DIFFUSION AND ABSORPTION
OF IONS IN PLANT TISSUE

II. TIME COURSE OF NH4-ABSORPTION BY CUT DISCS OF POTATO TUBER AT DIFFERENT TEMPERATURES

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1. Introduction

Though the phenomena described in part I are readily explained by the hypothesis put forward, the authors realize that several other explanations might be advanced.

In the first place, where no data are presented to prove the contrary the rates of absorption measured and supposed to represent active absorption might still contain a passive component not saturated during the prerinsing period. In other words a steady state might not yet have been established during this period and consequently the free space (W.F.S. + I.E.R.) might still have continued to equilibrate with the external solution during the experimental period.

In the second place, two binding sites may be involved in NH4-transportation, a specific one transporting NH4 only and having a high affinity for this ion and a general cation binding site transporting mono- as well as di- and trivalent cations but as far as NH4 is concerned with a much lower affinity than the specific site. A general cation carrier has been described for the yeast cell by CONWAY and DUGGAN (1956).

However, a close examination of the data renders the second explanation less likely. It appears from Fig. 6 (part I) that the addition of excess cation reduces the rate of NH4-absorption to a level not lower than about 0.5 of the standard value. In terms of competition for a general cation carrier this would mean that all NH4-absorption at this point proceeds by means of the specific site. So the relation between the rate of absorption and the concentration of NH4 in the presence of excess cation in the range of low NH4-concentrations should be of the Langmuir type. From Fig. 4A (part I) it is clear that even in the presence of 10 me/l Ca + 10 me/l K this is not the case.

To exclude the first possibility as well as to collect more direct experimental evidence for the existence of a concentration gradient in the free space of the disc during steady state absorption at 20° C, a series of experiments was performed in which the time course of NH4-absorption was studied at 20° C as well as at 0° C, i.e. under conditions of checked active absorption. These experiments will be described in the following sections.

2. MATERIAL AND METHODS

The technique used was essentially the same as described in part I. The diameter of the discs was 17 mm. To enlarge the decrease in concentration and to enable the measurement of absorption during periods as short as a few minutes, 60 instead of 30 discs were strung on one needle. The volume of the experimental solutions in each tube amounted to 75 ml.

All discs were first rinsed in flowing tap water for 4 days. Following this, the discs were immersed in tap water at 0° C for several hours. Then the experiment at 0° C was started with one half of the discs, meanwhile the other half was immersed in tap water at 20° C for $1\frac{1}{2}$ hours before starting the experiment at 20° C.

Before starting each experiment, the discs were given three 10 minute rinses in distilled water of the experimental temperature and then, after being blotted by a gentle rolling of the needles over dry filter paper, they were transferred to the experimental solution.

$(NH_4)_2HPO_4$-solutions buffered at pH = 6.0 with tris in a concentration of 170.8 mg/l were used. The use of this solution does not change the essential features of the absorption-concentration relation as discussed in part I.

NH_4-absorption was measured by estimation of the decrease in concentration of the experimental solution. Without proper precautions this procedure would have led to a larger decrease in concentration and consequently a smaller mean concentration the longer the absorption period. Therefore one or more mls of a concentrated $(NH_4)_2HPO_4$-solution were added to the tubes in the experiments at 20° C when the duration of the absorption period was one hour or longer. In this way variations in mean concentration between the tubes were kept well within 12 %.

An experiment in which the release of NH_4 was studied was performed in the following way. Three needles with 60 discs each, pretreated in the way described previously, were allowed to absorb NH_4 at 0° C in three tubes containing a 0.556 me/l $(NH_4)_2HPO_4$-solution. After 150 minutes the needles were blotted and transferred to three tubes containing a 10 me/l $CaCl_2$-solution of the same temperature. This solution was renewed at increasing intervals and its NH_4-content measured.

NH_4 was estimated in the way described in part I.

3. RESULTS

The relation between the amount of NH_4 absorbed and time at 0° C and at 20° C was studied for two NH_4-concentrations, viz 0.556 and 2.222 me/l. The experiments were performed in duplicate and their results averaged. They are represented in Figs 1 and 2 respectively.

At both concentrations and at 20° C as well as at 0° C there appears to be a very rapid initial phase of absorption completed in 4 minutes or less. At 0° C this rapid initial phase passes into a phase with a
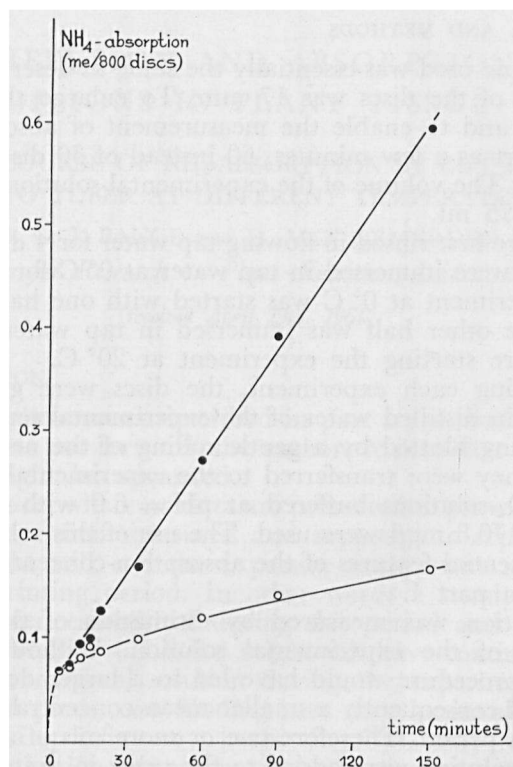
Fig. 1. Time course of NH$_4$-absorption at 0° C (open circles) and at 20° C (full circles) from a 0.556 me/l NH$_4$-solution at pH = 6.0 (mean of two experiments)

slower and gradually decreasing rate of absorption over a period of at least 150 minutes. However, at 20° C a steady rate of absorption is reached immediately after the rapid initial phase and is maintained over a period of at least 150 minutes.

In order to examine whether all NH$_4$-absorption at 0° C is essentially passive and consequently reversible, an experiment was performed in which the release of NH$_4$, after its preliminary absorption over a period of 150 minutes from a 0.556 me/l NH$_4$-solution at 0° C, was studied. The results are presented in Fig. 3. Again a very rapid initial phase of loss precedes a phase of slower and gradually decreasing NH$_4$-release. After 8 hours all NH$_4$ absorbed has been recovered.

4. DISCUSSION

The experiments described show that a prerinsing period of 30 minutes as used in the experiments of part I amply suffices to reach steady state conditions. At the same time they provide conclusive evidence for the existence of a concentration gradient inside the discs during active absorption under the prevailing conditions as will be clear from the following considerations.

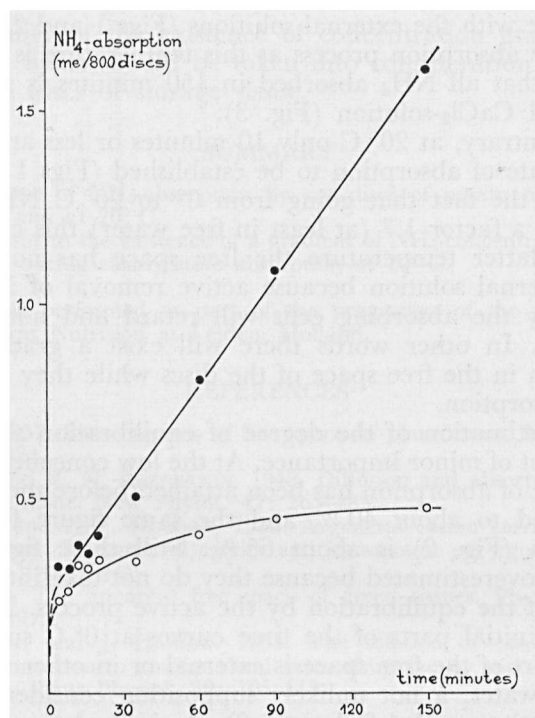At 0° C it takes 150 minutes or more for the free space of the discs

Fig. 2. Time course of NH_4-absorption at $0°$ C (open circles) and at $20°$ C (full circles) from a 2.222 me/l NH_4-solution at pH $= 6.0$ (mean of two experiments).
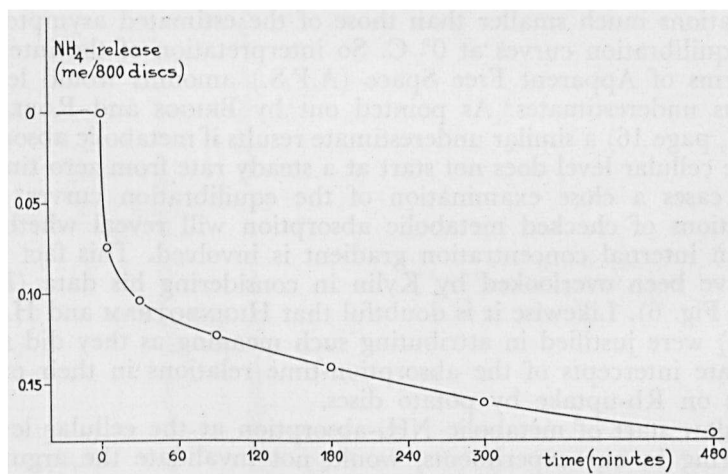

Fig. 3. Time course of NH_4-release to a 10 me/l $CaCl_2$-solution at $0°$ C and pH $= 6.0$ after absorption of 0.18 me NH_4 per 100 discs during a period of 150 minutes from a 0.556 me/l NH_4-solution at $0°$ C and pH $= 6.0$.

to equilibrate with the external solutions (Figs 1 and 2). The passive nature of the absorption process at this temperature is demonstrated by the fact that all NH_4 absorbed in 150 minutes is released again to a 10 me/l $CaCl_2$-solution (Fig. 3).

On the contrary, at 20° C only 10 minutes or less are required for a constant rate of absorption to be established (Figs 1 and 2). Even allowing for the fact that going from 0° to 20° C NH_4-diffusion is enhanced by a factor 1.7 (at least in free water) this can only mean that at the latter temperature the free space has not equilibrated with the external solution because active removal of NH_4 from the free space by the absorbing cells will retard and not enhance the equilibration. In other words there will exist a gradient of NH_4-concentration in the free space of the discs while they are in the act of active absorption.

A rough estimation of the degree of equilibration shows that the gradient is not of minor importance. At the low concentration (Fig. 1) a steady state of absorption has been attained before the equilibration has proceeded to about 40 % and the same figure for the higher concentration (Fig. 2) is about 65 %. Still these figures must be considerable overestimated because they do not take into account the retardation of the equilibration by the active process. Moreover, the rather steep initial parts of the time curves at 0° C suggest that an important part of the free space is external or in other words consists of adhering water, a not unlikely supposition considering the technique of blotting used (cf chapter 2). A similar steep initial rise is mentioned by BRIGGS (1957, page 321) for slices of carrot tissue and explained in essentially the same way. This circumstance would reduce the estimates mentioned above still further.

Another aspect of the results needs attention. The ordinate intercepts of the time course extrapolates at 20° C are at both concentrations much smaller than those of the estimated asymptotes of the equilibration curves at 0° C. So interpretation of the intercepts in terms of Apparent Free Space (A.F.S.) amounts would lead to serious underestimates. As pointed out by BRIGGS and ROBERTSON (1957, page 16) a similar underestimate results if metabolic absorption at the cellular level does not start at a steady rate from zero time. In such cases a close examination of the equilibration curves under conditions of checked metabolic absorption will reveal whether or not an internal concentration gradient is involved. This fact seems to have been overlooked by Kylin in considering his data (KYLIN 1960, Fig. 6). Likewise it is doubtful that HIGINBOTHAM and HANSON (1955) were justified in attributing such meaning as they did to the ordinate intercepts of the absorption-time relations in their experiments on Rb-uptake by potato discs.

A slow start of metabolic NH_4-absorption at the cellular level, if occurring in our experiments, would not invalidate the arguments presented for the existence of a concentration gradient because it would lengthen and not shorten the time needed for establishment of the steady state.

The possibility of the existence of concentration gradients inside the absorbing tissue should be taken into consideration especially in all work with discs of storage tissue.

SUMMARY

The time course of NH_4-absorption by cut discs of potato tuber tissue was studied at $0°$ C and at $20°$ C.

The results point to the existence of a gradient of NH_4-concentration inside the absorbing tissue during steady state absorption at $20°$ C.

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