the solution level. Roots were rinsed by dipping once in tap water; leaves and stems were washed with soap. After being dried (60° C) and weighed, the samples were ashed (500° C) and dissolved in 6 N HCl. Activity counts on these solutions were made in a well-type gamma scintillator and the results are given in table I.

The data show that substantial amounts of tagged iron moved into the tops of control plants but none was detectable in stems or leaves of plants from the bicarbonate cultures. Whether bicarbonate ion affects absorption of iron by roots or translocation from roots to tops is not clear. The fact that Fe* concentrations in roots of the bicarbonate-treated plants were lower than controls is at least consistent with the hypothesis that the effect was to reduce iron absorption by roots.

The results show that A: *Citrus sinensis* is among those plants that are sensitive to sodium bicarbonate, and B: the mechanism can be effective immediately upon exposure to the bicarbonate system.

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UPTAKE OF CALCIUM BY EXCISED BARLEY ROOTS [1,2]

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The term non-metabolic uptake has been used to describe the uptake of ions not directly involving metabolic activity (10). There is a great deal of uncertainty involved in the measurement of non-metabolic uptake and likewise in the measurement of metabolic absorption. When a non-metabolic uptake correction is applied to large absorption rates, the resulting errors are not appreciable and can usually be ignored. However, when the correction is of the same order of magnitude as the total uptake, evaluation of the metabolic component may be exceedingly difficult.

Ca has long been recognized to be a slowly absorbed ion (8, 18). The reported total Ca uptake by excised barley roots (7, 9, 12, 19) is approximately equal to the non-metabolic uptake for other cations (10). Consequently, it is impossible to obtain a reliable measure of the metabolic absorption rate of Ca from total uptake data alone. For this reason, this study was designed to investigate the nature of the Ca uptake by excised barley roots with particular emphasis on the evaluation of the non-metabolic and metabolic components.

Materials & Methods

All these experiments were conducted with 6-day-old excised barley roots of the variety Tennessee Winter, 1956 crop. The method of culturing root material was similar to that described by Jacobson et al (14). The excised roots were washed several times in distilled water prior to the experiment. After washing, the roots were centrifuged at 65 × *g* for 5 minutes. Representative samples of roots were weighed out and placed in bottles of the desired salt solutions which were kept at constant temperature in a water bath. Unless otherwise stated, 7 grams of roots in 7 liters of solution were used. The pH of the solutions was rigorously controlled by adding acid or base throughout the experimental period. The resulting changes in solution composition were negligible. All experiments were done at 25° C., unless otherwise indicated. The solutions were aerated continuously during the experiment.

Stock solutions of $Ca(OH)_2$ were prepared by allowing saturated solutions of this base to stand in closed pyrex bottles for several weeks. Just prior to the experiment, portions of the clear supernatent were drawn off. Care was taken to avoid exposure of the $Ca(OH)_2$ to CO_2.

At the end of the absorption period, the roots were collected by pouring the contents of the bottles through a fine mesh nylon screen. The roots were washed in a running stream of distilled water for 10 seconds. Great care was taken to standardize the washing pro-

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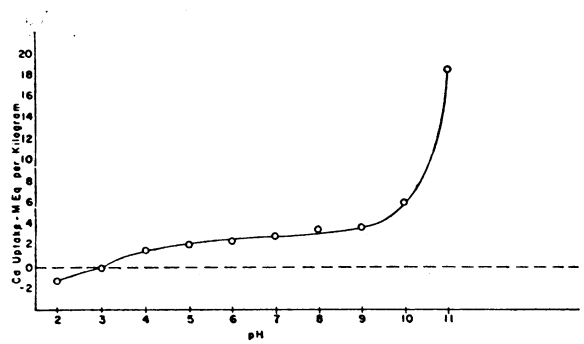
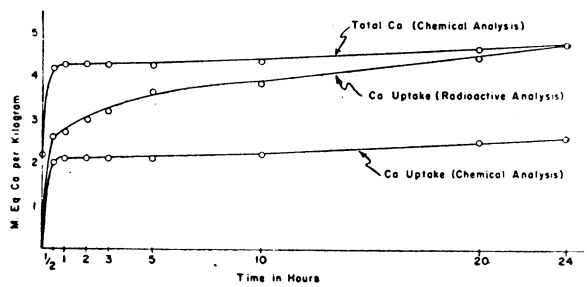
cedure to minimize variations in the non-metabolic uptake. The washed roots were dried in an oven overnight and ashed in a muffle at 600° C. K was determined by flame photometry on an acidified solution of the ash. Aliquots of the same unknown solutions were titrated with ethylenediamine tetraacetic acid to the Calcein endpoint for Ca (3).

Roots for organic acid analysis were frozen rapidly with liquid nitrogen at the end of the experiment. The material was lyophylized and organic acids were determined by ether extraction and titration (20). Inorganic ions were determined on ashed samples of the lyophylized material as described above.

When Ca^{45} was present in the samples, 1 ml of the unknown solution was placed in an aluminum planchet and dried in an oven. Duplicate planchets were prepared for each sample. Standards were prepared which had the same mass as the unknowns. Each planchet was counted twice in a windowless flow counter. Variations in count due to differences in mass and geometry were negligible. Counting rates were approximately ten times background.

Uptake data are expressed in meq per kilogram of fresh roots. These values represent the net change in content of the roots during the absorption period. Negative values indicate a loss of ions from the root. Initial contents of experimental material were 2.2 to 2.9 meq Ca and 11.2 to 13.4 meq K.





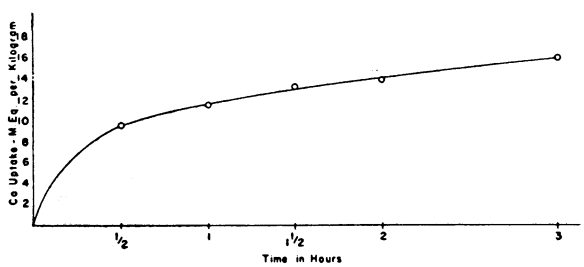


FIG. 1. The effect of pH on the uptake of calcium in 3 hours from 0.005 N $CaBr_2$

FIG. 2. The uptake of calcium as a function of time as measured by both chemical and radioactive analysis. The concentration of $CaBr_2$ was 0.005 N and the pH was 5.

FIG. 3. The uptake of calcium as a function of time from 0.005 N $CaBr_2$ at pH 11.

RESULTS & DISCUSSION

The effect of pH on the absorption of monovalent cations by barley roots has been studied in detail (11, 13). An experiment was designed to determine the effect of a wide range of pH on Ca uptake from a single salt solution. $CaBr_2$ and $Ca(OH)_2$ were combined so that the resulting solutions were 5 meq per liter in Ca at pH 11. These solutions were then adjusted to give a pH range from 2 to 11 by adding HBr. The uptake period was 3 hours. The results of this experiment are shown in figure 1.

It is apparent from figure 1 that there was little uptake of Ca below pH 9. Above pH 9 there was a rapid increase in Ca uptake. These data have not been corrected for non-metabolic uptake. The total uptake from pH 4 to 9 was of the same order of magnitude as the reported non-metabolic uptakes for other cations (10).

To obtain a measure of the non-metabolic uptake of Ca, experiments were conducted in which the metabolic absorption would be reduced to a low value. This was done by treatment with 2-4,dinitrophenol (DNP) and at low temperature. Since K is absorbed rapidly by this root material, the treatments were applied to K at the same time to measure the effectiveness of the treatments in inhibiting metabolic absorption. The uptake period was 3 hours and the concentration of Ca or K was 5 meq per liter. For the low temperature experiments the roots were submerged in distilled water at 0° C for 1 hour prior to the uptake period to eliminate any residual metabolic activity carried over from room temperature (15). The control and the DNP treatments were at 25° C. The pH was maintained at 5.0 throughout the experiment. The results are summarized in table I.

The results of table I indicate that most, if not all, of the Ca uptake by these roots was non-metabolic. It is difficult to conceive of a metabolic Ca absorption which is insensitive to both low temperature and DNP. If any metabolic component did exist, it was too small to be measured using this technique.

The conclusions drawn from the data in table I do not agree with those of Fried et al (6) and Chasson and Levitt (2). Chasson and Levitt found a rapid initial rise followed by a small linear increase with time up to 24 hours for the uptake of Ca^{45} by potato slices. Fried et al, using excised barley roots, have

TABLE I

EFFECT OF LOW TEMPERATURE AND DNP ON UPTAKE OF K & Ca

TREATMENT	TEST SOLUTION	CATION UPTAKE IN 3 HR AT pH 5, meq/kg
Control	KBr	23.5
0° C	KBr	3.0
10^{-6} M DNP	KBr	10.7
5×10^{-6} M DNP	KBr	0.9
10^{-5} M DNP	KBr	−0.4
Control	$CaBr_2$	2.0
0° C	$CaBr_2$	2.0
10^{-6} M DNP	$CaBr_2$	2.1
5×10^{-6} M DNP	$CaBr_2$	2.0
10^{-5} M DNP	$CaBr_2$	2.1

also reported a large initial uptake followed by a slow linear component for Ca^{45}. In both cases, the linear phase was interpreted to represent the steady-state metabolic absorption rate of Ca. The results of these investigators are open to serious objection, however. The uptake in both cases was measured using a radioisotope. If any appreciable isotopic exchange occurred with previously absorbed inert Ca, their conclusions would be in error. Such an exchange might be expected to occur, even over a longer period of time (16).

In an attempt to reconcile the differences between this study and the results of the above authors, an experiment was conducted using Ca^{45} to measure the uptake. In addition to counting the samples for Ca^{45}, total Ca was determined on the same samples by chemical analysis. Therefore, the results also give a measure of the isotopic exchange of Ca^{45} for inert Ca in the root. The uptake period varied from ½ hour to 24 hours and the pH was maintained at 5.0. The concentration of $CaBr_2$ was 5 meq per liter labeled with about 50,000 cps per meq. A parallel experiment was conducted at the same time in which the roots were given a 1-hour exposure to KBr after having been in the $CaBr_2$ solutions. The uptake of K was used as an indication of the metabolic activity of the roots. There was no reduction in the ability of the roots to absorb K, even after 24 hours in $CaBr_2$.

The total Ca content of the roots and the uptake of labeled Ca and inert Ca as a function of time are shown in figure 2. The striking feature of these curves is the large discrepancy between labeled Ca uptake and inert Ca uptake. Since there can be no doubt about the interpretation of the chemical analyses, these results point to a large isotopic exchange of Ca^{45} for initially present inert Ca. Furthermore, it is apparent that it took at least 24 hours for all the Ca in these roots to come to complete isotopic equilibrium.

The high initial uptake of Ca as measured by chemical analysis indicates a large non-metabolic component. Even though there is a small positive slope in the time curve after 1 hour, the rate of Ca increase is only about 0.1 meq per 5 hours. Two other long term experiments showed even slower rates. The small increase with time does not necessarily imply a metabolic process. It may be a reflection of exchange of Ca for Mg. Mg analyses indicated a slow loss of Mg over the entire time period. An alternative explanation is that growth may have occurred during the 24 hour uptake period. In addition to using Ca for new cell constituents, growth may have caused an increase in the total non-metabolic component. An increase in the non-metabolic uptake of K with time has been reported for similar barley root material (10).

Although the uptake of Ca at pH 5 by this tissue appears to be largely non-metabolic, an experiment was conducted to determine whether or not the isotopic exchange of Ca^{45} was a metabolic process. The experimental conditions were similar to those for figure 2. The time period was 3 hours and the concentration of DNP was 10^{-5} M. A KBr treatment was included to test the effectiveness of the DNP. The results showed that 10^{-5} M DNP completely inhibited the absorption of K. The effect of DNP on the isotopic exchange of Ca is shown in table II. These results show that the isotopic exchange of Ca was not affected by 10^{-5} M DNP. From this it would appear that Ca uptake and the isotopic exchange of Ca were both largely non-metabolic processes under the conditions of these experiments.

In light of the largely non-metabolic nature of the Ca uptake at pH 5, the uptake of Ca at high pH was investigated more thoroughly. Figure 3 shows the uptake of Ca as a function of time from a solution containing 5 meq $CaBr_2$ per liter at pH 11. Although the uptake curve is not linear, the continual increase with time suggested that the Ca uptake at this pH may have been an active process. At pH 11 the uptake of Br (about 1 meq/kg) can all be accounted for as non-metabolic uptake. Therefore, Br cannot serve to balance the uptake of Ca at this pH. Ordinarily, this root material balances excess cations absorbed by the production of organic acid anions (12) and this relationship may be used to measure metabolic absorption of a cation (10). The balancing of excess Ca uptake at pH 11 by organic acids would show that Ca was being metabolically absorbed at this pH. An experiment was designed to test this possibility.

TABLE II

EFFECT OF 10^{-5} M DNP ON ISOTOPIC EXCHANGE OF Ca

TREATMENT	3 HR IN 0.005 N $CaBr_2$ AT pH 5		
	TOTAL Ca BY CHEMICAL ANALYSIS meq/kg	Ca UPTAKE BY CHEMICAL ANALYSIS meq/kg	Ca UPTAKE BY Ca^{45} ANALYSIS meq/kg
Control	5.0	2.1	4.1
10^{-5} M DNP	5.0	2.1	4.1

Roots were treated in 5 meq $CaBr_2$ per liter at pH 11 for 3 hours. A parallel KBr treatment at pH 11 was included to give a measure of the ability of the roots to form organic acid anions at this high pH. The results of the KBr treatment indicated that the excess K absorbed at this pH was indeed balanced by organic acid anions. The Ca uptake from the $CaBr_2$ treatment was 17.2 meq per kilogram and the increase in organic acids was only 2.4 meq per kilogram. It appears, therefore, that excess Ca uptake at pH 11 did not elicit equivalent organic acid anion production, even though the tissue was capable of producing them. The loss of K and Mg from the roots during the $CaBr_2$ treatment was only 3.2 meq per kilogram and cannot account for the discrepancy.

The precipitation of $CaCO_3$ on or within the root was considered as a possible explanation of the large Ca uptake at pH 11. Although care had been taken to exclude external CO_2, the respiration of the roots may have supplied sufficient CO_2 to precipitate $CaCO_3$. To check on this possibility, 50 grams of roots were placed in 18 liters of 0.005 N $CaBr_2$ at pH 11. At the end of a 3-hour treatment, the roots were transferred to a closed vessel fitted with a KOH well. The system was evacuated and sufficient 1 N H_2SO_4 was introduced to cover the mass of roots. The system was kept closed and was occasionally agitated by swirling. At the end of 48 hours, the KOH was removed and titrated for the presence of carbonate. The results indicated that no more than 2 meq of Ca per kilogram of roots could be accounted for as $CaCO_3$. It is entirely possible that this figure is too large, since the strong acid treatment may have liberated CO_2 by decarboxylation of organic compounds.

A sample of roots which had been in a $CaBr_2$ solution at pH 11 for 3 hours was transferred to distilled water for 30 minutes. Out of a total Ca uptake of 18.4 meq per kilogram there was a loss of 12.3 meq. It would appear, therefore, that a large fraction of the Ca uptake at high pH is easily lost to distilled water.

Conclusions

Most, if not all, of the Ca uptake by this barley root material in the physiological pH range was non-metabolic. Ca has a marked influence on the absorption of other ions (5, 11, 19, 22). To be effective Ca must be present during the absorption process. Pretreatment of the roots with Ca is ineffective. This lack of a carry-over effect and the largely non-metabolic nature of the Ca uptake suggests that the action of Ca is in the surface region of the cell. As a result of this action, the permselectivity of the cell is greatly enhanced (11). The most logical place for this permselective barrier is at the plasmalemma. Such a barrier would afford the cytoplasm an excellent means of protection from the external medium. In addition, the properties of the permselective region could impart a large degree of specificity to the over-all ion absorption process. It is postulated here that this region is relatively impermeable to Ca.

Denying access of large amounts of Ca to the cytoplasm may benefit the cell. It has been reported that Ca inhibits many of the enzyme systems found in cells, particularly those which require Mg (4). Skeen (21) has shown Ca salts to coagulate the cytoplasm when they are introduced into the vacuole. On the other hand, Ca has been shown to be a requirement for certain enzymes (4). However, if this Ca were bound tightly by those enzymes in vivo, the amount of free Ca ions inside the cell might be kept low.

It has long been recognized that a continuous supply of Ca is essential for plant growth. In this respect, the utilization of Ca by growing tissue is undoubtedly closely associated with metabolic activity. However, in light of the results of this study, it is doubtful that Ca is actively absorbed by mature barley root cells and accumulated into the vacuoles. If the concentration of undissociated Ca in the cytoplasm or vacuole never exceeded the external Ca concentration, there would be no necessity for the direct expenditure of metabolic energy to explain the entry of Ca into the cell. It is conceivable that Ca ions, after slowly leaking past a relatively impermeable region into the cell, may become rapidly immobilized. The presence of Ca oxalate crystals in the vacuoles of some plant species (17) suggests this possibility.

The rather rapid equilibration of Ca^{45} with a substantial part of the inert Ca initially present in the roots indicates that much of the initial Ca may be located external to the cytoplasm. This hypothesis is also supported by the largely non-metabolic nature of this equilibration. A fraction of the initial Ca also appears to be readily exchangeable for other cations (10). Almost half the initial Ca in these roots may be in this exchangeable form, since that amount is easily lost to a solution containing 5 meq KBr per liter. If any of the remaining initial Ca is present as Ca pectates in the cell walls, then the amount of Ca inside the cytoplasm and vacuoles of these roots might easily be less than 1 meq per kilogram.

Except for perhaps that Ca which elicits the small increase in organic acids, Ca uptake at high pH was also largely non-metabolic. It is suggested that there are groups present in the barley root containing dissociable hydrogen which are capable of holding Ca at these high pH values. The Ca would be released by hydrolysis from these groups when the roots are placed in distilled water. Apparently, K is not held to any large extent by these groups since there was good agreement between K uptake and organic acid increase at pH 11. The nature of such groups and their significance, if any, to the mechanism of ion absorption is not clear.

The largely non-metabolic nature of Ca uptake by excised barley roots raises the question of how intact plants obtain their Ca. Some species of plants are known to take up large amounts of Ca. The grasses, however, are characterized by their low Ca requirement. It may be that the rate of metabolic absorption of Ca is sufficient to supply the needs of

the barley plant but is too small to be measured in relatively short-term experiments. It might be argued that the roots used in these experiments were already saturated with Ca and no further metabolic absorption was possible. This seems unlikely, however, in view of the low initial Ca status of these roots. Another possibility to account for the observed uptake of Ca by intact plants is surface migration through the root cortex to the transpiration stream. According to this hypothesis, an ion may move from the external solution along surfaces into the xylem without ever being actively absorbed. This may be more important for an ion like Ca than for other ions since much of the Ca appears to be associated with the cell surface region. There may be fixed negative charges in the cell wall as well as in the plasmalemma and Ca could migrate along these charged surfaces. Upon reaching the xylem, the Ca could be released by hydrolysis and carried upward in the transpirational stream. If these fixed charges are associated with phosphatides (1) and pectic substances, this type of movement could be rather specific for certain ions of which Ca may be the most important. Furthermore, the rate of surface migration of Ca through the cortex of the root need not parallel the rate of water movement through this region.

The complete equilibration of Ca^{45} observed in this study suggests use of caution when measuring absorption by means of an isotope. Furthermore, regardless of the location of Ca in the root or the mechanism by which it arrived there, it is clear that the uptake of Ca is a reversible process.

Summary

The uptake of Ca by 6-day-old excised barley roots appears to be largely non-metabolic. The Ca uptake at pH 5 was found to be insensitive to low temperature and dinitrophenol. The uptake of Ca^{45} was a reflection of isotopic exchange for initially present inert Ca in the root. This equilibration process was not affected by dinitrophenol.

There was a large uptake of Ca by this material at pH 11. This uptake was also largely non-metabolic. Only a small fraction of the Ca uptake at high pH could be accounted for by an increase in organic acids or by precipitation as $CaCO_3$.

It is postulated that much of the Ca in young barley roots is associated with the cell surface region. It is proposed that the Ca which is active in influencing the absorption of other ions is localized on this surface.

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