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The Polar Transport of Auxin and Vein Patterns in Plants [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1981 **295**, 461-471
doi: 10.1098/rstb.1981.0154

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Phil. Trans. R. Soc. Lond. B **295**, 461–471 (1981)
Printed in Great Britain

The polar transport of auxin and vein patterns in plants

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[Plate 1]

The hormone auxin is transported through many plant tissues with a definite velocity. It is thought that certain channels, or pumps, located at the basal ends of cells, are responsible for the hormone's transport. It is also known that auxin will induce veins when applied to suitable tissues. T. Sachs has suggested that it is the flow of the hormone that induces vessels. He suggests that discrete strands form because the transport capacity of a pathway increases with the flux that that pathway carries, leading to a canalization of flow. I cast this in the form of a more specific hypothesis: I suppose the permeability for the transport of auxin through the basal plasmalemma of a cell (by means of whatever kind of pump or channel) to increase with flux. I then show that discrete veins will form provided that the transport permeability increases rapidly enough with flux, and provided that the movement of auxin is not too polar, in the sense that there is a substantial amount of diffusive movement of auxin in addition to polar transport. The same hypothesis offers an explanation for the loops of veins found under certain conditions.

1. Introduction

The plant hormone auxin, or indole acetic acid, is transported in a polar fashion through many plant tissues (Goldsmith 1977). Auxin is also involved in the development of veins, both in the repair of existing veins and in the initiation of new xylem in certain cambial tissues (Jost 1942; Jacobs 1952; Sachs 1978). Sachs (1969) has suggested that veins form along the paths taken by auxin as it flows through tissues. To explain the localized paths of veins, he has proposed that the efficiency of transport of auxin along a path increases with the auxin flux that that path carries. So a path will become a better sink for the hormone as it carries a greater flux. In this way, certain preferred transport channels should appear, somewhat after the fashion of rivers in a landscape.

As auxin is transported in a polar manner, one expects to see evidence of this in the path taken by veins, and this is indeed found. Veins will follow the direction of auxin transport (Sachs 1969), even when this requires a detour, imposed by the presence of grafts of different polarity (Sachs 1968). Now one can explain the transport of auxin by assuming that there are pumps or channels of some kind, localized at one end of each cell, which move auxin in a polar manner between cells. Suppose that these pumps or channels increase in number, or become more effective, as the flux of auxin through a cell increases. Could this explain the formation of discrete veins? I show here that such a mechanism will generate satisfactory vein patterns provided that certain conditions are met. These concern the sizes of permeabilities for auxin transport between cells, and the way that these vary with the auxin flux.

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2. Flux-dependent auxin transport

If labelled auxin is briefly applied to a segment of a suitable plant tissue, a pulse is found to travel down the segment in the direction from the original apex to the base (Goldsmith 1967). The pulse spreads, but its mean has a definite velocity, often in the range 1.0–1.5 cm/h. I shall assume here that the movement of auxin involves two stages. First, a transport mechanism for transfer between cells, perhaps of the kind proposed by Rubery & Sheldrake (1974); and secondly, intracellular diffusion. Suppose the flux F between two cells depends only on the concentrations at the adjacent ends, with b denoting that at the basal end of one cell and a denoting that at the apical end of the next cell, according to the law

$$F = pb + q(b - a), \tag{1}$$

where p and q are constants with the dimensions of permeabilities.

This flux law covers a considerable variety of possible mechanisms, and would apply in particular to the 'chemiosmotic' theory of auxin transport (Rubery & Sheldrake 1974; Raven 1975). One can show that, with such a flux law, a pulse of auxin would move with a definite velocity (Mitchison 1980*b*; Goldsmith *et al.* 1981). From a variety of experiments, in which the dependence of this velocity on cell length is investigated (Wangermann & Mitchison 1981), and the velocity is changed by centrifuging cells (Cande *et al.* 1973), one can estimate values for the constants of transport (Mitchison 1981). Reasonable values are $p = 4 \times 10^{-4}$ cm/s and $q = 10^{-4}$ cm/s or less.

It is likely that auxin diffuses both through the cytoplasm of a cell, and partly through its vacuole, the tonoplast (the membrane surrounding the vacuole) being moderately permeable to auxin (Mitchison 1981). In the ensuing analysis of vein formation, I shall assume at first that the tonoplast is so permeable to auxin as to be 'invisible', so we may treat the cell as a single compartment. Later (see §5), I shall consider what happens at the other extreme, where the tonoplast is completely impermeable.

Suppose we have a two-dimensional sheet of cells. The flow pattern within the sheet is what concerns us, so let us represent a cell by a rectangle of length L and width W, and ignore the depth dimension (figure 1*b*). Let F be the flux per unit cross-sectional area across an end face of the cell. Then

$$F = (a - b)\, D/L, \tag{2}$$

where D is the diffusion constant for movement through the whole cell interior.

I now come to the key assumption. Suppose that the permeability p for forward movement between cells increases with flux. Can this lead to the formation of preferred pathways for auxin transport through a tissue?

Imagine that auxin is being transported down a sheet of cells, represented for convenience as a rectangular array (figure 1*a*). Suppose that initially there is a uniform flow of auxin down the sheet, with a line source above and a line sink below where zero concentration is maintained. Let us slightly perturb this flow pattern, with the result that one file of cells carries slightly more flux than its neighbours. Suppose that the increase in transport efficacy causes the concentration of auxin to fall in this file. If auxin diffuses in through the lateral walls of cells, then the file will begin to act as a sink for auxin in neighbouring tissues. As it draws more flux to itself, the transport permeability in its transverse walls will increase further, and in this fashion an autocatalytic cycle will be generated. The condition for this to happen is that increasing the flux in the file of cells should actually lower the concentration there.

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Consider therefore a file of cells, with a flux F entering at the apical end, and a sink of zero concentration maintained at the basal end. Let the concentration at the top end of the nth cell be a_n, and at the bottom end b_n. Then, from (1) the flux is given by

$$F = pb_n + q(b_n - a_{n+1}). \tag{3}$$

The equation for diffusion inside the cell is given from (2) as

$$F = (a_n - b_n)\, D/L. \tag{4}$$

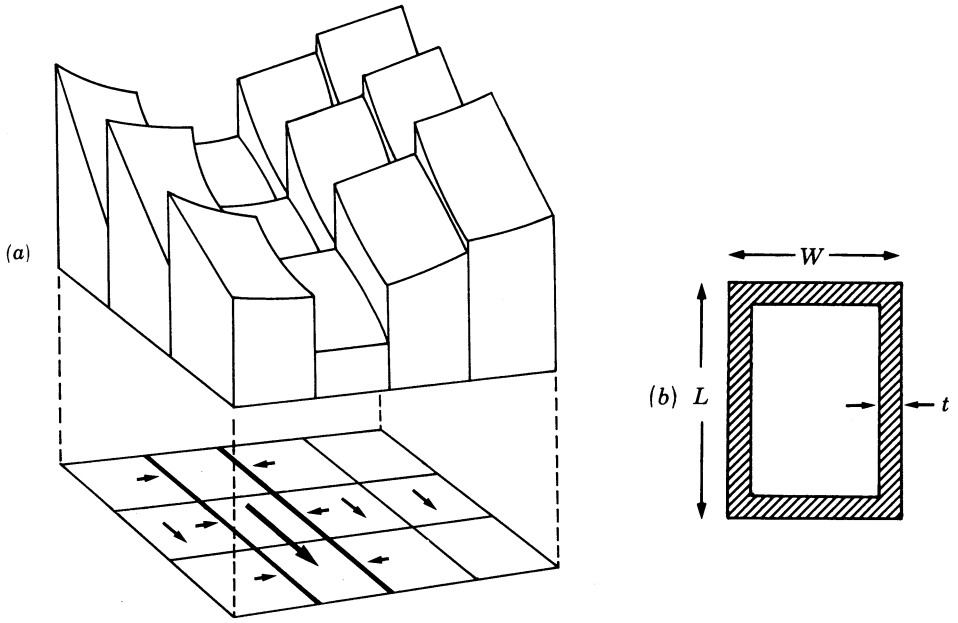

FIGURE 1. (*a*) This shows a small array of cells, with the auxin concentration inside them depicted graphically in the upper part, and the flow patterns of auxin beneath on the cell grid. The main direction of auxin flow is shown by the larger arrows, and one file (heavy boundary) carries a larger flux than its neighbours. With appropriate assumptions about dependence of transport permeabilities on flux, the concentration in this file will fall, and auxin will diffuse in from neighbouring cells. This lateral movement into the file is shown by the small arrows. (*b*) The dimensions of a cell are shown. The cytoplasm (shaded) has thickness t, and the overall cell length and width are L and W, respectively.

Suppose that the Nth cell is a sink with $b_N = 0$. Then the solution of these difference equations is

$$a_n = FL/D + b_n; \quad b_n = (1 + qL/D)\,(F/p)\,(1 - (1 + p/q)^{n-N}). \tag{5}$$

Now it is reasonable to assume that p/q is fairly large. So for cells far from the sink, where $(N-n)$ is large, we can neglect the term $(1+p/q)^{n-N}$, and write simply

$$a_n = FL/D + b_n; \quad b_n = F(1 + qL/D)/p. \tag{6}$$

There will be net inward diffusion into a cell in the file from its lateral neighbours if the mean concentration $\frac{1}{2}(a_n + b_n)$ falls as F rises. That is,

$$\tfrac{1}{2}(a_n + b_n) = F(1 + qL/D)/p + FL/2D \tag{7}$$

must fall as F increases. If p increases only as the first power of F, then the mean given by (7)

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will increase with F. For the mean to fall, p must increase as a higher power of F (compare Mitchison 1980*a*).

Let us assume that p increases quadratically with F. Now we cannot simply set $p = kF^2$, with k a constant. For it turns out that this will lead to instabilities of auxin concentration due to local inhomogeneities in the concentration gradient (see Mitchison 1980*a*). To avoid this behaviour, p must change slowly relative to diffusive equilibration, and this can be expressed formally by setting

$$\partial p/\partial t = \epsilon(kF^2 - p), \tag{8}$$

where ϵ is a small constant.

This is a suitable law for the flux dependence of p when the flux F is given by (1). However, this assumes that transport has a fixed direction. If we have measured F as the flux from the basal end of one cell (where the cytoplasmic auxin concentration is b) to the apical end of the next (concentration a), then $F > 0$. If the direction of flux changes, so $F < 0$, then we have the law $F = -pa + q(a-b)$, which gives a new meaning to p. Equation (8) cannot be used to define the changes of permeability if we wish the system to behave continuously under conditions where the direction of flux changes abruptly. Instead, consider two adjacent cells, labelled 1 and 2, with concentrations a_1, a_2 in the cytoplasm near their interface. Let the flux F measured in the direction from cell 1 to cell 2, be given by

$$F = p_1 a_1 - p_2 a_2. \tag{9}$$

Now put

$$\left.\begin{aligned} \frac{1}{\epsilon}\frac{\partial p_1}{\partial t} &= (kF^2 + q - p_1) && F > 0; \\ &= q - p_1 && F < 0; \\ \frac{1}{\epsilon}\frac{\partial p_2}{\partial t} &= (kF^2 + q) - p_2 && F < 0; \\ &= q - p_2 && F > 0. \end{aligned}\right\} \tag{10}$$

This is a suitable general definition for flux dependence of transport permeability, and this is what I have used for computer simulations, to be described later. When the flux has a fixed direction for a long time, one of p_1, p_2 will tend to q, and the other p will be governed by the simpler equation, (8).

Despite their seeming complexity, (9) and (10) have a natural meaning. By definition, polar transport involves an asymmetry in the transport mechanism. For instance, according to the chemiosmotic theory of auxin transport (Rubery & Sheldrake 1974; Raven 1975), auxin enters cells from the acidic extracellular space in the form of the lipophilic undissociated acid, which freely passes through membranes. But auxin exists in the cytoplasm as the anion, which can only leave the cell through specialized channels. It is assumed that these channels are present in the basal area of the plasmalemma, giving a polar movement in the basal direction. We can now interpret p_1 as the anion permeability at the plasmalemma of cell 1, and p_2 that of cell 2. Then (10) says that the flux leaving a cell through a region of plasmalemma regulates the activity or density of anion channels there.

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3. Conditions for veins to form

Suppose now that we perturb a nearly uniform flow down a sheet of cells. Under what conditions will preferred channels emerge? I shall make several simplifying assumptions to analyse this problem. First, I assume that changes in permeabilities specified by (10) occur so slowly relative to diffusion that we may assume diffusive equilibrium inside cells. Secondly, I assume that the concentrations in cells are initially given by (6), and I ignore that region near to the sink where the exponential term in (5) is significant. Thirdly, I consider only the simplest mode of destabilization possible, where adjacent files of cells carry alternately larger and smaller fluxes. This is the first mode to appear (compare Mitchison 1980*a*).

In the initial uniform state, before perturbation, there will be no lateral flux between cells. Then, if the perturbation is small, and because p depends quadratically upon F, we may ignore changes in p in the lateral walls, and suppose that the lateral flux is given by $F = q(c_1 - c_2)$, where c_1, c_2 are the auxin concentrations in the adjacent cells. Now there is initially a linear gradient inside cells. Let the difference in the concentration at each point inside the cell from its initial value in the linear gradient be $c(x)$, where x is the distance measured along the cell's axis from the apical end. As concentrations in neighbouring files are perturbed an equal amount in opposite directions, the efflux at the point x is $F = 2qc(x)$. I shall ignore any diffusion gradients at right angles to the cell's axis, so the problem is essentially one-dimensional. The concentration inside the cell is then given by

$$(\tfrac{1}{2}W)\,Dc_{xx} = 2qc, \tag{11}$$

where the subscripts denote partial derivatives.

Suppose that the flux leaving the basal face of the nth cell is perturbed from its initial value F to $F + F_n$, where cells are counted in the direction of the initial flux. Similarly, let $a + a_n$, $b + b_n$ be the perturbed values of the concentrations at the apical and basal ends, respectively, of the nth cell. Then we can solve (11) and show that

$$\left.\begin{aligned} a_n &= c(0) = K_1 F_{n-1} - K_2 F_n, \\ b_n &= c(L) = K_2 F_{n-1} - K_1 F_n, \end{aligned}\right\} \tag{12}$$

where $K_1 = (L^*/D)\coth(L/L^*)$, $K_2 = (L^*/D)\operatorname{cosech}(L/L^*)$, and

$$L^* = (WD/4q)^{\frac{1}{2}}. \tag{13}$$

The appropriate equation for specifying p is (8), because the flux through the basal ends of cells will always be in the same direction for small perturbations. If we let $p + p_n$ be the perturbed value of p at the basal end of the nth cell, the linearized version of (8) is

$$\mathrm{d}p_n/\mathrm{d}t = \epsilon(2kFF_n - p_n), \tag{14}$$

and the perturbed version of (1) is

$$F_n = p_n b + p b_{n+1} + q(b_{n+1} - a_n). \tag{15}$$

We now look for solutions to (12), (14) and (15) of the form $F_n = Aw^n \cos(zn)\,\mathrm{e}^{ft}$, $p_n = Bw^n \cos(zn)\,\mathrm{e}^{ft}$. On substituting, we find that $w = (1 + p/q)^{-\frac{1}{2}}$, and

$$f + \epsilon = (2\epsilon Fkb)/\{1 + K_1(p + 2q) - 2K_2 q \cos z/w\}. \tag{16}$$

The boundary conditions require $\cos Nz = 0$, so with the simplest mode in this direction,

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$z = \pi/2N$. As we are implicitly assuming N to be large, to justify neglecting the nonlinear gradient near to the sink, we may assume that z is small, and put $\cos z = 1$. On substituting for K_1 and K_2, we find the condition for the solutions to grow with time, or $f > 0$, is

$$p + 2q < 2q(1 + p/q)^{\frac{1}{2}} \operatorname{sech}(L/L^*) + \{D/L^* + 2qL/L^*\} \tanh(L/L^*). \quad (17)$$

From (13), $D/L^* = 4qL^*/W$, and we can then express (17) in terms of the ratio p/q. Putting $a = (2L/L^* + 4L^*/W) \tanh(L/L^*)$, and $b = \operatorname{sech}(L/L^*)$, we find that

$$p/q < 2b^2 + a - 2 + 2b(b^2 + a - 1)^{\frac{1}{2}}. \quad (18)$$

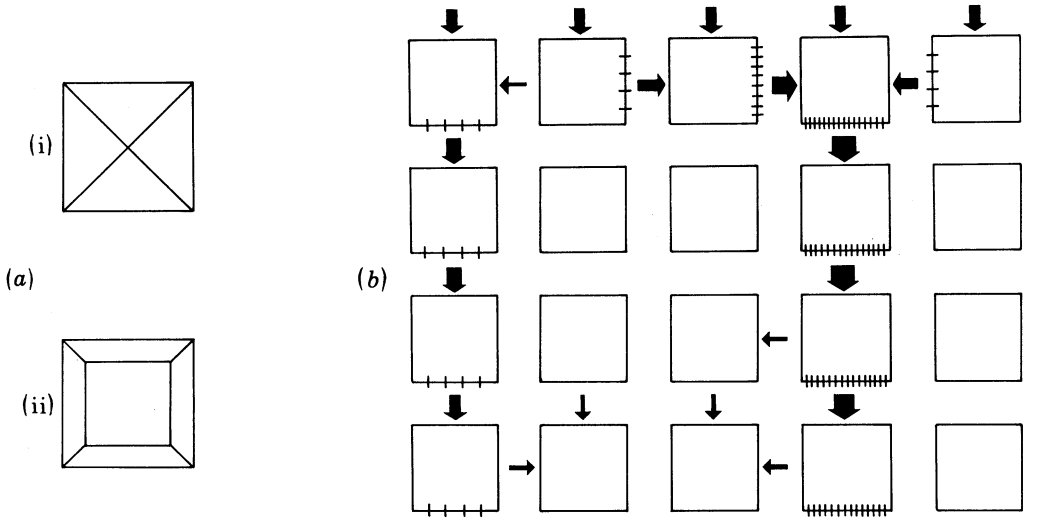

FIGURE 2. (*a*) For computer simulations, cells are divided into four internal compartments. If we assume the tonoplast is a negligible permeability barrier, then auxin diffuses equally freely through the whole interior of the cell, and the four sectors in (i) partition the interior into a crude grid. If the tonoplast is completely impermeable, then we interpret the four compartments as subdivisions of the cytoplasm, as shown in (ii). (*b*) Here, the outcome of a computer simulation is shown, in which an initially uniform flow from top to bottom of the diagram was slightly perturbed. Channels of preferred flow developed. These are indicated by the arrows. (For notational convenience, their thickness is proportional to the square root of the flux.) The density of channels, or equivalently, the permeability p_{12} for leaving cell 1 and entering cell 2 (see equation (9)), is indicated by the number of strokes across the wall in question. (Again, for notational convenience, the number of strokes is proportional to the square root of p_{12}). The thickness of the cell wall space between cells has, of course, been exaggerated.

This sets a limit on the polarity, measured by p/q, that is allowable if veins are to form. A similar inequality was obtained for a simpler model of polar transport in Mitchison (1980*a*). Clearly, q cannot be too small, since this permeability regulates the entry of auxin through lateral walls of cells. As an example, suppose the cell is twice as long as it is wide, so $L/W = 2$. Then as we vary L/L^*, the right-hand side of (18) has a minimum at $L/L^* = 2.5$, with $p/q < 7.0$. For larger or smaller L/L^* the right-hand side of (18) is larger. For instance, with $L/L^* = 10$, $p/q < 18.8$, and with $L/L^* = 0.1$, $p/q < 13.6$. A realistic value of L^* is obtained by putting $L = 200$ μm, $W = 100$ μm, $D = 7 \times 10^{-6}$ cm²/s (see Larsen 1955), $q = 10^{-4}$ cm/s, in which case (13) yields $L^* = 132$ μm. Then $L/L^* = 1.5$, which gives $p/q < 8.1$, not far from the minimum, as it happens.

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There are many variants of this analysis. For instance, the permeability for auxin entering along the side walls may be different from q, say q'. Then we obtain exactly the same inequality, (17), but with $L^* = (WD/4q')^{\frac{1}{2}}$ instead of (13).

To illustrate the generation of veins, I have made a simple computer model of an array of cells, in which polar transport between cells is combined with diffusion within them. I have treated diffusion within cells only coarsely. Each cell is a square, subdivided into four triangular segments (figure 2*a*(i)), with a symmetric permeability d for moving between those segments which are in contact, corresponding to intracellular diffusion. The movement of auxin between cells is assumed to obey (1), with p and q varying with flux in the manner previously described. The analogue of (17) assumes the simpler form

$$(p-2q)/d < 1+(1+p/q)^{\frac{1}{2}}-p/2q. \tag{19}$$

Figure 2 shows the result of perturbing an array of cells, initially with a uniform flow from top to bottom. Here, I have set $d = 0.4$, $q = 0.06$, with $F = 15$, $k = 10^{-3}$, $\epsilon = 10^{-2}$, giving $p = 0.225$ from (10). It can be checked that these values of p, q and d satisfy (19). Taking $L = 100$ μm, and $D = 7\times10^{-6}$ cm²/s, we find that these computational constants correspond to $p = 4\times10^{-4}$ cm/s and $q = 10^{-4}$ cm/s.

After a 1% perturbation of the initial values of p, the flow pattern destabilizes and two channels develop, one carrying a much larger flux than the other (figure 2*b*). For more elaborate patterns of this kind, generated by a diffusion-based model, see Mitchison (1980*a*).

4. Vein loops

One intriguing feature of vein formation is the occurrence of loops (see figure 3). These are seen when a piece of a storage tissue is cut out and left for some time, preferably with an artificial supply of auxin at the apical end. Then loops are frequently found, usually near the basal end of the tissue (T. Sachs, personal communication). These can be interpreted as a diagnostic test for an underlying polar mechanism. If veins are indeed formed by the flow of a substance, then a circular flow could not be maintained by diffusion alone, but would need some kind of polar transport. Intuitively, it is reasonable that a cycle of cells can maintain a circulating flow. We can now check the conditions for the flow to be self-catalysing.

Suppose that every cell has concentration a at the end where auxin enters in the circulating flow, and concentration b at the other end. Let us assume that auxin neither enters nor leaves the ring of cells. Let the flux through each cell in the ring be F. Then from (2) and (3) we have

$$F = D(a-b)/L = pb+q(b-a), \tag{20}$$

and conservation of the total amount of auxin in the ring implies that

$$a+b = C \tag{21}$$

for some constant C.

If we wish circulation to increase, then (8) requires that

$$\frac{1}{\epsilon}\frac{\partial p}{\partial t} = kF^2-p > 0.$$

Eliminating a and b from (20) and (21), we have

$$p = 2F(1+qL/D)/(C-FL/D),$$

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and using this expression for p in (21) we obtain as the condition for escalating circulation

$$kF(C-FL/D) > 2(1+qL/D). \tag{22}$$

When this is an equality, we have

$$F = CD/2L \pm \tfrac{1}{2}\{(CD/L)^2 - 8(D/L+q)/k\}^{\frac{1}{2}}. \tag{23}$$

These roots are real when

$$C^2 > 8L(1+qL/D)/kD, \tag{24}$$

which is the condition for there to be a range of values of F for which the flux increases and vein loops form.

This condition explains why loops occur at the basal end of a piece of storage tissue. Transport carries auxin to the basal end, where its concentration rises. In fact, steep concentration gradients are observed at the free basal ends of segments supplied with an apical auxin source (Wangermann 1974). As such a gradient forms, back-diffusion will decrease the basally directed flux, so p will fall in the basal faces of cells. We therefore expect the following type of auxin distribution in the tissue: a low concentration at the apical end, then, moving basally, a sigmoidal region of steep concentration increase, followed by a plateau at the basal end, where cells have lost their original polarity. In this plateau, fluctuating, random flow patterns can develop. When the concentration, C, is large enough to satisfy (24), these can become self-reinforcing flux circuits.

The formation of loops in this manner does not happen by destabilization, and is therefore formally unlike vein formation. For veins, given appropriate conditions, a perturbation of a uniform flow grows in amplitude until channels form. This is true on matter how small the perturbation is. For loops, the perturbation must exceed a certain minimum. However, this minimum may be very small, so that intrinsic noise would suffice to initiate loops. The formal difference may therefore be of no practical significance.

To see how large a perturbation is needed, consider first what happens when the condition (24) is just satisfied. From (23), $F = \{2(q+D/L)/k\}^{\frac{1}{2}}$, and taking $p = kF^2$ we obtain $p = 2(q+D/L)$. With $D = 7\times10^{-6}$ cm^2/s, $L = 100$ μm, and $q = 10^{-4}$ cm/s, we find that $p = 9\times10^{-4}$ cm/s, which is a large value for this permeability (larger in fact than our estimate of $p = 4\times10^{-4}$ cm/s for the basal walls of cells during unperturbed transport). It seems unlikely that such large polarities would often form by chance. However, suppose C is, say, five times larger than this minimum value. Then, from (23), $p = 0.02(q+D/L)$, and with the same values of q, D and L we have $p = 9\times10^{-6}$ cm/s. It is now quite plausible for this small permeability to occur by a chance fluctuation in the distribution of transport channels. So we conclude that a moderate increase in concentration beyond the minimum specified by (24) produces conditions where loops should arise spontaneously.

5. Conclusions

I have shown that, under appropriate conditions, a uniform flow of auxin, transported down a sheet of cells, is inherently unstable, and will break down into channels carrying a larger flux than the background. These channels may be regarded as the precursors of veins. For this destabilization to occur, the transport permeability p must increase with flux in a suitable manner; for instance, p may vary quadratically with flux. Moreover, the polarity of

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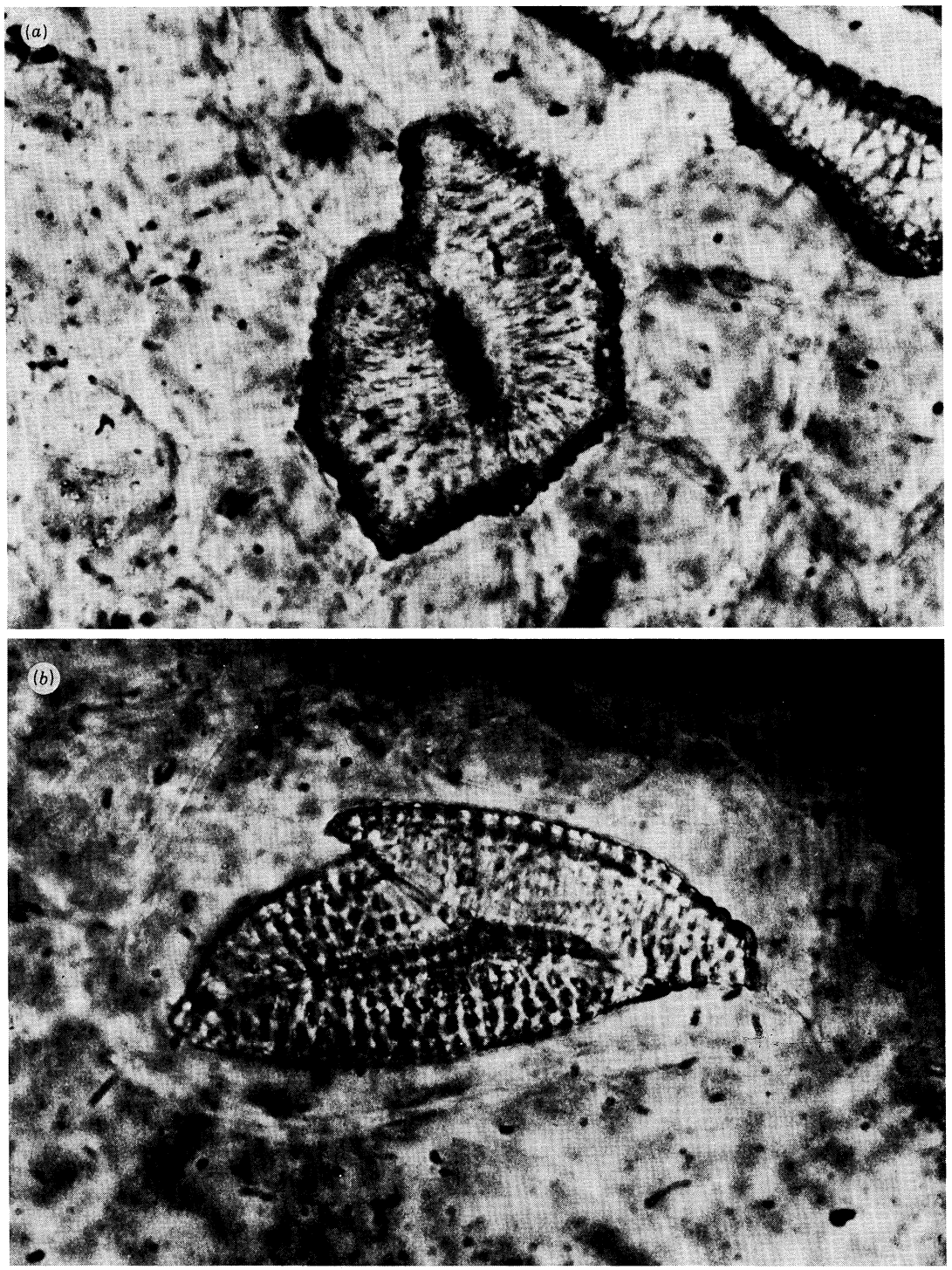

FIGURE 3. Two vein loops, one with two mature xylem vessels, the other with three, obtained by keeping small pieces of radish root with a supply of auxin at their original apical end. This is a technique that Professor T. Sachs kindly demonstrated to me.

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transport, measured by the ratio of the polar permeability p to the diffusive permeability q (see equation (1)), must not be too large. More precisely, there is an upper bound for p/q (equation (18)) that depends upon, among other things, the length L and width W of the cell. When $L/W = 2$, for instance, we must have $p/q < 7$. This inequality is satisfied by plausible values of p and q.

I have assumed that the interior of a cell is all one homogeneous compartment from the point of view of auxin movement. However, one can argue that the tonoplast may be fairly impermeable to auxin (Mitchison 1981). Consider, therefore, what happens when the tonoplast is completely impermeable. Auxin will be channelled through the layer of cytoplasm (figure 1*b*), which is typically quite thin, having a width t of a few micrometres. If the channels by which auxin leaves the cell are clustered where the flux is largest, they will presumably be at their highest density near to the lateral walls of the cell. Then we may, in effect, treat the cytoplasmic strip by the lateral walls as a very elongated 'cell', of length L and width t. Inequality (18) then prescribes an upper bound for p/q, which can be much higher than that for a homogeneous cell interior. For instance, with the constants previously used ($D = 7 \times 10^{-6}$ cm^2/s, $q = 10^{-4}$, $L = 200$ μm) and with a cytoplasmic width t of 5 μm, we find that $p/q < 35$. So the conditions are more propitious for vein formation when cells have impermeable tonoplasts.

One pleasing feature of the kind of mechanism that I have considered is that it not only explains the occurrence of loops of vein, but shows why these should tend to appear in regions of high auxin concentration. Essentially, this is because the polar movement due to transport must overcome back-diffusion, both between and inside cells. And while diffusive fluxes increase with the concentration gradient, those due to polar transport increase with the absolute value of auxin concentration.

I have implicitly assumed that there is a mechanism for measuring the auxin flux leaving a cell. However, it is not entirely clear what kind of mechanism this would be. If we follow Rubery & Sheldrake (1974) in supposing that there are anion channels by which auxin leaves the cell, then we might suppose that it was enough to measure the auxin efflux through these channels. However, this will not do. For, with the assumptions of the chemiosmotic theory, auxin will return to the cell by passive diffusion (as the undissociated acid) from the acidic environment outside, and there will be diffusive recycling, even where there is no net flow. One possibility is that auxin does not enter a cell freely as the undissociated acid, but needs special channels of some kind both to enter and leave a cell. This might avoid a certain lack of economy inherent in the present formulation of the chemiosmotic theory. For, if auxin enters cells as the undissociated acid, then about half the molecules should re-enter the cell that they have just left, so half the energy used for transport would be wasted.

Another way of measuring flux would be by reference to the concentration gradient produced inside the cell by the flux. If channels tended to congregate in that part of the cell where auxin concentration were lowest, then the desired kind of flux-dependence of polar transport could be brought about. However, it would not suffice for channels to be present at a given point in some inverse relation to the auxin concentration there, as this would change a file carrying a large flux from a sink into a source. Instead, some mechanism operating over the extent of the whole cell would seem to be needed, to ensure clustering of channels in the region of lowest concentration and a depletion elsewhere.

I have considered vein formation in relatively mature tissues of the plant. Veins may be

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induced in mature leaves by auxin (Jost 1942), but not in very young leaves (Young 1954). Sachs has postulated that the same kind of flux mechanism operates in young leaves, but involves the flow of a different signal. Now vein patterns in young leaves do not reveal any obvious polarity, but often make complex networks with strands running in different directions. In spite of this, vein loops of the kind I have described are never seen, and would presumably be an undesirable feature. I have therefore suggested (Mitchison 1980*a*) that the putative early signal may move by diffusion, which would prohibit circulation and vein loops. At a later stage, when the distances become too large for diffusion, polar transport would be appropriate. It is here that we would expect vein formation to be controlled by the flow of auxin, in the manner I have proposed.

I thank Professor T. Sachs for helpful conversations, and Professor F. H. C. Crick and the Salk Institute for support during the preparation of this paper.

References (Mitchison)

Cande, W. Z., Goldsmith, M. H. M. & Ray, P. M. 1973 Polar auxin transport and auxin-induced elongation in the absence of cytoplasmic streaming. *Planta* **111**, 279.
Goldsmith, M. H. M. 1967 Movement of pulses of labeled auxin in corn coleoptiles. *Pl. Physiol.* **42**, 258.
Goldsmith, M. H. M. 1977 The polar transport of auxin. *An. Rev. Pl. Physiol.* **28**, 439.
Goldsmith, M. H. M., Goldsmith, T. H. & Martin, M. H. 1981 Mathematical analysis of the chemiosmotic polar diffusion of auxin through plant tissues. *Proc. natn Acad. Sci. U.S.A.* **78**, 976.
Jacobs, W. P. 1952 The role of auxin in the differentiation of xylem round a wound. *Am. J. Bot.* **39**, 301.
Jost, L. 1942 Über Gefässbrücken. *Z. Bot.* **38**, 161.
Larsen, P. 1955 Growth substances in higher plants. In *Modern methods of plant physiology* (ed. K. Paech & M. V. Tracey), vol. 3, p. 565. Berlin, Heidelberg and New York: Springer-Verlag.
Mitchison, G. J. 1980*a* A model for vein formation in higher plants. *Proc. R. Soc. Lond.* B **207**, 79.
Mitchison, G. J. 1980*b* The dynamics of auxin transport. *Proc. R. Soc. Lond.* B **209**, 489.
Mitchison, G. J. 1981 The effect of intracellular geometry on auxin transport. I. Centrifugation experiments. *Proc. R. Soc. Lond.* B (In the press.)
Raven, J. A. 1975 Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. *New Phytol.* **74**, 163.
Rubery, P. H. & Sheldrake, A. R. 1974 Carrier-mediated auxin transport. *Planta* **118**, 101.
Sachs, T. 1968 The role of the root in the induction of xylem differentiation in peas. *Ann. Bot.* **32**, 391.
Sachs, T. 1969 Polarity and the induction of organized vascular tissues. *Ann. Bot.* **33**, 263.
Sachs, T. 1978 Patterned differentiation in plants. *Differentiation* **11**, 65.
Wangermann, E. 1974 The pathway of transport of applied indolylacetic acid through internode segments. *New Phytol.* **73**, 623.
Wangermann, E. & Mitchison, G. J. 1981 The dependence of auxin transport on cell length. *Pl. Cell Environ.* (In the press.)
Young, B. S. 1954 The effects of leaf primordia on differentiation in the stem. *New Phytol.* **53**, 445.

Discussion

D. E. Hanke (*Botany School, University of Cambridge, U.K.*). I was surprised that Dr Mitchison has not included in his scheme the well known autocatalytic effects of auxin on auxin uptake and the polar transport of auxin. As is known, the inward diffusion of protonated auxin and outward diffusion of auxin anion would soon cease if the protons carried into the cytoplasm thereby were not pumped out by plasma-membrane proton pumps, providing the energy to drive auxin uptake and polar transport. The activity of these plasma-membrane proton pumps is very strongly stimulated by auxin in stem and coleoptile tissues and this stimulation is probably the basis of the autocatalytic effect.

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One advantage of a model which included autocatalysis would be that, in contrast to Dr Mitchison's model, the 'drainage channels' would have *higher* concentrations of auxin than the tissue around, as is known to be required for cell elongation and differentiation into xylem or wound vessel members.

G. J. Mitchison. I agree that proton pumps may act so as to increase auxin uptake and transport. The problem is to make this sufficiently localized in space. The presence of protons in the extracellular space will increase the uptake of auxin into all cells, and not just those of a preferred channel. In fact, under certain conditions, extrusion of protons may encourage auxin to spread away from a file carrying a large flux. I therefore believe that this kind of autocatalysis cannot explain vein formation, though it might enter indirectly into the kind of scheme I am proposing (which is also autocatalytic, by the way).

On the question of differentiation of cells into xylem or wound vessel members, I believe the stimulus for this is likely to be auxin flux, not concentration. As my argument on vein loops shows, high concentrations will often bring about a large flux, and the experimental conditions may not enable one to distinguish between these two. Having said this, I would point out that, although I have assumed that the concentration falls in a strand as the flux increases, this need only be true in the early stages of strand formation. At a later stage, the anion channels (or their activity) may cluster at the basal end of a cell and be depleted from the lateral walls; and because of the latter fact, auxin may flow into a strand even when the concentration there is higher than in surrounding cells. So the metaphor of a 'drainage channel' should not be pressed too far.

A. R. Sheldrake (1*a*, *Magnus Street, Newark-on-Trent, Notts., U.K.*). The 'drainage pattern' model of vein formation that Dr Mitchison has suggested seems to depend primarily on growth centres and their distribution. Would he regard the vein pattern in a particular type of leaf as a general *consequence* of the pattern of growth in this leaf, i.e. as a consequence of its morphogenesis, just as the drainage pattern of a river system is a consequence of the general topography?

G. J. Mitchison. I have talked mostly about the flow pattern of auxin, but have implicitly assumed that there are definite sources and sinks, the sources presumably being in young leaves. I also assume that in the earliest stages of vein development in leaves there are sources of some other signal (see Discussion, and Mitchison 1980*a*). I would conjecture that sources of this other signal are also growth centres, and that the distribution of these centres is determined by a mechanism distinct from that which controls vein formation. Of course a vein, once formed, may in turn influence this mechanism, and affect the distribution of centres. But my guess – and it is no more than this – is that vein patterns are indeed largely a consequence of the pattern of growth.

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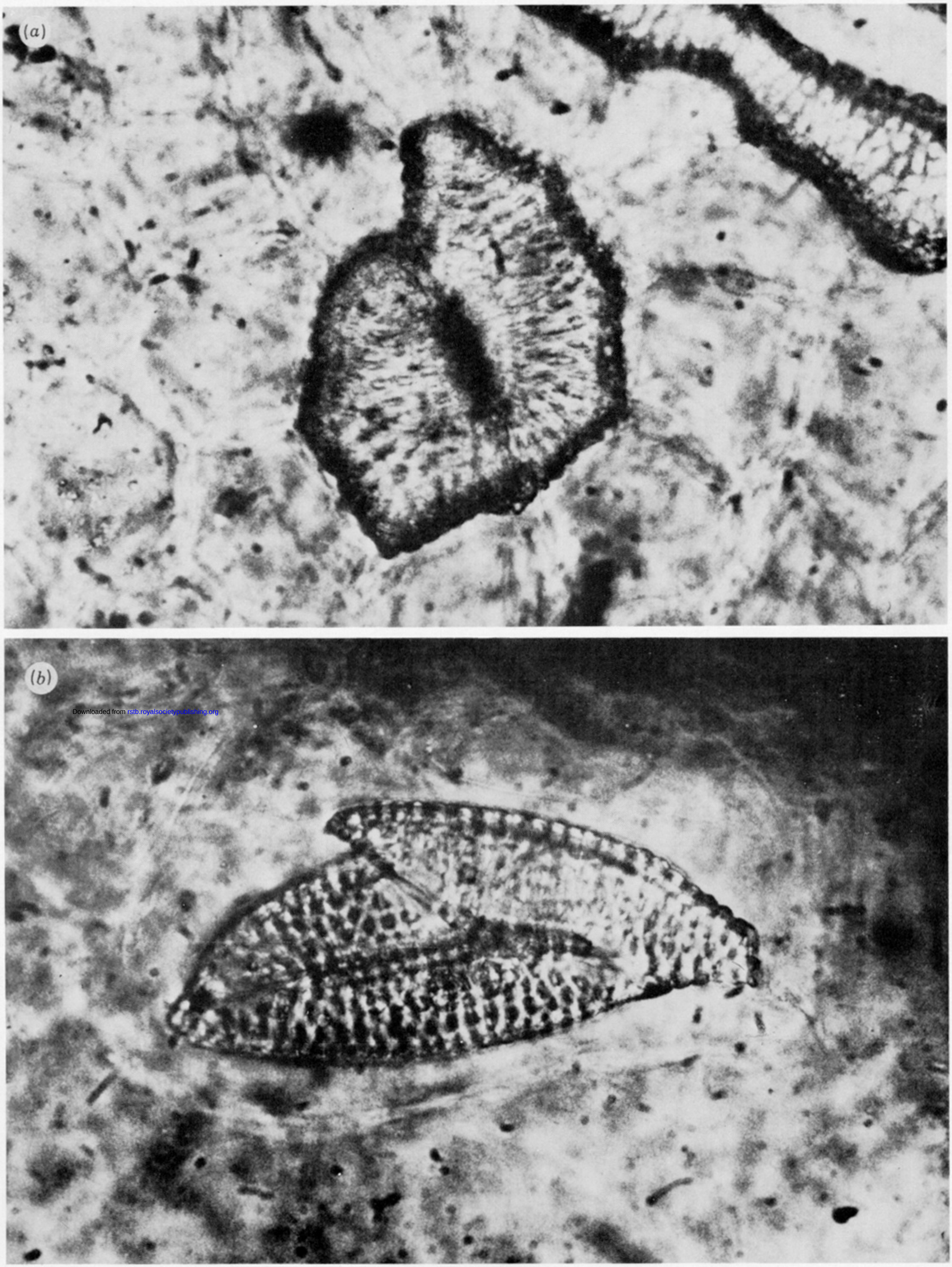

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FIGURE 3. Two vein loops, one with two mature xylem vessels, the other with three, obtained by keeping small pieces of radish root with a supply of auxin at their original apical end. This is a technique that Professor T. Sachs kindly demonstrated to me.

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