maxima near 7400 Å undergoes a reaction with another molecule in darkness.

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INHIBITION OF ION ABSORPTION AND RESPIRATION IN BARLEY ROOTS[1,2]

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Since most current theories of ion absorption postulate the existence of metabolically produced ion carriers (18), studies of the effect of respiratory inhibitors on ion absorption have assumed increasing significance. Machlis (13) found that cyanide, azide, malonate and iodoacetate inhibited bromide absorption by barley roots. Lundegårdh (10) inhibited chloride absorption in wheat roots with iodoacetate. He also inhibited chloride and nitrate absorption with fluoride. Lundegårdh (11) and Milthorpe and Robertson (16) inhibited salt absorption with cyanide. Weeks and Robertson (27) found salt absorption in carrot tissue to be sensitive to carbon monoxide and the inhibition to be photoreversible. Robertson et al (20) linked salt absorption in carrots to oxidative phosphorylation by inhibition of absorption with 2,4-dinitrophenol (DNP). The literature cited strongly suggests the involvement of the Krebs cycle in ion absorption.

Most of the studies of the effect of inhibitors on ion absorption have been carried out by investigating the effect on either anion absorption or total salt absorption as measured by the conductivity method (16). Few, if any, studies have been undertaken to investigate the effect of inhibitors on cation and anion absorption simultaneously.

Differences or similarities in the metabolism associated with the absorption of K and Br ions in excised barley roots were examined by studying the effect of various inhibitors on K and Br absorption and on respiration. This was carried out in order to determine how closely glycolytic and Krebs cycle activity could be linked to the two ion absorption mechanisms. The roles of terminal oxidases and of phosphorylation also were studied by the use of inhibitors.

Materials and Methods

All experiments were carried out with excised roots from 6-day-old barley seedlings (variety Atlas 46). Seedlings were grown in a very dilute nutrient solution in the dark as described earlier (8) with the omission of the peroxide treatment. The excised roots were rinsed in distilled water and were centrifuged for 5 minutes at $65 \times g$. Weighed portions were then placed into various solutions.

Except where indicated otherwise, the temperature of growth and experiment was 26° C. The temperature of the bath in which respiration was measured was 25.5° C. All solutions used for absorption studies were aerated by compressed air except as indicated.

For pretreatments, root to solution ratios of 3 to 5 gm/l were used. In KBr solutions, 2 gm/l were used. In Warburg vessels 0.5 gm of root material was placed into 2.5 ml of water.

Potassium salts were used throughout this work, both in pretreatments and in subsequent treatments. After each treatment, roots were rinsed and centrifuged before being weighed out for the next treatment.

At the conclusion of the experiment, the roots

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after each treatment were rinsed in distilled water and either were stored at −18 ° C until lyophillized or they were dried overnight at 60° C.

Potassium was determined in ashed aliquots of the dried and ground root material either by precipitation as cobaltinitrite followed by ceric sulfate titration (7) or by flame spectrophotometry. Bromide was determined as described earlier (7). Respiration, i.e., oxygen uptake, was determined in a standard Warburg assembly as described by Umbreit et al (26) using distilled water. At the conclusion of the absorption experiments, respiration measurements were made for at least 30 minutes on samples of the treated roots.

Sugar was extracted with 80 % ethanol in a soxhlet extractor and total reducing sugar determined by the method of Hassid (6).

The inhibition data are presented as percentage of control. These were determined form the slopes of ion content—time equations obtained in KBr solution following inhibitor pretreatment or obtained in the presence of the inhibitor and KBr where there was no pretreatment. The slopes are based on 6 points in KBr solution in most cases and were determined by the method of least squares. In most of the experiments reported here, pretreatment with inhibitor alone was used to avoid ion competition effects. Since in most of the experiments described here, potassium was absorbed from solutions containing slowly absorbed anions, sulfate was used as a control in order to have the same concentration of K present together with a slowly absorbed anion. In other experiments with this root material, differences in the rate of K absorption due to differing accompanying non-toxic anions were less than 15 %. This included absorption in the presence of rapidly as well as slowly absorbed anions.

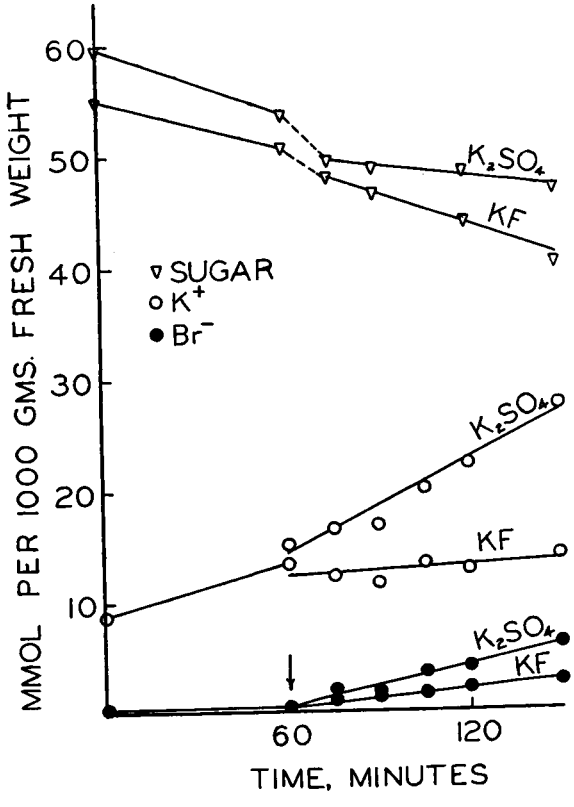

FIG. 1. Effect of 0.05 *N* KF pH 6.3 pretreatment and of 0.05 *N* K_2SO_4 pH 6.0 pretreatment, each followed by 0.005 *N* KBr pH 6.0, on total reducing sugar, K and Br content as function of time. Transfer indicated at arrow.

TABLE I

EFFECT OF INHIBITOR TREATMENT ON ION ABSORPTION AND RESPIRATION OF EXCISED BARLEY ROOTS

INHIBITOR	% OF CONTROL		
	K UPTAKE	BR UPTAKE	O_2 UPTAKE ††
0.02 *N* Fluoride *	53.0	53.1	65.8
0.04 *N* Fluoride *	3.0	40.0	40.5
0.0005 *N* Iodoacetate	63.5	69.8	...
0.001 *N* Iodoacetate	19.0	35.1	76.0
3×10^{-5} M Arsenite *	71.5	67.9	91.5
1×10^{-4} M Arsenite *	39.6	25.3	91.5
2×10^{-4} M Arsenite *	24.8	12.5	78.2
0.0025 *N* Fluoroacetate	49.2	51.6	76.2
0.005 *N* Fluoroacetate	28.3	36.0	69.2
0.01 *N* Fluoroacetate	17.1	40.9	58.8
0.01 M Trans-aconitate	68.0	66.6	...
0.02 M Trans-aconitate	37.2	53.3	60.0
0.005 M Malonate	60.7	58.2	70.0
0.01 M Malonate	8.4	29.7	45.0
0.001 M Cyanide *	63.5	50.5	70.5
0.002 M Cyanide *	26.2	26.0	64.8
90 % CO-10 % O_2 dark **	21.5	48.8	76.0
95 % CO-5 % O_2 dark **	−13.0 ‡	2.5	51.5
95 % CO-5 % O_2 light **	92.0	100.0	71.5
5.4×10^{-7} M Dinitrophenol **	40.5	61.3	...
1.1×10^{-6} M Dinitrophenol **	10.0	32.7	100.0
5.4×10^{-6} M Dinitrophenol **	−22.3 ‡	5.6	...
5×10^{-6} M Arsenate †	67.7	65.7	81.0
2×10^{-4} *N* Arsenate *	26.3	27.4	64.8

* pH 6.0 to 6.5.
** No pretreatment, inhibitor with KBr.
† pH 5.6.
†† Measured in distilled water at conclusion of experiment.
‡ Negative value indicates loss from roots.

RESULTS

FLUORIDE AND IODOACETATE: Inhibition of ion absorption was carried out by means of the glycolytic inhibitors, fluoride and iodoacetate. Roots were pretreated with 0.05 *N* KF or 0.05 *N* K_2SO_4 at pH 6 for 1 hour followed by 0.005 *N* KBr only at pH 6. Figure 1 shows that sugar utilization following fluoride pretreatment is not inhibited. On the other hand, potassium absorption is inhibited 92 % and bromide absorption is inhibited 57 %.

Table I shows the effect of various inhibitors on K absorption, Br absorption and oxygen consumption expressed as percent of control (the smaller the percent of control, the greater the degree of inhibition).

TABLE II

CHANGE IN CONTENT OF POTASSIUM AND BROMIDE OF 0.02 *N* POTASSIUM FLUORIDE TREATED ROOTS AS INFLUENCED BY INTERMEDIATE TREATMENT

INTERMEDIATE TREATMENT	MEQ/KG FRESH WT		
	ABSORPTION IN INTERMEDIATE SOLUTION	ABSORPTION IN 0.005 *N* KBr	
	K	K	Br
Pyruvate	3.9	0.2	5.3
L-Glutamate	7.8	0.6	6.2
Succinate	5.2	4.7	5.1
L-Malate	7.4	...	5.6
L-Glutamate + succinate	5.1	10.7	6.8
L-Glutamate + L-malate	4.6	2.6	6.2
Sulfate	3.7	3.6	7.0

Pretreatment: 0.02 *N* KF, pH 6.3, 2 hrs.
Intermediate Treatment: 0.02 *N* potassium salts, pH 4.5, 2 hrs.
Final Treatment: 0.005 *N* KBr, pH 4.55, 2 hrs.

All of the treatments listed are 2-hour pretreatments at pH 4.5 except as indicated in the footnotes. Following 0.02 *N* KF pretreatment, K absorption and Br absorption are inhibited equally and respiration is inhibited less than absorption. At the higher concentration (0.04 *N*), K absorption is inhibited much more than Br absorption and the inhibition of respiration is about the same as the inhibition of Br absorption.

Since Slater and Bonner (23) showed that fluoride can inhibit succinic dehydrogenase as well as enolase, bypassing a 0.02 *N* fluoride block was tried with pyruvate and other organic acids. Roots were pretreated with 0.02 *N* fluoride at pH 6.3 for 2 hours. The roots were then transferred for an additional 2 hours into solutions of 0.02 *N* potassium salts at pH 4.5 (intermediate treatment). This was followed by 2 hours in 0.005 *N* KBr at pH 4.5. The results given in table II indicate that glutamate and malate were quite effective in supporting K absorption during the intermediate treatment and that glutamate plus succinate supported the greatest K absorption subsequent to its use. The superior effect of sulfate compared to the other treatments with respect to Br absorption indicates further the divergence in behavior to inhibitors between the K and Br absorption mechanisms. In this experiment, glutamate was used in conjunction with other Krebs cycle acids to remove by transamination the oxalacetate which, if accumulated, would inhibit malic and succinic dehydrogenases. This technique was used by Swingle et al (25).

Table I shows the results of iodoacetate pretreatments on absorption and respiration. The results are similar to those with fluoride except that respiration is inhibited less. Experiments designed to overcome iodoacetate inhibition of absorption were carried out as with fluoride and a similar pattern of behavior was obtained.

ARSENITE: Arsenite, which inhibits electron transport from α-keto acids was tested. Table I shows that Br absorption is inhibited more than K absorption at all levels of inhibition. Respiration is only slightly affected.

FLUOROACETATE AND TRANS-ACONITATE: The use of fluoroacetate and trans-aconitate permits the Krebs cycle to be blocked at aconitase as shown by Buffa et al (1) and Saffran and Prado (21) respectively. Massey (14) later reported that trans-aconitate also inhibits fumarase.

The data of table I show that as the concentration of the fluoroacetate increases, the inhibition of K absorption increases more rapidly than the inhibition of Br absorption. Respiration is inhibited less than absorption.

Data obtained in experiments designed to overcome fluoroacetate inhibition using Krebs cycle acids are given in table III. During the intermediate treatment succinate induces the greatest absorption of K, followed by glutamate and fumarate. Citrate is relatively inactive in supporting absorption. Glutamate and malate exhibit some residual effect in the final period. The causes for the low immediate effect of malate and the subsequent fumarate effect as well as the relative ineffectiveness of citrate are not known.

Table I shows that trans-aconitate has an effect on absorption similar to fluoroacetate. An experiment designed to overcome the effects of trans-aconitate inhibition, carried out like that described in table III, produced a pattern similar to that obtained with fluoroacetate inhibition.

MALONATE: Table I demonstrates that 0.005 M malonate pretreatment produces equal inhibition of absorption of K and Br but that 0.01 M pretreatment causes K absorption to be inhibited more than Br absorption. Respiration is also inhibited at both concentrations.

Roots, pretreated with 0.01 M malonate, were subjected to either sulfate or succinate intermediate

TABLE III

CHANGE IN CONTENT OF POTASSIUM AND BROMIDE OF 0.01 *N* POTASSIUM FLUOROACETATE TREATED ROOTS AS INFLUENCED BY INTERMEDIATE TREATMENT

INTERMEDIATE TREATMENT	MEQ/KG FRESH WT		
	ABSORPTION IN INTERMEDIATE SOLUTION	ABSORPTION IN 0.005 *N* KBr	
	K	K	Br
Citrate	1.2	−0.1	3.6
L-Glutamate	4.2	2.3	4.4
Succinate	4.8	1.4	3.4
Fumarate	3.4	−2.0	2.9
L-Malate	0.8	3.3	4.0
Sulfate	1.0	1.4	3.5

Pretreatment: 0.01 *N* potassium fluoroacetate, pH 4.5, 2 hrs.
Intermediate Treatment: 0.02 *N* potassium salts, pH 4.5, 2 hrs.
Final Treatment: 0.005 *N* KBr, pH 4.80, 2 hrs.

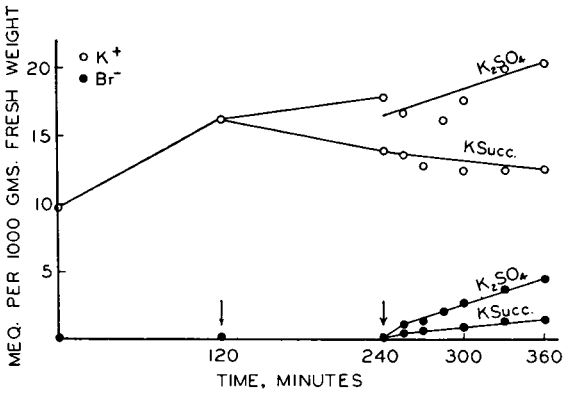

FIG. 2. Effect of 0.04 M K succinate treatment and of 0.03 N K_2SO_4 treatment, each followed by 0.005 N KBr, on K and Br content of 0.01 M K malonate pretreated roots as function of time. Roots removed from malonate at first arrow and placed in KBr at second arrow. All solutions single salt at pH 4.5.

treatment in an attempt to reverse inhibition by competition. Figure 2 shows a time curve of K and Br content in the period following such treatment. With 0.04 M succinate a definite enhancement of inhibition was noted, especially with reference to K absorption. Even in the final KBr solution, the roots lost potassium. Lower concentrations of succinate caused less deleterious effect than the higher concentrations of succinate (17). The results of treatments with other acids and a lower concentration of succinate during the intermediate period are summarized in table IV.

CYANIDE AND CARBON MONOXIDE: Machlis (13) and Lundegårdh (11, 12), among others, showed that potassium cyanide, which inhibits metallic oxidases, inhibited anion absorption while affecting respiration to a much less extent.

TABLE IV

CHANGE IN CONTENT OF POTASSIUM AND BROMIDE OF 0.01 M POTASSIUM MALONATE TREATED ROOTS AS INFLUENCED BY INTERMEDIATE TREATMENT

INTERMEDIATE TREATMENT	MEQ/KG FRESH WT		
	ABSORPTION IN INTERMEDIATE SOLUTION	ABSORPTION IN 0.005 N KBr	
	K	K	Br
Citrate	3.2	3.8	4.7
L-Glutamate	2.3	1.4	2.9
Succinate	2.3	3.0	3.2
Fumarate	5.1	3.4	4.7
L-Malate	6.6	3.7	5.3
Sulfate	4.5	1.7	4.6

Pretreatment: 0.01 M potassium malonate, pH 4.5, 2 hrs.
Intermediate Treatment: 0.02 N potassium salts, pH 4.5, 2 hrs.
Final Treatment: 0.005 N KBr, pH 4.65, 2 hrs.

Roots were pretreated wth cyanide at pH 6 for 2 hours. These pretreatments differed from the previous ones in several respects. Distilled water was aerated continuously in Pyrex bottles for 3 or 4 days prior to the experiment. Potassium cyanide was added and the pH was adjusted just before the roots were placed into the solution. After the roots were in position, the bottles were sealed and mixing was attained by inverting the bottle several times at 10-minute inervals for a 2-hour period. A the end of this time, the roots were treated as previously.

Table I shows that 0.001 M KCN causes a subsequent inhibition of bromide absorption which exceeds that of K absorption. At 0.002 M KCN the inhibitions are equal.

Experiments were carried out with CO in order to have a more specific inhibitor for cytochrome oxidase.

Carbon monoxide-oxygen and nitrogen-oxygen mixtures were prepared just before use by the evacuation technique of Umbreit et al (26). These experiments were not pretreatment types, i.e., the gas mixture was present with the KBr solution used. Portions of the gas-free solutions were displaced from stoppered bottles, after roots had been placed into them, by the desired gas. The sealed bottle was then placed in a shaker either in the dark or between banks of fluorescent lights. The lights, consisting of equal numbers of daylight and blue tubes, provided 400 fc at the outer face of the bottle and 250 fc at the inner face. Respiration measurements were made on roots in distilled water in Warburg vessels in the presence of these gases. For the illumination period of the respiration experiments, a 300-watt tungsten lamp provided 400 fc at the vessels and daylight and blue fluorescent lights provided 70 fc.

The results are given in table I. In the dark, 90 % CO caused K absorption to be inhibited more than Br absorption. With 95 % CO in the dark, K was lost from the roots. In the light, 95 % CO caused practically no inhibition of absorption of either ion.

Respiration data are given in figures 3 A and 3 B and table I. In the dark, 90 % CO caused a partial inhibition of respiration, but no inhibition in the light. In 95 % CO, reversal of respiratory inhibition by light was not complete, i.e., respiration was 72 % of the control.

2,4-DINITROPHENOL AND ARSENATE: The effect of phosphorylation uncoupling on ion absorption was studied by the use of DNP and KH_2AsO_4. Since the low concentration of DNP required for inhibition would rule out ionic competition during absorption, DNP was added at several concentrations to 0.005 N KBr at pH 4.5. The absorption and respiration data are presented in table I. At all concentrations, K absorption was affected more than Br absorption. At 5.4×10^{-6} M, K loss occurred and Br uptake was nil. At 1.1×10^{-6} M DNP, O_2 uptake was unaffected.

Arsenate pretreatments at 5×10^{-6} N at pH 6.1 and 2×10^{-6} N at pH 5.6 were carried out. Table I shows that arsenate inhibits subsequent K absorption

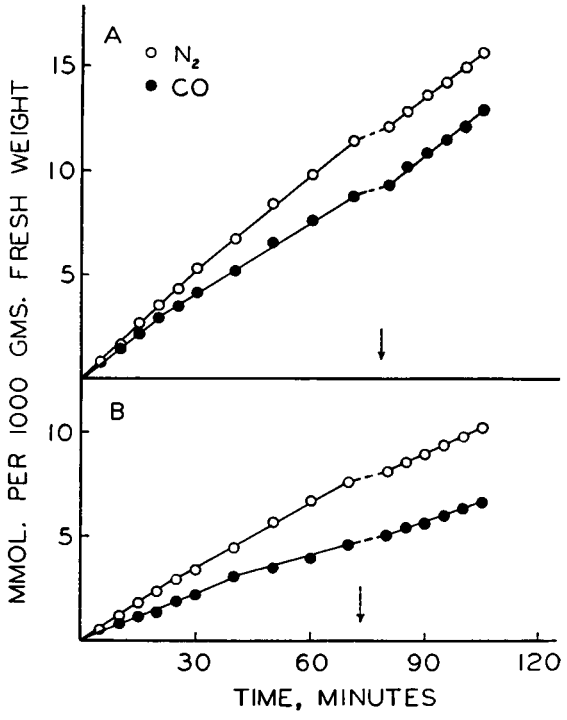

FIG. 3 A. Effect of 90% CO-10% O_2 and of 90% N-10% O_2 on oxygen uptake in distilled water. Light turned on at arrow.

FIG. 3 B. Effect of 95% CO-5% O_2 and of 95% N-5% O_2 on oxygen uptake in distilled water. Light turned on at arrow.

and Br absorption equally. There is also some inhibition of respiration at both concentrations.

DISCUSSION

The inhibition of absorption by substances which inhibit glycolysis and the Krebs cycle, indicates that these pathways supply the intermediates or energy necessary for both potassium and bromide absorption in barley roots.

The fact that respiration is inhibited much less than absorption by iodoacetate, arsenite and fluoroacetate and lesser differentials are produced by fluoride trans-aconitate and malonate treatments, suggests three possible interpretations. There may be parallel sets of qualitatively similar enzyme systems which possess different properties or which occur at different sites in the cell. Secondly, there may be non-glycolytic and non-Krebs cycle pathways of respiration which cannot, however, support ion absorption. This is suggested in part by the observation that sugar metabolism following fluoride treatment appears to be non-glycolytic and non-aerobic in nature. Thirdly, there may be alternate pathways for respiration just over those steps which involve ion absorption. Probably all three alternatives exist.

Coxon (2) postulated multiple loci for enzymes. Millerd (15) showed in plant tissues that particulate sites, i.e., mitochondria, possess complete sets of the Krebs cycle enzymes. It is possible that some of these sites could be involved in ion absorption as well as other cell activities and some could be involved only in the latter.

The differential between inhibition of K absorption and inhibition of Br absorption also suggests a separation yet parallelism between the enzymes associated with the K and Br absorption mechanisms. At levels of inhibition between 50 and 90%, (i.e., 50 to 10% of control) fluoride, iodoacetate, fluoroacetate, transaconitate and malonate exert a greater effect on K absorption than on Br absorption. Arsenite, on the other hand, inhibits Br absorption more.

The relative ineffectiveness of pyruvate in overcoming fluoride inhibition of absorption may be due in part to a low activity of the pyruvate because of the high environmental pH in relation to the pyruvic acid pK of 2.5 (22). It was found that fluoride pretreatment inhibited the absorption as well as the metabolism of radioactive pyruvate (17). The closer proximity of the environmental pH to the pK of the Krebs cycle acids favor their activity and this may explain their greater degree of effectiveness in overcoming fluoride inhibition.

The effect of fluoride on ion absorption, therefore, is attributed primarily to the inhibition of enolase and secondarily to the inhibition of succinic dehydrogenase. The partial effectiveness of Krebs cycle acids in alleviating inhibition of absorption together with the common observation that anaerobiosis does not inhibit glycolysis but does inhibit absorption suggests that enolase is involved in absorption to the extent of controlling the rate of entry of substrates into the Krebs cycle.

Iodoacetate results are interpreted similarly except that the chief enzyme involved is probably triosephosphate dehydrogenase.

The results of the fluoride and iodoacetate inhibitions indicate that some step or steps of the Krebs cycle or closely associated reactions are essential to ion absorption.

The data on overcoming fluoroacetate and malonate inhibitions suggest that the bypassing of blocks at aconitase and succinic dehydrogenase can maintain absorption. The malonate studies indicate that succinate, which is quite effective in overcoming fluoride and fluoroacetate inhibitions and therefore is not toxic when metabolized, probably cannot displace malonate from succinic dehydrogenase and instead exerts a blocking action on other enzymes. The deleterious effect of glutamate may be due to interference with the normal utilization of α-ketoglutarate (19). Machlis (13) also reported that succinate failed to reverse malonate inhibition of respiration and had little effect on inhibited bromide absorption. The effectiveness of malate in overcoming malonate inhibition of absorption in the work reported here is presumably due to malate acting as a substrate for reactions which involve the absorption of ions. This suggests either that only certain series of steps are involved, i.e.,

those steps presumably free to react in common under both conditions of inhibition, or that several alternate sequences of steps can support absorption.

The inhibition of cytochrome oxidase by CO or KCN would result in blocking the Krebs cycle due to the linkage of succinic dehydrogenase to cytochrome oxidase. The observed inhibited respiration in 95 % CO in the light may be due to inhibition of copper enzymes (9) which apparently are not involved in ion uptake. The unequal inhibition of K and Br absorption by CO indicates that although the absorption of both ions is mediated via cytochrome oxidase, separate absorption mechanisms exist as previously indicated. The divergence in behavior between CN and CO on the relative inhibition of K and Br absorption may be due either (1) to the effect of CN not only on cytochrome oxidase but also on cytochrome c and other enzymes and compounds or (2) to the existence of the oxidase in two forms which possess different properties with respect to complexing with carbon monoxide and cyanide. The role of cytochrome oxidase may be primarily to govern the activity of the Krebs cycle rather than to act directly as ion carriers as suggested by Lundegårdh (12).

In addition to the more or less specific requirement for Krebs cycle activity, oxidative phosphorylation may participate in ion absorption as shown by the effect of DNP. Apparently the two absorption mechanisms, i.e., for K and for Br, are differentially sensitive to this substance. Substrate phosphorylation, as indicated by arsenate inhibition, also appears to participate in ion absorption. The lack of differential effect in this case suggests that the two sites are equally sensitive to arsenate or that the reaction affected occurs in common to both mechanisms.

The role of phosphorylation is of some interest since according to Goldacre (5) the major role of metabolism in absorption is to provide adenosinetriphosphate (ATP) which directly governs a postulated ion carrying activity of proteins. According to Lundegårdh (12) oxidative phosphorylation maintains the diffusion barrier in cells. Alternatively, ATP or similar compounds may be involved in ion absorption by, for example, reacting with intermediates of the Krebs cycle to form ion carriers or to destroy them. According to this view metabolism is needed both to provide ATP and to provide intermediates which interact to function as ion carriers. Steward and Street (24) speculated that phosphorylated energy-rich nitrogen compounds function as carriers. These are produced by oxidative metabolism.

If ions are carried by intermediate unstable compounds of the Krebs cycle, or if ATP is utilized during absorption, the act of absorption may stimulate the cycle. This would occur because in a sense the ions would be cofactors in oxidative metabolism. This may be a function of ions which may be otherwise physiologically inactive after being absorbed. There may exist alternate routes of metabolism which cannot support ion transport and perhaps such processes as growth, but can support cell maintenance.

The differing effects on K and Br absorption noted here may display different patterns in other plants or in barley with other ion pairs. The work of Epstein and Hagen (4) on cation competition and the work of Epstein (3) on anion competition during absorption suggest that ions may be bound at similar or different sites depending on the ionic species. If these sites have enzymes which are not identical in properties, differential effects by inhibitors should become apparent for them. On the other hand, ions bound at the same site should show identical behavior to inhibitors, provided similar enzymes are involved.

Summary

Pretreatment with potassium salts of fluoride, iodoacetate, transaconitate, fluoroacetate and malonate, inhibits subsequent K and Br absorption equally at less than 50 % inhibition. Pretreatment with these salts inhibits potassium absorption more than bromide absorption above this level.

Potassium arsenite pretreatment inhibits subsequent Br absorption slightly more than K absorption at all levels of inhibition.

Potassium cyanide pretreatment inhibits subsequent Br absorption more than K absorption at low levels of inhibition (more than 50 % of control) but inhibits both equally at 75 % (25 % of control).

When 2,4-DNP is added concomitantly with KBr, K absorption is inhibited more than Br absorption. Potassium arsenate pretreatment inhibits K and Br absorption equally at several levels.

Carbon monoxide (90 %) inhibits concomitant K absorption more than Br absorption in the dark. This inhibition of both K and Br absorption is almost completely reversed by light.

In all cases of inhibition except 95 % CO in the light, respiration was depressed less than ion absorption.

Various Krebs cycle acids can overcome fluoride and fluoroacetate inhibition. Malonate inhibition is enhanced by succinate but overcome by malate.

It is postulated that separate but parallel mechanisms exist for the absorption of K and Br ions.

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THE EFFECT OF DIFFERENT IRON AND MANGANESE NUTRIENT LEVELS ON THE CATALASE AND CYTOCHROME OXIDASE ACTIVITIES OF GREEN AND ALBINO SUNFLOWER LEAF TISSUES[1]

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From a physiological standpoint, it is difficult to assess separately the role of iron and manganese in plant nutrition because these elements have been shown to be mutually antagonistic. Shive et al (16, 20, 21) and Twyman (25, 26) have reported that a high nutrient level of manganese has a depressing effect on the absorption of iron from nutrient solutions and upon the maintenance of a high level of water-soluble iron in plant tissues, inducing symptoms of iron deficiency chlorosis. High nutrient levels of iron were likewise shown to have a depressing effect on the absorption of manganese from nutrient solutions and upon the maintenance of a high level of water-soluble manganese in plant tissues, although to a much lesser degree than the effect of manganese on iron. Oulette (14) found that the severity of manganese toxicity symptoms in soybeans decreased as the nutrient level of iron was increased in the substrate. Other instances of manganese-induced iron deficiency have been reported by Sideris and Young (18), Sideris (17) and Hewitt (8, 9, 10, 11) for several plants.

Sideris and Young (18) and Twyman (26) have suggested that manganese-induced iron deficiency in plants is a result of substitution of manganese for two hydrogen atoms or for iron in porphyrin molecules. In vitro substitution of iron by manganese in purified horseradish peroxidase with resulting feeble peroxidatic activity has been reported by Gjessing and Sum-

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