

# INTERACTIONS IN THE ABSORPTION OF AMMONIUM, POTASSIUM, AND SODIUM IONS BY WHEAT ROOTS

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## I. INTRODUCTION

It is a well-known fact that with respect to the active absorption of ions most plant roots are highly selective. This selectivity, however, is often not absolute. Many investigations have shown that distinct interactions in the absorption of ions can be demonstrated. The absorption of monovalent cations in particular has been studied extensively in this connection (COLLANDER, 1937, 1941; EPSTEIN and HAGEN, 1952; SUTCLIFFE, 1956, 1957; FRIED and NOGGLE, 1958; BANGE, 1959; and BANGE and OVERSTREET, 1960).

Although selectivity and interaction in the absorption of ions are generally explained in terms of the carrier hypothesis, and our results will also be explained on this basis, there is no agreement in the literature concerning the mechanism of these phenomena.

SUTCLIFFE (1956, 1957) distinguished two possible explanations of ion selectivity and interaction:

1) There are several types of carriers, each specific for one single ion or a group of ions.

2) There is only one type of carrier capable of transporting all ions, but this carrier exhibits distinct preferences when a choice is available.

The first interpretation is supported especially by EPSTEIN and HAGEN (1952), FRIED and NOGGLE (1958), BANGE (1959), BANGE and OVERSTREET (1960), and EPSTEIN (1961) in investigations on interactions in the absorption of alkali ions. Their conclusions are based on the consideration that when two or more ions compete for absorption via the same mechanism, the rate of absorption of each ion will be reduced in the presence of one or more of the other ions, the extent of inhibition being dependent on the concentration of the different ions and the affinity of each ion for the absorption mechanism.

The second hypothesis mentioned above was developed by SUTCLIFFE (1957). In Sutcliffe's opinion, if two ions compete for the same mechanism, one ion may inhibit, fail to affect, or even stimulate the absorption rate of the other ion, dependent upon the effect of the concentration of both ions on the absorption rate and the preference exhibited by the mechanism. This preference may vary strongly for different plant species (SUTCLIFFE, 1956, 1957).

To obtain a better insight into the mechanism of ion selectivity and interaction, more research is required. It is also essential, as distinct from what is the case in some investigations in this field of research, to consistently determine the rate of absorption of all the ions involved in the interaction. The aim of the present investigation is therefore to analyse the mutual interactions in the absorption of  $\text{NH}_4$ , K, and Na in order to throw more light on this mechanism.

In past investigations concerning interactions in the absorption of cations, little attention has been paid to the  $\text{NH}_4$  ion. Only BANGE and OVERSTREET (1960) report for excised roots of barley an inhibition of the Cs absorption by  $\text{NH}_4$ . The lack of knowledge about  $\text{NH}_4$  in this field of research is probably due mainly to the difficulty of determining (e.g. by means of the Kjeldahl analysis) the absorbed amount of  $\text{NH}_4$  as an increase in the nitrogen content of the tissue because the increase is so small in relation to the total nitrogen content of the tissue. Determination of the absorption rate from the decrease in concentration caused by absorption has the disadvantage that the concentration is not constant during the experiment. To avoid these difficulties, the continuous flow technique as applied by VAN DEN HONERT (1933) was used in the present investigation. This technique has the additional advantage that only the active absorption is measured, the rate of absorption being only measurable a considerable time after the start of the experiment. Corrections with respect to the rapid equilibrations which appear during a short period after the transfer of the roots to the experimental solution are therefore not required.

In contrast to much other work on interactions in the absorption of ions, the present investigation could not be limited to the determination of the rate of absorption in relation to the concentration of the ions involved in each other's absence and presence because it was not clear which factor is responsible for the interactions, the concentration in the experimental solution of the inhibitor or the rate of absorption of the inhibiting ion. To make a choice possible, an attempt was made to vary the rate of absorption of the inhibitor at the same concentration. To that end, a number of experiments was first carried out in roots with different nitrogen contents. Based on the generally known fact that the rate of absorption is not the same in all regions of the root, the  $\text{NH}_4\text{-K}$  and the  $\text{NH}_4\text{-Na}$  interaction by different parts of the root was determined next. These experiments showed that the ratio "rate of absorption inhibitor/decrease in the rate of absorption inhibited ion" always was nearly constant. To obtain more data about this, the effect of the pH and the temperature, as variable factors, was also included in the investigation since it is well known that the rate of absorption is frequently clearly affected by both. The rate of absorption of the different ions in relation to pH was also determined, in order to study possible interactions between the H ion and the three other ions.

## II. MATERIAL AND METHODS

### a) GROWING OF PLANTS

The wheat roots used in this investigation (*Triticum aestivum* L., summer wheat, commercial strain "Peko") were raised from two batches of seed, one of the 1958 harvest (experiments with intact plants) and the other of the 1960 harvest (experiments with excised roots). The percentage of germination was small in the fall of the year of harvest but increased gradually during the winter and spring of the next year. In order to obtain comparable plant material, all plants were grown in an air-conditioned room (temperature 25° C, relative humidity 60–70 %).

#### *Intact plants*

A quantity of grains was disinfected in a 1 %  $\text{HgCl}_2$  solution for two minutes and then rinsed in running tap water for about 20 minutes. Next, the grains were washed in well-aerated distilled water for 24 hours. Then 150 grains showing signs of germination were selected and laid out on a stainless steel grid (Fig. 1) in three groups of 50. The grids were placed on 800 ml glass beakers filled with 500 ml culture solution, the level being just below the grid. The nutrient solution (chemicals from B.D.H., quality "Analar", dissolved in distilled water) was the same as that used by WOODFORD and GREGORY (1948), and had the following composition:

$\text{Ca}(\text{NO}_3)_2 \cdot 3 \text{H}_2\text{O}$	0.102 mM	$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	0.098 mM
$\text{KNO}_3$	0.277 mM	$\text{KH}_2\text{PO}_4$	0.151 mM



Fig. 1. Stainless steel grid on a 800 ml glass beaker.

The solution was completed by the addition of Fe (10 mg/l culture solution, as the ferric complex of sequestric acid), of micronutrients (A-Z solution according to HOAGLAND and SNYDER, 1933, 1 ml/l culture solution) and of Mo (0.2 mg/l culture solution, as ammonium molybdate).

The units of 50 plants were held in the dark for 72 hours. During that time the grains were covered with a piece of filter paper moistened by two small strips of filter paper inserted in the solution. The sets were then placed in an artificial-light chamber containing four incandescent lamps (Philips, Comptalux 150 W) and a high-pressure mercury lamp (Philips, HO 2000, 450 W). The heat of the lamps was screened by a one cm thick layer of tap water flowing over a glass pane mounted just below the lamps. The distance between plants and lamps was about 45 cm. The light was switched off daily between 1 a.m. and 9 a.m. The nutrient solution of the plants in the artificial-light chamber was renewed each day, except on Sunday. All beakers were aerated continuously and rigourously.

In spite of the constancy of the growing conditions the fresh weight of the roots per set of plants varied within rather wide limits (from about 5.5 to 9.5 g).

When the plants were 14 days old the three units as such were transferred to the continuous flow apparatus.

#### *Excised roots*

In many respects the method of culturing was similar to that described by ULRICH (1941) and JACOBSON *et al.* (1950) for barley. Fifty grams of the grain were disinfected and rinsed in flowing tap water as described above, and then washed in well-aerated demineralized water for 24 hours. They were next spread on a stainless steel screen (size 30 × 25 cm) covered with a piece of coarse gauze, two edges of which were in contact with the solution. The screen was supported by a perspex frame and the whole placed in a plastic tray filled with four litres of  $2 \times 10^{-4}$  M  $\text{CaSO}_4$  solution. The level of the solution was kept at about 1.5 cm under the screen. In order to keep the grains sufficiently moist, a second piece of gauze was spread

on top of them, again with two edges in the culture solution. The solution was continuously aerated.

The plants were allowed to grow for 6 days in the dark. During that time the  $\text{CaSO}_4$  solution was renewed twice. Seedlings raised in this manner are typical low-salt plants (HOAGLAND and BROYER, 1936).

About 45 minutes before the start of the experiment the roots were excised just below the gauze and washed thoroughly for 30 minutes in three changes of aerated, demineralized water of about three liters each. To remove adhering water, the roots were wrapped in gauze and centrifuged for five minutes in a A.E.G. basket centrifuge (radius basket 12 cm, velocity about 600 rpm).

Portions of 2 to 5 g were weighed to an accuracy of 0.01 g and transferred to the apparatus.

#### b) CONTINUOUS FLOW APPARATUS

The continuous flow apparatus used in this investigation (Fig. 2<sup>1</sup>) was a simplified modification of the apparatus designed by VAN DEN HONERT (1933) for his experiments on phosphate absorption by sugar cane. The experimental solution dripped from a Mariotte flask A

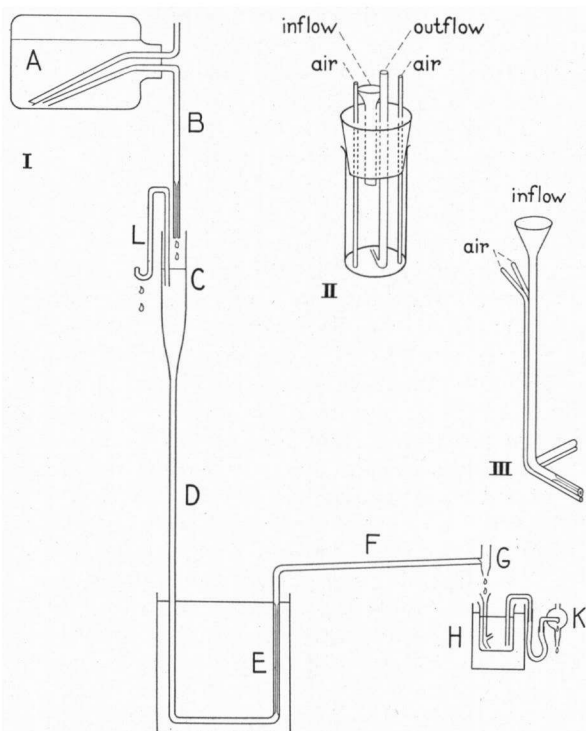


Fig. 2. Continuous flow apparatus. I. General view. Not drawn to scale. II. Root vessel specially designed for experiments with excised roots. III. Detail: Inflow element specially designed for experiments with intact plants.

lying on a stand about two meters high, through a glass tube B into a wider tube C. In order to reduce the rate of flow, tube B terminated in a 5 to 7 cm long capillary.

From the wide tube C the experimental solution flowed through D into the long capillary tube E and from there through F into a wider vertical tube G open at both sides. From G it dripped into the root vessel H. By keeping the rate of supply from the Mariotte flask to the wide tube C somewhat higher than the rate of flow through the system C-D-E-F-G and removing the excess supply by means of an overflow siphon L, the level in C was kept constant. The temperature of the solution flowing through the capillary tube E was kept constant by a water-bath, and was held at 26° C in all experiments.

The rate of flow in the apparatus depended on two factors:

1) The difference in height between the level of the solution in tube C and the dripper G. In order to make the rate of flow as independent as possible of small fluctuations in the level in C, this difference in height was rather large (about 75 cm). Within certain limits the rate of flow could be adapted to needs by moving the adjustable overflow siphon L upward or downward.

2) The length and width of the capillary tube E. These dimensions were chosen in such a way that with the siphon L in its intermediary position the rate of flow in the apparatus amounted to about 100 ml/h.

The height of the level of the solution in the root vessel for intact plants and excised roots (glass beakers of 800 ml and 250 ml respectively) was adjusted by means of the overflow siphon K in such a way that the volume of the solution in the root vessels amounted to about 500 ml and 85 ml respectively.

In order to mix the entering solution with the solution already present in the root vessel, in the experiments in intact plants the solution dripping from G flowed through a tube which was funnel-shaped at its upper end. This tube ended at the bottom of the root vessel in two 1 cm long capillaries just over the outlets of two air tubes (Fig. 2<sup>III</sup>). In the experiments with excised roots the entering solution dripped into the root vessel via a short, funnel-shaped tube which was kept in place by means of a perforated rubber stopper together with two air tubes and the overflow siphon (Fig. 2<sup>II</sup>). A special device for mixing the entering solution with the solution already present did not appear necessary because of the small volume of the solution in the root vessel and the rigorous aeration. The root vessel was placed in a water thermostat set at the required temperature.

As a result of absorption by the roots, the concentration of ions will be lower in the solution discharged from the root vessel than in the entering solution. Depending upon the rate of flow and the volume of the solution in the root vessel, a steady state will be established after a given time.

According to BECKING (1956) the time required to reach a steady state with no roots in the root vessel is:

$$t = \frac{A}{v} \cdot \ln \frac{E - c_0}{E - c}$$

in which  $A$  = volume of the solution in the root vessel,  $v$  = rate of flow of the entering solution,  $E$  = concentration of the entering solution,  $c_0$  = initial concentration in the root vessel, and  $c$  = concentration in the root vessel at time  $t$ .

If  $A = 500$  ml and  $v = 100$  ml/h (the average conditions in the experiments with intact plants) then 16.1 hours are required to reach the steady state for 96 % ( $c = 0.96 E$ ). In the experiments with excised roots, the average values for  $A$  and  $v$  being 85 ml and 120 ml/h respectively, this time is only 2.3 hours. According to Becking, the presence of ion-absorbing roots always accelerates the establishment of the steady state. In connection with these factors the duration of the experiments was fixed for intact plants at about 18 hours and for excised roots at about 2.3 hours.

### c) EXPERIMENTAL TECHNIQUE

The  $\text{NH}_4$ , K, and Na salts used in this investigation (B.D.H. "Analar") were dissolved in distilled water (experiments with intact plants) or demineralized water (experiments with excised roots). The anion was phosphate in all cases. No other salts were added.

To increase the buffer capacity, the experimental solution contained the organic base trishydroxymethylaminomethane at a concentration of 4.4 mM/l. By adding some phosphoric acid, the pH of the experimental solution was adjusted at such a value that the pH in the root vessel, after establishment of the steady state, had a value between 5.7 and 6.5. The average pH in the various series of experiments varied from 5.9 to 6.0 for intact plants and from 5.9 to 6.3 for excised roots. This method was also used in the experiments in which the absorption rate was studied in relation to pH (pH range from about 4.5 to 8.3). Unless otherwise stated, the temperature in the root vessel was maintained at 20° C. During the experiments the intact plants were exposed to two incandescent lamps (Philips, Comptalux 150 W) at a distance of about 80 cm.

At the end of each experiment the rates of supply and discharge of the solution in the root vessel were determined by measuring the time required to fill a 50 ml graduated flask. In the experiments with intact plants the rate of outflow always was somewhat lower than the rate of inflow, owing to transpiration by the plants and evaporation of the solution. The difference seldom amounted more than 2 ml/h, however. With excised roots the rates of inflow and outflow differed much less, of course.

If the concentration of the samples in the graduated flasks are known, the rate of absorption can be calculated as follows:

$$\begin{array}{cccccc} \text{rate of} & \text{rate of} & \text{concentration of} & \text{rate of} & \text{concentration of} \\ \text{uptake} = & \text{inflow} \times & \text{supplied solution} & - & \text{outflow} \times & \text{discharged solution} \\ (\text{me/h}) & (\text{l/h}) & (\text{me/l}) & & (\text{l/h}) & (\text{me/l}) \end{array}$$

In order to check the steady-state condition, in a number of experiments a second sample was taken later (for intact plants 2 to 3 hours, for excised roots about 30 minutes) for comparison with the first sample.

In the experiments with intact plants a steady-state condition for  $\text{NH}_4$  could only be reached if, about 6 hours before the start of the experiment, the nutrient solution was replaced by a solution of ammonium phosphate at a concentration of 2.0 me/l. At the start of the experiment the root vessel was filled with an ammonium phosphate solution of 1.4 me/l. For the sake of the comparability of the results of the different series of experiments, the same procedure was followed in the series where K was the only ion to be absorbed. To avoid the presence of interfering quantities of  $\text{NH}_4$  after establishment of the steady state, the concentration of ammonium phosphate in the root vessel at the start of the experiment was only 0.4 me/l in this K series. With respect to the K absorption, no difficulties arose with regard to the steady-state condition.

In the experiments with excised roots, to the contrary, no special pre-treatment was necessary to reach a steady state in the  $\text{NH}_4$  absorption. At the start of each experiment the root vessels were filled with a solution identical with the supply solution. In a few experiments, very small deviations from the steady-state condition appeared. The K absorption rate, however, showed a slight increase during the experimental period in a number of experiments. In general, this increase appeared to be somewhat higher at high than at low pH values. At a pH of 6.0 the deviation from the steady-state condition is small, so that the relation between absorption rate and concentration was not affected. The K absorption rate, to the contrary, is therefore perhaps somewhat more dependent on pH than the experimental results suggest. The Na absorption created no difficulties with regard to the establishment of the steady-state condition.

Portions of 2 to 5 g of excised roots were used per experiment, according to the rate of absorption to be expected. The experimental results for one batch of roots were found to be highly reproducible. In spite of the constancy of the growing conditions, the capacity for absorption of different batches of roots sometimes varied widely, however. To avoid the spread in the experimental data owing to this variation in absorption capacity, the "standard technique" which VAN DEN HONERT (1933) used in his experiments on the phosphate absorption of sugar cane was applied here. Unless otherwise stated, in all series of experiments for each batch of roots the "unit of absorption rate" was determined, i.e. the rate of absorption of one ion or a combination of ions under standard conditions of concentration, temperature, and pH. The absorption rate of the same ion or combination of ions by roots from the same batch under other conditions is expressed as a fraction of this "unit of absorption rate":

$$\text{relative rate of absorption} = \frac{\text{absorption rate/h-kg fr. wt. not under standard conditions}}{\text{absorption rate/h-kg fr. wt. under standard conditions}}$$

In a few series of experiments the rate of absorption of one ion is expressed in the "unit of absorption rate" determined by another ion.

The standard conditions, varying according to the kind of experi-



ment, will be given where required in each series of experiments separately. In the graphs, a circle enclosing a dot represents the rate of absorption obtained under the standard conditions in the relevant series of experiments. A dotted line indicates that the spread in the experimental data is too large to plot a reliable curve.

The experimental results obtained in intact plants are expressed as the rate of absorption/h-unit of 50 plants.

#### d) ANALYTICAL METHODS

The  $\text{NH}_4$  concentration was determined according to the Nessler colorimetric method as used by ALLPORT (1947) (cf. BECKING, 1956). The measurements of colour intensity were performed at a wavelength of  $440\text{ m}\mu$  by means of a Unicam absorptiometer (Model SP 400). For the determination of K and Na, a flame photometer (Beckman, Model Du) was used, adjusted to a wave length of  $767\text{ m}\mu$  and  $589\text{ m}\mu$  respectively.

### III. EXPERIMENTAL RESULTS

#### a) INTERACTIONS IN THE ABSORPTION OF $\text{NH}_4$ , K, AND Na AS AFFECTED BY CONCENTRATION

The absorption curves of  $\text{NH}_4$ , K, and Na in the absence of each other will be described first. The results of the experiments in which the mutual interactions in the absorption of  $\text{NH}_4$ , K, and Na were investigated in three combinations of two ions each, will then be given. Two series of experiments were run with the same combination of ions: in one series the concentration of one ion was varied and the other was held constant, and in a second series these conditions were reversed. Lastly, in a single series the Na absorption rate in the presence of  $\text{NH}_4$  and K together was compared with that in the presence of these ions separately.

The  $\text{NH}_4$ -K interaction was studied in intact plants. The series of experiments on the two other interactions were done in excised roots. To look into the question of the extent to which the two sets of results may be compared qualitatively, in a few cases the same relation was investigated in intact plants and in excised roots.

##### 1) *Absorption of $\text{NH}_4$ in relation to the $\text{NH}_4$ concentration in the absence of K and Na (intact plants). Fig. 3*

The relation found between the rate of absorption and concentration to about  $0.2\text{ me/l}$  can be represented by a curve resembling a Langmuir adsorption isotherm. At low concentrations a very strong dependence on concentration appears, which gradually passes into almost complete independence at higher amounts of  $\text{NH}_4$ . Above a concentration of about  $0.2\text{ me/l}$ , the curve diverges from the Langmuir relation; in spite of the large spread the results suggest, as will also be shown in some other series of experiments, a small decrease in absorption rate followed by a further rise which appears to be proportional to the concentration in the range studied.

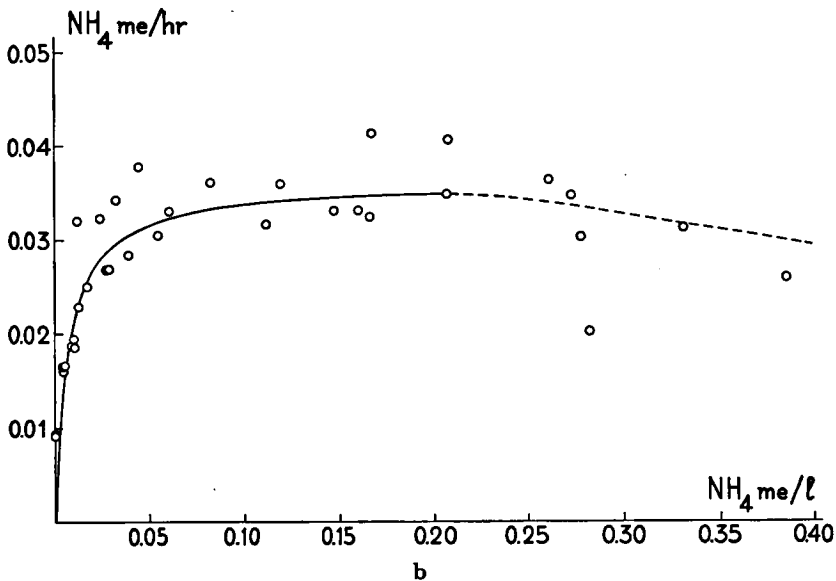
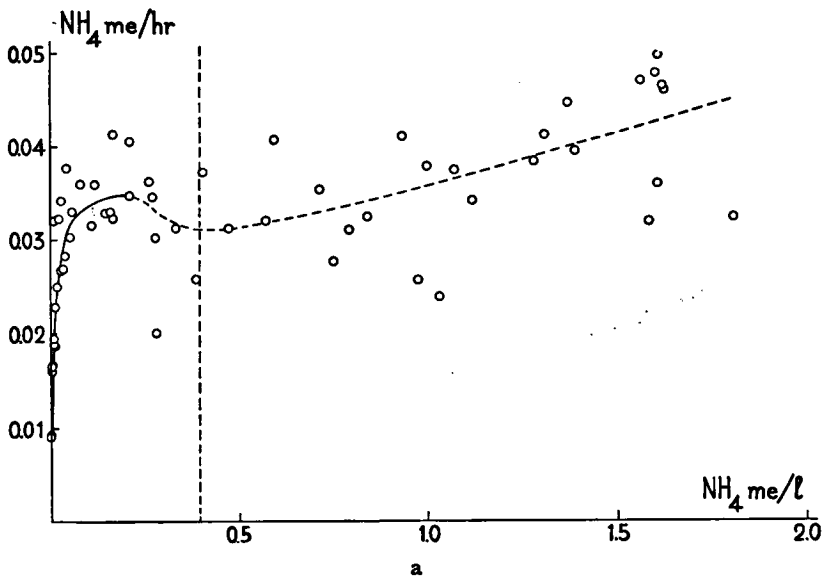


Fig. 3. a. Absorption of  $\text{NH}_4$  in the absence of K and Na in relation to  $\text{NH}_4$  concentration (intact plants); b. The same as a, but with the concentration given on a larger scale to show the initial part in detail.

- 2) *Absorption of K in relation to the K concentration in the absence of  $\text{NH}_4$  and Na (intact plants and excised roots). Figs. 4 and 5*

In the experiments in intact plants the rate of the K absorption becomes also at low concentrations (though not as low as for  $\text{NH}_4$ ), more or less independent of concentration (Fig. 4). As for  $\text{NH}_4$ , the K curve does not flatten wholly at high concentrations but shows another rise proportional to the concentration in the range studied. In the concentration range from about 0.7 to 1.2 me/l the data again suggest a slight dip in the curve which reflects a small decrease of the uptake rate.

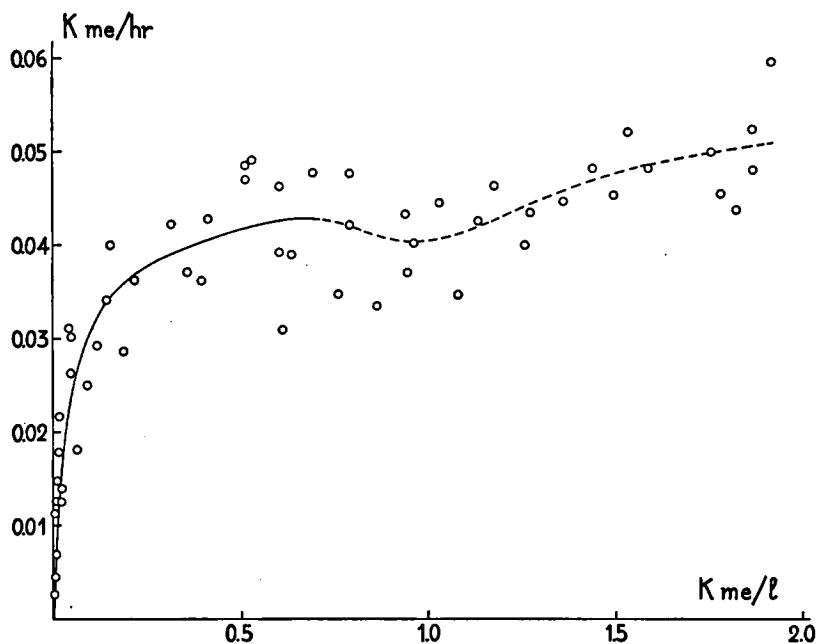


Fig. 4. Absorption of K in the absence of  $\text{NH}_4$  and Na in relation to K concentration (intact plants).

In excised roots the rate of the K absorption was determined in a much smaller concentration range (Fig. 5). It is striking that even at much lower concentrations than is the case for intact plants, the absorption rate is practically independent of concentration. Here too, the curve does not flatten markedly at higher concentrations.

- 3) *Absorption of Na in relation to the Na concentration in the absence of  $\text{NH}_4$  and K (excised roots). Fig. 6*

Fig. 6 shows that at least in excised roots the Na absorption rate only becomes more or less independent of concentration at much higher concentrations when compared with K. The Na curve seems to flatten at higher concentrations.

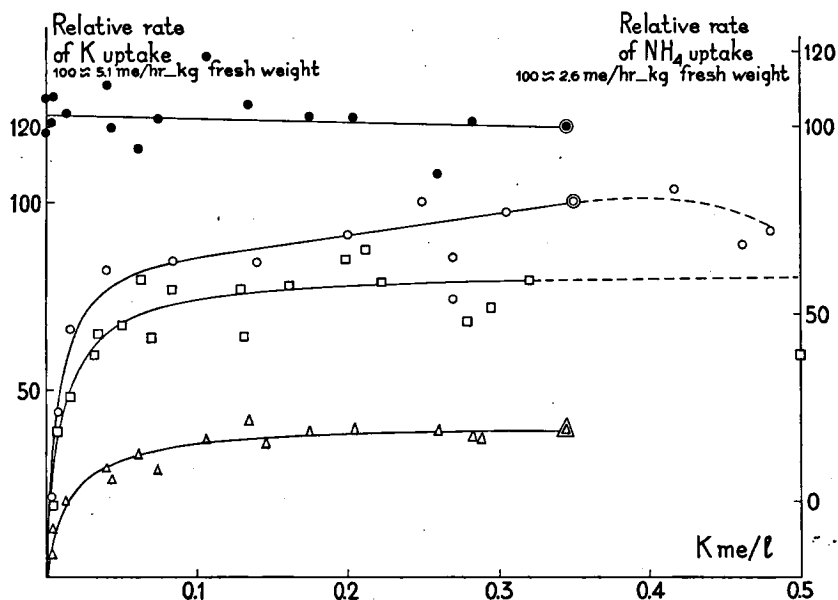


Fig. 5. Absorption of K in the absence of  $\text{NH}_4$  and Na at a pH of about 6.3 ( $\circ$ ) and about 4.7 ( $\square$ ), and absorption of K and  $\text{NH}_4$  present together ( $\triangle$  and  $\bullet$  respectively) at a constant  $\text{NH}_4$  concentration of about 0.11 me/l in relation to K concentration (excised roots). The "unit of absorption" for the K absorption at a pH of about 4.7 is that for the K absorption in the absence of  $\text{NH}_4$  and Na at a pH of about 6.3 ( $\circ$ ).

#### $\text{NH}_4$ -K INTERACTIONS

- 4) *Absorption of  $\text{NH}_4$  and K in relation to the  $\text{NH}_4$  concentration at a constant K concentration (intact plants). Fig. 7*

The presence of K at a constant concentration (about 0.34 me/l) does not influence the  $\text{NH}_4$  absorption rate in the range studied (compare Fig. 3). The K absorption rate, however, is decreased by  $\text{NH}_4$  to about 40 % as compared with this rate in the absence of  $\text{NH}_4$ . The decrease of the K absorption rate and the rate of the  $\text{NH}_4$  absorption show the same relation to the  $\text{NH}_4$  concentration, the  $\text{NH}_4$  absorption rate being always somewhat larger than the decrease in the K absorption rate. Like the  $\text{NH}_4$  absorption rate in the absence of K, the experimental results suggest a small decrease in the  $\text{NH}_4$  absorption rate at concentrations higher than about 0.15 me/l in the range studied.

- 5) *Absorption of K and  $\text{NH}_4$  in relation to the K concentration at a constant  $\text{NH}_4$  concentration (intact plants and excised roots). Figs. 8 and 5*

Unless otherwise stated, the  $\text{NH}_4$  concentration was always about 0.11 me/l. In agreement with the results of the preceding experimental series, even high concentrations of K have very little influence on

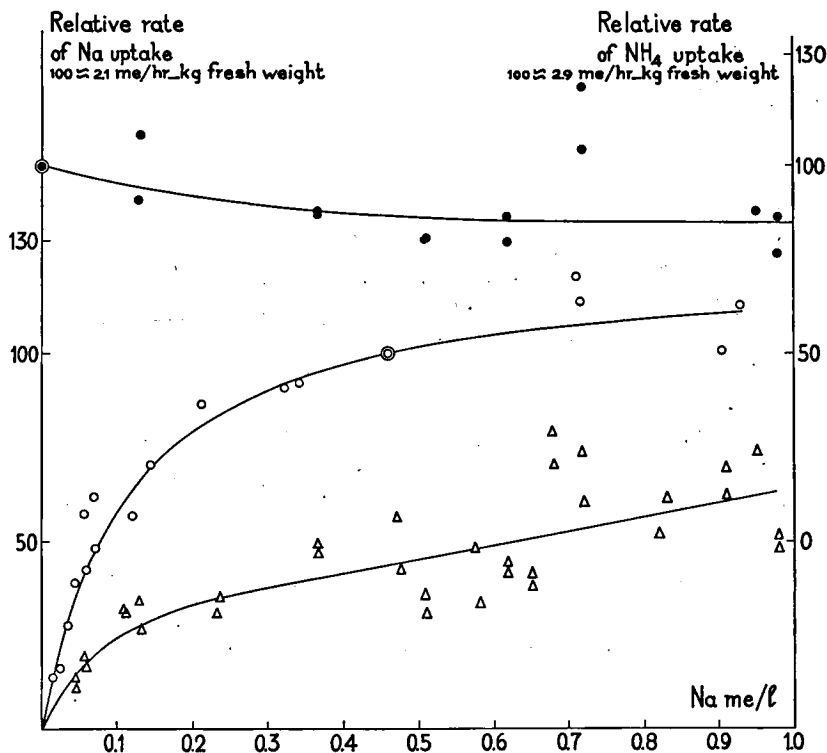


Fig. 6. Absorption of Na in the absence of  $\text{NH}_4$  and K ( $\circ$ ) and absorption of Na and  $\text{NH}_4$  present together ( $\triangle$  and  $\bullet$  respectively) at a constant  $\text{NH}_4$  concentration of about 0.10 me/l in relation to Na concentration (excised roots). The "unit of absorption" for the Na absorption at a constant  $\text{NH}_4$  concentration is that for the Na absorption in the absence of  $\text{NH}_4$  and K ( $\circ$ ).

the  $\text{NH}_4$  absorption rate by intact plants (Fig. 8). The rate of the K absorption in the concentration range studied is to the contrary, — dependent on concentration — 40–60 % lower than when no  $\text{NH}_4$  is added (compare Fig. 4). The relation between the rate of absorption and the concentration at low concentrations is practically the same in both cases. The K absorption curve does not flatten again at higher concentrations, but shows a rise practically proportional to the concentration in the range studied. This increase is about the same as that shown by the K curve in the absence of  $\text{NH}_4$ , but here the curve shows no dip reflecting a decrease in absorption rate.

The experiments with excised roots give the same result (Fig. 5). The K absorption rate is again 60–70 % lower than that in the absence of  $\text{NH}_4$ , whereas the relation to the concentration is about equal in both cases. However, the curve shows a distinct flattening at higher amounts of K. As in intact plants, the  $\text{NH}_4$  absorption rate is hardly affected at increasing K concentrations.

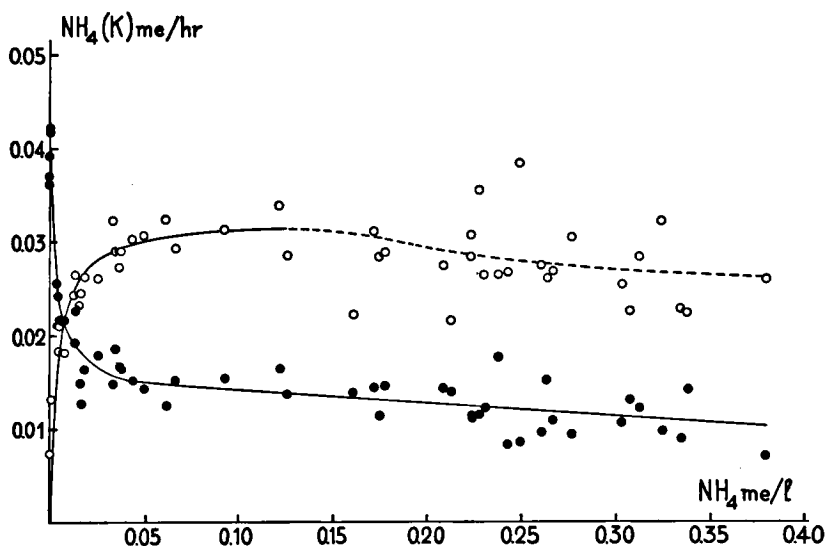


Fig. 7. Absorption of  $\text{NH}_4$  and K present together (○ and ● respectively) at a constant K concentration of about 0.34 me/l in relation to  $\text{NH}_4$  concentration (intact plants).

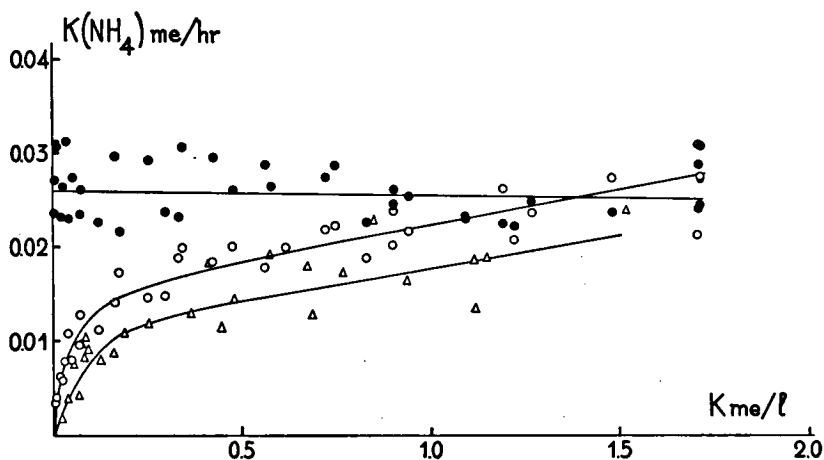


Fig. 8. Absorption of K and  $\text{NH}_4$  present together (○ and ● respectively) at a constant  $\text{NH}_4$  concentration of about 0.11 me/l and absorption of K from a mixed solution of K and  $\text{NH}_4$  (△) at a constant  $\text{NH}_4$  concentration of about 1.6 me/l in relation to K concentration (intact plants).

The inhibition of the K absorption by intact plants is somewhat stronger at a much higher  $\text{NH}_4$  concentration (about 1.6 me/l) than in the presence of about 0.1 me/l  $\text{NH}_4$  (Fig. 8). The inhibition at this high  $\text{NH}_4$  concentration is relatively stronger at low K concentrations. For higher amounts of K the absorption curve rises almost equally in both cases. It was not possible to determine the  $\text{NH}_4$  absorption rate accurately here, the quantity absorbed being too small with respect to the total amount present.

#### $\text{NH}_4$ -Na INTERACTIONS

- 6) *Absorption of  $\text{NH}_4$  and Na in relation to the  $\text{NH}_4$  concentration at a constant Na concentration (excised roots). Fig. 9*

The  $\text{NH}_4$  absorption rate in the presence of Na at a constant concentration (about 0.49 me/l) shows the familiar picture once more: a strong increase at low concentrations gradually passes into almost complete absence of dependence on concentration at higher  $\text{NH}_4$  concentrations. At least at the standard  $\text{NH}_4$  concentration (0.116 me/l) used in this experiment, the  $\text{NH}_4$  absorption rate is hardly affected by Na.

The Na absorption behaves like that of K in series 4, the decrease of the Na absorption rate caused by  $\text{NH}_4$  being about 50 % of the Na absorption rate in the absence of  $\text{NH}_4$ . The decrease and the  $\text{NH}_4$

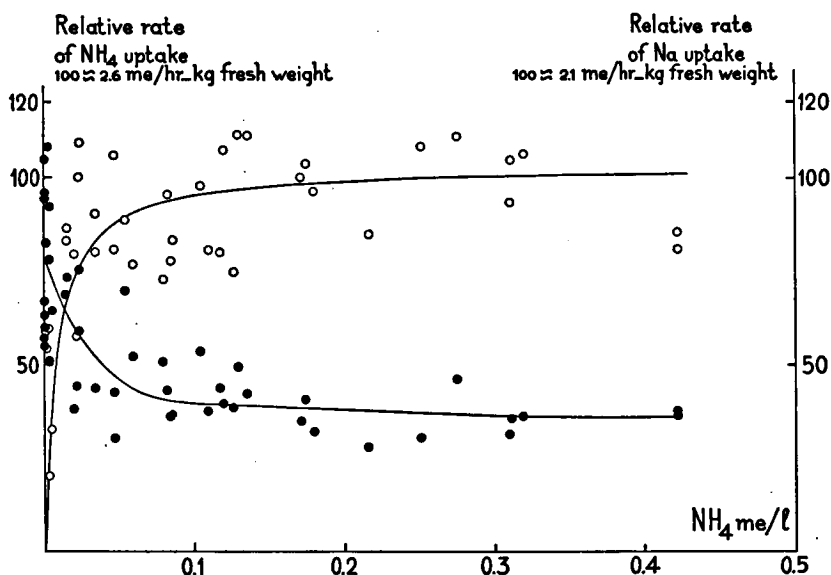


Fig. 9. Absorption of  $\text{NH}_4$  and Na present together (○ and ● respectively) at a constant Na concentration of about 0.49 me/l in relation to  $\text{NH}_4$  concentration (excised roots). The "unit of absorption" for the  $\text{NH}_4$  as well as the Na absorption is the  $\text{NH}_4$  absorption in the absence of K and Na at a concentration of about 0.12 me/l.

absorption rate again show the same relation to the  $\text{NH}_4$  concentration. The decrease of the Na absorption rate is about  $2\frac{1}{2}$  times smaller than the  $\text{NH}_4$  absorption rate at the Na concentration used in this series.

7) *Absorption of Na and  $\text{NH}_4$  in relation to the Na concentration at a constant  $\text{NH}_4$  concentration (excised roots). Fig. 6*

In agreement with the experimental results in the preceding series, the Na absorption rate at a constant  $\text{NH}_4$  concentration (about 0.10 me/l) is strongly decreased (depending on concentration, 40–60 % of the uptake rate in the absence of  $\text{NH}_4$  in the range studied). The shape of this Na curve is not quite the same as that in the absence of  $\text{NH}_4$ . The dependence on concentration is the same in both cases to a concentration of about 0.3 me/l. Above this concentration the resemblance disappears. The experimental results suggest that at this Na concentration, the Na curve determined at a constant  $\text{NH}_4$  concentration gradually shows a small rise which is proportional to concentration in the range studied. The  $\text{NH}_4$  absorption rate is hardly, if at all, affected by increasing Na concentrations.

#### K-Na INTERACTIONS

8) *Absorption of K and Na in relation to the K concentration at a constant Na concentration (excised roots). Fig. 10*

The rate of the K absorption in the presence of Na at a constant concentration (about 0.49 me/l) is 15–25 % lower than that in the

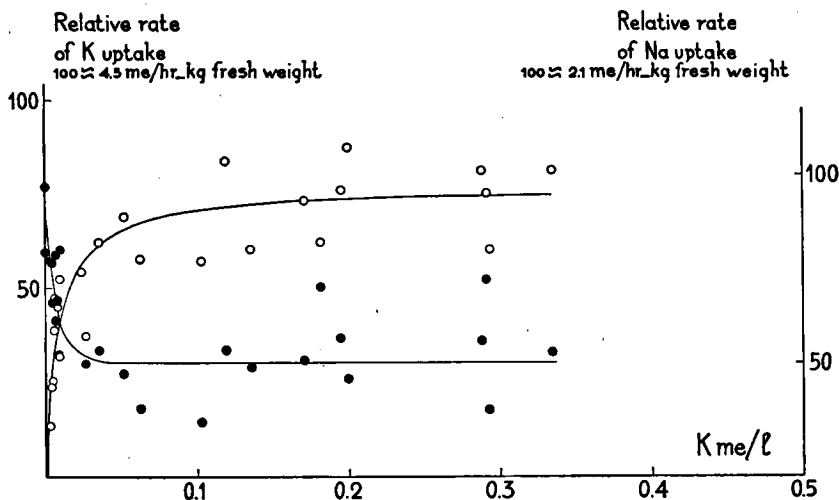


Fig. 10. Absorption of K and Na present together (○ and ● respectively) at a constant Na concentration of about 0.49 me/l in relation to K concentration (excised roots). The "unit of absorption" for the K as well as the Na absorption is the K absorption in the absence of  $\text{NH}_4$  and Na at a concentration of about 0.35 me/l.



absence of Na in the range studied (compare Fig. 5). Na does not affect the relation between the K uptake rate and the concentration at low K concentrations. However, the K curve seems to flatten at higher concentrations, in contrast to the curves found in the absence of  $\text{NH}_4$  and Na (series 2). The Na uptake rate simultaneously determined is decreased by K to practically the same level as by  $\text{NH}_4$ , the decrease of the Na absorption rate and the rate of the K absorption showing about the same relation to the K concentration. As for the  $\text{NH}_4$ -Na interaction, the K absorption rate at this Na concentration is much larger (about 4 times) than the decrease of the rate of the Na absorption.

9) *Absorption of Na and K in relation to the Na concentration at a constant K concentration (excised roots). Fig. 11*

The pattern of the Na absorption rate in relation to concentration in the presence of K at a constant concentration (about 0.06 me/l) is the same as the one obtained when  $\text{NH}_4$  is added. The Na curves found in both series differ very little. The experimental results suggest that K decreases the Na absorption rate somewhat less than  $\text{NH}_4$  (compare Fig. 6). As is the case for the rate of the  $\text{NH}_4$  absorption, increasing the Na concentration has little effect on the K absorption rate.

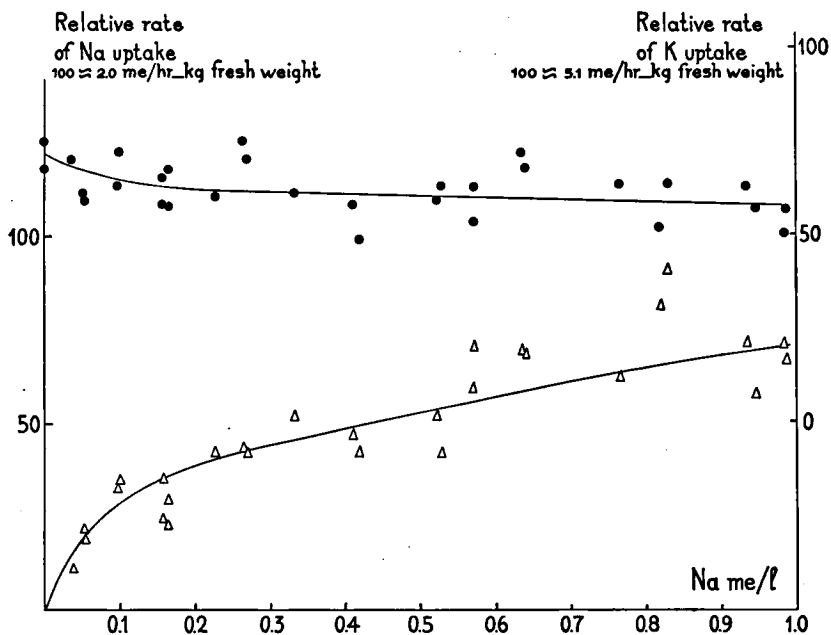


Fig. 11. Absorption of Na and K present together ( $\Delta$  and  $\bullet$  respectively) at a constant K concentration of about 0.06 me/l in relation to Na concentration (excised roots). The "unit of absorption" for the Na as well as the K absorption is the Na absorption in the absence of  $\text{NH}_4$  and K at a concentration of about 0.47 me/l.

10) *Absorption of Na in the presence of  $\text{NH}_4$  and K together (excised roots).*  
Fig. 12 and Table 1

This experiment was performed at only one concentration of Na,  $\text{NH}_4$ , and K (about 0.48, 0.11, and 0.12 me/l respectively). Fig. 12 and Table 1 show the results of five identical experiments, separate batches of roots being used in each experiment. For the sake of comparison, the Na absorption rate in each experiment was determined in the presence of K and  $\text{NH}_4$  separately and together. The Na absorption rates do not differ much in the three cases. In agreement with what was stated above, the rate of the Na absorption in all the experiments appears to be affected somewhat more by  $\text{NH}_4$  than by K (Fig. 12). The experimental results also suggest that the largest

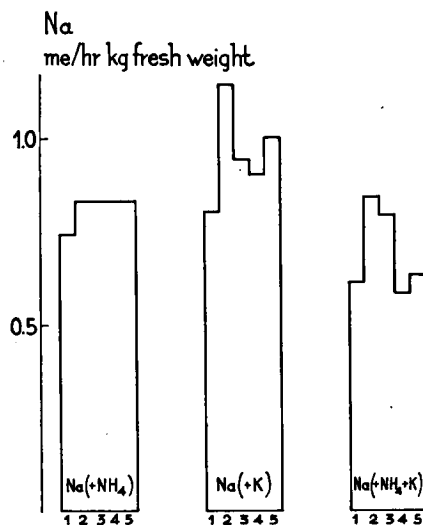


Fig. 12. Absorption of Na in the presence of  $\text{NH}_4$  and K separately and together at a constant Na,  $\text{NH}_4$ , and K concentration of about 0.48, 0.11, and 0.12 me/l respectively (excised roots). (For the sake of comparison, the Na absorption in the absence of  $\text{NH}_4$  and K is about 1.9 me/h-kg fr. wt. at the concentration used here.)

decrease of the Na absorption rate appears if  $\text{NH}_4$  and K are added together.

Table 1 shows the  $\text{NH}_4$  and K absorption rates in the three cases determined simultaneously. These results seem to suggest that the rate of the  $\text{NH}_4$  absorption may be somewhat lower if K and Na are added together than in the presence of Na only.

Summarizing the essentials of the experimental results of the preceding series, it may be said that:

I. The K absorption rate is strongly decreased by  $\text{NH}_4$  and the Na absorption rate by  $\text{NH}_4$  and K. Conversely, K has very little

TABLE 1.

Rates of absorption of  $\text{NH}_4$  and K in five identical experiments using solutions of  $\text{NH}_4 + \text{Na}$ ,  $\text{K} + \text{Na}$ , and  $\text{NH}_4 + \text{K} + \text{Na}$  at a constant  $\text{NH}_4$ , K, and Na concentration of about 0.11, 0.12, and 0.48 me/l respectively. (For the sake of comparison it should be mentioned that the  $\text{NH}_4$  and K uptake rates in the absence of other cations amount to about 2.9 and 3.9 me/h–kg fr. wt. respectively.).

	Experiment (batch of roots)	Cations present in the experimental solution		
		$\text{NH}_4 + \text{Na}$	$\text{K} + \text{Na}$	$\text{NH}_4 + \text{K} + \text{Na}$
Rate of $\text{NH}_4$ uptake (me/h–kg fr. wt.)	1	2.84		2.04
	2	3.19		2.65
	3	3.15		2.26
	4	2.32		2.40
	5	2.60		2.75
Rate of K uptake (me/h–kg fr. wt.)	1		3.14	1.41
	2		3.78	1.71
	3		3.10	1.29
	4		2.80	1.43
	5		3.23	1.50

effect on the  $\text{NH}_4$  absorption rate. The same is true for Na with regard to the absorption rates of  $\text{NH}_4$  and K.

II. The inhibiting ion, even at high concentrations, does not completely inhibit absorption of the inhibited ion.

III. The decrease of the absorption rate of the inhibited ion and the rate of absorption of the inhibitor show the same relation to the concentration of the inhibiting ion. The absolute magnitude of the decrease, however, does not always equal the magnitude of the absorption rate of the inhibiting ion.

IV. In spite of a clear effect on the absolute magnitude of the rate of absorption, the nature of the relation between rate of absorption and concentration of the inhibited ion is not influenced by the inhibiting ion.

b) INTERACTIONS IN THE ABSORPTION OF  $\text{NH}_4$  AND K BY ROOTS DIFFERING IN PRE-TREATMENT

11) *Interactions in the absorption of  $\text{NH}_4$  and K by roots differing in nitrogen content (excised roots).* Fig. 13

In this experimental series the decrease of the rate of the K absorption by  $\text{NH}_4$  was determined in roots differing in  $\text{NH}_4$  absorption capacity owing to differences in their nitrogen content.

High-nitrogen roots were obtained by growing the plants in a  $\text{Ca}(\text{NO}_3)_2$  solution of 2.0 me/l. Comparable plant material with a low nitrogen content was raised in a solution of  $\text{CaCl}_2$  of 2.0 me/l. For the rest, the method for growing the plants and the experimental technique did not deviate from those described in Chapter II.

The Figure shows the results of four identical experiments. The

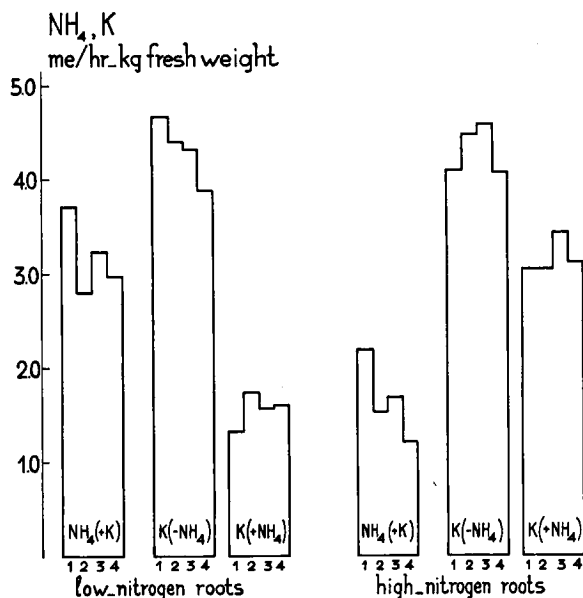


Fig. 13. Interactions in the  $\text{NH}_4$  and K absorption by low- and high-nitrogen roots at a constant  $\text{NH}_4$  and K concentration of about 0.11 and 0.12 me/l respectively (excised roots).

K absorption rate with no addition of  $\text{NH}_4$  and the absorption rates of  $\text{NH}_4$  and K present together were determined in each experiment at only one concentration of  $\text{NH}_4$  and K (about 0.11 and 0.12 me/l respectively) in roots with a high nitrogen content from one batch as well as in low-nitrogen roots, also from one batch.

The  $\text{NH}_4$  absorption rate by the roots grown in the  $\text{Ca}(\text{NO}_3)_2$  solution is about 50 % lower than that of the low-nitrogen roots. On the other hand, the K absorption rate in the absence of  $\text{NH}_4$  is about equal in both cases. The K absorption rate is decreased by  $\text{NH}_4$  much less in roots with a high nitrogen content than in low-nitrogen roots. It is striking that in both cases the ratio between the  $\text{NH}_4$  absorption rate and the decrease of the rate of the K absorption is the same.

c) INTERACTIONS IN THE ABSORPTION OF  $\text{NH}_4$  AND K AND OF  $\text{NH}_4$  AND Na IN DIFFERENT PARTS OF THE ROOT

12) *Interactions in the absorption of  $\text{NH}_4$  and K in different parts of the root (excised roots).* Fig. 14

From about 14 g excised material, each root was divided in two pieces, the length of the top part being about 2.4 cm. The remaining part, varying widely in length, was also divided in two. Any lateral roots were removed. Owing to the time needed to divide all roots

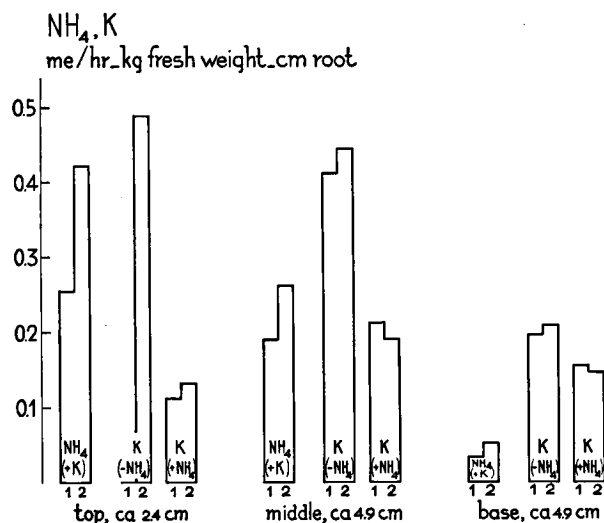


Fig. 14. Interactions in the  $\text{NH}_4$  and K absorption by different parts of the root at a constant  $\text{NH}_4$  and K concentration of about 0.15 and 0.14 me/l respectively (excised roots).

in three parts (about two hours), the average washing time in demineralized water before the start of the experiment was much longer than for the other experimental series. For each part of the root the K absorption rate in the absence of  $\text{NH}_4$  and the rates of absorption of  $\text{NH}_4$  and K present together were again determined at only one  $\text{NH}_4$  and K concentration (about 0.15 and 0.14 me/l respectively). The experiment was carried out in duplicate, separate batches of roots being used in each experiment. The total weight of the top parts of the roots is small (about 2.6 g). The absorption rate to be measured is consequently small, so that the results concerning the top parts of the roots are not very reliable. In addition, the determination of the K absorption rate in the absence of  $\text{NH}_4$  in the top part failed in the first experiment. In the Figure the absorption rate is expressed in me/h-kg fr. wt.-cm root, i.e. the amount absorbed per cm root in one hour by the total amount of top, middle and basal parts respectively of one kg roots (fr. wt.).

The capacity of the root to absorb both  $\text{NH}_4$  and K (in the absence of  $\text{NH}_4$ ) decreases from the top to the base of the root. The  $\text{NH}_4$  absorption rate in the basal part is small, while the K absorption rate in the presence of  $\text{NH}_4$  is considerable in spite of a clear decrease brought about by  $\text{NH}_4$ . The ratio " $\text{NH}_4$  absorption rate/decrease K absorption rate" in the three parts is about the same, and amounts to about unity, which is in reasonable agreement with the results of series 4.

13) *Interactions in the absorption of  $\text{NH}_4$  and Na in different parts of the root (excised roots). Fig. 15*

The methods used in this series are the same as those described above for series 12. The Na absorption rate where no  $\text{NH}_4$  was added and the absorption rate of  $\text{NH}_4$  and Na present together were again determined at only one  $\text{NH}_4$  and Na concentration (about 0.12 and 0.50 me/l respectively) in each root part separately. The experiments were again run in duplicate, separate batches of roots being used in

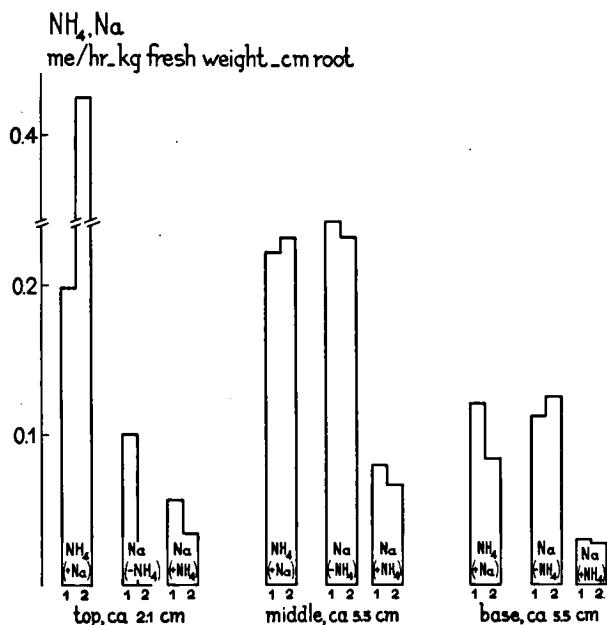


Fig. 15. Interactions in the  $\text{NH}_4$  and Na absorption by different parts of the root at a constant  $\text{NH}_4$  and Na concentration of about 0.12 and 0.50 me/l respectively (excised roots).

each experiment. The results concerning the top part are again not very accurate. In spite of the small Na absorption rate — which a determination is not very accurate at this concentration — the results with respect to the other two parts differ very little in both experiments. In the second experiment the determination of the Na absorption rate in the absence of  $\text{NH}_4$  failed for the top part of the root.

Only the following conclusions are justified: In each part of the root,  $\text{NH}_4$  decreases the Na absorption rate strongly, but the inhibition is never complete. The ratio " $\text{NH}_4$  absorption rate/decrease Na absorption rate" in the middle and basal part of the root is about the same within the limits of the accuracy of the experiment.

d) INTERACTIONS IN THE ABSORPTION OF  $\text{NH}_4$ , K, AND Na AS AFFECTED BY pH

All experiments on this relation were carried out with excised roots. The pH was varied from about 4.5 to 8.3. In six experimental series the pH effect on the absorption rate of  $\text{NH}_4$ , K, and Na separately and combined in pairs of two ions each was determined successively. The  $\text{NH}_4$ , K, and Na concentrations were always about 0.11, 0.34, and 0.48 me/l respectively. These concentrations lie in the concentration range in which the absorption rate is hardly at all affected by changes in concentration, at least at the pH value used in the preceding experiments.

Finally, the relation between the rate of absorption and the concentration of K was determined at a low pH value (about 4.7).

14) *Absorption of  $\text{NH}_4$  in relation to pH in the absence of K and Na.* Fig. 16

The pH affects the  $\text{NH}_4$  absorption rate clearly, the uptake rate being about 30 % lower at pH 4.5 than that at pH 6.0. A rise in pH from about 6.0 to 7.5 causes an increase in the absorption rate of only about 10 %. Above a pH of about 7.5 the experimental results suggest a rather strong rise in absorption rate.

VAN DEN HONERT (1953), VAN DEN HONERT and HOOYMANS (1962), and LYCKLAMA (personal communication) explain this peculiar effect at high pH values as follows. At a rise of the pH in the solution of  $\text{NH}_4$ , increasing amounts of undissociated  $\text{NH}_4\text{OH}$  will be present.

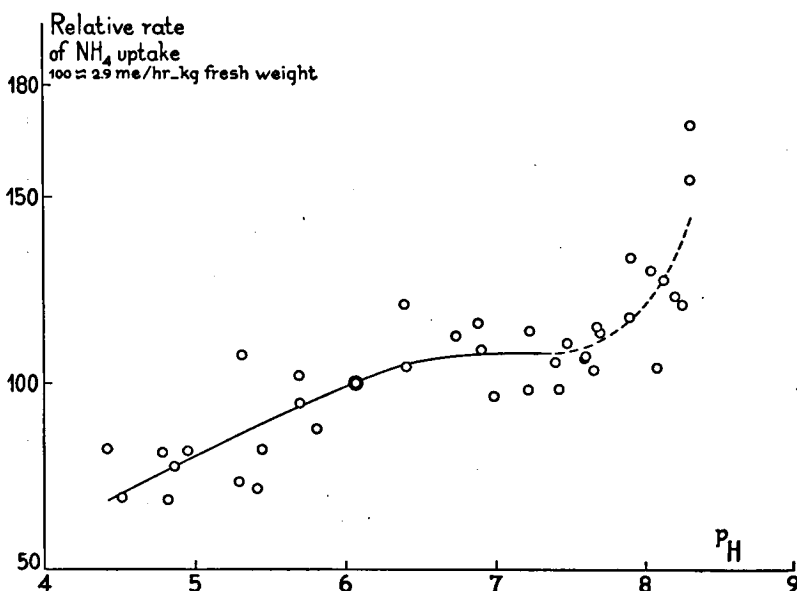


Fig. 16. Absorption of  $\text{NH}_4$  in the absence of K and Na at a constant  $\text{NH}_4$  concentration of about 0.10 me/l in relation to pH (excised roots).

This undissociated  $\text{NH}_4\text{OH}$  is supposed to diffuse into the cells in which it may be fixed by a process which does not limit the rate of absorption.

15) *Absorption of K in relation to pH in the absence of  $\text{NH}_4$  and Na.* Fig. 17

The pH curve for K in the absence of  $\text{NH}_4$  and Na resembles that for  $\text{NH}_4$  in the preceding series. At low pH values a clear decrease in the K uptake rate appears (to about 65 % of the absorption rate at pH 6.0), the absorption rate above a pH of about 6.0 being affected only to a small extent. A strong increase in absorption rate above a pH of about 7.5, as was found for  $\text{NH}_4$ , does not appear here.

As stated above (p. 154), the K absorption rate in a number of experiments deviated slightly from the steady-state condition. This effect being somewhat stronger at high than at low pH values in general, the absorption rate above a pH of about 6.0 is possibly somewhat more strongly dependent on pH than Figure 17 suggests.

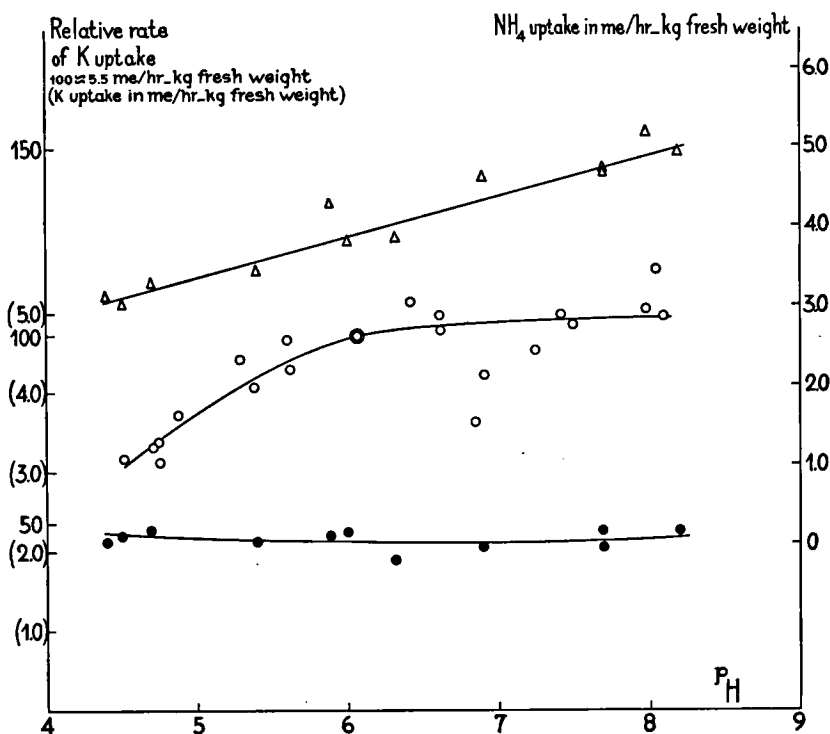


Fig. 17. Absorption of K in the absence of  $\text{NH}_4$  and Na ( $\circ$ ) and absorption of  $\text{NH}_4$  and K present together ( $\Delta$  and  $\bullet$  respectively) at a constant  $\text{NH}_4$  and K concentration of about 0.10 and 0.32 me/l respectively in relation to pH (excised roots).



16) *Absorption of Na in relation to pH in the absence of  $\text{NH}_4$  and K.* Fig. 18

This pH relation differs little from that for  $\text{NH}_4$  and K. At a pH of 4.5 the rate of absorption is about 35 % lower than at a pH of 6.0. In spite of the considerable spread of the experimental data, it seems

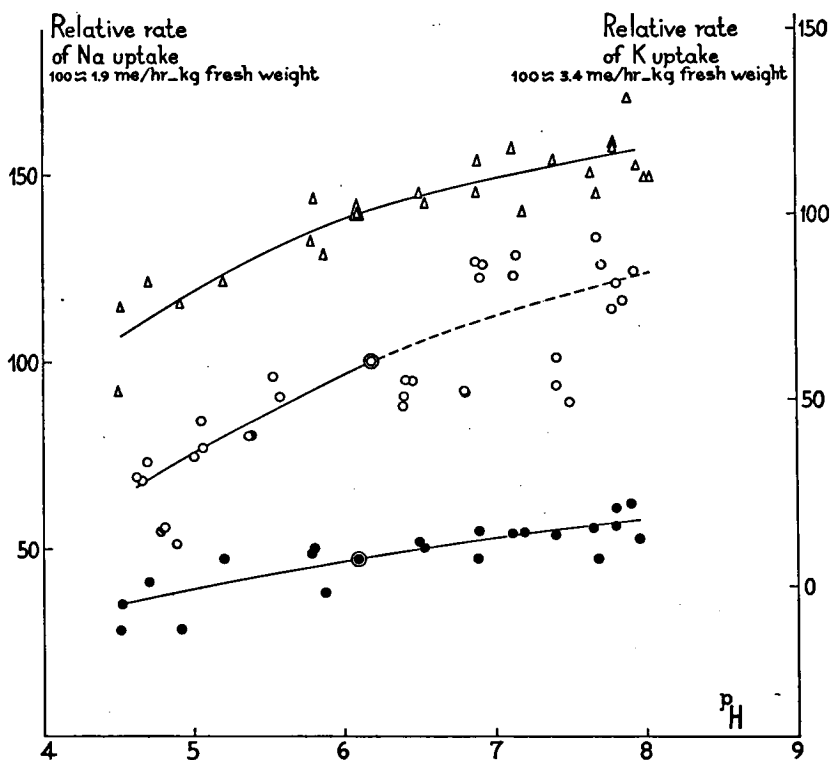


Fig. 18. Absorption of Na in the absence of  $\text{NH}_4$  and K ( $\circ$ ) and absorption of K and Na present together ( $\Delta$  and  $\bullet$  respectively) at a constant K and Na concentration of about 0.36 and 0.48 me/l respectively in relation to pH (excised roots).

likely that above a pH of about 6.0 the absorption rate is somewhat more strongly pH-dependent than for  $\text{NH}_4$  or K in the absence of other cations.

17) *Absorption of  $\text{NH}_4$  and K present together in relation to pH.* Fig. 17

In this series the absorption rate at the different pH values is not expressed as a fraction of the absorption rate under standard conditions as was the case in all other experiments with excised roots. The rate of uptake is here given in me/h-kg fr. wt.

In contrast to the relation between the pH and the absorption rate in the absence of K, the  $\text{NH}_4$  uptake rate in the range studied increases practically proportionally to the pH. Below a pH of about 6.0, the

influence of pH is equal in both cases, but although the  $\text{NH}_4$  absorption rate in the absence of K is almost unaffected by the pH in the range from about 6.0 to 7.5, it seems likely that this does not hold for the relation studied here.

The K absorption rate in the presence of  $\text{NH}_4$  unfortunately again showed a small increase in relation to time. The curve is therefore probably not quite reliable. In spite of this, it seems rather certain that the K absorption rate in the presence of  $\text{NH}_4$  is almost unaffected by pH.

18) *Absorption of  $\text{NH}_4$  and Na present together in relation to pH.* Fig. 19

The pattern of the  $\text{NH}_4$  absorption rate in the presence of Na in relation to pH equals that of  $\text{NH}_4$  in the presence of K within the limits of the accuracy of the experiments. It is not clear to what

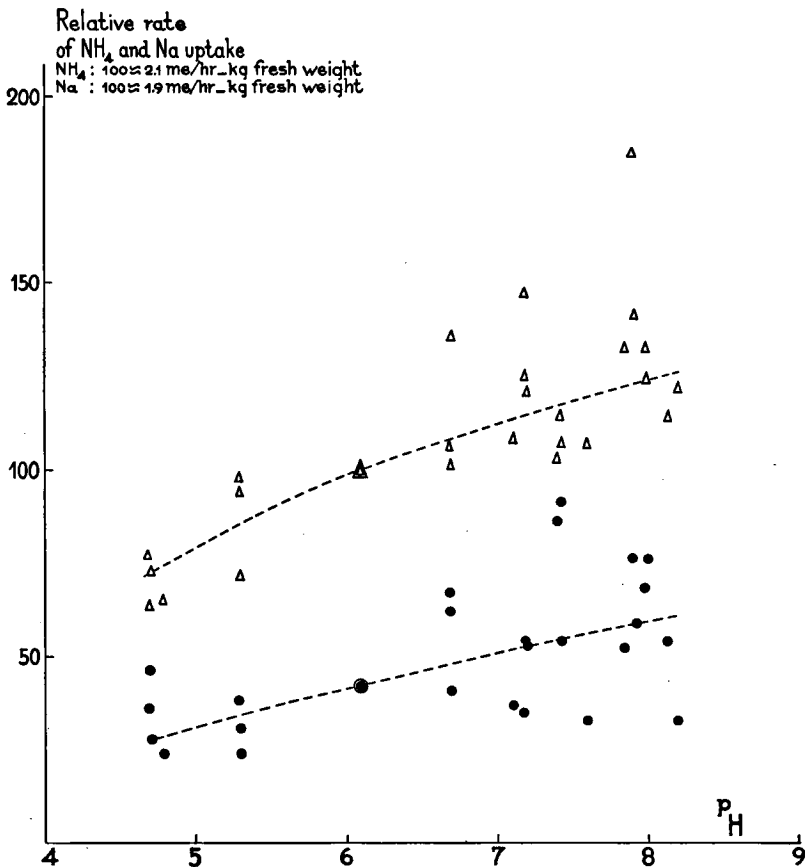


Fig. 19. Absorption of  $\text{NH}_4$  and Na present together ( $\Delta$  and  $\bullet$  respectively) at a constant  $\text{NH}_4$  and Na concentration of about 0.14 and 0.48 me/l respectively in relation to pH (excised roots).

extent the shape of the  $\text{NH}_4$  curve in this series and series 17 is also affected by an extra uptake of undissociated  $\text{NH}_4\text{OH}$  at high pH values.

Considering the spread in the experimental data, the Na absorption rate in the presence of  $\text{NH}_4$  is equally affected by pH whether or not  $\text{NH}_4$  is added.

19) *Absorption of K and Na present together in relation to pH.* Fig. 18

The K absorption rate in the presence of Na is affected by the pH in the range from about 4.5 to 6.0 to the same extent as in the absence of  $\text{NH}_4$  or Na. Above a pH of about 6.0 this agreement disappears. The K uptake rate in the absence of  $\text{NH}_4$  or Na being hardly at all affected by pH, the curve determined here shows a clear rise at higher pH values. For the rest, the same reservation applies here as in series 15 with respect to the small increase in the rate of K absorption in relation to time.

Like  $\text{NH}_4$ , K does not show an influence on the pattern of the Na absorption rate in relation to the pH.

20) *Absorption of K in relation to the K concentration in the absence of  $\text{NH}_4$  and K at a low pH value.* Fig. 5

A pH value of about 4.7 was used throughout this series. The curve has the same shape as most of those encountered in Part a) of this chapter. The absorption rate at all concentrations in the range studied is 10–20 % lower than that determined at a pH value of 6.3. It is striking that the K uptake rate in both series is about equally dependent on concentration. The results of this series, however, suggest that the absorption rate is almost completely independent of concentration at higher concentrations.

e) INTERACTIONS IN THE ABSORPTION OF  $\text{NH}_4$  AND K AS AFFECTED BY TEMPERATURE

All experiments in these series were carried out with excised roots. The temperature was varied from 2.5° C to 34° C. The absorption rate in relation to the temperature was determined successively for K without addition of  $\text{NH}_4$  or Na and for K and  $\text{NH}_4$  present together. The  $\text{NH}_4$  and K concentrations amounted to about 0.10 and 0.31 me/l respectively in all experiments.

21) *Absorption of K in relation to temperature in the absence of  $\text{NH}_4$ .* Fig. 20

Temperatures in the range from about 5° C to 25° C clearly affect the rate of the K absorption, the uptake rate being at 5° C only about 15 % of that at 24° C. It should be observed that in this range the slope of the curve increases at rising temperatures. Because of the spread of the experimental data the curve is difficult to plot above about 27° C. Nevertheless, it may be concluded that above 25° C the temperature affects the K absorption rate only slightly.

22) *Absorption of  $\text{NH}_4$  and K present together in relation to temperature.*  
Fig. 20

In the range from about  $3^\circ \text{C}$  to  $23^\circ \text{C}$ , temperature affects the  $\text{NH}_4$  absorption rate much less than it does the K uptake rate in the absence of  $\text{NH}_4$ . This is shown by the fact that at  $5^\circ \text{C}$  the  $\text{NH}_4$

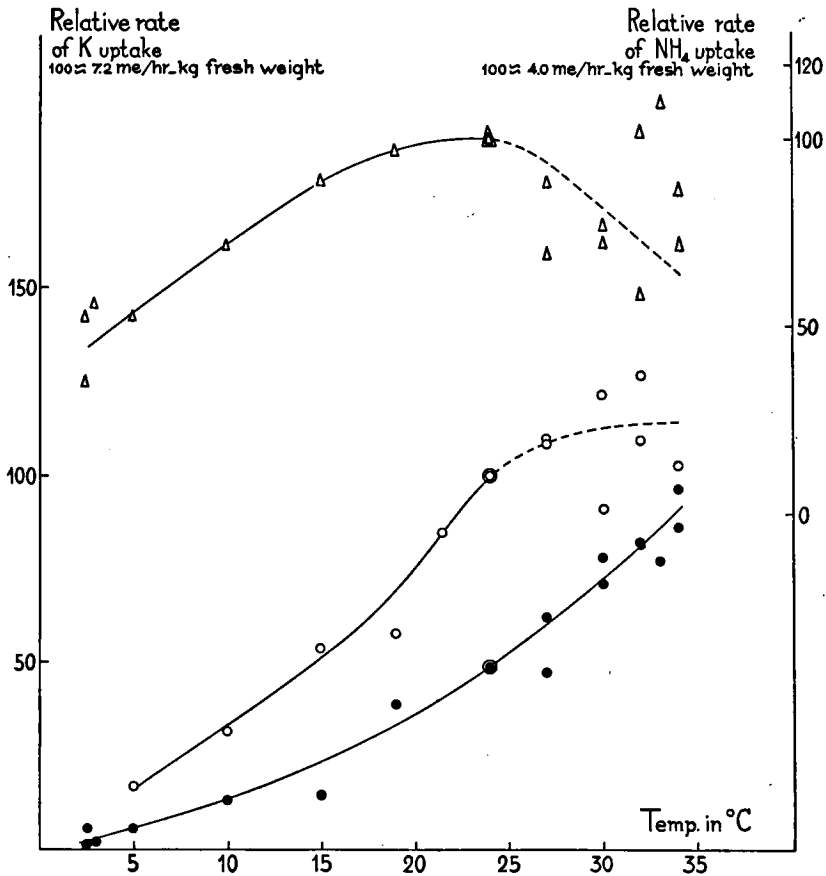


Fig. 20. Absorption of K in the absence of  $\text{NH}_4$  and Na (○) and absorption of  $\text{NH}_4$  and K present together ( $\Delta$  and ● respectively) at a constant concentration of about 0.10 and 0.31 me/l respectively in relation to temperature (excised roots).

absorption rate is only 50 % lower than at  $24^\circ \text{C}$ . Besides, in contrast to the K curve described above, the slope of the  $\text{NH}_4$  curve in this range decreases at rising temperatures. It is striking that the curve shows a distinct optimum at about  $23^\circ \text{C}$ . Above that temperature a decrease of the  $\text{NH}_4$  absorption rate appears. As for K in the absence of  $\text{NH}_4$ , the spread in the experimental data is rather large at temperatures above about  $24^\circ \text{C}$ .

Although up to about 24° C the slopes of the absorption curves for K in the presence and the absence of NH<sub>4</sub> in relation to the temperature differ strongly, both curves show a distinct agreement in this range, namely the increase in the slope is about the same in both cases at rising temperatures. However, this agreement is lost above about 24° C. While the K absorption curve in the absence of NH<sub>4</sub> flattens, the K uptake rate with addition of NH<sub>4</sub> continues to show a strong increase at rising temperatures in the range studied.

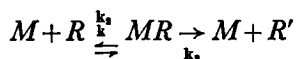
#### IV. DISCUSSION

A. Ion absorption in relation to time is characterized by a brief interval of rapid initial equilibrations (phase of passive absorption) followed by a slower steady-state absorption dependent on metabolism (phase of active absorption). In the present investigation only steady-state rates of NH<sub>4</sub>, K, and Na uptake were determined. However, for NH<sub>4</sub> it cannot be ruled out that at least part of the absorption is less directly dependent on metabolism, the rate of the NH<sub>4</sub> absorption being considerable even at low temperatures, as distinct from the K absorption rate (Fig. 20). The rate of the Na absorption in relation to temperature was not determined in this study.

The rate of absorption in relation to concentration in the present investigation always shows roughly the same pattern: a rapid increase at low concentrations passes into a more or less complete independence of concentration at higher concentrations (Figs. 3–11). A similar relation has been demonstrated for both cations and anions by many investigations (HOAGLAND *et al.*, 1928; VAN DEN HONERT, 1933, 1936; OLSEN, 1950; EPSTEIN and HAGEN, 1952; EPSTEIN, 1953, 1960, 1961; EPSTEIN and LEGGETT, 1954; VAN DEN HONERT and HOOYMANS, 1955; FRIED and NOGGLE, 1958; BANGE, 1959; BANGE and OVERSTREET, 1960; HANDLEY *et al.*, 1960; and MIDDLETON *et al.*, 1960).

VAN DEN HONERT (1933, 1936) compared the asymptotical shape of the absorption curve for phosphate found by him in sugar cane with the curve representing the adsorption to a surface in relation to concentration. In his hypothesis of the "conveyer belt" (later known as "carrier hypothesis") based on this resemblance, in which the ion absorption is represented as a revolving belt system, the binding to the units of the revolving belt (to the "carriers") is considered as an adsorption process.

JACOBSON *et al.* (1950) formulated the carrier hypothesis as a chemical reaction:



in which  $M$  = ion,  $R$  = carrier,  $MR$  = ion-carrier complex, and  $R'$  = carrier chemically somewhat transformed.

Analogously to the Michaelis-Menten equation for enzyme reactions, EPSTEIN and HAGEN (1952) derived for this reaction:

$$v = \frac{[M] \cdot V_{\max}}{[M] + K_m} \quad (1)$$

in which  $v$  = rate of absorption,  $V_{\max}$  = maximal rate of absorption, and  $K_m = \frac{k_2 + k_3}{k_1}$  = Michaelis-Menten constant = concentration at which  $v = \frac{1}{2} V_{\max}$  = half-value.

With a few exceptions, the concentration curves found in the present investigation can be described rather well by this Michaelis-Menten equation, at least in the range of low concentrations. In some cases (Figs. 3, 4, and 8) the absorption rate in relation to concentration was investigated in a much wider concentration range. As distinct from the expectations in accordance with the Michaelis-Menten equation, the rate of uptake continued to increase with a further rise in concentration. BANGE and OVERSTREET (1960) found similar curves for the absorption of Cs and MIDDLETON *et al.* (1960) for K in barley. They suppose two separate absorption mechanisms, one already working at maximal capacity at low concentrations and a second one, which being far less effective, becomes important only at high concentrations. FRIED and NOGGLE (1958) also assume for barley two distinct carriers for each of the cations Rb, K, Na, and Sr, the absorption via the one dominating at low and via the other at high concentrations. The data about this phenomenon supplied by this investigation in wheat is not sufficiently extensive to justify such a conclusion. In view of the fact that steady-state rates of absorption were determined, the possibility that the continuing increase in the absorption rate is passive at higher concentrations is less likely, especially in excised roots. On the basis of their results, BANGE and OVERSTREET (1960) do not support this view either.

In the kinetic analysis of the carrier hypothesis given above it is taken for granted that the concentration of the carrier  $R$  is constant during the experimental period. An increase of this carrier concentration dependent on the concentration of the experimental solution, however, can also explain the gradual increase in the rate of uptake obtained at higher concentrations. It is not considered possible here to choose between these hypotheses: more research is required for a better understanding of this phenomenon.

The calculated Michaelis-Menten constants ( $K_m$ ) and the maximal rates of absorption ( $V_{\max}$ ) of all concentration curves determined in this investigation are given in Table 2. These values are based exclusively on the data obtained in the range of low concentrations where the curves can be described rather well by the Michaelis-Menten equation. The calculated values of the maximal rate of absorption in a number of experiments are consequently lower than the rate of uptake determined at considerably higher concentrations.

TABLE 2.

$K_m$  and  $V_{max}$  values of all absorption rate versus concentration curves determined in this investigation.

Ion	Figure	Experimental series	Intact plants		Excised roots	
			$K_m$ (me/l)	$V_{max}$ (me/h-plant unit)	$K_m$ (me/l)	$V_{max}$ (me/h-kg fr. wt.)
$NH_4$ (—K, —Na) . . . . .	3	1	0.007	0.036		
$NH_4$ (+K) . . . . .	7	4	0.004	0.033		
$NH_4$ (+Na) . . . . .	9	6			0.008	2.7
K (— $NH_4$ , —Na) . . . . .	4	2	0.035	0.042		
id. (pH 6.3) . . . . .	5	2			0.008	4.7
id. (pH 4.7) . . . . .	5	20			0.010	4.2
K (+ $NH_4$ , 0.1 me/l) . . . . .	8	5	—	—		
id. . . . .	5	5			0.015	2.1
K (+ $NH_4$ , 1.6 me/l) . . . . .	8	5	0.125	0.018		
K (+Na) . . . . .	10	8			0.009	3.5
Na (— $NH_4$ , —K) . . . . .	6	3			0.115	2.4
Na (+ $NH_4$ ) . . . . .	6	7			0.115	1.1
Na (+K) . . . . .	11	9			0.115	1.2

No  $K_m$  and  $V_{max}$  values are given for the curves deviating from the Michaelis-Menten relation at low concentrations. In connection with the deviations from the ideal Michaelis-Menten relation discussed above, it will be clear that not too much importance may be attached to the exactitude of the values given, the more so in view of the unavoidable spread in the experimental results.

In the results concerning the rate of absorption in relation to concentration, one point requires special attention. In some experimental series (series 2 and 5) the same relation was studied in both intact plants and excised roots. Quantitatively, large differences appear (Table 2). Although this fact cannot be explained satisfactorily, it should be noted that the plant material for the experiments using intact plants and excised roots was raised from seeds harvested in different years and the technique of growing the plants also differed in the two cases. Further, little is known about whether the process of absorption is affected by the excision of the roots.

Qualitatively, no clear differences were found (Figs. 4, 8, and 5). This result does not agree with the results obtained by SUTCLIFFE (1956), who found for the K-Na interaction that the preference exhibited by barley roots for K compared with Na is much less marked in excised roots than in intact plants. The experimental periods in Sutcliffe's work, however, varied from 24 to 96 hours, while this time interval in the present experiments with excised roots was about 2.3 hours. It appears from Sutcliffe's data that the difference in selectivity between intact plants and excised roots is considerably less marked in the experiments with a 24 hour interval than at longer experimental periods. It is not ruled out, therefore, that for barley too qualitative differences in the K-Na selectivity between excised

roots and intact plants do not appear at still shorter experimental periods.

B. Although, as appeared from the above, the results on the rate of absorption in relation to concentration cannot always be easily fitted into the carrier hypothesis, an attempt will nevertheless be made to explain the interaction phenomena found here in terms of this hypothesis.

One of the most salient properties of these interactions is that the decrease in uptake rate of the inhibited ion present at a constant concentration and the rate of absorption of the inhibitor show the same relation to the concentration of the inhibiting ion. The question consequently arises, which factor is responsible for the inhibition, the concentration of the inhibitor at the surface of the cell or the rate of uptake of the inhibiting ion. In the first case the interaction must occur at the first binding to the carrier (hypothesis I), whereas in the latter case it must be assumed that the interaction appears after the first binding of the ions to be absorbed, i.e. in a further phase of the absorption process proper or during subsequent metabolic reactions in which the ions participate (hypothesis II).

Starting from the first hypothesis, it must be deduced that since the inhibitions are never complete (Figs. 7, 9, and 10) two carriers are involved in the absorption of K as well as Na in the range of low concentrations. One mechanism of each pair is specific for the absorption of K and Na respectively, mutual interferences being consequently impossible. Both the other carriers are able to transport  $\text{NH}_4$  and K as well as Na and may be identical. The K (Na) uptake via this last mechanism can be inhibited by  $\text{NH}_4$  ( $\text{NH}_4$  and K) completely. It cannot be derived from these results whether more than one mechanism is also involved in the absorption of  $\text{NH}_4$ . BANGE (1959) distinguished in maize two K absorption mechanisms which were also effective at low concentrations. One was specific for K, the other showed no distinction between K and Na.

For hypothesis II, only one mechanism each need be assumed for the absorption of  $\text{NH}_4$ , K, or Na. Based on the phenomenon that the inhibitions as found are never complete, two possibilities can be imagined.

In the first place (hypothesis II<sup>a</sup>) it cannot be ruled out that such a type of inhibition, possibly occurring via metabolic reactions, does not lead to 100 % inhibition.

On the other hand (hypothesis II<sup>b</sup>) it is possible that only in certain root or cell compartments there is 100 % inhibition, while no inhibition appears in the rest of the root or cell. It is then supposed that, e.g. for the  $\text{NH}_4$ -K interaction in some parts of the root or cell,  $\text{NH}_4$  inhibits the K absorption completely. In other parts, to the contrary, no inhibition appears owing to different physiological conditions. This conception rests on the well-known fact that large morphological and physiological differences are found within the root. In this connection it should be stated that, according to HANDLEY *et al.*



(1960), Na uptake in maize is clearly not metabolic in the first 1.8 mm of the root top. It appeared to these authors, however, that the relation between the rate of absorption and the concentration in the first 1.8 mm as well as in parts further from the top is exactly the same. In the latter parts at least some of the absorption appeared to be dependent on metabolism.

The postulated compartments of the root can be imagined as longitudinal regions of different lengths reckoned from the top. If the region in which the K absorption is localized is longer than that of the  $\text{NH}_4$  absorption, then part of the K absorption is unaffected by  $\text{NH}_4$ . It is also conceivable that similar compartments are present either within the cell or as radial regions in the tissue.

A strong argument against hypothesis I is that if this hypothesis were correct, it would have to be purely a matter of chance that both absorption mechanisms, the specific and the non-specific, had the same affinity for the ion to be absorbed in the case of both K and Na, within the limits of the accuracy of the experiment (Figs. 4, 8, 5, 6, and 11;  $K_m$  values in Table 2). On the other hand, the fact that these affinities are always the same supports hypothesis II. The K half-values of both absorption mechanisms in the investigation of BANGE (1959) mentioned above differed greatly.

The experimental results of series 10 (Fig. 12), in which the uptake rates of Na in the presence of  $\text{NH}_4$  and K separately and together were compared, fit well with hypothesis I, which supposes two absorption mechanisms for Na. For, it is clear in this conception that part of the Na absorption is affected by neither  $\text{NH}_4$  nor K. All the same, these results and hypothesis II do not necessarily conflict. With respect to hypothesis II<sup>a</sup> this becomes clear from the fact that the decrease in the Na absorption rate by  $\text{NH}_4$  and K separately cannot be cumulative, since the K absorption rate itself is also strongly decreased by  $\text{NH}_4$ . These results can also be explained on the basis of the compartment theory.

Another strong argument for hypothesis II is the following: the physiological conditions of the root are changed by increasing the internal nitrogen content in such a way (series 11, Fig. 13) that the  $\text{NH}_4$  absorption rate at a given  $\text{NH}_4$  concentration is sharply decreased with a higher nitrogen content of the root. Thus the uptake rate of  $\text{NH}_4$  was decreased, while the concentration was the same in both cases. The ratio " $\text{NH}_4$  uptake rate/decrease rate of K absorption caused by  $\text{NH}_4$ " is almost equal in both cases. However, the rate of the K absorption in the absence of  $\text{NH}_4$  undergoes practically no change. The experiments in which the  $\text{NH}_4$ -K and the  $\text{NH}_4$ -Na interactions were investigated in different parts of the root (series 12 and 13, Figs. 14 and 15) give similar results. The ratio " $\text{NH}_4$  absorption rate/decrease rate of uptake of K" is always roughly the same, the  $\text{NH}_4$  rate of absorption in different parts of the root differing considerably. The same holds for the ratio " $\text{NH}_4$  uptake rate/decrease rate of Na absorption". The only deviation from the experiments using roots of different nitrogen content is that the rate of absorption

of K and Na respectively in the absence of the other ions also differs widely in the three parts.

In order to fit these results in with hypothesis I (i.e. two absorption mechanisms involved in the K absorption) it must be assumed that the decrease in absorption capacity of the non-specific mechanism due to an increase in nitrogen content is compensated by a rise in the capacity of the specific mechanism: the total rate of K absorption in the absence of  $\text{NH}_4$  is not affected. This must be rejected as being unlikely.

On the other hand, these phenomena fit in with hypothesis II<sup>a</sup> logically. This hypothesis implies a relation between the rate of absorption of the inhibitor and the inhibition of the absorption of the inhibited ion. A constant ratio between the rate of absorption of the inhibitor and the decrease in the absorption rate of the inhibited ion fits in well with this hypothesis. In these terms, the fact that the absorption rates of K in the absence of  $\text{NH}_4$  are equal in roots of different nitrogen content is not striking.

For the same reasons, the results of the experiments using roots of different nitrogen content also fit in with the idea of root or cell compartments evolved above (hypothesis II<sup>b</sup>). It must then be assumed that the decrease in the magnitude of the compartment where the inhibitions occur, is compensated by the increase in the magnitude of the other compartment where the K absorption is not inhibited by  $\text{NH}_4$ .

In the concept of regions of absorption of different lengths for the three ions, a shortening of the region of the  $\text{NH}_4$  absorption, such as might be due to the influence of an increased nitrogen content leads to a decrease in the  $\text{NH}_4$  uptake rate and to an increase of the K absorption rate unaffected by  $\text{NH}_4$ .

In the experiments on the  $\text{NH}_4$ -K and the  $\text{NH}_4$ -Na interactions in three different parts of the root it was seen that in both the top and middle parts of the root, part of the K and Na absorption is not affected by  $\text{NH}_4$  (Figs. 14 and 15). These results conflict with the hypothesis which attempts to explain the unaffected K and Na uptake with the help of absorption regions of different lengths for the three ions. It should be noted, however, that owing to the complicated morphological and physiological conditions in the root top, too much importance may not be attached to the data obtained for that part of the root. In addition, the length of the roots used in these experiments differs widely. Strictly speaking, this argument consequently holds only if the relative length of the postulated absorption regions is the same in roots of different lengths. The two other possibilities i.e. the compartments are present within the cells and the compartments are present as radial regions in the tissue remain.

It is not surprising that the individual rates of absorption of  $\text{NH}_4$  and of K and Na in the absence of  $\text{NH}_4$  are not equal in different parts of the root (Figs. 14 and 15). It is a well-known fact that the absorption capacity is not the same in different regions of the root. The absorption capacity of the base of the root per cm length is

usually lower than that of the top part, in agreement with most of our results.

In the experiments discussed above the ratio between the uptake rate of the inhibitor and the decrease in the rate of absorption of the inhibited ion always has the same value irregardless of differences in the absorption rate of the inhibitor at the same concentration. Differences in the rate of absorption of the inhibiting ion at a given concentration can also be reached by varying such external factors as pH and temperature (Figs. 16–20). The ratio “rate of  $\text{NH}_4$  or K absorption/decrease Na absorption rate” does not appear to vary much within the limits of the accuracy of the experiment, independent of the absolute magnitude of the  $\text{NH}_4$  or K uptake rate (Figs. 18 and 19). The same is not true, however, for the  $\text{NH}_4$ -K interaction when the pH and the temperature are varied (Figs. 17 and 20).

With regard to the pH effect on the  $\text{NH}_4$ -K interaction it should be noted that the deviations appearing in the ratio “ $\text{NH}_4$  absorption rate/decrease rate of K uptake” especially at low pH values may not be real, in view of the fact that the steady-state condition was not always reached in the experiments on K absorption in the absence as well as in the presence of  $\text{NH}_4$  (see p. 154). However, the largest deviations from the steady-state condition were found for the most part at higher pH values. Further, it appears from comparison of the curves representing the rate of K absorption in the absence of  $\text{NH}_4$  in relation to the concentration at pH values of about 6.3 and about 4.7 (Fig. 5) that the decrease in the rate of K absorption at low pH values is not always so large as indicated by Fig. 17.

The ratio “ $\text{NH}_4$  uptake rate/decrease rate of K absorption” decreases rather sharply from about 2.5 at about 5° C to about 1.0 at about 25° C (Fig. 20). In view of the spread in the experimental data, a correct value for this ratio cannot be given above the latter temperature. In the most extreme case, a certain rise in the value of the ratio may not be ruled out. The high values at low temperatures may be explained by the assumption, already mentioned above, that part of the  $\text{NH}_4$  absorption is less directly dependent on metabolism and is not involved in the interaction process. It may be expected that such an absorption will depend on temperature much less and in a quite different way than the other part of the absorption. This assumption is founded on the fact that the rate of  $\text{NH}_4$  uptake at about 3° C is still 50 % of that at 24° C.

If at above 25° C the ratio “rate of  $\text{NH}_4$  uptake/decrease K absorption rate” really increases again with a simultaneous decrease of the  $\text{NH}_4$  absorption rate, this may be explained by the suggestion that at increasing temperatures a process is stimulated which decreases the rate of absorption of  $\text{NH}_4$  and K (at least in the absence of  $\text{NH}_4$ ) in different ways. In the case of the K uptake it is also possible that at a high rate of absorption a process which is connected with the uptake and practically independent of temperature, at least above 25° C, becomes the rate-limiting factor of the absorption. It would then be clear, why above 25° C the rate of K absorption in the

absence of  $\text{NH}_4$  hardly increases at all, whereas the K uptake rate in the presence of  $\text{NH}_4$ , being much lower, still shows a strong rise in that temperature range.

Summarizing, it may be concluded that the variations which sometimes appear in the ratio "absorption rate of inhibitor/decrease rate of uptake of the inhibited ion" under the influence of pH or temperature may on reasonable grounds be attributed to secondary factors.

Do the results of the experiments on the effect of pH and temperature on the absorption rate give any indication which of the two hypotheses must be preferred? With regard to the effect of pH on the absorption process, some authors (JACOBSON *et al.*, 1950; OLSEN, 1953; FAWZY *et al.*, 1954; NIELSEN *et al.*, 1955; HAGEN and HOPKINS, 1955; JACOBSON *et al.*, 1957; FRIED and NOGGLE, 1958; and MURPHY, 1959) explain this phenomenon as a competition of H with cations or OH with anions for the same site on the carrier. It is clear that in this case the effect of the H and OH ion results in an increase of the half-values of the ions involved. Although most of the authors mentioned above have not investigated whether changes in half-value actually appear with variation in pH, HAGEN and HOPKINS (1955) and FRIED and NOGGLE (1958) did demonstrate changes in half-value for the uptake of  $\text{H}_2\text{PO}_4$  and  $\text{HPO}_4$  and FRIED and NOGGLE (1958) for the absorption of Rb, K, Na, and Sr under the influence of pH. VAN DEN HONERT and HOOYMANS (1955), to the contrary, found for nitrate in maize the same half-value at two different pH values.

If the aforesaid explanation of the pH effect on the rate of ion absorption is true, it is reasonable to suppose that starting from hypothesis I the half-values of both mechanisms for the same ion change in a different measure as a result of variation in pH. It is self-evident that different half-values for the K uptake rate in the absence and the presence of  $\text{NH}_4$  would be a strong argument in favour of hypothesis I. It has really been shown that pH values below about 6.0 do affect the K absorption rate in the absence of  $\text{NH}_4$  and hardly affect it at all in the presence of  $\text{NH}_4$  (Fig. 17). But from Fig. 5 and Table 2 follows that the decrease in the K uptake rate in the absence of  $\text{NH}_4$  at low pH values is not the result of a change in half-value but of a decrease in the maximal absorption rate. This fact consequently rules out the possibility of distinguishing whether there are two K carriers involved in the absorption. Since the rate of Na absorption in relation to pH is not affected by  $\text{NH}_4$  and K (Figs. 18 and 19), a priori a two-carrier system for the absorption of Na cannot be demonstrated in this way. Because it is here uncertain where and how the pH interferes with the absorption process, no conclusions may be drawn as to whether the uptake of the inhibited ions occurs via one or two absorption mechanisms.

Up to now no shifts in half-value have been found as an effect of temperature (VAN DEN HONERT and HOOYMANS, 1955; KAHN and HANSON, 1957; and LYCKLAMA, personal communication). Therefore no attempt was made here to demonstrate a twofold absorption mechanism for the inhibited ions by means of a change of the tem-

perature. For the rest, so little is known about how temperature interferes with the absorption process that no support can be offered for either hypothesis by the data obtained in these experiments.

Summarizing the present discussion as a whole, we come to the conclusion that where the experiments make a choice between the hypotheses possible, this choice favours hypothesis II over hypothesis I. To restate the essentials of hypothesis II:

- 1) The decrease in the rate of absorption of the inhibited ions is not determined by the concentration of the inhibitors in the experimental solution but by the rate of uptake of the inhibiting ions.
- 2)  $\text{NH}_4$ , K, and Na are each absorbed via a single mechanism.

C. Little attention has been paid in the foregoing to the interaction mechanism proper. We shall therefore now examine the question of whether the hypotheses advanced by some authors to explain the interaction phenomena in the uptake of ions can be applied to the results of the present investigation (summarized on p. 164-165), basing the discussion on the two points just mentioned.

It should be observed that the fact that the inhibition is never complete does not complicate the explanation of the interaction mechanism according hypothesis II<sup>b</sup>, because a priori this compartment hypothesis may provide a reasonable elucidation. On the other hand, this phenomenon is a point which requires a closer discussion in terms of hypothesis II<sup>a</sup>.

EPSTEIN and HAGEN (1952) investigated in excised roots the influence which K, Cs, Na, and Li exert on the rate of Rb absorption. Based on the formulation of the carrier hypothesis given above in accordance with JACOBSON *et al.* (1950), the analysis of the results was made by means of three types of inhibition adopted from enzymology. These three types of inhibition are:

- 1) Competitive inhibition. This type of inhibition appears when two ions  $M_1$  and  $M_{11}$  compete for the same site on the carrier  $R$ . Both ions decrease each other's rate of uptake dependent on their concentrations and their affinities for binding to  $R$ . The maximal absorption rate of the two ions is not affected, while the half-values shift to higher values. This type of inhibition has been found many times for monovalent as well as polyvalent cations and anions (EPSTEIN and HAGEN, 1952; EPSTEIN, 1953; EPSTEIN and LEGGETT, 1954; MENZEL, 1954; MENZEL and HEALD, 1955; LEGGETT and EPSTEIN, 1956; FRIED and NOGGLE, 1958; BANGE, 1959; and EPSTEIN, 1961).

- 2) Non-competitive inhibition. In this case the inhibiting ion  $M_1$  combines with the carrier, irrespective of the presence of ion  $M_{11}$ , that is, it combines with both  $R$  and  $M_{11}R$  at a site different from the one to which  $M_{11}$  combines with  $R$ . The maximal rate of absorption of ion  $M_{11}$  is decreased by  $M_1$ . The half-value for the inhibited ion, however, is not affected by rising concentrations of  $M_1$ .

- 3) Un-competitive inhibition. The only distinction with the non-competitive inhibition is that the inhibiting ion  $M_1$  only combines with  $M_{11}R$  and not with  $R$ , again at a site different from that to which

$M_{11}$  combines with  $R$ . The maximal rate of uptake of  $M_{11}$  is again decreased. The half-value of the absorption rate of the inhibited ion in this type of inhibition also shifts to lower values at increasing concentrations of ion  $M_1$ .

According to the competitive type of inhibition, the decrease in the rate of absorption of ion  $M_1$  present at a constant concentration and the absorption rate of  $M_{11}$  show the same relation to the concentration of ion  $M_{11}$ . This also applies in the reverse case.

The absorption of the inhibiting ion  $M_1$  is not involved in Epstein and Hagen's discussion of the non-competitive and un-competitive types of inhibition. Although conceivably ion  $M_1$  may inhibit the absorption of ion  $M_{11}$  without itself being transported, we nevertheless assume that ion  $M_1$  is normally absorbed.

From the principle of non-competitive inhibition it then follows that the rate of absorption of the inhibitor  $M_1$  is not affected by increasing concentrations of  $M_{11}$ . According to the un-competitive type of inhibition, to the contrary, the rate of absorption of  $M_1$  increases at rising concentrations of ion  $M_{11}$ . If ion  $M_{11}$  is present at a constant concentration, the decrease in the uptake rate of ion  $M_{11}$  brought about by the inhibiting ion  $M_1$  in the non-competitive inhibition and the rate of absorption of  $M_1$  show the same relation to the concentration of  $M_1$ . This is not true in the un-competitive type of inhibition.

Epstein and his collaborators (EPSTEIN and HAGEN, 1952; EPSTEIN, 1953; and EPSTEIN and LEGGETT, 1954) claim to have shown in some instances the last two types of inhibition or intermediate forms of them in barley.

If in the  $\text{NH}_4\text{-K}$  and  $\text{NH}_4\text{-Na}$  interactions  $\text{NH}_4$  is the inhibiting ion and in the  $\text{K-Na}$  interaction it is  $\text{K}$ , then the fact that the decrease in the absorption rate of the inhibited ion present at a constant concentration and the rate of absorption of the inhibitor show the same relation to the concentration of the inhibitor and the fact that the half-value of the inhibited ion is the same in the presence as well as in the absence of the inhibited ion, are in striking agreement with the properties of the non-competitive inhibition. In addition, it appears from the results found here that the rate of absorption of the inhibitor is hardly, if at all, affected by the inhibited ion.

Unfortunately, Epstein and Hagen's hypothesis cannot be used to explain the interaction phenomena found in the present study for the following reason: the inhibition in accordance with these three types of interaction is determined by the concentration and not by the rate of absorption of the inhibitor. This conflicts with the conclusion on page 183 i.e. hypothesis II.

A general objection to the experiments of Epstein and his collaborators and to the conclusions derived from them is that in studies of the mutual interactions in the absorption of two ions, the rate of absorption of only one of the two ions was determined in most cases. In the investigation made by EPSTEIN and HAGEN (1952) of the interactions between  $\text{Rb}$  and a number of other alkali ions, only the

absorption rate of Rb was measured. It becomes clear that this may lead to erroneous conclusions if we imagine, for instance, that in the  $\text{NH}_4\text{-K}$  interaction studied here only the rate of the  $\text{NH}_4$  uptake had been determined. Useful conclusions cannot be drawn from investigations into the interaction phenomena in the absorption of ions unless the uptake rates of all ions involved in the interaction are determined.

SUTCLIFFE (1956, 1957) studied the interactions in the absorption of alkali ions in storage-tissue discs and barley roots. For most the material he investigated, it appeared that the same amount of, or even more, K but much less Na was absorbed from a mixture of K and Na at equal concentrations than from solutions of the single salts at the same concentration as in the mixed solution. For red beet tissue the reverse was true. Thus, this type of interaction shows a high degree of correspondence with the results obtained in the present investigation.

To explain his results Sutcliffe starts from the supposition that, on the whole, in the absorption of alkali ions only one general type of carrier is involved which shows a different measure of preference for the various alkali ions. Apart from the fact that the author's concept is not quite clear, it is certain that in his explanation too the interactions are supposed to occur at the first binding to the carrier. This again is contrary to hypothesis II.

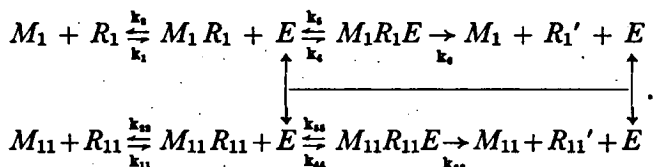
It is clear from the above that the interaction phenomena found in the present study can only be fitted in with a hypothesis according to which the interactions do not occur at the first binding to the carrier but at a later stage of the absorption process or during subsequent metabolic reactions in which the ions participate.

BANGE (1962), in an extensive study of the carrier hypothesis postulates, in terms of this hypothesis, a type of interaction in which the interactions indeed occur in a later stage of the absorption process, namely the breakdown reaction of the ion-carrier complex. If we start from the simple case, conceived in terms of the carrier hypothesis, that the breakdown of the ion-carrier complex  $MR$  occurs via an enzyme  $E$ , it is clear, as Bange states, that in the conventional concept the amount of  $E$  does not limit the rate of the breakdown reaction. This means that  $E$  is present in excess as compared to the amount of  $MR$  or that the affinity between  $MR$  and  $E$  is low in comparison with the amount of  $MR$ . In this view, interaction phenomena could only appear in the phase of the absorption process where the binding of the ion to the carrier takes place. As stated above, in that case the inhibition is determined by the concentration of the inhibitor.

Bange states, however, that these are arbitrary assumptions. If we suppose that the total amount of  $E$  is of the same order of magnitude as, or less than, the total amount of  $MR$ , then it is clear that if two or more ions each form a complex compound with their own carrier, the complex compounds being broken down with the help of the same

enzyme  $E$ , interaction phenomena will also appear. In that case the interaction occurs in a further stage of the absorption process proper consequently. On closer study Bange's interaction hypothesis appears to contribute to the understanding of the interaction phenomena found in the present investigation.

BANGE (1962) gives an extensive kinetic analysis for the case that the total amount of  $E$  ( $= [E_t]$ ) is small with respect to the total amount of carrier ( $= [R_t]$ ). If  $M$  = ion,  $R$  = carrier,  $MR$  = ion-carrier complex,  $E$  = enzyme,  $MRE$  = ion-carrier-enzyme complex, and  $R'$  = carrier somewhat transformed by the enzyme reaction, applies for two competing ions (indices 1 and 11 respectively):



If the forming reaction of  $MR$  is rapid with respect to the breakdown reaction of  $MR$ , it can be derived that, if the ions are present separately:

$$v_1 = \frac{[M_1] \cdot V_{\max 1}}{[M_1] + H_1} \quad (2) \quad \text{and} \quad v_{11} = \frac{[M_{11}] \cdot V_{\max 11}}{[M_{11}] + H_{11}} \quad (22)$$

and if the ions are present together:

$$v_1^{11} = \frac{[M_1] \cdot V_{\max 1}^{11}}{[M_1] + H_1^{11}} \quad (3) \quad \text{and} \quad v_{11}^1 = \frac{[M_{11}] \cdot V_{\max 11}^1}{[M_{11}] + H_{11}^1} \quad (33)$$

( $v$  = rate of uptake,  $V_{\max}$  = maximal rate of uptake,  $H$  = half-value,  $v_1^{11}$  = rate of uptake of ion  $M_1$  in the presence of a constant amount of ion  $M_{11}$ , and conformable meanings of  $v_{11}^1$ ,  $V_{\max 1}^{11}$ ,  $V_{\max 11}^1$ ,  $H_1^{11}$  and  $H_{11}^1$ ) in which

$$V_{\max 1} = \frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2} \quad (2a), \quad V_{\max 11} = \frac{k_{611} \cdot [R_{11t}] \cdot [E_t]}{[R_{11t}] + K_{22}} \quad (22a),$$

$$H_1 = \frac{K_1 \cdot K_2}{[R_{1t}] + K_2} \quad (2b), \quad H_{11} = \frac{K_{11} \cdot K_{22}}{[R_{11t}] + K_{22}} \quad (22b),$$

$$V_{\max 1}^{11} = \frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \quad (3a),$$

$$H_1^{11} = \frac{K_1 \cdot K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)}{[R_{1t}] + K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \quad (3b),$$



$$V_{\max 11}^1 = \frac{k_{66} \cdot [R_{11t}] \cdot [E_t]}{[R_{11t}] + K_{22} \cdot \left( \frac{[R_{1t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)} \quad (33a),$$

and

$$H_{11}^1 = \frac{K_{11} \cdot K_{22} \cdot \left( \frac{[R_{1t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)}{[R_{11t}] + K_{22} \cdot \left( \frac{[R_{1t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)} \quad (33b).$$

$$\left( K_1 = \frac{k_2}{k_1}, K_2 = \frac{k_5 + k_6}{k_4}, K_{11} = \frac{k_{22}}{k_{11}}, \text{ and } K_{22} = \frac{k_{55} + k_{66}}{k_{44}} \right)$$

It is clear that these equations lead to curves of the same shape as the equations showing the rate of absorption in relation to concentration in accordance with the conventional concept (EPSTEIN and HAGEN, 1952).

How far can the  $\text{NH}_4\text{-K}$ ,  $\text{NH}_4\text{-Na}$ , and the  $\text{K-Na}$  interactions be explained with the help of this hypothesis? To start with, the fact that in Bange's concept the ions between which interactions appear are absorbed via separate mechanisms, in addition to the supposition that the interaction occurs only in a later stage of the absorption process, agrees with the conclusions drawn in the preceding discussion.

Bange concludes from this type of conception some important properties which, the interaction phenomena being qualitatively equal in the three cases, will only be tested here for the  $\text{NH}_4\text{-K}$  interaction. In the formulas given above, for the absorption rates of ion 1 and 11 should be read the absorption rate of  $\text{NH}_4$  and K respectively.

a) For two competing ions a decrease in the maximal rate of uptake appears, combined with a rise in half-value. The magnitude of the changes in the maximal absorption rate and the half-value depend on the values of the various constants in the formulas. The data obtained in the present investigation apparently do not agree with this property. Neither the maximal rate of  $\text{NH}_4$  uptake nor the half-value of the  $\text{NH}_4$  absorption rate changes under the influence of K (Figs. 3, 7, 8, and 5; Table 2).  $\text{NH}_4$  does indeed distinctly decrease the maximal rate of the K absorption, but it does not noticeably affect the half-value (Figs. 4, 8, and 5; Table 2). Nevertheless, if it is supposed that we have to do with an extreme case, namely  $[R_{11t}] \ll K_{22}$ , the phenomena found in this study fit in with Bange's hypothesis excellently. This means that the total concentration K carrier ( $[R_{11t}]$ ) is small with respect to the half-value of the reaction between the K-carrier complex  $M_{11}R_{11}$  and enzyme E.

The maximal rate of  $\text{NH}_4$  uptake in the presence of K at a constant concentration is, in that case (formula 3a):

$$V_{\max 11}^1 = \frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \approx \frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2}.$$

The half-value of the  $\text{NH}_4$  absorption rate in the presence of K too becomes (formula 3b):

$$H_1^{11} = \frac{K_1 \cdot K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)}{[R_{11t}] + K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)} \approx \frac{K_1 \cdot K_2}{[R_{11t}] + K_2}.$$

The maximal rate of  $\text{NH}_4$  absorption and the half-value of the  $\text{NH}_4$  absorption rate do not appear to be noticeably affected by K.

With respect to the maximal rate of K uptake it is clear that it is decreased under the influence of  $\text{NH}_4$  present at a constant concentration (formula 33a).

The half-value of the rate of the K absorption in the presence of  $\text{NH}_4$  is (formula 33b):

$$H_{11}^1 = \frac{K_{11} \cdot K_{22} \cdot \left( \frac{[R_{11t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)}{[R_{11t}] + K_{22} \cdot \left( \frac{[R_{11t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)} \approx K_{11}.$$

It is clear that under the condition  $[R_{11t}] \ll K_{22}$  the half-value of the K absorption rate in the absence of  $\text{NH}_4$  also approaches to  $K_{11}$  (formula 22b). Consequently the half-value of the rate of the K absorption does not change noticeably under the influence of  $\text{NH}_4$ .

b) Contrary to the types of inhibition of EPSTEIN and HAGEN (1952), the absorption of one ion is never inhibited completely at increasing concentrations of the other ion. By the same token, a limit is imposed upon the increase in half-value. Therefore, part of the absorption of each of two ions competing in accordance with Bange's interaction principle cannot be affected by the other ion. The results of the present investigation fit in strikingly with this. The K uptake is only partly inhibited by increasing  $\text{NH}_4$  concentrations (Fig. 7). With respect to the half-value of the K absorption rate, this condition is of course satisfied because no noticeable shift appears (Figs. 4, 8, and 5; Table 2).

c) If one of two competing ions is present at a varying concentration and the other at a constant concentration, the decrease in the uptake rate of the ion present at a constant concentration and the rate of absorption of the ion present at a varying concentration show the same relation to the concentration of the latter ion. It was indeed found that at a varying  $\text{NH}_4$  concentration and a constant K concentration the half-value of the decrease in the K absorption rate appears at the same  $\text{NH}_4$  concentration as that of the rate of the  $\text{NH}_4$  uptake itself (Fig. 7). It has not been demonstrated that the reverse (constant  $\text{NH}_4$  and varying K concentration) is also true because the  $\text{NH}_4$  absorption rate is not noticeably affected by K (Figs. 8 and 5).

As stated, the ratio "absorption rate of the inhibitor/decrease absorption rate of the inhibited ion" at each value of the absorption rate of the inhibitor, is the same for roots differing in nitrogen content

as it is for different parts of the same roots. It may be supposed that the same is also true at various pH and temperature values. This is not a general property of the type of inhibition postulated by Bange but a consequence of the special condition  $[R_{11t}] \ll K_{22}$ . For, in that case ( $[M_1] \gg K_1$ ):

$$\begin{aligned} \frac{V_{\max 11} - V_{\max 11}^1}{V_{\max 1}^1} &= \frac{\frac{k_{66} \cdot [R_{11t}] \cdot [E_t]}{[R_{11t}] + K_{22}} - \frac{k_{66} \cdot [R_{11t}] \cdot [E_t]}{[R_{11t}] + K_{22} \cdot \left( \frac{[R_{1t}]}{K_2} \cdot \frac{[M_1]}{[M_1] + K_1} + 1 \right)}}{\frac{k_6 \cdot [R_{1t}] \cdot [E_t]}{[R_{1t}] + K_2 \cdot \left( \frac{[R_{11t}]}{K_{22}} \cdot \frac{[M_{11}]}{[M_{11}] + K_{11}} + 1 \right)}} \approx \\ &\approx \frac{k_{66} \cdot [R_{11t}]}{k_6 \cdot K_{22}} \quad (4). \end{aligned}$$

From this it follows that if the term  $[R_{11t}]$  is constant, the whole ratio is constant. The experimental results indeed show that the rate of the K uptake in the absence of  $\text{NH}_4$  is the same for roots differing in nitrogen content (Fig. 13). The amount of  $R_{11t}$  must consequently also be the same. The K absorption rate in the absence of  $\text{NH}_4$ , however, differs strongly in different parts of the root (Fig. 14). In view of the condition derived above (4), it must be assumed in this case that the differences are caused by a change in the concentration of the enzyme  $E_t$  and not by a change in the amount of  $R_{11t}$ .

In the above it is taken for granted that the decrease in the absorption rate under the influence of factors such as internal nitrogen content, etc., represents only a decrease in the maximal rate of uptake. If we realize the following, it will be clear that in the main this supposition was justified: a decrease in the maximal rate of uptake is caused by a decrease in either  $[E_t]$  or  $[R_t]$  or in both  $[E_t]$  and  $[R_t]$  (see e.g. formula 2a). In the first case no change in half-value will occur, while in the last two cases the half-value will increase (compare e.g. formulas 2a and 2b). However, the concentrations of the inhibiting and the inhibited ion in the experiments concerned were chosen in such a way that if the half-values are considerably higher the rate of uptake is still practically independent of concentration at these concentrations.

The interaction mechanism given in Bange's hypothesis also implies that when several ions are present simultaneously, in principle the absorption of one ion cannot be inhibited completely by the other ions combined. The result that the Na uptake is inhibited only a little more by  $\text{NH}_4$  and K together than by  $\text{NH}_4$  and K separately agrees with this (Fig. 12). Since sufficient data are not available on the interaction phenomena appearing in the presence of  $\text{NH}_4$ , K, and Na simultaneously, we shall not go into this point further.

It should be stressed that Bange's hypothesis is of course only a

sketchy and simplified idea of what takes place in the cell. In spite of this restriction, the essentials of the phenomena observed in the present investigation fit in logically with the hypothesis. Nevertheless, we must not lose sight of the fact that hypothesis II<sup>b</sup> (the compartment hypothesis) can also give a plausible explanation of these phenomena. As concluded on page 180, the compartments must be present either within the cell or as radial regions.

One result in particular is not easily explained by Bange's hypothesis: in spite of distinct differences in absorption rate, the K uptake rate in the presence of about 0.1 me/l  $\text{NH}_4$  and in the absence of  $\text{NH}_4$  shows the same relation to the K concentration. However, the half-value of the K absorption rate in the presence of about 1.6 me/l  $\text{NH}_4$  is much higher than in the absence of  $\text{NH}_4$  (Fig. 8, Table 2). This fact may be explained by assuming that, in addition to the interaction of  $\text{NH}_4$  and K via the breakdown process of the ion-carrier complex,  $\text{NH}_4$  competes to some small extent with K for binding on the K uptake mechanism, in accordance with the principle of the competitive inhibition (EPSTEIN and HAGEN 1952). The affinity of  $\text{NH}_4$  for the K mechanism must in that case be so small that only at high  $\text{NH}_4$  concentrations a significant shift in the relation between the rate of K uptake and K concentration can be shown. In view of the large spread in the experimental data on K in the presence of  $\text{NH}_4$  at high concentrations, a closer quantitative approximation of this phenomenon is not possible. It should also be realized that the high  $\text{NH}_4$  concentration chosen here lies in the concentration range in which the deviation from the theoretical relation becomes appreciable (Fig. 3).

Although the different interactions appearing in wheat between  $\text{NH}_4$ , K, and Na can reasonably be explained in accordance with Bange's hypothesis, a further test of this concept in other material is required.

Apart from the question of which hypothesis gives the best explanation for the results of the present study, it is in any case clear from the present investigation that the interpretation must not be thought of as interactions occurring at the first binding to the carrier of the ions to be absorbed but in a later phase of the absorption process.

#### SUMMARY

The mutual interactions in the absorption of  $\text{NH}_4$ , K, and Na studied in part in intact wheat plants and in part in excised wheat roots were investigated by means of the continuous flow technique. The phenomena found are:

- 1) The K absorption is strongly inhibited by  $\text{NH}_4$ , while conversely K has little, if any, effect on the rate of  $\text{NH}_4$  absorption.
- 2)  $\text{NH}_4$ , even at much increased concentrations, does not completely inhibit the  $\text{NH}_4$  absorption.
- 3) The decrease in the K absorption rate caused by  $\text{NH}_4$  at a constant K concentration and the  $\text{NH}_4$  uptake rate both show the same relation to the  $\text{NH}_4$  concentration.
- 4) In spite of the fact that it has a distinct effect on the absolute magnitude of the K absorption rate, the nature of the relation between the K uptake rate and the K concentration is not influenced by  $\text{NH}_4$ .

5) Points 1 to 4 also apply to the  $\text{NH}_4\text{-Na}$  and the  $\text{K-Na}$  interaction.  $\text{NH}_4$  and K both act as inhibitors with respect to Na.

6) The  $\text{NH}_4$  absorption rate at a given  $\text{NH}_4$  concentration is strongly decreased by a rise in the nitrogen content of the root, whereas the ratio "rate of  $\text{NH}_4$  uptake/decrease K absorption rate caused by  $\text{NH}_4$ " undergoes no change. The rate of the K absorption in the absence of  $\text{NH}_4$  is not affected by the nitrogen content.

7) The ratio " $\text{NH}_4$  absorption rate/decrease K uptake rate" is also always the same in different parts of the root in spite of differences in absolute magnitude of the rates of absorption of all three ions between the different parts. The same holds for the ratio " $\text{NH}_4$  uptake rate/decrease Na absorption rate".

8) The ratio "absorption rate inhibitor/decrease rate of uptake of the inhibited ion" in each of the three interactions is, where studied in the present experiments, also the same at different pH and temperature values.

The explanation of these results is based on the carrier hypothesis. It is shown that:

a) One mechanism is involved in the absorption of  $\text{NH}_4$ , K, and Na individually.

b) The absorption rate of the inhibiting ion and not the concentration of the inhibitor in the experimental solution determines the inhibitions found.

It is therefore considered that the interactions do not appear at the first binding to the carrier of the ions to be absorbed but in a later phase of the absorption process.

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