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THE ABSORPTION OF LI AND CA BY BARLEY ROOTS [1])

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1. INTRODUCTION

Recently two different, and partly contradictory, explanations have been given for the interaction of lithium and calcium in absorption by barley roots (EPSTEIN, 1960; JACOBSON *et al*, 1960).

Calcium, which is known to stimulate in low concentrations the absorption of some monovalent cations e.g. of potassium and rubidium (VIETS, 1944; OVERSTREET, JACOBSON and HANDLEY, 1952) suppresses to a great extent the absorption of lithium, even when its concentration in the outer solution amounts to only one hundredth of the concentration of lithium.

EPSTEIN (1960), investigating the interaction of calcium and lithium, found that Ca inhibited lithium absorption competitively. He concluded from his experiments that the competition between the two ions is for specific sites on carriers responsible for absorption.

JACOBSON *et al* (1960) stated that "the presence of calcium almost completely inhibits the absorption of lithium". These authors suggested the following explanation. Lithium absorption will take place in two stages. The first stage is the diffusion of Li through a barrier situated in the cell wall or at the outer protoplasmic membranes. This barrier, formed or induced by Ca constitutes a bottle neck which slows down the inward diffusion of lithium and hydrogen ions but which does not interfere with the absorption of potassium. The second stage of the cation absorption was not dealt with in their paper. Since the competitive inhibition found by Epstein and the concentration-independent inhibition of Jacobson et al are difficult to reconcile it was thought of interest to investigate the interaction of Ca and Li in absorption once more.

2. MATERIAL AND METHODS

Barley roots of the variety "Union Zomergerst" were used for the absorption experiments.

The grains were soaked for 24 hours in aerated water, after which they were transferred to cheese cloth which was stretched over a solution of 2×10^{-4} molar of $CaSO_4$ in a 1 liter beaker. The solution just touched the cheese cloth; it was constantly aerated. The deve-

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loping plants were kept for 7 days in a dark room at 21° C. The solution was renewed once, after 3 days. About 12 hours before the experiments were carried out, the roots were cut and immersed in continuously aerated, de-ionized water.

Lithium was applied as LiCl and calcium as $CaCl_2$. The pH of the solution was about 5.8–6.0. Whenever needed, the pH was adjusted by adding LiOH to the LiCl solutions. At the end of the experiment the roots were washed under running de-ionized water for 1 minute, dried at 90° C for 24 hours and ashed at 600 °C. The ash was dissolved in 10 ml of acidified water (2 cc HCl + 8 cc H_2O) and the Li content was then determined by means of a flame photometer.

Each sample consisted of about 50 mg. of dried root material, which corresponds to about 1 g. of fresh roots.

Experiments were usually conducted in 3–4 replications; each point in the graphs represents the mean of these replications.

3. Experiments and results

3.1. *The effect of Ca on the absorption of Li*

Epstein's experiments, with the addition of a few modifications, were repeated first. The results were identical with those of Epstein.

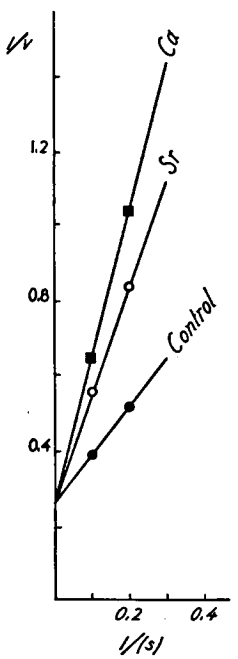

Fig. 1. Competitive inhibition of lithium absorption by calcium and by strontium. (S) = Li concentration in m. moles. V = Li absorption in μ moles. Absorption time: one hour. Calcium concentration: 5 m. moles; strontium concentration: one m. mole.

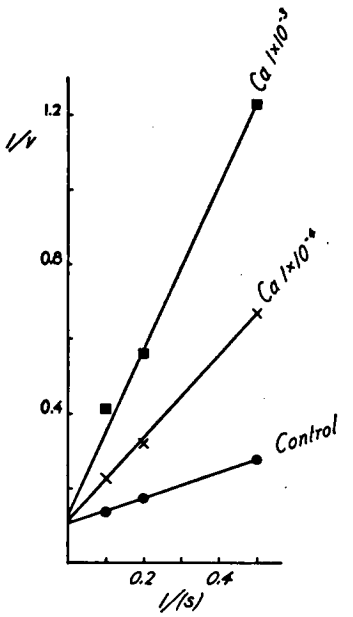

Fig. 2. Competitive inhibition of lithium absorption by calcium. (S) = Li concentration in m. moles. V = Li absorption in μ moles. Absorption time: three hours.

In Fig. 1 an additional calcium treatment of 5 m eq./l. was given, a concentration that, in the experiments of JACOBSON *et al* (1960), blocked lithium absorption nearly completely.

With all calcium concentrations investigated in the present experiments lithium absorption depended on the concentration of the lithium, even in solutions where the lithium: calcium ratio was approximately 1 : 1. In Fig. 2 a three hours absorption period instead of absorption during one hour was investigated.

Since the results might be affected by the pretreatment of the roots, in one experiment (Fig. 3) the Li-Ca interaction was investigated with roots which had been grown on a Hoagland solution. The results were similar to those of the previous experiments: plotting reciprocally absorption versus concentration revealed a competitive inhibition of lithium absorption by calcium.

3.2. *The effect of calcium, strontium and magnesium on the absorption of lithium*

In these three experiments the absorption of Li from a pure LiCl-solution (10 millimoles) and from a Li-solution to which 1 millimole of Ca, Sr, or Mg was added, was investigated.

Plotting the reciprocals of absorption and concentration revealed a competitive inhibition of Li absorption by strontium (Fig. 1) and an uncompetitive inhibition by magnesium (Fig. 3).

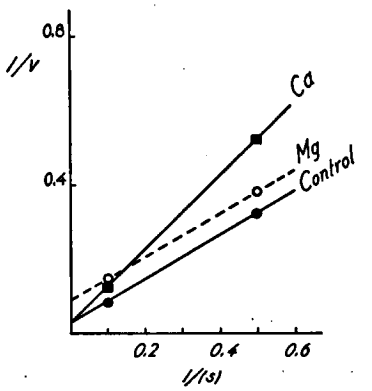

Fig. 3. Inhibition of lithium absorption by calcium and by magnesium by roots of plants that had been grown on Hoagland's solution instead of on calcium sulphate. (S) = Li concentration in m. moles. V = Li absorption in μ moles.

3.3. *The effect of temperature on lithium absorption*

Fig. 4 shows the absorption of lithium at 0° C and at 30° C. The absorption rate remains constant for 3 hours. The lithium concentration was 10 millimoles.

Q_{10} was determined from the absorption at 20° C and at 30° C with roots from the same batch of plants. The absorption period was

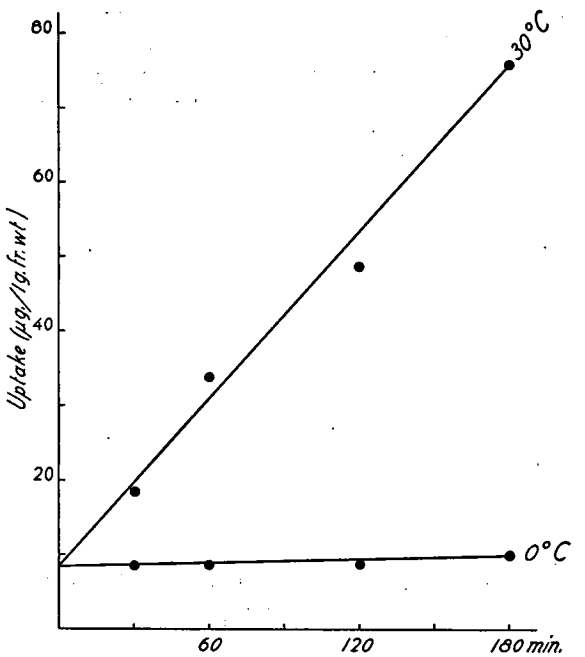

Fig. 4. The time course of Li absorption at 30° C and at 0° C.

TABLE 1

Lithium absorption, in μg/g. fr. weight, by roots from a solution containing 40 millimoles of LiCl and from a solution containing 40 millimoles of LiCl and 1 millimole of $CaCl_2$.

	Li	Li + Ca	Ratio Li/Li + Ca
20° C . . .	30.9	22.7	1.36
30° C . . .	77.3	53.3	1.45
Q_{10}	2.50	2.34	

120 minutes. In these experiments the Li concentration was 40 millimoles. In a previous experiment it was found that at this concentration the uptake is not limited by the concentration of lithium of the solution. In the same experiment lithium uptake was determined at 20° C and at 30° C from solutions that contained 40 millimoles of lithium and, in addition, one millimole of calcium. Table 1 gives the results. The ratio of the uptake from a solution of lithium and of lithium + calcium is approximately equal at 20° C and at 30° C, and so is the Q_{10}.

3.4. *The relationship between metabolic and non-metabolic absorption*

At 0° C a rapid initial absorption of lithium takes place; it is followed by a much slower uptake. Calcium decreases the initial rapid uptake, but the rate of the subsequent slow uptake is not altered by the presence of calcium ions (Fig. 5 and 6). Thus, it appears that interference of calcium with lithium absorption occurs in two different phases of the absorption process, viz. in the rapid initial absorption and, at temperatures higher than 0° C, in the subsequent absorption phase. Since the latter has a relatively high Q_{10} it is assumed that its rate depends on metabolism.

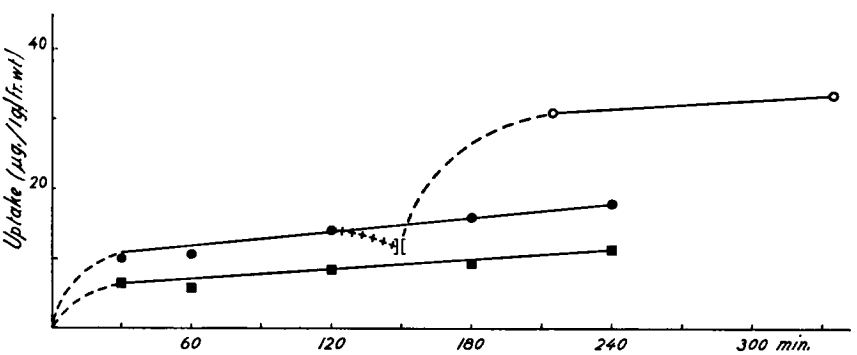

Fig. 5. Lithium absorption at 0° C from a pure lithium solution of 10 m. moles and from a solution containing 10 m. moles of lithium and one millimole of calcium.
Black dots = roots continuously in lithium solution at 0° C.
++++++ = roots transferred to water at 30° C and at][back to Li solution at 0° C. (= open circles).
Black squares = roots continuously in a Li + Ca solution at 0° C.

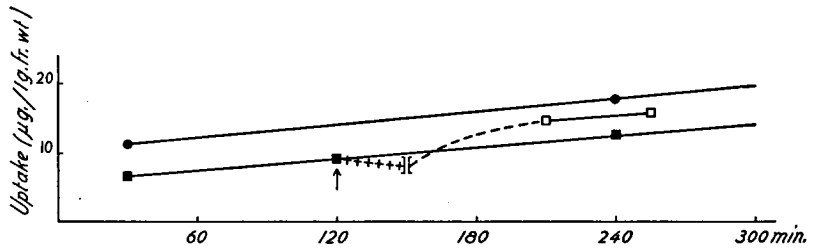

Fig. 6. Lithium absorption from a pure lithium solution of 10 m. moles and from a solution containing 10 m. moles of lithium and 1 m. mole of calcium.
Black dots = roots continuously in lithium solution at 0° C.
Black squares = roots continuously in Li + Ca solution at 0° C.
++++++ = roots after 120 minutes transferred to water at 30° C. and at][back to Li + Ca at 0° C. (open squares).

The rate of the rapid initial absorption is independent of temperature and, hence, this process is considered to be non-metabolic. The relationship between the two phases of the absorption was studied in the following way. Roots were bathed in a lithium solution at 0° C. At definite intervals samples were taken for the determination of lithium uptake. After a certain period a portion of the roots was rapidly rinsed with water and transferred to water at 30° C for 30 minutes. At the end of this time the roots were returned to the lithium solution at 0° C. Another portion of the roots remained in the lithium solution at 0° C throughout the uptake period. The results of these experiments are shown in Figs. 5, 6 and 7. In the water at 30° C the roots lose part of the lithium which was absorbed at 0° C previously. When the roots are returned to the lithium solution at 0° C a second stage of rapid uptake of lithium takes place. The quantity of lithium taken up in this second rapid uptake is usually larger than that of the initial rapid uptake. It is approximately equal to the quantity of lithium the roots contained just prior to their transfer to water at 30° C.

These results may be interpreted as follows. The rapid absorption of lithium at 0° C is caused by adsorption of the lithium ions to sites in the cell or in the cell wall. Upon transfer of the roots to water at 30° C part of the adsorbed lithium ions is lost to the medium. The rest is removed from the adsorption sites and transported to other places by a mechanism which is driven by metabolism. When the roots are returned to the lithium solution at 0° C lithium ions are again adsorbed to the empty sites, causing a second rapid absorption. When the lithium treatment at 0° C is interrupted by immersion of the roots in water at 0° C, instead of in water at 30° C, for half an hour, no rapid absorption of lithium takes place when the roots are returned to the lithium solution at 0° C. (Fig. 8).

In water at 0° C lithium ions may diffuse outwards into the water, but the metabolic processes which are responsible for the transport of the lithium ions from the original adsorption sites to other places

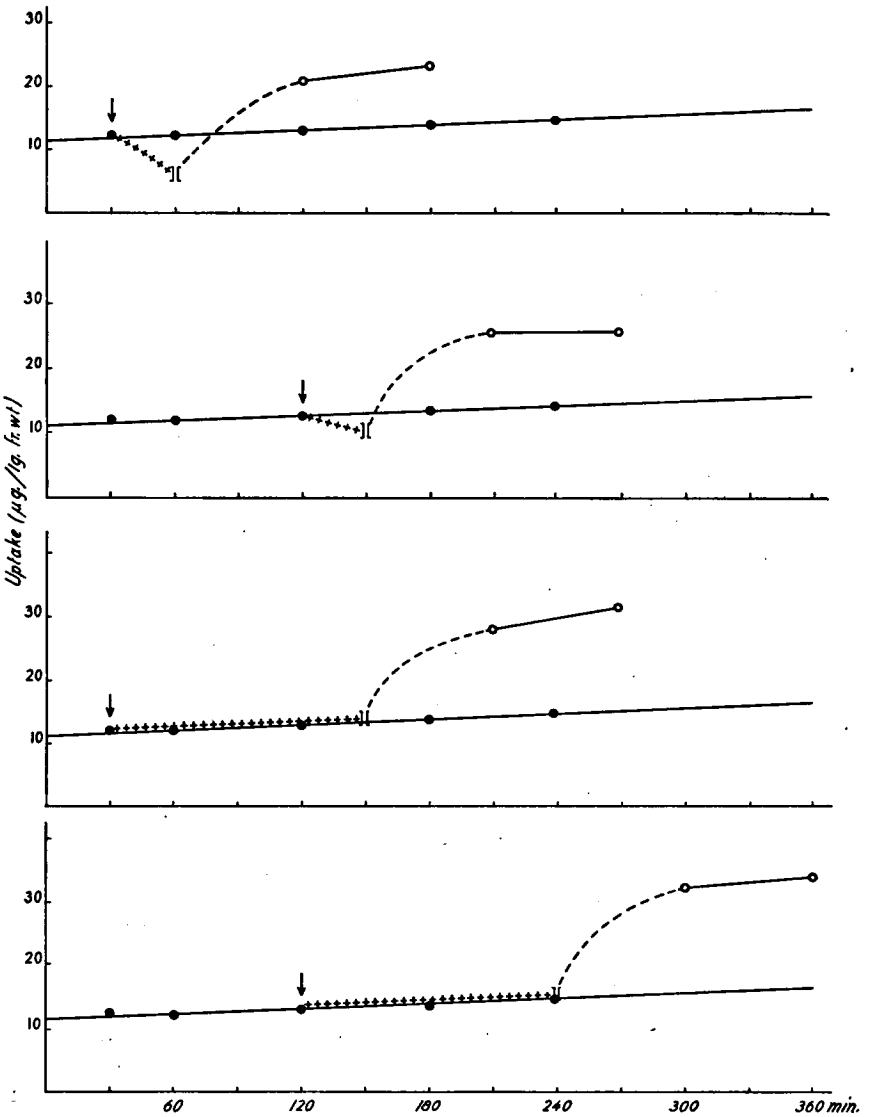

Fig. 7. Effect of interruption of the lithium absorption at 0° C. by a treatment with water at 30° C at different moments after the beginning of the experiment (30 min. and 120 min.) and for a different length of time. Explanation as in Fig. 5.

have stopped at this temperature. Therefore, most of these sites are not emptied and no second rapid uptake occurs when the roots are returned to the lithium solution at 0° C.

3.5. *The slow uptake at* 0° *C*

A phenomenon worth attention is the gradual increase of the lithium content of the roots in LiCl solutions at 0° C. It was tentatively

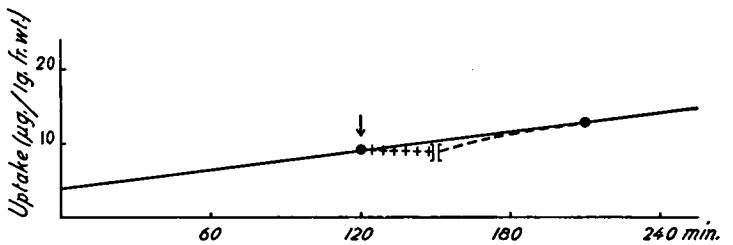

Fig. 8. Lithium absorption from a lithium solution of 10 m. moles at 0° C. +++ = roots transferred to water at 0° C. and at] [back to the lithium solution.

assumed that this was due to an increase in number of the sites which can be occupied by the lithium ions that are adsorbed at 0° C. It may be asked if these sites are functional in the metabolic absorption process at 30° C, so that their increase at 0° C will be followed by an increased metabolic absorption rate at higher temperatures. This was tested as follows; Roots were bathed in a solution of lithium chloride of 10 millimols per liter at 0° C. At certain intervals two samples of the roots were removed simultaneously from the solution. In one sample the lithium content was determined at once, the other sample was first bathed in a solution of lithium chloride of the same concentration at 30° C for one hour. After that its lithium content was determined too. The difference in Li content of the two batches of roots was the lithium absorbed at 30° C.

If the gradual increase of the lithium content at 0° C was due to an increase of sites that are functional in the metabolic uptake at higher temperatures i.e., carriers, it could be expected that the following uptake at 30° C would be highest with roots that had been in LiCl at 0° C for the longest time. The results (Fig. 9) do not confirm the hypothesis: absorption at 30° C is the same, whether the roots had been previously in lithium at 0° C for a long time or for a short period.

An experiment in which the rubidium absorption of the roots at 30° C was determined after different periods in water or in LiCl at 0° C gave a similar result (Table 2): the pretreatment did not affect the subsequent uptake at 30° C.

TABLE 2

The effect of a pretreatment of the roots in water and in a Li solution at 0° C on the subsequent absorption of Rb^{86}. (Absorption period 2 hours).

Pretreatment at 0° C	Rb absorption at 30° C
30 minutes in Li solution	16.5 moles [1]
30 minutes in Li solution followed by 150 minutes in water. . . .	18.1 moles
180 minutes in Li solution	15.8 moles [1]
30 minutes in water.	14.0 moles [1]
180 minutes in water	16.1 moles [1]

[1]) No significant difference between treatments.

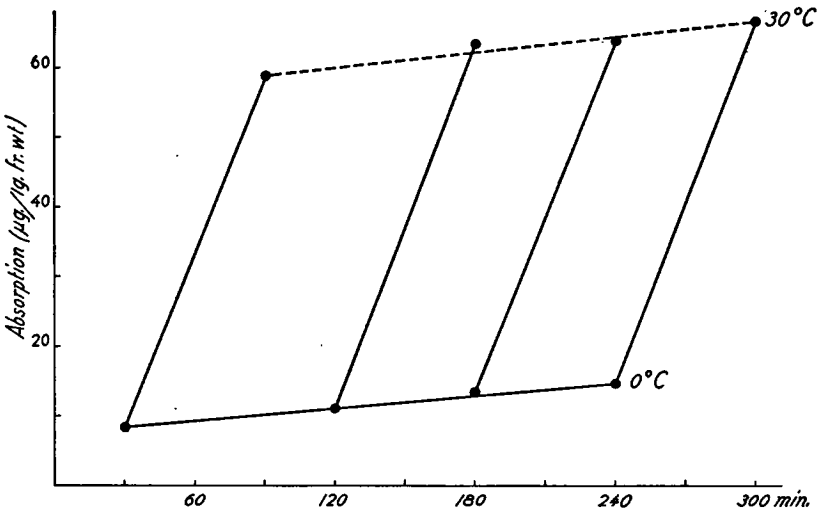

Fig. 9. Lithium absorption at 30° C. during 60 minutes of roots that had been kept for increasing periods in lithium solution at 0° C. Concentration of the lithium solution: 10 m. moles.

3.6. *The effect of pH*

In this experiment the non-metabolic uptake of Li^+ at pH 6.2 and at pH 8.2 was compared. In order to keep the pH of the solutions fairly constant, the solutions were renewed every 30 minutes. Absorption periods were 30, 90 and 180 minutes and, in order to exclude the possibility of metabolic absorption, the experiment was carried out at 0° C.

The results, given in Fig. 10, show that at pH 8.2 the Li content of the roots was higher than at pH 6.2.

4. Discussion

Epstein and Hagen (1952) and Helder (1952) have demonstrated that the Michaelis-Menten equation of enzyme kinetics may be used to describe the absorption of ions by plant cells. The former authors applied the transformation of Lineweaver and Burk (1934) for

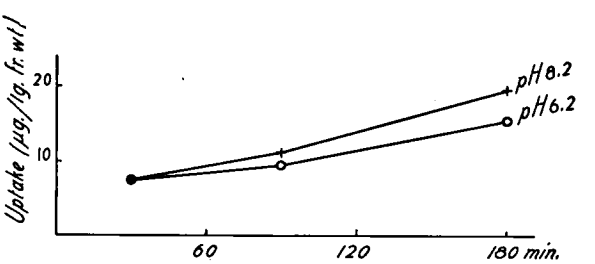

Fig. 10. The uptake of lithium at 0° C. from a Li solution at pH 6.2 and at pH 8.2.

enzyme-substrate dissociation, in which the reciprocals of the concentration and of the reaction velocity are plotted to the interaction of cations in the absorption process by barley roots. With this method, the line representing the uptake of a single species of cations and the line which gives the absorption of the same cation, but in the presence of a competitive inhibitor, have different slopes but the same intercept on the ordinate.

The results of the present experiments, plotted in this way, yield precisely this type of graph, thus proving the existence of a competitive inhibition of lithium absorption by calcium and thus confirming EPSTEIN's results (1960).

A further support for the existence of a competition between lithium and calcium was found in the fact that Li-absorption was competitively inhibited by strontium and uncompetitively by magnesium. EPSTEIN and LEGGETT (1954) found that Ca inhibited strontium absorption competitively whereas magnesium inhibited it non-competitively. Thus, strontium and calcium compete with lithium in the same way, or for the same sites, as the former ions compete mutually.

Inhibition of absorption of lithium ions by calcium also was reported by JACOBSON *et al.* (1960). These authors found an almost total inhibition of the uptake of lithium from a Li solution of 5 m.eq. per liter if Ca-ions were added to the same concentration. They concluded from experiments on the interaction of Li, Ca and K that calcium creates a barrier, presumably at some outer surface of the cell, by which the access of lithium ions to the absorption sites is almost completely blocked. In their paper there is no indication of the lithium uptake being competitively inhibited by calcium. From a second publication on this subject, from the same laboratory (JACOBSON *et al*, 1961), it may be derived that at concentrations of calcium lower than 5 m. eq. per liter the degree of inhibition of lithium ions depends on the calcium concentration.

The present experiments do not allow any conclusions as to the place in the cell where the interaction of lithium and calcium takes place. However, as generally a metabolic and a nonmetabolic absorption are distinguished it may be asked in which of these absorption processes the competitive interaction between lithium and calcium occurs.

The present experiments do not seem to give much support to the idea of a calcium-imposed barrier. The effect of such a barrier would be to diminish the access of lithium ions to the sites of the metabolic absorption process, thus decreasing the influence of metabolism on the absorption. In that case increasing the metabolism, e.g. by raising the temperature, should have less effect on the absorption rate in a solution of lithium and calcium than in a pure lithium solution. Where Li concentration in the outer solution does not constitute a limiting factor and the rate of Li absorption is regulated by metabolism, an increase in temperature would result in a Q_{10} of about 2–3. If the interference of Ca in the absorption of Li is due to competition on

the carrier Q_{10} should have the same value. On the other hand, in case Ca forms, or induces the formation of, some kind of mechanical screen (increasing selectively the resistance of cell membranes to diffusion of Lithium ions?) the increase in temperature will affect only the diffusion of the Li ions through this barrier, and the Q_{10} of the process will be of a much lower magnitude (1.3–1.5). However, the results of section 3 show that the Q_{10} of the absorption is practically the same, whether calcium is present or not. From this it may be concluded that the decrease of the lithium absorption by calcium is due to an effect on the metabolic absorption process and not to a decreased access of the lithium ions to the sites of the absorption process.

The results of section 4 (Fig. 5) show that also in non-metabolic uptake calcium decreases the uptake of lithium ions. The rapid initial uptake of Li at 0^0 C is smaller in Li + Ca solution than in a pure solution of Li. The rate of the following slow uptake does not differ in both solutions. The two lines in Fig. 5, representing the lithium content of the roots versus time seem to be parallel and repeated this behaviour in consecutive experiments.

There may be some doubt whether the rapid initial uptake at 0° C is metabolic or non-metabolic. The capacity for such a rapid uptake is regenerated in de-ionized water at higher temperatures (Figs. 5, 6, and 7), but this does not imply that the uptake itself is metabolic. LATIES (1959) described a rapid chloride uptake at 0° C the capacity for which also was generated at higher temperatures. From the shape of the curve, representing this rapid uptake, Laties named the phenomenon "absorption shoulder". Though it resembles superficially the rapid uptake of lithium at 0° C, the "absorption shoulder" should not be confused with this uptake. First Laties' "absorption shoulder" is abolished during prolonged incubation in water at 0° C, whereas the capacity for the rapid uptake of lithium is not destroyed under these circumstances (Fig. 7). Secondly, in Laties' experiments, the time curve of chloride absorption at 30° C starts linearly from the origin, whereas the absorption line for lithium at 30° C intercepts the ordinate (Fig. 4). In our experiments, this incipient uptake of Li is approximately the same at 0° C and at 30° C; this fact might point to its being a non-metabolic uptake.

Laties ascribes the "absorption-shoulder" to the metabolic formation of an excess of absorption capacity (carrier precursor), which at 0° C is used up in the rapid initial uptake of chloride. After transition of the roots from a higher temperature to water at 0° C absorption of chloride cannot take place but, nevertheless, the absorption capacity formed at the high temperature disappears. Contrary to this, in the present experiments, the capacity for rapid uptake of lithium is preserved at 0° C as long as it is not used for the uptake of lithium ions. It is possible to see that the Li content of the roots after the various transfers is a sum of two components. The first is the amount which was actively absorbed while the metabolic uptake took place at 30° C. The second is the non-metabolic adsorption which suits well each time

the increase in adsorbing sites. The interpretation of the rapid uptake as empty sites created by the removal at 30° C of lithium ions to other places in the cells seems to be very attractive, and implies that the rapid uptake itself is a non-metabolic process.

After the initial rapid uptake at 0° C the lithium content of the roots continues to increase slowly. A new rapid absorption may be induced by immersing the roots in water at 30° C for a short time and then returning them to the lithium solution at 0° C. It should be pointed out again that the quantity of lithium taken up in a rapid absorption is about equal to the quantity the roots had taken up at 0° C previously (Figs. 5, 6, 7). The longer the preceding slow uptake has lasted the higher is the rapid uptake following the incubation in water at 30° C. This shows that the slow uptake at 0° C is not just a metabolic uptake which is slowed down by the low temperature. A better interpretation is probably that the number of sites to which lithium ions are absorbed increases continuously. According to BRIGGS *et al* (1958), and WILLIAMS and COLEMAN (1950), such an increase might be explained by the ionization of a hypothetic acid (HA), caused in the long run by the higher pH of the external solution. Such a dissociation increases the amount of free A and, therefore, causes an increase in the absorption capacity. The difference in the uptake at pH 8,2 and at pH 6,2, found in the present investigation, indicates that this interpretation may apply to our case. This may explain the relationship between the time during which the slow uptake has proceeded and the size of an induced rapid absorption.

The inhibition of the uptake of lithium by calcium occurs in metabolic absorption as well as in non-metabolic uptake. As the carriers are supposed to be of a similar type of compound (HA) to that forming the D. F. S., it may be asked if these processes constitute subsequent steps of one mechanism of uptake. The fact that the interaction of lithium and calcium is similar in both processes seems to support such an assumption. On the other hand, the experiments of section 5 show that, under the conditions of the experiments, the rate of lithium uptake or of rubidium uptake at 30° C is independent of the quantity of lithium that was absorbed at 0° C previously, i.e., independent of the number of adsorption sites. This might mean that the sites at which the lithium ions are adsorbed at 0° C do not constitute a necessary intermediate stage in the metabolic absorption at 30° C.

SUMMARY

1) The effect of calcium on the uptake of lithium by excised barley roots was investigated. The absorption of lithium was competitively inhibited by calcium. Strontium inhibited the uptake of lithium competitively, magnesium inhibited it uncompetitively.

2) No support was found for the hypothesis of a calcium imposed barrier which inhibits the absorption of lithium.

3) The Q_{10} of the lithium absorption process between 20° C and 30° C was 2.3–2.5 whether calcium ions were present or not.

4) At 0° C a rapid initial uptake of lithium takes place followed by a slow absorption. The capacity for rapid uptake at 0° C may be regenerated by bathing

the roots in water at 30° C for a short time. This capacity was not destroyed during incubation, even for prolonged periods, in water at 0° C. The rate of lithium uptake at 30° C was not influenced by the quantity of lithium that was previously absorbed at 0° C.

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REFERENCES

BRIGGS, G. E., A. B. HOPE and M. G. PITMAN. 1958. Journ. Exp. Bot. **9**: 128–141.
EPSTEIN, E. 1960. Nature **185**: 705–706.
———— and C. H. HAGEN. 1952. Plant Physiology **27**: 457–476.
———— and J. E. LEGGETT. 1954. Amer. Journ. Bot. **41**: 785–791.
HELDER, R. J. 1952. Acta Bot. Neerl. **1**: 361–434.
JACOBSON, L., D. P. MOORE and R. J. HANNAPEL. 1960. Plant Physiology **35**: 352–358.
————, R. J. HANNAPEL, D. P. MOORE and MICHAEL SCHAEDE. 1961. Plant Physiology **36**: 58–61.
LATIES, G. C. 1959. Proc. Nat. Acad. Sci. U.S.A. **45**: 163–172.
LINEWEAVER, H. and D. BURK. 1934. Journ. Amer. Chem. Soc. **56**: 658–666.
OVERSTREET, R., L. JACOBSON and R. HANDLEY. 1952. Plant Physiology **27**: 583–590.
SUTCLIFFE, J. E. 1957. Journ. Exp. Bot. **8**: 36–49.
VIETS, F. G. 1944. Plant Physiology **19**: 466–480.
WILLIAMS, D. E. and N. T. COLEMAN. 1950. Plant and Soil **2**: 243–256.
WINTER, H. 1961. Acta Bot. Neerl. **10**: 341–393.