

IRREVERSIBLE EQUILIBRATION OF BARLEY ROOTS WITH Na^+ IONS AT DIFFERENT EXTERNAL Na^+ CONCENTRATIONS

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SUMMARY

1. Roots of decapitated barley plants were allowed to equilibrate with Na^+ solutions of different concentration. The kinetic characteristics of this process were examined.
2. Attainment of the saturation level appeared to involve not only strong reduction of the net flux but also of the influx as measured with $^{22}\text{Na}^+$.
3. The final Na^+ content of the tissue depended on the external Na^+ concentration. Reckoned from a certain level of internal Na^+ the relationship was the same as for the initial rate of Na^+ uptake.
4. Transfer of roots saturated at a low Na^+ concentration to a more concentrated Na^+ solution brought about a resumption of Na^+ absorption until a level characteristic of the new concentration had been attained. On the other hand transfer of the material from a higher to a lower concentration after saturation did not involve appreciable loss of Na^+ from the tissue.
5. A kinetic model is proposed to explain these phenomena.

1. INTRODUCTION

Recently, VALLÉE (1969a, b) reported an intriguing phenomenon in CrO_4^{2-} (a "physiological isotope" of SO_4^{2-}) absorption by *Chlorella* cells. She observed that after incubation of the cells for several hours in CrO_4^{2-} solutions of different strength an equilibrium level was attained that varied with external concentration in about the same way as the initial rate of CrO_4^{2-} absorption, although at the same time the absorption was essentially irreversible.

The purpose of the work reported here was to determine whether a similar phenomenon occurs in the establishment of equilibrium levels of the monovalent cations, more especially of Na^+ , in barley roots. From similar studies with the monovalent cations and with the Cl^- ion JACKSON & STIEF (1965) and JACKSON & EDWARDS (1966) concluded that the equilibrium level in barley roots is independent of external concentration and in fact is established by the influx and efflux rates approaching equality. However, for K^+ ions the correctness of this conclusion has been disputed by JOHANSEN *et al.* (1970).

Evidence is presented to show that the phenomenon reported by Vallée is of a more general occurrence and a hypothesis is put forward to explain this paradoxical condition of "irreversible equilibrium".

2. MATERIAL AND METHODS

2.1. Plant material

For the experiments we used decapitated plants of barley (*Hordeum vulgare* L. cv. 'Herta') grown as follows. After the grains had been allowed to germinate in the dark for three days as described by BANGE & VAN IREN (1970), the young seedlings were transferred to PVC grids (25 seedlings per grid) and raised further in the light of fluorescent lamps (Philips TLF 40W/33) on two PVC tanks each filled with 18 l of a nutrient solution containing 0.05 mM each of $\text{Ca}(\text{NO}_3)_2$, MgSO_4 , KNO_3 and KH_2PO_4 . The nutrient solution was circulated continuously through both tanks with the aid of small pumps. The light regime was 16/24. On the fourth day after transfer to the grids the seedlings were decapitated just above the grain and rinsed for one minute in running demineralized water before being used in the experiments. The root fresh weight per 25 seedlings was about 2 g.

This material was preferred to excised roots raised on a dilute CaSO_4 solution for experiments of long duration (24 hours or longer) because it was expected to be less subject to possible exhaustion of metabolites or damage by unbalanced experimental solutions. Decapitation was necessary to eliminate the complications arising from upward transport of ions. There was no bleeding from the cut stumps in the experimental solutions used.

2.2. Experimental procedure

The sets of plants were placed either separately on top of polyethylene bottles containing 10 l of the aerated experimental solutions or distributed over two 18-litres PVC tanks through which an additional 50 or 120 l of experimental solution was circulated with the aid of small pumps. Absorption periods were concluded with a one-minute rinse in demineralized water. All experiments were performed at 25°C.

Further details concerning the separate experiments are given in the legends to the figures.

2.3. Isotope techniques and analytical procedures

Influx rates of Na^+ were determined in solutions labeled with $^{22}\text{Na}^+$. The isotope content of the roots was established by packing them in small test tubes and counting under conditions of constant geometry in a well-type scintillation counter.

Preparation of the roots for flamespectrophotometric analysis was as described by HOOYMANS (1964). Dilutions were chosen such that the final Na^+ concentrations were below 0.6 mM.

3. RESULTS

In roots pretreated in a 0.1 mM Na_2SO_4 solution during 41 hours and in which net Na^+ absorption had fallen to a low level (*fig. 1*, upper curve), the rate of

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Table 1. Effect of pretreatment of roots with Na^+ on the rate of Na^+ influx. Absorption of $^{22}\text{Na}^+$ from a labeled solution of 0.1 mM Na_2SO_4 in the presence of 0.1 mM $\text{Ca}(\text{HCO}_3)_2$ (pH about 7) over a period of 6 hours was compared for roots pretreated during 41 hours in a similar but unlabeled solution and non-pretreated roots (controls). The experiment was run in triplicate.

	counts/min	mmoles. $\text{kg}^{-1}.\text{h}^{-1}$
Pretreated roots	1 971	0.21
Controls	22 776	2.56

Na^+ influx, too, as measured with $^{22}\text{Na}^+$ appeared to be strongly reduced to about 8% of the control value (table 1). So, as in CrO_4^{2-} uptake by *Chlorella*, equilibration of the tissue with the external solution proceeds by a reduction of the influx rate and not by an increasing rate of efflux counterbalancing a constant rate of influx. A reduction of Na^+ influx in roots pretreated with Na^+ was also reported by PITMAN *et al.* (1968).

In the next experiment the relation between the amount of Na^+ absorbed and time was studied for two external concentrations, viz. 0.180 and 0.041 mM Na^+ , over a period of 52 hours (fig. 1). In complete accordance with the results of Vallée the equilibrium level attained after about 24 hours proved to be a function of the external concentration. By the same token, transfer to the higher concentration of plants equilibrated at the lower concentration for 28 hours induces a resumption of the uptake up to the Na^+ level characteristic of the higher concentration. Plants equilibrated at the higher concentration on the contrary lose hardly any Na^+ upon transfer to the lower concentration.

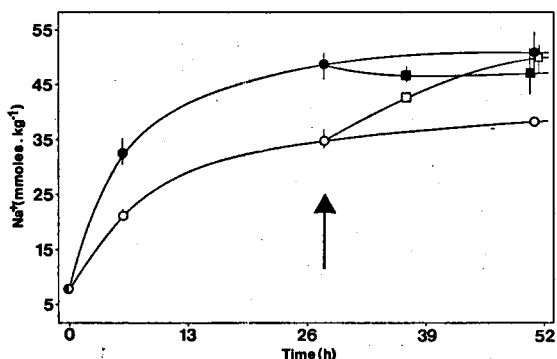


Fig. 1. Relation between amount of Na^+ absorbed and time, for external concentrations of 0.09 mM (full circles) and 0.02 mM (open circles) Na_2SO_4 in the presence of 0.1 mM $\text{Ca}(\text{HCO}_3)_2$ at a pH of about 7. The original root-to-solution ratio was 1 g/4.6 l for the lower and 1 g/2.3 l for the higher concentration. The arrow indicates the moment at which one group of the plants was transferred from the higher to the lower concentration (full squares) or vice versa (open squares). The experiment was performed in triplicate.

The irreversibility of the equilibration confirms the conclusion drawn from the isotope experiment that saturation does not involve the appearance of a strong efflux of Na^+ from the tissue. Furthermore, the immediate resumption of the absorption upon transition from the lower to the higher concentration proves that despite the reduced influx the uptake mechanism as such is completely unimpaired.

Another remarkable observation by Vallée is that in her plant material the equilibrium level of CrO_4^{2-} depended upon the external CrO_4^{2-} concentration in the same way as the initial absorption rate. To check this point for barley plants, the equilibrium levels of Na^+ attained after 30 hours in different Na^+ concentrations were compared to the uptake rates from similar concentrations during the first few hours. This procedure seems justified because in this material, unlike the situation in low-salt excised roots and despite the relatively low Ca^{2+} concentration, a complicating biphasic nature of the initial part of the time curve is indistinct, if not absent. Inspection of the curves of *fig. 2, A* and *B*, reveals some common features which were subjected to a more exact comparison by plotting the equilibrium levels attained at different concentrations against the corresponding initial absorption rates. If the two curves have a comparable shape, a straight line through the origin should result. For CrO_4^{2-} uptake by *Chlorella* (VALLÉE 1969a: data from paragraph III B 4, *pp.* 152/153) this proved to be true, but for barley the straight line obtained does not pass through the origin (*fig. 2 C*). This means that the two curves are only of similar shape provided the curve of *fig. 2 A* is considered to have as abscissa the horizontal $y = 20 \text{ mmoles Na}^+/\text{kg} =$ the ordinate intercept of the line of *fig. 2 C* (see dashed line in *fig. 2 A*).

4. DISCUSSION

The results obtained in this study seem to leave no doubt that the phenomena first described by Vallée for *Chlorella* are not restricted to this unicellular alga. The fact that a similar irreversible equilibration appears to exist in barley roots as well offers a challenge to reconcile the phenomenon with the model conceived for cellular transport of monovalent cations on the basis of former kinetic work (HOOYMANS 1964; BANGE & HOOYMANS 1967). As will be shown below, the phenomenon proves to fit in with the model.

In brief, the essence of the concept is that, in so far as ion transport is concerned, the cell compartments are not strictly in series as advocated by many authors (see *e.g.* PITMAN 1963) but in a more parallel arrangement by the existence of a direct pathway between the cell surface and the vacuolar membrane. Along this pathway ions may move directly from the uptake sites at the plasmalemma through the cytoplasm to the tonoplast. The exact structural nature of this pathway is irrelevant for these considerations. More recent evidence in favour of such a concept has been presented by MACROBBIE (1969) in work with the algae *Nitella* and *Tolypella*. PALLAGHY *et al.* (1970) have likewise met with difficulties in interpreting some of their kinetic data in terms of a strictly serial

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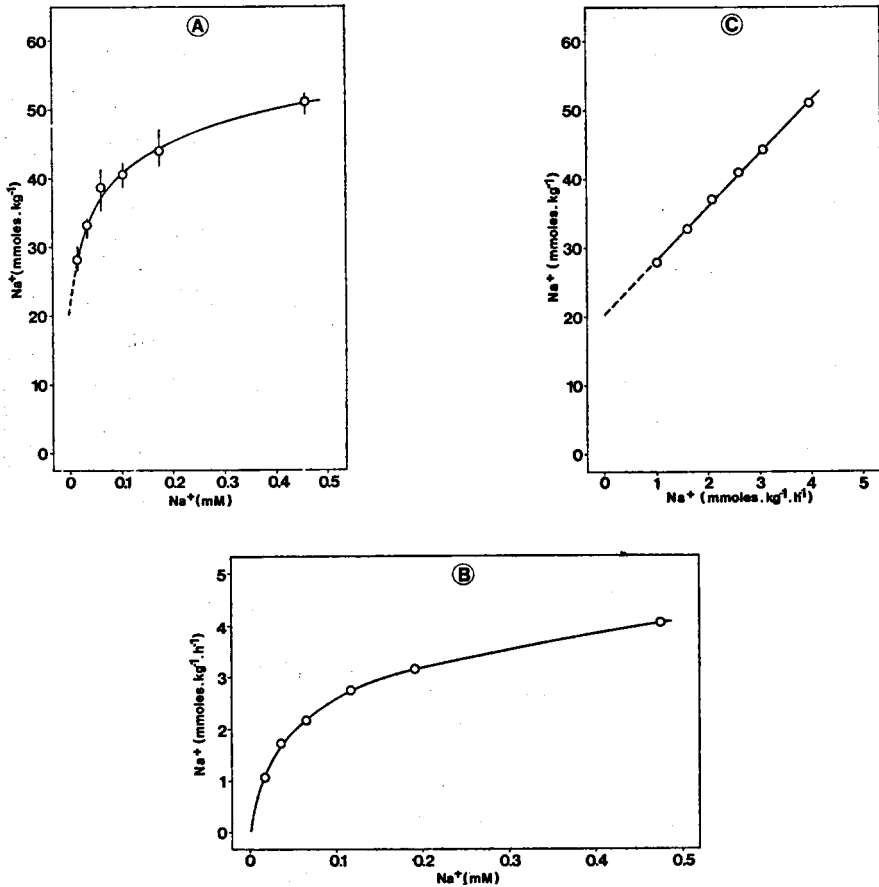


Fig. 2. (A) Equilibrium levels of Na^+ reached after 30 hours in Na_2SO_4 solutions of different concentration in the presence of 0.1 mM $\text{Ca}(\text{HCO}_3)_2$ at a pH of about 7. Root-to-solution ratio 2 g/10 l. Concentrations plotted are final values measured after 30 hours' absorption. The experiment was run in triplicate. For dashed line, see text. (B) Initial rates of Na^+ uptake from Na_2SO_4 solutions of different concentration in the presence of 0.1 mM $\text{Ca}(\text{HCO}_3)_2$ at a pH of about 7. At each concentration plants were allowed to absorb for different lengths of time, up to 260 minutes. The slope of the time line was determined from a total of seven experimental points for each concentration. Root-to-solution ratio 2 g/10 l for the three lower and 2 g/5 l for the three higher concentrations. (C) Equilibrium levels of Na^+ at different external concentrations as derived from the drawn line in fig. A, plotted against the corresponding initial absorption rates as derived from the drawn line in fig. B.

arrangement of the cellular compartments of root cells.

An important feature of the concept is that this transfer "track" is not considered as closed; in other words, during passage through the protoplasm there may be either net release of ions absorbed at the plasmalemma from the track to this phase or, conversely, net uptake of ions present in the protoplasm onto the track followed by transfer to the vacuole. The actual direction in which the ions will move in a special case depends on the apparent relative affinities of the track and the protoplasm for the ionic species involved and, for a given ionic species, on the degree to which both systems have become saturated with the ions.

Experiments on Na^+ uptake in the absence and presence of Ca^{2+} (HOOYMANS 1964) have demonstrated that during transport of ions from the protoplasm to the vacuole by the track mechanism net absorption of ions by the same mechanism at the plasmalemma is temporarily reduced or even completely inhibited. The effect has proved not to be due to Na^+ absorption being compensated for some time by an independent release of Na^+ ions to the medium but to involve also a temporary reduction of Na^+ influx as measured with $^{22}\text{Na}^+$ (BANGE, unpublished results). An obvious condition for the occurrence of this phenomenon is that the rate-limiting step in vacuolar absorption be in the ion-release reactions at the tonoplast.

An internal supply of Na^+ ions to the track may arise not only from protoplasmatic ions derived from the medium as in the experiments of HOOYMANS (1964) but also from ions flowing back from the vacuole into the protoplasm. Evidence for the existence of a vacuolar efflux has been presented for all kinds of cells (MACROBBIE & DAINTY 1958a, b; DIAMOND & SOLOMON 1959; PITMAN 1963; ETHERTON 1967). It seems not too far-fetched to suppose that this vacuolar efflux increases with increasing vacuolar Na^+ content.

Within this concept of the arrangement of compartmental fluxes the paradoxical phenomenon of irreversible equilibration can be explained. Prolonged exposure of the roots to a low Na^+ concentration, which does not saturate the absorption mechanism, will result in an increase of the vacuolar Na^+ concentration and concomitant Na^+ efflux. In the protoplasm the ions leaked out of the vacuole will be picked up by the transfer track and returned to the vacuole at the cost of the absorption of external Na^+ ions. When, at a certain vacuolar concentration, the efflux has reached a value at which the degree of internal loading of the transfer track equals the extent to which the track was loaded at the outer cellular surface by the prevailing external concentration, Na^+ influx into the cell from the outer solution will stop. At higher external concentrations the vacuolar concentration will be able to rise to a higher level before vacuolar efflux counterbalances the higher Na^+ absorption from the external solution. So transfer of roots equilibrated at a low Na^+ concentration to a higher concentration will result in a resumption of the absorption. On the other hand, roots equilibrated at a high Na^+ concentration will be able to retain their Na^+ against lower external concentrations.

It has been shown that plotting of the equilibrium levels attained at different external concentrations against the corresponding initial absorption rates –

which within this concept also represent the vacuolar efflux rates at equilibrium – results in a straight line. This rectilinear relationship strongly suggests that the vacuolar efflux has the character of a free diffusion whose rate is determined by the activity gradient between the vacuole and the protoplasm, in accordance with the reported smallness of the electrical potential difference between these compartments (*cf.* ETHERTON & HIGINBOTHAM 1960; SPANSWICK & WILLIAMS 1964). In the case of *Chlorella* CrO_4^{2-} activity in the protoplasm would be negligible whereas in barley roots the protoplasmatic Na^+ activity at equilibrium would amount to the equivalent of about 20 mM.

The fact that equilibration of *Chlorella* cells in solutions of CrO_4^{2-} and of barley roots in media containing Na^+ have striking features in common suggests a considerable degree of conformity in the subcellular management of ion transport in both organisms despite their large evolutionary distance. Whether or not the model presented in this paper provides the correct explanation of these phenomena, will be shown by future work.

ACKNOWLEDGEMENT

The authors are indebted to Miss P. van der Duyn Schouten for technical assistance.

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