

INTERACTIONS IN THE ABSORPTION OF POTASSIUM, SODIUM, AND AMMONIUM IONS IN EXCISED BARLEY ROOTS

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ABSTRACT

A kinetic study of the interactions in the uptake of NH_4^- , K-, and Na-ions by excised barley roots was performed using the continuous flow technique.

In all cases the absorption of one ion was inhibited by the presence of a second ion, but the extent of the inhibition was limited. The presence of an inhibiting ion consistently resulted in an increase of the half-value of the uptake vs. concentration relation of the inhibited ion.

The difficulties encountered in the interpretation of these results in terms of ion competition are discussed. On the other hand, it is shown that the characteristic features of the interaction are readily reconcilable with the concept of carrier competition.

INTRODUCTION

In a recent reconsideration of the carrier theory of ion transport one of us (BANGE, 1962) suggested an extension of its usual kinetic formulation (cf. EPSTEIN and HAGEN, 1952). It was shown that this extension was useful not only in the explanation of the variability repeatedly found in the value of the Michaelis-Menten constant of absorption curves but also provided the base for a hypothesis concerning a new type of competition called 'carrier competition' as opposed to the 'ion competition' proposed by EPSTEIN and HAGEN (l.c.).

Recent data collected by one of us (TROMP, 1962) on the competition of NH_4 , K, and Na in wheat roots could be satisfactorily explained by assuming that the individual carriers of these ions compete for a common enzymatic breakdown reaction. On the other hand, it appeared difficult to understand the phenomena observed in terms of one of the types of ion competition.

Nevertheless, it was deemed desirable to provide a wider experimental basis for the phenomenon of carrier competition, the more so as the results obtained with wheat roots could be understood quantitatively only if a special theoretical condition was satisfied. It will be shown below that the competition pattern of NH_4 , K, and Na in barley roots seems to provide a more general example of the phenomenon of carrier competition. At the same time, a broader interpretation of the concept of carrier competition will be discussed.

MATERIAL AND METHODS

a. *Plant material*

All experiments were performed with excised roots of *Hordeum vulgare*, cultivar 'Herta'.

Seven days old barley seedlings were grown in essentially the same way as described for wheat by TROMP (1962). Briefly 50 grams of seed were disinfected for 2 minutes in a 1 % HgCl_2 -solution, rinsed in flowing tap-water, and subsequently soaked in aerated demineralized water for 24 hours. They were then spread over a piece of aseptic gauze supported by a stainless steel screen mounted on a perspex frame. The frame was placed in a PVC tray containing 4 litres of aerated 2×10^{-4} M CaSO_4 -solution, the level of this solution being at about 1.5 cm below the screen and the aseptic gauze dipping in the solution at all sides of the frame. The tray was placed in an air-conditioned growing chamber (temperature 25°C) for 6 days. During this time the CaSO_4 -solution was renewed twice.

About 45 minutes before the start of an experiment the roots were excised just below the gauze and washed in three changes of demineralized water for 10 minutes each. They were then centrifuged in a basket centrifuge for 5 minutes at a low speed. Portions of 3 grams were weighed out to an accuracy of about 0.01 grams to be used in the experiment.

b. *Experimental technique*

The continuous flow technique was used throughout. An extensive description of this technique as applied here has been given by TROMP (1962). Samples of the discharged solutions were collected about 2 hours after the start of the experiment. A steady state of uptake was established after this period of time as was demonstrated by the identical concentrations of samples taken 2 and $2\frac{1}{2}$ hours respectively after the beginning of the experiment.

The cations were supplied as phosphates. To increase the buffering capacity of the experimental solutions 'tris' was added at a concentration of 1.75 mM and the pH adjusted to the value required with the aid of phosphoric acid. Experimental pH values varied between 5.5 and 6.5 but in most cases ranged between 5.7 and 6.2. All experiments were performed at 20°C .

To reduce the experimental variability due to differences in absorption capacity of different batches of roots, the 'standard technique' as introduced by VAN DEN HONERT (1933) was used: in each experimental series the rate of absorption of an ion was expressed in terms of the rate of absorption of the same ion under the—arbitrarily chosen—standard conditions of the experimental series in question. The average values of the standard absorptions in the separate series were used to connect quantitatively all the relations determined. In the graphs, standard uptakes are indicated by a double circle, triangle, or square.

Concentrations are expressed as milliequivalents per litre (me/l).

c. Analytical procedures

The Nessler method was used for the colorimetric estimation of NH_4 (cf. ALLPORT, 1947; see also BECKING, 1956).

K- and Na-concentrations were determined with a Beckman Model DU flame spectrophotometer.

EXPERIMENTAL RESULTS

The interactions in the absorption of K and Na, of K and NH_4 , and of NH_4 and Na were each studied in 4 series of experiments, viz.:

1. rate of absorption of ion A in relation to its concentration in the absence of ion B;
2. rate of absorption of ions A and B in relation to the concentration of ion A (= inhibited ion) at a constant concentration of ion B (= inhibiting ion);
3. rate of absorption of ion B in relation to its concentration in the absence of ion A;

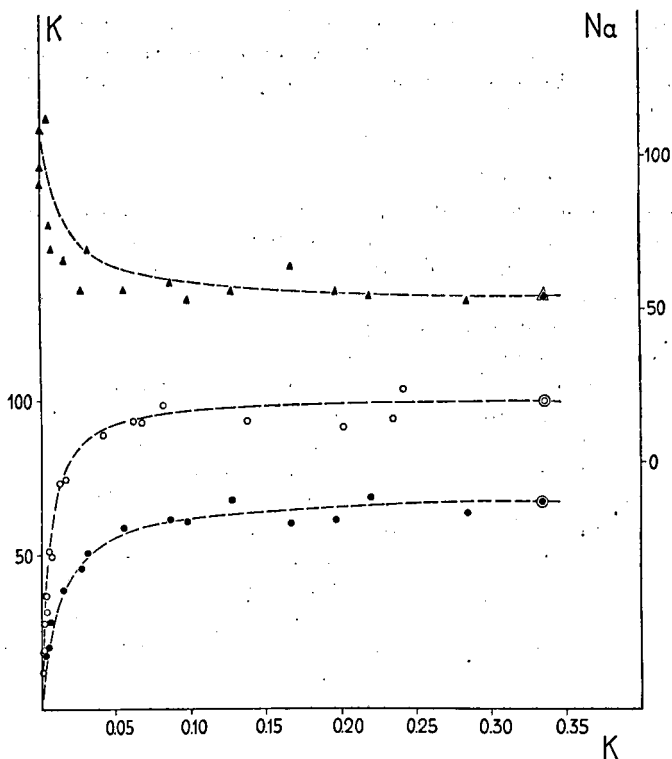


Fig. 1, A. Relative rates of K- and Na-absorption as a function of the K-concentration (in me/l) of the medium. Open circles = K-absorption in the absence of Na, solid circles = K-absorption in the presence of a constant Na-concentration (= 0.450 me/l), solid triangles = concomitant Na-absorption. For broken lines, see text.

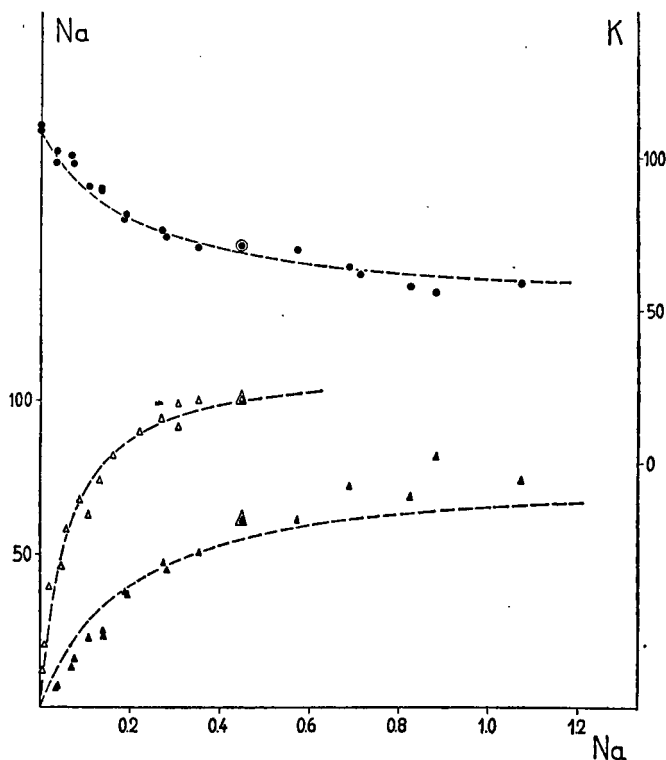


Fig. 1, B. Relative rates of Na- and K-absorption as a function of the Na-concentration (in me/l) of the medium. Open triangles = Na-absorption in the absence of K, solid triangles = Na-absorption in the presence of a constant K-concentration (= 0.334 me/l), solid circles = concomitant K-absorption. For broken lines, see text.

4. rate of absorption of ions B and A in relation to the concentration of ion B (= inhibited ion) at a constant concentration of ion A (= inhibiting ion).

For the K-Na interaction the results of these 4 series are presented in Fig. 1, A and B; for the K-NH₄ interaction in Fig. 2, A and B; and for the NH₄-Na interaction in Fig. 3, A and B. The main features may be summarized as follows:

a. In the absence of an inhibiting ion the rate of absorption of all 3 ion species at rising concentrations becomes increasingly independent of concentration. The absorption curves may be characterized by their 'half-values', i.e. those concentrations at which the rate of uptake amounts to half the maximal value attained at relatively high concentrations.

b. In the presence of a constant amount of an inhibiting ion the maximal rate of absorption of the inhibited ion is lower than in its absence. At the same time, the rate of absorption of the inhibiting

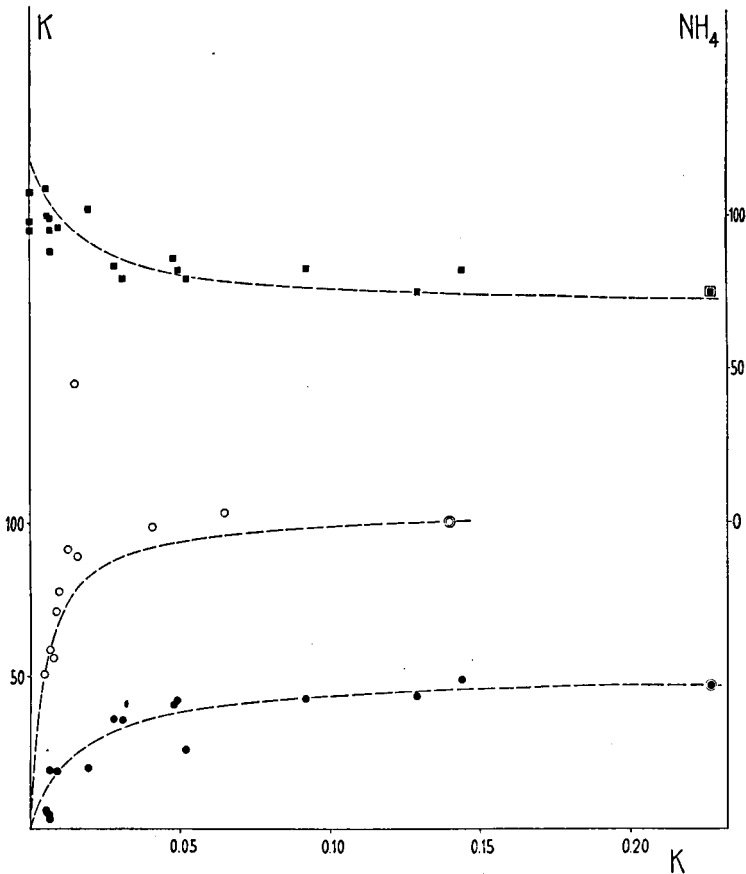


Fig. 2, A. Relative rates of K- and NH_4 -absorption as a function of the K-concentration (in me/l) of the medium. Open circles = K-absorption in the absence of NH_4 , solid circles = K-absorption in the presence of a constant NH_4 -concentration (= 0.318 me/l), solid squares = concomitant NH_4 -absorption. For broken lines, see text.

ion is lowered at increasing concentrations of the inhibited ion but only to a limited extent.

c. In the presence of a constant amount of an inhibiting ion the 'half-value' of the absorption curve of the inhibited ion shows a consistent shift to higher values. Within the limits of the experimental error this 'half-value' equals the 'half-value' of the decrease in the absorption rate of the inhibiting ion or, in other words, the absorption curves of the inhibiting and the inhibited ion are each other's image although the absolute scale is different.

Although these features are evident in all cases, the quantitative effects are not equally strong for the different ions. Especially the rate of NH_4 -absorption appears to be less sensitive to the presence of K- and Na-ions than K- and Na-absorption are to the presence

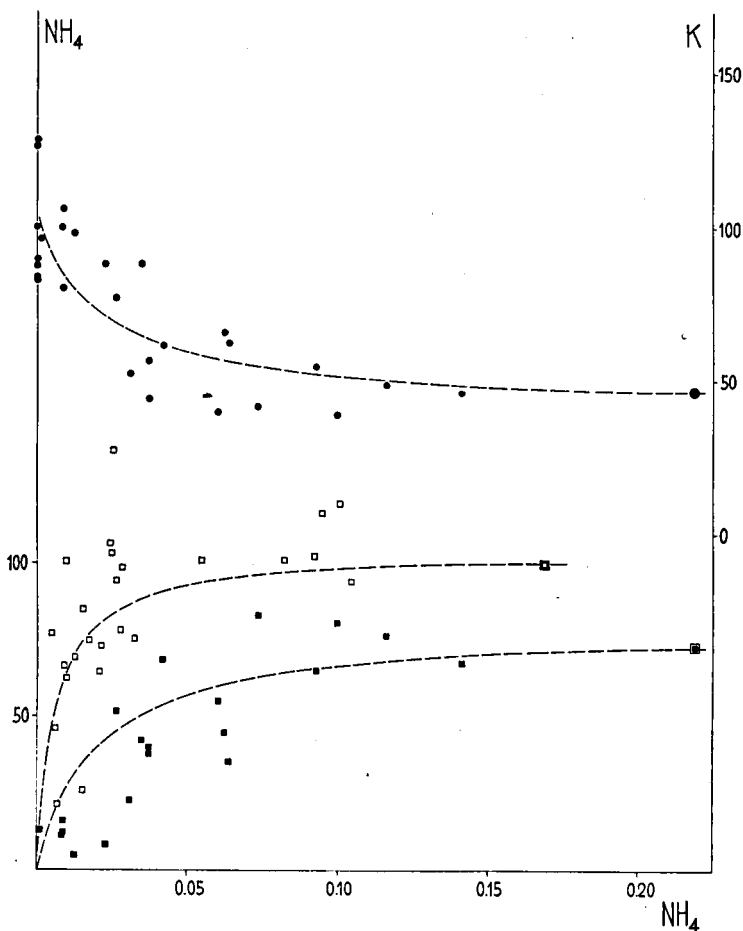


Fig. 2, B. Relative rates of NH₄- and K-absorption as a function of the NH₄-concentration (in me/l) of the medium. Open squares = NH₄-absorption in the absence of K, solid squares = NH₄-absorption in the presence of a constant K-concentration (= 0.120 me/l), solid circles = concomitant K-absorption. For broken lines, see text.

of NH₄-ions (Figs. 2 and 3). On the other hand, K-absorption is lowered by the presence of Na-ions to about the same degree as is Na-absorption by the presence of K-ions (Fig. 1). At the same time, no large differences in the ratio: half-value in the absence/half-value in the presence of the inhibiting ion were demonstrated in the different combinations of ions.

The variability of results obtained with the continuous flow technique is usually somewhat larger than with other techniques and therefore in most cases the running of more than one experimental series is necessary to establish a relationship. In these series the inhibitions were not always equally strong. For the Na-NH₄ inter-

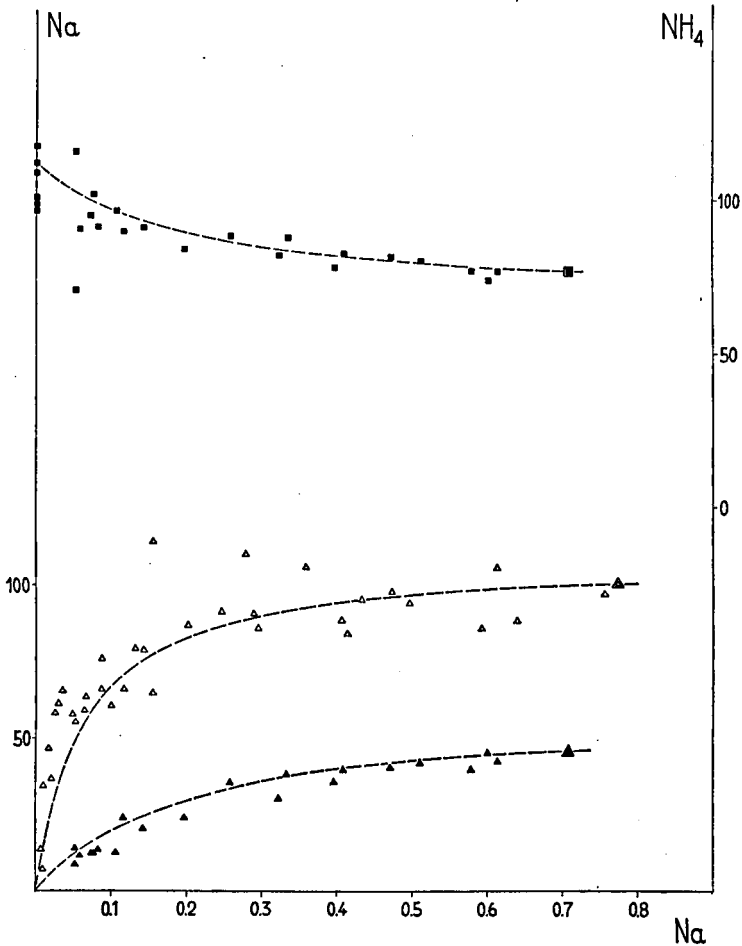


Fig. 3, A. Relative rates of Na- and NH_4 -absorption as a function of the Na-concentration (in me/l) of the medium. Open triangles = Na-absorption in the absence of NH_4 , solid triangles = Na-absorption in the presence of a constant NH_4 -concentration (= 0.151 me/l), solid squares = concomitant NH_4 -absorption. For broken lines, see text.

action this variable behaviour led to the observation that the more the Na-absorption was affected by the presence of NH_4 , the less concomitant NH_4 -absorption was reduced by the presence of Na (Fig. 4, A and B). In Fig. 3, A and B, as in Figs. 1 and 2, the results of the different series have been combined and averaged.

DISCUSSION

The first question we are confronted with is whether or not these results can be explained in what may be referred to as the classical theory of ion competition (EPSTEIN and HAGEN, 1952). Notwithstanding the fact that the number of carriers present as well as the

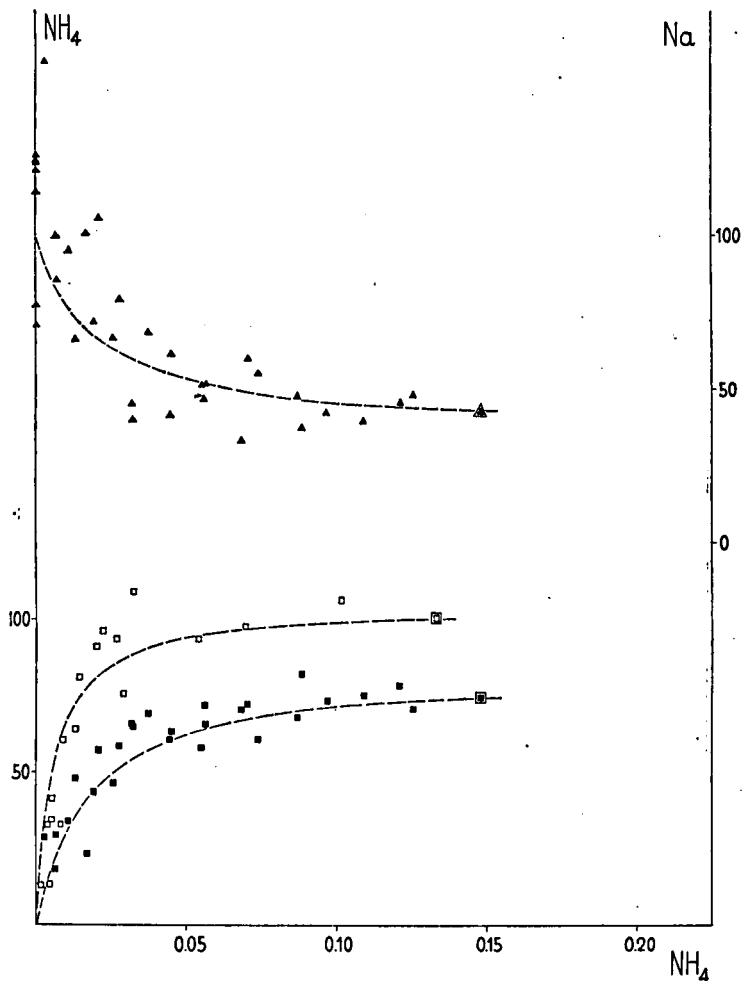


Fig. 3, B. Relative rates of NH₄- and Na-absorption as a function of the NH₄-concentration (in me/l) of the medium. Open squares = NH₄-absorption in the absence of Na, solid squares = NH₄-absorption in the presence of a constant Na-concentration (= 0.720 me/l), solid triangles = concomitant Na-absorption. For broken lines, see text.

degree of their specificity and the type of competition involved may be introduced as variables, the resulting pliability does not produce a satisfying and unforced picture of the uptake system operative in this material.

The most salient feature of our results is the incompleteness of all inhibitions. If we confine ourselves for the moment to—say—the K–Na interaction (Fig. 1), the implication is that at least one site with a high preference for K and at least one site with a high preference for Na must be involved. Thus the most simple situation conceivable

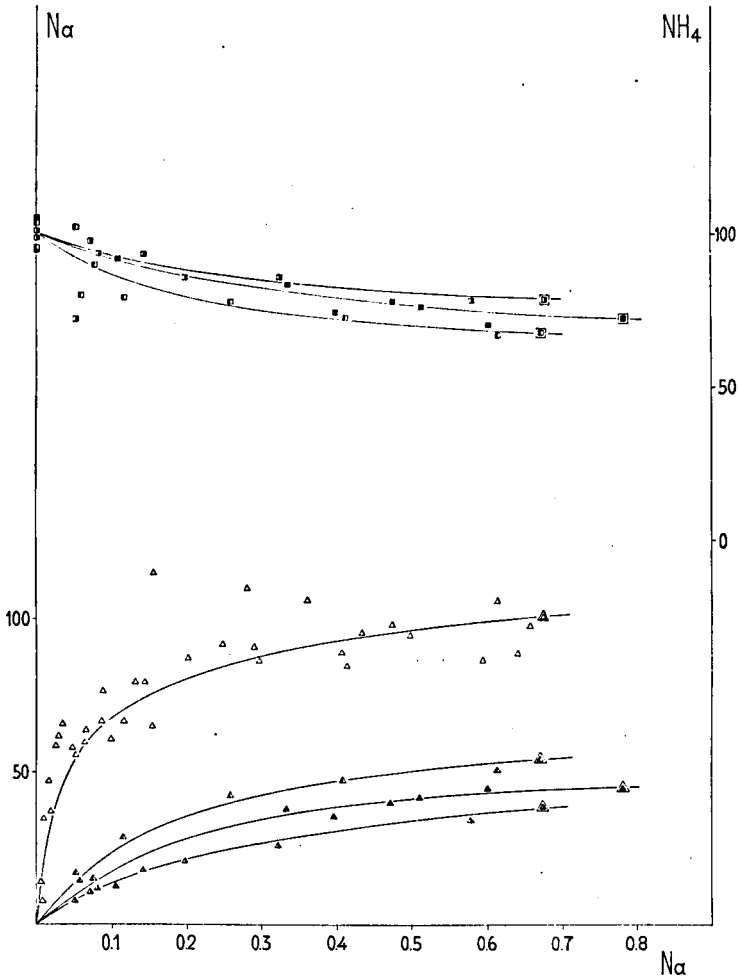


Fig. 4, A. The same experiment as in Fig. 3, A, but with the results of the individual series (half-solid and solid squares and triangles respectively) plotted separately. Drawn lines have no theoretical background.

is a combination of 2 sites, one preferring K to Na and the other preferring Na to K. So K is transported by both sites in the absence of Na but as soon as Na is present, K is expelled from one site and its transport becomes limited to the site with the high preference for K. The same holds for Na. From a qualitative point of view, no objections can be raised to this picture. However, difficulties arise from quantitative considerations. The relation between K-concentration and rate of K-absorption by the site with the high preference for Na may be obtained by subtraction of the amount of K absorbed in the presence of Na from the amount absorbed in the absence of Na at the different K-concentrations. The half-value of this relation

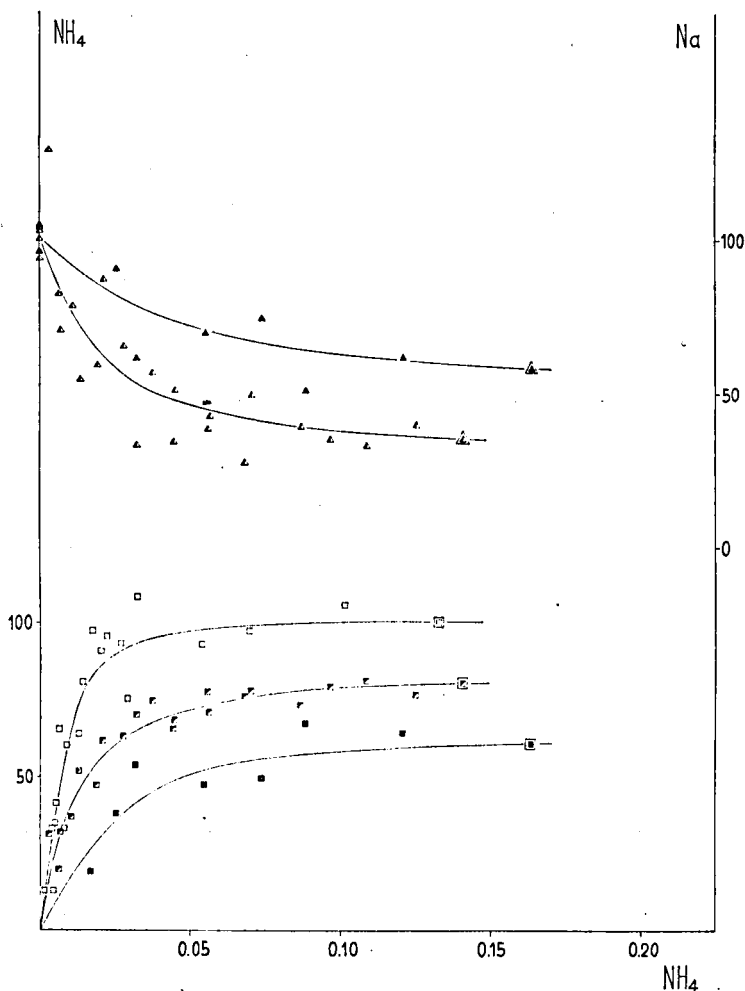


Fig. 4, B. The same experiment as in Fig. 3, B, but with the results of the individual series (half-solid and solid squares and triangles respectively) plotted separately. Drawn lines have no theoretical background.

will not be far from 0.01 me/l (Fig. 1, A). On the other hand, Na-uptake by this site proceeds with a half-value on the order of 0.2 me/l (Fig. 1, B). Because in the classical theory half-values are supposed to reflect real affinities between ion and carrier, this theory does not explain why in a solution of 0.45 me/l Na + 0.33 me/l K the K-ion is expelled quantitatively from a site to which it must have an about 20 times higher affinity than the expelling Na-ion. Therefore, this scheme must be rejected as unlikely.

An alternative explanation might be based on a system of 3 sites, two of which are wholly specific and one common. In this concept the decline of—say—Na-uptake at rising K-concentrations (Fig. 1, A)

would be due to the increasing competitive power of the K-ion with regard to the common site. However, this picture leaves unanswered the question of why K-uptake itself is still inhibited at a K-concentration at which the expulsion of Na from the common site has apparently been completed. This difficulty could be overcome by the assumption that besides the two specific sites not one but two common sites are involved in K- and Na-absorption, one preferring K to Na and the other Na to K. However, any explanation based on a competition of K and Na for common sites seems irreconcilable with the fact that the affinity of these sites for K as apparent from the half-values of the concentration curves in Fig. 1, A, is so much higher than for Na (cf. Fig. 1, B) that in a mixture of 0.33 me/l K and 0.45 me/l Na no measurable competitive inhibition of K-absorption by Na could actually occur. For these reasons we are inclined to maintain some reserve with respect to the explanation given by EPSTEIN (1961) for this type of competition behaviour.

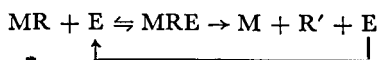
EPSTEIN and HAGEN (1952) introduced two additional types of inhibition into ion absorption as opposed to the competitive type of inhibition discussed above, viz. the uncompetitive and non-competitive type. These inhibitory mechanisms have been claimed for enzymes, but in our opinion the evidence presented for their existence in ion uptake (EPSTEIN and HAGEN, *l.c.*) is not conclusive. Leaving this question aside for the moment and applying the principle to our data on K-Na interaction, we come to the picture of two transporting systems, both of which are able to transport Na as well as K, albeit at different sites. However, in one system the presence of Na at the Na-site inhibits the transport of K at the K-site and in the other system the presence of K at the K-site inhibits the transport of Na at the Na-site. As long as we restrict ourselves to the interaction of two ions only, this concept seems to offer an explanation for the data presented even though the consistent increase of the half-values in the presence of the inhibiting ion remains a purely accidental feature of the system. However, the picture becomes less attractive when the mutual interactions of the three ions are considered. In this case we are forced to assume either the existence of 3 pairs of transporting systems of the type described above for K- and Na-transport or, alternatively, the existence of two transport systems, both of which are equipped with 3 sites carrying the 3 ionic species and exhibiting an inhibition sequence of—say—NH₄ inhibits K inhibits Na and Na inhibits K inhibits NH₄ respectively. Besides their complexity, both alternatives have the already mentioned disadvantage of not explaining the consistent upward shift of the half-values in the presence of an inhibiting ion. Additional objections arising from quantitative considerations may be raised to the second alternative.

However, rather than enlarging upon systems whose merits seem theoretical rather than practical, we should like to consider the results presented in the light of the hypothesis of carrier competition.

In a reconsideration of the assumptions underlying the conventional formulation of steady state carrier kinetics in ion absorption studies, one of us (BANGE, 1962) pointed out the objections to the supposition that the amount of ion transported is directly proportional to the amount of ion-carrier complex present as expressed by the formula:

$$v = k_3 \cdot [\text{MR}]$$

in which v is the amount of ion transported in unit time, k_3 the velocity constant, and $[\text{MR}]$ the concentration of the ion-carrier complex. This supposition implies that the disintegration of MR is a spontaneous monomolecular reaction. The objection is that without additional assumptions it cannot be understood why the complex should disintegrate at one side of the membrane and be stable at the other. The hypothesis was therefore introduced that the complex is broken down by means of a limited quantity of some enzyme E:



This hypothesis, taken together with the assumption that the respective carriers of—say—the alkali cations may be chemically so related as to be broken down in the same enzymatic reaction, contained the elements for a new type of competition to be posed, as ‘carrier competition’, against ‘ion competition’. For the elaboration of the kinetics of this type of competition the reader is referred to the original paper.

The main qualitative features of carrier competition may be summed up as follows:

1. In both the absence and the presence of an inhibiting ion of constant concentration the relation between ion concentration and rate of uptake is represented by an equation of the type:

$$v = A \cdot c / (B + c)$$

in which v is the rate of uptake, c the ion concentration, and A and B constants.

2. In the presence of a constant concentration of an inhibiting ion the maximal rate of uptake of an ion is always lower than in the absence of the inhibitor, but not even in the presence of a relative excess of the inhibitor does this rate fall below a certain limiting value.

3. The presence of a constant concentration of an inhibitor always entails an increase in the half-value of the rate of uptake vs. concentration relation of an ion, but this increase too comes to an end with increasing concentrations of the inhibitor.

4. In the presence of a constant concentration of an inhibitor the rate of uptake of an ion at rising concentrations is directly proportional to the decrease in the rate of uptake of the inhibitor.

These features are identical with the characteristics of the graphs obtained in the present work as described in the preceding chapter. From a qualitative point of view, therefore, our results are readily explained in terms of carrier competition. Do our results also answer the quantitative requirements of theory?

In the elaboration of the quantitative aspects of the hypothesis, obviously some simplifying assumptions had to be made or are implied. On the other hand, it has already been demonstrated in the preceding chapter that in the various experimental series run

to establish a relationship, the competition behaviour was not always identical. Moreover, the experiments extended over a period of about two years and consequently different harvests of barley seed were involved. These circumstances should be taken into account in an evaluation of the degree of agreement between theory and experiment.

The procedure adopted to test the applicability of formulae (4) and (5) from the previous paper (BANGE, 1962) to the interaction between ions M_1 and M_{11} was the following. Of the 6 quantities involved ($H_1, H_{11}, H_1^{11}, H_{11}^1, V_{\max 1}/V_{\max 1}^{11}$, and $V_{\max 11}/V_{\max 11}^1$), 4 were estimated from the experimental results (e.g. $H_1, H_1^{11}, H_{11},$ and $V_{\max 11}/V_{\max 11}^1$). The remaining 2 (H_{11}^1 and $V_{\max 1}/V_{\max 1}^{11}$) could then be calculated from formulae (4) and (5) respectively. The choice of the quantities to be computed is completely arbitrary because the values taken from the graphs may be varied within certain limits to give the best general fit of all curves involved.

From the half-values and V_{\max} -ratios, the quantities $K_1, K_{11}, R_{1t}/K_2$, and R_{11t}/K_{22} may be calculated with the aid of the formulae:

$$K_1 = \frac{(V_{\max 1}/V_{\max 1}^{11}) \cdot H_1^{11} - H_1}{(V_{\max 1}/V_{\max 1}^{11}) - 1}$$

and

$$R_{1t}/K_2 = \frac{V_{\max 1}}{V_{\max 1}^{11}} \cdot \frac{H_1^{11} - H_1}{H_1 \cdot [(V_{\max 1}/V_{\max 1}^{11}) - 1]}$$

and analogous expressions for K_{11} and R_{11t}/K_{22} .

By the same token, from the interaction of the ions M_{11} and M_{111} the value of K_{111} and R_{111t}/K_{222} may be calculated. With these values known, the interaction between ions M_1 and M_{111} can be predicted quantitatively as will be clear from a consideration of the expressions for $V_{\max 1}^{111}, V_{\max 111}^1, H_1^{111}$, and H_{111}^1 which are analogous to equations (3a) and (3b). For the same reason as mentioned above, the choice of the cation pair whose interaction is to be predicted, is completely arbitrary.

The results of this quantitative test are represented by the broken lines in Figs. 1, 2 and 3. Although there are some deviations, the general fit appears to be reasonably good. So, from the quantitative point of view the agreement between theory and experiment is also satisfactory. The inference seems justifiable that the competition behaviour described may be adequately explained in terms of carrier competition.

A marked influence of Ca on the ratio in which K and Na are absorbed from a mixture of the two ions has been reported by JACOBSON *et al.* (1961) and by EPSTEIN (1961). This effect has also been observed in our laboratory. Jacobson and his coworkers assume that a common carrier is involved in the K- and Na-absorption by barley roots and ascribe the effect of Ca on the interaction to a differential influence on the accessibility of the common site to Na ions on the one hand and K-ions on the other. Epstein, to the contrary, concludes that in K- (Rb-)uptake as well as in Na-absorption two carrier sites are involved, one of which is largely in-

different to the other ion as long as Ca is present while the other site may be common to both ions. Thus, in Epstein's view Ca is essential for the maintenance of the selectivity of the absorption mechanisms.

Although no Ca was added to our experimental solutions, the absence of a strong inhibiting effect of Na on K-absorption suggests the presence of at least some Ca. With the high root/solution ratio used (3 grams in 80 ml) and notwithstanding the continuous removal of solution from the experimental vessels, Ca could have been present due to its release from the roots. JACOBSON *et al.* (1961) have stressed the importance of the root/solution ratio in this connection. In fact, the varying results with respect to the degree of mutual inhibitions observed in the Na-NH₄ series (Fig. 4) may with some certainty be attributed to a concomitant variation in the concentration of released Ca provided Ca has a similar effect on the interaction of these ions as on the competition between K and Na. In any further refinement of the continuous flow technique, this factor will have to be taken into account.

At the moment we cannot offer a plausible alternative to the hypotheses put forward by Jacobson *c.s.* and by Epstein. A shift in the ratio of the total amount of carrier available for the transport of each of the ions would be an attractive assumption were it not that in this case the rate of uptake of the separate ions would have to be affected at least to some extent by the presence of Ca as well. However, a recent study of the effect of Ca on the uptake of alkali cations in barley roots performed in this laboratory (HOOYMANS, 1964) with due precautions to eliminate any secondary effects, has shown that steady state K- and Na-uptake are completely indifferent to the absence or presence of Ca. At the same time, this observation seems to weigh against the interpretations given by Jacobson *c.s.* and by Epstein: if Ca affects either the accessibility or the integrity of sites, in our view a change in the absorption rate of at least one of the separate ions in the presence of Ca must be expected as well. Therefore, more experimental work seems required to clarify this intriguing aspect of the alkali cation interaction.

CONCLUDING REMARKS

We have pointed out the in our view considerable difficulties encountered in interpreting our results in terms of ion competition. Similar difficulties were noted by TROMP (1962) for the alkali cation interactions in wheat roots. For the moment it seems safe to conclude that if real ion competition is operative, the essentials of the type of competition involved must be such as to escape our knowledge. On the other hand, naturally, we do not consider the hypothesis of carrier competition proven by the evidence presented. Whether the hypothesis will have to be accepted or rejected seems to depend for the present first of all on whether or not a reasonable explanation for the Ca-effect can be found.

In this connection we should like to emphasize that the concept of carrier competition may easily be extended to the effect that any

common restriction in either the breakdown or resynthesis of different carriers is liable to entail similar effects as calculated for the more narrowly-defined condition of a common 'carrierase'. For, the essential factor in this type of competition can be shown to be the apparent reduction in the amount of effective carrier under the influence of concomitant transport of a second ion. So, not only a limitation in the amount of a common enzyme available for carrier breakdown but also, for instance, a restriction in the amount of energy available for reconstruction of the ion-trapping systems may be the keystone on which the observed inhibitions are based.

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