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THE ROLE OF CALCIUM IN THE ABSORPTION OF ANIONS AND CATIONS BY EXCISED BARLEY ROOTS

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CONTENTS

ABSTRACT

The effect of Ca on the uptake of the anion Br and the cations K (Rb) and Na was investigated, with emphasis on its relation to time.

Absorption of the Br ion is stimulated in the presence of Ca ions. This stimulation is relatively stronger at low concentrations than at higher ones, and manifests itself without a lag-phase. The effect of the Ca ion on Br uptake is independent of temperature; it appears at 1.5° C as well as at 20° C. Moreover, this stimulation is more or less independent of the pH in the range between 3.8 and 7.0. Further, the stimulating effect on Br uptake is not specific for the Ca ion because other cations such as K, Na, and Li also increase the Br absorption albeit to a less extent than the divalent Ca ion. The view is advanced that cations screen the negative charges on the cell surface, as a result of which more absorption sites come within the reach of the Br ion.

The effect of the Ca ion on Na uptake is manifested at a pH of 5.3 in the form of a reduction in the total amount of Na entering the roots. It was found that the Na uptake can be divided into two fractions, a steady-state Na uptake and an additional Na uptake taking place only over a limited period, viz. during the first two hours of the experimental time, and probably consisting of the adsorption of Na to cytoplasmatic sites. This additional Na uptake is entirely or partially eliminated by Ca, but, quite to the contrary, the steady-state Na uptake is not at all influenced by Ca. Evidence is advanced indicating that the effect of Ca on this additional Na uptake is based not on competition for negative adsorption sites in the cytoplasm but on a reduction of the permeability of the outer plasma membrane under the influence of the Ca ion, by which admittance to the cytoplasmic sites behind this membrane is denied to the Na ion.

The influence of the Ca ion on the K uptake at pH 5.4 manifests itself as inhibitory, stimulatory, or neutral depending upon the K concentration and the experimental time. At low K concentration (0.01 m.e./l), analogous to the situation with Na, there is an additional K uptake of limited extent and duration which is inhibited by Ca. As in the case of Na, this additional K uptake is assumed to consist

of an adsorption of K ions to negative adsorption sites situated behind a membrane, the permeability of which is decreased by Ca. The steady-state K uptake is in itself not Ca-sentitive. At higher K concentrations (0.2 m.e./l) the additional K uptake is no longer abolished by the presence of Ca. The fact that in the absence of Ca at higher K concentrations (0.2 m.e./l and higher) the uptake rate diminishes quite considerably after a few hours is to be seen as the result of an efflux of K coming into operation as the K content of the root increases.

The results are discussed in relation to the interpretations presented in the literature with respect to the influence of the Ca ion on ion uptake. An attempt is made to localize the various physiological processes involved in cation uptake at the cellular level.

CHAPTER I

INTRODUCTION

A stimulating effect of Ca and other polyvalent cations on the rate of ion uptake by roots was first reported by VIETS (1944). This stimulation concerned the uptake of both cations and anions. A similar stimulation has since been reported by many authors. However, the explanations suggested for the increase of the rate of ion uptake by Ca differ widely. They may be summarized as follows:

a. Ca has been claimed to play an important part in carrier-mediated ion transport. The following possibilities have been proposed:
   Ca enhances the formation or turnover of the carrier (MARSCHNER 1961).
   Ca functions as a co-factor in the formation of the carrier (JACOBSON, OVERSTREET, KING and HANDLEY 1950).
   Ca increases the rate of break-down of the ion-carrier complex (OVERSTREET, JACOBSON and HANDLEY 1952; NIELSON and OVERSTREET 1955; FAWZY, OVERSTREET and JACOBSON 1954; LEGGETT 1956).
   Ca enhances the affinity between carrier and ion (KAHN and HANSON 1957; TANADA 1962).
b. In the presence of Ca the stability of the structure of the protoplasm is increased, in consequence of which the absorbed ion is bound in a more stable form (OVERSTREET 1957).
c. Ca influences the permselectivity of a barrier situated at the cell surface, through which the ions must migrate to reach the absorption sites. The stimulating effect of Ca is considered as a blocking of the entrance of interfering ions (JACOBSON, MOORE and HANNAPEL 1960; JACOBSON, HANNAPEL, MOORE and SCHAEDLE 1961; WAISEL 1962).
d. Ca enhances the formation of mitochondria, which are considered to play an important role in ion accumulation (FLORELL 1956).
e. Ca protects RNA, of importance in the membrane for the transport of ions, against break-down by endogenous enzymes (HANSON 1960).
f. Concerning the anion, Ca has been supposed to decrease the negative charge of the root surface so that the anion meets less resistance when it moves inside the root (ELGABALY 1962).

The primary purpose of this investigation was to study the influence of Ca ions on the uptake of anions, in particular the Br ion. Since the most plausible explanation for the stimulating effect of Ca on Br uptake appeared to lie in the presence of negatively-charged sites in the region of the cell surface where the first binding in the active uptake process is supposed to occur, it was necessary to investigate the influence of Ca on cation uptake as well, to make sure that no similar stimulation of cation uptake occurs. Here the complication was encountered that in contrast to anion uptake, cation uptake measured over a period of six hours in the absence of Ca is usually not linear with time. This complication, in its turn, complicated the effect of Ca: in some cases a reduction of the total amount of cation uptake over a period of six hours was found, in some cases an increase. In spite of this secondary complication, however, it could be concluded that Ca has no influence on the process of active uptake as such. Thus, the interpretation given for the stimulating effect of Ca on the uptake of anions is not contradicted.

On the other hand, the study of these secondary complications led to results which may be of importance for better understanding of the way in which the different cell compartments are involved in the ion absorption process.

Chapter II

MATERIAL AND METHODS

With only one exception, excised root material from barley (*Hordeum vulgare L.* "Herta") was used for these experiments. In many respects the method of culturing was similar to that described by Ulrich (1941) and Jacobson et al. (1950). Fifty grams of seeds were rinsed in running tap water for about one hour and then soaked for 24 hours in 600 ml demineralized water with continuous aeration at 25°C. Following this, the seeds were washed several times with demineralized water. Then they were spread on a stainless steel screen (size 30 × 25 cm) covered by a piece of coarse gauze and supported by a perspex frame. The whole was placed in a plastic tray filled with $4^1/_2$ litres of 5×10^{-4} M $CaSO_4$ solution. The level of the solution was kept at about 1.5 cm below the screen. The edges of the gauze were in contact with the solution which was continuously aerated. The plants were allowed to grow for 6 days in the dark. The temperature was maintained at 25° C and culture solution replaced after three and five days. When the plants were 7 days old, the roots were excised just below the gauze and washed thoroughly for 30 minutes in three changes of about 4 litres each of aerated, demineralized water. After washing, the roots were wrapped in gauze and centrifuged in a basket centrifuge (radius basket 12 cm, velocity about 600 rpm) for 5 minutes to remove the adhering water. Equal portions of root material, varying from 1 to 18 grams depending on experimental time and concentration, were placed in 20 polyethylene bottles, each con-

taining 10 litres of the experimental solution. During the absorption period the solutions were aerated with compressed air from the laboratory supply and kept at 20° C (experiments with Br) or 25° C (experiments with K, Rb, and Na) by a water thermostat. The pH of the solutions was approximately 5.3. With the root to solution ratio used, the pH did not change appreciably during the experiment. At the end of the experiment the roots were separated from the solution by means of a filter of nylon mesh, washed with running demineralized water for one minute, and transferred to a crucible. When Br was the experimental ion, 2 ml of CaO suspension (1.8 gram in 100 ml H_2O) was added to the roots. In the case of the cations this addition was omitted. Subsequently the roots were dried at 100° C and ashed at 560° C. For estimation of the cations the ash was dissolved in ten drops of concentrated HCl followed by addition of 20 ml water. Then the solution was heated on a hot plate ($\pm$ 80° C) for 20 minutes, filtered into a volumetric flask, and made up to volume (50 or 100 ml). The concentration of the cations was determined by means of a flame photometer (Beckman Model D.U.) at the wave lengths 767 mμ (K), 589 mμ (Na), and 780 mμ (Rb).

When Br was the experimental ion, the ash was dissolved in 15 ml cold acidified water (0.3 N HNO_3), filtered into a volumetric flask, and made up to 25, 50, or 100 ml. Br concentration was determined colorimetrically according to the gold chloride method described by ALLPORT (1947). To 15 ml solution was added 0.5 ml HNO_3 (4N) and 3 ml of a 0.5 % aqueous solution of gold chloride ($H(AuCl_4)$ H_2O Merck). The measurement of colour intensity was performed at a wave length of 500 mμ by means of a Unicam absorption meter (Model SP 400).

Any deviations from the procedure described here are mentioned in the separate chapters.

In one experiment intact plants with a high Br content were used. For this purpose the seeds were germinated as described. When the plants were two days old, 40 were selected and placed on a stainless steel grid. The grids were placed on 800 ml glass beakers filled with 500 ml of a solution of 5×10^{-4} M $CaSO_4$ and 10^{-2} M KBr, the level of the liquid being kept just below the grid. All beakers were aerated continuously. The nutrient solution of the plants was renewed daily. When the plants were 7 days old, they were used for the experiment.

CHAPTER III

RESULTS OF EXPERIMENTS ON BROMIDE UPTAKE

As a first approach to the nature of the influence of Ca on anion uptake, the relation between Br absorption and time was studied in the absence and presence of Ca.

For this purpose the bottles were filled with an experimental solution containing 0.05 m.e./l KBr, with and without addition of 10 m.e./l $CaSO_4$. The Br uptake of the roots was measured after

increasing time intervals. This experiment was repeated with 5 m.e./l KBr in the solution. The results are given in Figs. 1 and 2. The data indicate that the rate of Br absorption is constant over the whole experimental period of six hours. In the presence of Ca there is a strong stimulation of Br uptake. This stimulation by Ca ions is relatively much larger at the low Br concentration than at the higher one.

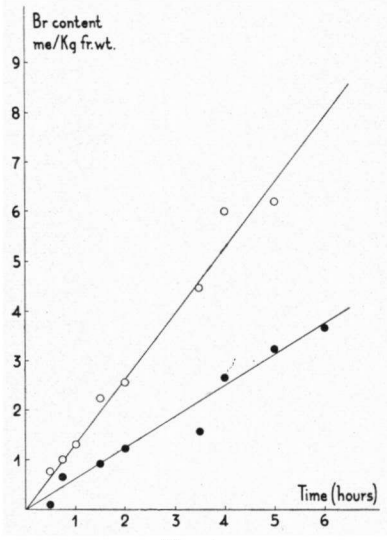

Fig. 1

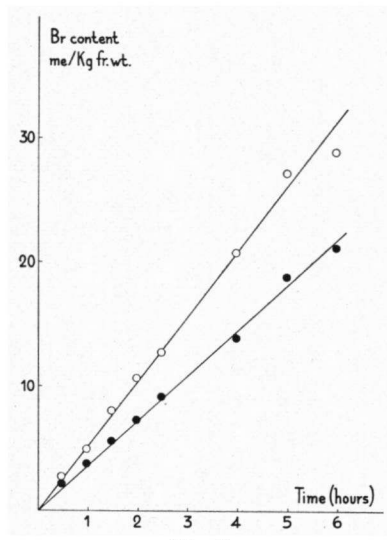

Fig. 2

Fig. 1. Relation between Br uptake from a 0.05 m.e./l KBr solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) Root/solution ratio: 3 gr/10 l.

Fig. 2. Relation between Br uptake from a 5 m.e./l KBr solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) Root/solution ratio: 3 gr/10 l.

Does this stimulation set in immediately after the addition of Ca ions or is there a "lag-phase" between the moment of addition and the starting of this stimulating effect? To answer this question the foregoing experiment was repeated but with low Br concentration in the experimental solution (0.02 m.e./l KBr) and shorter absorption periods (maximum 30 minutes). The stimulating effect of Ca ions on Br uptake manifests itself instantaneously, as can be seen from Fig. 3.

In the next experiment the rate of Br absorption was determined in the absence and presence of 10 m.e./l $CaSO_4$ at a temperature of 1.5° C. The Br concentration in the solution was 1 m.e./l (Fig. 4). At this temperature too, the absorption is stimulated by Ca ions throughout the absorption period.

The influence of the concentration of Br on the rate of Br absorption was studied in the absence and presence of 10 m.e./l $CaSO_4$. The

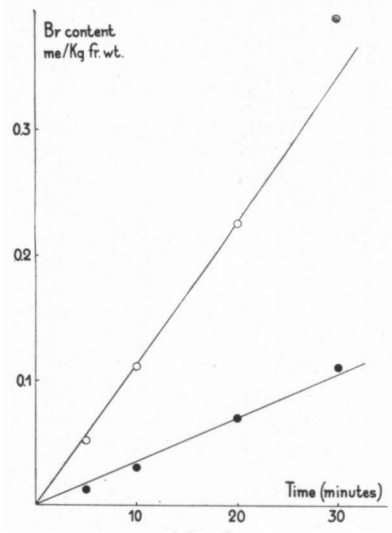

Fig. 3

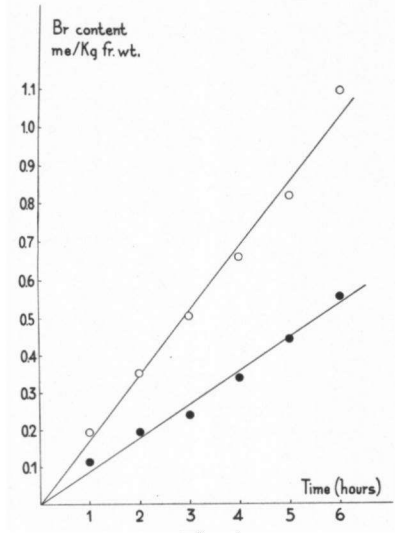

Fig. 4

Fig. 3. Relation between Br uptake and time during a half hour's immersion in a 0.02 m.e./l KBr solution in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.).
Root/solution ratio: 18 gr/10 l.

Fig. 4. Relation between Br-uptake from a 1 m.e./l KBr solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) at a temperature of 1.5° C.
Root/solution ratio: 20 gr/10 l.

experiment was carried out over a six-hour absorption period (Fig. 5). Over the whole range of concentrations used (0–5 m.e./l) the rate of Br absorption is dependent on the Br concentration in the experimental solution. The relation between absorption rate and concentration does not resemble a Langmuir adsorption-isotherm. The shape of the curve suggests that two mechanisms are involved in Br uptake. When the nearly linear increase of the rate of Br uptake above a concentration of about 1 m.e./l is tentatively ascribed to a second absorption mechanism, the relation between concentration and rate of absorption by the first mechanism can be obtained by means of a simple subtraction (see Fig. 5a). The resulting curve closely resembles a Langmuir adsorption-isotherm. It then appears that the first mechanism undergoes considerable stimulation in the presence of Ca ions, which is relatively highest at the lowest Br concentrations. On the other hand, the second mechanism is more or less unaffected by the presence of Ca ions.

This experiment was repeated at a temperature of 1.5° C (Fig. 6). Although the absolute rate of uptake is much lower than at 20° C, the general shape of the curves has undergone little change.

At a low Br concentration in the solution, the part taken by the

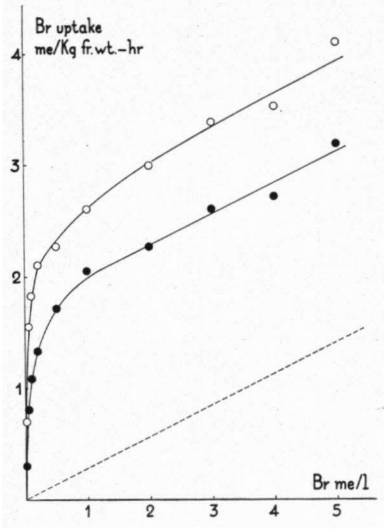

Fig. 5

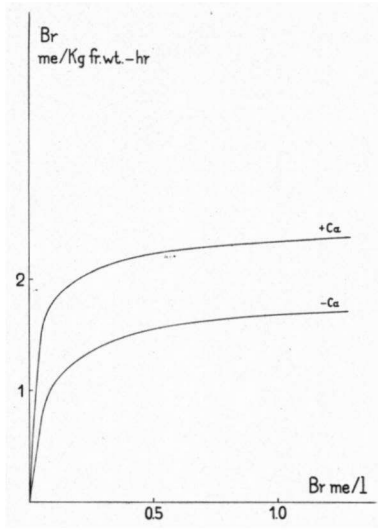

Fig. 5a

Fig. 5. Relation between rate of Br uptake from KBr solutions and concentration in the absence and presence of 10 m.e./l $CaSO_4$ (● and o resp.) Experimental time: 6 hours. Root/solution ratio: 3 gr/10 l. The broken line represents the rate of Br absorption by the second absorption mechanism (see text).

Fig. 5a. Relation between Br concentration and rate of Br absorption as calculated for the low-concentration mechanism in the absence and presence of Ca (see text).

second mechanism in the total uptake is relatively small. Since the following experiments were performed at low Br concentrations in the experimental solutions, the second mechanism can henceforth be left out of consideration.

The stimulating effect of Ca ions on Br uptake appears to be non-specific. This is apparent from the data of the following experiment, in which the influence of the monovalent cations Na, K, and Li and of the divalent cation Ca on Br uptake was studied over a cation concentration range of 0.1 to 10 m.e./l. To exclude the influence of the accompanying cation K on the Br uptake as much as possible,the Br uptake was determined from a solution with low Br concentration (0.05 m.e./l). The absorption period was one hour. The results given in Fig. 7 point to a similar stimulation of the Br uptake by the cations K, Na, and Li, but at the same time the stimulating effect of the monovalent cations is much lower than was observed for Ca ions under the same conditions.

The following experiment was designed to elicit whether or not the stimulating effect of Ca on the one hand and of the monovalent cations on the other are based on one and the same mechanism. The rate of absorption of Br was determined in KBr solutions (0.05 m.e./l) to which 0.2 m.e./l $CaSO_4$ and amounts of Na_2SO_4 ranging from

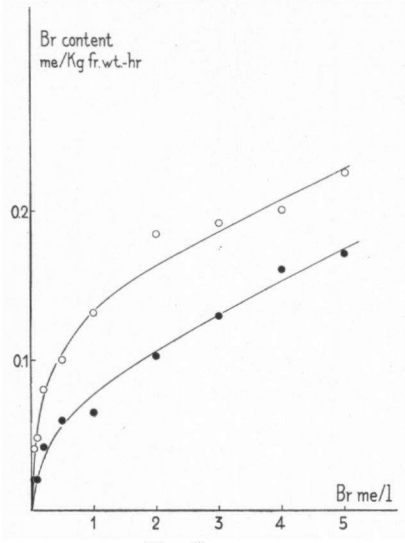

Fig. 6

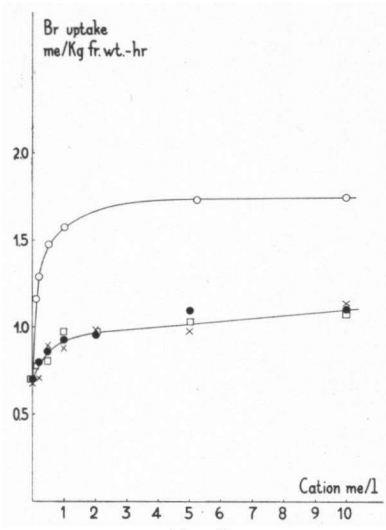

Fig. 7

Fig. 6. Relation between Br uptake from KBr solutions and concentration in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) at a temperature of 1.5° C. Experimental time: 6 hours. Root/solution ratio: 20 gr/10 l.

Fig. 7. Rate of Br absorption from a 0.05 m.e./l KBr solution in the presence of increasing concentrations of $NaSO_4$, K_2SO_4, Li_2SO_4, and $CaSO_4$ (× ● □ ○ resp.). Experimental time: 1 hour. Root/solution ratio: 14 gr/10 l.

0 to 50 m.e./l had been added. The absorption period was one hour (Fig. 8). It may be concluded from the results that in the presence of Ca the effect of Na ions on Br uptake fails to appear. Therefore, the effect of Ca and Na ions must be based on the same mechanism.

JACOBSON and co-workers (1961) stated that the effect of cations other than Ca on ion absorption might be ascribed to an indirect effect. They supposed an exchange between Ca ions in the roots and the pertinent cations in the solution, resulting in a release of Ca ions which can then occupy sites that are important for permeability. To exclude this possibility here, the roots were given the following pretreatment with a K_2SO_4 solution before the start of the experiment. Ten grams of barley roots were immersed in 10 litres of aerated solution containing 10 m.e./l K_2SO_4 for 30 minutes. The roots were then collected and transferred to a new solution of the same composition, again for 30 minutes. After this treatment the roots were collected and washed for 1 minute with demineralized water. The Br uptake by these pretreated roots in the presence of 10 m.e./l K_2SO_4 was then compared with the corresponding Br uptake of unpretreated roots. The results are shown in Table 1.

On the basis of Jacobson's hypothesis it must be expected that the Ca ions bound to occupy the sites important for permeability, are removed by pretreatment of the roots with K ions. The result of this

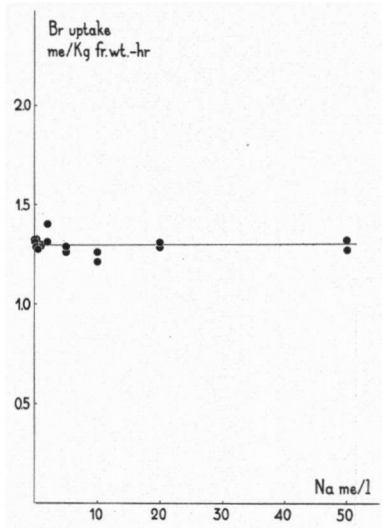

Fig. 8. Rate of Br absorption from a solution of 0.05 m.e./l KBr + 0.2 m.e./l $CaSO_4$ in the presence of increasing concentrations of Na_2SO_4. Experimental time: 1 hour. Root/solution ratio: 12 gr/10 l.

experiment shows that the stimulating effect of K ions on Br uptake was not altered by this treatment. It could be argued that the release of Ca ions from the root might continue after the pretreatment. However, a simple calculation shows that the quantity of Ca ions the pretreated roots would have to release to bring about the observed stimulation is beyond any real possibility. The stimulating effect on Br uptake by 10 m.e./l K is equivalent to that brought about by 0.1 m.e./l Ca. Consequently, the effect would require the release of 300 m.e. Ca/kg of roots (10 grams of roots were placed three times in 10 litres of a solution containing 10 m.e./l K). This quantity is too far out of proportion to be considered seriously. It follows that the stimulating effect on Br uptake by Ca ions is not specific, although there is a quantitative difference between Ca ions and the monovalent cations.

In the next experiment the influence of pH on the Ca effect was studied. The experimental solution contained 0.05 m.e./l Br and the uptake period was one hour. To obtain a pH range from 3.8 to 7.0,

TABLE 1

Pretreatment	Composition of experimental solution	Uptake m.e. Br/kg fr. wt.–hr.
———	0.05 m.e./l KBr	0.568
———	0.05 m.e./l KBr + 10 m.e./l K_2SO_4	0.920
2 × 30 min 10 m.e./l K_2SO_4	0.05 m.e./l KBr + 10 m.e./l K_2SO_4	0.893

drops of a dilute solution of either H_2SO_4 or NaOH were added to the experimental solution. For pH values above 7.0, so much alkali, and thus cation, had to be added that the risk that this extra quantity of cations would also influence the Br uptake became real. For this reason only the pH range between 3.8 and 7.0 was studied. The results of this experiment are shown in Fig. 9. It appears that not only do changes in pH have little effect on Br uptake but also that the influence of Ca is relatively independent of pH.

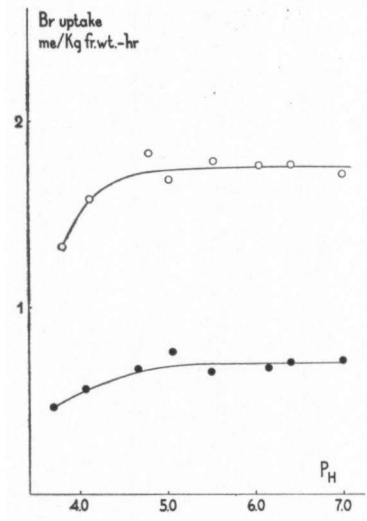

Fig. 9. Rate of Br absorption from a 0.05 m.e./l KBr solution in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) in relation to external pH. Experimental time: 1.5 hour. Root/solution ratio: 10 gr/10 l.

To obtain data about a possible outflux of Br ions, an experiment was performed in which barley with a high Br content was used. Intact plants were used in order to avoid a possible leakage of Br ions out of the cut surfaces. The plants were cultivated in the dark as described in Chapter II. The roots of these plants were rinsed three times with demineralized water and then placed for 30 minutes in 200 ml of aerated demineralized water with a pH of 5.3. Br ions could not be demonstrated in the surrounding solution after this interval. The Br content of the roots as assayed at the end of the experiment amounted to 36.1 m.e. Br/kg roots. Thus no evidence supporting an outflux of Br ions is provided by this experiment.

Discussion

It is apparent from the results that the uptake of Br ions remains in a steady state at least over a period of six hours. The concentration curve suggests that two mechanisms are involved in the uptake of Br ions.

Bözörményi and Cseh (1964) also concluded from a study of the

competition between the Br and the Cl ion in excised wheat roots that at least two mechanisms are involved in the uptake of these halides.

The rate of Br uptake is increased by Ca ions. This phenomenon cannot be ascribed to a general state of Ca deficiency of the roots because the plants were cultivated in a 1 m.e./l $CaSO_4$ solution. In addition, the instantaneity of the effect of Ca on the uptake of Br ions by roots washed with demineralized water shows that the Ca effect on Br uptake must be localized at the surface of the cell.

K, Na, and Li ions are also able to increase the rate of Br uptake. This effect appeared not to be due to Ca ions released from the roots by exchange with the monovalent cations used.

In a study of the influence of Ca on the uptake of other cations JACOBSON et al. (1960) suggested that Ca modifies the cell surface in such a way that for some of the cations a barrier is created. According to their idea, this barrier is located between the experimental solution and the carriers, and determines the relative amounts of the cations to reach their absorption sites. The stimulating effect of Ca is considered to be essentially a blocking of the influx of interfering ions. To apply this explanation to the stimulating effect of Ca ions on Br uptake would require competition between the Br ion and some other anion. The anions to be considered at a pH below 7.0 are the SO_4-ion (the pH was adjusted with H_2SO_4) and the HCO_3'-ion. Quite apart from the question of whether or not these ions have a competitive effect with respect to the Br ion, the relative influence of Ca on the Br uptake appeared to be nearly constant in the pH range of 4.5 to 7.0, notwithstanding the fact that different concentrations of both anions were present at these pH values. Another objection to the application of this hypothesis to the case of the Br ion is that a barrier-creating effect similar to that assumed for the Ca ion though less strong, must be attributed to the K, Na, and Li ions. Such an effect of these ions is less probable.

Next, it may be asked whether stimulation by Ca of the biochemical processes leading to the formation of carrier could explain the increase of the absorption rate, as suggested for the Cs uptake by MARSCHNER (1961). From the data presented it is clear that the extent of the stimulation of Br uptake by Ca at 20° C does not increase during the experimental time, i.e. no measurable time interval is needed for the Ca to reach its maximal effect, the same is true for the experiment at 1.5° C. It follows that the stimulation of carrier formation would also occur instantaneously at 1.5° C, a temperature at which the rate of biochemical processes is extremely slow. Therefore, it is not very probable that an increase of carrier formation is responsible for the stimulation of the Br uptake by Ca ions.

An increase of the affinity between the Br ion and its carrier under the influence of Ca, as supposed by KAHN and HANSON (1957) for K uptake by maize and by TANADA (1962) for Rb uptake by mung bean, does not explain why the maximum uptake is also enhanced under the influence of Ca, at least if carrier kinetics as proposed by EPSTEIN and HAGEN (1952) are accepted.

DAVIES and WILKINS (1951), Sutcliffe (1954), HELDER (1958) and others found an outflux of ions in various experimental material. BRIGGS (1957) has demonstrated that in the experiments of Davies and Wilkins and Sutcliffe, concerning the uptake of KBr by carrot and beet tissue slices respectively, the radio-activity in the vacuole increased and in the solution decreased more rapidly than is in agreement with the absorption of K ions as estimated with chemical methods. This leads to the conclusion that the net uptake is the sum of an influx into and an outflux of ions out of the vacuole.

An outflux of PO_4-ions influenced by Ca ions, could be demonstrated with mature maize plants cultivated in a Knop solution (unpublished results). When the Knop solution was replaced by distilled water, a considerable outflux of PO_4-ions from the roots could be shown. When Ca ions were added to the distilled water, this outflux of PO_4-ions decreased considerably. In accordance with this observation, the Ca effect on Br uptake in barley roots might be based on a decrease of a Br outflux under the influence of Ca ions, resulting in an increase of net Br uptake. However, even when the content of the roots amounted to 36 m.e. Br/ kg fr.wt. it was impossible to demonstrate a Br outflux. Moreover, the relation between the rate of Br absorption and time is not in agreement with this interpretation. The stimulating effect of Ca ions is already manifest at the very start of the experiment, before a sufficient quantity of Br can have accumulated in the roots. This is demonstrated most clearly in the experiments in which short absorption periods and low Br concentrations were used (Fig. 3). Even at 1.5° C, when the uptake is exceptionally small, the Br uptake is enhanced in the presence of Ca ions. Hence, the experimental results give little support to this hypothesis.

If a zone with fixed negative charges were located between the experimental solution and the carrier, the Br ion could only reach the carrier by diffusion through this layer. In this case the stimulating effect of Ca ions on Br uptake might be attributed to a screening of these negative charges by Ca with the consequence that the rate of Br diffusion, and thus the rate of Br absorption are enhanced. If at 20° C the rate of uptake were limited by a preliminary diffusion process, this limiting factor would for the most part be eliminated by lowering the temperature to 1.5° C because the rate of diffusion is so much less affected by temperature than the active uptake process. In consequence, the relative effect of Ca would diminish when the active uptake is strongly inhibited by lowering the temperature. According to our results, however, the Ca effect on Br uptake is not smaller at 1.5° C than it is at 20° C.

Thus, no evidence supporting this interpretation is provided by the experimental data.

Yet it is a well-known fact that many negative charges are present in part of the free space of the roots, the so-called Donnan Free Space or Ion Exchange Region. Therefore, at a certain Br concentration in the solution, the Br concentration in this zone of negative charges will be lower than in the solution. Cations, especially polyvalent cations,

will screen these negative charges, as a result of which the Br concentration in this zone is enhanced. If the carrier is loaded with Br ions deriving from this zone, the lower Br concentration prevailing in it, rather than the Br concentration prevailing in the solution, might determine the extent of saturation of the carrier. According to this hypothesis and in accordance with the observation, the cations, especially the polyvalent ones, will increase the Br uptake by means of an increase of the Br concentration prevailing near the carrier sites. However, this interpretation must be rejected on the ground that the Ca effect persists in the range of Br concentrations in which the Ca-sensitive uptake mechanism (cf. page 512, under Results) has evidently already been saturated. This would not be the case if the effective Br concentration at the carrier were limiting the rate of uptake.

As mentioned in the Introduction, ELGABALY (1962) concluded that the electro-negative charge of the root is responsible for the effect of cations on the uptake of anions; in accordance with the view of Lundegårdh, energy derived from metabolism would be necessary to overcome the electrostatic repulsion. VERVELDE (1952) correctly pointed out that the passage of anions through the boundary between root and medium does not require a special energy supply, in spite of the negative root potential, because there is no jump of the electrochemical potential at the boundary.

There is still another conceivable way in which electro-negative charges in the neighbourhood of the sites of the absorption mechanism could inhibit the uptake of anions. Part of the total amount of carrier may be inaccessible to the Br ions as a result of electrostatic repulsion by negative charges in the close neighbourhood of the carrier sites. Screening of these charges by cations, of which Ca is the most effective, would lead to an increase in the amount of carrier involved in the uptake, and so to an increase in the maximal absorption rate. There are several arguments in favour of this hypothesis: the stimulating effect of Ca ions on Br uptake manifests itself without a lag-phase, the relative extent of the stimulation is not decreased by lowering the temperature, and the stimulating effect on Br uptake is not specific for Ca ions, i.e. other cations have a stimulating effect as well. Moreover, the cation involved must be present in the experimental solution; pretreatment with the cation has no effect (VIETS 1944).

As already pointed out, the presence of Ca ions was found to have the strongest effect at low Br concentrations, which is reflected in a decrease of the half-value of the uptake mechanism active at low Br concentrations under the influence of Ca. According to the theory of BANGE (1962), an increase in the amount of carrier should lead to a decrease of the half-value. However, it should be noted that in Fig. 5 the concentration curve obtained in the absence of Ca is complicated by the fact that simultaneously with Br concentration the concentration of the accompanying K ion also increases, and with it its stimulating effect on Br absorption.

The suggested interpretation involves the condition that Ca does not stimulate cation absorption in the same way. On the other hand,

it should be emphasized that the interpretation given for the stimulating effect of Ca on Br uptake does not imply a decrease in cation uptake in the presence of Ca if only the accessibility of the sites for cation uptake is ensured under all circumstances. VIETS (1944), OVERSTREET et al. (1952), JACOBSON et al. (1960), MARSCHNER (1961) and others have shown that the uptake of cations is influenced by the presence of Ca ions, at one time in the form of an inhibition, at another in the form of a stimulation. Therefore we must ascertain whether the nature of these influences supports or contradicts our hypothesis.

CHAPTER IV

RESULTS OF EXPERIMENTS ON SODIUM UPTAKE

The time course of Na absorption from a solution of 0.2 m.e./l Na_2SO_4 was determined in the absence and presence of 10 m.e./l $CaSO_4$ over a 6-hour uptake period. Fig. 10 shows that the rate of Na uptake in the absence of Ca is not constant throughout the experimental time. Initially, the overall rate of Na absorption shows a gradual decline with time but after about two hours a steady state is reached and maintained for the rest of the experimental period. In the presence of 10 m.e./l Ca, the rate of Na uptake is approximately

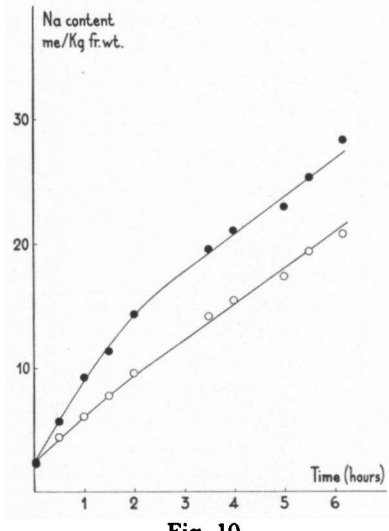

Fig. 10

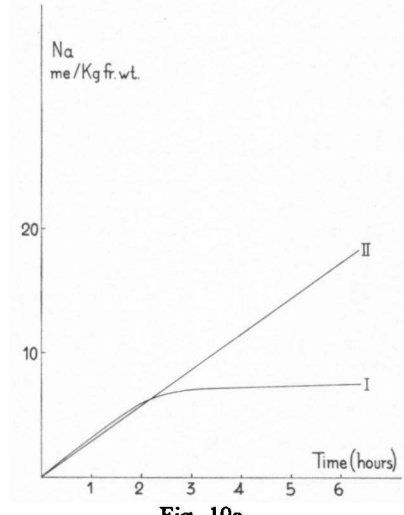

Fig. 10a

Fig. 10. Relation between Na absorption from a 0.2 m.e./l Na_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and o resp.). Root/solution ratio: 3 gr/10 l.

Fig. 10a. Separation of the curve for the absorption of Na in the absence of Ca (Fig. 10) into a component of limited duration (I) and a steady state component (II).

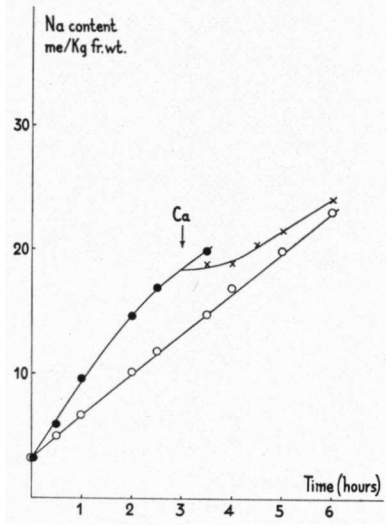

Fig. 11. Relation between Na absorption from 0.2 m.e./l Na_2SO_4 solution and time in the absence (●) and presence (○) of 10 m.e./l $CaSO_4$. After 3 hours part of the roots absorbing Na in the absence of Ca was transferred to the solution containing Ca. (×) Na content of the roots after transfer. Root/solution ratio: 3 gr/10 l.

constant over the whole period of 6 hours and equals the rate of steady-state uptake of Na ions in the absence of Ca.

The uptake curve of Na in the absence of Ca can be tentatively resolved into two fractions: Fraction I, in which the rate of uptake shows a gradual decline with time and approaches zero after about two hours; and Fraction II, in which the rate of uptake appears to be strictly linear with time, i.e. in a steady-state, and equals the rate of Na uptake in the presence of Ca (Fig. 10a).

The following experiments were performed to ascertain whether Fraction I of the Na uptake is exchangeable in a simple way against Ca ions of the medium. The preceding experiment was repeated with the difference that after an experimental interval of three hours the roots present in the solution without Ca were taken out and transferred, without rinsing, to a solution containing 10 m.e./l Ca in addition to 0.2 m.e./l Na. The result of this experiment is presented in Fig. 11. The transfer of the roots to the solution containing Ca induced a temporary stagnation of the Na uptake after which the steady-state uptake was resumed. However, the fact that not even a temporary decrease in the Na content of the roots occurred, raised some doubt with respect to the obvious assumption that an exchange of Na versus Ca was involved. This question can be elucidated experimentally by taking due precautions that after the addition of Ca no reabsorption of exchanged Na by the steady-state mechanism can occur. Therefore, in the next experiment the steady-state mechanism was inhibited by anaerobiosis after transfer of the roots to the $CaSO_4$ solution. Fig 12

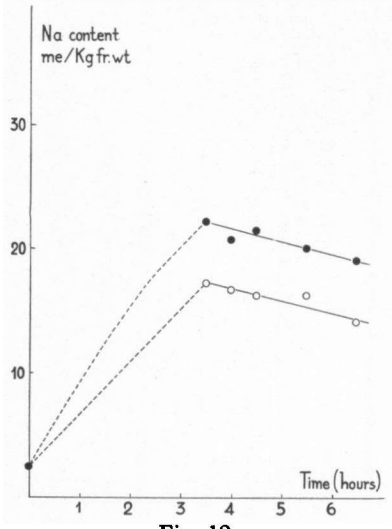

Fig. 12

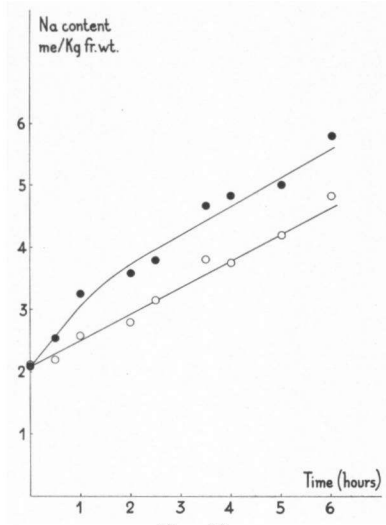

Fig. 13

Fig. 12. Na content of roots in relation to time of immersion in an anaerobic solution of 10 m.e./l $CaSO_4$ after $3^1/_2$ hours absorption from an aerobic 0.2 m.e./l Na_2SO_4 solution in the absence (●) and presence (○) of 10 m.e./l $CaSO_4$. Root/solution ratio: 3 gr/10 l.

Fig. 13. Relation between Na absorption from a 0.2 m.e./l Na_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) at a temperature of 1° C.
Root/solution ratio 3 gr/10 l.

shows the behaviour of the Na content of the roots over a period of three hours after they had been transferred from the 0.2 m.e/l Na_2SO_4 solution with and without 10 m.e./l $CaSO_4$ to a $CaSO_4$ solution through which nitrogen gas was bubbled. The Na content of the roots decreases to some degree, but this appears not to be attributable to an exchange between Na and Ca ions because the two sets of roots behave very similarly. This indicates that the slow decrease must be considered as a general effect of the N_2 atmosphere and is unrelated to the presence of Ca. Therefore, it is clear from these data that the Na ions of Fraction I do not enter the solution as the result of a simple exchange against Ca ions. An effect similar to that obtained with N_2 was found at low temperature.

The following experiment was designed to establish the temperature sensitivity of Fraction I. The rate of Na uptake from a solution containing 0.2 m.e./l Na_2SO_4 with and without the addition of 10 m.e./l $CaSO_4$ was determined over a 6-hour period at a temperature of 1°C. It was found that not only was the steady-state uptake much lower than at 25° C but the amount of Na of Fraction I was also considerably reduced (Fig. 13).

The time relation of Na absorption from a solution containing 5 m.e./l Na with and without the addition of 10 m.e./l Ca resemble those

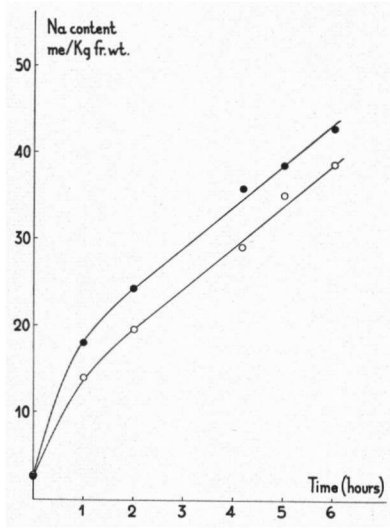

Fig. 14

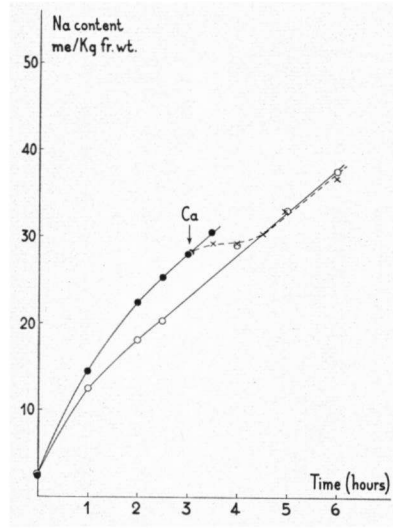

Fig. 15

Fig. 14. Relation between Na absorption from a 5 m.e./l Na_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and o resp.). Root/solution ratio: 3 gr/10 l.

Fig. 15. Relation between Na absorption from a 5 m.e./l Na_2SO_4 solution and time in the absence (●) and presence (o) of 10 m.e./l $CaSO_4$. After 3 hours part of the roots absorbing Na in the absence of Ca was transferred to the solution containing Ca. (×) Na content of the roots after the transfer. Root/solution ratio: 3 gr/10 l.

obtained at a Na concentration of 0.2 m.e./l Na (Fig. 14). However, in this case, also in the presence of Ca, a steady-state Na uptake was reached only three hours after the start of the experiment. Apparently Ca ions do not fully repress the Na uptake into Fraction I. When the same time relations were determined at 1° C (Fig. 16), the curve representing the Na uptake in the presence of Ca appears to be approximately linear.

It is apparent from the data presented in Figs. 10 and 14 that the Na uptake into Fraction I, at both low and high Na concentrations is completed after about three hours. The Na absorption occurring after this period is due to the steady-state uptake mechanism. As a consequence, it is possible to study the relation between the rate of Na absorption by the steady-state mechanism and the Na concentration in the solution only after an uptake period of three hours. To establish this relationship, the Na uptake in the absence of Ca was measured over two experimental periods, viz. three and five hours. The steady-state uptake over two hours could be obtained by subtraction. Fig. 17. shows how the total absorption of the Na ion within absorption periods of three and five hours, the calculated rate of steady-state uptake over a period of two hours, and the size of Fraction I depend upon

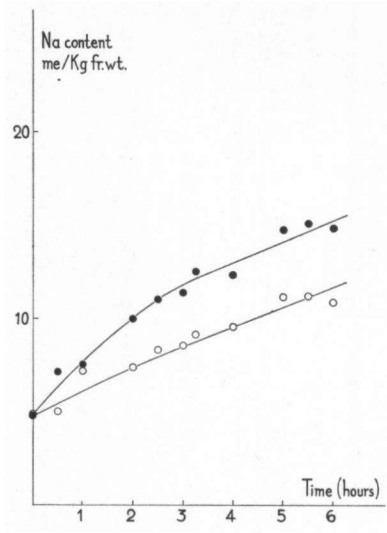

Fig. 16

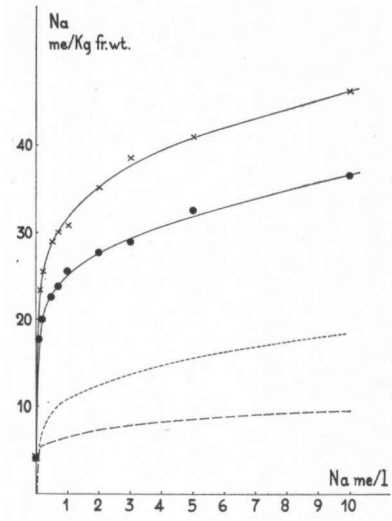

Fig. 17

Fig. 16. Relation between Na absorption from a 5 m.e./l Na_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) at a temperature of 1° C.
Root/solution ratio: 3 gr/10 l.

Fig. 17. Rate of Na absorption from a Na_2SO_4 solution as affected by concentration. (● experimental period 3 hours, × experimental period 5 hours). The broken line (– – – –) represents steady-state Na uptake over 2 hours in relation to Na concentration and was obtained by subtracting the 5- and 3- hour absorption lines. The dotted line (. . . . . .) represents the amount of Na present in Fraction I in relation to Na concentration and was obtained by calculating steady-state Na uptake for a period of 3 hours and subtracting this amount from overall Na uptake in 3 hours.
Root/solution ratio 3 gr/10 l.

the Na-concentration. The size of Fraction I was calculated by subtracting of the steady-state uptake from overall uptake during the first three hours of the absorption period. The rate of Na absorption in the steady state does not reach a maximum when the Na concentration of the experimental solution is increased from 0.2 to 10 m.e./l, but the increase above a Na concentration of 1 m.e./l is relatively small. Therefore, in contrast to what has been supposed for Br, any additional absorption mechanism operating at higher concentrations, e.g. at a Na concentration of 5 m.e./l, will play a role of only minor importance in the total uptake.

The amount of Na in Fraction I shows a sharp initial rise with increasing outward Na concentration, followed by a gradually declining increase. However, within the range of concentrations studied the amount of Na in Fraction I does not attain a maximum.

Discussion

The most important conclusion to emerge from the experiments discussed so far is that the rate of steady-state Na uptake, as proceeding after an absorption period of about three hours, is not influenced by Ca ions, at an Na concentration of either 0.2 m.e./l or 5 m.e./l in the solution.

It is true that the presence of Ca ions brought about a reduction of the total amount of Na taken up, just as in the experiments of Jacobson, Moore and Hannapel (1960), but the course of the time curves shows that the phenomenon cannot underlie an impediment of the acces of Na to the carrier sites as suggested by these authors. For, the influence of Ca concerns only an additional Na uptake of limited extent (at an Na concentration of 0.2 m.e./l, about 5 m.e./kg fr. wt.) and limited duration (approximately two hours).

With respect to this additional Na uptake, denoted here as Fraction I, two questions must be answered:

1) Can this fraction be identified with an amount of Na present in a certain cell compartment?

2) Can the influence of Ca on the uptake of this fraction be explained in terms of competition between Ca and Na ions for adsorption sites in this cell compartment?

Concerning the first question, the following considerations are relevant. On the basis of quantitative considerations it is clear that a simple diffusion of Na ions into the water free space (WFS) of the roots cannot represent Fraction I because this fraction contains 5 m.e./Na /kg fresh weight of roots at a Na concentration of 0.2 m.e./l in the solution. Moreover, the Na content of Fraction I does not increase proportionally to the concentration (Fig. 17). At the same time, it is clear that the amount of Na of Fraction I cannot be identified with Na adsorbed on cell-wall material for not only does it show a strong dependence on temperature (at 1° C the Na content of Fraction I is only 20 % of the content at 25° C) but also it is not released from the root by the presence of Ca. Considering the further behaviour of this fraction, the obvious assumption would seem to be that it consists of Na adsorbed to cytoplasmatic constituents.

As to the second question, conclusive evidence has been presented in the preceding chapter for the view that the Na ions of Fraction I are not exchanged by Ca ions. Therefore, the inhibiting effect of Ca on this fraction of the Na uptake cannot be based on a competition between Ca and Na ions for common exchange sites. A possible explanation for the observed Ca effect may be sought in a reduction of the permeability of the plasma membrane. In the absence of Ca, the Na ions may be able to pass this membrane, in whatever way, to saturate the binding sites of Fraction I located behind it. The degree of this saturation will depend upon the concentration of the Na ion in the medium. Ca ions may decrease the permeability of the plasma membrane in such a manner that the Na ions are no longer able to

pass it in the same way, thus making the binding sites of Fraction I inaccessible to them.

The temperature sensitivity of the amount of Na present in this fraction might be understood by the assumption that its filling is closely linked to temperature-sensitive plasma streaming. It seems conceivable that the cations do not move freely to the binding sites in the protoplasm but that these binding sites move, as it were, to the cations along with the streaming protoplasm. At low temperature protoplasmic streaming is retarded and the circulation of the plasmatic constituents may be incomplete. As a result, a number of binding sites may be no longer attainable for Na ions. An alternative explanation is that the temperature sensitivity depends upon a marked reduction in the permeability of the membrane at low temperatures. Since, however, the temperature sensitivity concerns not so much the rate of equilibration of this fraction as its degree of saturation, the first possibility seems the more likely one. A third possibility might be found in a difference in the number of binding sites present in the cytoplasm at 25° C and 0° C.

To explain the depletion of Fraction I after the membrane has been made impermeable to Na ions by Ca (Fig. 11), it must be assumed that the Na ions in this fraction are removed by the steady-state absorption mechanism. Thus, after the addition of Ca, initially most of the binding sites of this transport system will be saturated by Na ions derived from Fraction I, but gradually an increasing number of sites will become available again for the transport of external Na. After Fraction I has been entirely depleted, the rate of Na absorption from the external solution is restored to its original value. It even seems possible that in the absence of Ca, steady-state Na transport is mediated by the Na ions absorbed in Fraction I. It is noteworthy that after the addition of Ca the absorption lines converge (Figs. 11 and 15), demonstrating that the rate of Na uptake is identical whether the carrier is loaded in Fraction I or in the external solution. Since the Na concentration in Fraction I must be much higher than in the external solution, the effective degree of saturation of the carrier is presumably determined not so much by the chemical as by the electrochemical potential.

When the experimental solution contains 5 instead of 0.2 m.e./Na (Fig. 14), Na absorption in the presence of Ca, too, is not linear with time. The fact that at 1° C the time relation at this Na concentration is linear proves that in this case, too, the additional Na uptake during the first two hours at 25° C does not represent an uptake into the Donnan Free Space.

The inability of Ca to inhibit the filling of at least part of Fraction I in this case, might be tentatively ascribed to an antagonistic effect on membrane permeability of the rather considerable amount of Na ions in the experimental solution, or to the circumstance that an incomplete membrane impermeability to Na ions in the presence of Ca is brought out only at a higher gradient of the Na concentration. However, an alternative possibility is that in the presence of Ca,

Na ions bound to the steady-state mechanism pass to part of the adsorption sites of Fraction I. In this case the degree of saturation of the sites of Fraction I may be supposed to be determined by the amount of ion-carrier complex available. Consequently, the phenomenon may only occur to an observable degree when this amount exceeds a certain value. When, in the absence of Ca, part of the saturation of Fraction I has not been mediated by the steady-state mechanism, the addition of Ca (Fig. 15) will result in a removal of Na ions from Fraction I until the degree of its saturation is again in equilibrium with the prevailing concentration of ion-carrier complex. In this connection it should be borne in mind that according to the theory of BANGE (1962) the amount of ion-carrier complex may rise without a proportional increase in the rate of carrier-mediated ion transport.

Summarizing, it is concluded that steady-state Na absorption is in no way influenced by the presence of Ca ions. At the start of the absorption period and in the absence of Ca, an additional Na uptake is superimposed upon steady-state uptake. Depending upon the concentration of the Na ions in the solution, this additional Na uptake is completely or partially abolished by Ca. The quantity of Na involved cannot be identified with exchangeable Na adsorbed on cell-wall material, but is most probably located in the cytoplasm behind a membrane which permeability to Na ions depends to a higher degree upon the presence of Ca ions.

CHAPTER V

RESULTS OF EXPERIMENTS ON POTASSIUM UPTAKE

As a first approach to the influence of Ca on K absorption, K uptake from a solution of 0.01 m.e./l K_2SO_4 (pH 5.3) in the absence and presence of 10 m.e./l $CaSO_4$ was determined over a 6-hour period (Fig. 18). The curves resemble those obtained with Na when an experimental solution of 0.2 m.e./l Na was used. For the first few hours the K uptake is larger in the absence than in the presence of Ca, but thereafter the rate of uptake is independent of the presence of Ca.

The uptake in the absence of Ca can again be divided into a steady-state absorption and an additional uptake which comes to an end after about three hours. As in the case of Na, this additional uptake will be indicated by the term Fraction I.

The uptake of K is somewhat unsettled during the first hour of the experiment. This is probably due to the relatively high K concentration present in the roots. As is seen in the following experiment, the corresponding Rb uptake (Fig. 19) did not show such behaviour.

The following experiment was designed to establish whether the K ions of Fraction I are reversibly exchangeable with Ca ions from the medium. In this experiment the Rb ion was used as a "physiological" isotope of the K ion (Menzel and Heald 1955). The roots were immersed in a solution of 0.01 m.e./l Rb_2SO_4 for three hours, collected,

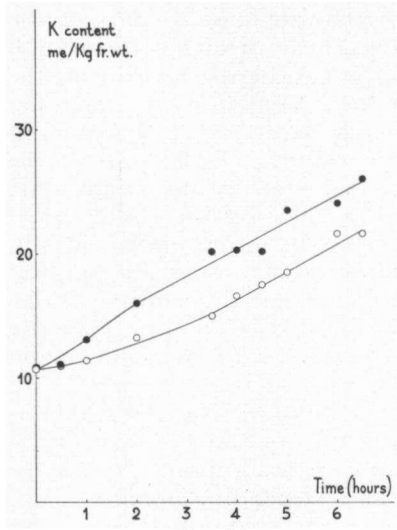

Fig. 18

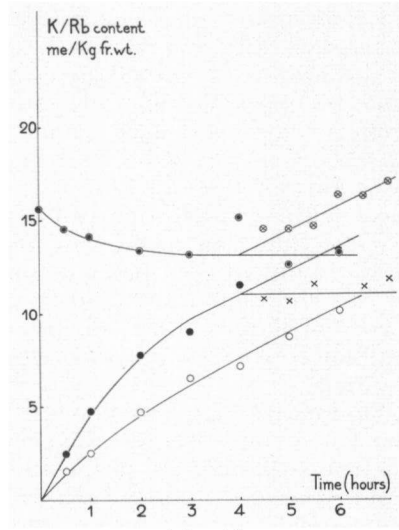

Fig. 19

Fig. 18. Relation between K absorption from a 0.01 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.). Root/solution ratio: 1 gr/10 l.

Fig. 19. Relation between Rb absorption from a solution of 0.01 m.e./l Rb_2SO_4 and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) • K content of the roots during Rb uptake in the absence of Ca.
After 4 hours part of the roots was transferred from the Rb solution lacking Ca to an equivalent K_2SO_4 solution containing 10 m.e./l $CaSO_4$. ⊗ K content of the roots during K uptake, × Rb content of the roots during K uptake. Root/solution ratio: 1 gr/10 l.

and transferred without rinsing to a solution containing 0.01 m.e./l K_2SO_4 and 10 m.e./l $CaSO_4$. During the next three hours the K uptake and the Rb content of the roots were measured. For the sake of comparison, the time course of Rb absorption from a 0.01 m.e./l Rb_2SO_4 solution in the presence and absence of 10 m.e./l $CaSO_4$ was determined over a 6-hour absorption period. In this last series the K content of the roots was measured as well. The results are shown in Fig. 19.

It is evident from these data that Rb is not released from the roots. It should be noted that the presence of an excess of competing K ions is bound to restrict to a minimum any reabsorption of Rb ions released from the roots by exchange against Ca ions. An important point is that the uptake of K, which occurs at approximately the same rate as the uptake of Rb, starts without a lag-phase, i.e. there is no stagnation of the K(Rb) transport out of the solution into the root after the addition of Ca ions, as was observed in the case of Na.

The time relation of K uptake from a solution containing 0.2 m.e./l K (pH 5.3) is shown in Fig. 20. This curve differs in two important aspects from the curves already dealt with. First, the absence or

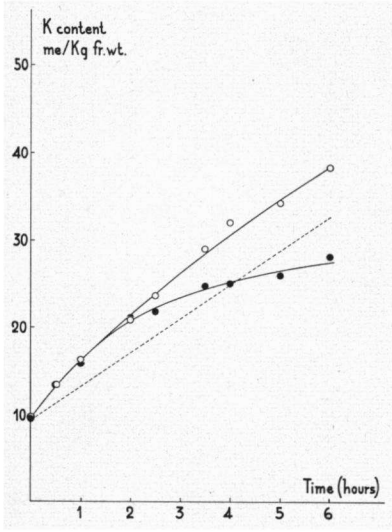

Fig. 20

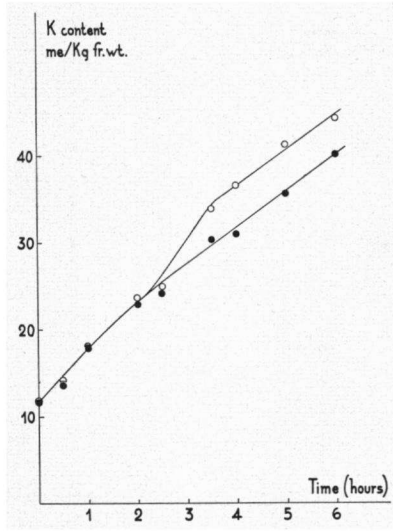

Fig. 21

Fig. 20. Relation between K uptake from a 0.2 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.).
For the sake of comparison a straight line (– – – –) has been drawn through the point of origin of the curves on the ordinate and parallel to the 3–6 hours portion of the time curve for K absorption in the presence of Ca.
Root/solution ratio: 3 gr/10 l.

Fig. 21. Relation between K uptake from a 0.2 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.) at pH 6.8.
Root/solution ratio: 3 gr/10 l.

presence of Ca ions makes no difference for the rate of K uptake during the first hour. Second, the rate of K uptake in the absence of Ca does not remain constant but shows a gradual decrease after about two hours.

This experiment was repeated at pH 6.8 (Fig. 21). The pH was adjusted by addition of a few drops of a 17 % solution of the organic base trishydroxymethylaminomethane ("tris"). The course of the K uptake in the absence of Ca appears to be identical to the K uptake in the presence of Ca at pH 5.3. Moreover, at pH 6.8 the rate of K uptake is the same in the presence and absence of Ca, except for a discontinuity in the absorption rate in the presence of Ca after about two hours. However, this discontinuity is of short duration (± one hour), and in no way influences the rate of K uptake during the next three hours.

Thus, it appears that at pH 5.3 the H ion concentration is directly or indirectly responsible for the decrease in the rate of K absorption in the absence of Ca, and also that this effect of H ions is counteracted by the presence of Ca. The decrease in the rate of K uptake at pH 5.3 cannot be attributed to permanent damage to the uptake mechanism

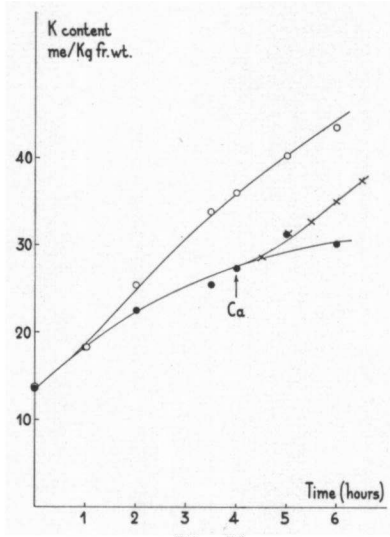

Fig. 22

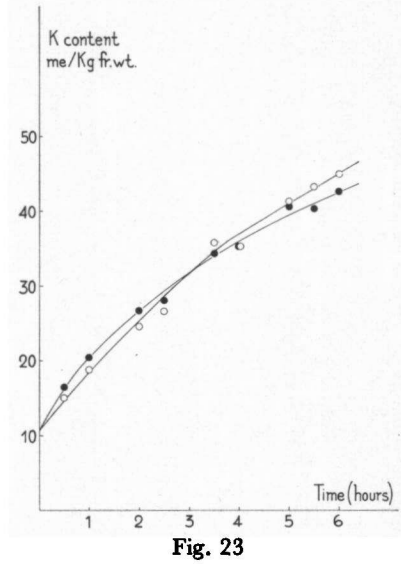

Fig. 23

Fig. 22. Relation between K uptake from a 0.2 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.). After 4 hours part of the roots absorbing K in the absence of Ca was transferred to the solution containing Ca. × K content of the roots after the transfer.

Fig. 23. Relation between K uptake from a 5 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.). Root/solution ratio: 3 gr/10 l.

because the effect could be shown to be of a reversible nature. For this purpose the roots were immersed for four hours in a solution (pH 5.3) containing 0.2 m.e./l K without the addition of Ca ions and then transferred without rinsing to a solution containing in addition 10 m.e./l Ca. The controls were allowed to absorb K from the same solution in the absence and presence of Ca over a 6-hour period. The results are given in Fig. 22. Shortly after the roots have been placed in the solution containing Ca ions the rate of uptake becomes equal to that of roots absorbing K in the presence of Ca from the start of the experiment. It is evident from these data that the effect of the H ion concentration cannot be due to irreversible damage to the uptake mechanism.

In addition to the presence of Ca and increase of the pH from 5.3 to 6.8, the decline in the rate of K uptake after a 2-hour absorption period can also be checked by using higher K concentrations in the experimental solution. Thus, at a K concentration of 5 m.e./l, the rate of K uptake in the absence of Ca at pH 5.3 starts deflecting from the absorption rate in the presence of Ca only after about three hours (Fig. 23).

The next experiment was designed to determine whether the decrease in the rate of K uptake of the roots from a solution of 0.2

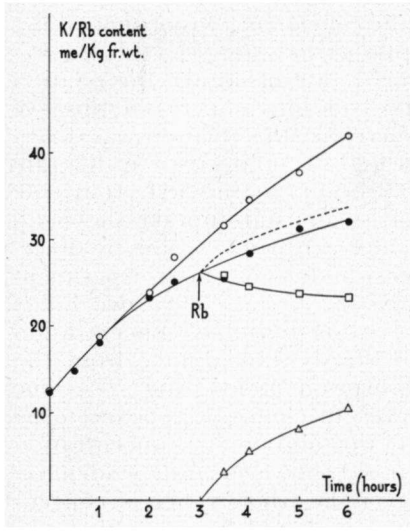

Fig. 24

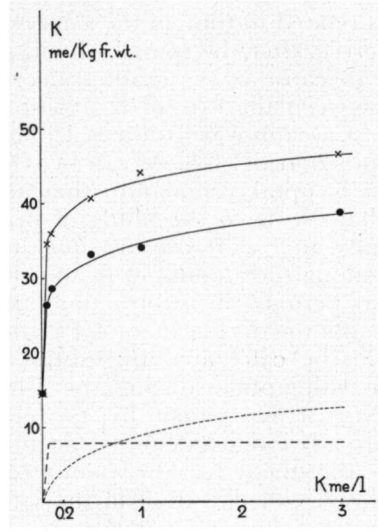

Fig. 25

Fig. 24. Relation between K uptake from a 0.2 m.e./l K_2SO_4 solution and time in the absence and presence of 10 m.e./l $CaSO_4$ (● and ○ resp.).
After 3 hours part of the roots absorbing K in the absence of Ca was transferred to an equivalent Rb_2SO_4 solution lacking Ca. △ Rb content of the roots during Rb uptake, □ K content of the roots during Rb uptake. The dotted line has been obtained by adding the curves for the K and Rb content of the transferred roots. Root/solution ratio: 3 gr/10 l.

Fig. 25. Rate of K absorption from a K_2SO_4 solution as affected by concentration. (● experimental period 3 hours, × experimental period 5 hours).
The broken line (– – – –) represents steady-state K uptake over 2 hours in relation to K concentration and was obtained by subtracting the 5- and 3-hour absorption lines. The dotted line (. . . . . .) represents the amount of K present in Fraction I in relation to K concentration and was obtained by calculating steady-state K uptake for a period of 3 hours and subtracting this amount from overall K uptake in 3 hours. Root/solution ratio 3 gr/10 l.

m.e./l K in the absence of Ca at pH 5.3 could be ascribed to an increasing outflux of K ions accompanying the increasing K content of the roots. For this purpose, after an absorption period of three hours, when the rate of K uptake already shows a considerable decrease, the 0.2 m.e./l K_2SO_4 solution was replaced by a Rb solution of the same strength and the rate of Rb uptake from this Rb solution as well as the K content of the roots determined over the next 3 hours. For the sake of comparison, other portions of the roots were allowed to take up K from a 0.2 m.e./l K solution in the absence and presence of 10 m.e./l Ca over a period of 6 hours. The data shown in Fig. 24 indicate that the initial rate of Rb uptake approximates the rate of steady state K uptake in the presence of Ca. Simultaneously, there is a considerable decrease of the K content of the roots. As expected, the algebraic sum of Rb uptake and K loss during this 3-hour period

is related to time in the same way as the K content of roots not transferred after the initial 3-hour absorption period.

Because of the gradual decrease in the rate of uptake the relation between the rate of K absorption and concentration in the absence of Ca cannot be studied at pH 5.3. Therefore, this relation was determined at pH 6.8. As can be seen from Fig. 21, no decrease in the rate of K uptake similar to that at pH 5.3 occurs at this pH. It is true that owing to the filling of Fraction I, steady-state uptake shows up only after a few hours, but this difficulty can be overcome by determining the amount of K absorbed over 3-hour and 5-hour experimental periods. By subtraction, the steady-state uptake over two hours is obtained. The size of Fraction I as a function of K concentration can be calculated by subtracting the steady-state uptake from the overall uptake during the 3-hour absorption period (Fig. 25). The rate of K uptake by the steady-state mechanism appears to be already independent of concentration in the solution at a concentration of 0.2 m.e./l K. The amount of Fraction I, however, is dependent on the K concentration in the solution over the whole range of concentrations studied. For the sake of clarity, it should be noted that this curve is not entirely comparable to the same relation obtained for Na because the latter was established at pH 5.3, while former was determined at pH 6.8.

Discussion

In the absence of Ca and at low K concentration (0.01 m.e./l) K uptake like Na uptake, shows two phases. The same phenomenon is shown by the Rb absorption from a 0.01 m.e./l Rb_2SO_4 solution. In analogy to the case of Na, this behaviour suggests that K enters the roots via two uptake mechanisms, viz. the mechanism responsible for steady-state absorption (Fraction II) and a mechanism responsible for the penetration of K to electro-negative adsorption sites (Fraction I).

If, in complete analogy with Na uptake, K uptake into Fraction I is tentatively envisaged as the binding of K to cytoplasmatic sites, the inhibitory effect of Ca may once more be ascribed to either a competitive action of Ca with respect to these binding sites or a decrease in the permeability of the plasma membrane to K ions in the presence of Ca. These two possibilities will be examined successively on the basis of the experimental results.

It appears (Fig. 19) that the Rb ions of Fraction I are not released from the root when, after the filling of Fraction I has been completed (i.e. after 3 hours), Ca is added to the solution. So no simple exchange between Rb and Ca at the binding sites is demonstrated. Reabsorption of released Rb is improbable because of the excess of K ions added together with the Ca ions. Moreover, a lag-phase in the K/Rb uptake after addition of Ca, such as was found in the case of Na, did not appear. This excludes the possibility that exchange does occur but does not manifest itself by transport of the exchanged ions via the steady-state mechanism, resulting in a temporary decrease of the

rate of absorption from the solution. That the absence of a release of Rb ions after the addition of Ca might be explained by a high specificity of the binding sites of Fraction I for Rb must be rejected because it leaves unexplained the fact that Ca does prevent the binding of Rb when it is added simultaneously with the Rb. Therefore, the experimental data do not support the view that the inhibitory effect of Ca on Fraction I of the K uptake in a 0.01 m.e./l K solution must be ascribed to competition between Ca and K ions for the binding sites of Fraction I.

The second hypothesis supposes Ca to prevent the K ions from reaching the electro-negative adsorption sites of Fraction I by reducing the permeability of the intermediate membrane. This hypothesis provides a simple explanation of the results shown in Fig. 18. The absence of a lag-phase in the K uptake when the roots are transferred from a 0.01 m.e./l Rb solution lacking Ca to a 0.01 m.e./l K solution containing Ca, can be explained by the assumption that unlike Na "internally-bound" Rb or K is not transported by the steady-state uptake mechanism, so that after the access of K to the binding sites of Fraction I has been blocked by Ca, these sites remain saturated by Rb or K. This difference in the behaviour of K and Rb as compared to Na could be based on a relatively stronger binding of K and Rb to the cytoplasmatic binding sites, preventing transfer to the steady-state mechanism as in the case of Na. Such a difference in power to combine with cell components between K and Na ions has been reported by ROBERTS, ROBERTS and COWIE (1949) for E. Coli. LING (1952) explained the difference in binding within the cell between K and Na ions in terms of the smaller hydration shell of the K ions, enabling this ion to approach adequately the fixed negative charge of phosphorylated compounds to be held by the Coulomb forces. The strongly hydrated sodium would be only weakly held.

The rate of K absorption from a solution containing 0.2 m.e./l K is not affected by the presence of Ca during the first few hours of the experiment (Fig. 20), although the course of the time curves indicates that Fraction I is present in this case too. In accordance with this result, it was established by Meyer (unpublished data) in this laboratory that Ca is unable to prevent the uptake of Rb into Fraction I at comparable concentrations.

It must be considered unlikely that in the presence of only slightly higher K concentrations in the solution (0.2 in stead of 0.01 m.e./l) Ca ions are unable to inhibit the penetration of K ions to the cytoplasmic sites by the same mechanism. In this case and in analogy with what has been supposed for Na, the saturation of the cytoplasmatic binding sites might be explained by the hypothesis that they can also be saturated by K supplied by the steady-state absorption mechanism if only the concentration of the K ion-carrier complex exceeds a certain value. The phenomenon seems to occur for both Na and K. The fact that it becomes manifest at different concentrations of these ions in the medium may be related to a difference in the affinity of the K and Na ions for the respective carrier mecha-

nisms on the one hand and the cytoplasmatic binding sites on the other. One difficulty that is not explained by this hypothesis is the fact that at a concentration of 0.01 m.e./l K there is no depletion of the cytoplasmatic sites by the steady-state uptake mechanism after the addition of Ca (Fig. 19), notwithstanding the circumstance that in the presence of Ca this mechanism is unable to supply K ions to Fraction I.

The decrease in the rate of K uptake starting after two hours when absorption proceeds from a solution of 0.2 m.e./l K at pH 5.3 in the absence of Ca must presumably be attributed to an outflux of K ions because of the following considerations. The fact that the effect appears after absorption has proceeded for some time suggests it to be related to the increased K content of the roots. This assumption is supported by the observation that no decrease of the uptake rate becomes apparent within the experimental period when the solution contains 0.01 m.e./l K. In this case the rate of K uptake is only about half the maximal rate. Therefore, it will take longer to reach the "threshold" value at which the outflux of K ions starts. Obviously, this threshold concentration must also be considered in connection with other processes going on in the roots, for example the rate of further transport of the absorbed K ions.

Additional evidence for an outflux of K is provided by the observation that at a K concentration of 0.01 m.e./l the rate of uptake is higher than the rate of K uptake from a 0.2 m.e./l solution after a five-hour absorption period. Furthermore, the tendency of the rate of uptake to decrease sets in later (Fig. 23) when the K concentration in the solution is increased to 5 m.e./l. This may be envisaged as a consequence of the fact that it will take longer before the concentration gradient from inside to outside attains the critical value at which a K outflux sets in. It should be borne in mind that the rate of steady-state absorption at a concentration of 5 m.e./l K does not exceed its value at a concentration of 0.02 m.e./l.

During the first hours of the experiment shown in Fig. 23, the overall K uptake in the absence of Ca was somewhat higher than in the presence of Ca. This may be safely ascribed to the increasing importance of non-specific free space adsorption at this K concentration.

The experimental results presented in Fig. 24 do not contradict the assumption that a K outflux accompanies K uptake. The course of the Rb uptake shows that the absorption capacity of the roots has not been affected, because after transfer of the roots to the Rb solution, the initially rate of Rb uptake equals the steady state K uptake in the presence of Ca. Therefore, the decrease in the rate of absorption of both K and Rb in the Ca-free solutions must be ascribed, most probably, to a concomitant outflux increasing with the concentration of the ion in the cell and resulting in a decrease of the net uptake. In this connection it should be stated that leakage of K ions out of the cut surface of the roots is unlikely. In the first place, the K uptake at pH 6.8 is linear with time. Secondly, there is no indication of such leakage in the Na and Br experiments.

In the absence of Ca, there is no outflux of K at pH 6.8. Presumably, this outflux is possible only when, under the influence of the concentration of H ions, the membrane permeability for K ions has been increased, an effect which is counteracted by the presence of Ca.

The membrane concerned in this phenomenon is in all probability not the tonoplast but the plasmalemma. This is suggested by the observation (Fig. 22) that within a period of less than half an hour the addition of Ca results in an acceleration of the uptake process. The slow penetration into the plasma of K and Na makes it less likely that Ca ions could reach the tonoplast so quickly.

The explanation given for the decrease in the rate of K uptake in the absence of Ca at weakly acid pH is in complete accordance with the recent findings of JACKSON and ADAMS (1963). These authors found an efflux of K when the barley roots have absorbed about 10 m.e. K/kg fr. wt.

The experiments with K show once more the importance to establish the relation of the Ca effect to time. If, following OVERSTREET, JACOBSON and HANDLEY (1952), the uptake period had been restricted to three hours, in full accordance with their results, an inhibitory effect of Ca at a K concentration of 0.01 m.e./l K, a stimulating effect at a K concentration of 0.2 m.e./l, and only a small effect at a concentration of 5 m.e./l had been found. However, the interpretation given by these authors becomes unlikely when the present time curves are taken into account.

Summarizing, the K uptake appears to be composed of three separate processes. Firstly, a process responsible for the steady-state K uptake which is not influenced by Ca and has been supposed to proceed by means of a carrier. The two other processes are both sensitive to Ca: a K uptake which may be considered as the filling of a certain cell compartment, possibly the cytoplasm, and a K outflux which apparently depends upon the concentration of H ions in the solution and reveals itself when the K concentration in the roots has reached a certain value.

CHAPTER VI

GENERAL DISCUSSION

The influence of Ca on the uptake of anions and cations in these experiments could not be traced to a single principle such as carrier formation, inhibition of an efflux, etc. The stimulation of Br uptake by Ca has been attributed here to a more effective screening of electronegative charges in the immediate neighbourhood of the carrier sites by which more such sites become accessible to Br ions and thus available for uptake. It has been pointed out that the same mechanism need not lead to a decrease in cation uptake if only more effective screening of the negative charges involved is not disadvantageous to the accessibility of the cation carriers. The results of the Na experiments indicate that Na uptake is not influenced by Ca in a way comparable

to that for Br uptake. The actual difference between Na uptake in the presence and absence of Ca appears not to be due to a difference in steady-state uptake but to concern only an additional uptake of Na ions during the first few hours of the experimental time, simultaneously with the steady-state uptake.

An obvious question is wether the electro-negative sites affecting the uptake of the Br ions are somehow connected with the additional phase of the cation uptake. This question must be answered in the negative for two reasons. In the first place apparently no measurable time interval is required for the cations to saturate the electro-negative sites involved in anion uptake; the additional phase of the cation uptake, on the other hand, requires two to three hours. In the second place, the accessibility of the mechanism responsible for anion absorption is hampered to relatively the same extent at low as at high temperatures, whereas the additional phase of cation uptake appears to be considerably sensitive to temperature. These differences are so striking that the electro-negative sites which have been assumed to be involved in the additional phase of cation uptake, cannot be identical in character and localization with the electro-negative sites involved in Br uptake. The combination of easy and rapid accessibility and temperature independence clearly shows that the electro-negative charges involved in Br uptake are a true part of the free space. On the other hand, the temperature effect excludes the possibility that any true free space component is responsible for the additional phase (Fraction I) of cation uptake. The conclusions drawn in Chapters IV and V, i.e. that the effect of Ca on this additional phase is not based on a simple exchange, is in full agreement with this statement.

MacRobbie and Dainty (1958) and Diamond and Solomon (1959) studied the isotopic exchange of cations in relation to time in various seaweeds and were led to distinguish three exchange phases in these experiments. It may be considered as beyond doubt that the electro-negative sites affecting Br uptake are part of their rapid phase, which they localize in the cell wall or, in any case, at the outside of the plasma membrane. The question of whether the filling of the less easily accessible cell compartment, supposed to be responsible for Fraction I of cation uptake, can be identified with the second exchange phase of these authors (which also required an interval of a few hours and was localized by them in the cytoplasm as well) is difficult to answer. Not only was the nature of the objects quite different in the two series of experiments but also their nutritional state: a dynamic equilibrium with the environment in the case of Nitella and a low salt state in the case of barley.

The presence in the cell of the cations of Fraction I does not appear to be an essential condition for the operation of the steady-state uptake mechanism of the cations concerned because, for example, the rate of Na absorption after completion of the additional phase of uptake (in the absence of Ca) equals the rate of uptake in the absence of this phase (in the presence of Ca). The same may be observed

for K uptake from an experimental solution of 0.01 m.e./l K in the presence and absence of Ca ions. It is striking that BANGE and OVERSTREET (1960) did not observe an additional phase of uptake lasting about two hours in Cs absorption by barley roots at a concentration of 0.01 m.e./l CsCl. The Cs uptake in their experiments was linear with time during a 3-hour uptake period. On the other hand, BANGE and VAN GEMERDEN (1963) found that the rate of Cs uptake was not constant, but showed a decrease after about eighty minutes. This is in accordance with our results, just as is their observation that overall Cs uptake is inhibited by the presence of Ca, especially at low Cs concentrations. It is true that Bange and Overstreet used a more or less complete nutrient solution for cultivating their roots, whereas Bange and Van Gemerden grew their plant material in a dilute $CaSO_4$ solution, as was done in the experiments dealt with here. However, use of a complete culture solution did not alter the behaviour of the barley roots used in these experiments (Meyer, unpublished results). Therefore, it must be assumed that varietal differences are responsible for the difference in behaviour. Thus, emphasis is placed once more upon the danger of generalizing from results obtained with a single object.

From both the experiments with Na and those with K, it could be concluded that competition for the electro-negative sites of Fraction I between the Ca ions on the one hand and the Na or K ions on the other is not likely.

The experimental results appeared to be readily explained by the assumption that the effect of Ca on both Na and K uptake is based on the reduction of the permeability of a membrane bordering the cell compartment involved in the uptake of Fraction I. This interpretation of the Ca effect is borne out by the conclusion of MOORE, JACOBSON and OVERSTREET (1961) that the Ca ions influencing the uptake of other ions are located at the cell surface. However, the value of this agreement is reduced by already-mentioned difference in the behaviour of the experimental material. Owing to the fact that the steady-state uptake is not affected by the addition of Ca, it seems safe to conclude that the first step in the uptake of the ions by the active mechanism cannot be located behind this membrane. If, as has been supposed, Fraction I is located in the cytoplasm, or part of it the most obvious interpretation seems to be that this membrane is the plasma-lemma. The active uptake mechanism must then be located in the plasmamembrane. The picture emerging here is at variance with a location of ion carriers not in but behind a Ca-sensitive permselective barrier, as proposed by JACOBSON and co-workers (1960).

At low Na and K concentrations in the solution the uptake into Fraction I and the steady-state uptake may be taken as separate and additive processes. However, at higher Na and K concentrations, the steady-state uptake mechanism has been tentatively supposed to be involved in the filling of this fraction. This supposition implies that the filling precedes and is not mediated by the rate-limiting step in true steady-state uptake. In terms of Bange's theory, this must be

understood in such a way that the transfer of K or Na from the ion-carrier complex to the cytoplasmic sites can occur directly and without mediation of the rate-limiting enzymatic break-down of the ion-carrier complex. This supposition is in complete agreement with the observation that the removal of Na from Fraction I after addition of Ca (Fig. 11) proceeds at the cost of the rate of Na absorption from the external solution. By the same token, the view that Na ions may pass the plasmalemma in the absence of Ca ions, either in the free state or temporarily bound to certain membrane components, implies that the Na ions transported by the steady-state uptake mechanism are eventually released behind a second membrane less permeable to Na ions. It would therefore appear that the rate-limiting enzymatic breakdown of the Na ion-carrier complex proceeds in some internal membrane, perhaps the tonoplast. The same applies to K ions, with the difference that with higher rates of uptake the membrane involved is apparently unable to keep all the accumulated K, so that a K efflux results.

With higher concentrations of K (Fig. 20) or Na (Fig. 14) in the solution, the Ca effect on Fraction I decreases. Because of the lack of experimental data, it cannot be excluded completely that this phenomenon should be ascribed to an antagonistic action between these monovalent cations and Ca. It should be noted in this connection that the behaviour of the monovalent cations in this respect is difficult to ascertain experimentally on account of the many possible complications (e.g. ion competition, carrier competition, etc.) which make it extremely difficult to obtain accurate and unequivocal data on this point. On the other hand, in the case of K the phenomenon manifests itself at a concentration as low as 0.2 m.e./l K. The low K/Ca ratio prevailing under these experimental conditions makes an antagonistic action between K and Ca ions less likely (cf. Booy and Bungenberg de Jong 1956).

It has been supposed above (cf. Chapter V) that the absence of a Ca effect during the initial phase of K uptake at a K concentration of 0.2 m.e./l and higher is not to be ascribed to the inability of Ca to prevent K to penetrate through the plasmalemma when the K concentration of the solution is enhanced. For, if Ca is unable to prevent the penetration of K from the outside into Fraction I, it cannot be seen why Ca should be able to abolish the outflux of K unless the Ca as well as the pH effect on K efflux were located at the tonoplast. With respect to the Ca ion, this assumption would be at variance with the interpretation given for its blocking effect on the entrance of the monovalent cations into the cytoplasmatic compartment (Fraction I). For, it seems unlikely that Ca would not block its own entrance into the cytoplasm by lowering the permeability of the plasmalemma. A penetration of H ions as far as the tonoplast seems hardly reconcilable with the buffering capacity of the cytoplasm. Therefore, the effect of Ca and pH on K efflux must also be localized at the plasmalemma.

The occurrence of a K efflux at low pH values which is inhibited

by Ca, has already been described by Jacobson, OVERSTREET, KING and HANDLEY (1950) and by FAWZY, OVERSTREET and JACOBSON (1954). However, these authors did not interpret this phenomenon in terms of an effect of Ca on membrane permeability.

VIETS (1944) has shown that the stimulating effect of Ca on K uptake decreases with higher external K concentrations. This phenomenon may be connected with the observation (Fig. 23) that at higher K concentrations the moment at which the time relations in the absence and presence of Ca start to diverge is delayed. If, as suggested, this divergence is based on an efflux of K in the absence of Ca, the delay observed is in accordance with the finding of Jacobson, Overstreet, King and Handley that an increase of external K concentration suppresses K outflux.

In accordance with our observation, RAINS and co-workers (1964) found a progressive decrease of the uptake capacity of barley roots at low pH values and in the absence of Ca and, at the same time, no influence of Ca concentrations up to 10 mM on Rb absorption from a 0.1 m.e./l RbCl solution.

It does not seem impossible that the stimulating effect of Ca on Cs uptake, reported by MARSCHNER (1961) for Cs concentrations lower than 5 m.e./l is also based on an antagonistic action between Ca and H ions as described here. Taking into account the pH of 5.4 prevailing in the experiments of this author, the time relation of uptake in the absence of Ca may also have diverged from linearity at concentrations lower than 5 m.e./l. On the other hand, in the experiments presented here the decrease of the rate of K uptake from a 0.2 m.e./l K solution under the influence of H ions usually manifests itself after an experimental time of about two hours, whereas in Marschner's experiments the entire experimental period was usually only two hours. However, it cannot be completely excluded this was due to the existence of a stimulating effect of Ca on cation uptake of a different nature than that reported in this study.

Using roots of Phaseolus aureus, TANADA (1962) found a strong stimulation of the uptake of Rb by Ca ions from zero time. However, the procedure adopted by him for rinsing the roots after the uptake period (washing for ten minutes in a K solution of a concentration as high as 10 m.e./l) introduces an unsettling element into his experiments which makes comparison with our results rather senseless.

The discussion of the experimental results has led to the conclusion that the effect of Ca on anion uptake on the one hand and cation uptake on the other is based on two completely different mechanisms, namely screening of electro-negative charges in the close neighbourhood of the carrier sites in the former case and lowering of the permeability of the plasmalemma in the latter. Because it seems very likely that the electro-negative charges involved in the stimulation of anion absorption by Ca are membrane charges, both effects may be described in general terms of an interaction between Ca and the plasmalemma. On the other hand, it should be emphasized that the negative

sites involved in the Ca effect on Br uptake may be, but are not necessarily identical with the sites responsible for the permeability effects of Ca operative in cation uptake.

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