

Absorption of Chloride by Barley Roots: Kinetics and Selectivity^{1, 2}

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In studying the salt relations of plants, particular importance attaches to sodium chloride, the predominant salt of the oceans and as well the main salt of the earth, in the arid and semi-arid regions of the world. The sharp distinctions plants make in regard to the absorption and translocation of sodium and potassium, and of chloride and other anions, present problems of intrinsic interest in cellular physiology. Furthermore, research into these problems is of vital importance because irrigation agriculture in the arid and semi-arid regions of the world represents the greatest single potential for increasing the world's food supply in the near future, and the cell-physiological mechanisms governing the salt relations of plants must be investigated if plant nutritional problems posed by salinity are to be solved.

In previous experiments, the kinetics of Cl absorption by excised barley roots have been examined (4). When the external concentration was varied from 0.005 mM Cl (0.18 ppm) to 50 mM (1800 ppm), at least 2 absorption mechanisms were shown to exist. One of these, mechanism 1, has a high affinity for Cl (Michaelis constant $K_m = 0.014$ mM) and operates at essentially the maximal theoretical rate at an external concentration of 0.1 mM. At higher concentrations, up to 50 mM, another mechanism comes into play which itself is not homogeneous (4). In wheat roots, also, the mechanism of Cl absorption is heterogeneous (1). In the present work the high-affinity mechanism 1 has been investigated in respect to kinetics, selectivity, and the role of metabolism.

Materials and Methods

Seeds of barley, *Hordeum vulgare*, var. Arivat, were germinated and seedlings grown as described earlier (6). Root samples (0.50 g fr wt) were prepared and short-term absorption experiments performed as before (4, 8). Experimental solutions contained KCl or NaCl, the chloride being labeled with Cl^{36} (Cl^*). In addition, the experimental solutions usually contained 0.5 mM $CaSO_4$ (6). Exceptions are mentioned in connection with certain experiments. The volumes of the experimental solutions ranged from 2000 ml for the lowest Cl concentrations used (0.005 mM) to 250 ml for the highest

(0.2 mM). The volumes were such that the decline in the concentration of Cl of the experimental solutions, due to withdrawal by the roots, did not exceed 2.5 % of the initial concentration. Except as noted, the temperature was 30° and the pH (unbuffered) 5.6 ± 0.2 . For adjustment of the pH, H_2SO_4 was used. Stock solutions of inhibitors were adjusted to the same pH before use.

At the end of the absorption period (20 min except in the time experiments), Cl^* in the outer space of the tissue was removed by a 30-minute exposure to a cold (8°) solution of 5 mM KCl, 0.5 mM $CaSO_4$ (8). Aliquots of an aqueous extract of the tissue were either pipetted into counting cups, dried, and counted with a thin-window gas-flow counter, or pipetted into vials containing the scintillation solution given by Nissen and Benson (14) and counted in a Tricarb 314 liquid scintillation spectrometer. The 2 procedures gave identical results. Calculations of amounts of Cl absorbed were based on measurements of the radioactivity of aliquots of the experimental solutions done in the same manner as those of the tissue extracts.

Experimental Results

Time Course and Rates of Absorption. Figure 1 shows the results of an experiment in which absorption of Cl from a 0.10 mM solution of KCl was followed as a function of time. Absorption proceeds at a constant rate from zero time, the regression extrapolating to zero absorption at zero time. After 60 minutes, 1 set of root samples was rinsed for 1 minute and then transferred to a second solution. The rinsing and second solutions, both at 30°, were identical in chemical composition with the solution in which the samples were kept before, but the Cl was unlabeled. At intervals, samples were removed and rinsed with water to discontinue the experimental period. Only a very slight fraction of the previously absorbed labeled Cl was lost to the unlabeled solution in the second period (open symbols), while Cl continued to be absorbed (solid symbols for the 60–120 min period).

Similar results were obtained with NaCl instead of KCl, except that the actual rate of Cl absorption from NaCl is about 50 % higher than it is with KCl. In these experiments $CaSO_4$ was present at a concentration of 0.5 mM. Omitting the Ca salt lowers the rate of absorption (cf. fig 2), but in this case, also, absorption is a linear function of time for at least 60 minutes.

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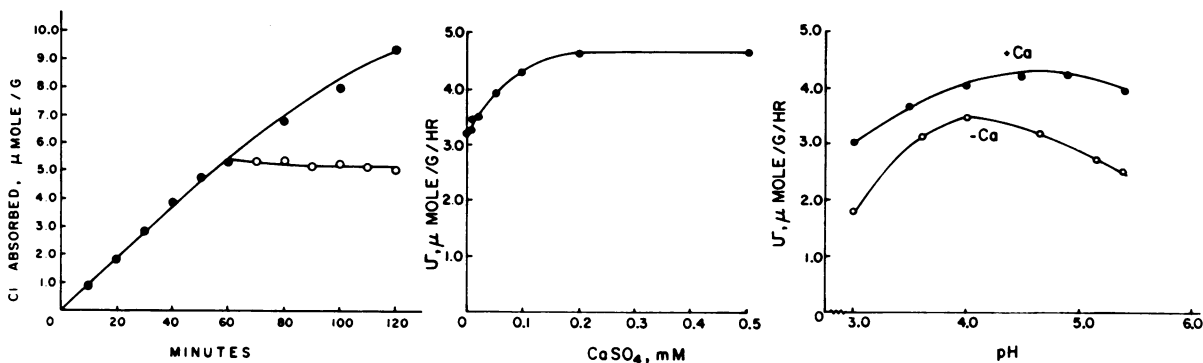


FIG. 1. Absorption of Cl from 0.10 mM KCl, 0.5 mM CaSO_4 , as a function of time, and the effect of transferring the tissue to a chemically identical solution in which the Cl was unlabeled. Temperature, 30° throughout. Solid symbols, tissue in solution of labeled Cl; open symbols, tissue in unlabeled solution. "Cl absorbed" refers to labeled Cl.

FIG. 2. Rate of absorption of Cl from 0.05 mM KCl as a function of the concentration of CaSO_4 .

FIG. 3. Rate of absorption of Cl from 0.05 mM KCl as a function of pH and the effect of Ca (0.5 mM CaSO_4). pH adjusted with H_2SO_4 .

The Effects of Ca and of pH. Figure 2 shows the effect of increasing concentrations of CaSO_4 on the rate of Cl absorption from 0.05 mM KCl. On the basis of this and other experiments (also cf. refs. 6, 16) 0.5 mM CaSO_4 was routinely included in experimental solutions except where the effect of omitting it was being studied. The effect of varying the pH is shown in figure 3. With Ca included at 0.5 mM, there is little effect between pH 5.6 and 4.0, but at lower pH values rates of Cl absorption are diminished. In the absence of Ca, the rate of absorption shows a maximum at pH 4.0. The concentration of KCl was 0.05 mM.

The Effects of the Concentration of Cl and of Other Anions. When the concentration of KCl is varied over the range 0.005 to 0.20 mM, the rate of absorption is found to be a function of the external concentration of Cl according to Michaelis-Menten kinetics (fig 4). The Michaelis constant, K_m , for Cl absorption in the presence of 0.5 mM CaSO_4 was 0.013 mM. Omission of Ca has the effect of greatly lowering the apparent affinity of the transport mechanism for Cl, the Michaelis constant being 0.046 mM in the absence of Ca. The calculated theoretical maximum rate of absorption, V_{\max} , is nearly the same in both cases. Very similar results are obtained

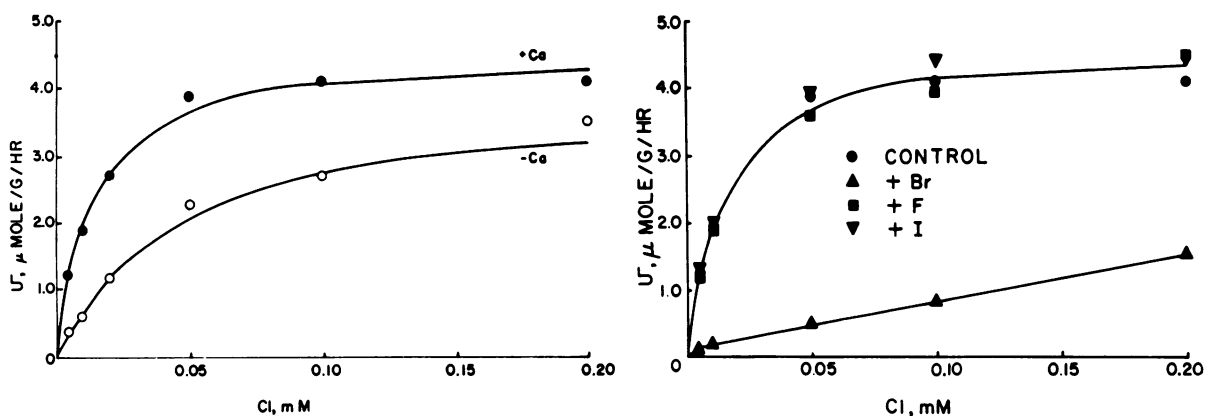


FIG. 4. Rate of absorption of Cl as a function of the concentration of KCl, with and without 0.5 mM CaSO_4 . The lines are plots of the Michaelis-Menten equation, with the following parameters; +Ca, $K_m = 0.013$ mM, $V_{\max} = 4.6$ $\mu\text{mole/g per hr}$; -Ca, $K_m = 0.046$ mM, $V_{\max} = 4.0$ $\mu\text{mole/g per hr}$. The parameters were calculated by the least square method from Lineweaver-Burk plots of the data.

FIG. 5. Rate of absorption of Cl as a function of the concentration of KCl, and the effect of 1.0 mM KBr, KI, and NaF, respectively. Concentration of CaSO_4 , 0.5 mM. The lines are plots of the Michaelis-Menten equation, with the following parameters: Control, + I, and + F, $K_m = 0.014$ mM, $V_{\max} = 4.7$ $\mu\text{mole/g per hr}$; + Br, $K_i = 0.036$ mM, $V_{\max} = 4.7$ $\mu\text{mole/g per hr}$. The parameters were calculated by the least square method from Lineweaver-Burk plots of the data.

with the Na salt, except that actual rates of Cl absorption are consistently higher by about 50% in that case, as noted above.

The effects of other halide ions on the absorption of Cl were examined in several experiments. Figure 5 shows the results of an experiment in which the rate of absorption of Cl was studied over the 0.005 to 0.20 mM range of concentrations of KCl, in the absence of other halides and in the presence of 1.0 mM F, Br, and I, respectively. (The actual concentration of the F ion must have been lower, because of the presence of Ca, but was in any case well in excess of the low concentrations of Cl in the experiment.) These halides were also offered as the potassium salts except F which was added as NaF. The lines are plots of the Michaelis-Menten equation. The constants, V_{max} and K_m , were calculated by the least square method from Lineweaver-Burk (double-reciprocal) plots of the data. For the upper line, the values used for this calculation were the means of the control, plus F, and plus I values. The Michaelis constant, K_m , for Cl absorption was 0.014 mM, and the inhibitor constant, K_i , for Br was 0.036, suggesting that the Cl-Br transporting sites have an affinity for Cl about 2.5 times higher than their affinity for Br. The calculated maximal velocity, V_{max} , of Cl absorption was identical in the presence and absence of Br (4.7 μ mole/g per hr), evidence that the inhibition by Br is strictly competitive.

In striking contrast, F and I had no effect. This held even at the lowest Cl concentration used, 0.005 mM, where the concentration of the added halides exceeded the Cl concentration by a factor of 200.

The indifference of this mechanism of Cl absorption to F and I ions is further brought out in the results of an experiment shown in table I. The rate

Table I. *Effects of Other Halides on the Rate of Absorption of Chloride by Excised Barley Roots*
The concentration of Cl was 0.05 mM

Added halide	Rate of Cl absorption, μ mole/g per hr				
	0	Conc of added halide, M			
		10^{-6}	10^{-5}	10^{-4}	10^{-3}
None	3.87				
F		3.70	4.14	3.45	3.81
I		3.52	3.87	4.03	4.02
Br		3.73	3.58	2.05	0.45

of absorption of Cl from a 0.05 mM solution was reduced to 11.6% of the control by 1.0 mM Br, but F and I were without significant effect at any concentration tested.

The Role of Metabolism. Lowering the temperature drastically decreases the rate of Cl absorption, as shown in table II. The concentration of KCl was 0.05 mM in this experiment. The data were plotted and estimates of the Q_{10} made on the basis of the

Table II. *Effect of Temperature on the Rate of Absorption of Chloride by Excised Barley Roots*

Rate of Cl absorption, μ mole/g per hr	Temperature, degrees				
	6.8	11.5	25.3	30.4	40.3
	0.24	0.77	3.06	4.19	4.62

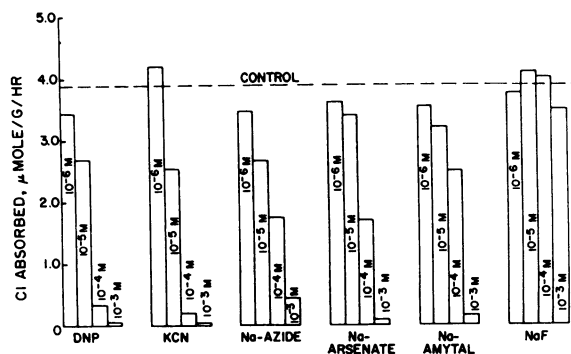


FIG. 6. Rate of absorption of Cl from 0.05 mM KCl, 0.5 mM $CaSO_4$, as influenced by inhibitors.

plot. Between 10° and 20° , the Q_{10} was 3.3, and between 20° and 30° it was 2.1.

That absorption of Cl is metabolically mediated is further brought out in figure 6 showing the effects of several inhibitors on absorption of Cl from 0.05 mM KCl. At concentrations of 10^{-5} M and higher, all inhibitors with the exception of NaF caused large reductions in Cl absorption.

Discussion

As is the case with absorption of K (7), absorption of Cl by barley roots proceeds via at least 2 mechanisms (4). One of these operates at nearly the maximal rate at an external Cl concentration of 0.1 to 0.2 mM. The second mechanism does not come into effect except at much higher concentrations, 0.5 mM and above (4). When experiments include only the high range of concentrations, the observed rates of absorption represent the sums of the rates of 2 or more transport mechanisms. Effects of various agents on absorption are blurred, under these conditions, because the several mechanisms do not necessarily respond in the same way to a given agent. An example drawn from experiments on K absorption will illustrate this point.

It used to be thought that the rate of absorption of K is higher when the rapidly absorbed Cl ion is the counterion than with the much more slowly absorbed SO_4 as the counterion (10). These conclusions were based on experiments done at relatively high concentrations. From recent experiments (7) it is clear, however, that the high-affinity mechanism 1 of K absorption is entirely indifferent to the anion being Cl or SO_4 . On the other hand, mechanism 2

of K transport, which operates at concentrations of about 1 mM and higher, is almost totally inhibited with SO_4 instead of Cl as the anion (7). This all-or-nothing response to the anion became averaged out, in the earlier experiments done at high concentrations only and without regard to the heterogeneity of the absorption process, resulting in the conclusion of a merely moderate, quantitative difference between rates of K absorption from KCl and K_2SO_4 , respectively. This experience drives home the point that in experiments on ion absorption, the existence of more than one mechanism of absorption of a given ionic species must be recognized and steps be taken to analyze separately the effects of various experimental agents on each.

In the present investigation, the absorption of Cl was studied in experiments in which the Cl concentration never exceeded 0.20 mM, so that mechanism 1 only was operative. Not till the Cl concentration is raised to about 0.5–1.0 mM does the rate of Cl absorption rise above the level attained at 0.1–0.2 mM, evidence that mechanism 2 is beginning to make a contribution to the total observed uptake.

At all concentrations tested, the rate of absorption is constant from zero time for 60 minutes. Internal concentrations of Cl reach values greatly in excess of the external concentration. If it is assumed that the Cl absorbed by 1 g barley roots is in solution in 1 ml water, the accumulation ratio (the internal concentration divided by the external one) achieved in the experiment shown in figure 1 was 53 at the end of 60 minutes. This experiment was done at 0.10 mM Cl, at which concentration the rate of absorption is maximal. At lower concentrations, much higher accumulation ratios are obtained. For example, at 0.005 mM Cl, the accumulation ratio reaches a value of about 240 in 1 hour (figs 4, 5).

The failure of previous'sy absorbed labeled Cl to be lost when unlabeled Cl is substituted for labeled Cl in the solution shows that Cl transport is essentially unidirectional under these conditions (open symbols of fig 1). The very slight loss observed may have occurred from the xylem, via the cut end. Even when the tissue is much more severely preloaded with Cl than was the case in this experiment, subsequent isotopic exchange is very slight. For example, tissue absorbed Cl from 0.2 mM KCl, 0.5 mM CaSO_4 for 3 hours in an experiment similar to that shown in figure 1. At that point it had absorbed 11.0 μmoles Cl, and the rate of absorption was declining. Upon transfer to a chemically identical but unlabeled solution at the same temperature (30°) it lost 6.8% of the labeled Cl during the first 90 minutes after the transfer, and there was no further loss during the remainder of the 180-minute desorption period.

In response to increasing concentrations of CaSO_4 , the rate of Cl absorption rises to a maximum at 0.2 mM CaSO_4 (fig 2). In this experiment, the concentration of CaSO_4 was increased up to 10 mM (not shown in fig 2), without any further effect

on the rate of absorption of Cl (cf. 4). Absorption in the presence of Ca should be looked upon as the normal, physiological condition (cf. 6, 16). The lower rates of absorption when Ca is omitted or present at low concentrations are a reflection of impairment of the transport mechanism due to inadequate concentrations of Ca. Addition of Mg instead of Ca had a similar effect, although in previous work Mg did not substitute for Ca as an agent which maintains the normal operation of membrane transport (6).

The experiment shown in figure 3 shows the importance of Ca in rendering the mechanism of Cl absorption relatively indifferent to the external pH. This function of Ca has been described in relation to the absorption of cations (9, 12, 16), and the more general conclusion has been drawn that the presence of Ca in the external solution is essential for selective cation transport (6, 16). The evidence of figure 3 shows Ca to be equally significant in anion absorption, specifically, in stabilizing the mechanism of the absorption of Cl against changes in external pH.

The experiments presented in figure 5 and table I demonstrate that the halide transport mechanism 1 under discussion here is highly specific for Cl and Br ions. Competition between Cl and Br in a common transport mechanism has been shown before (1, 3, 5, 11). Of greater interest, however, is the failure of F and I ions to influence the rate of Cl absorption, because it reveals the high degree of selectivity of this transport mechanism for Cl and Br ions. The mechanism is shown to have extremely low affinity for F and I ions.

This conclusion is in complete accord with recent work by Venkateswarlu et al. (17) on absorption of Cl and F by barley roots. They found in experiments in which the initial external concentration of these ions varied from 0.01 to 1 mM that absorption of Cl was metabolically mediated and high accumulation ratios were achieved. In striking contrast, F was not metabolically accumulated, and what little F uptake occurred was solely by diffusion into the outer space of the tissue. Others have also observed that from dilute solutions, *Gramineae* take up very little F (15) and I (1).

The results of the present work, together with those just mentioned, all lead to the conclusion that the mechanism which effects the absorption of Cl and Br from dilute solutions (1 mM and less) has very little affinity for F and I; it does not transport these ions, nor is its operation when transporting Cl or Br influenced by them. In experiments on the essential role of Cl in plant nutrition it has also been found that Br acts as a metabolic analog of Cl, within limits, while neither I nor F can substitute to any extent (2, 13).

The results shown in table II and figure 6 show that absorption of Cl is metabolically mediated. This is not surprising in view of the fact that Cl and Br ions were extensively used in the classical investigations of Hoagland (10) and others which led to the

recognition that ion absorption by plant cells is a metabolic process. The results are included here mainly because at the concentration of Cl used in the present experiments (0.05 mM), the effects of the inhibitors on only mechanism I of Cl transport are shown, unobscured by their possibly quite different effects on the mechanism which becomes effective at higher concentrations of Cl (4). The results concerning the effect of F obtained in this experiment parallel those of the experiment shown in table I.

Summary

Absorption of Cl by excised roots of barley, *Hordeum vulgare*, var. Arivat, was a strictly linear function of time for at least 60 minutes. In the absence of Ca, the rate of Cl absorption was lower than in the presence of Ca at 0.5 mM, and more greatly influenced by the pH of the solution. Transport of Cl is essentially unidirectional (inward).

Over the concentration range 0.005 to 0.20 mM Cl, the rate of absorption of Cl followed Michaelis-Menten kinetics with a Michaelis constant of 0.013 mM in the presence, and 0.046 mM in the absence of Ca. In all other experiments, Ca was routinely included in the experimental solutions at 0.5 mM.

Absorption of Cl was competitively inhibited by Br; the inhibitor constant, K_i , for Br 0.036 mM. The 2 other halides, F and I, were tested at concentrations ranging from 10^{-6} to 10^{-3} M and found to be without effect on the rate of Cl absorption.

In the presence of 2,4-dinitrophenol, KCN, Na-azide, Na-arsenate, and Na-amytal, absorption of Cl was inhibited. Low temperatures also reduced the rate of absorption.

The results are discussed with special reference to the high selectivity of the mechanism for transport of Cl and Br vis-à-vis F and I ions.

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Literature Cited

1. BÖSZÖRMENYI, Z. AND E. CSEH. 1964. Studies of ion uptake by using halide ions changes in the relationships between ions depending on concentration. *Physiol. Plantarum* 17: 81-90.
2. BROYER, T. C., A. B. CARLTON, C. M. JOHNSON, AND P. R. STOUT. 1954. Chlorine—a micronutrient element for higher plants. *Plant Physiol.* 29:526-32.
3. BUDD, K. AND G. G. LATIES. 1964. Ferricyanide-mediated transport of chloride by anaerobic corn roots. *Plant Physiol.* 39: 648-54.
4. ELZAM, O. E., D. W. RAINS, AND E. EPSTEIN. 1964. Ion transport kinetics in plant tissue: complexity of the chloride absorption isotherm. *Biochem. Biophys. Res. Commun.* 15: 273-76.
5. EPSTEIN, E. 1953. Mechanism of ion absorption by roots. *Nature* 171: 83-84.
6. EPSTEIN, E. 1961. The essential role of calcium in selective cation transport by plant cells. *Plant Physiol.* 36: 437-44.
7. EPSTEIN, E., D. W. RAINS, AND O. E. ELZAM. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. *Proc. Natl. Acad. Sci.* 49: 684-92.
8. EPSTEIN, E., W. E. SCHMID, AND D. W. RAINS. 1963. Significance and technique of short-term experiments on solute absorption by plant tissue. *Plant Cell Physiol.* 4: 79-84.
9. FAWZY, H., R. OVERSTREET, AND L. JACOBSON. 1954. The influence of hydrogen ion concentration on cation absorption by barley roots. *Plant Physiol.* 29: 234-37.
10. HOAGLAND, D. R. 1940. Salt accumulation by plant cells, with special reference to metabolism and experiments on barley roots. *Cold Spring Harbor Symp. Quant. Biol.* 8: 181-94.
11. HOAGLAND, D. R., A. R. DAVIS, AND P. L. HIBBARD. 1928. The influence of one ion on the accumulation of another by plant cells with special reference to experiments with *Nitella*. *Plant Physiol.* 3: 473-86.
12. JACOBSON, L., D. P. MOORE, AND R. J. HANNAPEL. 1960. Role of calcium in absorption of monovalent cations. *Plant Physiol.* 35: 352-58.
13. JOHNSON, C. M., P. R. STOUT, T. C. BROYER, AND A. B. CARLTON. 1957. Comparative chlorine requirements of different plant species. *Plant Soil* 8: 337-53.
14. NISSEN, P. AND A. A. BENSON. 1964. Liquid scintillation counting of plant roots. *Intern. J. Appl. Radiation Isotopes* 15: 505-07.
15. PETERS, R. AND M. SHORTHOUSE. 1964. Fluoride metabolism in plants. *Nature* 202: 21-22.
16. RAINS, D. W., W. E. SCHMID, AND E. EPSTEIN. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. *Plant Physiol.* 39: 274-78.
17. VENKATESWARLU, P., W. D. ARMSTRONG, AND L. SINGER. 1965. Absorption of fluoride and chloride by barley roots. *Plant Physiol.* 40: 255-61.