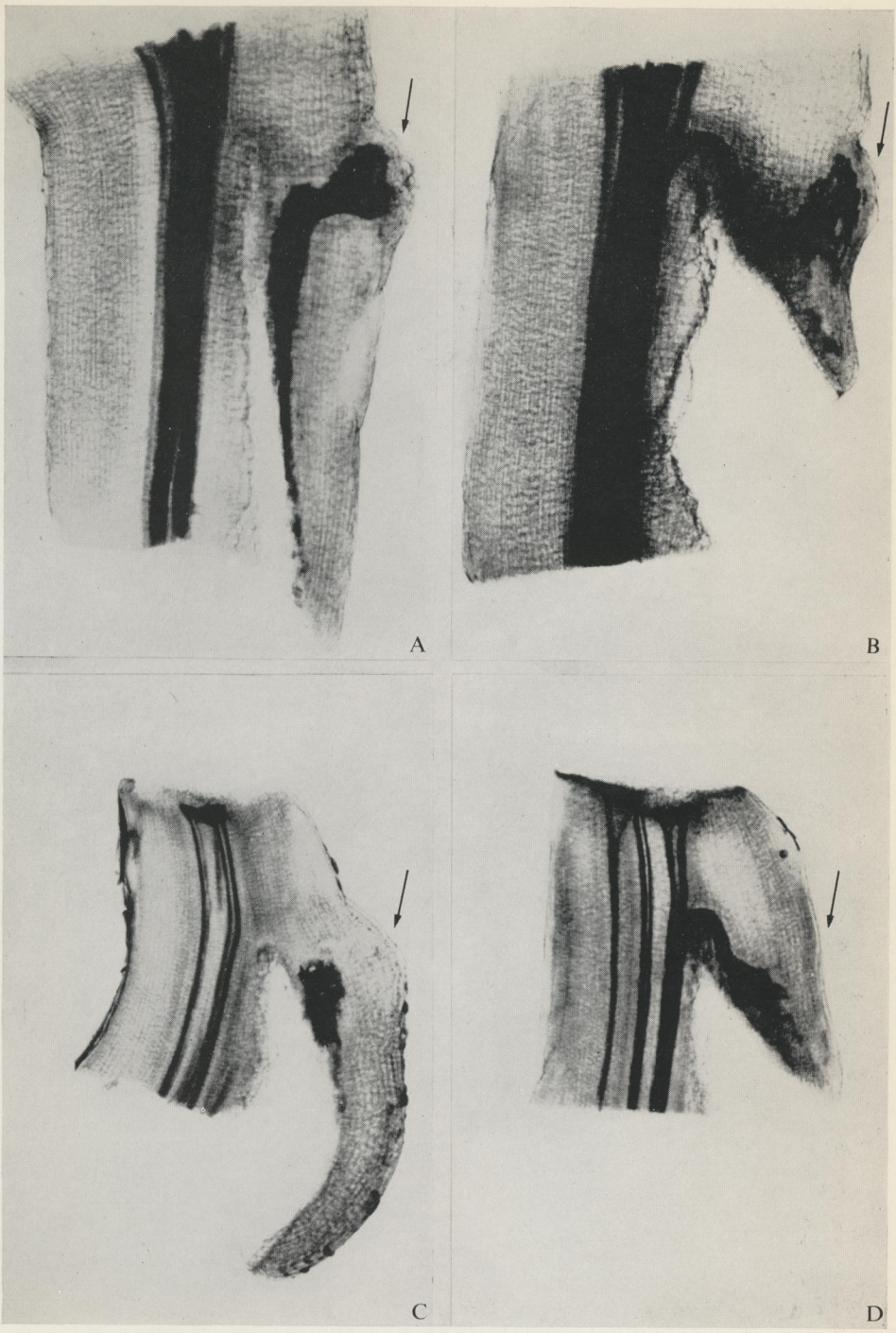
Experiments on the influence of the polarity of parenchyma tissue on the direction of newly induced xylem strands. This type of experiment is explained diagrammatically in Fig. 3; it



T. SACHS



T. SACHS



T. SACHS

demonstrates the relative influence of tissue polarity and the vascular system as a sink for auxin on the differentiation of new xylem strands. For further explanation see text.

A. A 1 per cent solution of IAA was applied laterally to the root of a pea seedling, the shoot apex of which was removed. A cut was made separating the source of auxin from direct contact with the vascular cylinder. A flap of tissue was present below the point of auxin application and the strand of auxin was directed downwards, ending blindly.

B. As in A, except that the tissue below the point of auxin application was removed. The induced xylem strand connected directly to the vascular cylinder.

C and D. As in A and B, except that epicotyl tissue was used and the concentration of auxin was 0·1 per cent.