CALCIUM AND OTHER POLYVALENT CATIONS AS ACCELERATORS OF ION ACCUMULATION BY EXCISED BARLEY ROOTS

FRANK G. VIETS, JR.

(WITH ELEVEN FIGURES)

The effects of one ion on the absorption of another has merited the attention of physiologists for almost a century. The investigations in this field (**15**), however, have usually been concerned with the analysis of plants grown in different media or with plants or cells subjected for short periods generally to high concentrations of salts with little regard for the metabolism of the tissue. Growth experiments are so complicated by secondary factors that they are of little value in the study of the primary phases of absorption. Short term experiments with inactive tissues and high concentrations of salt (passive absorption) have led to the theory of antagonism between monovalent and divalent cations.

The technique of growing barley roots (**6**) capable of rapid absorption and accumulation of mobile ions against concentration gradients during intensive aerobic metabolism offers a new and valuable method for the study of ion relationships. The object of this paper is to present data on the absorption of K and Br by barley roots as influenced by various polyvalent cations and to discuss them in relation to current theories of solute absorption. A brief account has been published by the author (**17**).

Material and methods

The method of growing root material and general technique of experimentation developed by HOAGLAND and BROYER (**6**) have been followed in these investigations. Sacramento barley was grown for about 4 weeks in a complete culture solution with a limited supply of nutrients. The roots were then excised, subjected to various absorption treatments, frozen, and later thawed and the sap expressed for analysis. These roots, low in total salt, high in sugars and capable of rapid accumulation of K and Br were in no sense deficient in Ca. Hence, these experiments cannot be compared with those in which Ca supply was deliberately restricted in order to study Ca as a permeability factor.

The plants were grown in the fall, winter, and spring months and hence the root weight, salt content, and capacity for salt accumulation fluctuated as indicated by HOAGLAND (**5**), but it is believed that this variable does not invalidate any of the conclusions. Modifications in technique necessary for certain experiments will be noted later. In all cases where very dilute (0.0001 N) KBr solutions were used the roots were immersed in tanks holding at least 40 liters of solution in order to provide enough total salt so that the concentration would not appreciably diminish during the course of the absorption. The pH of the experimental solutions varied within narrow limits (5.5 to 7.0), a range exerting little effect on ion accumulation (**7**).

Results

GENERAL FEATURES OF THE CALCIUM EFFECT

Several experiments have shown that the classical concept of antagonism of Ca and K in absorption does not apply to barley roots accumulating salt from dilute solutions. The results of a typical experiment are shown in figure 1. Sets of roots were immersed in a series of 0.005 N KBr solutions containing $CaBr_2$ varying from 0.0001 N to 0.5 N with 0.005 N KBr serving as a control. After 8¼ hours the roots immersed in 0.3 N and 0.5 N $CaBr_2$ had lost 10 and 20 per cent. of their original fresh weights, respectively. The lower absorption and the loss in weight was probably due to unfavorable osmotic effects. Roots from the other solutions were normal in appearance and showed no significant change in weight. The significant results of this experiment are that $CaBr_2$ increased the amount of K absorbed by about 100 per cent. and the Br absorption by more than 150 per cent., the increase depending on the $CaBr_2$ concentration. The absorption of Ca was small and the Ca concentration in the expressed sap was always lower than that of the external medium except for the 4 most dilute solutions. Several experiments of this kind have shown the same favorable effect of Ca on absorption, whether the data be evaluated on the basis of the expressed sap or on the total root ash. The concentration of the Ca, K, and Br remaining in the press cakes was of the same general magnitude as that of the expressed saps [Compare BROYER and HOAGLAND (**3**)].

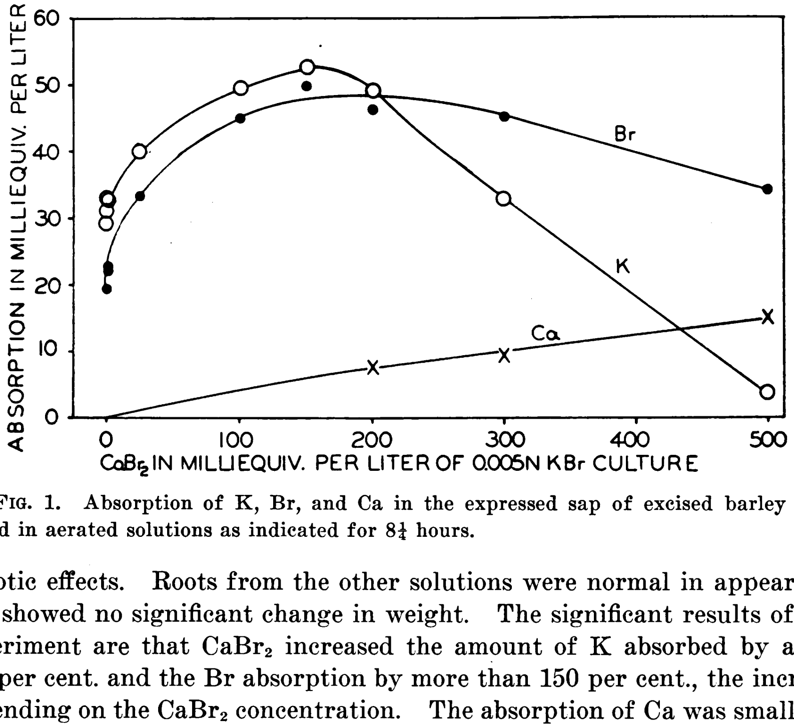

FIG. 1. Absorption of K, Br, and Ca in the expressed sap of excised barley roots placed in aerated solutions as indicated for 8¼ hours.

This experiment is of interest in view of the contentions of MAZIA (**14**), working with Elodea leaf cells, that Ca had a higher capacity to compete for positions on the protoplasmic membrane than K and thus antagonized the entrance of K. The above experiment shows that a Ca : K molecular ratio of 15 : 1 produced the largest absorption of K and a ratio of 30 : 1 gave as large absorption as KBr alone.

Another experiment using a range of $Ca(NO_3)_2$ concentrations up to 0.1 N in a standard solution of 0.005 N KNO_3 has shown that Ca associated with a different anion than Br is also able to increase the absorption of K by about 80 per cent. A similar effect was obtained with solutions of $CaSO_4$ up to 0.025 N in a solution of 0.005 N K_2SO_4. The increase in the K absorption without a corresponding absorption of anion was compensated in the expressed sap by an increase in the total organic acid anions as previously shown by ULRICH (**16**) for the differential absorption of cation and anion. The total organic acids were calculated from the titration curves of the expressed sap according to the method of ISAACS and BROYER (**10**).

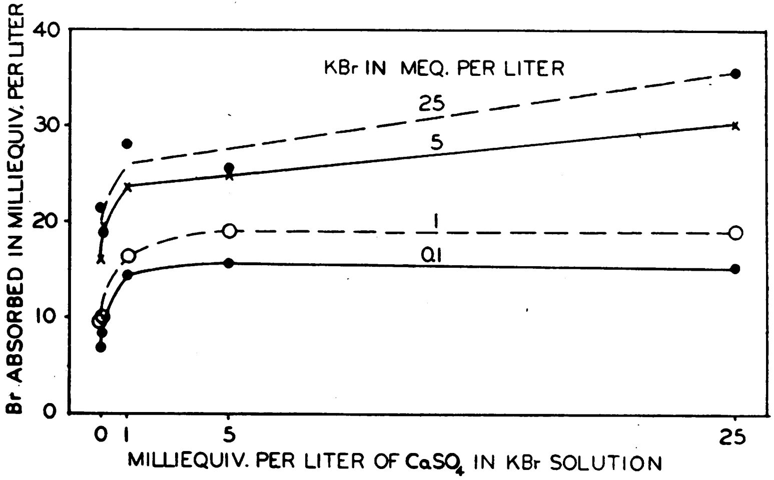

FIG. 2. Bromide absorbed in the expressed sap of barley roots immersed for 10 hours in KBr solutions at different $CaSO_4$ concentrations.

In the foregoing experiments 0.005 N K solutions were used in conjunction with variable concentrations of Ca and the solution contained only one species of anion. It was conceivable that the high and variable concentration of anion might influence in some way the absorption from the constant amount of K used. Hence, the experiment shown in figure 2 was performed in which different concentrations of $CaSO_4$ were used in conjunction with various levels of KBr. The data for Br absorption show that the Ca ion is significant in the enhanced absorption rather than a high common anion concentration (fig. 1). Also apparent is the fact that 0.001 N $CaSO_4$ gave marked increases in absorption of Br at all KBr concentrations. It thus appears that the concentration of Ca is of importance rather than any particular ratio of Ca to K and Br.

These experiments indicate that the traditional concept of antagonism of Ca for monovalent ion absorption from dilute solutions cannot be applied to accumulating barley roots of the type used in these experiments. They may further indicate that if the first step in the accumulation of ions is an adsorption on certain adsorption positions of the membrane as postulated by many investigators (**2**, **4**, **13**, **14**) then either Ca is unable to compete for these positions or else it increases in some unknown way the total number of positions thereby obscuring the competitive effects. The view that Ca is unable to compete with K for adsorption positions is consistent with investigations on ion exchange between roots and media. JENNY and OVERSTREET (**11**) found that Ca bentonite or Ca salts of equivalent concentration of Ca did not remove as much K from barley roots by exchange as did the corresponding NH_4, K, and Na sols or solutions. Similarly, BROYER and OVERSTREET (**4**) found that radioactive K in roots would not exchange for Ca in $CaSO_4$ solution whereas it would exchange readily for non-radioactive K in the solution.

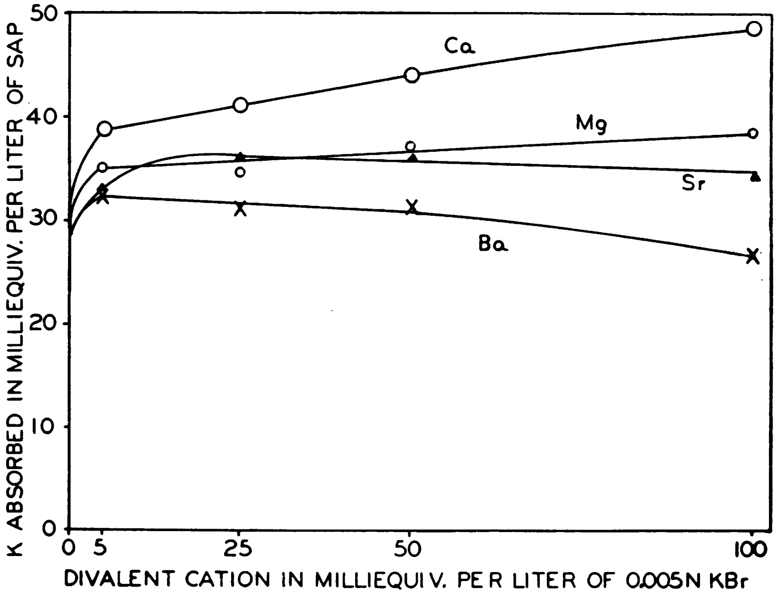

FIG. 3. Influence of various divalent cations on the absorption of K in the expressed sap of roots kept in the solutions indicated for 8¼ hours.

EFFECTS OF OTHER POLYVALENT CATIONS

Since Ca promoted absorption of K and Br it was of interest to learn if other ions like Mg, Sr, Ba, and possibly Al might also be effective and if the relative effect of these ions could be arranged in a series corresponding to their known physico-chemical properties.

A preliminary experiment using 0.005 N solutions of $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, and $Ba(NO_3)_2$, respectively, containing 0.005 N KNO_3 showed that these divalent cations could also accelerate K accumulation, the magnitude of the increase being 7 to 11 m.eq. per liter of additional K absorbed in the expressed sap of the roots.

A second series using $MgBr_2$, $CaBr_2$, $SrBr_2$, and $BaBr_2$, respectively, in a range of concentrations from 0.005 N to 0.1 N in a solution 0.005 N with respect to KBr gave marked increases in the amount of Br absorbed; but only Ca, Mg, and Sr increased the K absorbed over that of the controls as shown in figure 3. The addition of $BaBr_2$ had little effect on the amount of K absorbed. There were no visible signs of root injury and the roots showed no significant changes in weight.

One of the foregoing experiments showed that the absorption of Ca is quite small in relation to the K and Br absorbed concomitantly; but it was possible that Sr, Ba, and Al, which are toxic to the growth of plants, could be absorbed in sufficient quantities so that their deleterious effect might mask their favorable effect on the plasma membrane. Therefore, an experiment using short periods of time and low concentrations (0.001 N) of Sr, Ba, and Al ions in order to minimize their absorption was conducted. The data for K and Br absorption are shown in figures 4 and 5, respectively. These curves show that even in low concentrations these ions are effective, but that the differences between ions are of insufficient magnitude for any arrangement in a series. Another experiment using 0.001 N $Al_2(SO_4)_3$ in a solution of 0.050 N KBr has shown that K absorption is increased by 10 and Br by 20 per cent. over the controls.

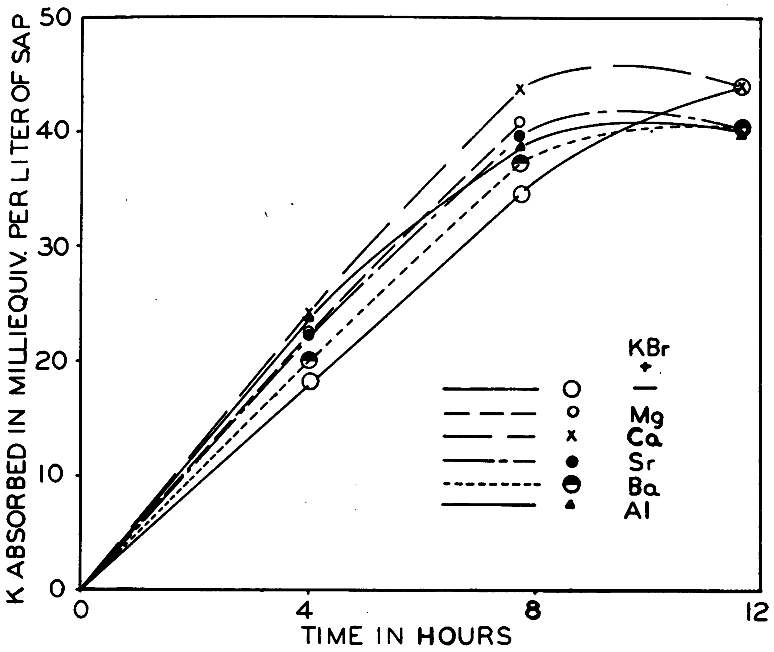

FIG. 4. Potassium absorbed in the expressed sap of barley roots from 0.005 N KBr as modified by dilute concentrations of polyvalent cations.

These experiments, in general, show that Ca of the cations tested is the most efficient accelerator of accumulation, but that others can also perform the same function. Any attempt to arrange them in a series must involve careful consideration of the many variables and utilization of new and improved techniques.

It was of interest to know if these cations were performing independent or some common functions. A series of solutions were made in which the $CaSO_4$ concentrations varied from 0 to 0.025 N in a solution of 0.005 N KBr and the $MgSO_4$ concentrations varied in the same series from 0.025 N to 0 in an inverse order. Thus the solution always contained 0.005 N KBr and a total concentration of $CaSO_4$ and $MgSO_4$ amounting to 0.025 N with a varying ratio of Ca to Mg. The results for K and Br absorbed from the solutions as shown by the expressed sap are plotted in figure 6. This experiment indicates that Ca and Mg are performing the same function and that the total increase in absorption depends upon the relative proportion and efficiency of each constituent cation.

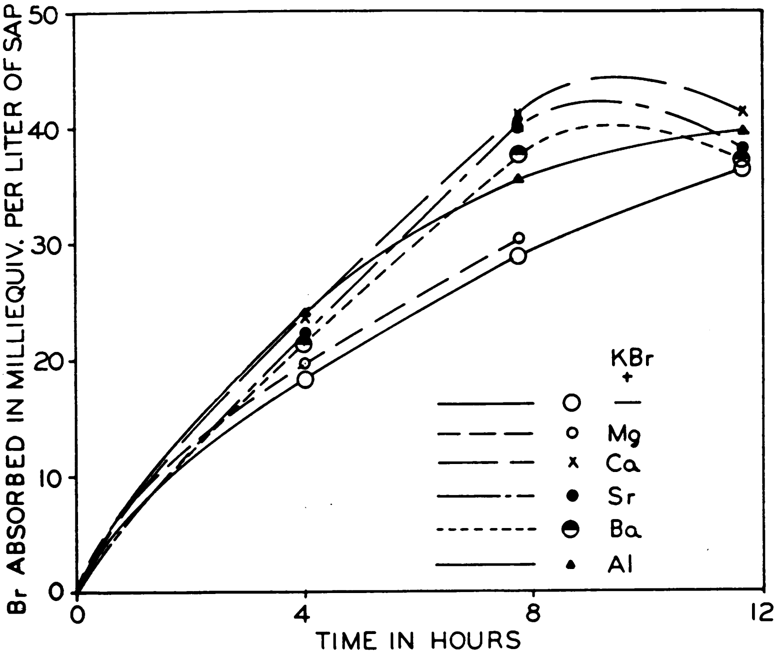

FIG. 5. Bromide absorbed in the expressed sap of barley roots treated as indicated in figure 4.

This mutual replaceability of Ca and Mg may account for the failure of growth experiments of the type of LUNDEGARDH (**12**) to show a significant effect of Ca and Mg on K absorption. When the effect of Mg is being studied, the solution usually contains abundant Ca bringing the total divalent cation into a region on the absorption curves where the effect of increasing divalent cation has little effect. The result is that the effect of Mg as a factor in absorption may appear insignificant. The same may be true when Ca is the variable.

THE POLYVALENT CATION EFFECT AND METABOLISM

Polyvalent cations may conceivably affect either the oxidative mechanism supplying the energy for accumulation—the "pump-like" mechanism of **Hoagland** and **Broyer** (**8**)—or they may exert their effect primarily on the plasma membrane so that ions accomplish more readily the initial step in absorption.

Preliminary experiments indicated that there was no significant effect of Ca or Mg in the KBr solution on the rate of CO_2 production (**17**). However, **Machlis** and **Broyer** (unpublished data) studying the respiration of segments of primary barley root in a Fenn respirometer found a significant increase in both the O_2 uptake and the CO_2 respired due to the presence of Ca in the KBr absorption medium. **Machlis** also found that respiration was increased by 0.015 N $CaSO_4$ as compared with respiration of comparable root segments in distilled water. Several experiments of the writer have also shown that the increased absorption of K salts due to Ca is accompanied by a greater loss of total sugars (fig. 8). What proportion of the increased rate of respiration and sugar loss is a primary effect of Ca and how much is due to the increase in total salt absorbed is not clear. [See **Hoagland** and **Steward** (**9**) and **Lundegardh** (**13**) for a discussion of salt absorption in relation to respiration.]

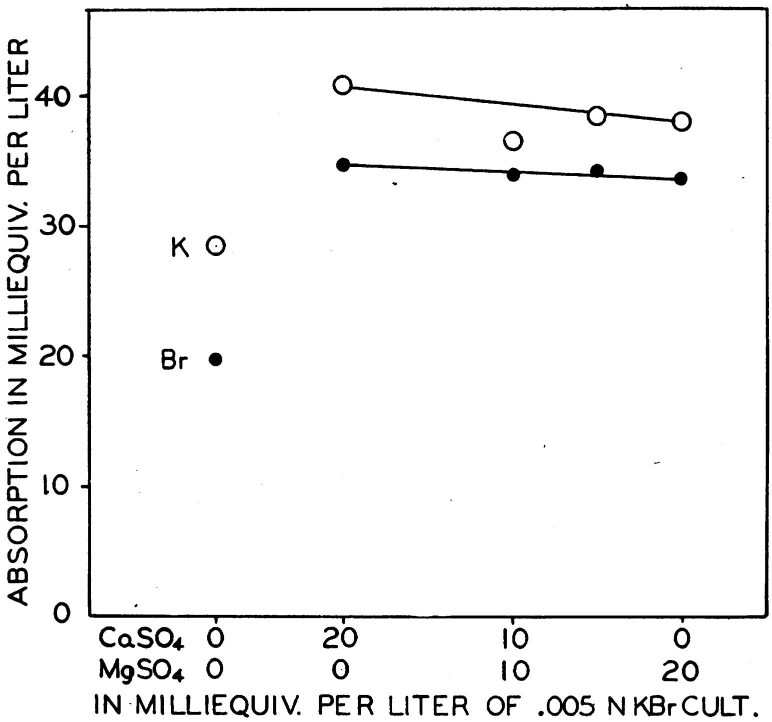

FIG. 6. Absorption of K and Br in the sap of excised roots immersed in the solutions indicated for 10 hours.

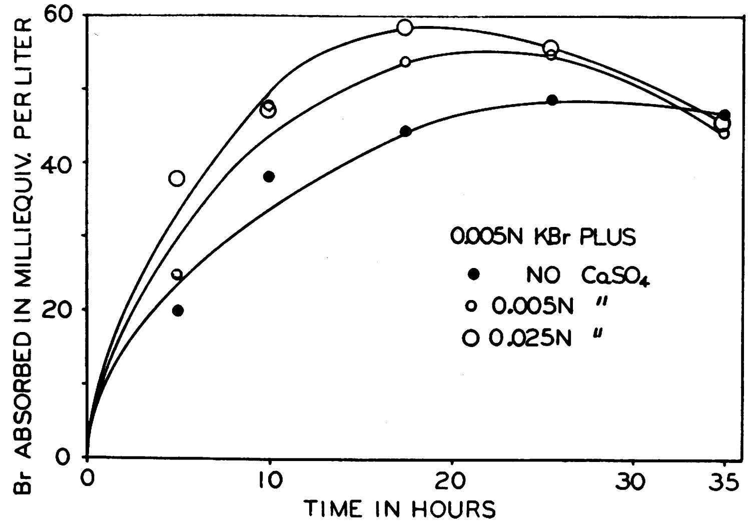

FIG. 7. Bromide absorbed in the expressed sap of barley roots immersed in the solutions indicated.

Additional evidence that Ca may increase the rate of metabolism was obtained in a time study of K and Br absorption from KBr and from KBr plus $CaSO_4$ solutions as shown in figure 7 for Br absorbed and in figure 8 for loss of total sugars. The data for K absorption were quite similar to the data for Br absorption. In figure 7 it is apparent that not only the rate but the total capacity for accumulation by excised roots is increased by the presence of Ca ions in the absorption medium.

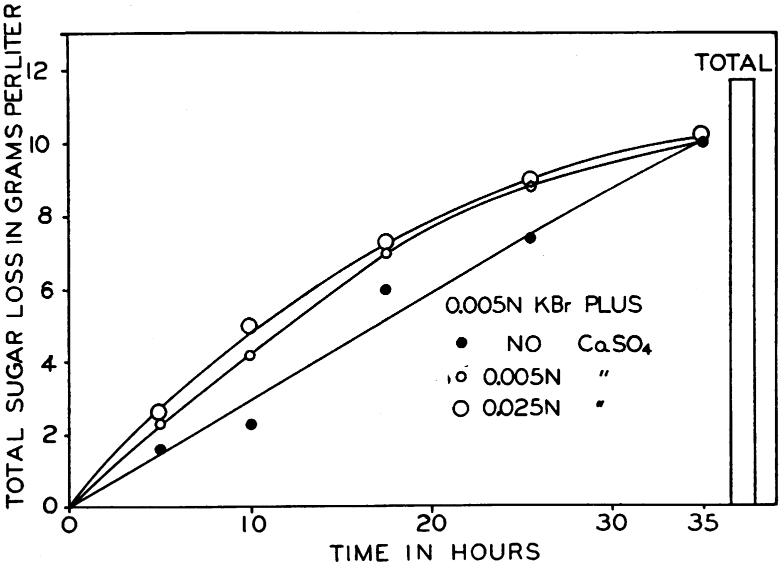

FIG. 8. Sugar loss in the expressed sap from roots immersed in the solutions indicated.

If Ca were acting as a limiting factor in metabolism, and as a result, roots responded to its presence during concurrent absorption of ions, it was conceivable that roots with higher initial Ca content at the time of the experiment would not respond to additional Ca during accumulation. In other words, the Ca effect might merely be a fortuitous result of the way in which the roots were originally grown.

To explore this possibility plants were grown during the preliminary period at three Ca levels. Subsequently the absorption of K and Br into the sap from KBr solutions and from the same solutions supplemented with $CaSO_4$ was measured. The data are shown in figure 9. The roots containing an initial supply of 4.2 m.eq. of Ca per liter were grown in the standard one-half strength HOAGLAND's solution used for the growth of roots in all of the foregoing experiments. The "low Ca" roots were grown concurrently in a solution containing only one-fourth as much Ca. The N as NO_3 associated with the Ca usually used in making the solutions was derived from NH_4NO_3. "High Ca" roots were grown in the standard solution almost saturated with $CaSO_4$. At the end of the growth period this solution contained a concentration of Ca twice that of the expressed sap.

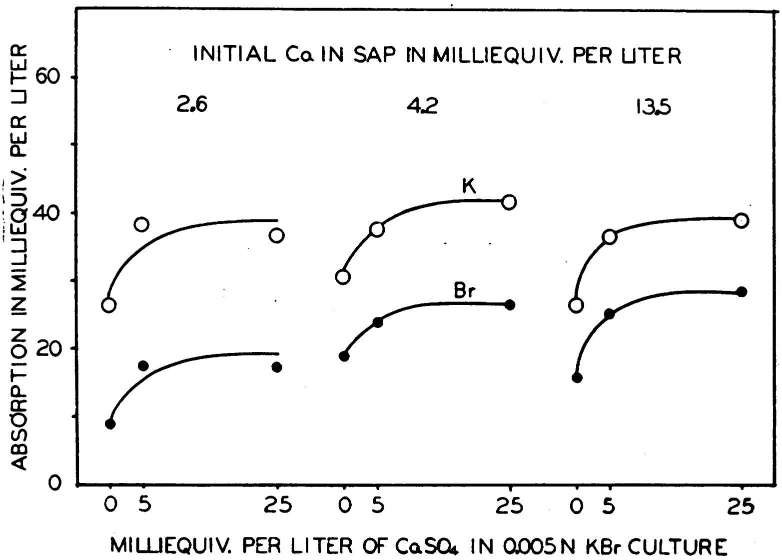

FIG. 9. Absorption of K and Br in the expressed sap of barley roots grown at various Ca levels and then placed in the solutions indicated for 12 hours.

Figure 9 shows that the excised roots with different Ca contents possess capacities of the same magnitude to absorb KBr from the single salt solution, and that the roots grown at all Ca levels respond to the presence of Ca in the solution during concurrent K and Br absorption. The analyses of the ashed press cakes for total K, Ca, and Br added to the calculated results for total K, Ca, and Br in the sap (cc. × m.eq./cc.) showed that the conclusions would have been substantially the same if the experiment had been appraised on the basis of the ashed, unpressed roots.

Another experiment in which the roots were excised and then placed in aerated 0.025 N $CaSO_4$ for 4 hours and then transferred to 0.005 N KBr for periods ranging from 2 to 7½ hours indicated that pretreatment with Ca had no residual effect on subsequent K and Br absorption, as compared with roots kept in aerated distilled water for the 4-hour pretreatment period.

The capacity of roots to absorb salt from KBr was to a large extent independent of the Ca status of the sap, within the limits used here; also, the roots responded almost quantitatively the same to the presence of Ca in the solutions during concurrent absorption of K and Br. These two facts may be evidence that the Ca effect is largely confined to the protoplasmic membrane and is not a direct effect on the oxidative "pump-like" mechanism. This explanation is suggested by the evidence that other ions, some of them toxic and not known to be essential for growth, are capable of performing the same function, at least to a limited extent.

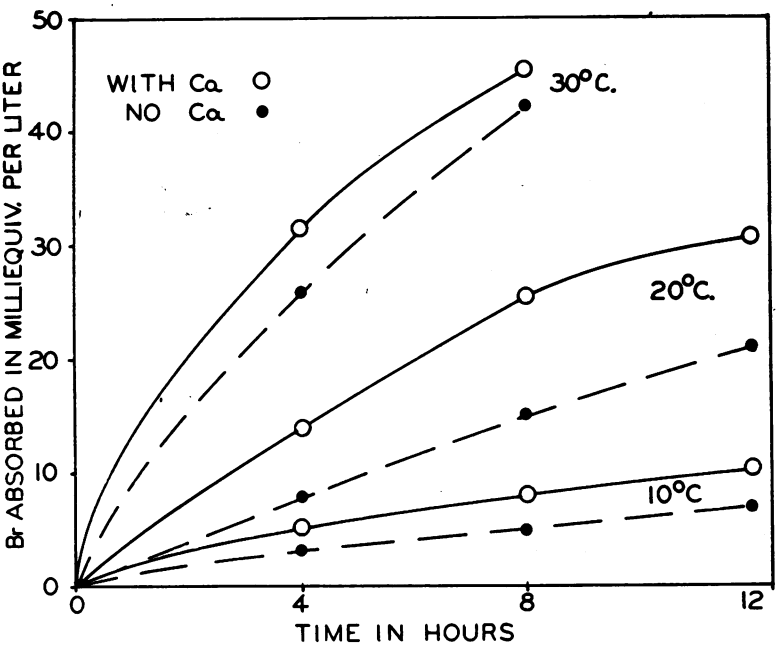

FIG. 10. Bromide absorbed in the expressed sap of excised barley roots immersed in 0.005 N KBr with and without $CaSO_4$ at the controlled temperatures indicated.

RATE AND KIND OF METABOLISM IN RELATION TO THE CA EFFECT

The experiments reported above were conducted at solution temperatures ranging from about 18° to 24° C. It was conceivable that the Ca effect was apparent at only a certain range of temperatures and that at greater extremes the influence of Ca would not be apparent. Figure 10 shows the absorption of Br in the expressed sap of roots kept for various periods in aerated 0.005 N KBr solutions with and without 0.025 N $CaSO_4$ at controlled temperatures of 10°, 20°, and 30° C. These data show that the effect of Ca is apparent throughout a wide range of temperatures. It can be safely predicted from the results of other experiments that as the absorption temperatures approaches 0° C. the effect of Ca would disappear because the absorption of Br from 0.005 N KBr would also become negligible. The results for K absorption likewise showed the same favorable effect of $CaSO_4$ throughout this range of temperatures.

HOAGLAND and BROYER (8) have shown that active aerobic metabolism is essential for the absorption of ions even from high concentrations of KBr. Roots in which aerobic metabolism was arrested by cold, KCN, or N_2 treatment did not absorb K or Br in appreciable quantities. They interpreted this impermeability as possibly existing at the vacuolar membrane. It was therefore of interest to know if roots immersed in high concentrations of KBr with and without $CaSO_4$ would show significant differences in absorption if aerobic metabolism was arrested. Figure 11 shows the Br absorbed by roots subjected to N_2 scrubbed through alkaline pyrogallol to remove traces of oxygen and also by roots immersed in 0.005 N KCN. These results indicate that Ca does not increase the permeability of root systems to high concentrations of bromide if aerobic metabolism is arrested. The scarcely significant effect of Ca in the aerated solutions on bromide absorption may be due to the high KBr concentration used.

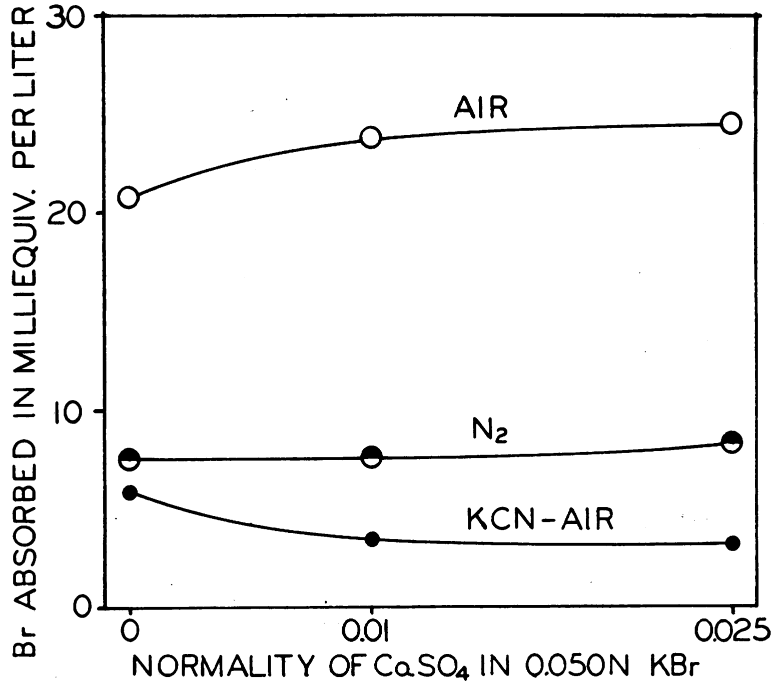

FIG. 11. Bromide absorbed in the expressed sap of roots immersed in the solutions indicated for 10 hours and aerated as noted.

Discussion

Many experiments with excised barley roots have shown that Ca accelerates K and Br accumulation throughout a wide range of concentrations of both the Ca and the KBr. Al, Sr, Mg, and Ba are also able to produce the same effect, but any arrangement in a series of efficiencies depends upon the experimental conditions of time, concentration, etc. Calcium appears to be the outstanding promoter of absorption, since its effect is the greatest and is exerted over the widest range of concentrations.

The effect of polyvalent cations in the presence of KBr apparently increases the rate of respiration, the loss of sugar, and the rate and total capacity for salt accumulation. These effects may be evidence that these cations are modifying the oxidative mechanism providing the energy for accumulation.

On the other hand the wide diversity of cations, some of which are definitely toxic in growth experiments, that are able to produce the effect and that are substitutable one for the other with varying degrees of efficiency indicates that the primary effect may be either on the plasma membrane or on a surface metabolism intimately related to the permeability of the plasma membrane. Supporting evidence for this view is that roots respond to Ca during concurrent K and Br absorption regardless of their initial Ca content. Likewise, pretreatment of the roots with $CaSO_4$ for a brief period has no residual effect on K and Br absorption.

Although polyvalent cations may not effect oxidative metabolism directly, nevertheless, active aerobic metabolism is essential to show their effect. Experiments in which aerobic respiration was arrested show that Ca does not let K and Br diffuse into the roots to equal the high external concentration. The rate of aerobic metabolism, however, may vary within wide limits without affecting the magnitude of the Ca effect to an appreciable extent as shown by the temperature experiments. This dependence of the Ca effect upon aerobic metabolism does not necessarily demand that it is an effect upon the "pump-like" mechanism, but merely that it is one link in the chain of reactions transporting salt from the external medium to the vacuole.

Hoagland and **Broyer** (**8**) have suggested that the vacuolar membrane is essentially impermeable at all times to moderate diffusion gradients and that the metabolic machinery of aerobic metabolism is involved in moving ions across this membrane. The results herein are consistent with this view since Ca was not effective in increasing absorption under anaerobiosis even when K and Br could have moved along diffusion gradients if the system as a whole had been permeable.

One explanation of the data presented here is that polyvalent cations exert their effect directly upon the protoplasmic membrane. This surface effect due either to the presence of the polyvalent cation or to its absorption in the surface might conceivably be an effect on some phase of metabolism concerned with permeability [compare **Blinks**, **Darsie**, and **Skow** (**1**)], as dissociation of membrane components, configuration of protein chains, hydration of colloids, etc. This surface effect would demand that during rapid accumulation the resistance to ion movement offered by the protoplasmic surface is a link in relative minimum in the chain of links involved in the complex mechanism of salt accumulation from single salt solutions. Under anaerobiosis some other link in the chain becomes limiting and no effect of polyvalent cations is apparent.

LUNDEGARDH (**13**) found that mixtures of $CaCl_2$ and KCl gave more negative root potentials than did KCl alone, the decrease in potential being related to the amount of Ca in the solution. He gives as one possible explanation that Ca increases the dissociation of the membrane phosphatides, thereby giving more positions for ionic exchange. Exchange studies with radioactive isotopes in the presence and absence of Ca might confirm or deny this postulate. If Ca does increase the exchange capacity it could conceivably increase the rate of movement across the membrane because of the increase of active area. The difficulty with interpretations based on potentials is that their true relationship to absorption is not known as pointed out by HOAGLAND and STEWARD (**9**).

With the data and knowledge now at hand it is impossible to separate the effect that polyvalent cations may have on oxidative metabolism from their effects on the physical structure and metabolism of the membrane. HOAGLAND and BROYER (**8**) have directed attention to the difficulties of separating metabolism from permeability in salt accumulation by complex tissues like barley roots.

These results with excised roots are not comparable with the absorptions of plants growing in solutions or soils over extended periods of time where the rate of absorption may be modified by the availability of ions to the roots and by translocation and growth in the plant.

Summary

1. A systematic study of K and Br absorption as influenced by polyvalent cations has been made with the excised roots of barley seedlings capable of accumulating large amounts of K and Br in short periods of time.

2. Contrary to the concept of antagonism deduced from experiments conducted usually with high concentrations of salt on material incapable of active aerobic metabolism, these investigations have shown that a variety of polyvalent cations accelerate K and Br absorption, Ca being the most effective. A ratio greater than 30 Ca ions to 1 K ion in the solution was necessary before K absorption could be depressed below the absorption from pure KBr.

3. Ca, Mg, Sr, Ba, and Al ions were found to be effective in increasing the absorption of K and Br, but no regular series of efficiencies could be determined. Mixtures of $CaSO_4$ and $MgSO_4$ produced increases in absorption of the same magnitude as did these salts used alone with KBr. This indicates that the polyvalent cations are performing the same common function with different degrees of efficiency.

4. The influence of Ca on ion absorption in its relationship to the increased respiration and sugar loss is discussed and the difficulty of separating primary and secondary effects is mentioned.

5. Roots grown with different Ca supplies responded similarly to the presence of Ca in the KBr solution during subsequent ion absorption.

6. Absorption of potassium and bromide from relatively high concentrations of KBr could not be accelerated by Ca when the roots were deprived of oxygen or treated with KCN. The effect of Ca on potassium and bromide absorption is apparent over a wide range of temperatures.

7. Two interpretations of the data are discussed and the difficulties of separating metabolism from permeability in discussions of ion accumulation is reemphasized.

The writer wishes to express his appreciation to PROF. D. R. HOAGLAND for his suggestions and criticisms during the course of this research and to MR. T. C. BROYER for his suggestions and encouragement.

DIVISION OF PLANT NUTRITION
UNIVERSITY OF CALIFORNIA

LITERATURE CITED

1. BLINKS, L. R., DARSIE, M. L., JR., and SKOW, R. K. Bioelectric potentials in Halicystis. VII. The effects of low oxygen tension. Jour. Gen. Physiol. **22**: 255–279. 1938.
2. BROOKS, S. C. The accumulation of ions in living cells—a non-equilibrium condition. Protoplasma **8**: 389–412. 1929.
3. BROYER, T. C., and HOAGLAND, D. R. Methods of sap expression from plant tissues with special reference to studies on salt accumulation by excised barley roots. Amer. Jour. Bot. **27**: 501–511. 1940.
4. ————, and OVERSTREET, R. Cation exchange in plant roots in relation to metabolic factors. Amer. Jour. Bot. **27**: 425–430. 1940.
5. HOAGLAND, D. R. The plant as a metabolic unit in the soil-plant system. Essays in geobotany in honor of William Albert Setchell. Univ. of California Press. Pp. 219–245. 1936.
6. ————, and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
7. ————, and ————. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. **27**: 173–185. 1940.
8. ————, and ————. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. **25**: 865–880. 1942.
9. ————, and STEWARD, F. C. Metabolism and salt absorption by plants. Nature **143**: 1031–1032. 1939.
10. ISAACS, T. L., and BROYER, T. C. Application of the liquid extraction method for the determination of total organic acids in plant sap. Plant Physiol. **17**: 296–302. 1942.

11. JENNY, H., and OVERSTREET, R. Cation interchange between plant roots and soil colloids. Soil Sci. **47**: 257–272. 1939.

12. LUNDEGARDH, HENRIK. Die Nahrstoffaufnahme der Pflanze. Gustav Fischer. Jena. 1932.

13. ——————. Investigations as to the absorption and accumulation of inorganic ions. Ann. Agr. Coll. Sweden **8**: 233–404. 1940.

14. MAZIA, D. Binding of ions by the cell surface. Cold Springs Harbor Symp. Quant. Biol. **8**: 195–203. 1940.

15. STILES, W. Permeability. Pp. 212–227. Wheldon and Wesley Co. London. 1924.

16. ULRICH, A. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Amer. Jour. Bot. **28**: 526–537. 1941.

17. VIETS, FRANK G., JR. Effects of Ca and other divalent ions on the accumulation of monovalent ions by barley root cells. Science n.s. **95**: 486–487. 1942.