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THE ALKALI CATION CARRIER OF BARLEY ROOTS: A MACROMOLECULAR STRUCTURE?

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ABSTRACT

Excised barley roots were allowed to absorb Rb and Cs from single or mixed Rb- and Cs-chloride solutions of various concentrations in the presence of 10 me/l $CaSO_4$. Absorption periods of 3 and 6 hours respectively enabled the separate estimation of the steady rate of uptake setting in after about 3 hours and the additional amount of cation absorbed within the first 3 hours, which is supposed to represent cation bound to protoplasmatic binding sites. The following results were obtained.

1. The absorption and competition behaviour of Rb and Cs at different concentrations cannot be understood on the basis of the view that the binding sites involved in the absorption of these ions are separate and completely independent units.

2. In some important features there is a close though not absolute similarity between the effect of concentration on the steady rate of absorption of Rb and Cs after 3 hours, on the one hand, and on the additional amount of Rb and Cs absorbed within the first 3 hours, on the other.

The bearing of these results on our notions concerning the nature of the carrier mechanism is discussed, and a structural conception of the association between ion and carrier advocated.

1. INTRODUCTION

The kinetic approach applied in ion uptake studies has appeared useful in elucidating mechanisms underlying competition behaviour (e.g. BANGE, 1959; BANGE, TROMP and HENKES, 1965) or distribution patterns (e.g. BANGE and VAN VLIET, 1961), but it may seem questionable whether this type of work can contribute to the identification of "carrier" substances supposedly involved in the transport process. It seems to us that such doubt is unjustified. In this paper we will present kinetic data that, in our view, may contain an important clue to the nature of the cell constituents involved in alkali cation transport in barley roots.

The leading thought behind this work was that if ion carriers are relatively simple chemical compounds entering into a reversible binding reaction with the ions to be transported, then the competition between such ions as Rb and Cs must proceed strictly according to kinetic formulae analogous to those developed for enzyme reactions when two substrates compete for the same active site on an enzyme molecule (cf. EPSTEIN and HAGEN, 1952). It will be shown below that the kinetic behaviour of Rb and Cs uptake is much more complex than can be explained on the basis of any such relatively simple reaction scheme.

Another observation could be brought to bear upon the tentative conclusions drawn from this fact. With respect to some striking features, an unmistakable similarity was found in the dependence on external concentration of steady-state Rb and/or Cs absorption on the one hand and the amount of these ions present in a kinetically defined cell compartment as described by HOOYMANS (1964) and tentatively identified by her with the cytoplasm, on the other.

It will be brought out that in our view the evidence obtained suggests that in the binding and transport of the alkali cations a macromolecular structure rather than some relatively simple chemical compound is involved.

2. MATERIAL AND METHODS

2.1. *Plant material*

Excised roots of barley (*Hordeum vulgare* L. cultivar. "Herta") were used throughout these experiments.

Fifty grams of seed were disinfected for two minutes with a 1 % $HgCl_2$ solution and rinsed in flowing tapwater for three hours. They were then soaked for about 20 hours in aerated tapwater at 25° C, washed with demineralized water, and spread evenly on a stainless-steel screen covered by a piece of aseptic gauze and supported by a perspex frame. The frame was placed in a plastic tray containing about 4 litres of 2×10^{-4} M $CaSO_4$ solution, which was aerated continuously; the gauze dipped into the solution at all sides of the frame. The tray was placed in an airconditioned dark room (temperature 25° C, relative humidity about 70 %) for 7 days. During this period the $CaSO_4$ solution was renewed three times at regular intervals.

2.2. *Experimental procedure and analytical methods*

Before the start of an experiment the roots were cut at a point between the gauze and the steel screen and rinsed in three changes of 4 litres of aerated demineralized water for 10 minutes each. After washing, the roots were wrapped in gauze and centrifuged for three minutes at low speed to remove the adhering water.

Equal portions of root material, varying from 0.5 to 3.0 grams according to experimental conditions, were weighed out and transferred to polyethylene bottles containing 10 litres of experimental solution. Air was bubbled through the solutions and their temperature maintained at 25° C by a waterbath. At the end of the experimental period the roots were collected by pouring the solutions over a filter of nylon mesh, washed for 1 minute with flowing demineralized water, and transferred to crucibles.

After drying at 85° C, the roots were ashed at 560° C and the ash dissolved in diluted HCl. After digestion for a quarter of an hour on a hot plate, the solutions were filtered and the filtrate made up to 50 or 100 ml.

The concentrations of Rb, K, and Cs were estimated with a Beckman

Model DU flamephotometer at wave lengths of 780, 766, and 852 mμ respectively.

2.3. *Experimental solutions*

The alkali cations were supplied throughout as their chlorides. Apparently, the RbCl (CsCl) used was not completely free of contaminating Cs (Rb) since in some experiments in which only RbCl (CsCl) was present, a small increase in the Cs (Rb) content of the roots was demonstrated. To all experimental solutions, $CaSO_4$ (B. D. H. "AnalaR") was added in a concentration of 5×10^{-3} M. No special precautions were taken to control the pH of the solutions because it was not deemed desirable to add buffering substances. Under such circumstances the actual pH value may vary between 5.2 and 5.6.

3. Results

3.1. *Uptake of Rb and Cs in relation to time*

It has been demonstrated by Hooymans (1964) that the establishment of a steady state in alkali cation uptake in excised root material is complicated by two circumstances. In the first place, in the absence of Ca at pH values of about 5.5 the rate of K uptake shows a gradual decline with time which can be abolished by the addition of an appropriate amount of Ca to the experimental solutions. In the second place, except at very low concentrations after about 2½ hours the initial rate of K and especially Rb uptake in the presence of Ca changes rather abruptly into a new and lower value that is maintained for the rest of the experimental period.

To eliminate the first complication, we performed all experiments in the presence of 5×10^{-3} M Ca. The effectiveness of this procedure is demonstrated by Fig. 1 A, which shows that for Rb as well as for Cs after 2½ hours a constant rate of uptake sets in. This holds for separate Rb and Cs solutions (Fig. 1 A) as well as for mixtures (Fig. 1 B).

As to the second complication, which appears to be very pronounced in the case of Rb as well as of Cs, it was ascertained that in compliance with the conclusions of Hooymans (l.c.) the decline in the rate of uptake after about 2½ hours is not due to the onset of an efflux: when after 4 hours Rb is replaced by an equal concentration of K (Fig. 1 C) the Rb content of the roots remains at the level attained. The rate of uptake (in me/hr) setting in after about 2½ hours, and which for convenience we will refer to as steady-state uptake, was determined by subtracting total uptake over 3 hours from total uptake over 6 hours and dividing the number of milliequivalents obtained by three. The same data allow the estimation of the amount of additional cation uptake during the initial period of 2 or 2½ hours, by the subtraction of steady-state uptake from total uptake in the first period of 3 hours. For instance, when in 3 hours 16 and in 6 hours 25 me Rb/kg fresh weight of roots have been absorbed, the rate of steady-state uptake is $(25 - 16)/3 = 3$ me Rb/kg-hr and the

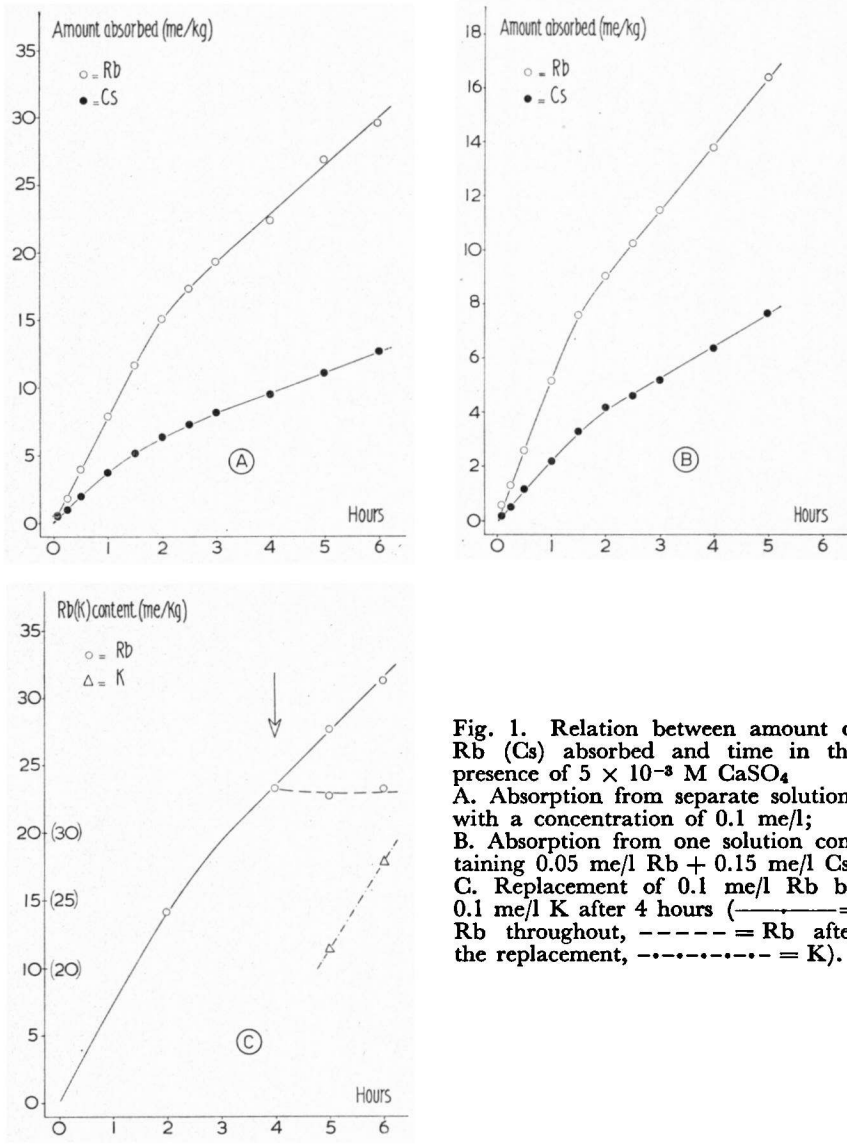


Fig. 1. Relation between amount o Rb (Cs) absorbed and time in the presence of 5×10^{-3} M $CaSO_4$
A. Absorption from separate solutions with a concentration of 0.1 me/l;
B. Absorption from one solution containing 0.05 me/l Rb + 0.15 me/l Cs;
C. Replacement of 0.1 me/l Rb by 0.1 me/l K after 4 hours (——•——= Rb throughout, – – – – – = Rb after the replacement, –•–•–•–•–•– = K).

excess Rb absorbed during the initial period ("fraction I" in the terminology of Hooymans) 16 — (25 — 16) = 7 me Rb/kg.

It should be noted that the excess amount of Rb and Cs absorbed during the first $2^1/_2$ hours has nothing to do with Rb or Cs present in the so-called A(pparent) F(ree) S(pace) of the tissue. Actually in the time curves shown neither a W(ater) F(ree) S(pace) nor a D(onnan) F(ree) S(pace) manifests itself by an intercept on the ordinate what may be ascribed to the relatively long final rinsing of the roots with demineralized water and the presence of excess divalent cation in the experimental solutions.

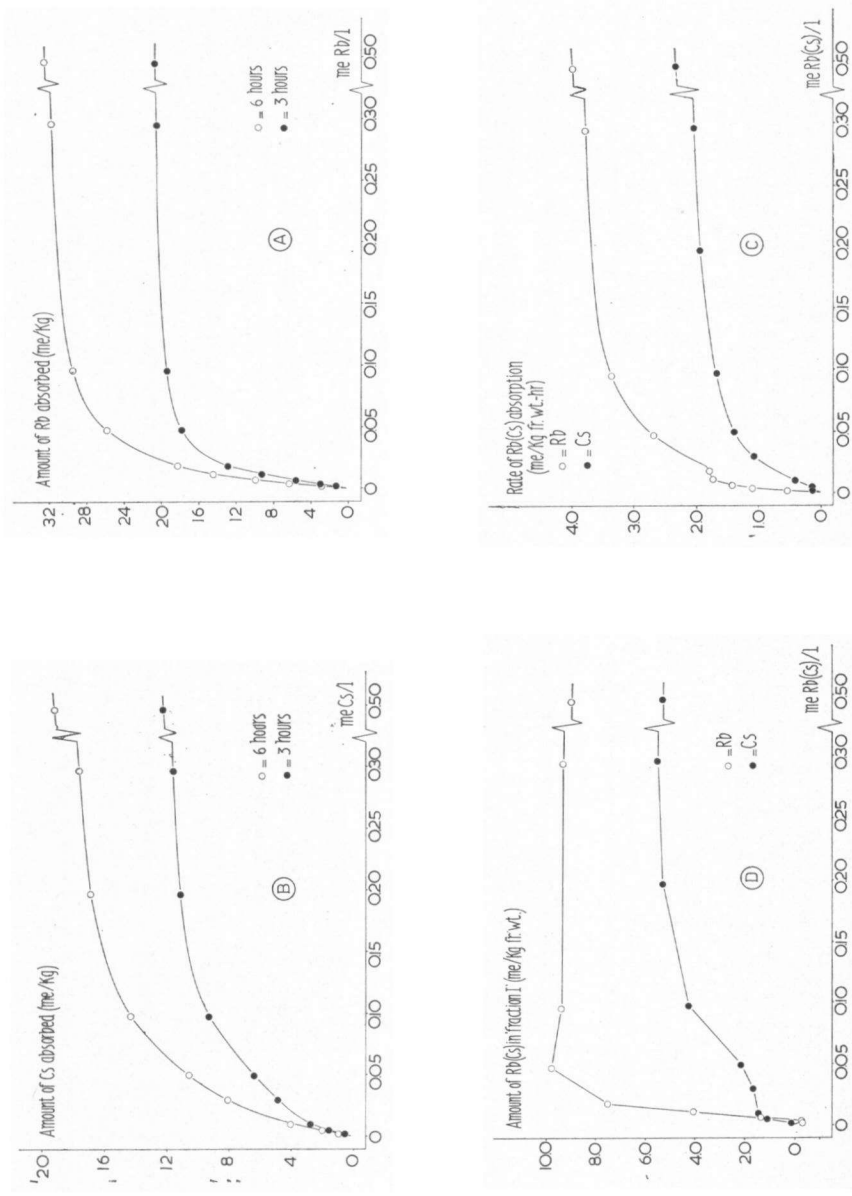


Fig. 2. The effect of concentration on the rate of steady-state Rb and Cs absorption and on the amount of Rb and Cs present in fraction I
A. Amount of Rb absorbed within 3 and 6 hours in the range of concentrations from 0 to 0.5 me/l. Data represent the average of 3 separate experiments;
B. The same for Cs;
C. Calculated rates of steady-state Rb and Cs absorption;
D. Calculated amounts of Rb and Cs present in fraction I.

3.2. *Uptake of Rb and Cs in relation to concentration*

The amount of Rb and Cs absorbed in 3 and 6 hours in the range of concentrations from 0 to 0.5 me/l is shown in Figs. 2 A and 2 B respectively. Calculated rates of steady-state absorption are given in Fig. 2 C, and calculated Rb and Cs contents of fraction I in Fig. 2 D.

The important features of these graphs are the following.

1. The relation between steady-state Rb uptake and Rb concentration (Fig. 2 C) cannot be described by some simple mathematical equation. The curve breaks up into a steep initial part and a portion rising more slowly to the level of saturation. Between these parts a point of inflection seems to occur in the curve, a feature that is manifest in all of the three experiments from which the data presented in Fig. 2 C have been averaged.
2. The same holds for the inflection point indicated at the lowest Cs concentrations (Fig. 2 C). Quite apart from this complication, the remarkable feature of the absorption curve for Cs is that the absorption level at saturation is nearly twice as low as for Rb.
3. With increasing concentration the amount of Rb as well as Cs in fraction I attains a saturation level (Fig. 2 D) that again for Cs is almost twice as low as for Rb. For Rb this saturation is reached at a much lower concentration than for Cs. No Rb is accumulated in fraction I at the lowest concentrations, which corroborates the observation of HOOYMANS (l.c.) that at low Rb concentrations Ca has the effect of straightening out the uptake versus time curves. A considerable part of the final amount of Cs present in fraction I seems to be accumulated at very low Cs concentrations.

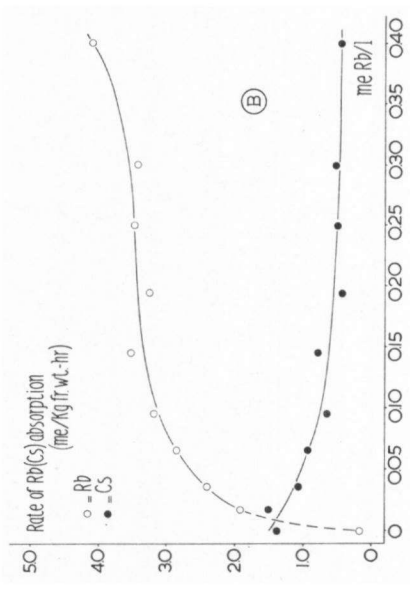
3.3. *Competition between Rb and Cs: varying Rb concentration, constant Cs concentration*

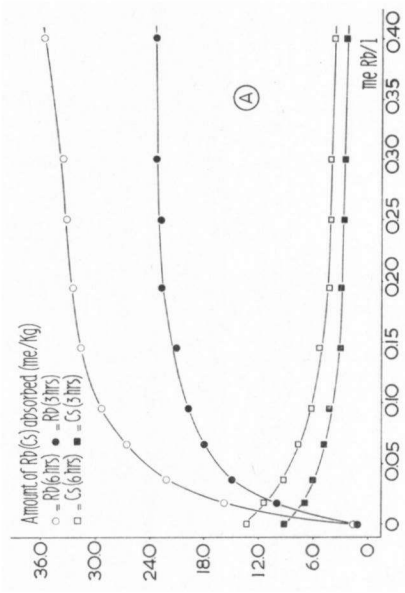
In a first series of competition experiments (Fig. 3, A, B, and C) the concentration of the inhibiting ion (in this case Cs) was maintained at 0.1 me/l, whereas the concentration of the inhibited ion (Rb) was varied from 0 to 0.4 me/l. Total uptake within 3 and 6 hours is shown in Fig. 3 A, the rate of steady-state absorption in Fig. 3 B, and the amount accumulated in fraction I in Fig. 3 C.

A direct comparison between Figs. 3 B and 2 C is hampered by the fact that for different batches of roots absolute rates of uptake may vary. Therefore, all rates of Rb as well as Cs absorption have been expressed as percentages of the rate of Rb uptake at a Rb concentration of 0.1 me/l in the presence of 0.1 me/l Cs (Fig. 5 A). Because of the larger variability in the results concerning the amount of Rb and Cs in fraction I, no attempt has been made to apply the same procedure to these data.

The salient features of the competition behaviour in the steady-state phase of absorption (Figs. 3 B and 5 A) are the following.

1. There is a marked but gradually declining inhibition of Cs absorption per unit increment of Rb concentration.
2. Rb uptake is not hampered by the presence of Cs at low





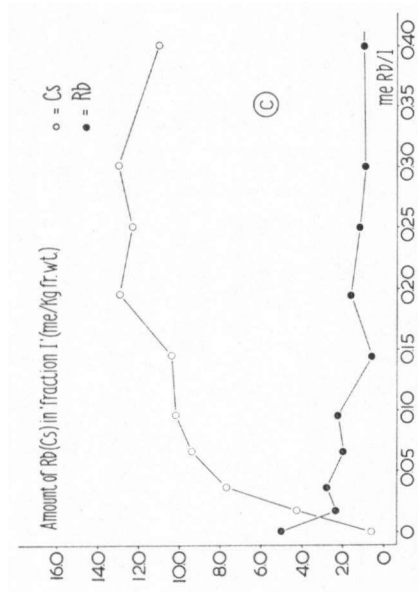


Fig. 3. Competition between Rb and Cs absorption from a solutian with a Cs concentration of 0.1 me/l and a Rb concentration varying from 0 to 0.4 me/l.
A. Amounts of Rb and Cs absorbed within 3 and 6 hours. Data represent the average of 3 separate experiments;
B. Calculated rates of steady-state Rb and Cs absorption;
C. Calculated amounts of Rb and Cs present in fraction I.

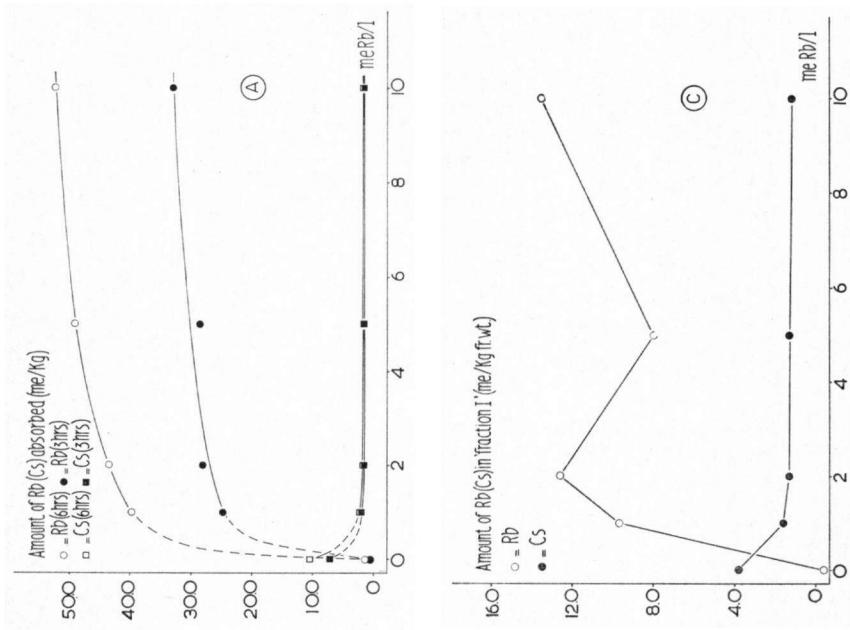


Fig. 4. Competition between Rb and Cs absorption from a solution with a Cs concentration of 0.1 me/l and a Rb concentration varying from 0 to 10 me/l
A. Amounts of Rb and Cs absorbed within 3 and 6 hours. Data are from a single experiment;
B. Calculated rates of steady-state Rb and Cs absorption. Small circles represent data from Fig. 3B reduced to the absolute scale of Fig. 4B by means of the ratio of absolute rates of uptake of Cs at 0.1 me/l in the absence of Rb;
C. Calculated amounts of Rb and Cs present in fraction I.

concentrations (0.015 me/l). As to the part of the curve above the inflection point, it is not so much the general shape as the relative saturation level attained in this concentration range that appears to be affected by the presence of Cs.

3. There is a close similarity between the competition behaviour of steady-state Rb and Cs absorption and the uptake of Rb and Cs into fraction I (Fig. 3, B and C).

In a second, not duplicated, experiment the Rb concentration was raised up to 10 me/l at the same constant Cs concentration of 0.1 me/l (Fig. 4, A, B, and C). In accordance with the disproportionate increment in Rb absorption at 0.4 me/l Rb in Fig. 3 B, another inflection

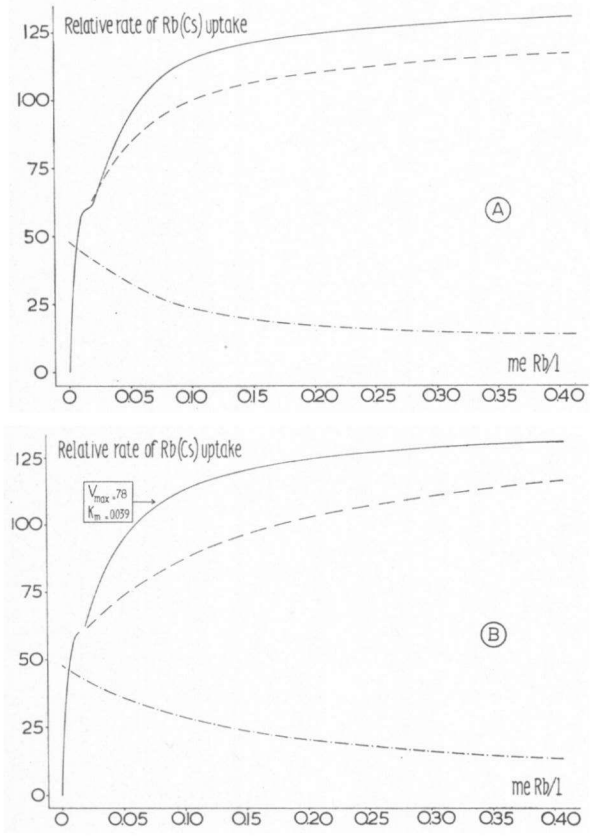


Fig. 5. A. Rate of Rb absorption at various concentrations in the absence (——) and presence (– – –) of 0.1 me/l Cs, and concomitant Cs absorption (–•–•–•–) on a comparable scale (see text);
B. Theoretical competition curves for the case of a two-site uptake mechanism for Rb, one of which is common to Cs and Rb. Denotations as in Fig. 5A. For further explanation, see text.

point appears to lead to a new and still higher saturation level of steady-state Rb absorption (Fig. 4 B). At the same time, Cs uptake sinks to the very low level of about 0.05 me/kg fresh weight per hour, which is maintained even at the highest Rb concentrations. The same feature is still more conspicuous in the amount of Cs present in fraction I (Fig. 4 C), thus demonstrating once more the impracticability of checking all Cs absorption by the supply of an excess of a competing ion. No marked increase in the Rb content of fraction I at high Rb concentrations is indicated (cf. Figs. 4 C and 3 C).

3.4. *Competition between Cs and Rb: varying Cs concentration, constant Rb concentration*

In the next series of competition experiments the concentration of Rb as the inhibiting ion was kept constant at 0.1 me/l, whereas Cs concentration was varied from 0 to 0.4 me/l (Fig. 6: data averaged from three separate experiments) and from 0 to 10 me/l (Fig. 7: data from a single experiment). Total uptake within 3 and 6 hours is shown in Figs. 6 A and 7 A, the rate of steady-state absorption in Figs. 6 B and 7 B, and the amount accumulated in fraction I in Figs. 6 C and 7 C. Finally, in complete analogy to Fig. 5 A, Fig. 8 A contains the data from Fig. 2 C and Fig. 6 B recalculated relative to the same standard uptake as used for Fig. 5 A.

The following features of the competition behaviour in the range of low concentrations (Figs. 6 B and 8 A) are noteworthy.

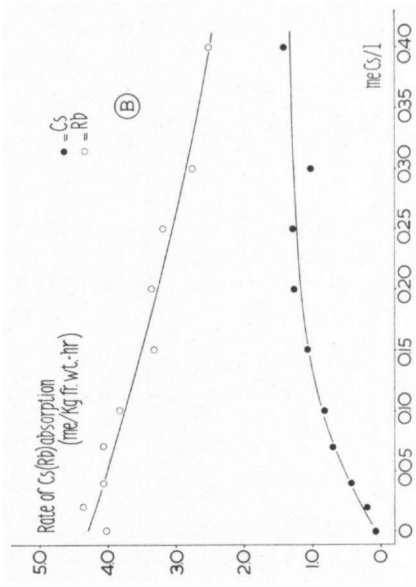
1. In the presence of a constant Rb concentration Cs uptake rises more slowly with concentration and to a lower relative saturation level than in the absence of Rb.

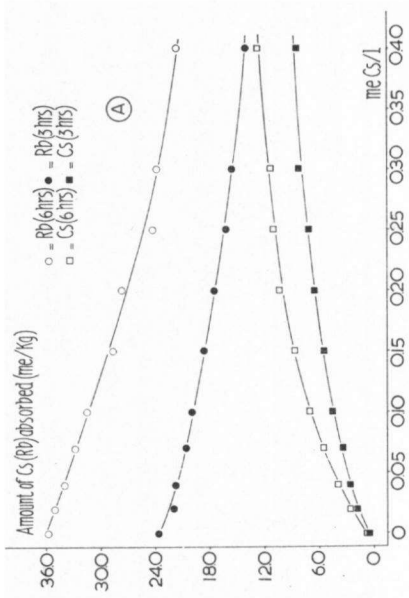
2. From a Cs concentration of 0.2 me/l to 0.4 me/l there is a marked reduction in the rate of Rb absorption without a concomitant rise in the rate of Cs absorption.

3. Competition for uptake into fraction I appears to proceed along lines similar though not identical to the characteristics of competition in the steady-state phase of absorption (Fig. 6, B and C).

When the Cs concentration is raised to 10 me/l, the rate of Cs absorption rises above the relative saturation level attained in Fig. 6 B, and reaches a new maximum (Fig. 7 B). Here too, an inflection point seems indicated at a concentration of about 0.5 me/l. At the same time, after an initial steep fall, Rb absorption keeps declining slowly in the range of concentrations from 2 to 10 me/l though unaccompanied by an equivalent rise in Cs uptake.

Again the same general features are apparent in the amount of Cs and Rb in fraction I (Fig. 7 C). However, even at the highest Cs concentrations the amount of Rb in fraction I is not reduced anywhere near so strongly as steady-state Rb absorption. The same phenomenon was encountered in Cs inhibition by excess Rb (see under § 3.3).





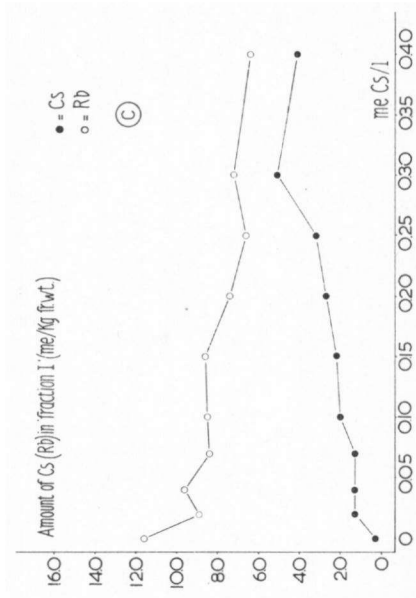
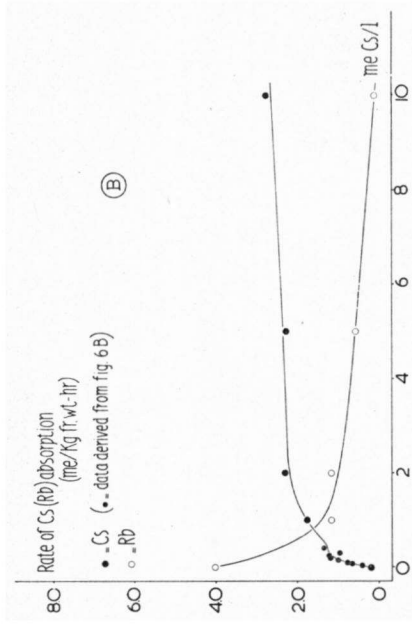


Fig. 6. Competition between Cs and Rb absorption from a solution with a Rb concentration of 0.1 me/l and a Cs concentration varying from 0 to 0.4 me/l
A. Amounts of Cs and Rb absorbed within 3 and 6 hours. Data represent the average of 3 separate experiments;
B. Calculated rates of steady-state Cs and Rb absorption;
C. Calculated amounts of Cs and Rb present in fraction I.



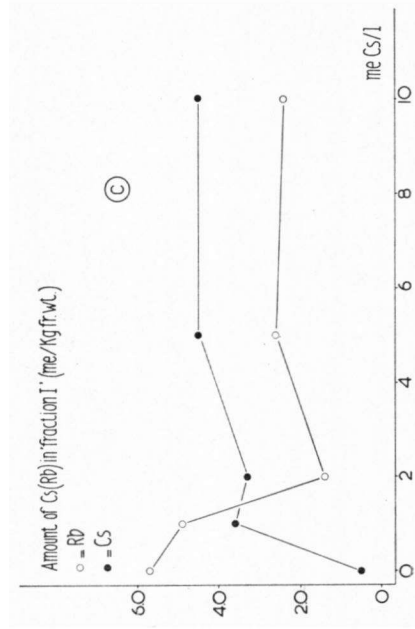


Fig. 7. Competition between Cs and Rb absorption from a solution with a Rb concentration of 0.1 me/l and a Cs concentration varying from 0 to 10 me/l
A. Amounts of Cs and Rb absorbed within 3 and 6 hours. Data are from a single experiment;
B. Calculated rates of steady-state Cs and Rb absorption. Small circles represent data drom Fig. 6B reduced to the absolute scale of Fig. 7B by means of the ratio of absolute rates of uptake of Rb at 0.1 me/l in the absence of Cs;
C. Calculated amounts of Cs and Rb present in fraction I.

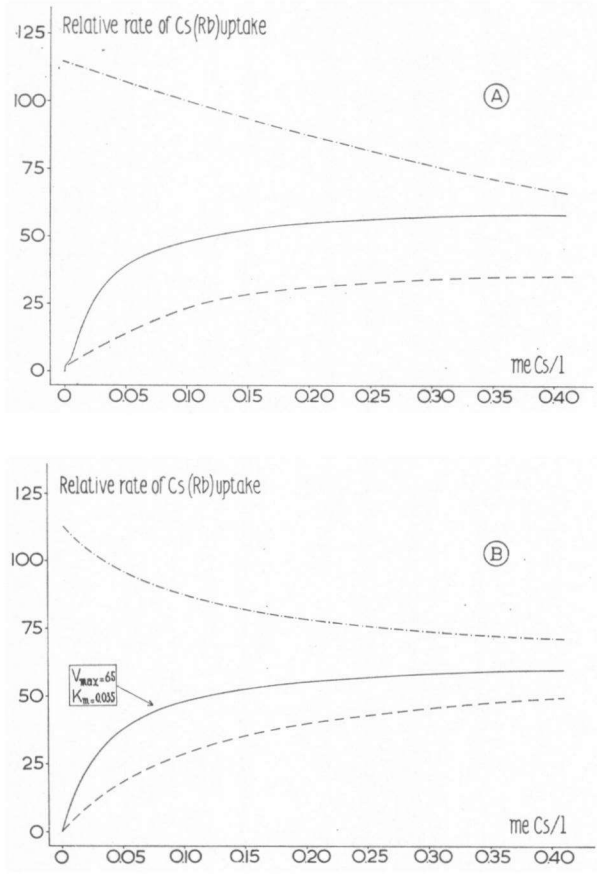


Fig. 8. A. Rate of Cs absorption at various concentrations in the absence (——) and presence (– – – –) of 0.1 me/l Rb, and concomitant Rb absorption (–•–•–•–) on a comparable scale (see text); B. Theoretical competition curves for the case of a two-site uptake mechanism for Rb, one of which is common to Cs and Rb. Denotations as in Fig. 8A.For further explanation, see text.

4. Discussion

4.1. *Kinetic considerations*

As mentioned in the Introduction, the main purpose of this work was to ascertain whether or not the absorption behaviour of Rb and Cs fits into a kinetic pattern corresponding to the initial binding of these ions to identical sites of a carrier mechanism. For plant roots the principles of carrier kinetics have been worked out by Epstein and Hagen (1952) to which paper may be referred for further details.

We at once meet the difficulty that no simple kinetic scheme is able to explain the occurrence of inflection points in the absorption curves for Rb and – to a less extent – Cs (Fig. 2 C). Even when two or more independent binding sites are involved in the absorption of an ion,

the slope of the relation between the over-all rate of uptake and concentration should decline continuously. It should be mentioned that similar inflection points have also been reported by other workers (EPSTEIN, 1964; ELZAM, 1964).

In the second place, we have to account for the fact that the rate of Cs uptake is only about one half of the rate of Rb uptake (Fig. 2 C). Two explanations present themselves.

1. Cs is transported by the same binding site as Rb but the rate of breakdown of the complex between the Cs-ion and the carrier is only about one-half of the corresponding value for Rb.
2. There are two binding sites, both of which transport Rb but only one of which transports Cs.

The first explanation is based on the assumption that only one common binding site is involved in Rb and Cs transport. It was demonstrated (Fig. 5 A) that at low Rb concentrations (up to about 0.02 me/l) Rb uptake is not inhibited by the presence of 0.1 me/l Cs. Taking into account the fact that Cs uptake is almost saturated at 0.1 me/l, in case of one common binding site this lack of inhibition should imply a sharp concomitant drop in Cs absorption. The actual decrease in Cs uptake is only about 15 %.

The alternative explanation seems more attractive because, as discussed under Material and Methods, § 2.2., the relation between Rb uptake and concentration consists of two distinct parts (Fig. 5 A). If it is assumed that the steep initial part reflects binding of Rb by a site with a high affinity to this ion but hardly any affinity to Cs, whereas the second part rising more slowly to relative saturation represents binding to a site with about equal affinities to Rb and Cs, not only the lower level of saturation of Cs absorption but also the lack of inhibition of Rb uptake at low Rb concentrations and the absence of a concomitant drop in Cs uptake in the competition experiment of Fig. 5 A might be explained. However, a close examination of the quantitative relations to be expected in case of the proposed mutual "competitive inhibition" reveals that such a mechanism cannot underly the experimental behaviour described (Figs. 5 B and 8 B).

The curves in Figs. 5B and 8B have been constructed with the use of the formula for competitive inhibition given by EPSTEIN and HAGEN (l.c.). The K_m and K_I values required were derived from the absorption curves in the absence of an inhibiting ion. On account of the inflection in the uptake curve for Rb (Fig. 5A), the line ($x = 0.019$) had to be used as an ordinate in the mathematical description of the second part of this curve.

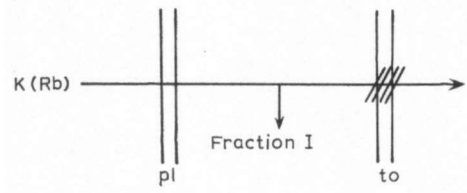
Quite apart even from such quantitative considerations, the considerable drop in Rb uptake in the competition experiment shown in Fig. 6 B (cf. Fig. 8 A) in the range of concentrations from 0.2 to 0.4 me/l Cs, unaccompanied by so much as half a proportional rise in Cs uptake, seems irreconcilable with any explanation based on a strictly competitive behaviour of the ions. The fact that absorption of Cs as well as Rb can be reduced to very low values by higher concentrations of the inhibiting ion (Figs. 4 B and 7 B) does not detract from this conclusion because the crucial question is whether

or not purely competitive effects alone are able to explain our results.

The inference seems justified that the characteristics of Rb and Cs interaction do not fit the rigorous pattern of competitive inhibition kinetics.

4.2. *Steady-state absorption and uptake into fraction I*

HOOYMANS (l.c.) has presented evidence in favour of the view that the uptake of cations into fraction I represents binding of the ions to cytoplasmatic constituents. Although differing in some details, essentially the same view is held by FOOTE and HANSON (1964) who observed a similar biphasic time curve in K uptake by soybean roots after removal of two-thirds of the calcium of the tissue by treatment with EDTA. HOOYMANS (l.c.) assumes that in the presence of Ca the supply of K(Rb) to these cytoplasmatic sites is mediated by the steady-state absorption mechanism, the rate-limiting step of the latter occurring at the tonoplast and not at the plasmalemma, according to the diagram:



pl = plasmalemma to = tonoplast /// = rate-limiting step

In the experiments presented above we pointed out the remarkable though not absolute similarity with regard to concentration dependence and competition behaviour existing between the rates of steady-state absorption of Rb and Cs on the one hand and the amounts present in fraction I on the other. It might be argued that the quantity of Rb or Cs accumulated in fraction I in the presence of Ca is not representative of a true state of equilibrium between environmental alkali cation concentration and cytoplasmatic sites because of the inhibiting effect Ca has on the saturation of these sites at Rb concentrations below about 0.02 me/l (cf. 3.2.). However true this may be, it does not detract from such parallel features in Figs. 2 C and 2 D as the attainment of saturation in the concentration range from 0.1 to 0.5 me/l for Rb as well as for Cs, and, above all, the saturation level being about twice as high for Rb as for Cs. In the competition experiments the total concentration of Rb + Cs exceeded 0.1 me/l from the outset, so in this case these effects mav have been absent or at least of minor importance.

The parallelism observed seems of some consequence for our notions concerning the nature of the substance supposedly involved in alkali cation transport. From Fig. 2 D it appears that as much as 10 me Rb per kg fresh weight of roots may be present in fraction I. If this fraction is correctly identified with uptake into the cytoplasm or parts of it and the amount of cytoplasm is estimated at roughly 10 % of

the fresh weight of the tissue, the cytoplasmatic Rb concentration proves to be 100 me/l. Therefore, the important conclusion to be drawn from this part of our observations seems to be that sites with binding characteristics closely akin to those of the cell components responsible for alkali cation transport, must be quite abundant in the cell.

4.3. *The possible nature of the substances involved in alkali cation transport*

Rather than raising doubts about the fundamental correctness of the concept of carrier-mediated cation transport, the discrepancies observed may serve the purpose of shaping its rather vague and simplified general formulation.

The anomalies revealed in the absorption and competition curves seem to make it inevitable that the idea of a system of binding sites working quite independently and without any mutual interference, is discarded. Although far from pretending to be able to give an alternative detailed explanation of the data presented, we hold the opinion that a better understanding of the facts observed becomes possible if two essential properties are attributed to the transport system, to be formulated as follows.

1. The separate sites binding the alkali cations are part of a larger unit (e.g. a protein molecule).
2. This unit does not have a structure so rigid as to remain unaffected by the presence or absence of ions at the binding sites as well as by the nature of the ions bound. In other words, binding fo an ion to one site may exert a strong influence on the structural properties determining the conditions under which similar or other ions are bound to adjacent sites.

It is noteworthy that in enzymology comparable "allosteric" effects have been claimed for the explanation of certain types of interaction between enzymes and their substrates or inhibitors (cf. MONOD, WYMAN, and CHANGEUX, 1965). In fact, the un- and non-competitive types of inhibition introduced into the field of ion absorption by EPSTEIN and HAGEN (1952) may be looked upon as oversimplified examples of the type of structural interferences we have in mind.

Is it feasible, for instance, that the fundamental feature underlying the uptake behaviour of Cs as compared to Rb, consists of such a structural interference of adjacent sites as to prevent their simultaneous association with a Cs-ion, thus explaining the lower saturation level of Cs absorption? And could the same allosteric effect be responsible for the difference in affinity with which successive portions of Rb-ions are bound, as judged from the complex character of the absorption curve? For the moment, any answers to these and similar questions would merely be speculation about the properties of a system for whose existence any more direct evidence is lacking. The same consideration holds with respect to the inflection points. Do the carriers occur in different structural states in the sense that one state having a fixed binding capacity is stable within a certain range of ion concentrations but breaks up into a different structural arrangement with a higher

binding capacity when the external concentration of the ion is raised?

The hypothesis that the alkali cation carrier is a molecular structure rather than some simple chemical compound, fits in with the conclusion drawn in the preceding paragraph that binding sites similar to those involved in alkali cation transport are not at all rare in the cell. The representation of the carrier as some mysterious chemical compound with unique properties, seems hardly reconcilable with a protoplasmatic concentration of identical or at least closely related substances as high as 0.1 M. It looks rather as though common cell components are involved, whose structural capacities for specific binding of ions as prevailing in the living cell, however, have not yet been fully disclosed. Recently, WIGGINS (1964) described a selective accumulation of K-ions by rat kidney slices, for which she holds the cell proteins responsible. Tempting though this assumption may be for our system too, because of the considerable evidence for a close linkage between ion transport and protein synthesis obtained from work with antibiotics, the direct proof of the nature of the molecular structure involved remains a challenge to further experimentation.

Emphasis on the importance of protoplasmatic structure in ion absorption dates back to 1957, when OVERSTREET put forward the view that the ion carrier is the living protoplast itself, of whose complicated and labile structure the ions absorbed are – either permanently or temporarily – going to make up an integral part. It is this view to which, in our opinion, the data presented lend support.

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REFERENCES

BANGE, G. G. J. 1959. Interactions in the potassium and sodium absorption by intact maize seedlings. Plant and Soil **11**: 17–29.

———, J. TROMP and S. HENKES. 1965. Interactions in the absorption of potassium, sodium, and ammonium ions in excised barley roots. Acta Bot. Neerl. **14**: 116–130.

——— and E. VAN VLIET. 1961. Translocation of potassium and sodium in intact maize seedlings. Plant and Soil **15**: 312–328.

ELZAM, O. E. 1964. Mechanisms of chloride absorption by excised barley roots. Plant Physiol. **39**: suppl. xli.

EPSTEIN, E. 1964. Kinetic evidence for complex between potassium and binding sites of carriers. Plant Physiol. **39**: suppl. xxxix.

——— and C. E. HAGEN. 1952. A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. **27**: 457–474.

FOOTE, B. D. and J. B. HANSON. 1964. Ion uptake by soybean root tissue depleted of calcium by ethylenediaminetetraacetic acid. Plant Physiol. **39**: 450–460.

HOOYMANS, J. J. M. 1964. The role of calcium in the absorption of anions and cations by excised barley roots. Acta Bot. Neerl. **13**: 507–540.

MONOD, J., J. WYMAN and J.-P. CHANGEUX. 1965. On the nature of allosteric transitions: a plausible model. J. Mol. Biol. **12**: 88–118.

OVERSTREET, R. 1957. Comment on the absorption of inorganic ions by root cells. Plant Physiol. **32**: 491–492.

WIGGINS, P. M. 1964. Selective accumulation of potassium ions by gel and kidney slices. Biochim. Biophys. Acta **88**: 593–605.