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Roots and the Delivery of Solutes to the Xylem

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Roots and the delivery of solutes to the xylem

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SUMMARY

The structural features of the pathways followed by solutes and water are described. The porous nature of the cell walls comprising the apoplasm is described and the difficulties in verifying the passage of water through different parts of the apoplasm are discussed. The endodermis is of ubiquitous occurrence and has two invariant characteristics, a girdle-like wall thickening, the Casparian band, and the attachment of the plasma membrane to the band. Suggestions are made concerning the constraints placed on the passage of materials in the stele by these structures. The hypodermis is also a very common structure which shares a number of properties seen in the endodermis. The implications of an apoplasmic barrier in the hypodermis are discussed.

The plasmodesmata are the key structural feature of the symplasmic pathway and recent information makes it clear that the size of the pores in the neck region can vary with the physiological state and position of tissues. The symplasmic pathway seems not to be interrupted by structural developments which make the endodermis an apoplasmic barrier of high resistance.

Recent information from transpiring plants indicates that the turgor pressure in cortical cells increases centripetally: there is, therefore an outwardly directed hydrostatic pressure gradient. The implications of these new findings for water and solute flows in the symplast are considered.

The final step in the radial transfer of materials is their release into the xylem. There is evidence that stelar tissues contain an H^+-translocating ATPase whose activity can be influenced by physiological factors. It is pointed out that there may be major changes in the concentration of K^+ in xylem sap during a day–night cycle which may influence the polarization of the cell membranes of xylem parenchyma and the opening of ion-channels. The xylem elements themselves are not always fully conductive, even when their final diameter has been reached. The protoplasts and cross walls may be more persistent than is usually assumed, especially in soil-grown roots.

Because of the low activity of Ca^{2+} in the cytoplasm and the discontinuity of compartments within cells which contain abundant free Ca^{2+}, this ion probably moves radially primarily by diffusion in the apoplasm. The transfer of Ca^{+2} across the endodermis is shown to depend on the activity of Ca^{2+} ATPase in the plasma membrane of the stelar side of the endodermis, emphasising once again the epithelial nature of this cell layer.

1. INTRODUCTION

Both convective and diffusive movements of materials occur within roots before the composition of the transpiration stream is established. These centripetal flows towards the xylem must involve passage through or around the cells of the root. Passage via the apoplasm, if it is sufficiently porous, would carry material around cells; some form of movement through the symplasm is probable because cells are interlinked by junctions, the plasmodesmata. This much has been evident for a long time, but physiologists, succumbing to the temptation of assigning particular functions to structures, have made many assumptions about the nature of the pathways and what is carried in them. Such assumptions are risky in the absence of quite basic information about the physical and chemical properties of structures presumed to be either channels for or barriers to, movement (see Canny, this volume). The truth is that our current perception of what materials move, and in which pathway, is based more on intuition than on information. Over the years it has become necessary to revise our view of the relations between structure and function as previous assumptions proved to be wrong. It is an interesting, but difficult, task to write the present paper because recent information on the properties of root cells in transpiring plants has revealed facts about the direction of hydrostatic driving forces in the root cortex which, if correct, seriously challenge a widely held view of solute movement in the symplasm (see § 3).

It is rather hard to avoid relating function and structure but an attempt is made in what follows to describe what we know about the possible pathways for movement and to arrive at a conclusion concerning the limiting parts of them. This chapter will deal only in passing with the strategies used by plants to

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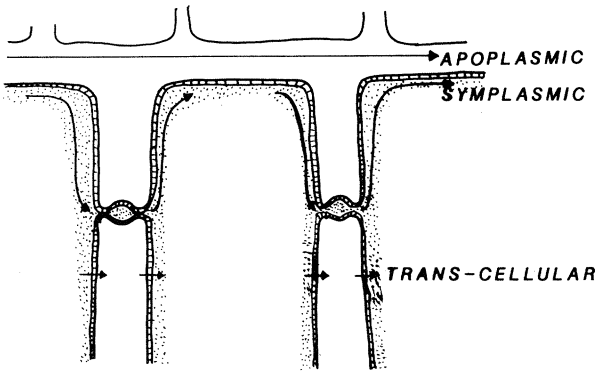

Figure 1. Scheme to illustrate the three modes by which water and solutes may move across the root radius.

acquire nutrients from soil and only the very broad outlines of membrane transport of individual components of the transpiration stream are given.

2. PATHWAYS IN WHICH MATERIALS MAY MOVE

If we consider a file of cortical cells arranged along the root radius and connected to cells of the stele via the endodermis, one can envisage three modes by which water and solutes could move down the file (figure 1). These ideas are very familiar, although the third, the cell-to-cell or *transcellular* pathway, is more often discussed by students of water relations that by those of ion transport. In general, the probable energy costs involved in successive uptake and release of ions at each step of the latter pathway (perhaps as high as 15–45 kJ mol^{-1} for anions at each step) are prohibitive. It has been shown recently that membrane transport of nitrate alone accounts for 5% of root respiration, and this value compares quite well with the calculated energy cost of transport across a single plasma membrane (Bloom *et al.* 1992). For water movement in the root of the transpiring plant, the 'cost' of the driving force involves quite different considerations and many authors have believed that the sum of all the hydraulic resistances in series in the transcellular pathway (figure 1) may be comparable with measurements of the overall resistance between the outside and the xylem (Steudle & Jeschke 1983; Jones *et al.* 1983).

(*a*) *The apoplasm*

(i) *The structure of cell walls*

During its construction, the cell wall increases in thickness as successive bundles of cellulosic microfibrils are laid down. Frequently, successive layers have a shift in the orientation of the bundles giving rise to a well defined, three-dimensional arrangement with pores both perpendicular to the wall surface and within the plane of the wall. The properties of this matrix, as it may influence ion uptake and migration, have been well reviewed by Sentenac & Grignon (1991). For the present discussion it is the physical porosity of the wall which is of interest. Figure 2 displays deep-freeze-etched walls from the cortex of

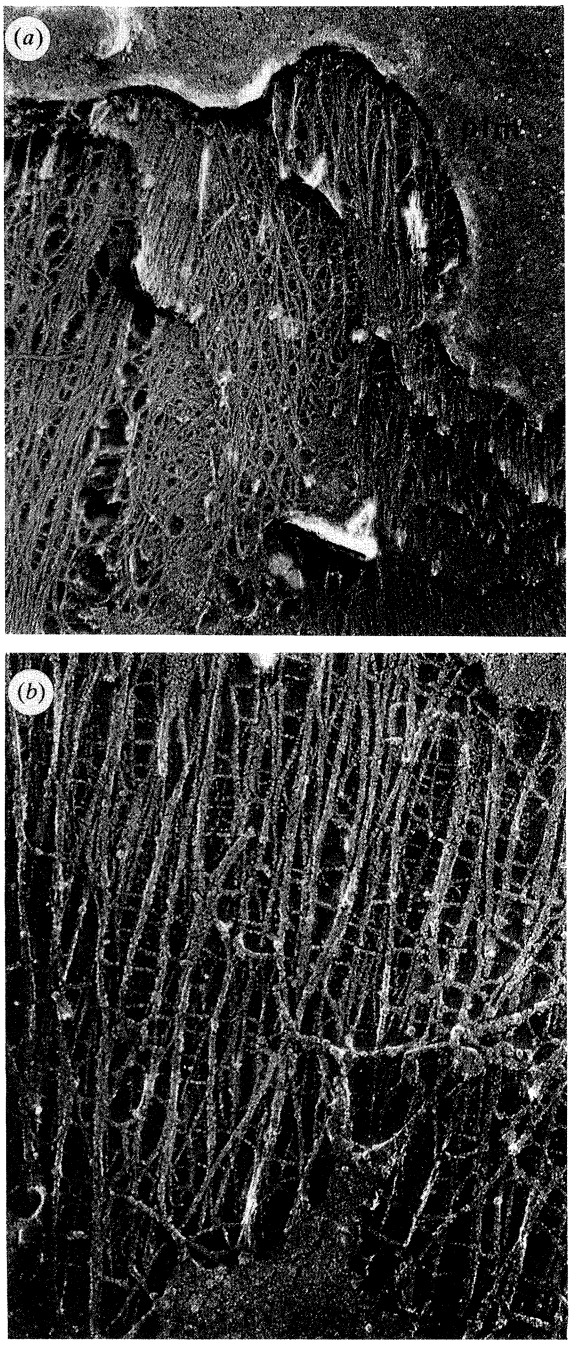

Figure 2. Cell wall in maize root tip cells. (*a*) The polylamellated structure comprises at least five layers each lying parallel to the plasma membrane (plm). Before freezing and etching the material was aldehyde-fixed and lightly digested with cellulase–pectinase mixture for 30 min (×60 000). (*b*) Higher magnification of one layer in the polylamellated wall; cross bridges between cellulose fibrils regularly spaced (×159 750). (Reproduced from Satiat-Jeunemaitre *et al.* (1992) by permission.)

young maize roots where, in addition to cellulosic fibrils (which in this case are oriented similarly in successive layers), short fibrillar bridges (16–19 nm long) regularly connect adjacent cellulose fibrils (Satiat-Jeunemaitre *et al.* 1992). The bridge fibrils are thought to be hemicellulose (McCann *et al.* 1990), and the ladder-like structure they produce stabilizes the wall. Inspection of figure 2 suggests that, in the plane

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perpendicular to the wall, there are gaps of at least 20 nm in diameter in any given wall layer. The gaps in successive layers are not aligned so that the effective porosity of the wall in this direction will be appreciably less than 20 nm. Attempts have been made to estimate this effective diameter by the provision of osmotica of varying molecular size to readily observed cells, such as root hairs, and to see whether hypertonic solutions cause normal plasmolysis (osmoticum penetrates the wall and enters periplasmic space) or cytorrhysis (osmoticum is excluded from the wall and the cell buckles as water is lost from the protoplast). The estimates of the limiting wall porosity obtained in this way by Carpita *et al.* (1979) are from 3–4 nm. The value is quite large enough to permit free movement of hydrated ions and organic metabolites from the outside solution to the plasma membrane of the underlying cell. However, biochemists find it hard to reconcile such narrow pores with either the release of enzymes, e.g. acid phosphatase (molecular mass *ca.* 100 kDa) or the uptake of markers conjugated with biotin by endocytosis (Horn *et al.* 1990). There may be a small number of larger pores to permit the movement of such bulky marcomolecules. The same suggestion had been made for movement in the plane of the wall (Passioura 1988). The resistance to water movement through channels increases with the fourth power of the radius (Poiseuille equation), thus a small number of larger pores could result in distinctly non-uniform movement of water in the radial direction (e.g. Brown *et al.* 1986). In addition to their narrow average diameter, the pores in the wall will be very tortuous so that the effective length of the apoplasm pathway would be much greater than the radius of the root or the simple perimeters of the cells in the path. Entry of water into such pores and passage along them requires that their surface is hydrophilic (Passioura 1988). Both the diameter of pores and their hydrophobicity are affected by the deposition of lignin and its precursors, the resistance to flow being greatly increased. Thus, in the endodermis and hypodermis such deposition among the cellulose fibrils of radial walls is assumed to decrease greatly the permeability of the apoplasm (see below).

(ii) *Water, rafts and dyes*

As there is no practical way to observe water moving through the apoplasm, the pathway and its limits are frequently inferred from so-called apoplasmic tracers. The pitfalls in such methods are caused by specific interactions between the tracers and structural elements of the pathway and because they may be much bulkier than water molecules or hydrated ions (see also Canny, this volume). Fluorescent dyes of different chemical nature may be shown to be either held up by the hypodermis in young maize root, or pass through it (Peterson *et al.* 1981), whereas both were excluded from the apoplasm by the hypodermis in onion roots (see Peterson 1989). Mass flow of water through the apoplasm may carry with it colloidal particles (generally anything greater than 3.5 nm is excluded) and, if these contain electron dense nuclei, the particle distribution can be seen readily in unstained material prepared for electron microscopical (EM) examination. Very convincing images of the exclusion of La by the Casparian band of the endodermis can be produced readily by this technique, an example being shown in figure 3. The inner part of the wall of the adjoining endodermal cells contains a dense deposit of La, but closer inspection reveals only isolated pockets of La in the wall of the adjoining cortical cell. The distribution shows where La accumulates but not necessarily the pathway along which it moves to get there. The picture certainly reinforces the impression that much La gets filtered out on the outer tangential face of the endodermis but does not really say that water cannot pass through the Casparian band. Only the positive presence of dyes and 'rafts' gives unequivocal information about where water must have passed or where water channels are continuous; their exclusion is open to many interpretations.

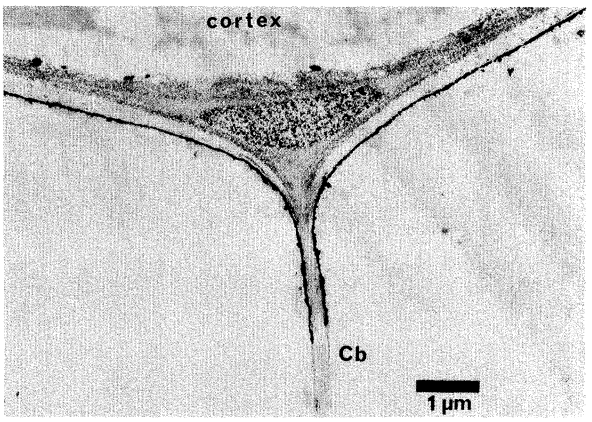

Figure 3. Accumulation of lanthanum tracer in the walls on the cortical side of the state I endodermis of a barley root. The tissue was fixed in glutaraldehyde but not stained with osmium. The Casparian band (Cb) in the radial wall seems to arrest the radial movement of lanthanum (×9100). (Micrograph kindly provided by Dr A. J. Wilson and Professor A. W. Robards.)

In theory, at least, nuclear magnetic resonance (NMR) imaging techniques may resolve the pathway for bulk water flow. For some time ^{3}H-NMR imaging has been used to reveal roots, and the water they contain, in dense volumes of soil (Bottomley *et al.* 1986). The NMR signal decays at different rates depending on the environment of the water molecules. It is possible to distinguish moving and stationary water in relatively large structures such as stems and roots (Brown *et al.* 1986) but, at present, the resolution of the method is not precise enough to study water movement through cell walls (Connelly *et al.* 1987), the most finely resolvable pixels of the image being equivalent to a volume of $5 \times 5 \times 100$ μm (G. Ratcliffe, personal communication).

(iii) *The endodermis*

For much of its length the xylem is composed of dead cells whose lack of a plasma membrane (PM) means that no control can be exercised on solutes entering or leaving the conduits (see, however, § 5).

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The composition of the transpiration stream does, however reflect the requirements of the shoot tissues and not that of the external medium. It is obvious that the shoot cannot influence this state of affairs directly so that, at some point, the continuity of the apoplasma of stele and cortex must be interrupted so that an appropriate selection of transported materials can be made. The endodermis is widely supposed to provide this barrier. It is a structure found in every species of plant examined and there appear to be two features of its design which are shared by all species examined in detail (see Clarkson & Robards 1975; Clarkson 1991).

In the radial wall of the differentiating endodermal cell, substances which stain positively for lignin and suberin are deposited in a girdle around the radial and transverse walls. It is presumed that these deposits greatly impede the flow of material in the plane of the wall (see figure 4). They appear in barley (Robards *et al.* 1973) and *Ranunculus* (Scott & Peterson 1979) just at the time when the first xylem elements are differentiating. An appreciation of the structure in three dimensions is greatly aided by the work of Karahara & Shibaoka (1992). When other walls of pea roots had been removed by enzymic digestion, the Casparian bands, which are held in common between contiguous endodermal cells, could be separated as a fine, but robust net exhibiting a powerful autofluorescence from its lignin precursors.

Karahara & Shibaoka (1992) also shed some light on another feature of the endodermis which has been recognized by microscopists for more than 50 years. When placed in hypertonic solutions, the plasmolysed endodermal cells maintain attachment between the PM and the Casparian band. As plasmolysis proceeds the protoplasm is left stretched out like a septum separating the inner and outer sides of the endodermal cell. This feature is of crucial importance in diverting flows of material in the apoplasm across the endodermal protoplast before progressing further (figure 4). In this way, the selective transport at the PM is assured. The attachment itself has always been a mystery, but it was known to be so secure that the most severe treatment would break the PM, leaving its remnants attached to the band. Karahara & Shibaoka (1992) have found three polypeptides in their isolated bands which are rare or absent in general cell wall preparations. They remain in place when fragments of adhering PM have been solubilised by Triton X-100 treatment; subsequent extraction with detergent (2% SDS) revealed the three characteristic polypeptides of 46, 30 and 20 kDa. Further investigation of the relationship between these polypeptides and the plasma membrane should lead to a better understanding of this essential design feature.

In common with the hypodermis, the endodermal cells enter, asynchronously, a second stage of development in which suberin lamellae are deposited over the entire wall surface. At this stage, the attachment of the PM to the Casparian band is broken and remnants of the PM can be seen buried beneath the lamellae (Robards *et al.* 1973); a normal pattern of plasmolysis is found once this development has occurred. Entry into this phase depends on the rate of root elongation, occurring nearer the root tip when growth is slow. It has been assumed that these suberin lamellae increase the resistance of the endodermal walls to the passage of water and solutes, thus impeding greatly any direct absorption from the apoplasm. This view is supported by evidence of major decreases in water uptake and calcium entry into the transpiration stream when this

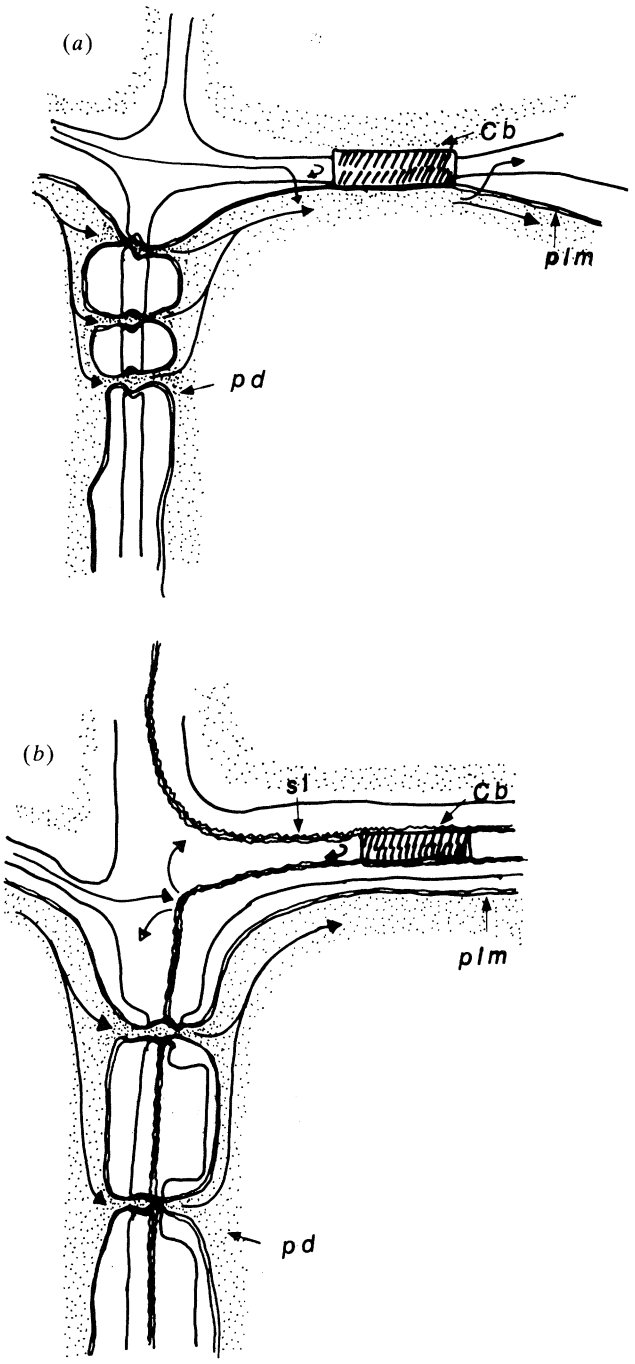

Figure 4. Schematic views of the endodermis. (*a*) A partly plasmolysed cell is shown to illustrate the attachement of the plasma membrane (plm) and the Casparian band (Cb). The cell is connected to its neighbours by plasmodesmata (pd). Only apoplasmic movement is blocked by the unique structure of the endodermis. (*b*) Representation of a state III cell with suberin lamellae (sl) in its walls. Movement from the apoplasm into the endodermal protoplast is blocked, but plasmodesmata remain open. The Casparian band is buried in the secondary wall, and the cell plasmolyses normally since the attachment to the plm is lost.

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development begins (Harrison-Murray & Clarkson 1973; Sanderson 1983; Robards *et al.* 1973).

(iv) *The hypodermis*

In many species the outer-most cortical layer(s) of roots become differentiated into a hypodermis. The anatomy of this structure is highly variable and its function is frequently assumed to be related to the environmental conditions experienced by the root periphery. In extreme cases, such as *Carex arenaria* – the sand sedge – the structure eliminates the transfer of materials between the root and the outside; multi-layered suberin lamellae are deposited and the cellular wall is impregnated with lignin or lignin precursors (Robards *et al.* 1979). The structure has these properties even in roots grown in solution culture. By contrast, *Zea mays* develops similar features (Peterson *et al.* 1982; Perumalla & Peterson 1986; Peter 1989; Clarkson *et al.* 1987), but water and ions are freely transferred from the solution to the xylem (Clarkson *et al.* 1987; Ferguson & Clarkson 1976) at rates which are comparable to those found in barley, which lacks a hypodermis (Sanderson 1983). Only when older parts of the root were exposed to humid air was their permeability to water and ions greatly reduced. Peterson (1989) points out that, during drying, roots with a hypodermis retain their cortical structure, with only the epidermal layer desiccating, while in those whick lack hypodermis the whole cortex dies back to the endodermis, e.g. barley (Clarkson *et al.* 1968) or *Rumex acetosella* (Scott 1928).

The modified radial and transverse walls in hypodermae are referred to as Casparian bands (Peterson *et al.* 1982; Peterson 1989) on the basis of (i) their position; (ii) their hydrophobic nature; and (iii) their apparent role in excluding apoplasmic tracers. The attachment of the plasma membrane to these walls, the essential feature of the endodermis, has been reported only once (Peterson & Emanuel 1983) and so there is reasonable doubt that the structure seen in the hypodermis is exactly equivalent in function to that of the endodermis. But generalization is risky because of variation in structure of the hypodermis between species. In onion roots, for example, the hypodermis is composed of long and short cells, which differ in their anatomy, particularly in older (>200 mm from the tip) parts of the root. All cells contained suberin lamellae but those in the short cells were less developed (Peterson *et al.* 1978; Wilson & Robards 1980). The short cells have denser cytoplasm, many more mitochondria than the long ones, and in some cases were seen to have secondary wall ingrowths which greatly expand the surface area of the PM (figure 5*b*). Transfer cells are uncommon in roots but their presence in other tissues indicates sites where large transfers of materials occur between the apoplasm and the symplasm (see Gunning & Pate 1969). Wilson & Robards (1980) commented that it seems anomalous for a possible transfer cell to possess suberin lamellae in its walls. This, and results from *Zea mays* (Clarkson *et al.* 1988) suggest that the presence of suberin lamellae should not be taken automatically to indicate reduced wall permeability. There has been an analysis of the composition of suberin from the cortex ($\equiv$ hypodermis) and stele ($\equiv$ endodermis) of maize roots. The compositions differed in several respects, particularly in the dominant chain length of hydroxy-fatty acids (the most abundant components) and in the much greater abundance of fatty alcohols in the hypodermis than in the endodermis (Pozuelo *et al.* 1984). The authors also found that in roots growing slowly in limiting Mg^{2+} supply, there was a dramatic increase in the thickness of suberin lamellae in both structures. This interesting work does not seem to have been followed up.

Where the hypodermis does act as an efficient apoplasmic barrier, water and solutes would be diverted into the hypodermal protoplast; the transport properties of the PM in these circumstances would be of great interest, and a start to such an investigation might be made using antibodies to the H^+-ATPase (see Samuels *et al.* 1992). Subsequent movements (figure 5) may occur in the cortical apoplasm, with a second diversion into the symplasm at the endodermis, or in the symplasm throughout. In figure 5 a 'passage' cell is included whose wall ingrowths suggest that flows across the hypodermis may not be uniform. This is also an under-exploited experimental proposition.

(*b*) *The Symplasm*

(i) *Plasma membrane transport*

The activity of proton translocating ATPases, coded for by a multigene family (Sussman 1992), is responsible for energising the transport of most of the solutes across the PM of various tissues. The precise significance of small differences of sequence, in the translated and upstream regions, of the different H^+-ATPases is not yet clear but the presumption is that they relate to tissue-specific expression and, possibly, to post-translational control of activity. Thus, although the biochemical properties of H^+ pumps in the stele and the cortex are very similar, they may be coded for by different genes and regulated in subtly different ways (see § 4).

Each of the major nutrient ions appears to cross the membrane in one direction or another, by specific mechanisms. In higher plants, at the time of writing, the only ion other than H^+, which is known to be transported directly by an ATPase is Ca^{2+}. As with H^+, the direction of active Ca^{2+} transport is from the cytoplasm to the outside (apoplasm or xylem). Other ion movements against gradients of electrochemical potential make use of the free energy of the proton gradient ($\Delta\bar{\mu}H^+$) across the plasma membrane in combination with specific transport proteins. Thus the anions, NO_3^-, $H_2PO_4^-$ and SO_4^{2-} are co-transported with H^+ in the inward direction by symports (Lüttge & Clarkson 1987) and Na^+ may move out of the cell coupled to the inward movement of H^+, an antiport (Hassidim *et al.* 1990). Ions diffusing down electrochemical potential gradients may enter or leave cells via channel proteins. Channels may be open or closed, depending on such factors as membrane potential cytosolic pCa or pH and other kinds of signal. Thus, depending on the electrical polarization of the PM,

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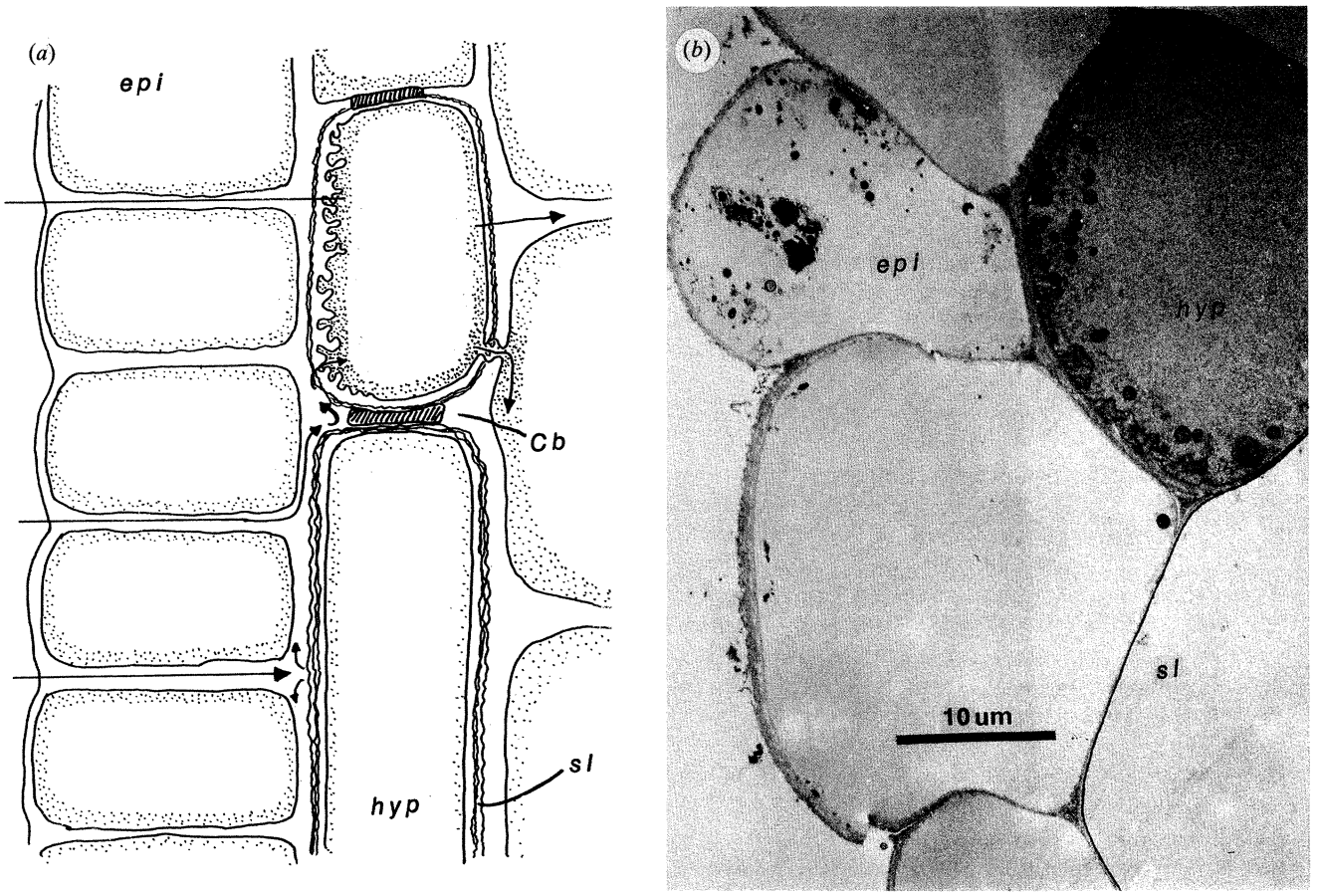

Figure 5. (*a*) Scheme to illustrate possible constraints on movements into the root apoplasm by a hypodermis with high resistance to flow in its radial walls (Cb) and with suberin lamellae (sl) in the walls. A 'passage' cell, such as described for onion roots by Wilson & Robards (1980), is included. (*b*) Transverse section at the epidermal–hypodermal junction of an onion root > 20 cm from the tip, showing a short 'passage' cell with wall ingrowths on its outer tangential face and numerous mitochondria. Note the absence of any features in the neighbouring long hypodermal cell. (Unpublished micrograph kindly provided by Dr A. Wilson and Professor A. W. Robards.)

inwardly or outwardly rectifying K^+ channels may open or close (Tester 1990); this behaviour is best known in stomatal guard cells (Blatt 1988; MacRobbie 1992)) but may be relevant to the xylem parenchyma.

(ii) *Buffering*

It is not possible in this article to do more than point to evidence which suggests that the cytoplasmic activity of some ions, H^+, Ca^{2+}, NO_3^- and $H_2PO_4^-$ is buffered, there being frequently a large store of nutrient ions in the vacuole and, in the case of Ca^{2+}, in the ER also. Sequestration and release of these ions, and of others such as SO_4^{2-} or Cl^- and K^+ for which evidence of precise regulation is less, tend to keep the cytoplasmic pool within a narrow range of concentration. If current views are correct, it is from this pool that the symplasmic flux of ions is derived. It is, therefore, interesting to note that the ratios of ions in the cytoplasmic pools may differ very markedly from those in the xylem sap (table 1). Admittedly, the values used to calculate these ratios are arbitrarily selected from a range of species but they make the point that the ionic composition of the xylem sap is more a reflection of the PM fluxes of ions than of the cytosolic compositions.

(iii) *Cytoplasmic streaming*

In certain cells, such as root hairs, streaming is readily observed and intuition suggests that this process must be important in the transport of materials within the cell. In root hairs of tomato and *Brassica napus* cytoplasmic streaming in root hairs has a velocity of 1–4 $\mu m\ s^{-1}$ (Clarkson *et al.* 1988). The diffusion coefficient (D) of ions in dilute aqueous solutions is approximately $1 \times 10^{-9}\ m^2\ s^{-1}$; a measure of the radial diffusion from a source is given by $\sqrt{(Dt)}$. Even

Table 1. *Possible ratios of ions in the cytosol of roots and xylem sap compared with ratios of plasma membrane fluxes*

(Ratios in cytosol based on published estimates: K^+ (100 mol m^{-3}), Leigh & Wyn Jones (1984), barley; Ca^{2+} (100 µmol m^{-3}), Clarkson *et al.* (1988), tomato; $H_2PO_4^-$ (~5 mol m^{-3}), Lee, Ratcliffe & Southon (1990), maize; NO_3^- (~5 mol m^{-3}), Zhen *et al.* (1991), barley.)

ion ratio	cytosol	xylem sap	PM flux
K^+/Ca^{2+}	10^6	30	10
K^+/NO_3^-	20	~1	≤1
$NO_3^-/H_2PO_4^-$	1	10	10

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if diffusion coefficients in cytoplasm are as low as one tenth of those in simple solutions, an ion will diffuse across a cell from its point of entry at a rate of $10\ \mu m\ s^{-1}$. The greater rate of diffusion than of streaming is, perhaps, counter-intuitive but is compatible with several experiments which show that disruption of actin microfilaments with cytochalasin B or D had little or no effect on symplasmic movement of ions (Glass & Perley 1979) or fluorescent dyes (Tucker 1987).

(iv) *Plasmodesmata*

Cells are usually linked by plasmodesmata and there is a broad correspondence between the flux of materials occurring between two cells and the frequency of plasmodesmata in their common wall. The dispute over whether plasmodesmata can or do act as channels for material movement is now resolved and current research, which is very vigorous, is aimed at understanding how or if the transport is regulated (Robards & Lucas 1990). The resolution of the dispute owes much to the work of Goodwin (1983) and Terry & Robards (1987) who observed the movement of fluorescently labelled small peptides from an injected cell to its neighbours. From these studies, exclusion limits, based on the Stokes radius of the peptide, indicated an effective diameter of 3 nm in the plasmodesmata of extrafloral nectaries of *Abutilon* (Terry & Robards 1987). Such a pore is large enough to allow unhindered transport of hydrated ions and metabolites. It is becoming clear, however, that the limiting pore size in plasmodesmata within an organ may vary from place to place (Erwee & Goodwin 1985), and with chemical or physical changes in the cells. It is relevant to note, in connection with later discussion, that plasmodesmatal channels can be shut down by injecting a cell with fluid so as to increase its turgor pressure. When this was done to leaf trichomes of *Nicotiana clevelandii*, the transport of co-injected dye (Lucifer Yellow) was eliminated by increases in turgor of 0.2 mPa and markedly impeded at smaller turgor pressures (Oparka & Prior 1992). Passioura (1988) speculated that plasmodesmata might function as clack valves if hydrostatic pressure built up on one side of the canal. The well-known association of the endoplasmic reticulum with the necks of the plasmodesmata may be involved in this process, becoming appressed against the mouth of the channels if hydrostatic pressure begins to force water through the plasmodesmata at unpermitted rates. The suggestion was first made by Barclay & Fensom (1984).

The structure of plasmodesmata is much more complex than that of a simple canal between adjoining cells (e.g. Ding *et al.* 1992). There are numerous plasmodesmatal proteins some of which have common epitopes with those from gap junctions in mammalian cells (Meiners & Schnidler 1987). Recent immunological studies show that there is cross reactivity between plasmodesmata and antibodies to gap junction connexin (Yahalom *et al.*, 1991) and there has been immunolocalization of actin microfilaments in the neck region between plasmodesmata and the ER (R. G. White *et al.* 1992). The full significance of this more complex structure for transport functions is not yet appreciated.

(v) *The endodermis*

The endodermis is connected to the cells of the inner cortex and those of the pericycle by frequent plasmodesmata (e.g. Warmbrodt 1986). A detailed study in barley roots revealed that there were more in the inner tangential wall than in the outer one, and thus there is a suggestion that symplasmic flow between the endodermis and the pericycle in barley is greater than across the endodermal–cortical boundary (Robards *et al.* 1973). This might be explained by a confluence of apoplasmic and symplasmic flows at the outer half of the endodermal cell (see figure 4*a*). Plasmodesmatal connections remain in place during and after secondary deposition of the suberin lamellae and thus the channels they provide may well be open in older parts of the root, formerly supposed to play no part in the absorptive process (see Scott & Priestley 1928).

3. TURGOR PRESSURE GRADIENTS IN ROOTS

When the micropressure probe is used to measure the properties of cortical cells of transpiring plants it has been found that the turgor pressure, P, of cells is greater in the inner cortex, by a substantial amount, than in cells at the root periphery (see figure 6). These results can be obtained by progressive insertion of the probe along a radial file of cells in the root cortex of *Mesembryanthemum crystallinum* (Rygol & Zimmermann 1990). The gradient, ΔP, but not the absolute values of P, was maintained in roots exposed to increasing levels of NaCl salinity. Earlier reports suggested that

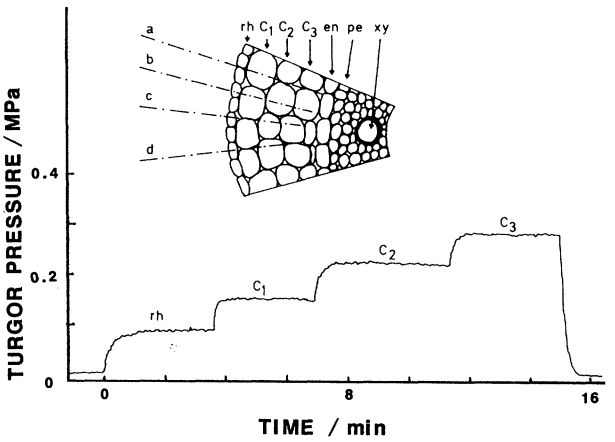

Figure 6. Typical turgor pressure gradient measured 25 mm from the tip of a root of *Mesembryanthemum crystallinum* during step-wise penetration of the tissue with the microcapillary of a pressure probe. The probe did not enter the endodermis and subsequent steps in the profile are, regrettably, unknown. The inset shows how the angle of insertion (a–d) of the probe needs to be well chosen. The example was from insertion b. (Reproduced from Rygol & Zimmermann (1990) with permission of the authors and publisher.)

there were no gradients of ΔP or osmotic potential, $\Delta\pi$, in roots of cereal plants (Steudle & Jeschke 1983; Jones *et al.* 1983, 1988) and thus, with negative gradients between the xylem and the outer cortex, the driving forces in both symplasm and apoplasm would be centripetal. It is now becoming clear that the use of very young plants, or of excised roots where there is little or no transpiration, almost certainly obscured this most important phenomenon. In *Aster tripolium*, grown with or without NaCl salinity (Zimmermann *et al.* 1992) and glycophytes *Zea mays* and *Hordeum vulgare* (Rygol *et al.* 1993), substantial turgor pressure gradients are either collapsed or evened out when transpiration is lowered or eliminated by root excision. This new information challenges our conception of the symplasm and the way in which flows are organized within it. With greater turgor in the cells of the inner cortex there is a hydrostatic pressure gradient outwardly directed, i.e. centrifugal, despite the fact that there is a net centripetal flow of water entering the transpiration stream. If plasmodesmata, which link cells along this gradient, are simply open channels it is hard to avoid the conclusion that water will pass through from them in a centrifugal direction.

It is clear that the gradient depends on transpiration but central to understanding the phenomenon is the establishment of a greater osmotic potential, π, in the inner cortical cells. There are two components to be considered. The thermodynamics of irreversible processes can be used to relate water and solute flows (equation 1).

$$J_s = (1 - \sigma)C_s J_v + \omega\Delta\pi + J_A, \qquad (1)$$

where: σ, reflection coefficient; ω, solute permeability coefficient; C_s, concentration of solutes; J_v, volume flow; J_s, solute flow; J_A, active component; and π, osmotic potential.

Where there is transpiration, the first term $(1-\sigma)C_sJ_v$, the solvent drag effect, may be large and is directed towards the xylem. At high transpiration rates, this drag will contribute to the solute concentration of the inner cortical cells since the rate of water entry into the xylem exceeds that of solutes. A second process J_A, which represents active loading of the symplast, also contributes to the solute concentration of cells in the pathway by transport from the apoplasm. At high transpiration rates one might envisage diffusible cations, e.g. K^+, being swept into the root apoplasm and being absorbed by inner cortical cells. At low transpiration, symplast loading might be more restricted to peripheral cortical–endodermal layers. The gradient of osmotic potential $\Delta\pi$ opposes the solvent drag effect. The equation predicts that the elimination of transpiration (i.e. $J_v = 0$) will allow $\Delta\pi$ to dissipate centrifugally. With *Aster tripolium* this is precisely what happened to the ΔP and $\Delta\pi$ gradient over a period of 15–20 min after roots were excised. If, however, the J_A component of equation (1) was eliminated by treatment with 2 mol m^{-3} KCN, a gradient of turgor pressure was maintained across the cortex for at least 30 min, even though the magnitude of π and P in each cell declined. Although transpiration is reduced by KCN in the long term, the solvent drag term in equation (1) would not have been eliminated.

For water flow, a gradient of solute potential $\Delta\pi$ will oppose the hydrostatic pressure gradient of ΔP. In *Aster tripolium*, $\Delta\pi - \Delta P$ and thus the net driving force for water is close to zero. This situation suggests a model in which there is a cycle of water movement in the root cortex, involving entry into the inner cortical cells from the apoplasm with centrifugal movement through the symplasm. In *Zea mays* it was found that $\Delta\pi > \Delta P$, and consequently there is a centripetal gradient of water potential. The water potential of outer cortical cells in transpiring plants was -0.36 mPa while that of the fourth rank of cortical cells was -0.45 mPa (Rygol *et al.* 1993). In either case it is hard to see how ΔP could fail to influence water movement through the plasmodesmata if they remain simple open canals. Bulk flow might be prevented if plasmodesmata closed in response to ΔP, and indeed there is sound evidence that this can happen (see § 2*b*(iv)). Closure would appear to prevent movement of solutes: it would have the effect of maintaining $\Delta\pi$ but would prevent centripetal solute movement. This is the crux of the problem created by these new data. At the risk of seeming to seek refuge in obscurity, it is worth pointing out again that much new ultrastructural and immunological information suggests plasmodesmata are much more complex than simple canals (§ 2*b*(iv)). How much of this complexity might serve to move solutes against $\Delta\pi$ remains to be discovered.

Ions diffuse in response to their particular electrochemical potential gradient, $\Delta\bar{\mu}_j$. At present there is no evidence about the cytoplasmic components of π in inner and outer cortical cells. Gradients in some ions, e.g. Ca^{2+} or $H_2PO_4^-$, are unlikely because of tight regulation (see § 2*b*(iii)). It should be remembered that fluid samples collected from individual cells by modified use of the micropressure probe (Malone *et al.* 1989) are largely, if not exclusively, of vacuolar origin and thus do not necessarily reflect the composition of the cytosol or symplasm. Compartments within the cell must, of course, be isotonic, but the cytosol could have its π raised by compatible solutes or metabolites. For instance, sugars or transported amino acids might accumulate in the inner cortex if their centrifugal diffusion was opposed by flow of water: this gradient would not affect the diffusion of ions within the symplast. Only reliable analysis of the cytoplasmic osmolytes can resolve this matter.

4. LOADING THE XYLEM

(*a*) *Regulation*

There appear to be independent controls over the loading of solutes into the xylem and the initial uptake into the symplast.

Inhibition of protein synthesis by the provision of amino acid analogues (e.g. azetidine carboxylic acid ≡ proline), caused a relatively rapid collapse of Cl^-- and ^{86}Rb-labelled K^+-entry into the xylem of barley roots but had much slower effects on uptake into the root tissue. Transport to the stele was not

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strongly influenced by the treatments, but release was, indicating that it depended on some rapidly-turning over protein distinct from those involved in uptake at the PM (Pitman *et al.* 1977).

Nutrient deficiency or large internal demand can influence specifically the loading into the xylem of the nutrient in deficit. There are a great many examples of this behaviour (Clarkson 1985). In barley, P_i deficiency in remotely located parts of the plants stimulated P_i uptake by a small number of donor roots supplied with P_i and not themselves deficient in P. Virtually all of the additional P_i uptake was loaded into the xylem ($\times 5$ the rate seen in P-replete plants), thus it seemed that the unloading of the symplast was regulating the uptake into the symplast (Drew & Saker 1984). A more recent experiment on *Zea mays*, where shoot:root ratio was manipulated by cooling shoot bases, clearly shows that K^+ translocation responded to 'demand' for K^+ and was regulated independently of K^+ uptake (Engels & Marschner 1992).

Xylem loading can be dissected from uptake in a mutant of *Arabidopsis thaliana*, *pho1*, which has a specific defect which greatly reduces the transfer of P to shoots but which does not affect root uptake (Poirer *et al.* 1991). Correction of this defect by phenotypic complementation should give a most important clue to a crucial step in xylem loading.

(*b*) *Mechanisms*

There is much less certainty about what is regulated than about the existence of regulation itself. The cells of the stelar parenchyma essentially reverse the processes involved in loading the symplast (see § 2*b*(i)). In theory, unloading can be explained by changing the balance of mechanisms responsible for influx and efflux (Hanson 1978). Efflux of NO_3^- or $H_2PO_4^-$ across the PM of cortical cells can always be detected and varies specifically with the intensity of N and P inputs or status of the cells. If these effluxes occur via anion-selective channels in the open state, we might think of efflux into the xylem as being dependent either on a greater number of such channels, relative to the H^+-symporter responsible for influx, or to their being more frequently in an open state. At present relatively little is known about the transport properties of stelar parenchyma cells. There is, however, persuasive evidence that they do possess an H^+-ATPase.

The xylem of root and stem segments can be perfused with solutions of defined composition (Okamoto *et al.* 1978; De Boer *et al.* 1983; Clarkson *et al.* 1984). Experiments of various kinds showed that there are two mutually opposed components of the electric potential difference between the xylem sap and the outside solution. Electrogenic activity at the xylem–symplasm boundary tended to depolarize the trans-root potential while that at the symport–outside boundary hyperpolarized it. This experimental evidence confirmed Hanson's (1978) idea that there might be 'back to back' proton pumps on opposite sides of the symplasm. The electrogenic activity revealed in the electrophysiological experiments seems very likely to be a proton pump because: (i) the sap coming out of the xylem in onion roots was strongly acidified by the addition of fusicoccin to the perfusion solution (Clarkson & Hanson 1986); (ii) purified PM isolated from stelar tissue of maize roots contains an H^+-translocating ATPase with properties *in vitro* closely resembling those of the cortex (Cowan *et al.* 1989, 1993); (iii) antibodies to the *Arabidopsis* and maize PM H^+-ATPase were intensely bound to the periphery of xylem parenchyma cells in barley roots (Parets-Soler *et al.* 1990; Samuels *et al.* 1992). In the former study it was most interesting to see that only the inner half of the endodermal plasma membrane reacted strongly with the antibody.

The behaviour of excised root systems may not always be a reliable guide to that of the intact plant and De Boer *et al.* (1983) needed considerable intellectual agility to explain how major variation arose in the value and the behaviour of the trans-root potential in different excised root systems of *Plantago media* and *Plantago lanceolata*. Roots were of two types; those in which the electrogenic activity at the xylem–symplasm boundary was engaged and those where it was not. The most important aspect of this paper is that various perturbations, e.g. O_2 partial pressure, could switch a given root from one state to another, showing that metabolic and other factors can have a specific effect on the activity of the H^+-pump at the xylem–symplasm boundary. In a recent paper, Cortes (1992) explored the consequences for ion transport in a model system, of proton pumping being low or absent in the inner cortex and stele.

Rates of ion-uptake and -loading into the xylem display some diurnal variation but this is far less than the fluctuation in water flow. Thus the concentration of ions in the xylem sap may vary in physiologically significant ways. The simplified illustration of this behaviour, based on a number of questionable assumptions, in table 2, indicates that a five-fold variation in $[K^+]$ could alter the direction of the electrochemical potential gradient (ΔE_{K^+}) so that during the night, if the membrane potential across the xylem symplast boundary remained constant, K^+ would diffuse into the stelar parenchyma. For comparison the calculation shows that the Ca^{2+} must always be pumped from the symplast into the xylem and that NO_3^- will diffuse into the xylem during the day or night. The latter would only be true if the electrical potential difference remained at the same value. Depolarization of the potential might eliminate or even reverse the direction of the diffusion gradient for NO_3^-.

A change in the xylem $[K^+]$ from 4 to 20 mol m^{-3} may well depolarize the membrane potential of the xylem parenchyma as it would in many types of cell. During perfusion of onion root segments a step up in $[K^+]$ over this range transiently increased the rate of release of labelled K^+ into the xylem from the stelar tissues (Clarkson & Hanson 1986). It was suggested that the increase $[K^+]$ may have depolarized the electrical potential difference across the xylem–symplasm boundary. Since that time much has been learnt about the behaviour of voltage-gated ion channels in

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Table 2. *Hypothetical effects of a diurnal change of transpiration rate on passive driving forces between xylem parenchyma and xylem sap*

(Assumptions: the electrical potential difference $E_{cx} = -58$ mV; the transpiration rate at night = 0.2 daytime rate; that ion loading into the xylem does not change.)

	concentration (mol m^{-3})			electrochemical potential gradient		
ion	cytoplasm	xylem	Nernst potential/mV	mV	kJ mol^{-1}	direction
K^+						
day	100	4	−81	+23	2.2	c→x
night	100	20	−41	−17	1.6	c←x
NO_3^-						
day	4	4	0	−58	5.2	c→x
night	4	20	−41	−17	1.6	c→x
Ca^{2+}						
day	0.0001	0.2	96	−154	29	c←x
night	0.0001	1	116	−174	33	c←x

plant cell membranes (see Tester 1990). Outwardly rectifying K^+ channels in guard cells opened quickly in response to jumps to more positive potential differences and were slow to close (Blatt 1988). K^+-dependent depolarisation of the xylem parenchyma may provoke increased activity of the proton pump and this affects H^+-cotransports. Evidently, what is needed to understand xylem loading is to learn more about the properties of the xylem parenchyma cells. Perfusion studies give only indirect evidence about what can happen to them. An alternative approach is to dismantle the system completely and study the properties of stelar protoplasts using patch clamping and transport assays (Ketchum *et al.* 1989). Such studies are underway in the laboratory of Professor K. Rashke (U. Göttingen, personal communication) and the technical feasibility of working with barley roots in this way has been mentioned in a brief report (Wegner & Raschke 1992). The work is encouraged by the broad correspondence between the electrical properties of guard cell protoplasts and the behaviour of guard cells *in vivo* (Hedrich & Schroeder 1989).

5. XYLEM MATURATION

During their transition from the living state to being fully conductive vessels, xylem cells pass through several stages. For simplicity these might be distinguished as a phase where the late maturing xylem (LMX) elements, first expand and develop lignified walls. At this stage they retain their protoplasts even though they are greatly enlarged. Subsequently the protoplasts disintegrate and the rather extended process of breaking down the xylem cross walls begins. These broad outlines have been known for a very long time in both dicot and monocot species, but transport physiologists paid relatively little attention to these facts until the work of McCully and her students brought them into prominence once more. When roots are grown in soil, or other soild support media, the maturation of LMX is spread along a greater length of root axis than when the same species are grown in water culture. Thus, in soil-grown soybean, *Glycine max*, the first LMX matured to open vessels at 170 mm from the root tip with additional LMX developing in more prominent regions of the root as it grew older (Kevekordes *et al.* 1988). Earlier it was shown that living xylem elements or vessels occluded by cross walls were to be found in the LMX of maize roots at distances of more than 200 mm from the tip of roots with a soil sheath attached to them but were absent at 150 mm and only present in some roots at 100 mm in roots without a soil sheath (St Aubin *et al.* 1986). The significance of these findings is that, in the apical zones of such roots, the water flow must be carried in the very much narrower early metaxylem and protoxylem elements where axial resistance would be far greater. Sanderson *et al.* (1988) found persistent remnants of cross walls in the apical 200 mm of barley roots grown in water culture. Flow through excised segments of these roots for a given pressure gradient could be predicted from the Poiseuille equation only if the dimensions of the single central LMX were ignored. The restriction of water flow to the narrow capillaries has the effect of attenuating the driving force related to leaf water potential, but Sanderson *et al.* (1988) concluded that a greater solute potential in the sap of the apical zone might counteract the attenuation of the leaf driving force.

These results are, to some extent, counter-intuitive as many direct observations of water uptake, using micropotometry, show that a maximum rate per unit root length can be measured in the region 50–100 mm from the tip in barley (Sanderson 1983) and in *Zea mays* a broad zone of relatively high rates of water uptake was found 40–150 mm from the tip of seminal axes (Clarkson *et al.* 1987). The steady-state water uptake may be determined by progressive increases in the resistance of the radial pathway for water movement across the cortex related to developments in the endodermis (Sanderson 1983) or the hypodermis.

The gradual development of the xylem vessels has the effect of increasing the effective cross section conducting system along the root axes. It should be remembered that all of the water collected by an axis and its branches must pass through the base of the

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root; thus its conductive cross section needs to become larger. In this the root resembles the widening of a river with many tributaries as it approaches its mouth.

The observations focus attention on the contribution of older root zones to the total water uptake by a plant (McCully & Canny 1989), especially in conditions where transpiration is great.

6. CALCIUM DELIVERY TO THE XYLEM

The free Ca^{2+} ions in the cytoplasm are held at a low resting concentration of around 10^{-7} M. A variety of surfaces and compartments bind Ca electrostatically or sequester it in massively greater amounts than remain as free Ca^{2+}, but exchange between the bound and free forms is very rapid and is kinetically 'invisible' in $^{45}Ca^{2+}$ washout experiments (Macklon 1984; P. J. White *et al.* 1992). The lumen of the ER and the vacuole are the most significant sites of sequestered calcium but these do not cross the plasmodesmata. Calcium can pass from one cell to another in two ways: from the small pool of mobile ions in the cytoplasm or by diffusion of Ca-chelates, e.g. Ca malate. The same problem is encountered in the phloem which has a very poor capacity for Ca^{2+} transport.

To explain the relatively large fluxes of Ca^{2+} into the transpiration stream, radial movement is usually assigned to the cortical apoplasm into which Ca^{2+} freely diffuses. As discussed earlier, the barrier properties of the endodermis appear to direct the flow of Ca^{2+} across the endodermal plasma membrane. In figure 7 this flux is assigned to calcium channels on the cortical side of the cell and is quantitatively matched by efflux through Ca-ATPase units on the other side. In reality the open channels may be far fewer in number than the more slowly acting pumps. When $^{45}Ca^{2+}$ uptake and exchange is studied in whole roots it can be seen that influx and efflux are almost equal. In *Secale cereale* (rye), for example, net uptake was only $\times 0.025$ the influx (P. J. White *et al.* 1992). These authors found that in transpiring plants influx can be as high as 3 μmol g^{-1} FW h^{-1}, a value comparable to K^+ influx, but that most of the Ca^{2+} is returned to the external solution, presumably by Ca^{2+}-ATPase pumping. There is, therefore, nothing very radical about the proposal in figure 5, except that channels and pumps are spatially separated on the cortical and stelar sides of the endodermal cell, respectively.

The proposed apoplasmic pathway for Ca^{2+} is equivalent to that followed by water. In barley (Sanderson 1983) and in *Cucurbita pepo* (marrow) (Clarkson 1981) suberization of the endodermal cell walls was correlated with equivalent major decreases of calcium and water movements into the xylem. Despite these similarities, the rate of transpiration is not necessarily correlated with Ca^{2+} movement across the endodermis (Atkinson 1991). Figure 7 makes it clear that this would be controlled by the activity of the Ca pumps. In pea plants, the $[Ca^{2+}]$ in the xylem sap displayed two marked diurnal peaks which were unrelated to the pattern of transpiration (Atkinson 1992). These authors showed that, in standardized

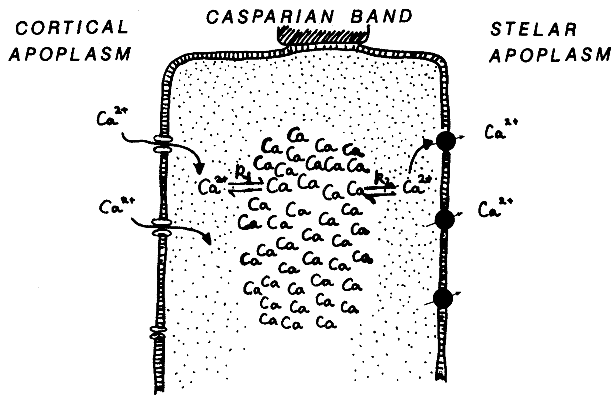

Figure 7. Schematic view of the endodermis as a calcium translocating epithelium. Calcium ions are admitted via channels from the cortical apoplasm and equilibrate with a very large pool of bound calcium. On the stelar side of the cell, Ca^{2+}-ATPase removes Ca^{2+} from the cytoplasm to the stelar apoplasm. The rate of transfer depends on the activity of the calcium pumps and the very rapid rate of release of Ca from its bound form to maintain a constant pool of free Ca^{2+}.

conditions, species within a family and varieties within a species had markedly different $[Ca^{2+}]$ in the xylem sap extracted by pressurising either roots or shoots in a Scholander chamber.

When mono-specific antibodies to the PM Ca^{2+}-ATPase become available it will be interesting to see whether or not calcium pumps are particularly abundant on the stelar side of the endodermal PM.

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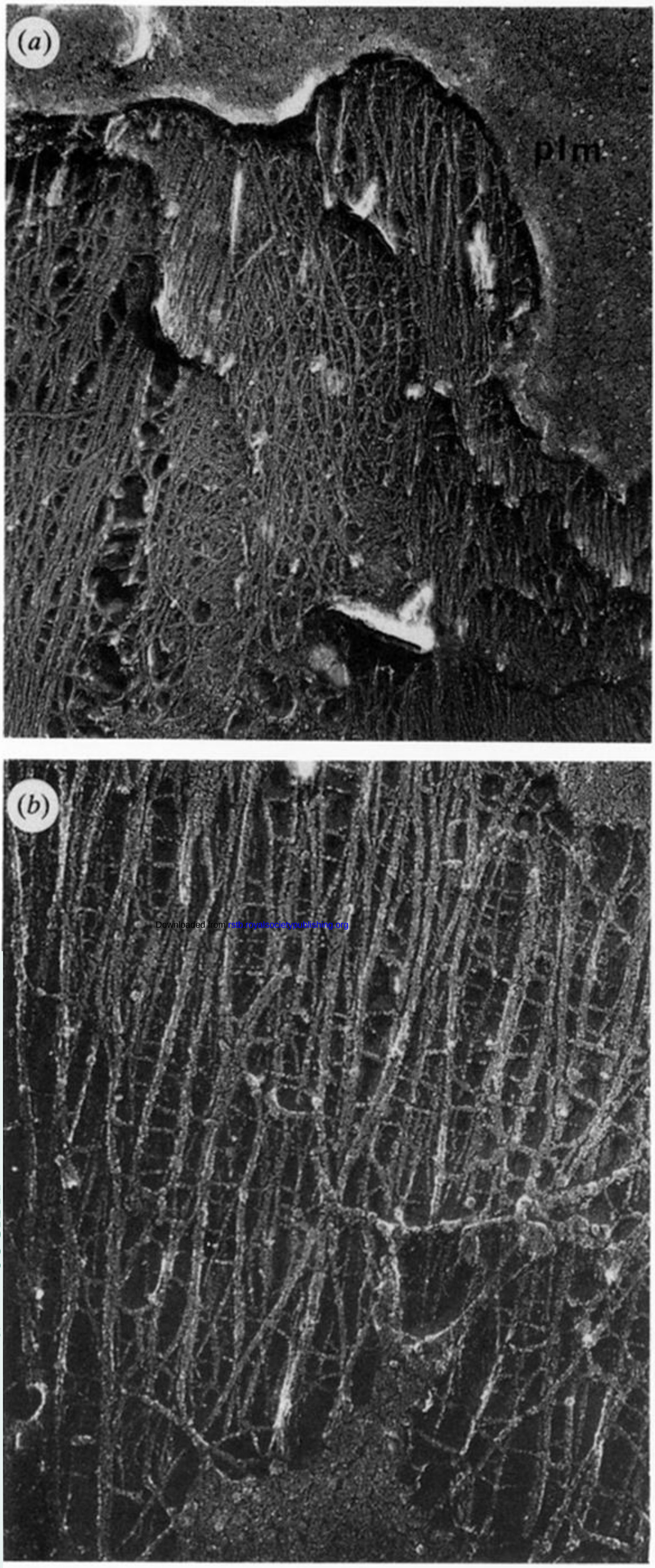
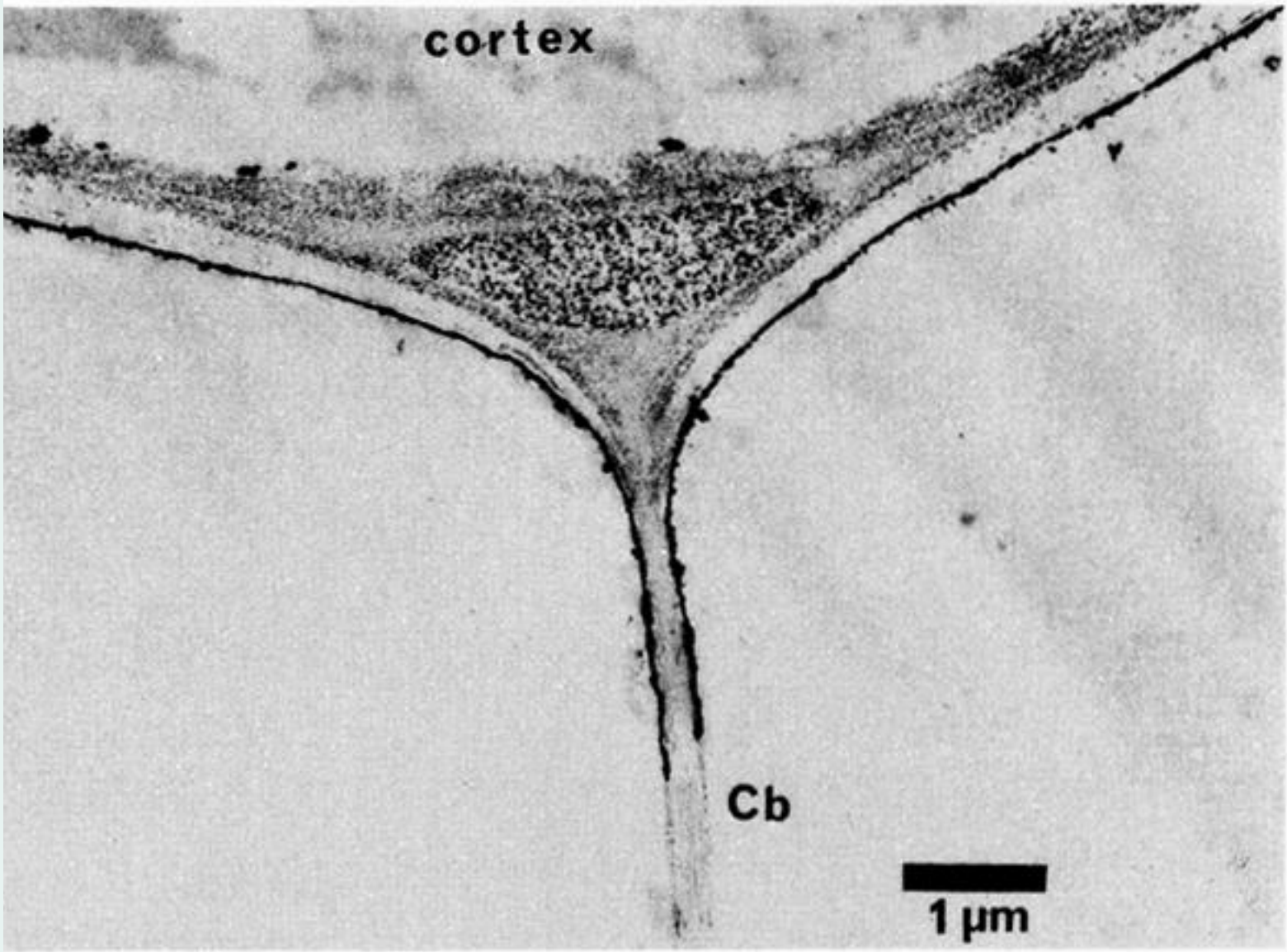
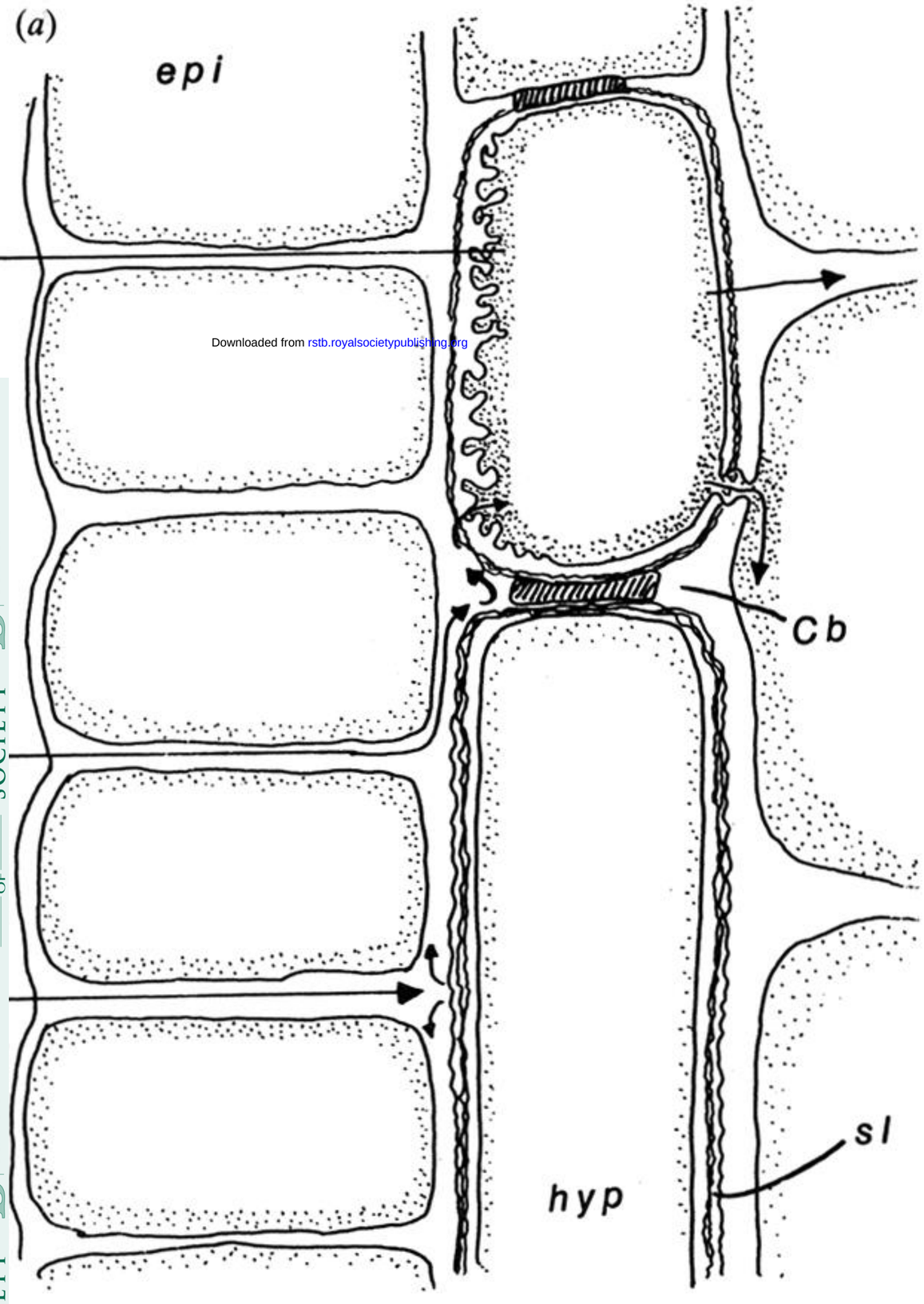


gure 2. Cell wall in maize root tip cells. (*a*) The lylamellated structure comprises at least five layers each ng parallel to the plasma membrane (plm). Before ezing and etching the material was aldehyde-fixed and htly digested with cellulase–pectinase mixture for 30 min 60 000). (*b*) Higher magnification of one layer in the lylamellated wall; cross bridges between cellulose fibrils gularly spaced (× 159 750). (Reproduced from Satiat-unemaitre *et al.* (1992) by permission.)



gure 3. Accumulation of lanthanum tracer in the walls on e cortical side of the state I endodermis of a barley root. ıe tissue was fixed in glutaraldehyde but not stained with mium. The Casparian band (Cb) in the radial wall seems arrest the radial movement of lanthanum (×9100). Micrograph kindly provided by Dr A. J. Wilson and ofessor A. W. Robards.)



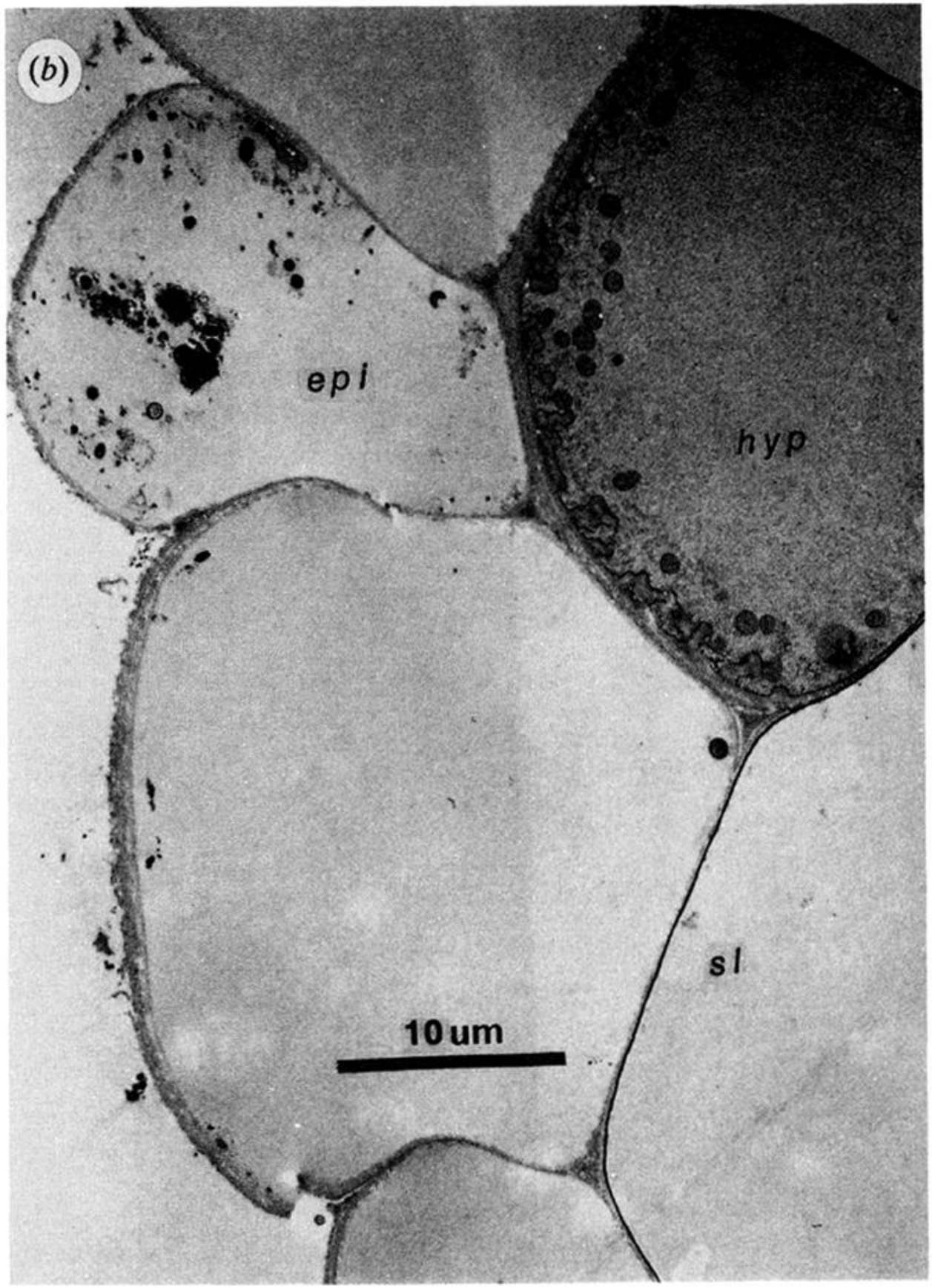


Figure 5. (*a*) Scheme to illustrate possible constraints on movements into the root apoplasm by a hypodermis with high resistance to flow in its radial walls (Cb) and with suberin lamellae (sl) in the walls. A 'passage' cell, such as described for onion roots by Wilson & Robards (1980), is included. (*b*) Transverse section at the epidermal–hypodermal junction of an onion root > 20 cm from the tip, showing a short 'passage' cell with wall ingrowths on its outer tangential face and numerous mitochondria. Note the absence of any features in the neighbouring long hypodermal cell. (Unpublished micrograph kindly provided by Dr A. Wilson and Professor A. W. Robards.)

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