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DIFFUSION AND ABSORPTION OF IONS IN PLANT TISSUE

I. OBSERVATIONS ON THE ABSORPTION OF AMMONIUM BY CUT DISCS OF POTATO TUBER AS COMPARED TO MAIZE ROOTS

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1. Introduction

Since the early studies of Steward, cut discs of potato tuber tissue have been used widely for experiments in the field of ion absorption. In the laboratory of the authors they have been used during the winter months especially when it has been difficult to grow maize plants of sufficient absorption capacity.

Striking differences became apparent in the behaviour of potato discs and maize with respect to the absorption of NH_4-ions under different conditions of concentration, pH and presence of other cations.

There is some evidence that the phenomena observed in potato discs are not restricted to that object, which does not function as an ion absorbing organ under normal conditions, but also occur in the roots of some plant species, and thus are of a more general importance for our understanding of the process of ion absorption.

2. Material and methods

a. *Plant material*

Maize plants (single cross D × 9) were grown in essentially the same way as described in an earlier paper (cf Van den Honert, Hooymans and Volkers 1955).

Discs of potato tuber tissue (commercial strain "Bintje") were prepared according to the procedure introduced by Steward (1930). In short, tissue cylinders with a diameter of 24 mm were cut with a cork borer and sliced with a slicer of simple construction to discs with a thickness of 2 mm. A hole with a diameter of 2.5 mm was cut in the centre of each disc with another cork borer.

After cutting the discs were strung on a knitting-needle each disc being separated from its neighbour by a porcelain bead. Each needle carried 30 discs.

The discs were rinsed in flowing tap water during 4 days before being used in the experiments. Between experiments they were placed again in flowing tap water. In this way they usually stayed healthy

[1]) The text of this paper was written by us as much according to Van den Honerts latest concepts as possible. Nevertheless we hold ourselves responsible for the contents. G. G. J. Bange and J. J. M. Hooymans.

for about 14 days during which time about 10 experiments were performed. Sets of discs that had lost their turgescence or caused some opalescence in the solution were discarded.

b. Experimental vessels

Maize plants were kept in cylindrical beakers the content of which varied from 250 to 1500 ml according to root development.

The experiments with potato discs were performed in cylindrical tubes with a diameter of 28 mm and a length of about 350 mm. The volume of the experimental solution in each tube was 120 ml throughout. At the bottom the tubes narrowed and here the air supply was connected.

Both beakers and tubes were aerated continuously during the experiments, the aeration served also for the purpose of stirring.

c. Maintenance of temperature and pH

The experimental vessels were kept in a waterbath maintained at $20 \pm 0.1°$ C. During the experiment the pH was checked frequently and, if necessary, adjusted in the way described earlier (cf VAN DEN HONERT, HOOYMANS and VOLKERS 1955, page 146). With freshly cut discs the pH usually remained constant but with older discs it tended to shift to the acid side.

d. Experimental procedure

Different plants or different sets of discs may vary considerably in their absorption capacity. To compensate for this, essentially the same technique as described in earlier papers was used (cf VAN DEN HONERT 1933 and VAN DEN HONERT and HOOYMANS 1955). Standard or unity uptake was defined as the rate of NH_4-absorption from a 0.5 me/l $(NH_4)_2SO_4$-solution in the presence of a modified Woodford and Gregory culture solution (see table 1) at a pH of 6.0 and a temperature of 20° C. Standard rates of uptake were determined for each plant or set of 30 discs before and in some cases after an experiment.

TABLE 1

Composition of the culture solution

K_2SO_4	0.277 me/l
KH_2PO_4	0.151 me/l
$CaSO_4$	0.204 me/l
$MgSO_4$	0.195 me/l

Transfer of plant tissue from one solution to another involves rapid initial equilibrations. Where steady state absorption was the subject of study all tissues were allowed an adjustment period of at least 30 minutes in a solution of the same composition as was to be used in the experiment proper.

The time during which the tissues were allowed to absorb ammonium from the solutions depended on the actual rate of uptake. Too large a decrease in concentration of the experimental solutions had to be avoided, and too small a decrease enlarged the experimental error. On the average the decrease amounted to 20 to 30 %. However, at high concentrations it had to be smaller to avoid too prolonged absorption periods.

The range between the concentration at the beginning and at the end of an experiment has been indicated by horizontal lines in Figs 1, 2, 3, 4 and 8.

e. NH_4-estimation

Ammonium was estimated according to the method described by ALLPORT (1947).

All concentrations are expressed in milliequivalents per litre (me/l).

3. RESULTS

a. NH_4-absorption as related to NH_4-concentration

In maize the relation between the rate of NH_4-absorption and NH_4-concentration in the presence of the culture solution (Fig. 1) has the characteristics of a Langmuir adsorption isotherm the absorption being largely independent of concentration at concentrations higher than 0.6 me/l. A halfvalue of about 0.11 me/l can be computed.

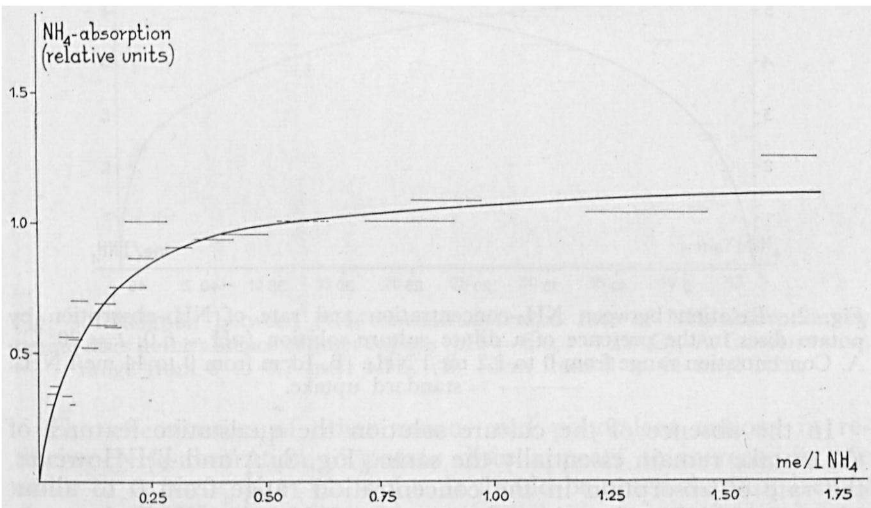

Fig. 1. Relation between NH_4-concentration and rate of NH_4-absorption by maize plants in the presence of a dilute culture solution (pH = 6.0, t = 20° C). ------ = standard uptake.

In striking contrast NH_4-absorption in potato discs in the presence of the culture solution (Fig. 2, A and B) does not attain a maximal value at so low NH_4-concentrations. Here a relatively steep initial rise in the concentration range from 0 to about 0.1 me/l grades into a slower rise at higher concentrations but a maximum is not reached until a concentration of about 30 me/l. The shape of the curve deviates from the simple Langmuir adsorption isotherm.

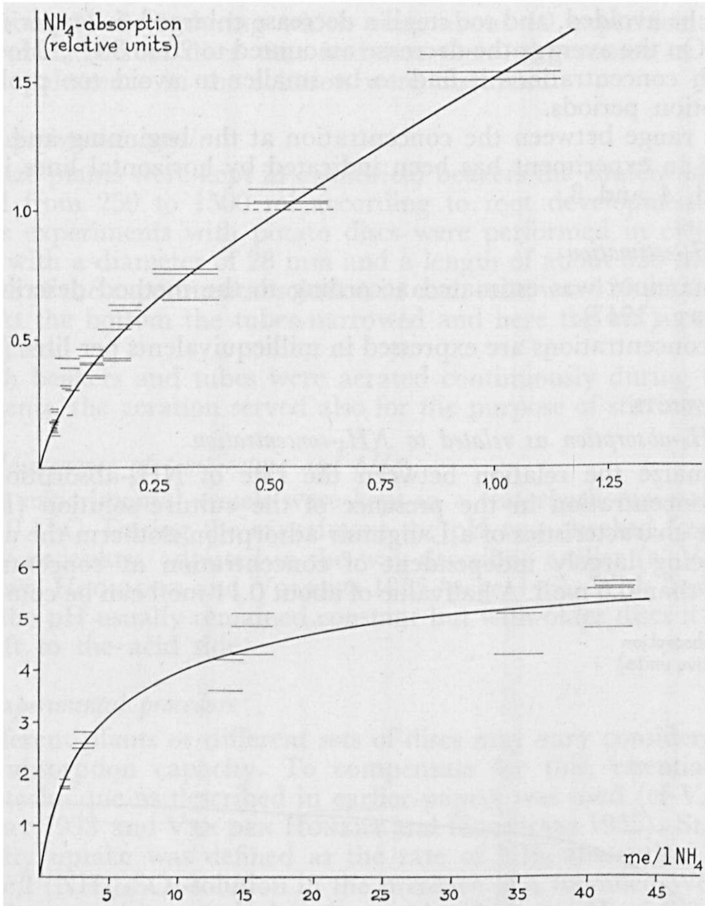

Fig. 2. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the presence of a dilute culture solution (pH = 6.0, t = 20° C). A. Concentration range from 0 to 1.2 me/l NH_4. B. Idem from 0 to 44 me/l NH_4. ------ = standard uptake.

In the absence of the culture solution the qualitative features of the uptake remain essentially the same (Fig. 3, A and B). However, the rate of absorption in the concentration range from 0 to about 10 me/l is higher than in the presence of the culture solution.

If the culture solution is replaced by a solution containing 10 me/l $CaCl_2$ + 10 me/l KCl, the same steep initial part is observed again (Fig. 4, A) but thereafter the curve rises more slowly and no maximum is attained within the range of concentrations used (Fig. 4, B).

b. Influence of other cations on NH_4-absorption

In maize the addition of increasing amounts of Ca (as $CaCl_2$) to a 0.5 me/l $(NH_4)_2SO_4$-solution affects the rate of NH_4-absorption to a much less extent than in potato discs as may appear from a comparison of Figs. 5 and 6.

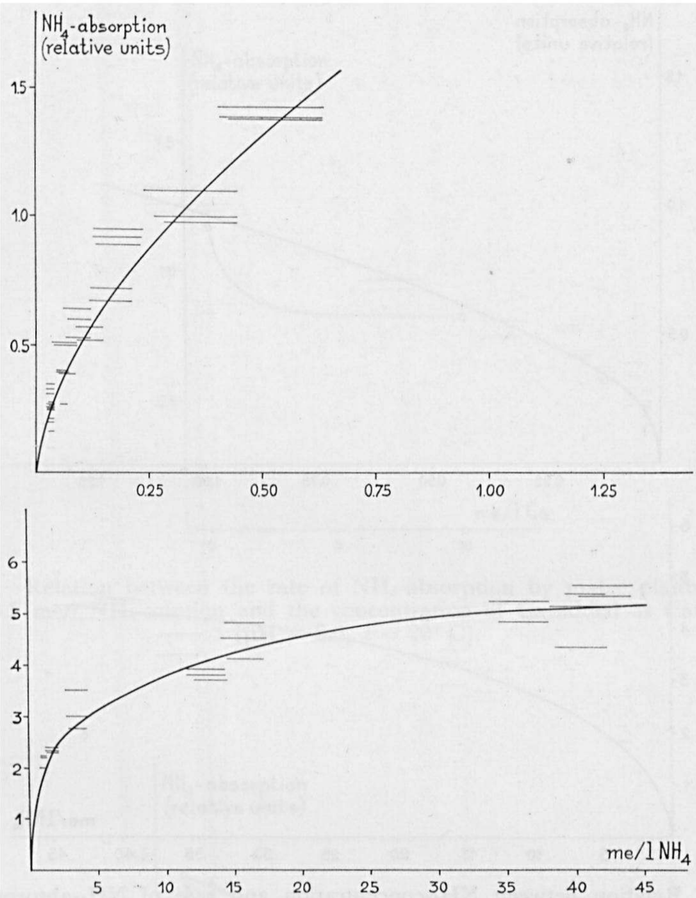

Fig. 3. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the absence of other cations (pH = 6.0, t = 20° C). A. Concentration range from 0 to 0.7 me/l NH_4. B. Idem from 0 to 44 me/l NH_4.

The effectiveness of other mono- and polyvalent cations in reducing NH_4-absorption in potato discs appears to increase in the order Li = Na < K < Ca < La. It should be noted that apparently the rate of NH_4-absorption does not fall below a certain level even with excess cation as is especially clear in the case of La.

c. Influence of pH on NH_4-absorption

The influence of pH on the rate of NH_4-absorption in maize and in potato discs shows some striking resemblances on the one hand and striking differences on the other.

In maize there is hardly any influence in the pH-range from 4.3 to 6.0 (Fig. 7). However, an increasing stimulation of NH_4-absorption is observed at pH-values above 6.0. The amount of the increase at a certain pH-value shows a linear relationship with the

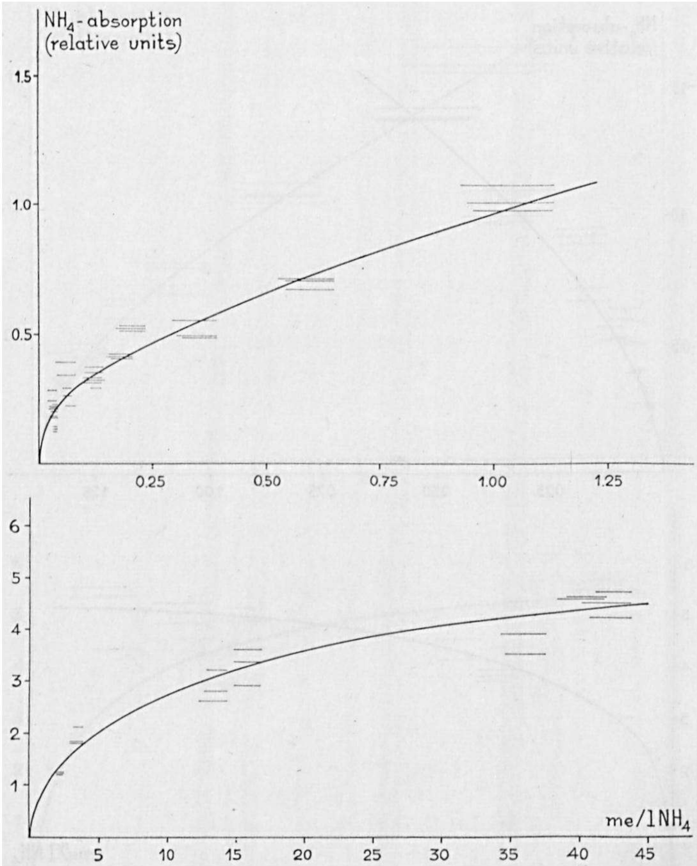

Fig. 4. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the presence of 10 me/l KCl + 10 me/l $CaCl_2$ (pH = 6.0, t = 20° C). A. Concentration range from 0 to 1.2 me/l NH_4. B. Idem from 0 to 44 me/l NH_4.

NH_4-concentration used. This is demonstrated by the fact that a straight line is obtained when the relations between NH_4-concentration and NH_4-absorption at pH = 6.0 and pH = 7.4 are subtracted (Fig. 8, dotted line).

The same behaviour with respect to pH-values above 6.0 is shown by potato discs irrespective of the presence of other salts in the $(NH_4)_2SO_4$-solution (Fig. 9).

In contrast, the behaviour of potato discs at pH-values below 6.0 strongly depends upon the composition of the solution (Fig. 9). If nothing is added to the $(NH_4)_2SO_4$-solution there is a marked fall in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0. This fall is completely checked by the presence of 10 me/l KCl + 5 me/l $CaCl_2$, the culture solution taking an intermediate position. In all cases, about the same level of NH_4-absorption is attained at pH = 4.0.

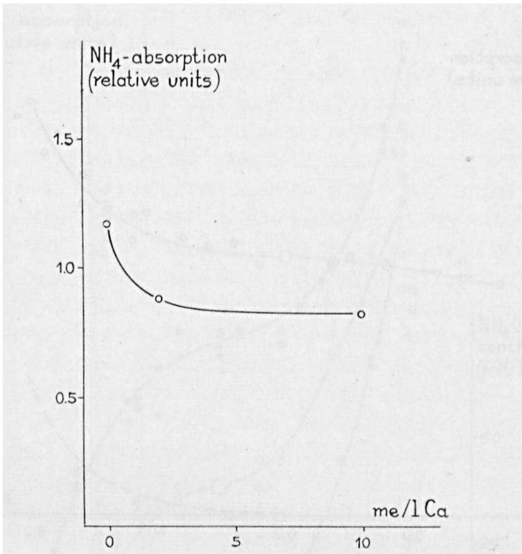

Fig. 5. Relation between the rate of NH_4-absorption by maize plants from a 0.5 me/l NH_4-solution and the concentration of Ca added as $CaCl_2$ (pH = 6.0, t = 20° C).

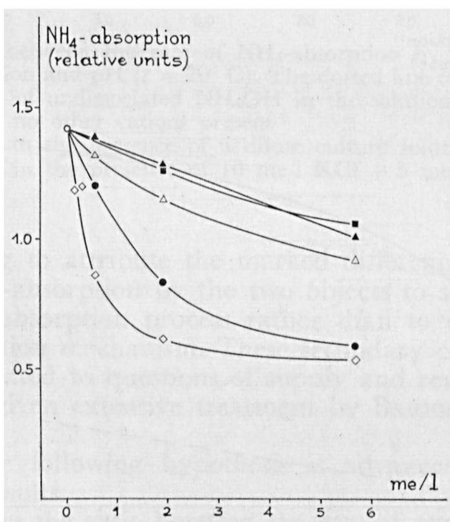

Fig. 6. Relation between the rate of NH_4-absorption by potato discs from a 0.5 me/l NH_4-solution and the concentration of mono-, di- and trivalent cations added as their chlorides (pH = 6.0, t = 20° C).

■ = Li ● = Ca
▲ = Na ◇ = La
△ = K

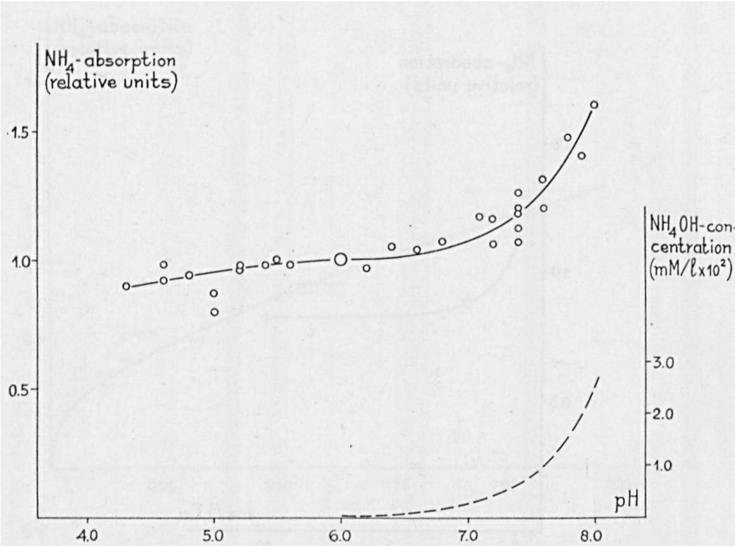

Fig. 7. Relation between the rate of NH_4-absorption by maize plants from a 0.5 me/l NH_4-solution and pH in the presence of a dilute culture solution (t = 20° C). The dotted line represents the amount of undissociated NH_4OH in the solution.

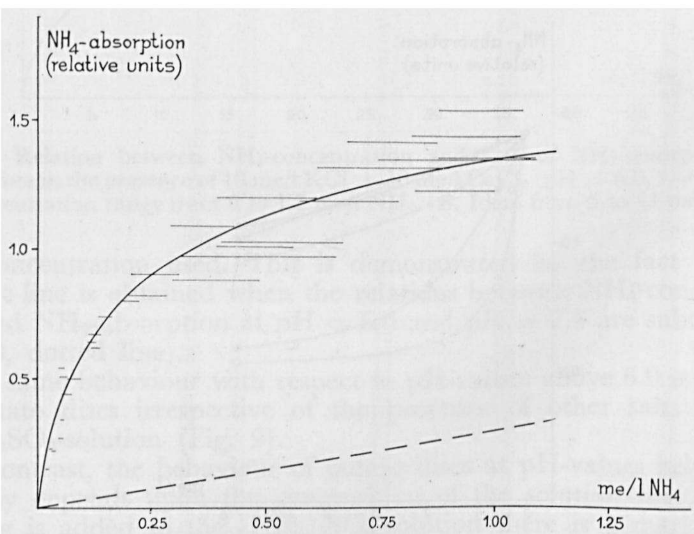

Fig. 8. Relation between NH_4-concentration and rate of NH_4-absorption by maize plants at pH = 7.4 in the presence of a dilute culture solution. The dotted line represents the difference between the rate of uptake at pH = 7.4 and at pH = 6.0 (as derived from Fig. 1).

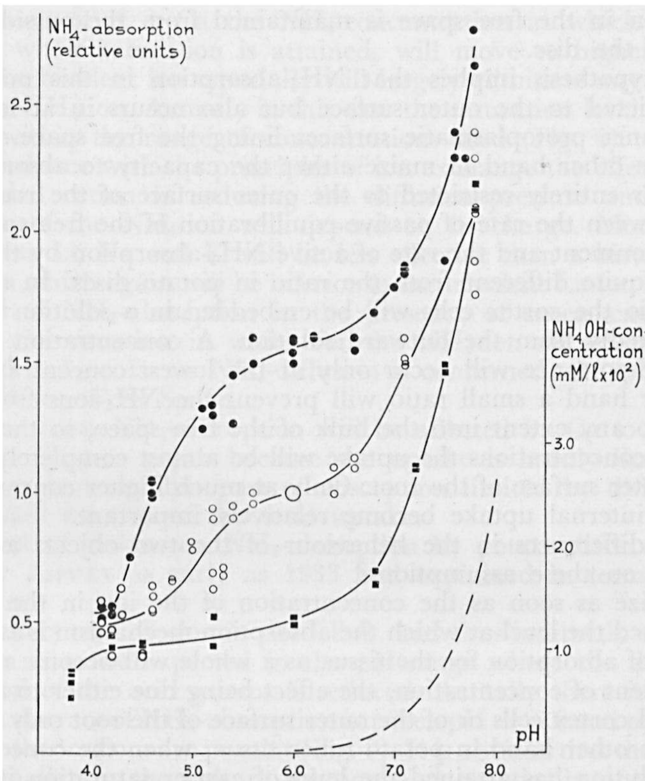

Fig. 9. Relation between the rate of NH_4-absorption by potato discs from a 0.5 me/l NH_4-solution and pH (t = 20° C). The dotted line represents the amount of undissociated NH_4OH in the solution.

● = no other cations present
○ = in the presence of a dilute culture solution
■ = in the presence of 10 me/l KCl + 5 me/l $CaCl_2$.

4. Discussion

It is tempting to attribute the marked differences in the characteristics of NH_4-absorption by the two objects to secondary complications in the absorption process rather than to differences in the primary absorption mechanism. These secondary complications conceivably are related to questions of supply and removal, a problem that has been given extensive treatment by Briggs and Robertson (1948).

Therefore the following hypothesis is advanced to explain the experimental results.

In potato discs the ratio between the rate of passive equilibration of the free space with the environment and the rate of active NH_4-absorption by the protoplasts is such that the NH_4-ions are removed too rapidly from the free space to allow its equilibration with the surrounding solution. In this case a steady state of absorption will be established for the disc as a whole in which a gradient of NH_4-concentration in the free space is maintained from the outside to the inside of the disc.

The hypothesis implies that NH_4-absorption in this material is not restricted to the outer surface but also occurs in at least part of the inner protoplasmatic surfaces lining the free space.

On the other hand in maize either the capacity to absorb ions is largely or entirely restricted to the outer surface of the root or the ratio between the rate of passive equilibration of the free space with the environment and the rate of active NH_4-absorption by the protoplasts is quite different from the ratio in potato discs. In case of a large ratio the cortex cells will be embedded in a solution differing not markedly from the outward solution. A concentration gradient of any importance will occur only at the lowest concentration. On the other hand a small ratio will prevent the NH_4-ions from penetrating to any extent into the bulk of the free space, so that at low external concentrations the uptake will be almost completely limited to the outer surface of the root. Only at much higher concentrations will the internal uptake become relatively important.

What differences in the behaviour of the two objects are to be expected on these assumptions?

In maize as soon as the concentration of the ion in the solution has reached the level at which the absorption mechanism is saturated, the rate of absorption for the tissue as a whole will become relatively independent of concentration, the effect being due either to a saturation of all cortex cells or of the outer surface of the root only (Fig. 1).

On the other hand in potato tuber tissue, when the concentration in the solution has attained the level of carrier saturation it will be only the outer surface of the disc that is able to absorb at its maximal rate. All inner surfaces will still be operating at a lower level. Only when the concentration rises further will the region of carrier saturation extend gradually from the outside to the inside of the disc until at last all inner surfaces absorb at their maximal rate. So the absorption curve will not become level after its initial steep rise but will go on rising with a gradually decreasing slope until for the disc as a whole saturation is attained (Figs. 2, 3 and 4).

The presence of a large number of fixed negative charges has been demonstrated for the free space of potato tuber tissue (Bange 1957). Consequently diffusion will proceed at least partly in an ionic exchange region (I.E.R.) and for this reason will show special features such as interaction with other cations and pH-effects.

The addition of mono- or polyvalent cations to the NH_4-solution will lower the NH_4-concentration in the I.E.R. and so reduce its diffusion pressure. Therefore in potato discs the rate of NH_4-absorption will rise more rapidly with concentration in the absence of other cations and more slowly in the presence of a high K + Ca-concentration than in the culture solution (Figs 2, 3 and 4). At high NH_4-concentrations, however, the difference due to the absence or presence of the culture solution will vanish owing to its relatively low concentration (Figs 2 and 3). On the other hand in the presence of a high concentration of K + Ca the NH_4-concentration at which for the disc as a whole saturation is attained, will move to higher values because the bivalent ion especially will largely eliminate any adsorption of the NH_4-ion. Actually in this case the saturation level was not attained within the range of concentrations used (Fig. 4).

In accordance with the principles of ion exchange the effectiveness of an added cation in reducing the diffusion pressure and so the absorption of the NH_4-ion will depend on its charge. Monovalent ions such as Li, Na and K will be less effective than a bivalent ion such as Ca which again will be inferior to the trivalent La-ion (Fig. 6). The level of NH_4-absorption in the presence of excess cation will represent the situation in which for the NH_4-ion all adsorption is eliminated and, as far as NH_4-diffusion is concerned, the whole free space is Water Free Space.

In maize roots NH_4-absorption will be affected by Ca to a much less extent (Fig. 5) either because the uptake is hardly limited by diffusion or because that part of absorption limited by diffusion is only a small fraction of the total uptake.

An effect of cations on NH_4-absorption by potato discs was described by ASPREY as early as 1933 and explained in terms of ion antagonism.

A pH-effect on diffusion in the I.E.R. and thus indirectly on NH_4-absorption is to be expected in the pH-range where the degree of dissociation of the acid groups is reduced. Actually there is a steep decline in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0 when no other cations are present (Fig. 9). It is obvious that the decline will be smaller in the culture solution and practically absent in a solution with a high K + Ca-content, the diffusion pressure of the NH_4-ion in these cases having been reduced already by the addition of cations.

In maize no such pH-effects will occur in the pH-range between 6.0 and 4.3 (Fig. 7).

The peculiar pH-effect at pH-values higher than 6.0 common to maize roots and potato discs (Figs 7 and 9) is supposed to stand in no relation to the diffusion phenomena described above but is tentatively explained in the following way.

With rising pH increasing amounts of undissociated NH_4OH will be present in solutions of NH_4-salts. Molecular NH_4OH is known to penetrate readily through the permeation barriers of the cell. The assumption therefore is that the effect is due to the diffusion of NH_4OH into the cells in which it is subsequently fixed by a process which is not rate-limiting.

This hypothesis is supported by two observations.

In the first place if it is assumed that the absorption of the NH_4-ion is independent of pH at pH-values higher than 6.0, all absorption above the level of pH = 6.0 appears to be proportional to the amount of NH_4OH present in the solution. This amount is indicated by the dotted lines in Figs 7 and 9.

In the second place it was demonstrated for maize that the extra absorption at pH = 7.4 is proportional to the concentration of the NH_4-salts in the solution (Fig. 8, dotted line) as the hypothesis requires.

It should be noted that a similar phenomenon was observed by HURD and SUTCLIFFE (1957) and by HURD (1958, 1959) in K-absorption from KCl-solutions by different kinds of storage tissue and by barley roots. The authors relate the effect to the increasing availibility of HCO_3-ions at higher pH-values. Their statement that the phenomenon was not observed in potato discs makes a common explanation, however tempting, less likely. It is true that in the experiments described above $(NH_4)_2SO_4$ and not NH_4Cl was used but the stimulation of the absorption by alkaline pH-values was not affected by the presence of excess Cl-ions (Fig. 9).

To return to the main subject under discussion, there is another question that deserves attention. A close inspection of the curves of Figs 3A and 4A reveals that absorption at the lowest concentrations is hardly affected by the presence of a high K + Ca-content in the solution. Moreover absorption in the interior of the disc only would not show the sharp changes in slope of the curve of Fig. 4A but rise more smoothly from the origin. The obvious explanation is that at the low concentrations an absorption component not mediated by cation sensitive diffusion becomes relatively important and that this component is identical with the absorption at the outer surface of the disc.

A rough estimation of the contribution of the outward surface to the total absorption capacity of the disc can be made by a tentative extrapolation of the slowly rising portion of the curve of Fig. 4A to the ordinate. This procedure yields a value of about 0.25. The total absorption capacity of the disc being about 5.2 the share of the outward surface can amount to not more than 5 %. In case of cells of a cubical shape this would mean that about 3 superficial layers would participate in the uptake provided the absorption capacity were evenly distributed over the surface of the absorbing cells. However, this is likely to be an underestimate because the absorption capacity may be supposed to decrease less abruptly to the interior of the disc.

Do the experiments described above contain any clue to the chemical nature of the fixed negative charges in the diffusion medium?

It was shown (Fig. 6) that the efficacy of added cations in reducing the rate of NH_4-absorption increases in the series

$$\mathrm{Li} = \mathrm{Na} < \mathrm{K} < \mathrm{Ca} < \mathrm{La}.$$

Comparison of this sequence with the ionspectra for sulphate, phosphate and carboxyl colloids (BOOY and BUNGENBERG DE JONG 1956) suggests that carboxyl rather than one of the other types of colloids are involved.

This conclusion is supported by the strong decrease in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0 (Fig. 9). Apparently the dissociation constant (pK) of the acidic groups is higher than 4.0, but due to the complexity of the system a satisfactory estimation from these indirect data is not possible. At any rate, the estimation seems too high for the much stronger acidic sulphate and phosphate groups.

Therefore a tentative identification of the dissociable groups with carboxyl groups of the cell wall pectin seems justified. This conclusion is in agreement with other evidence (LATIES 1959, page 94–95) though the discrepancy with the results of BANGE (1957, cf LATIES l.c.) remains a point to be elucidated.

SUMMARY

NH_4-absorption by maize plants and by cut discs of potato tuber was studied in relation to NH_4-concentration, pH and concentration of added mono- and polyvalent cations.

Striking differences in the behaviour of the two objects became apparent.

The results are tentatively explained on the assumption that during steady state absorption in potato discs a gradient of NH_4-concentration is maintained in the free space.

Several explanations for the different behaviour of maize roots are discussed.

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