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IONIC EQUILIBRATION OF THE SYMPLAST OF LOW-SALT BARLEY ROOTS

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SUMMARY

Evidence is presented in support of the view that during the initial phase (lasting 1 to 3 hours) of Rb^+ absorption by low-salt barley roots the root symplast comes close to equilibrium with the external Rb^+ solution. The characteristics of this state of equilibrium are:
1. proportionality between the equilibrium content and the initial rate of entry into the symplast;
2. reduction of influx which increases with the degree of saturation of the transport mechanism;
3. a lower degree of specificity in the internal control of influx than in external uptake;
4. temporary reduction of influx after a change of external conditions (from A to B) leading to a lower absorption rate, below the value prevailing after equal exposure to conditions B.
These results are discussed on the basis of a tetrameric transport model in which there is an interplay of movement of ions in the free and in the bound state but in which no special feedback mechanisms are operating.

1. INTRODUCTION

Progress in the understanding of the behaviour of the plant root as an ion absorbing organ requires unravelling of the complications arising from the partition of the root cells into two main compartments, viz. cytoplasm and vacuole. It is well established that in salt-saturated tissues ion exchange proceeds frequently as can be expected from a system the non-free space of which consists of two homogeneous ion pools in series of which the outer exchanges more rapidly than the inner. On the other hand, in low-salt barley roots cell compartmentation is considered to be involved in the gradual or even quite abrupt fall of the absorption rate to a new and steady level observed under appropriate conditions one to three hours after the start of uptake (cf. HOOYMANS 1964, 1971, 1975). This phenomenon is ascribed to a shift of the rate-limiting step in net ion transport from the plasmalemma o the tonoplast of the root cells. There is strong circumstantial evidence in favour of this interpretation.

1. External factors like the nature and concentration of the ion absorbed, the presence or absence of other ions in the external solution and temperature often differ in their effects on the initial and the subsequent more durable phase of ion absorption, as can be illustrated from the following observations:

a. Rb^+ as well as Na^+ uptake by excised roots is more sensitive to external concentration, presence of Ca^{2+} and temperature during the initial phase than thereafter (*figs. 1, 2* and *5*);

b. under appropriate conditions the effect of polyvalent cations on monovalent cat- and anion absorption by excised roots is limited to the initial phase (HOOYMANS 1964, 1971); at the same time all evidence points to location of this effect at the cell surface (HOOYMANS 1964; BANGE 1975);

c. differences in the external conditions can be such that ion uptake by excised roots is identical during the initial phase but varies thereafter (HOOYMANS 1975);

d. in barley roots Tl^+ ions are transported at K^+ sites but after a more or less comparable initial phase the rate of this process falls far below that for K^+ (and Rb^+) ions; addition of an equimolar concentration of Rb^+ to the external solution causes inhibition of Tl^+ uptake during the initial phase but considerable stimulation thereafter (BANGE & VAN IREN 1970).

2. At an external concentration of 0.1 mM Rb^+ accumulation into the roots of intact and decapitated plants is identical (HOOYMANS 1968). However, in the intact material there is, in addition, a considerable transfer of Rb^+ to the shoot after the initial phase whereas in the decapitated material under these conditions exudation of Rb^+ is negligible. This fact demonstrates that the capacity for net Rb^+ transport at the plasmalemma is not impaired after the initial phase. Therefore the reduction of net Rb^+ uptake in decapitated plants (and excised roots) after the initial phase must be due to the more restricted transport capacity of some interior membrane.

Thus the biphasic nature of the time curves of net ion transport in low-salt excised roots or decapitated plants is apparently due to a gradual filling of the cytoplasmic compartments (symplast) during the initial phase the rate of this process being limited at the plasmalemma. As the ion content of the symplast increases, the net uptake rate at the plasmalemma slows down and becomes equal to the rate at which ions are transferred from the symplast to the vacuoles of the root cells. Therefore during the second phase the net transport rate at the tonoplast determines the net rate of entry of ions into the root. On the other hand, in intact plants the rate-limiting step in uptake after the initial phase may remain at the plasmalemma – in case the overall rate of removal of ions from the symplast both at the tonoplast of the root cells and at the xylem vessels exceeds net uptake at the plasmalemma – or shift to these interior locations in the opposite case. Likewise in excised roots or decapitated plants a strictly linear time curve indicates that ion transport at the tonoplast of the root cells exceeds net ion transport at the plasmalemma (HOOYMANS 1971).

Another circumstance is relevant to the role of compartmentation of the root cells in the process of ion absorption by low-salt barley roots. It could be shown (BANGE 1977) that during the initial phase of Rb^+ transport in this material little, if any, transfer of Rb^+ from the symplast to the vacuole of the root cells does occur in the same way as there is hardly any translocation of Rb^+ to the shoots within this period. So it would appear that the symplast must reach a state of relative Rb^+ saturation before appreciable amounts of Rb^+ ions are released either to the vacuoles of the root cells or to the xylem vessels. This circumstance enables direct estimation from the time curves of the Rb^+

content of the symplast at relative saturation and thus the study of the factors controlling this amount.

The purpose of this paper is to report some observations made along these lines and to discuss the questions conjured up.

2. MATERIALS AND METHODS

2.1. Plant material

In most experiments excised roots of barley (*Hordeum vulgare* L. cv. *Effendi*) were used. They were grown for six days on a dilute $CaSO_4$ solution at 25°C and prepared for the experiments as described elsewhere (HOOYMANS 1964). In two experiments (*tables 3* and *4*) decapitated plants were used. This material was essentially of the same origin as the excised roots. Decapitation occurred just before pretreatment (*table 3*) or just before the start of influx measurement (*table 4*).

2.2. General procedure

Experiments were performed in a similar way as described by HOOYMANS (1964). In short, weighed portions of roots were placed in aerated solution at 25°C, the root to solution ratio being chosen such that depletion and pH changes (in unbuffered solutions) were minimized. In solutions buffered with bicarbonate pH was about 7; unbuffered solutions were slightly acid (pH about 5.4). After the uptake period roots and solution were separated and the roots washed during one minute in flowing demineralized water.

2.3. Influx measurements

Short-term influx measurements may be subject to considerable error if not properly corrected for free space uptake. Therefore the influx estimations were accompanied by an independent free space measurement involving exposure of the root material during 10 minutes to the solution used for influx estimation at 0°C. Because pretreatment may also affect free space values owing to Donnan effects, in the experiments of *tables 5*, *7* and *8* the free space determination was further refined by inclusion of a similar pretreatment of the roots as in the corresponding influx measurement. Both after influx and free space determination roots were rinsed in flowing demineralized water during one minute. Real influx values were then obtained by subtraction of the free space content. Only in two experiments (*tables 2* and *3*) the somewhat less accurate procedure of rinsing the roots in ice-cold unlabelled solution during 7 to 9 minutes after exposure to the labelled solution has been followed. Influx estimations extended over 12 to 13 minutes in the case of Rb^+ and Na^+ and over 20 minutes in the case of Cl^-.

2.4. Analytical and radiotracer procedures

The Rb^+ and Na^+ content of roots was assayed either by flame emission and flame absorption measurements after dry ashing of the material at 540°C and

dissolution of the ash in dilute HCl or by counting the ^{86}Rb and ^{22}Na content of the unprocessed roots in an automatic gamma counter. Cl^- was determined in aliquots of the aqueous extract of the roots by titration with a Cotlove Aminco automatic titrator or by assay of ^{36}Cl in a liquid scintillation counter.

2.5. Variability of results

Unless otherwise stated vertical bars in the graphs denote lowest and highest value measured (when exceeding the dimensions of the circles or squares). In the tables numbers between brackets denote the standard error of the mean. In all cases their values were sufficiently low to render calculations of significance unnecessary.

3. RESULTS

3.1. Initial rate of uptake and equilibrium content

A very intriguing phenomenon encountered in the study of the time course of ion absorption by low-salt barley roots, is the independence of the length of the initial phase on external factors that do influence the absorption rate. These factors include ion concentration (*fig. 1*), presence or absence of external Ca^{2+} (*fig. 2*), nature (Rb^+ or Na^+) of the ion absorbed (*figs. 3* and *4*) and temperature

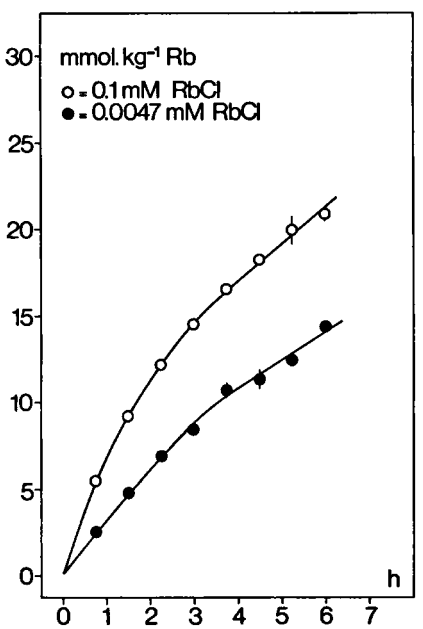


Fig. 1. Time course of Rb^+ absorption by excised roots from 0.1 mM RbCl (open circles) and 0.0047 mM RbCl (closed circles) in the absence of Ca^{2+}; experiment in duplicate.

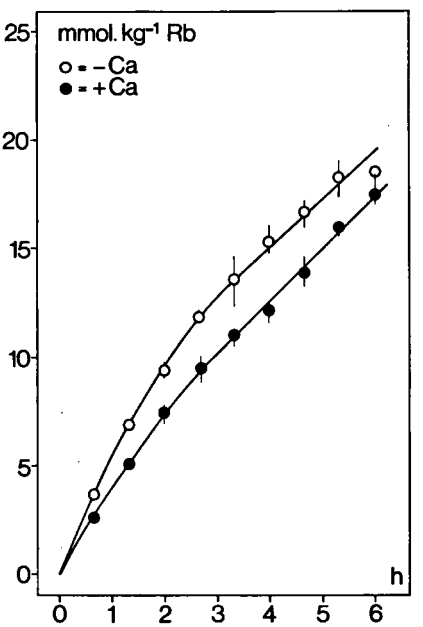


Fig. 2. Time course of Rb^+ absorption by excised roots from 0.02 mM RbCl + 0.1 mM $Ca(HCO_3)_2$ in the absence (open circles) and presence (closed circles) of 5 mM $CaSO_4$; experiment in triplicate.

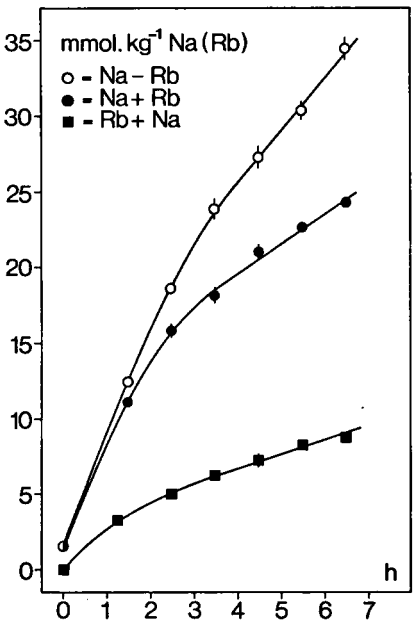
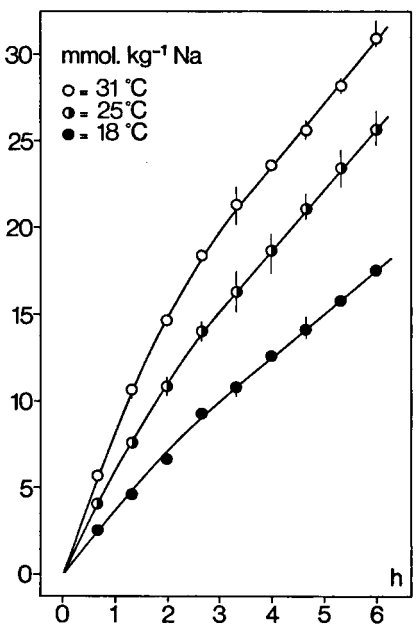


Fig. 3. Time course of Na^+ (circles) and Rb^+ (squares) absorption by excised roots from 0.05 mM Na_2SO_4 (open symbols) and 0.05 mM Na_2SO_4 + 0.005 mM Rb_2SO_4 (closed symbols) in the absence of Ca^{2+}; experiment in duplicate.



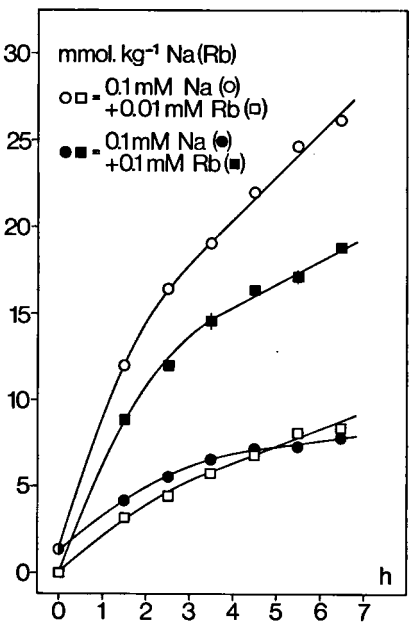


Fig. 4. Time course of Na^+ (circles) and Rb^+ (squares) absorption by excised roots from 0.05 mM Na_2SO_4 + 0.005 mM Rb_2SO_4 (open symbols) and 0.05 mM Na_2SO_4 + 0.05 mM Rb_2SO_4 (closed symbols) in the absence of Ca^{2+}; experiment in duplicate.

Fig. 5. Time course of Na^+ absorption by excised roots from 0.4 mM NaCl + 0.05 mM $Ca(HCO_3)_2$ at 18°C (closed circles), 25°C (half-open circles) and 31°C (open circles); experiment in triplicate.

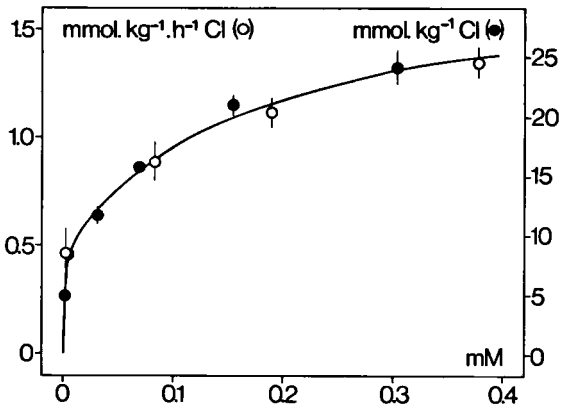


Fig. 6. Comparison of the initial rate of Cl^- uptake by excised roots (open circles, left ordinate) and the equilibrium level of Cl^- in the roots after 30 hours (solid circles, right ordinate) in solutions containing varying concentrations of NaCl + 0.1 mM $Ca(HCO_3)_2$; vertical bars denote s.e.m.

(*fig. 5*). At the same time it should be emphasized that, by causes unknown, in plant material that is not strictly comparable as to age and origin of the seed and pretreatment, the length of the initial phase may vary from about 1 to 3 hours. As mentioned in the introduction, the evidence indicates (BANGE 1977) that during the initial phase Rb^+ is accumulated mainly, if not exclusively, into the cytoplasm and not into the vacuole of the root cells. Therefore a fairly close proportionality must exist between the amount of Rb^+ present in the symplast of the root at the end of this phase and the initial absorption rate. Interestingly, a similar relationship was found between the overall root Na^+ content after equilibration with different external Na^+ concentrations and the initial rate of Na^+ absorption (NEIRINCKX & BANGE 1971) though in this case the straight line relating both quantities did not pass through the origin. A still more general prevalence of this principle in roots is evident from the results of a study of the equilibration of low-salt barley roots with external Cl^- ions at different concentrations. Again the overall Cl^- content at equilibrium and the initial rate of net Cl^- absorption appear to depend in an identical way on external Cl^- concentration (*fig. 6*, from Bange and Poelman, unpublished results). It could be established that in this experiment the anion equilibrated quite independently from the accompanying Na^+ ion.

Proportionality between content at equilibrium and initial uptake rate of a substance is a feature of 'pump and leak" systems in which the decrease of net uptake is due to simultaneous leakage out of the tissue in direct proportion to the internal content (STEIN 1967). However, as will be discussed in the next paragraph, in this simple interpretation another important and widely observed feature of the equilibration process, viz. the reduction of influx (PITMAN et al. 1968; VALLÉE 1969; JOHANSEN et al. 1970; CRAM & LATIES 1971; NEIRINCKX & BANGE 1971; CRAM 1973; GLASS 1975, 1976), remains unexplained.

3.2. The reduction of Rb^+ influx in response to increasing cytoplasmic Rb^+ content

Increase of the cytoplasmic Rb^+ content during the initial phase brings about a reduction of the rate of influx. In *table 1* the influx value at the start of the experiment has been compared for two concentrations to the value obtained 4.5 hours later, i.e. after the shift of the rate-limiting step in uptake to the interior of the cell. The possibility has to be excluded that these reductions are artifacts due to unstirred layer effects. If internal Rb^+ ions are able to leak out of the cell through the plasmalemma into the unstirred layer at the cell surface, they may compete there with Rb^+ ions of external origin for transport inward through the membrane and thus reduce uptake for the latter. To check this possibility the reduction of influx was studied at three different Rb^+ concentrations which, being saturating for the absorption mechanism, did not change the Rb^+ influx by more than about 10% but would dilute any leaking Rb^+ ions in the unstirred layer to a considerably different degree. *Table 2* shows that the reduction of Rb^+ influx is independent of external concentration in this range and thus not an artifact in the above sense.

The relative reduction of influx into the cytoplasm is less at non-saturating Rb^+ concentrations than when the absorption rate is nearly maximal within the range of "system I" (*table 1*). In roots saturated with Cl^- the same feature is encountered (*table 3*, from Bange and Karsters, unpublished results). In this case the inhibition of influx is all but abolished at the lower concentration.

Table 1. Rb^+ influx from 0.2 and 0.0037 mM RbCl solutions in excised roots before and after pretreatment during 4.5 hours in the same solution; experiment with 5 replicates.

	Influx ($mmol.kg^{-1}.h^{-1}$)	
	0.2 mM	0.0037 mM
initial	8.19 (0.11)	4.016 (0.308)
after 4.5 hours	3.02 (0.09)	2.532 (0.137)
ratio reduced/initial influx	0.369 (0.012)	0.630 (0.059)

Table 2. Reduction of Rb^+ influx in excised roots in the period from 0.5 to 3.5 hours after the start of uptake at three different external Rb_2SO_4 concentrations; experiment with 5 replicates.

Concentration (mM)	Influx ($mmol.kg^{-1}.h^{-1}$)		Ratio b/a
	after 0.5 h (a)	after 3.5 h (b)	
0.14	7.20 (0.23)	3.55 (0.10)	0.49 (0.02)
0.23	7.34 (0.26)	3.74 (0.06)	0.51 (0.02)
0.32	8.03 (0.20)	4.11 (0.07)	0.51 (0.02)

Table 3. Cl^- influx from a 0.05 and 1.0 mM NaCl solution respectively in decapitated plants pretreated during 24 hours after decapitation either in 2.10^{-4} M $CaSO_4$ or a NaCl solution of the same concentration as used in the influx measurement; experiment with 3 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)	
	0.05 mM NaCl	1.0 mM NaCl
$CaSO_4$ (2.10^{-4} M)	1.37 (0.04)	4.19 (0.23)
0.05 or 1.0 mM NaCl	1.21 (0.03)	1.37 (0.06)
ratio reduced/initial influx	0.886 (0.034)	0.330 (0.023)

3.3. Ion specificity of the reduction of influx

For Cl^- absorption CRAM (1973) has shown that not only internal Cl^- but also NO_3^- is able to provide the stimulus leading to reduction of Cl^- influx. Mutatis mutandis the same is true for net NO_3^- uptake (SMITH 1973). This fact is remarkable because the processes of Cl^- as well as NO_3^- uptake have a considerable degree of specificity.

Separate absorption mechanisms have also been claimed for K^+ (Rb^+) and Na^+ uptake in barley roots (BANGE et al. 1965). Therefore it was of some interest to investigate whether a high cytoplasmic Rb^+ content led to a reduced influx of Na^+ and vice versa. In the experiment represented in *table 4* intact plants grown in the dark were allowed to absorb Rb^+ during four hours and were then decapitated and exposed to either a Rb^+ or Na^+ solution for measurement of influx. It appeared that not only Rb^+ influx was reduced in comparison with the unpretreated controls but also Na^+ influx. On the other hand, in a comparable experiment no effect of Na^+ pretreatment on Rb^+ influx could be established (results not shown).

Table 4. Rb^+ influx from a 0.1 mM RbCl solution and Na^+ influx from a 0.4 mM NaCl solution in the presence of 0.05 mM $Ca(HCO_3)_2$ in roots of decapitated plants before and after pretreatment of the intact plants during 4 hours in 0.1 mM RbCl + 0.05 mM $Ca(HCO_3)_2$; experiment with 5 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)	
	Rb^+	Na^+
none	6.74 (0.27)	5.31 (0.22)
0.1 mM RbCl + 0.05 mM $Ca(HCO_3)_2$	4.15 (0.10)	3.55 (0.23)

3.4. Reversibility of the equilibrium between cytoplasmic and external Rb^+

When equilibrium has been attained between cytoplasmic and external Rb^+, a change of external conditions leads to resumption of the equilibration process in case the cytoplasmic equilibrium level of Rb^+ under the new as com-

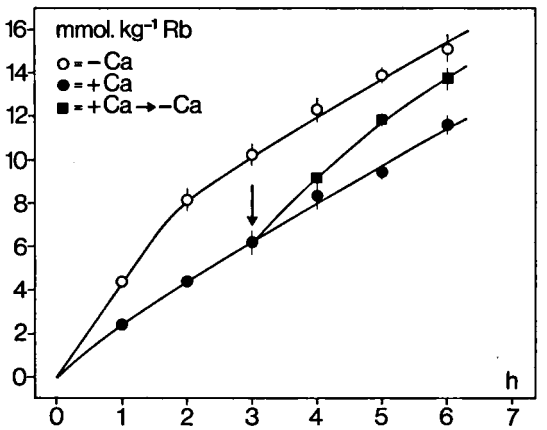


Fig. 7. Time course of Rb^+ absorption by excised roots from a 0.05 mM Rb_2SO_4 solution in the absence (open circles) and presence (closed circles) of 5 mM $CaSO_4$; after 3 hours part of the roots was transferred from the solution containing Ca^{2+} to the Ca^{2+}-free solution (closed squares); experiment in duplicate.

pared to the old conditions is higher. For example, in the experiment shown in *fig. 7* Rb^+ absorption in the presence of Ca^{2+} is enhanced at the moment Ca^{2+} is omitted from the external solution. Vacuolar accumulation being hardly sensitive to this factor under these conditions, the enhanced absorption must represent resumption of uptake into the cytoplasm. On the other hand, when the equilibrium level of Rb^+ in the cytoplasm fitting the new conditions is lower, there may or may not occur a shift to a new equilibrium. For instance, when after exposure of the roots during four hours to a Ca^{2+}-free Rb^+ solution, Ca^{2+} is added to the solution (*fig. 8*), there is no measurable temporary

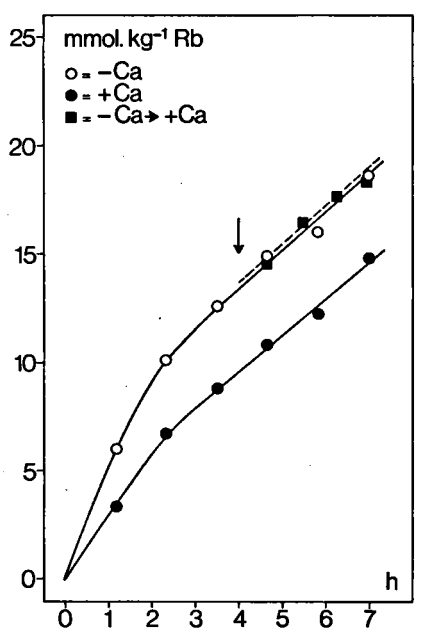


Fig. 8. Time course of Rb^+ absorption by excised roots from a 0.011 mM $RbHCO_3$ solution in the absence (open circles) and presence (closed circles) of 5 mM $CaSO_4$; after 4 hours part of the roots was transferred from the Ca^{2+}-free solution to the solution containing Ca^{2+} (closed squares); single experiment.

Table 5. Reduction of Rb^+ influx from a 0.012 mM RbCl + 5 mM $CaSO_4$ solution in excised roots pretreated during 4 hours in either 0.012 mM RbCl without Ca^{2+} or 0.012 mM RbCl + 5 mM $CaSO_4$; experiment with 4 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)
0.012 mM RbCl	1.44 (0.02)
0.012 mM RbCl + 5 mM $CaSO_4$	1.88 (0.01)

Table 6. Reduction of Na^+ influx from a 0.2 mM NaCl + 5 mM $CaSO_4$ solution in excised roots pretreated during 3.5 hours in either 0.2 mM NaCl without Ca^{2+} or 0.2 mM NaCl + 5 mM $CaSO_4$; experiment with 3 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)
0.2 mM NaCl	0.49 (0.18)
0.2 mM NaCl + 5 mM $CaSO_4$	2.69 (0.11)

Table 7. Rb^+ (Na^+) influx from a 0.005 mM RbCl (0.01 mM NaCl) solution in excised roots pretreated during 4 hours in either 0.005 mM RbCl (0.01 mM NaCl) or 0.10 mM RbCl (0.40 mM NaCl); experiment with 5 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)	
	Rb^+	Na^+
0.005 mM RbCl	2.10 (0.10)	
0.10 mM RbCl	1.52 (0.02)	
0.01 mM NaCl		0.805 (0.031)
0.40 mM NaCl		0.395 (0.066)

decline of the net rate of Rb^+ uptake. Nevertheless, at the moment of Ca^{2+} addition short-term Rb^+ influx falls about one quarter below the level prevailing after the same time when Ca^{2+} has been present from the start (*table 5*). Apparently this reduction is only of short duration. In comparable experiments on Na^+ uptake the addition of Ca^{2+} to the experimental solution brings about a sharp temporary decrease of net Na^+ absorption (HOOYMANS 1964) due to a remarkable reduction of Na^+ influx to a level far below the value obtaining after absorption during the same period in the presence of Ca^{2+} (*table 6*). Furthermore, when the reduction of the rate of Rb^+ uptake is accomplished by lowering of the external concentration, the rate of influx falls likewise below the value observed in roots that have been pretreated during the same period in the low instead of the high concentration (*table 7*). By the same token pretreatment at 25°C brings about a lower rate of Rb^+ influx at 9°C than pretreatment at 9°C (*table 8*).

Table 8. Influx of Rb^+ from a solution of 0.15 mM RbCl + 1 mM $CaCl_2$ + 0.1 mM Ca $(HCO_3)_2$ at 9 °C in excised roots pretreated during 4 hours either at 9 °C or at 25 °C in the same solution; experiment with 4 replicates.

Pretreatment	Influx ($mmol.kg^{-1}.h^{-1}$)
uptake solution at 9 °C	2.067 (0.097)
uptake solution at 25 °C	1.085 (0.013)

4. DISCUSSION

A crucial question that must be answered first, is whether or not the symplast reaches equilibrium with the external solution or, in other words, a state in which neither the continuing flux to the vacuoles nor the release to the xylem vessels interferes markedly with the maximum level the Rb^+ concentration in the symplast can attain under the prevailing conditions. In a number of earlier publications (HOOYMANS 1975, 1976; BANGE 1977) the inadequacy of the strictly serial model of cell compartmentation (PITMAN 1963) to explain kinetic data in low-salt barley roots has been emphasized. This conclusion is relevant to the problem at hand because, obviously, in a strictly serial arrangement of fluxes this state will not prevail before the whole cell has reached ion saturation. Whether or not the cytoplasmic phase is close to the state of equilibrium after the initial period, is elucidated by experiments with intact plants in which the rate of upward transport is reduced by checking leaf transpiration or shoot excision. It then appears (*fig. 1* in HOOYMANS 1968; *fig. 2* in BANGE 1977) that concomitant root accumulation of Rb^+ is not affected at all or even slightly reduced. So for Rb^+ a high degree of cytoplasmic equilibration after the initial phase seems realized. On the other hand, from similar experiments with K^+ (*figs. 1* and *2* in HOOYMANS 1969) and Cl^- (*fig. 4* in HOOYMANS 1971) the conclusion follows that in these cases equilibration is somewhat less complete.

At the moment our insight into the ionic relations of the cytoplasmic phase and its organelles is far too scanty to provide a base for the framing of a model from which the high degree of cytoplasmic equilibration, on the one side, and the intensive transit of ions, on the other, can be understood. For the same reason it is impossible to state over which membrane of the root cells flux equilibrium of Rb^+ is established during the initial period. Despite these limitations it seems justified to examine whether the characteristics of cytoplasmic equilibration can be fitted to a relatively simple model of membrane transport, the more so as they appear to resemble so much the characteristic features of whole cell equilibration. The tetramer model proposed by STEIN et al. (1973) for the Na^+-K^+-pump of animal tissues can serve the purpose when adapted to a less specific function (*fig. 9*). This model consists of four subunits half of which face the outside and half the inside of the membrane. Each of these subunits carries a binding site that, by a conformational change of the subunit, may be orientated either to the membrane surface or to an internal cavity in such a way that with respect to these alternatives adjacent (in the

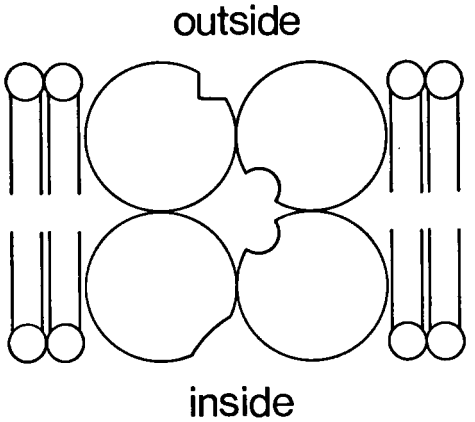


Fig. 9. Tetrameric transport protein (cf. STEIN et al. 1973).

plane of the membrane) subunits have always opposite and successive (perpendicular to the membrane) subunits identical orientations. Thus transport consists of the consecutive steps of binding of substrate to the outward site of an external subunit, movement of the site + substrate to the internal cavity, transition of the substrate to the site on the internal subunit, movement of the site + substrate to the inward surface of the membrane and release of the substrate. When substrate affinity is higher at the outward than at the inward membrane surface, the system is able to perform active transport. In the variant of the model used here substrate affinity to the sites on the inward membrane surface is supposed to be low relative to the substrate levels attained at equilibrium. The affinity of the sites in the internal cavity to the substrate is assumed to be identical. In this system a substrate particle transferred from the outer membrane surface to the interval cavity by a conformational change of the subunit involved ("transposition"), has an equal chance of being carried back to the outer surface as of being transported to the inner surface during the next transposition. Therefore even at saturating external concentrations the available transport capacity is only used half. Finally, spontaneous transformations may occur in the unloaded state of the system without expenditure of energy.

Interesting perspectives open when the principles outlined above are amplified with the not too far-fetched assumption that, in the hydrophilic environment of the subunits, ions are able to penetrate into the central cavity from the inside of the membrane by free diffusion. Introduction of this principle will be shown to be also of some use in the interpretation of the problems conjured up by the experiments described above.

1. Proportionality of equilibrium content and initial rate of uptake

As mentioned earlier (paragraph 3.1) this feature strongly suggests a "pump and leak" mechanism but leakage over the whole of the membrane thickness leaves other characteristics of the quilibration process unexplained. This difficulty

can be overcome by the hypothesis that leakage proportional to the ion concentration on the membrane inside, occurs only over part of the membrane thickness, i.e. to the internal cavity. In the model chosen net uptake will come to a stop when the rate of this leakage is twice the initial rate of net uptake (see Appendix, 1). When site affinities in the internal cavity are not identical, a proportionality factor other than 2 will prevail. Incidentally, without additional assumptions the mechanism proposed does not explain why after long-term exposure of roots to Na^+ solutions of varying concentration proportionality between initial rate of Na^+ uptake and final Na^+ content does not exist unless from this content a constant amount is subtracted (NEIRINCKX & BANGE 1971).

2. Reduction of influx

If the internal cavity were large with respect to ionic radii, ions transported into it from the exterior and those leaking into it from the interior would be able to exchange freely on the sites facing the cavity and no reduction of influx would result. With growing steric (and possibly also electrostatic) restriction the chance that ions entering the cavity simultaneously from both sides, will leave it on the side of entrance, will increase and a proportional reduction of influx will result (see Appendix, 2). When equilibrium has been attained at saturating external concentrations, there will be always two ions at a time in the cavity but under non-saturating conditions the chance that ions of external and internal origin meet in the cavity, will decrease with concentration and so will the reduction of influx.

3. Ion specificity of the influx reduction

When two ion species A and B have specific sites of their own for entry from the outside but discrimination at the sites in the respective cavities is less, flux equilibrium for both species will be determined by the internal level of A and B together and not of A, respectively B separately. Obviously there is not necessarily reciprocity in this respect: a high internal level of B may affect the accumulation of A to a much less degree than vice versa. Relative affinity of the sites in the cavity to the substrates involved will be the controlling factor in this regard.

4. Pretreatment effects

In the cytoplasm at least a small compartment must exist that, by its involvement in the transit of ions to either the cell vacuoles or adjacent cells and ultimately the xylem vessels, does not attain equilibrium with the environment. With respect to this compartment a strictly serial flux arrangement may exist governed by the relation (see Appendix, 1):

$$[c_o.k_3.R_t/2(K_o + c_o)] - D.c_i/2 = c_i.V_{max(i)}/(K_i + c_i)$$

in which c_i is the steady-state concentration in the transit compartment and K_i and $V_{max(i)}$ are the parameters of the transport system present at its inner boundary. In accordance with this scheme plasmalemma transport is more

sensitive to external factors such as ion concentration (*fig. 1*), presence or absence of Ca^{2+} (*fig. 2*) and temperature (*fig. 5*) than vacuolar transport. When, under a given set of conditions, a certain steady-state value of c_i has been established in the compartment and the roots are transferred to new conditions entailing a lowering of plasmalemma transport and thus of c_i, influx into these roots will fall temporarily below the level prevailing in the controls, i.e. roots that have been pretreated during an equal period under the new set of conditions. This phenomenon results from the temporary high value of the diffusional counterflux and thus of the resulting influx reduction before c_i has fallen to its new steady-state value. The factors governing the remarkable difference in response of net transport of Rb^+ on the one side and Na^+ on the other to the addition of Ca^{2+} after pretreatment in the absence of Ca^{2+} (see paragraph 3.4), to all probability root in the complexity of cytoplasmic compartmentation and, therefore, are not properly understood.

From the foregoing considerations it may be concluded that interplay of free diffusion and transport in the bound state in a tetrameric transport model provides a relatively simple base to explain the phenomena attended with ion equilibration of membrane-bounded compartments. In a previous publication on Na^+ equilibration of excised barley roots (NEIRINCKX & BANGE 1971) the short-circuiting of a cytoplasmic transport track by substrate particles leaking back from the accumulated pool in the vacuole into the protoplasm, was postulated. Essentially the same principle though restricted to the processes inside the membrane, underlies the explanation advanced in this paper.

A quite different approach to the problems attended with root equilibration, has been put forward by GLASS (1975, 1976, 1977). The feedback control of K^+ influx is visualized by this author as the result of allosteric effects of internal K^+ ions on the transport mechanism through four internal binding sites. No doubt along these lines an alternative explanation of the phenomena described above could be framed. Unfortunately, because of the flexibility of allosteric interpretations it will be difficult to develop discriminative criteria.

APPENDIX

1. Influx into the central cavity from the outside ($= v_o$) equals:

$$v_o = c_o . k_3 . R_t/(K_o + c_o)$$

in which c_o = external concentration, k_3 = rate of subunit transposition, R_t = total number of external subunits, and K_o = dissociation constant of the substrate-subunit complex. Influx into the central cavity from the inside by diffusion ($= v_D$) is supposed to be proportional to the internal substrate concentration ($= c_i$):

$$v_D = D.c_i.$$

Efflux from the cavity ($= v_e$) to either the outside ($= v_{eo}$) or the inside ($= v_{ei}$)

will be equal in case the dissociation constants of the sites on the external and internal subunit when facing the cavity, are equal:

$$v_{eo} = v_{ei}.$$

In the steady state influx into and efflux from the cavity cancel, so:

$$v_o + v_D = v_{eo} + v_{ei} = 2v_{ei}.$$

At low values of c_i the value of v_D is negligible and net uptake by the system equals:

$$v_{ei} = v_o/2.$$

On the other hand, at rising values of c_i net uptake will come to a stop when

$$v_{ei} = v_D = D.c_i = v_o,$$

and thus proportionality between c_i at equilibrium and the initial rate of uptake ($= v_o/2$) obtains.

2. A substrate particle entering the central cavity by free diffusion from the inside will initially bind to the site on the internal subunit. When simultaneously a substrate particle has been transported into the cavity from the outside by the external subunit, the particles will enter into a process of exchange. Now let it be assumed that, within the time interval between two transpositions, the chance for exchange is p ($p < 0.5$). At an external concentration $= c_o$ an ion that has entered the cavity from the outside, has a chance of:

$$(v_D/v_o).c_o/(c_o + K_o)$$

to come upon an ion that has penetrated into the cavity from the opposite site. In this case the radioactive fluxes out of the cavity after addition of label to the external solution will be in the proportion of:

$$v^x_{eo}/v^x_{ei} = (1-p)/p$$

and influx into the cell will amount to:

$$v_o - v^x_{eo} = v^x_{ei} = v^x_{eo}.p/(1-p) = p.v_o.$$

In the other case, i.e. when the particles are not present simultaneously, the influx into the cell equals $v_o/2$ (see sub 1). Therefore the total influx into the cell amounts to the algebraic sum of the respective products:

$$p.v_o.(v_D/v_o).c_o/(c_o + K_o) + [1-(v_D/v_o).c_o/(c_o + K_o)].v_o/2 =$$
$$[v_o.(c_o + K_o) - c_o.v_D.(1-2p)]/2(c_o + K_o)$$

and thus the ratio reduced/initial influx equals:

$$1-(1-2p).(v_D/v_o).c_o/(c_o + K_o).$$

So this ratio is smaller at lower values of p and at higher values of c_o and v_D. Under saturating conditions ($c_o \gg K_o$) and at equilibrium ($v_D = v_o$) the expression is reduced to 2p.

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