Plant Physiol. (1968), *43*, 747–755

Interpretation of the Dual Isotherm For Ion Absorption in Beet Tissue

C. B. Osmond[1] and George G. Laties

Department of Botanical Sciences and Molecular Biology Institute, University of California, Los Angeles, California 90024

Received March 14, 1967.

Abstract. Beet discs aged in 0.5 mM $CaSO_4$ develop a capacity to absorb K^+ and Cl^- from solutions of low concentration. The initial influx of these ions is described by a hyperbolic relationship with concentration in the range 0.01 to 0.5 mM KCl, which is identical with the system 1 absorption isotherm found in other tissues. A second hyperbolic isotherm, attributable to system 2, is found at higher concentrations (1–50 mM KCl).

When the transport of labeled ion to the vacuole is studied by wash-exchanging the bulk of the cytoplasmic label following the absorption period, it is noted that in the range of system 1, isotope influx to the vacuole increases with time as the concentration of labeled ions in the cytoplasm increases, while in the range of system 2, influx to the vacuole is constant from the beginning. Diminution of the cytoplasmic specific activity during radioisotope absorption by prefilling the cytoplasm with the analogous unlabeled salt, markedly reduces subsequent radioisotope uptake to the vacuole only in the range of system 1. These experiments suggest that the cytoplasm serves as a mixing chamber, and that the plasma membrane controls ion uptake to the tissue at low concentrations, indicating that the system 1 isotherm reflects ion movement into the cytoplasm through the plasma membrane. Flux experiments support this conclusion, showing that development with age of the system 1 isotherm corresponds to a quantitatively similar increase in plasma membrane influx in 0.2 mM KCl.

At higher concentrations the outer membrane no longer rate-limits entry of ions to the vacuole. Isotope influx under these conditions, described by the system 2 isotherm, presumably reflects movement across the tonoplast.

The initial rates of absorption of labeled ions by plant tissues over a wide range of concentrations show 2 or more hyperbolic isotherms which presumably represent carrier mechanisms having different apparent K_s values (5). Torii and Laties (23) recently suggested a spatial separation of the 2 principal ion absorption systems, which differ in their apparent affinities for ions by almost 3 orders of magnitude. On the basis of a comparison of the ion absorption isotherms in largely non-vacuolated root tips and proximal vacuolated cells, they proposed that system 1 (0.01–0.5 mM) reflects ion transport across the plasma membrane, whereas system 2 (1–50 mM) reflects transport across the tonoplast. Other distinctions, with regard to inhibitor sensitivity (12), counter ion effects (10) and organic acid synthesis (24), have supported this separation. The dual mechanism of ion transport, developed so extensively by Epstein (5, 6) was further explored in relation to symplastic transport (11, 13), with the further strengthening of the hypothesis of the spatial separation of systems 1 and 2. However, the principal basis for the postulated spatial separation remained one of comparison between vacuolate and non-vacuolate tissues. The experiments described here provide further information about the interrelation of the principal ion absorption processes reflected in the dual isotherm, in a more uniform tissue—the vacuolated parenchyma of beet storage tissue.

As a further extension of this hypothesis it was proposed that system 1 brings the ion concentration of the cytoplasm to a pseudo steady-state which determines the subsequent rate of uptake to the vacuole (23 *cf.* 17). It was suggested that at higher concentrations, diffusion of ions across the plasma membrane removes the restriction of system 1 transport, and permits more rapid uptake. These features of the hypothesis are readily investigated in aged storage tissue in which the ion fluxes in and out of the cytoplasm and vacuole respectively can be distinguished (15, 19, 25).

Initial influx rates of labeled ions to a slowly exchangeable phase of plant tissues are normally used to construct isotherms (4). Since influx may

[1] Present address: Research School of Biological Sciences, Institute of Advanced Studies, A.N.U., Canberra, Australia.

change with time (17), it may be important to consider both initial and subsequent rates. The slow loss of label attending to washing represents negligible efflux only if the internal specific activity is akin to that of the original labeled external solution. However, even "low-salt" tissues contain high levels of cations and appreciable chloride (8) in relation to the amount of radioactive ions absorbed in short term experiments, and since, as a consequence, the true efflux is unknown, net uptake or net flux must be measured independently by tissue analysis or change in external concentration (2). With these reservations in mind, we have attempted to relate initial influx characteristics, described by multiple isotherms, to ion fluxes into and between cellular compartments. Although the shape of an isotherm may indicate the nature of an absorption process (10), it has been cautioned that a hyperbolic isotherm does not necessarily indicate a specific carrier (1). This paper is primarily concerned with the location of absorption processes described by dual isotherms, not with the mechanisms involved.

Methods

Red beets (*Beta vulgaris* L.) were purchased at local markets and stored in moist vermiculite at 15° until use. Discs (10 mm × 1 mm) were cut from the outermost parts of the beet to contain as little vascular tissue as possible. These were used after several washes in 0.5 mM $CaSO_4$ (fresh discs), or after washing for at least 72 hours in repeated changes of $CaSO_4$ solution (aged discs). During washing the solution temperature was maintained below 20°, and sometimes 50 μg per ml streptomycin sulphate was added. These measures eliminated obvious bacterial contamination, and as far as could be judged, streptomycin had no effect on subsequent ion absorption (*cf.* 14).

For ease of handling during transfer between different solutions, batches of 5 or 7 discs were enclosed in small nylon net bags. KCl solutions (0.01–50 mM containing 0.5 mM $CaSO_4$) were labeled with ^{86}Rb, ^{42}K, or ^{36}Cl, and in some double labeling experiments, with ^{42}K and ^{36}Cl together. At the end of each uptake period, discs were briefly rinsed in deionized water, then stirred in 2 changes of unlabeled solution of the same concentration as the experimental solution, for a total of 30 minutes at 25°. Radioactivity in discs was assayed with a gas-flow GM tube. For counting, discs were blotted and dried onto aluminum planchets with several drops of 3 % Elvanol 51-05 polyvinyl alcohol as adhesive. Aliquots of the external solution were placed on planchets with the same number of untreated discs and dried, giving standards of approximately similar geometry and self-absorption characteristics. Absorption of ions was estimated on the basis of the radioactivity of the tissue and the specific activity of the ions in the external solution.

Initial influx (ϕ_i) was estimated from the slope of the curve of concentration of labeled ions in the tissue (C^*) against time during the first hour. This slope, which extrapolated to zero, was established by sampling replicate bags at 15 minute intervals.

Final influx (ϕ_I) was estimated from the final slope of the curve for C^* against time when the course of absorption was followed for 8 to 10 hours.

Net flux (ϕ_n) for potassium was measured by the change in external potassium concentration of solutions in which larger samples (15–20 discs, about 2 g) were shaken for longer time intervals (5–10 hr). Before measurement of net flux, the free space of the discs was equilibrated at each external concentration (0.01–50 mM) by treatment of discs in a large volume of solution for 2 hours. For net flux measurement, solution volume was adjusted to permit a measurable change in external concentration (about 20 %). Volume varied from 2 liter (0.01 mM) to 10 ml (10–50 mM). Potassium concentration was measured with a Perkin-Elmer Atomic Absorption Spectrophotometer. Concentrated solutions were diluted to less than 0.2 mM for measurement.

In pretreatment experiments samples of discs were stirred in a large volume (1–4 liter) of unlabeled KCl solution at the specified concentration and temperature, for the designated time. Samples were then rinsed in deionized water, and in 0.5 mM $CaSO_4$ for 5 to 10 minutes at room temperature before treatment in labeled solutions. Initial influx rates were measured as described above.

Time-constants for the loss of potassium from the cytoplasm were derived from measurements of label efflux into unlabeled solutions after pre-incubation of discs for up to 20 hours in highly radioactive solutions of the same concentration (19). Batches of discs (1–2 g) were removed from labeled solution at intervals, blotted, and shaken in successive changes of unlabeled solution (10 ml) at 2° or at 25°. Aliquots of these solutions were evaporated to dryness on aluminum planchets and counted as described above. Net flux was measured by change in the potassium concentration of the bathing solution during the final period of steady loss of radioactivity. The total tissue content and radioactivity of a given ion—which at the end of a prolonged wash-exchange interval yield essentially vacuolar values—were determined on 1:50 HNO_3 extracts.

The rationale and technique of efflux analysis in relation to compartmentation is as follows. Label is first washed from the free space, following which label from the cytoplasm, and then from the vacuole, is wash-exchanged to the external solution in distinct phases, in which loss of label is first-order. When the label lost at any time is subtracted from that in the tissue initially, a measure is had of residual tissue label. A semi-logarithmic plot of tissue label as a function of time yields a series of straight lines, the first describing loss from the free space, the second from the cytoplasm and the third from the vacuole. The values for the total residual

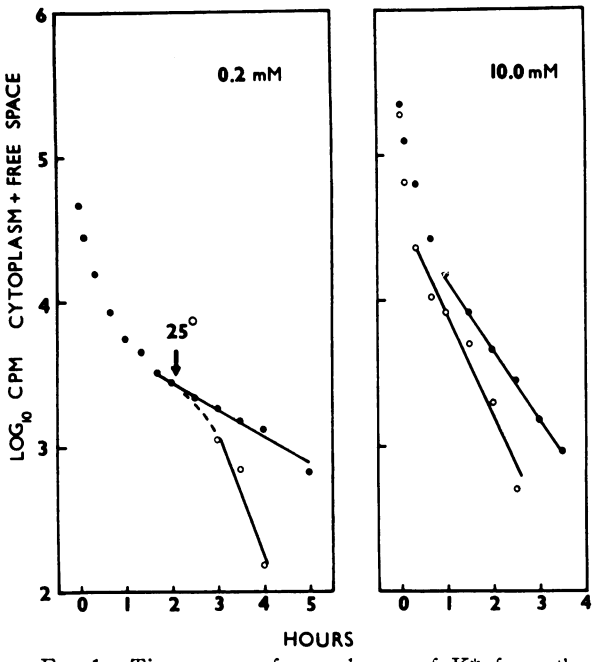

FIG. 1. Time course for exchange of K* from the "cytoplasm" and free space of beet discs at 2° (●) and 25° (○) after incubation in 0.2 mM K* Cl (8 hr at 2°) and 10.0 mM K* Cl (16 hr at 2°). External concentration the same during preincubation and wash-exchange. K_c = slope of linear portion and $t_{1/2} = 0.694/K_c$ [see (17)].

label in the early period of wash-exchange can be corrected for the vacuolar component—which is uniquely represented by the final straight segment of the semi-logarithmic plot after 6 to 8 hours—to yield the residual label in the free space and cytoplasm as a function of wash-exchange time. In figure 1, given as an example, the vacuolar contribution has been subtracted, and the linear segment depicted in the figure designates the cytoplasmic content. The slope of the line yields the time-constant, or $t_{1/2}$, for cytoplasmic exchange (see 17, 19).

Symbols used in the discussion and presentation of results are essentially those used by Pitman (19), and represent quantities measured as described above. They are defined as follows: C_o = external concentration (mM); C^* = concentration of absorbed ions in the tissue (μ-equiv/g fr wt, *i.e.* cpm/s_o/g fr wt); ϕ = flux (μ-equiv/g fr wt/hr), qualified by subscripts which indicate whether fluxes are initial (ϕ_i), final (ϕ_f) or net (n); and which specify the direction of individual fluxes between solution (o), cytoplasm (c), and vacuole (v) *e.g.* ϕ_{oc} = plasma membrane influx. s = specific activity (cpm/μ-equiv), qualified by subscripts o, c, and v referring to the above phases; A_c^* = apparent amount of isotope in the cytoplasm (cpm); Q_c = amount of an ion in the cytoplasm (μ-equiv); K_{co} = Michaelis constant (*i.e.* "affinity" constant^{-1}) for influx.

Results and Discussion

Isotherms and the Time-Course of Uptake. Figure 2 shows typical system 1 and system 2 isotherms for the absorption of K* and Cl* in fresh and aged beet discs. The rates plotted are initial influx values (ϕ_i) at room temperature. Temperature was constant in any one experiment, but varied from 20 to 25° between experiments. In fresh discs the system 1 isotherm (0.01–0.5 mM) was barely discernible, although a system 2 isotherm (1.0–50.0 mM) was present. Aging produced a clearly distinguished system 1 isotherm, and modified that of system 2. Double reciprocal plots of these data show approximate linearity in all cases, and indicate that aging results in a 20-fold decrease in the apparent K_{co} for K* and Cl* absorption in the range of system 1.

It is vital to know the location of labeled ions within cells after washing in unlabeled solution. Thirty minute washing at 25° removes more than 90 % of free space cation radioactivity. Maintenance of adequate calcium in the free space during aging and experimental treatment further minimizes errors due to free space carry-over of labeled cation when C_o is low. As deducible from figure 1, cation exchange from the cytoplasmic compartment has a $t_{1/2}$ of 20 to 30 minutes at 25°, and a $t_{1/2}$ at 2° of roughly 2.5 hours in 0.2 mM, and 1.5 hours in 10.0 mM solution. Similar exchange times have been measured for anions in beet and carrot (3, 19). Roughly half the label initially in the cytoplasm may therefore remain therein after a 30 minute wash-exchange at room temperature. However, further washing in unlabeled solution does not noticeably lower the residual radioactivity in discs after short absorption periods, suggesting that the proportion of the total radioactivity localized in the cytoplasm is relatively low, and considerably less than half of that absorbed. As label enters the cytoplasm, some fraction thereof constantly moves on into the vacuole. Almost half the label entering in the first hours may be in the vacuole (19), and efflux experiments such as those depicted in figure 1 have indicated that when discs have absorbed label from 0.2 mM K*Cl for from 3 to 18 hours, the label in the cytoplasm may not exceed 5 % of the total. The fraction to be found in the vacuole of course increases with absorption time. In any event residual label after 30 minute wash-exchange at room temperature in our experiments is predominantly in the vacuole, and this fact is crucial with respect to the interpretation of our observations.

The trend of influx with time is shown in figure 3 for a double labeling experiment where ^{42}K and ^{36}Cl fluxes were measured in the same discs. At very low external concentrations (0.01 mM, fig 3) influx to the vacuole increases with time. With increasing external concentration a family of curves is obtained akin to the left member of figure 3, in

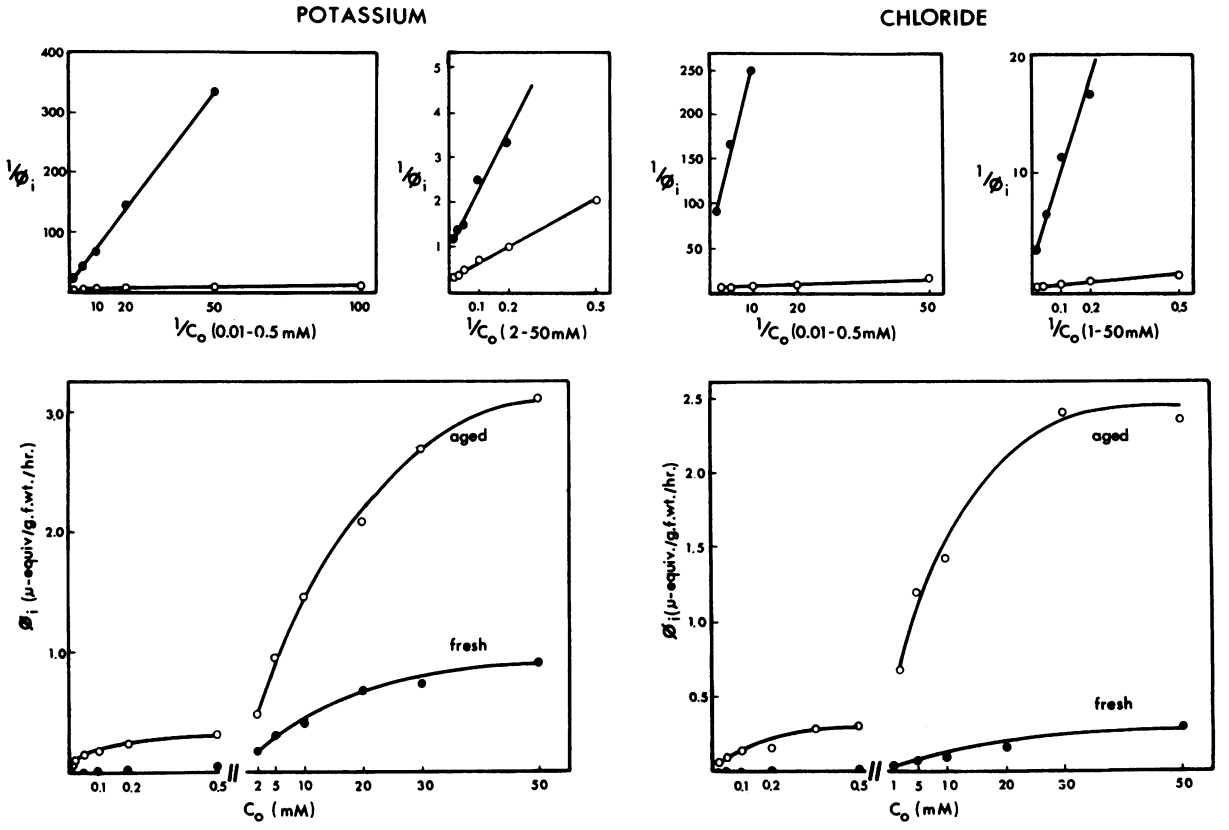

FIG. 2. Isotherms and double reciprocal plots of initial influx for potassium and chloride against external concentration (C_o) in fresh (●) and aged (○) beet discs.

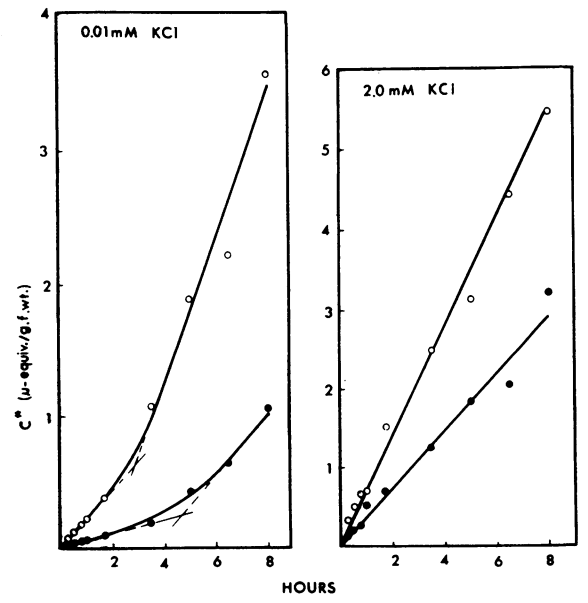

FIG. 3. Time course of isotope uptake (C^*) for ^{42}K (○) and ^{36}Cl (●) in aged beet discs from 0.01 mM and 2.0 mM KCl at 25°. Discs wash-exchanged 30 minutes in unlabeled solution at the same respective concentrations at 25°.

which the time for the achievement of maximal influx decreases inversely with C_o. At relatively high concentrations (2.0 mM, fig 3) influx is constant from the beginning. It is noteworthy that within the range of system 1, the maximal influx achieved ultimately is very similar regardless of the external concentration. Thus in figure 4, while initial influxes (ϕ_i) yield a typical hyperbolic isotherm with C_o, the final influx, ϕ_f, which is roughly constant with time, is much less affected by

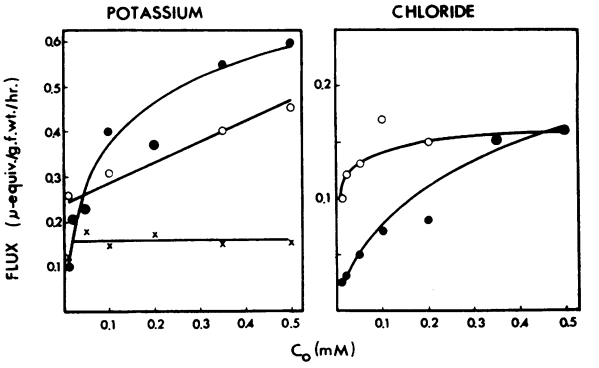

FIG. 4. System 1 isotherms for initial influx (●), final influx (○), and net influx (X) for potassium and chloride in aged beet discs.

C_o. An additional point of particular significance is that the value of C^* at the time of pronounced upward deflection of the time-course curve, is the same at all external concentrations in the range of system 1. The point of upward deflection is taken to be the point of intersection of tangents drawn to the initial and final portions of the time-course curve (fig 3). On the basis of this convention, C^* at the point of inflection is 0.2 to 0.4 μeq per g fresh weight for ^{36}Cl, and 0.8 to 1.2 μeq per g fresh weight for ^{42}K.

The meaning which we impute to these observations is that the maximal influx to the vacuole is achieved when the cytoplasmic concentration is maximal, and that roughly the same maximal cytoplasmic concentration is reached ultimately at all values of C_o in the range of system 1. As has been mentioned, the time, T, required to reach this concentration varies inversely with the external concentration, and is taken as the time of upward deflection of the time-course curve as noted above. On the assumption that the cytoplasmic concentration rises through the time, T, at low external concentrations, it is to be expected that at any time, t, less than T, C^* will vary with t/T independently of C_o. Figure 5, which was constructed from curves such as those on the left of figure 3, through the range 0.01 to 0.2 mM, verifies the prediction. Although filling of the cytoplasm may be expected to be essentially hyperbolic with time, C^* is linear with t/T in figure 5 simply because measurements have been confined to the early, roughly linear log of the filling time-course curve (*cf.* fig 9).

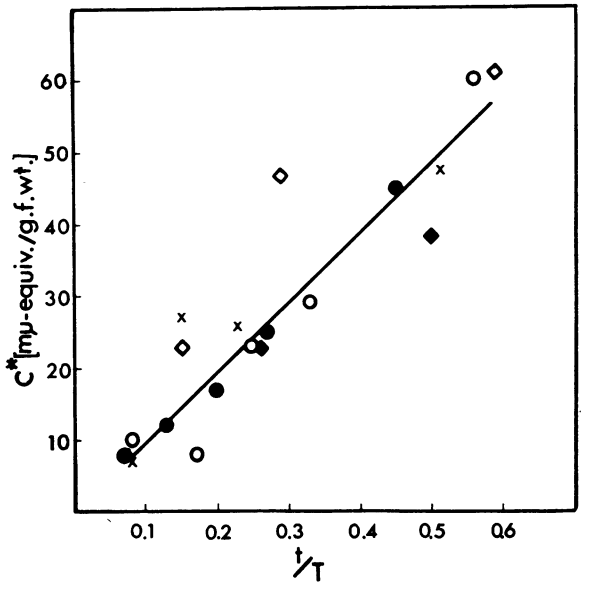

FIG. 5. ^{36}Cl uptake by aged beet discs as a function of the elapsed fraction by (t/T) of time, T, to reach cytoplasmic steady state. Data calculated from curves similar to those shown in left member of figure 3 when C_o = 0.01 mM (●). 0.02 mM (○). 0.05 mM (X), 0.10 mM (◇), and 0.2 mm (◆).

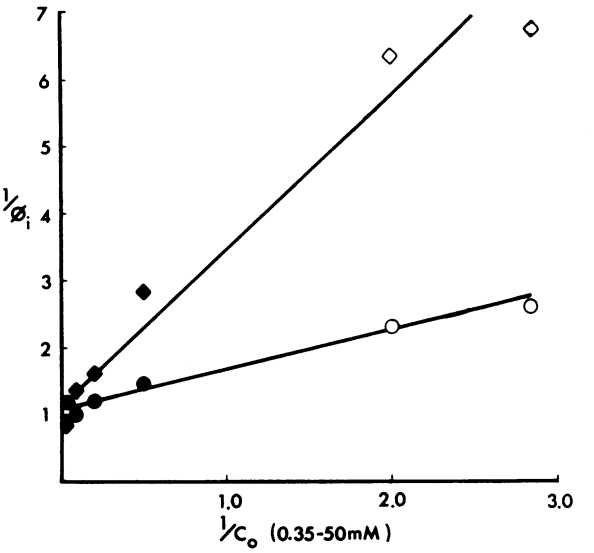

FIG. 6. Double reciprocal plot of system 2 initial influx (closed symbols) and system 1 influx (open symbols for potassium (○) and chloride (□) in aged beet discs.

When the steady state cytoplasmic concentration has been attained, influx (ϕ_i in fig 3) presumably reflects the rate of tonoplast transport. Although the external concentration in the low range continues to exert some influence on influx when the cytoplasm has once been filled, the influence is much less than when the cytoplasm is empty. As noted in figure 4, ϕ_i throughout the range of system 1 approaches the maximal values of ϕ_i obtained at external concentrations of 0.3 to 0.5 mM. As anticipated, the maximal influx values noted in figure 4 are similar to the extrapolated low rates for system 2, shown in figure 6, where double reciprocal plots of the system 2 isotherms for potassium and chloride are plotted together with the double reciprocal values for the maximal ϕ_i rates of system 1. At higher concentrations, *e.g.* at 2.0 mM (fig 3) ϕ_i is constant from the beginning, suggesting that neither movement through the plasma membrane nor cytoplasmic ion concentration is rate-limiting.

Pretreatment Experiments. In the range of system 1 the influx of labeled ions to the vacuole in aged discs is severely reduced by pretreatment in unlabeled solutions. System 1 was examined at 0.2 mM KCl, while 10.0 mM KCl served to investigate system 2. Figure 7 shows the influence of pretreatment in 0.2 mM and in 10.0 mM KCl on the subsequent influx of ^{42}K and ^{36}Cl from 0.2 mM KCl solution. Each point is the mean of duplicate samples and the results are typical of many experiments. At 25°, pretreatment results in a subsequent sharp reduction in ϕ_i which declines to a steady level after 2 hours of pretreatment. The time for half maximal effect of pretreatment on the subsequent ϕ_i for potassium and chloride was 30 to 45 minutes in all experiments. Pretreatment at low temperature (2°)

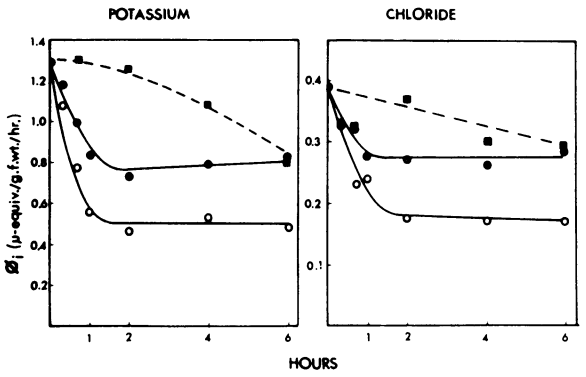

FIG. 7. Initial influx for potassium and chloride as estimated from influx of labeled ion from 0.2 mM ^{42}l ^{36}Cl in aged beet discs as a function of time in unlabeled pretreatment solution. Pretreated in 0.2 mM KCl, 25° (●); 10.0 mM KCl, 25° (○) and 10.0 mM KCl, 2° (■).

in 10.0 mM KCl produced a smaller and much slower reduction in ϕ_i (half-time 5–7 hr). Figure 7 shows that pretreatment in solutions of higher concentration (10.0 mM) at 25°, however, further depresses ϕ_i. The reduction of label influx by pretreatment in unlabeled solutions is reversible. When discs were returned to potassium-free solution after several hours treatment in unlabeled KCl, the subsequent ϕ_i for potassium, based on label uptake to the vacuole, increased with incubation time in K-free solution to about the initial level (fig 8). This reversal, like the pretreatment effect, is temperature sensitive.

In contrast to influx in the range of system 1, influx in the range of system 2 (10.0 mM) is less sensitive to pretreatment in unlabeled solutions, even when high pretreatment concentrations are used (table I). Although the effect of pretreatment on ϕ_i at system 2 concentrations was smaller, temperature sensitivity and reversibility were similar to that found for influx in the range of system 1.

Pretreatment involves more than free space saturation, as shown by the temperature sensitivity and half-time for maximal effect ($t_{1/2}$ about 45 min at 25°, *ca.* 5 hr at 2°), which is several times longer than free space exchange in this tissue. Salt saturation of the tissue with related large changes in overall flux (2) is an unlikely explanation of the consequences of pretreatment, since 1 to 2 hour pretreatment in 0.5 mM KCl, which gives maximum pretreatment effect, results in approximately but 1 % increase in tissue ion content. The time-constants of the pretreatment effect are significantly very similar to those quoted above for isotope exchange from the cytoplasmic compartment during exchange experiments of the type shown in figure 1. Figure 9 shows that the compartment which exchanges as "cytoplasmic" is more rapidly filled with isotope from 0.2 mM K*Cl at 20° than at 2° and that the half-times for this filling are again similar to those found for the pretreatment effect. These observations agree with the temperature and concentration sensitivity of the pretreatment effect shown in figures 8 and 9, and with the data of others (3, 19).

Table I. *Percentage Depression of ϕ_i for Potassium as Measured by Radioactive Potassium Uptake following Pretreatment in Unlabeled Solutions at Room Temperature*

The data are the means from different experiments.

Pretreatment		Conc during uptake	
Conc	Time	0.2 mM	10.0 mM
mM	*Hr*	*% Depression*	
0.5	5	65	6
0.5	10	67	14
0.5	12	82.5	20.5
10.0	12	98	58
50.0	12	99	70

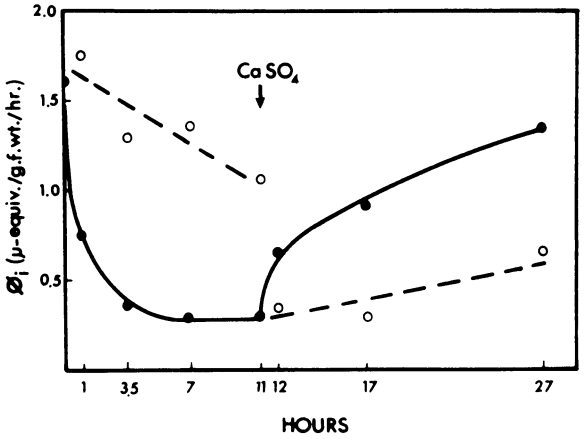

FIG. 8. Initial influx of potassium as estimated from influx of ^{42}K from 0.2 mM ^{42}KCl as a function of time in pretreatment solution (0.5 mM KCl) at 20° (●) and 2° (○). After 11 hours at 20° (arrow) the pretreatment solution was replaced by the original washing solution (0.5 mM $CaSO_4$) at 20° (●) and 2° (○).

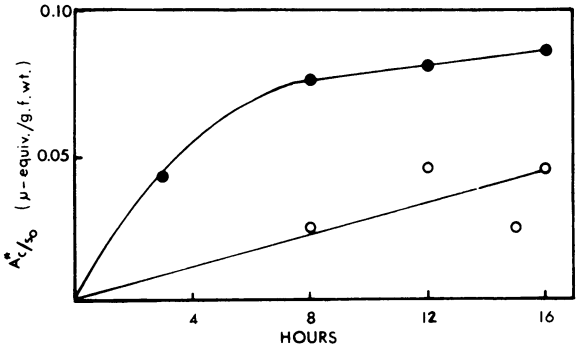

FIG. 9. Estimates of the amount of absorbed potassium (A_c^*/s_o) in the cytoplasmic phase of beet discs after different times in labeled 0.2 mM ^{42}KCl at 20° (●) and 2° (○). Data for (●) from a single experiment, those for (○) from several experiments.

Thus the pretreatment effect can reasonably be identified with the filling of a cytoplasmic phase having a wash-exchange half-time of something over 30 minutes at 20°. If the cytoplasmic phase is prefilled with unlabeled ions, the specific activity of entering labeled ions will be lessened, and uptake of isotope to the vacuole will consequently be diminished. Prefilling should not alter the influx of labeled ions to the cytoplasm. At high external concentrations (10 mM), the cytoplasm reflects the concentration and specific activity of the external solution in a short time, and as a consequence prefilling is of much less influence. Again it should be emphasized that since label moves to the vacuole at the same time that the cytoplasm is being labeled, and since a 30 minute wash-exchange removes a goodly part of the cytoplasmic label, the residual tissue label is predominantly vacuolar. Because isotope influx to the vacuole from solutions in the range of system 1 is very sensitive to the level of unlabeled ions in the cytoplasm, it follows that the primary barrier to ion absorption at these concentrations must be exterior to the cytoplasm. These data again suggest that ion absorption at low concentrations (system 1 isotherm) reflects ion movement across the plasma membrane. In the range of system 2, isotope influx is less sensitive to the influence of pretreatment, suggesting that ion movement across the plasma membrane is not rate-limiting.

Changes in Potassium Fluxes with Aging. As shown in figure 2, there is a 20-fold change in the magnitude of the system 1 isotherm in beet discs during aging. In view of the consistent implications of the foregoing experiments that system 1 mediates plasma membrane transport, we examined K* flux rates directly from 0.2 mM solution to determine whether plasma membrane influx showed a more pronounced response to aging than did the other fluxes. Analyses were carried out in the manner of Pitman (19). While all fluxes increase with aging, the most pronounced change, a 40-fold increase, was associated with the plasma membrane influx (table II). Isolated steles of corn roots, which show the same development of the system 1 isotherm with aging as do beet discs, also show a sharply enhanced plasma membrane influx (13). Thus, direct flux measurements confirm that influx in the range of system 1 reflects ion transport across the plasma membrane.

While the relative changes in flux values with aging are quite reliable in table II, the absolute values may not be in all cases. Final efflux values as calculated from the values of other fluxes [Pitman (19), equation 9] did not match experimentally observed final effluxes. It is likely we over-estimated the final efflux rate-constant (the high Ca/K ratio, and the low K concentration in the external solution may be pertinent factors in this connection), and it is consequently likely that both Q_c and the tonoplast fluxes are over-estimated as well. Withal, a large efflux is a reality, since ϕ_n is considerably less than ϕ_I (ϕefflux = $\phi_I - \phi_n$). It is noteworthy that for reasons given by Pitman (19) fluxes are frequently measured at low temperature, as in table II. While low temperatures in all likelihood enrich the contribution of passive flux components, there is no reason to presume that all fluxes at 2° are entirely passive (*cf.* 17, 18, 19).

Table II. *Individual Potassium Fluxes in Beet Discs Aged in 0.5 mM $CaSO_4$ for Various Times*

External concentration 0.2 mM KCl, 2°.

Time after cutting	Q_c[1] (μ-equiv/g fr wt)	Flux [2] (μ-equiv/g fr wt/hr)			
		ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}
Hr					
	0.14	0.01	0.05	0.04	0.09
6	0.10	0.01	0.04	0.03	0.06
	0.31	0.04	0.03	0.15	0.13
54	0.20	0.06	0.02	0.11	0.07
	2.20	0.35	0.24	0.60	0.49
102	2.08	0.38	0.27	0.62	0.52

[1] Q_c = "cytoplasmic" ion content.
[2] ϕ = fluxes between solution (o), "cytoplasm" (c), and vacuole (v). Direction shown by order of subscripts.

Concluding Remarks

The development with age of a dual isotherm for ion influx in beet is of interest for several reasons. In the comparative sense, the dual isotherm described here has characteristics essentially similar to those found for potassium and chloride in vacuolated tissues of other higher plants (5). In addition, the development of dual isotherms parallels the well known increase in ion absorption capacity with age in storage tissues. The isotherms are thus related to changes in the balance of ion fluxes (25), to the characterization as cytoplasmic of a second non-free space compartment for ions in addition to the vacuole (15, 25), and to the complex changes in metabolism that accompany aging of discs.

Experiments described above strongly indicate that the dual isotherms in beet reflect spatially separated ion absorption processes as deduced from earlier corn root experiments (23, 24). Increased influx with time, measured as described, is difficult to reconcile with a model involving the operation in parallel of 2 or more carriers at a single membrane. Nor is reduced label influx following pretreatment in unlabeled KCl readily interpreted on such a model. Rather, a metabolically controlled change of isotope concentration in an intermediate compartment is implied. The data can best be interpreted on the basis of a serial model for ion absorption processes in plant cells (3, 19), and flux estimates, based on this model, confirm that the increased influx with age from 0.2 mM KCl (system 1 isotherm) is due principally to changes in plasma membrane influx.

Our experiments suggest that in tissues aged for some time in the absence of absorbable salt the cytoplasm is relatively empty. This presumption lies at the very heart of our interpretation of the basis of dual isotherms. On the assumption that in the cells of higher plants the plasma membrane is operationally more permeable than the tonoplast [*cf.* (17, 18)], we suggest that at low external concentrations the system 1 isotherm, constructed from initial influx measurements, describes the filling of the cytoplasm, upon which transport to the vacuole is dependent. Our presumption regarding the relative permeabilities of the plasma membrane and tonoplast fits well with the observation that the cytoplasm is filled to approximately the same level at all external concentrations in the range of system 1. Filling simply requires more time at low concentrations (figs 3, 4, 5). When the cytoplasm has once been filled (*i.e.* when a maximal cytoplasmic concentration has been achieved, which is determined by the various flux rate-constants and compartment concentrations) further ion transport to the vacuole remains a function of the external concentration, albeit a new function. Thus when the cytoplasm is full, the isotherm for system 1 as we know it disappears (fig 4), and the system 2 isotherm subsequently describes absorption.

It is for this reason that in the years before the dual nature of the absorption process was recognized, when absorption studies more often than not were carried out at concentrations above 5 mM, the plotting of the reciprocal of the absorption rate against the reciprocal of the external concentration produced straight lines, in accordance with the original formulation of Epstein [see (5, 6)]. That is to say, no correction was made for the contribution of system 1, and, as it develops, none was necessary!

Filling of the cytoplasm can be accomplished relatively slowly, at low concentrations (0–0.2 mM), or rapidly at high concentration (*e.g.* 10 mM). In the latter instance system 2 is demonstrably operative virtually at once. In the former case system 2 is also operative, since transport to the vacuole invariably involves system 2, but the rate is controlled by system 1.

In all tissues so far examined the noticeable change from system 1 to system 2 absorption of monovalent ions occurs at about 1.0 mM (5). At lower concentrations the rate of transport by system 1 exceeds the diffusion rate across the plasma membrane. While the ascendancy of diffusion across the plasma membrane with increasing concentration may simply reflect the intrinsic permeability of the plasma membrane, it is possible that the permeability may increase with the external concentration of monovalent ions, as does the permeability of the plasma membrane of the marine red alga, *Griffithsia* (G. P. Findlay, personal communication). In *Nitella translucens* maintained in solutions containing 0.1 mM $CaCl_2$, plasma membrane resistance drops markedly when KCl concentration is raised above 1 mM, and K^+ influx, previously maximal with respect to concentration, increases, yielding a system 2 isotherm (R. M. Spanswick, personal communication). The concept of diffusive entry of ions into the cytoplasm at high external concentration is supported by the finding that part of the cytoplasm may behave as free space under these conditions (20). Flux measurements for potassium show that plasma membrane influx is much larger than tonoplast influx when C_o = 5 to 40 mM (3, 19). The relative values at 0.2 mM are set out in table II, but attention has been called to the uncertainty of these values on an absolute basis. Membrane potential measurements in higher plant tissues suggest passive movement of potassium and chloride at the plasma membrane at high concentrations (7, 16). More recent experiments (21) which provide evidence for an active potassium influx pump at the plasma membrane of barley roots at system 1 concentrations (0.5 mM) do not resolve the active or passive nature of this process at higher concentrations (5.0 mM).

The properties of the transport processes reflected in the system 1 and system 2 isotherms have not been investigated in the present experiments. Further inhibitor studies and potential measurements may reveal more of the mechanisms involved. It is interesting, however, that net potassium flux and final chloride influx are quantitatively similar in the system 1 range (fig 4). These fluxes, which presumably represent tonoplast transport, may be related to a salt transfer mechanism of the type suggested in *Nitella* (18) and in other beet experiments (22).

Acknowledgment

This work was generously supported by an Atomic Energy Commission contract. One of us (C.B.O.) is further indebted to the George Murray Travel Fund, University of Adelaide, for a travel grant. We extend our thanks to Dr. H. Nishita, Laboratory of Nuclear Medicine and Radiation Biology, U.C.L.A., for the use of his facilities for potassium analysis, and Drs. E. A. C. MacRobbie and W. J. Cram, Botany Department, University of Cambridge, for much helpful discussion.

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