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Potassium cycling in *Helianthus*: ions of the xylem sap and secondary vessel formation

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SUMMARY

Recent work has shown that dyes travelling in the transpiration stream in dicotyledon leaves become concentrated in the vessels of the finest veins where water enters the symplast. Such concentrations are called sumps. Using X-ray microanalysis of frozen-planed *Helianthus* leaf tissues, this paper investigates whether natural ions in the transpiration stream behave similarly and become concentrated in the fine veins. The most abundant ion in the xylem sap was potassium (K): concentrations of up to ~ 200 mM were found in some vessels of some leaf veins. Occurrence of such high concentrations was irregular and unrelated to vein order, leaf age, time of day or transpiration conditions. High K concentrations were not especially characteristic of the fine veins, and it appears that sumps are not formed (as with the dyes) by selective uptake of water to the symplast: absence of K sumps is probably the result of uptake of K by the cells surrounding the vessels. The origin of K was sought in the stem, where evidence was found that differentiating secondary vessel elements accumulate very high concentrations (~ 500 mM) of K, releasing it into mature open vessels when they mature themselves. I propose the hypothesis that the K in the leaf vessels is derived from the K of the maturing secondary vessel elements of the stem. It arrives irregularly because the vessel maturation is spasmodic and the destiny of the released K depends upon the particular downstream connections of the new vessel to leaf traces. I further propose that the K in the leaf veins is taken up by bundle sheath cells and phloem parenchyma cells, and part of it is returned via the phloem to the cambium of the stem where it may be used again to provide osmoticum in the expansion of newly differentiating secondary vessel elements. When high concentrations of ions are present in vessels that are embedded in tissues whose cell walls have non-zero reflection coefficients (low diffusivities), osmotic pressures would develop in them. Such pressures may counteract tensions in the xylem sap generated by transpiration, and help to account for the small values of these tensions measured recently with the xylem-pressure probe.

1. INTRODUCTION

Mineral ions absorbed by roots travel to the shoot in the xylem with the transpiration water. The accepted understanding of this traffic is that the ions would leave the roots in dilute solution (< 10 mM, e.g. Gollan *et al.* 1992), and during their passage through the stem they may be dispersed through the permeable cell walls of the stem parenchyma. There they may be accumulated by parenchyma cells or by the more specialized and localized transfer cells (Pate 1975). Such transfer cells are found mainly at nodes. The solution arriving at the leaves would thus be more dilute than that which left the roots. In the leaves there is a separation of the transpired water from solutes, which must either accumulate there, be excreted from the leaf surface (Arens 1934; Tukey 1970), or be returned to the stem by translocation in the phloem (Phillis & Mason 1942; Jeschke *et al.* 1987). This widely accepted view of the progress of the xylem sap needs modification in the light of recent work.

Until recently, it has been assumed that the course of the transpiring sap beyond the xylem of the leaves is a flow of solution through open, permeable cell walls so that each mesophyll cell is bathed in the transpiration stream (e.g. Aston & Jones 1976; Maier-Maercker 1980, 1983). It is expected that evaporation of the water would leave the ions at increased concentration in the cell walls, especially those near the sites of evaporation. It was these enhanced concentrations that Aston & Jones (1976) and Maier-Maercker (1980) were seeking, but these assumptions have been shown as invalid, at least for fluorescent dyes (Canny 1990*a*, 1993). Dyes in the transpiration stream do become concentrated in the leaves by a factor of hundreds to thousands, but not at the sites of water evaporation. They become concentrated at places where water crosses cell membranes and enters the symplast, leaving the dye behind in the apoplast. These sites of concentration are termed 'sumps', and the places where the water enters the symplast are called 'flumes' (Canny 1990*a*): sumps are evidence of the location of flumes. In dicotyledon leaves the sumps are inside the vessels of the smallest veins (Canny 1990*b*), and in grass leaves they are in intercellular spaces inside the parenchyma sheath of the minor veins (Canny 1988; O'Dowd & Canny 1993). In 1991 I proposed another mechanism by which sumps may be formed in the smallest vessels at the apex of a wedge of connected vessels in transpiring organs. In a second revision of the

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traditional view, cell walls have been shown not to be the open, flow spaces of the model described above. Dye molecules move in the walls by diffusion, and at rates that are orders of magnitude less than in water (Canny 1990*c*; Canny & Huang 1994). So once a sump is formed, the dye moves away from the sump in the cell wall apoplast by diffusion, unaffected by the flow of the transpiration water (Canny 1990*c*).

This study began first as an attempt to discover whether the natural solutes of the xylem sap behaved in the same way and to the same extent as the dyes, becoming concentrated in sumps in the same places. The second aim was, if possible, to discover whether these solutes (like the dyes) diffused slowly in the cell walls. The method chosen was that of X-ray micro-analysis of the frozen leaves of *Helianthus* in the cryoscanning electron microscope: by this technique ion contents of individual vessels can be measured. The method is not very sensitive, however, so that concentrations of 10 mM or less (typical for ions leaving the root in the xylem sap) would be below the threshold of measurement. But if higher concentrations were formed at sumps, as they were for dyes, these should be easily detected. A preliminary account of some of these results has already been published as part of a review (Canny 1993), showing that concentrations of potassium in the range 100–200 mM can be found in some vessels in leaves, along with calcium, phosphate and sulphate in the range 30–70 mM. This paper presents full experimental details and results of these observations, showing that the high ion concentrations are not formed in the same way as the dye sumps, and proposing a hypothesis for the origin of the K concentrations. The question of the rates of diffusion of these ions in cell walls has not yet been approached.

The different sizes of vein in the leaf are referred to by numbers from V1 for the midrib to V5 for the smallest veins, using the scheme set out by Wang & Canny (1985). Veins V1 to V3 have raised ridges of tissue projecting below the lamina and contain at least one vessel of large diameter (> 15 μm). Veins V4 and V5 lie wholly within the thickness of the lamina, and contain only vessels of small diameter (< 10 μm).

2. MATERIALS AND METHODS

Two species of the genus *Helianthus* were used: *Helianthus annuus* L. and *Helianthus tuberosus* L. (grown in pots in the greenhouses of Carleton University and in the field at the Experimental Farm of Agriculture Canada, Ottawa, respectively. The plant of *H. tuberosus* was flowering, and used for the systematic study of concentrations in the different sizes of vein in a single leaf. Twenty-one variously aged *H. annuus* plants (ranging in height from 40–200 cm) were sampled at different times of the day and used to study the ranges of concentration found in leaves of different plants and different ages. Two plants of *H. annuus* 160 cm high were used to investigate the concentrations in vessels of the stems.

Early trials showed that when samples were cut from leaves in air many of the vessels in the preparations were empty of fluid. Accordingly, in later experiments the samples were cut from the leaves under water, reducing greatly the number of embolized vessels. No differences were found (outside the limits of the usual variability) between analyses of leaves prepared wet or dry. A leaf was severed from the plant, transferred to water, and its petiole immediately recut under water. Then, with the leaf immersed in water, small pieces of petiole, or of lamina (*ca.* 4×10 mm) containing the chosen order of vein, were cut out with sharp scissors. These pieces were blotted and either frozen at once for storage under liquid nitrogen, or mounted in a slit in a stub with Tissue Tek and frozen in the stub. For the analysis of the stem xylem, intact stem pieces of selected internodes were frozen in baths of liquid nitrogen, and then dissected into smaller pieces under liquid nitrogen. Preparation, observation and analysis of the tissues, and calibration of the elemental concentrations, used the procedures detailed in Canny & Huang (1993) and Huang *et al.* (1994). Briefly, freezing of leaf pieces was in nitrogen slush (-210 °C). Stored pieces retrieved for later analysis were mounted, still frozen, on stubs with Tissue Tek. The frozen pieces were planed flat to reveal transverse sections of the veins, etched very lightly while observed in the column of the scanning electron microscope, coated with aluminium, and returned to the microscope for observation and EDX-analysis with the Be-window (Huang *et al.* 1994).

The threshold of detection for an elemental peak in a sample is determined by the random fluctuations in the background Bremsstrahlung at the position of the peak and the built-in smoothing progamme that calculates the magnitude of this background, which it subtracts from the peak to estimate the percentage ratio. Because in samples containing none of the measured elements the programme produced some negative values for peaks down to -1%, it was arbitrarily decided to record values only for peaks with values $\geqslant +1\%$. On this criterion, the limits of detection of the elements vary somewhat with the thickness of the aluminium coating in the range 10–20 mM. Concentrations less than this threshold are not necessarily zero, and are represented in the tables and figures by the symbol ∅, and the threshold level is indicated. Because of the overlap of the potassium K_b peak into the calcium peak, the threshold of detection of calcium also varies with the potassium content and the ∅ value quoted for calcium does not apply if the spectrum contained high potassium. The instrument measures elemental concentrations. It is likely, but by no means certain, that the elements measured are present as ions, and the calibrations were made on ionic solutions. To emphasize this point, the symbols for the elements are written without their ionic valencies.

3. RESULTS

Examples of the images and analyses obtained are shown in figures 1 and 2 and tables 1 and 2. The midrib in figure 1 had a high proportion of embolized vessels because it was prepared in the first stage of the study when leaves were cut from the plants in air. Vessel elements were classified as living and

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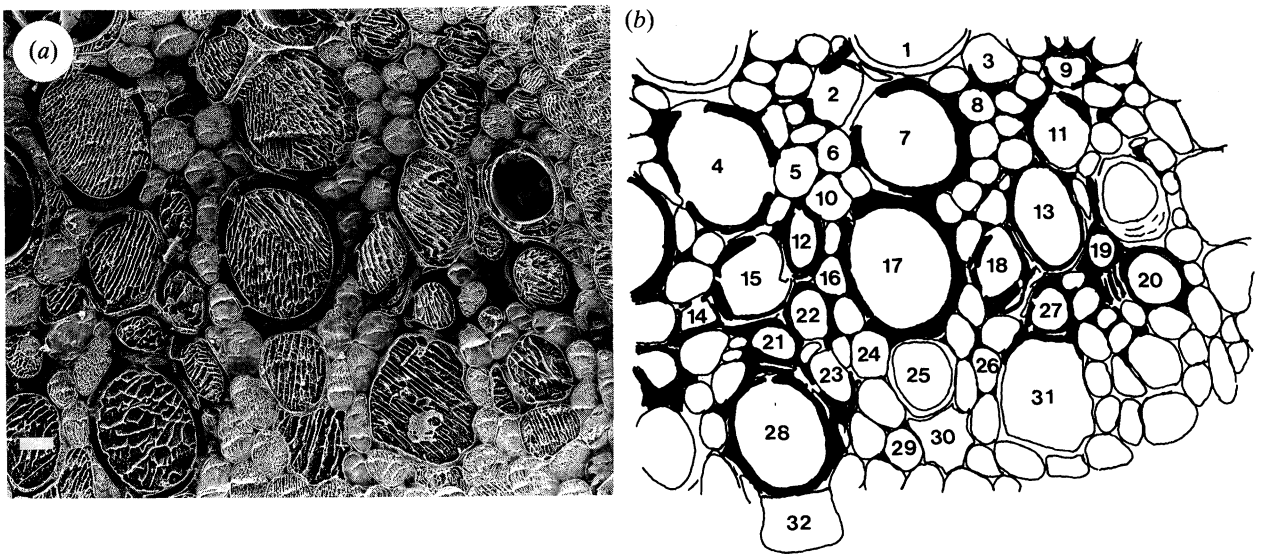

Figure 1. (*a*) Frozen planed face of part of the V1 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and xylem vessels, some with air embolisms, and most filled with sap. EDX microanalyses were made of the contents of the cells of the face indentified in figure 1*b*. Bar = 10 μm. (*b*) Drawing of the face shown in figure 1*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 1.

Table 1. *Measured concentrations of elements* (mM) *in xylem sap of three cell classes of a V1 vein of* H. annuus *leaf shown in figure* 1

(Cell numbers refer to the cells numbered in figure 1. The value ⌀ is the threshold of detection for each element.)

	P	Cl	K	Ca
	mM			
⌀ value	11	10	13	10
cell no.				
open vessels				
1	13	38	90	48
2	15	41	84	25
3	34	34	77	31
4	52	38	131	37
7	29	49	111	26
9	28	34	74	24
11	40	33	88	22
12	35	36	77	28
13	39	40	107	42
14	17	33	82	23
15	32	43	95	24
17	11	42	82	22
18	43	34	111	27
19	29	40	81	21
20	28	42	89	24
22	15	38	93	22
23	21	40	102	23
27	31	47	100	27
mean, s.d. ($n = 18$)	28±11	39±5	93±15	28±7

Table 1—*cont.*

	P	Cl	K	Ca
	mM			
⌀ value	11	10	13	10
cell no.				
differentiating vessels				
21	⌀	55	324	12
25	11	210	337	⌀
28	11	165	313	9
31	11	273	347	⌀
32	58	127	347	⌀
mean, s.d. ($n = 6$)	22±21	160±75	320±36	⌀
xylem parenchyma				
5	122	17	70	⌀
6	80	31	53	11
8	38	15	22	⌀
10	96	28	56	⌀
16	50	23	83	⌀
24	13	23	83	⌀
26	18	35	119	⌀
29	40	25	190	⌀
mean, s.d. ($n = 8$)	57±39	26±7	85±51	⌀

differentiating, or open and conducting, partly by their position (formed abaxially, maturing adaxially) and partly by their ionic contents. The wall thickening is not a reliable guide to maturity except for the very immature elements. Using these criteria, there were very few cells that could not be classified unambiguously as living vessel elements, dead vessels or xylem parenchyma. The analyses made on the designated cells are grouped in table 1 into those for dead, open vessels, living differentiating vessel elements and xylem parenchyma and exemplify several features found in all the preparations.

1. Open vessels contained substantial K concentrations (here, a mean of 93 mM).

2. Differentiating vessel elements contained high K concentrations (here, 320 mM), and the highest Cl concentrations found in any cells of the plants (here, 160 mM). Cell 21, which by its thickened wall and

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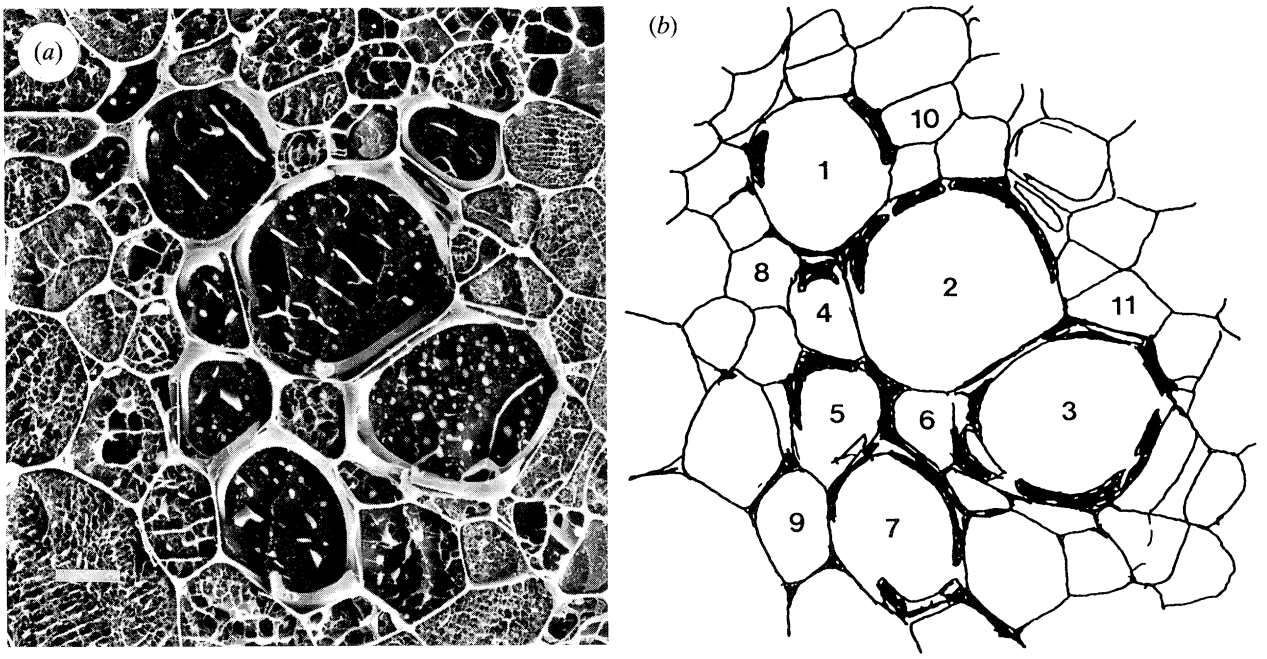

Figure 2. (*a*) Frozen planed face of a small bundle of a V3 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and xylem vessels. EDX microanalyses were made of the contents of the cells of the face indentified in figure 2*b*. Bar = 10 μm. (*b*) Drawing of the face shown in figure 2*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 2.

Table 2. *Measured concentrations of elements (mM) in xylem sap of vessels in the small bundle of a V3 vein of* H. annuus *leaf shown in figure* 2

(v denotes vessel; difv denotes differentiating vessel; xp denotes xylem parenchyma. Cell numbers refer to the cells numbered in figure 2. The value ⌀ is the threshold of detection for each element)

		P	Cl	K	Ca
		mM			
⌀ value		16	16	16	16
type	cell no.				
v	1	⌀	⌀	44	20
v	2	20	⌀	57	19
difv	3	⌀	64	227	⌀
v	4	21	⌀	48	16
v	5	31	⌀	60	16
xp	6	56	⌀	147	⌀
xp	8	50	⌀	111	22
xp	9	22	⌀	99	⌀
xp	10	26	⌀	86	⌀
xp	11	62	⌀	166	⌀

position might well be open and conducting, is shown as still alive by its ionic contents.

3. Xylem parenchyma cells contained K concentrations that were lower than those in differentiating vessel elements, and often higher than those in open vessels, or (as here) about equal to them.

4. Calcium was usually undetectable in living cells but present in open vessels at concentrations from 20–50 mM. Sodium and N (NO_3^-) are not detectable with the Be-window.

5. Of the elements detectable with the Be-window which might be present, Mg and Si were never detected and S occurred occasionally at levels just above the threshold of detection (but has not been included in the tables).

6. The standard deviations on means of small samples of similar cells could be as small as 10% of the mean, but were usually in the range 20–30% of the mean. This variation is probably due to inherent differences beween cells. The errors due to the technique are less than this. In table 1, the predominant cations in the open vessels could be balanced by undetected NO_3^-. The P is unlikely to be present as a di- or trivalent anion, which would be precipitated by the high level of Ca. In other veins (see table 3) there was little or no P.

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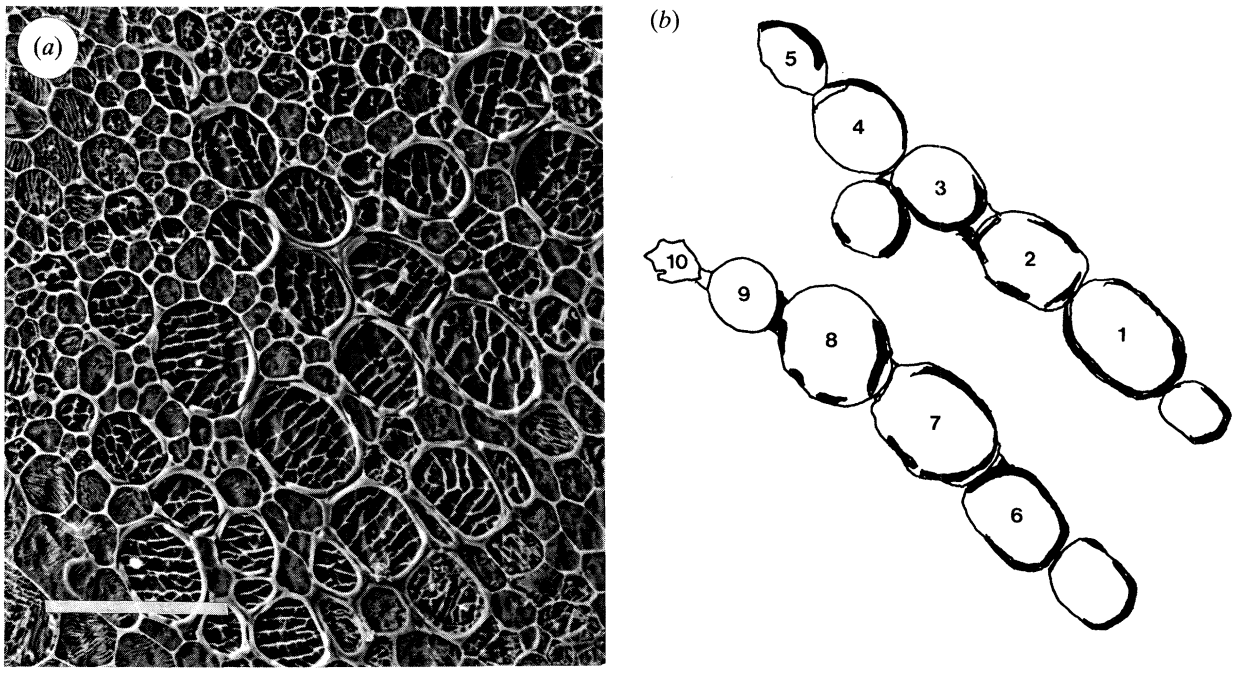

Figure 3. (*a*) Frozen planed face of part of the V1 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and arcs of metaxylem vessels. EDX microanalyses were made of the contents of the cells of the face indentified in figure 3*b*. Bar = 100 μm. (*b*) Drawing of the arcs of metaxylem vessels shown in figure 3*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 3.

Table 3. *Measured concentrations of elements in xylem sap of vessels in two arcs of metaxylem ending in protoxylem vessels in a V1 vein of* H. annuus *leaf shown in figure 3*

(px denotes protoxylem. Cell numbers refer to the cells numbered in figure 3. The value ⌀ is the threshold of detection for each element.)

	P	Cl	K	Ca
	mM			
⌀ value	18	16	18	17
cell no.				
upper file				
1	26	⌀	90	⌀
2	27	⌀	58	17
3	19	⌀	72	28
4	⌀	⌀	85	13
mean, s.d.			76 ± 14	
5(px)	41	⌀	109	28
lower file				
6	⌀	⌀	78	⌀
7	⌀	⌀	59	⌀
8	⌀	⌀	57	⌀
9	⌀	⌀	75	⌀
mean, s.d.			67 ± 11	
10(px)		⌀	123	⌀

Similar relations are seen on a smaller scale in figure 2 and table 2, giving analyses from a small vascular bundle in a V3 vein. Here the only differentiating vessel, Cell 3, was conspicuous by the high K and Cl concentrations. Phosphorus was low in the open vessels, and their K concentrations were below those of the adjacent xylem parenchyma cells.

An example of the distinctions made possible by this technique is a demonstration of the postulated sumps (Canny 1991) at the apex of wedges of metaxylem illustrated in figure 3 and table 3. Two arcs of metaxylem vessels in the midrib of a sunflower leaf ended in protoxylem vessels (cells 5 and 10). The dominant ion present in the vessels was K, and in both arcs the concentration in the terminal protoxylem vessels was higher than the mean level in the four metaxylem vessels, in the upper arc 140 %, in the lower 180 %. The upper arc contained considerably more P and Ca than the lower. This common composition of the contents of vessels within an arc and the difference

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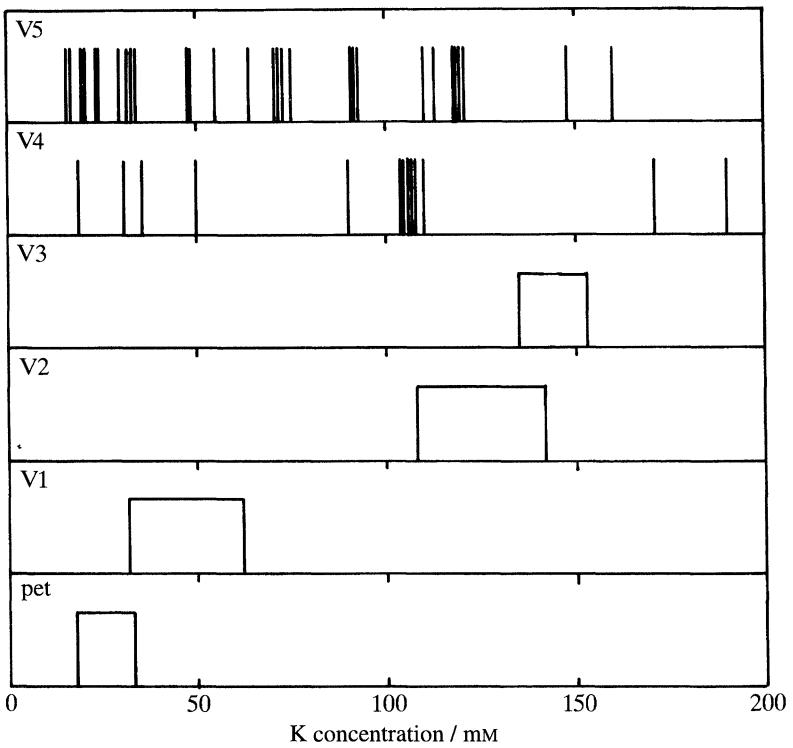

Figure 4. Bar graphs of the K concentrations in vessels of the petiole and five vein types of a single leaf of *Helianthus tuberosus*. Single lines represent measurements of single vessels in V4 and V5 veins. The wide bars in the graphs for the petiole and V1 to V3 veins represent mean concentrations in several vessels, centred on the mean, and 2 × s.d. wide.

in composition between neighbouring arcs, is shown in other examples below.

The search for the more general formation of sumps of the natural ions in the fine veins, prompted by the behaviour of the dyes, did not quickly yield a clear answer. The variation in ionic content of vessels between veins, between leaves, and between plants was large and clear patterns were not discerned. The search was further hampered by the limited sampling possible with the cryoanalytical technique (a limitation imposed by the time required to collect a spectrum from each vessel, the few specimens that could be analysed in a day and the high daily cost of operating the instrument). It is necessary to convey the extent of this variation.

A detailed study was made of the vessel contents in the different vein orders of a single leaf of *H. tuberosus*, and the results are presented in figure 4. This consists of six bar graphs of K concentrations measured in the petiole and five vein orders of the leaf. In the petiole and V1 to V3 veins the measurements are displayed by bars centred on the mean value measured and two standard deviations wide. For V4 and V5 veins each vein contains so few vessels (4 or 2), that averaging is useless, and the bars represent measurements in individual vessels. The absence of values at the limit of detection is real, not due to the omission of values equal to ∅. All vessels contained measureable quantities of K. The figure could be interpreted as giving some support to the hypothesis of sump formation in fine veins because the mean values increase from the petiole through V1 to V3. But in the finest veins, though there are some high values, the concentrations are spread over the whole range. This could be explained by adding to the hypothesis the likely proposition that the finest veins are the sites of retrieval of K from the transpiration stream.

However, this pattern is not sustained when the analyses from the vein orders of all the 21 other leaves studied are displayed in the same manner (figure 5). Here the convention of displaying means by wide bars has been abandoned because the number of means is too great. The bars in the graphs of petiole and V1 to V3 represent means, and those in the V4 and 5 graphs are mostly single vessels. The branched bars represent means from different bundles in the same vein section. The pattern of increasing concentration with smaller vein size seen in figure 4 is no longer apparent, and can certainly not be taken as a generalization for the pooled data. The large number of V5 veins with values less than 50 mM suggests that these veins may indeed be the main sites of retrieval of K. Not only was there no consistent relation between K concentration of vessels and vein order, but no relation was found with time of day, age of leaf, or plant growth conditions. The ions of the xylem sap were not behaving like the dyes and becoming concentrated by loss of water into the symplast in the finest veins. And yet the K concentration in the vessels of the leaves was much higher than expected, higher than in the sap as it comes from the roots (commonly in the range 50–150 mM) and reaching almost to 200 mM. Such concentrations in vessels should have important influences on the functioning of leaves. The search for the source of the high concentrations had to be directed elsewhere.

The occurrence in petioles of some vessels with K

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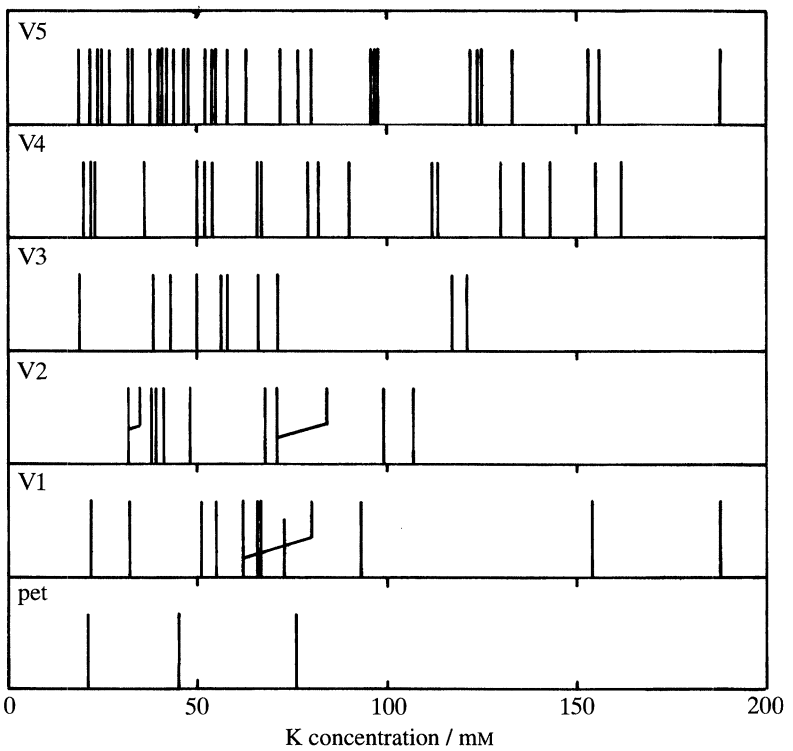

Figure 5. Bar graphs of the K concentrations in vessels of the petiole and five vein types of 21 leaves of *Helianthus annuus*. Bars represent single vessels in V4 and V5 veins, and mostly the means of several vessels in individual veins for the petiole and V1 to V3 veins. Branched bars indicate different mean concentrations in different vascular strands of the same vein.

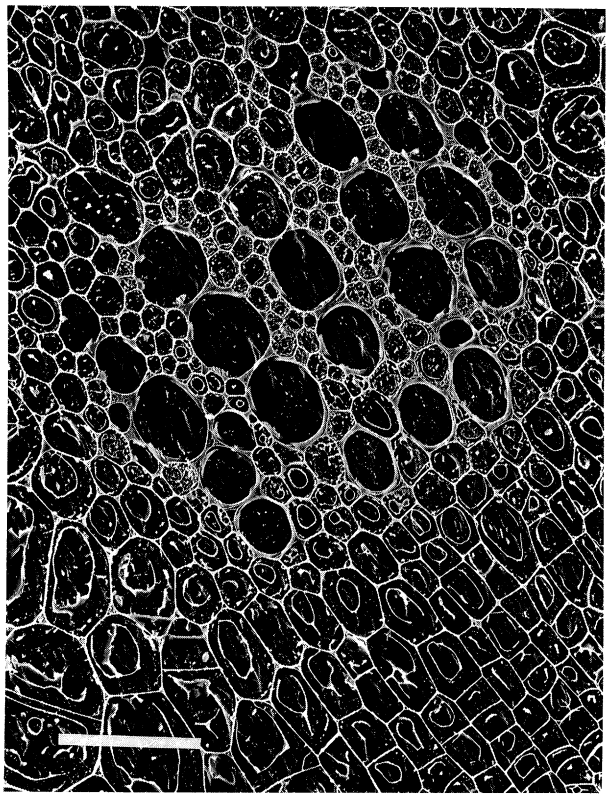

Figure 6. Planed frozen face of a vascular strand in the first internode of three expanded internodes above the cotyledons of a stem of *Helianthus annuus*. This strand shows mature metaxylem with no secondary xylem differentiating. Analyses of the vessel contents revealed no elements detectable above the threshold concentration. Bar = 100 μm.

concentrations as high as 80 mM (see figure 5) suggested that the source of the K might be somewhere in the stem: analyses were made of vessel contents in several internodes of stems frozen intact. Some stem bundles consisted of only metaxylem vessels (see figure 6) and registered no detectable K in their vessels. Other bundles showed the initiation of secondary xylem production from the interfascicular cambium (see figure 7). Analyses of these vessels showed two features already noticed in the leaves: (i) the differentiating vessel elements contained high K and Cl; and (ii) the contents of the open elements were different in different arcs. The analyses from part of the xylem of figure 7 are shown diagrammatically in figure 8. Open vessels are shown by continuous circles, differentiating vessels by dashed outlines. Within each cell the millimolarities of K and Cl are written ([K], [Cl]). The two left arcs of open vessels contained very little ionic solute; the right arc of three open vessels contained substantial K and no detectable Cl. The living vessel elements at the base of each arc contained high K, and in some cells, Cl.

A more abundant field of differentiating vessels in part of a bundle of an internode is shown in figure 9. In each arc the K and Cl increased from the youngest differentiating vessel to a peak: this peak may occur in the cell immediately before the first open vessel. There was then an abrupt fall (as in the centre arc from 545–43 mM K) at the first open vessel. Or the peak may be two cells before the first open vessel, when there is a smaller downward step (as in the arc to the left of centre from 400 mM K, 126 mM Cl to 186 mM K, 30 mM Cl), before the open vessel with 47 mM K and undetectable Cl.

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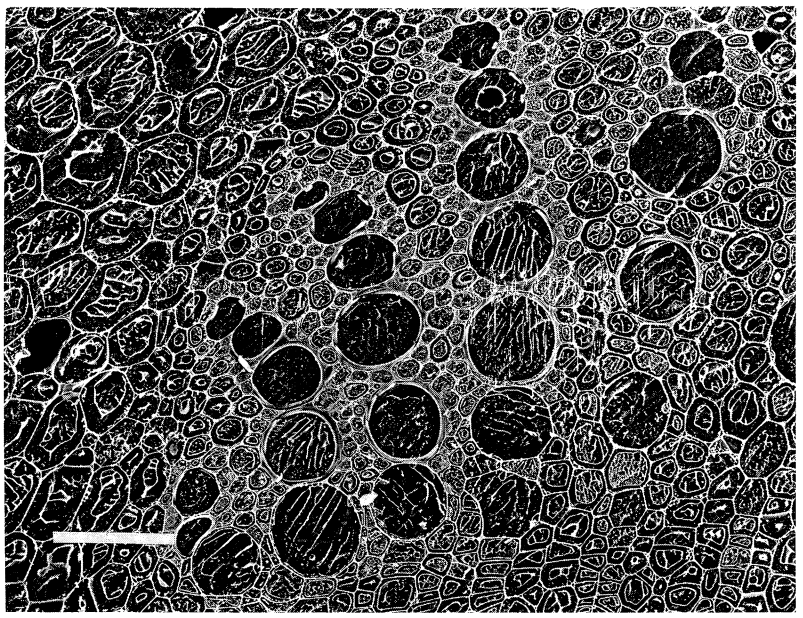

Figure 7. Planed frozen face of a vascular strand in the third (youngest elongated) internode of three expanded internodes above the cotyledons of a stem of *Helianthus annuus*. This strand shows vessel elements differentiating from the interfascicular cambium to form secondary vessels. Bar = 100 μm.

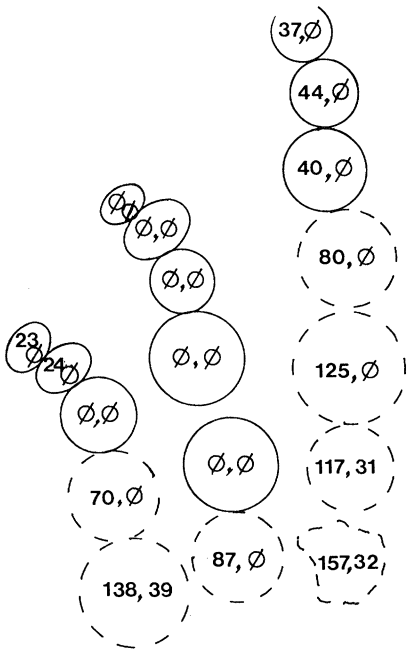

Figure 8. Diagram of the vessels analysed in the face shown in figure 7 (×200). Mature vessels indicated by continuous outlines, immature elements by dashed outlines. In each analysed cell the concentrations (mM) of K and Cl are written: [K], [Cl].

In a large field of developing and mature secondary vessels in the internode shown in figure 10 the characteristic K content of individual arcs is clearly seen. In the first three arcs from right to left the open vessels had respectively, 30–40 mM K, undetectable K and 26–40 mM K. The remaining four arcs had K concentrations that were undetectable, or just above the detection limit in the vessel(s) next to the last undifferentiated vessel element. The K and Cl contents during early stages of vessel formation from cambial derivatives are illustrated in figure 11, in which three or four arcs of vessels are followed from the cambium to the early metaxylem. Chloride content of the elements rose from undetectable at the cambium to 50–70 mM in the late stages of vessel expansion, and fell again as the element reached maturity. In all four figures (8–11), and indeed in all vessels measured, the Cl in the open vessels was undetectable. No P was detected in any of the open vessels shown in figures 8–11, in contrast to those of figures 1–3 and tables 1–3.

4. DISCUSSION

Analysis of the sap bleeding from sunflower stems cut off just above the roots (Gollan *et al.* 1992) gave values for the ionic concentrations leaving the roots as (mM) : K^+ 4.4; Ca^{2+} 0.7; Na^+ 0.06; NO_3^- 7.3; PO_4^{3-} 0.6; Cl^- 0.4; SO_4^{2-} 0.4. Of these ions, Na^+ and NO_3^- were not measurable by the present technique. The others were found concentrated up to 100-fold in some of the vessels of some of the leaves.

The one certain conclusion from the present observations is that K and the other ions of the transpiration stream do not behave like dyes introduced to the stream. They do not form sumps in the finest veins at the sites where the dye experiments suggest most water crosses cell membranes through flumes into the symplast. A likely explanation of this difference between the behaviour of the natural and foreign solutes in the stream, is that the plasmalemmas of the cells bordering the vessels, the xylem parenchyma and bundle sheath cells, have transport systems that admit the natural ions to the symplast, but do not admit the dyes. In the terminology introduced by Canny (1990*a*), there are 'chutes' for the ions but not for the dyes. This explanation is fully in accord with the evidence that the main natural constituent, K, is recovered from the stream in the leaves and re-exported to the stem by the phloem (Jeschke *et al.* 1987). This recovery process is operating in the

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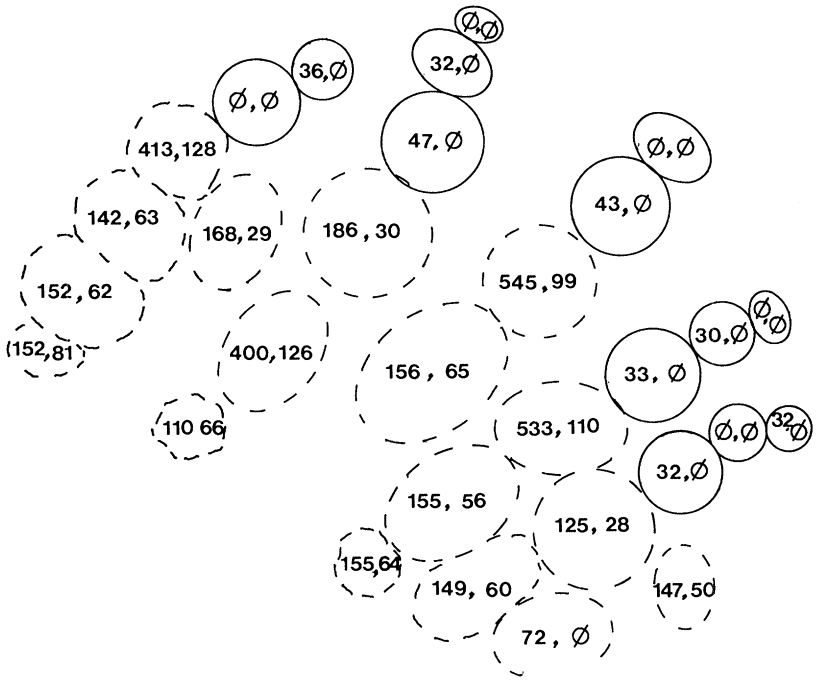

Figure 9. Diagram of the face of a vascular strand in the youngest elongated internode of a stem of *Helianthus annuus* (× 170), showing differentiating vessel elements (dashed line), and mature vessels (solid line). In each analysed cell the concentrations (mM) of K and Cl are written: [K], [Cl].

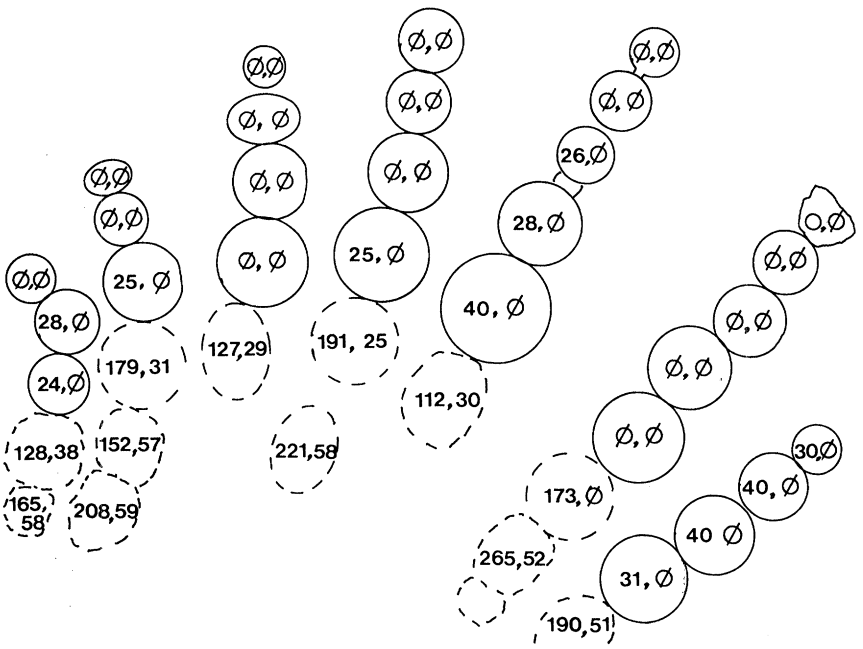

Figure 10. Diagram of another strand in the same face as figure 9 (× 140). In each analysed cell the concentrations (mM) of K and Cl are written: [K], [Cl].

opposite direction to the concentrating process that results from the separation of water from the stream by evaporation. The balance between the two processes can be followed on the whole leaf scale in figure 4, where K becomes more concentrated in the vessels as it moves through the petiole, V1, V2 and V3 veins, but in the smaller veins beyond V3 the concentration drops again as K is removed into the symplast through chutes. The concentrating effect of water loss is seen in the larger veins. The effect of the ion uptake to the symplast on the concentration remaining in the vessels increases as the vessels become smaller, as the surface to volume ratio becomes greater, and as the residence time of the stream in them becomes longer. The fact that this rational pattern disappears when the data from many leaves are pooled (figure 5) is easily accounted for if the concentration of K entering the leaves varies over a wide range, as indeed it does. In so far as the high concentrations of ions in leaf vessels may be produced by evaporative separation of water, this appears to occur in the large veins (V1–V3), not in the smallest veins (V4–V5) as for the dyes. But this

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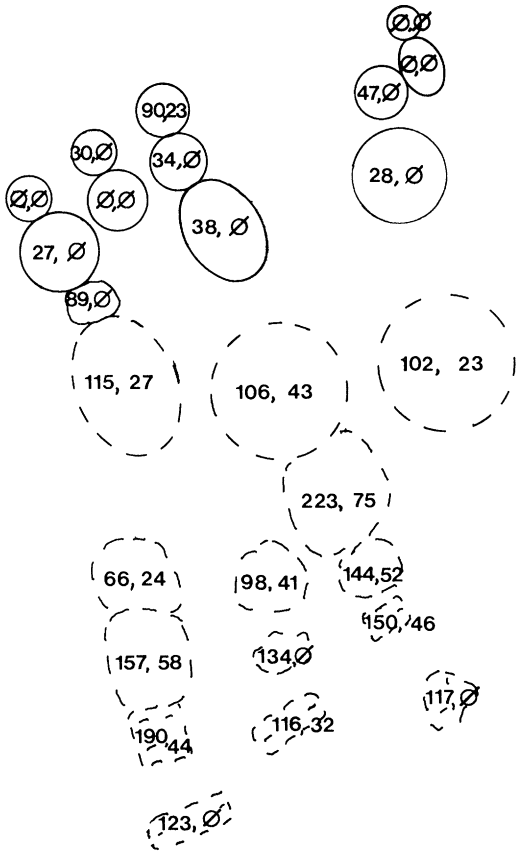

Figure 11. Diagram of the face of a vascular strand in the second of six elongated internodes above the cotlyledons of a stem of *Helianthus annuus* (×190), showing differentiating vessel elements (dashed line), and mature vessels (solid line). In each analysed cell the concentrations (of K and Cl are written: [K], [Cl].

increase in concentration of K in the leaf vessels by evaporation cannot explain the high levels of K which enter the petiole in the first place, nor the erratic occurrences of the high and low K concentrations, unrelated to the state of the plant or the environmental conditions. Some other mechanism must be operating.

I propose the following hypothesis to explain both the occurrence of high concentrations of K in vessels of leaves, and the erratic distribution of these concentrations within and between leaves. I suggest that one origin of the high concentrations of K is the developing vessel elements in the stem. As shown in figures 9, 10 and 11, as the vessels differentiate from cambial cells the K increases from around 100 mM to as high as 500 mM, and the volume of the vessel element increases several times. Indeed, it is most likely that the rising K concentration generates much of the increasing osmotic pressure to swell the vessel, and expand it among the surrounding parenchyma cells. Then, when the vertical series of elements matures to make a vessel by the dissolution of their end walls, and the new vessel becomes part of the flow apoplast, the K will be released into the transpiration stream as a pulse of half-molar solution which will travel with the stream towards the leaves. This released K will spill over into the adjacent mature vessel in the same arc which is connected directly to the new vessel through pits in their common side walls, and from there into the next adjacent vessel, and so on down the arc of touching vessels along the stem radius to the protoxylem. Thus all the vessels in a radial arc might be expected to have a common K composition (perhaps decreasing away from the source at the abaxial end), whereas the compositions in neighbouring arcs may be different. This is seen in figures 8 and 10. The K concentration in a given arc would depend on the recent history of vessel maturation in that arc proximal to the site of observation. If no vessel had matured recently in the arc, no K would have been released and the K concentration would not be detected. A recent maturation in an arc a short distance below should produce a high concentration of K in that arc, up to a maximum of about half the peak concentration inside the immature vessel (say a maximum of ~ 200 mM). This estimate of half is based on the approximate volume of the small mature vessels in the arc relative to the volume of the large differentiating vessel in preparations like that of figure 9.

The pattern of distribution of the released pulse of K will depend on the connections that the parent arc of vessels makes with other xylem elements in the distal direction. The arc may be part of a leaf trace, and the pulse of K would move quickly and undispersed out into the vein system of a leaf. Or the pulse may travel through several nodes of the stem, presumably dispersing and becoming more dilute before it enters a leaf. Thus the spasmodic occurrence of K concentrations in petiolar vessels in the range 30–80 mM would be explained.

Within the leaf, again depending upon the particular connections of this segment of xylem, the K pulse may enter a single vascular strand of the petiole and move into a confined area of the lamina, or it may be more generally distributed through all the orders of vein size to much of the lamina. Thus the pattern of K concentrations in a particular sample of the vessels of the leaf veins may depend little on the age, size or activity of that leaf. Instead they may derive from the activity of the developing secondary xylem in proximal parts of the stem, being determined by the particular vascular connections of the sampled portion of the lamina, through the petiole and leaf traces, to the stem.

A similar mode of generation of further inputs of K to the stream would be expected in the leaf, in developing metaxylem or secondary xylem in the petiole and larger veins. As shown in figures 1 and 2 and tables 1 and 2, a similar pattern of high K concentration in differentiating vessel elements, and consequent release to the mature vessels can be expected, though less frequently than in the stem. But if less frequently the effect may be more intense, because the volume into which the pulse is released is smaller than in the stem. So the K concentration in the vessels of the leaf may be augmented both by vessel maturation and by the evaporative loss of water, both causes producing their major effects in the V1 to V3 veins.

The proposed hypothesis would apply to plants with secondary xylem, and therefore not to the monocotyledons. A similar high concentration of K in

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developing late metaxylem vessels of maize roots was shown by McCully *et al.* (1987). This K must be released to the transpiration stream when the vessel matures, but the contribution from maturing vessels of the stem and leaves in maize, or any other monocotyledon, has not been investigated.

Inputs of K to the transpiration stream from dicotyledon roots come in part from developing xylem vessels in a manner closely parallel to the process in the sunflower stem. McCully (1994) has shown that in soybean roots, the high levels of K found in immature primary vessel elements (~ 110 mM) are exceeded by the levels in vessel elements developing from the cambium (~ 190 mM). The combination of vessel-element-derived K and K transported from soil to mature xylem (by whatever mechanism), does not produce a concentration in the stream of sunflower as it enters the stem of more than about 5–10 mM.

This hypothesis would provide a possible detailed description of some of the whole plant traffics of K measured in white lupin by Jeschke *et al.* (1987). In plants with 16 stem leaves and a terminal inflorescence, K was carried upwards in the xylem, moved into the leaves at all levels, and out of the leaves in the phloem in almost an equal amount (except in the apical four leaves, where about a third of the xylem-borne K was used for leaf growth). The xylem to phloem transfer would occur in the finest veins of the leaves, fairly directly from the two or four small-diameter vessels, through xylem and phloem parenchyma cells to sieve tubes. The phloem-borne K travelled downwards, re-entered the xylem stream and cycled back up the stem. In fact, the concentration of K in the xylem sap increased upwards. One path for this phloem to xylem transfer would, according to my hypothesis, be from phloem to cambium in the stem (and larger veins), accumulation in selected cambial derivatives to provide the osmotic force to swell them into large vessel elements, and release of this large volume of half-molar K to the transpiration stream when the vessel element matures. The zones of most active phloem to xylem transfer would be those in which secondary thickening was most active. In the lupins, the larger transfers are shown in the top four internodes and in the central stem region (Jeschke *et al.* 1987, figure 4*a*).

If the proposed hypothesis is accepted as a possible basis for further exploration of K movements in dicotyledons, a number of predictions can be made which may be tested with the present data or in future experiments.

1. Metaxylem strands of the stem composed of only mature vessels, and with no new vessels developing, should have sap with low K concentrations. This has been illustrated in figure 6.

2. At the level in a stem xylem strand showing only early stages of vessel development, where none or few of the developing elements have heavily thickened walls (sign of approaching maturity), there should be intermediate levels of K concentration. Maturing vessels in such strands must be some distance proximal in the older part of the stem, and K pulses released from them may be expected to have dispersed somewhat before reaching the mature vessels at this level. Such relations may explain the different K concentrations in the open vessels of the three arcs in figure 8. The two arcs with only young immature vessels have low K. The right-hand arc with more-mature vessels has high K.

3. In the larger veins of leaves (petiole to V3), different strands of xylem, having connections to different zones of the stem, may be expected to have different levels of K concentration in their sap. This has been illustrated by the branched bars in figure 7, and has been observed in other preparations not presented here.

4. In woody species, the K concentration of sap in leaf vessels should be especially high in spring when vessel differentiation is most active, and low in late summer when wood growth has ceased.

5. Species with wide vessels, requiring large volumes of concentrated K for the osmotic engines to expand the vessel elements and push smaller cells aside, may have larger pools of recycling K than species with narrow vessels, and their leaves may have higher ash (K) contents.

The presence in leaf vessels of a solution containing ~ 200 mM cations with accompanying anions has osmotic implications. Such a solution could produce an osmotic pressure of ~ 8 bar through a semipermeable membrane. What it does in fact in the leaf will depend on the properties of the tissues that surround it.

If the cell wall apoplast surrounding the vessels in the bundle sheath and mesophyll is freely accessible to this solution (as many assume), the solution is not surrounded by a semipermeable membrane and the main effect of the solutes will be to reduce the turgor of the cells by 8 bar. Any measurement of leaf water potential by pressure chamber or psychrometer will contain this component as a substantial apoplastic osmotic potential,which, if allowance is not made for it, will exaggerate the estimated xylem tension.

On the alternative view of the cell wall apoplast to which my investigations have driven me (Canny 1990*a*, 1993) the implications of the solutes in the xylem sap are more complex. As mentioned in the Introduction, this study diverged from an attempt to find whether the walls of leaf parenchyma cells had low diffusivities for the natural ions, as for the higher molecular weight dyes (5–600 Da), and the question remains unresolved. In the absence of data, one may assume that the ion diffusivities in cell walls may be reduced relative to diffusivities in water by proportions similar to those of the dyes, namely 1/100 to 1/10000. Low values of solute diffusivity in the cell wall apoplast (Canny 1990*c*; Canny & Huang 1994) mean that even in the absence of cell membranes surrounding the solution, the reflection coefficient of the cell wall apoplast will not be zero. The solution will generate an osmotic pressure in the xylem, not as high as +8 bar but up to approximately half of this. The dead xylem would be a compartment capable of generating +4 bar of positive pressure above atmospheric (+5 bar absolute). This is not a new idea. Braun (1984) has put forward much anatomical evidence that the xylem parenchyma of trees is constructed to secrete sugars and other solutes into the vessels, and provided

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experimental evidence that water absorption by young trees that are not transpiring may be driven by such an osmotic force. His arguments have lacked the key element, the non-zero reflection coefficient of the cell wall apoplast which makes the vessels into an osmotic compartment.

Positive pressures in the vessels producing guttation are a well known feature of many plants (Haberlandt 1914) and, despite much ingenious theorizing (see Schwenke & Wagner 1992), the force generating these pressures remains mysterious. Positive pressures in vessels to expel air and repair embolisms appear to be a universal requirement (Tyree & Sperry 1989; Sperry 1991), especially in deciduous species in spring. The flow of maple sap is a famous example. It seems also that such pressures are a feature of some species each night, after a day's vigorous transpiration in which many vessels become embolized (Tyree & Sperry 1989). The osmometer formed by a vessel containing solutes would be an effective unit in the presence of regained high water status to provide such pressures, to expel air, compress bubbles and redissolve gases.

Pressure in a static fluid is transmitted throughout the whole volume of the fluid. The pressure generated by a local high concentration of salts in a leaf vessel would be transmitted as a hydrostatic pressure all through the continuum of xylem sap connected to this vessel. Perforation plates between one vessel and the next, being permeable to water, would transmit this static pressure. It may thus be possible to think of the Cohesion Theory for the ascent of sap operating rather by reducing a positive pressure to around zero absolute pressure (-1 bar relative to atmospheric), than operating at tensions several to many bars negative on the absolute scale. This would be consistent with the measurements made recently by Zimmermann and coworkers with the xylem-pressure probe (Zimmermann *et al.* 1993). They showed that the pressure in vessels in many plants, from herbs to trees, and at heights of up to 30 m were in the range zero to $+1$ bar (absolute), and that the high tensions believed to be required by the Cohesion Theory were not present in the vessels. When the fluid is flowing, the pressures would no longer be uniform because gradients of pressure must exist to drive the flow but the whole system, from roots to leaves, might still operate at pressures closer to atmospheric.

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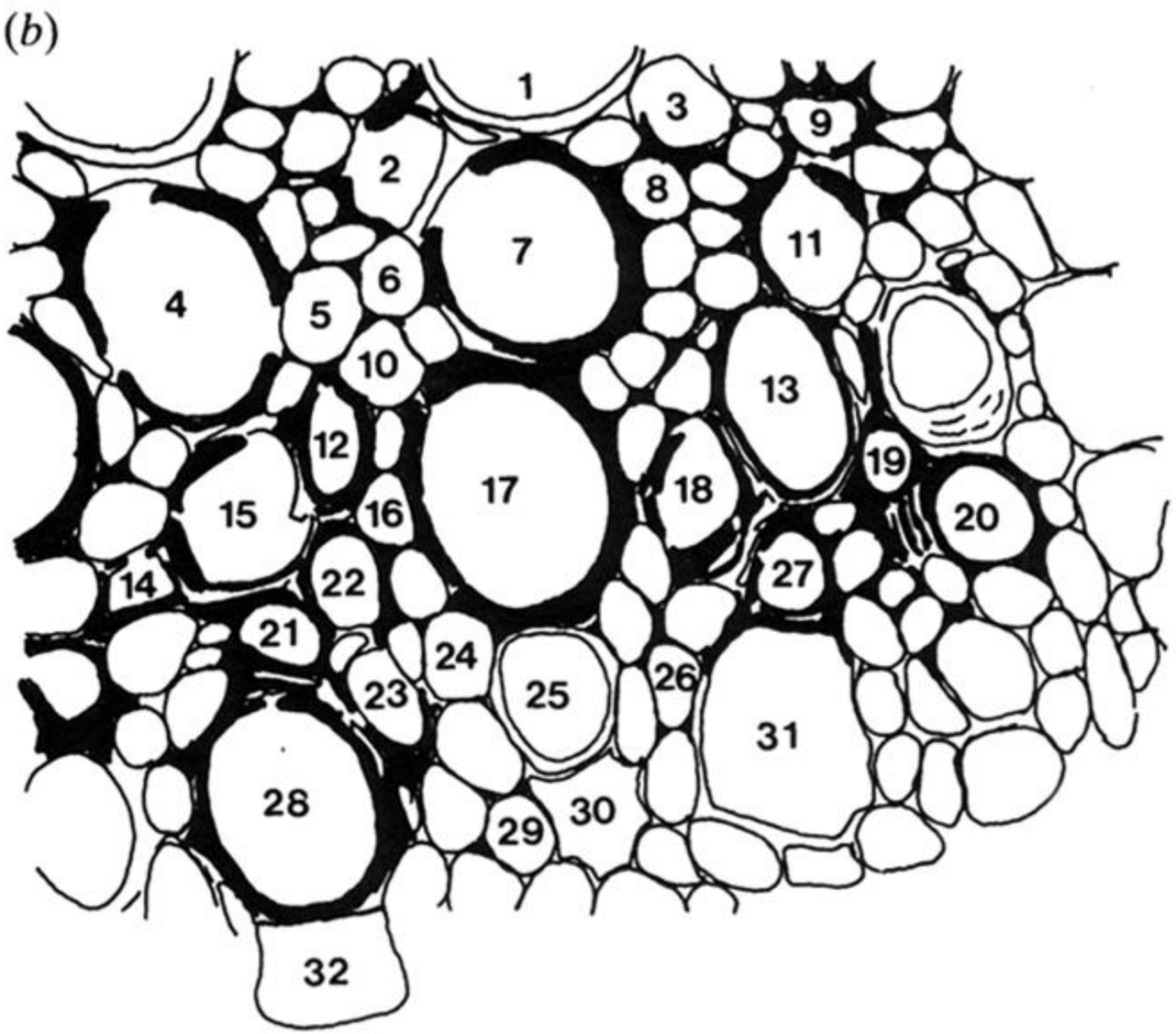

Figure 1. (*a*) Frozen planed face of part of the V1 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and xylem vessels, some with air embolisms, and most filled with sap. EDX microanalyses were made of the contents of the cells of the face indentified in figure 1*b*. Bar = 10 μm. (*b*) Drawing of the face shown in figure 1*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 1.

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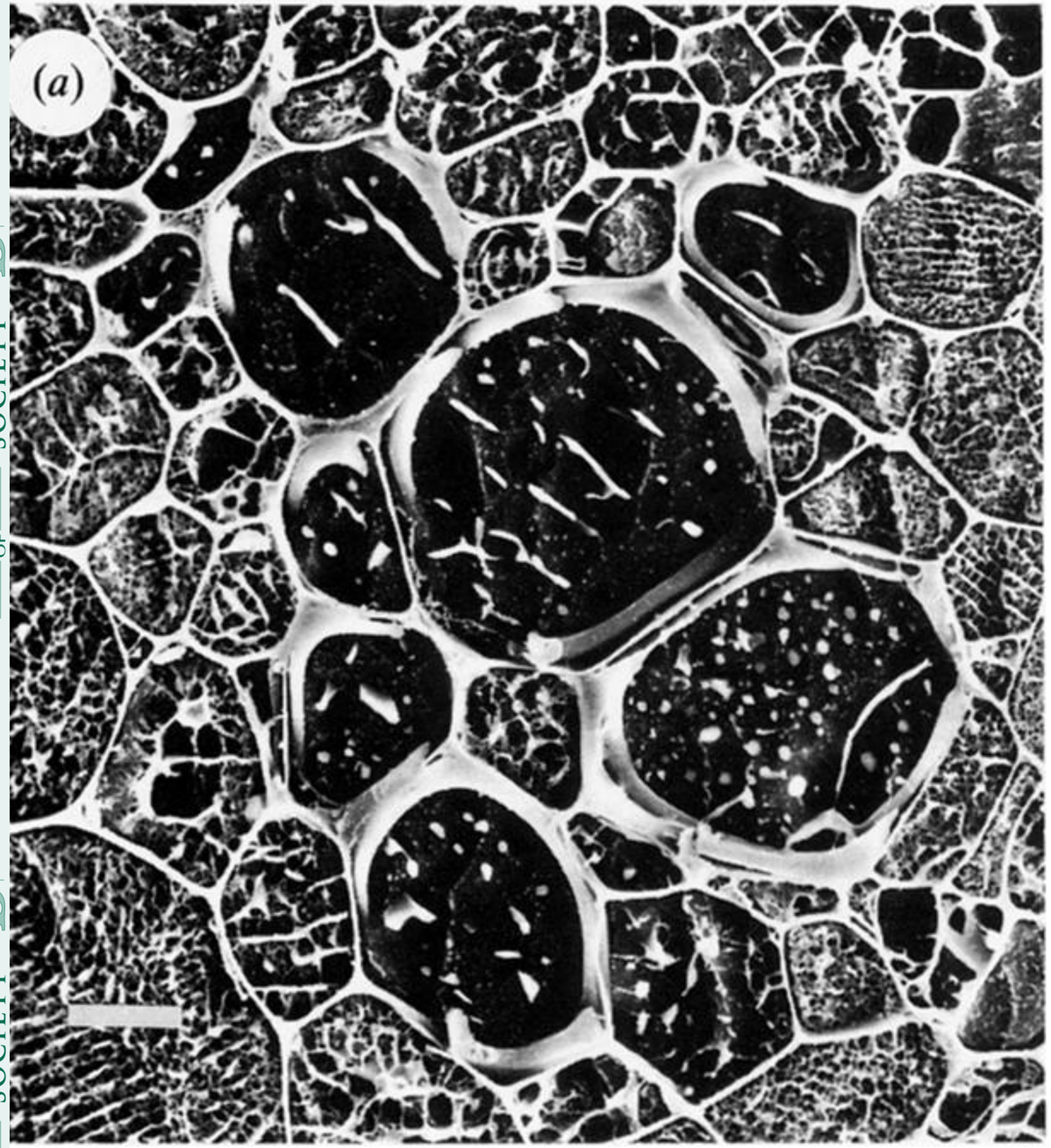

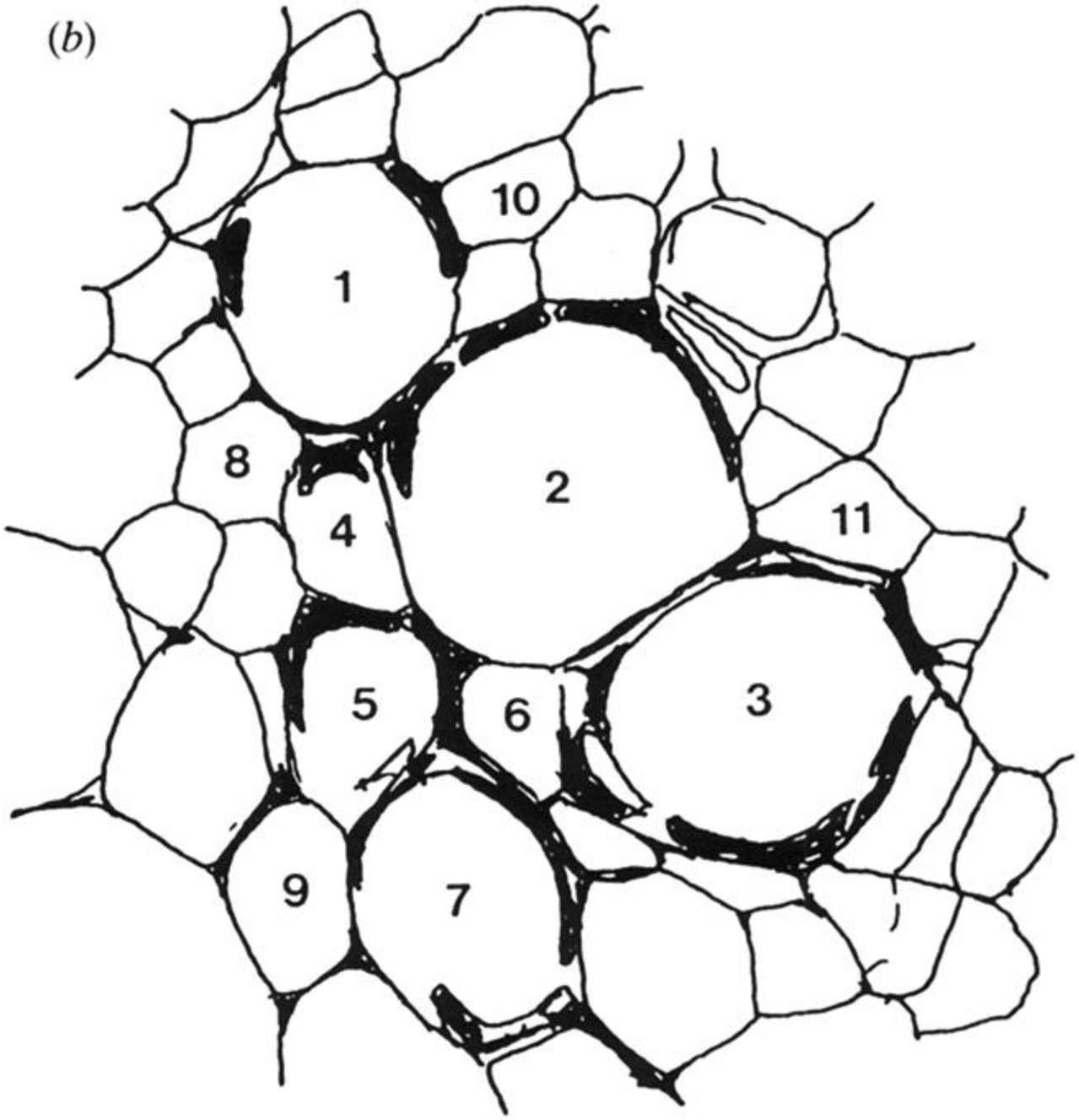

Figure 2. (*a*) Frozen planed face of a small bundle of a V3 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and xylem vessels. EDX microanalyses were made of the contents of the cells of the face indentified in figure 2*b*. Bar = 10 μm. (*b*) Drawing of the face shown in figure 2*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 2.

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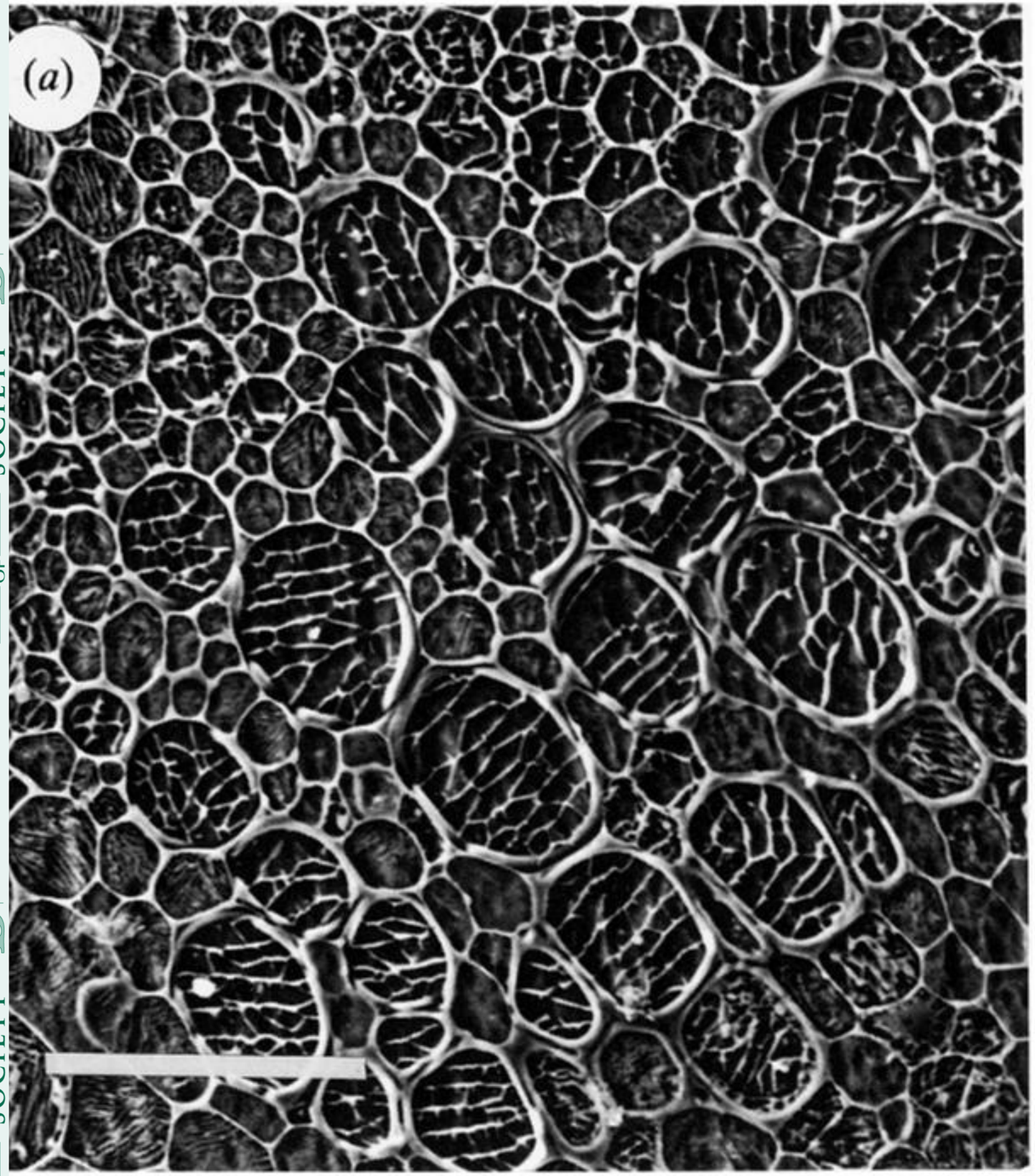

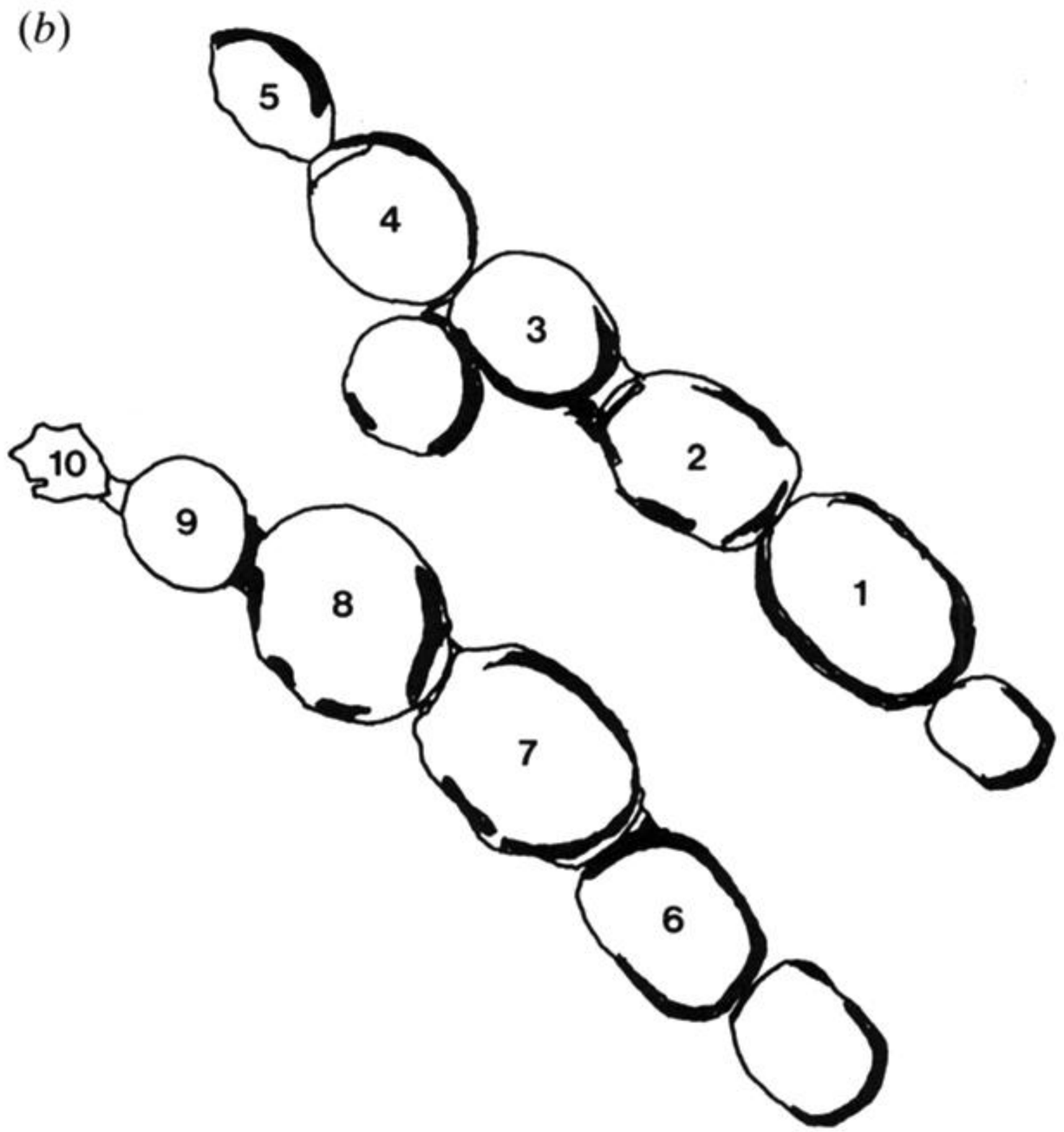

Figure 3. (*a*)Frozen planed face of part of the V1 vein of a leaf of *Helianthus annuus* showing xylem parenchyma and arcs of metaxylem vessels. EDX microanalyses were made of the contents of the cells of the face indentified in figure 3*b*. Bar = 100 μm. (*b*) Drawing of the arcs of metaxylem vessels shown in figure 3*a* with numbers identifying the cells whose contents were analysed. The cell type and elemental concentration for each cell is listed in table 3.

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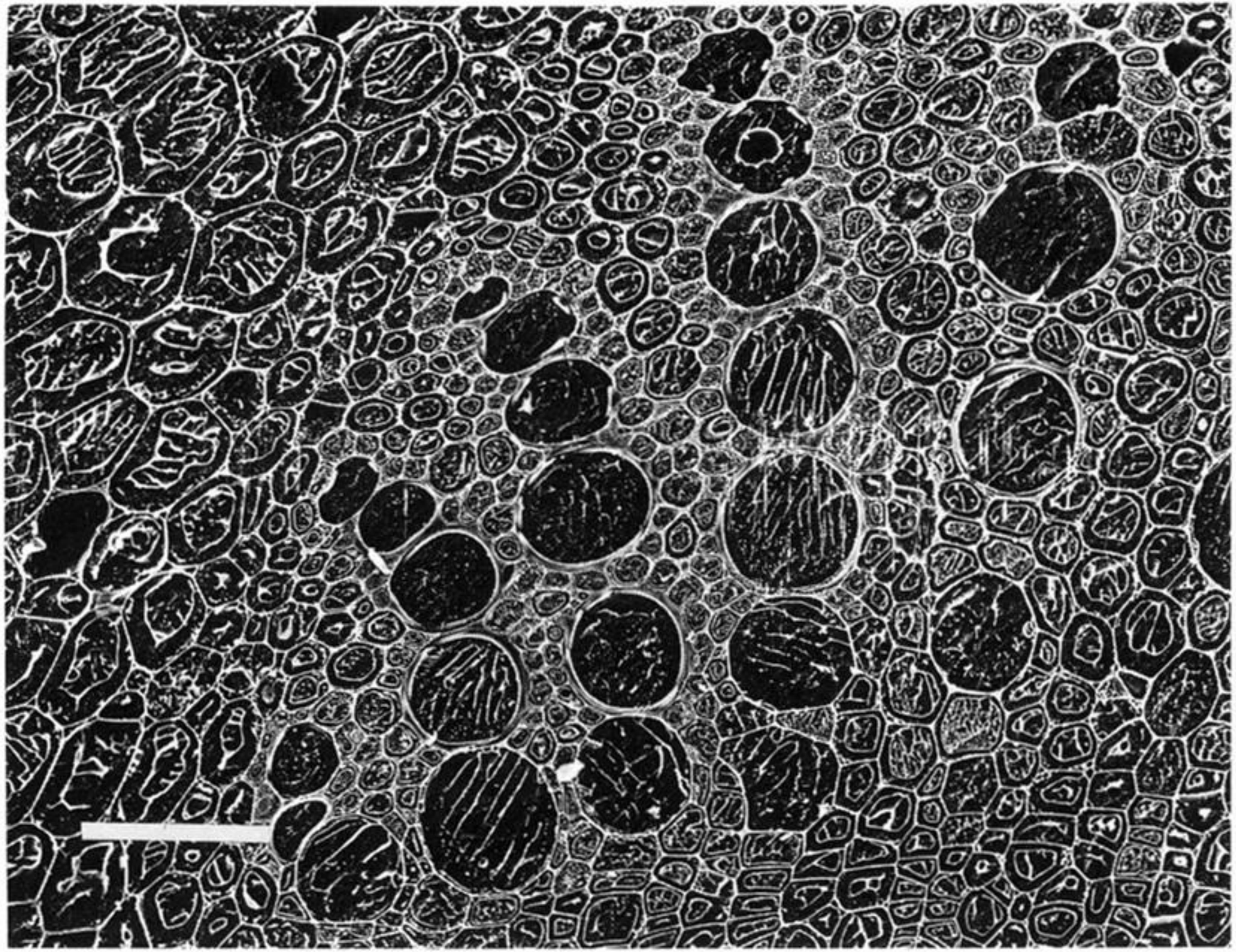
gure 6. Planed frozen face of a vascular strand in the first ternode of three expanded internodes above the cotyledons a stem of *Helianthus annuus*. This strand shows mature etaxylem with no secondary xylem differentiating. Analyses the vessel contents revealed no elements detectable above e threshold concentration. Bar = 100 μm.



gure 7. Planed frozen face of a vascular strand in the third (youngest elongated) internode of three expanded ternodes above the cotyledons of a stem of *Helianthus annuus*. This strand shows vessel elements differentiating from e interfascicular cambium to form secondary vessels. Bar = 100 μm.

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