Effects of Calcium, Indoleacetic Acid, & Distance From Stem Apex on Potassium & Rubidium Absorption by Excised Segments of Etiolated Pea Epicotyl [1, 2]

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The enhancing effect of Ca on K uptake by excised roots has been known for some time (25) and has been referred to as the Viets effect; however, whether or not such an enhancement occurs in stem tissue has not been established. Indoleacetic acid (IAA) may enhance Rb uptake by stem segments (11) and this effect in Jerusalem artichoke tuber tissue has been attributed to an increase in capacity of the tissue for cation exchange (8) rather than accumulation. The fact that each, Ca and IAA, may enhance cation absorption suggests that both may play some important role in cellular ion exchange or ion transport. One obvious possibility is that they separately or together may be involved in the formation, or activity, of an ion carrier; however, other explanations are possible (7) and the present study was done in an attempt to ascertain whether or not Ca would enhance K accumulation by pea stem segments and if so, what the effect of auxin is on this system.

Materials & Methods

The tissues used in this study were excised segments of epicotyls of the third internode of 7-day etiolated pea seedlings, *Pisum sativum* L. var. Alaska. The seedlings were grown in complete darkness in a moist incubator at 25 C. They were grown in a well drained flat of vermiculite moistened frequently with half-strength Hoagland's nutrient solution (12).

The stem tissues were cut into 0.5, 1, or 2 cm lengths as indicated for each experiment; only the upper 2 cm portion was used unless otherwise noted.

Potassium and rubidium uptake was measured by use of Rb^{86} as a tracer. The tissue samples were wet ashed by adding concentrated nitric acid and gently heating to dryness twice or more; the samples were then counted using a thin-window Geiger tube. Under the conditions of these experiments Rb^{86} appears to serve as a suitable label for K and the estimates of K uptake are based on this technique. However, in certain tests not reported here K^{42} was used to measure K uptake. The results support the idea that Rb^{86} influx rates closely approach those for K (15).

The solutions used in the experiments reported here were unbuffered, and the pH was not adjusted during the treatment period. However, the initial pH was adjusted on concentrated stock solutions by additions of KOH or HCl as required to give an initial pH value uniform between treatments. Initial pH values used ranged from 4.8 to 5.8 in separate tests. Preliminary experiments on solution pH changes of KCl solutions with initial values adjusted to give a range of 4.6 to 6.8 showed a rise from lower, and a reduction from higher pH values; the net result in one such test was a final pH of 6.1 to 6.4 with no significant difference in Rb-labeled uptake; similar results have been found by others (23). Other tests on pH change alone showed that the final pH was quite uniform after 48 hours at 1 mM/l and 10 mM/l KCl; the higher concentration gave a lower pH (5.0–5.2). A similar test with $CaCl_2$ (10 mM/l) plus KCl (1 mM/l) yielded the same result (initial pH 3.95–8.60; final pH 4.60–4.90). It seemed apparent that under the conditions of the experiments reported here the tissues tend to regulate the pH within certain limits. Since pH was not continuously controlled or monitored, the H ion cannot be completely eliminated as a variable. However, the writers have satisfied themselves in subsequent experiments using buffered solutions that the salient conclusions of this report hold true also in buffer systems.

In view of the nature of the cation uptake process (1, 17, 18) both exchange (apparent free space) and accumulation were measured in most experiments. The procedure was as follows. The freshly excised segments—of the size and distance from the apex as required by the test—were placed in distilled water for 1 to 2 hours to wash off the cut surfaces and to equilibrate in water content. The segments were then blotted and weighed in samples of suitable size—usually 20 segments per sample, but in some cases 30

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or 40—and then placed in the experimental solution in an Erlenmeyer flask. The volume of the solution was 25 ml for experimental periods of 4 hours or less but 50 ml (or more) for longer periods. During the experimental period the flasks were kept on a shaker and oscillated at a rate of about 100 cycles per minute. At the end of the experimental period the tissues were rapidly removed by dumping the flask contents into a sieve. The segments were given about 30 seconds' rinsing with a fine jet of distilled water to remove solution on the surface and were then blotted lightly and dumped into the exchange solution at ca. 2 C for 1 hour. The exchange solution was, unless stated otherwise, of the same solute content as the experimental solution except that it contained no radioactive label. The tissue sample was then removed, blotted, weighed, and wet-ashed by heating to dryness twice or more in concentrated nitric acid prior to counting; this value is reported as ion accumulation. The exchange solution was evaporated to dryness for counting; this assay is reported as ion exchange.

The exchange as measured in this study is believed to correspond approximately with the value frequently referred to as apparent free space, AFS (1, 17) or wall space (18).

Assays of total K and Ca were made with a Coleman flame photometer; the standard solutions contained equivalent amounts of K, Na, and Ca as the Cl salts.

Results

► Time Course of Rb Uptake: Freshly excised segments of etiolated pea epicotyl normally show a capacity for growth and for salt absorption. The time course of both growth and Rb uptake of 2 cm segments is shown in figure 1 for a 24-hour period in RbCl solution (5 meq/l). During this period Rb uptake approaches—but does not attain—a constant influx rate. However, as could be expected, during the more rapid growth phase there is a relatively rapid uptake of Rb from the RbCl solution.

► Relationship of Rb & K Uptake & Exchange: Several experiments were conducted to test the validity of using Rb^{86} as a tracer for K uptake and to measure the time course of exchange. In figure 2 may be seen the accumulation and exchange over a range of concentration of RbCl and KCl solutions each of which was labeled with Rb^{86}. Assay of the tissue samples of each series shows no significant difference between Rb^{86} uptake in either KCl or RbCl solutions; this in turn strongly suggests identical uptake and exchange rates for Rb and K by pea epicotyl segments. (Experiments not reported here using double labeling with K-42 and Rb-86 confirm the idea that within the limits of the experiments here Rb^{86} is a suitable tracer for K.)

► Measurement of Readily Exchangeable Fraction of K Uptake: The technique for distinguishing between accumulation and exchange used in this study is based on the fact that cation uptake consists of at least two time phases. There is an initial rapid uptake phase, phase 1, followed by a slower relatively steady uptake stage. Since phase 1 uptake apparently reaches an equilibrium relatively quickly, the tissues which have been in a labeled experimental solution when transferred to a similar unlabeled solution quickly lose the readily exchangeable tracer. The time course of this loss by exchange is shown in figure 3 for pea tissue, samples of which had been exposed previously to each of two concentrations of KCl and then transferred to identical unlabeled solutions for exchange at two temperatures. It is readily apparent that there is a very rapid initial exchange essentially completed within a half hour. Following this there is a steady slow loss phase over the remainder of the 4-hour period. If phase 2 uptake rate is constant from zero time, extrapolation of the slope back to zero time should give a value close to the phase 1 equilibrium amount of the ion (10); however, for present purposes this correction seems unnecessary and has not been made in the data to follow.

► Effect of Calcium in Short-Term Tests on Potassium Accumulation, Loss to Water, & Exchange: In the case of corn roots Ca enhances K uptake within 3 hours whereas with soybean roots K uptake was depressed (15). The pea epicotyl segments studied here behaved like the soybean roots in short-term experiments. However, with longer experiments, as will be noted below, pea segments gave the Viets effect.

The influence of Ca on various K uptake fractions is shown in table I. In this experiment a range of KCl concentrations labeled with Rb^{86} was used as shown in table I. In addition, following the 4-hour uptake period, the segments were blotted, weighed, and immersed in H_2O for 1 hour at 2 to 4 C. Following this the segments were transferred for 1 hour to an unlabeled solution of the same constitution as that in which uptake occurred. Ca, at 10 meq/l greatly depressed each of the fractions of uptake; the effect on total K uptake and the accumulation is also shown graphically in figure 4 for the first (upper) centimeter segment of pea epicotyl. Calcium depressed the K accumulation fraction and the loss by exchange to KCl solutions. In part these effects may be seen graphically in figure 4.

The fraction of K uptake which is rapidly removeable from the tissue may readily be shown to consist of two components: one which is lost rapidly to water and a second which is retained in water but may be displaced by exchange with an external cation. The effect of Ca on these fractions is shown in table II, the figures of which were calculated from the data shown in table I. It is apparent that Ca not only depressed total uptake of K but it also slightly increased loss to water when calculated on the basis of the percentage of total K uptake.

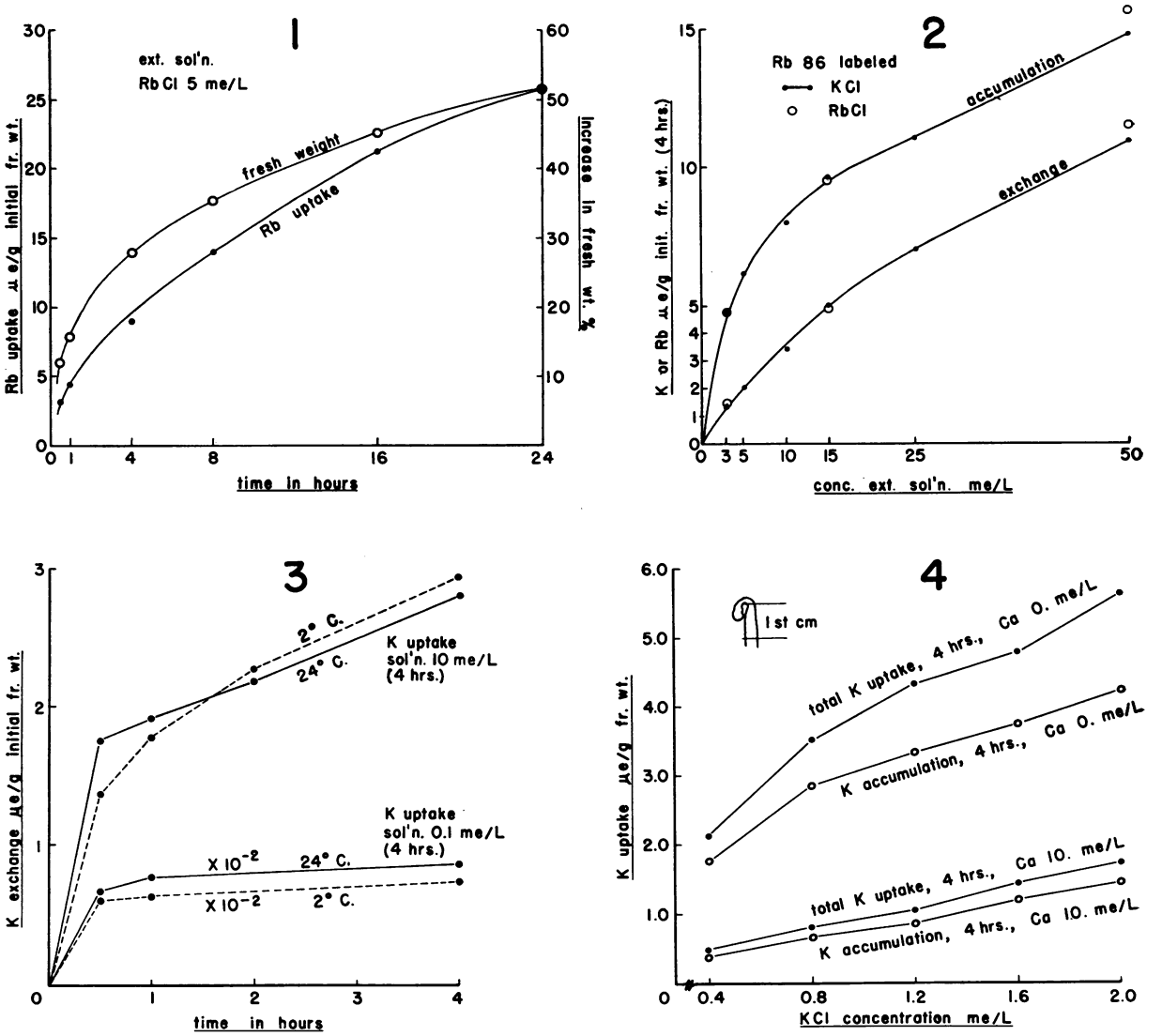


Fig. 1. The time course of Rb^{86} uptake and of increase in fresh weight of excised pea segments in 5 meq/l RbCl solution.

Fig. 2. The relationship of accumulation and exchange of both KCl and RbCl as measured by Rb^{86} tracer.

Fig. 3. The time course of exchange at 2 C and 24 C of segments pretreated in one lot for 4 hours in 0.1 meq/l labeled KCl and in a second lot for 4 hours in 10 meq/l labeled KCl. The exchange solution concentration was unlabeled and of the same concentration as the uptake solution.

Fig. 4. The accumulation and exchange of K by 1st cm segments (*uppermost*) in the absence and presence of Ca.

In table II it is shown that a fraction of the K taken up by the tissue is readily lost to water; this fraction which is not bound and is readily diffusible presumably corresponds to the water free space, WFS. The fraction not readily lost to water may be presumed to occupy sites on non-mobile anions which release cations by exchange; thus, this fraction corresponds to the Donnan free space, DFS (1, 17), at least qualitatively. From the data in table II it is suggested that the presence of Ca in the uptake solution resulted in a slight increase in the percentage of K subsequently lost to water. In contrast with this the presence of Ca in the subsequent exchange solution resulted in the loss of a smaller percentage of the K taken up. These values—6 % in the 1st (upper) cm segments and 5 % in the 2nd cm segments—suggest that the presence of Ca in the external exchange solution prevents some loss of K relative to the absence of Ca, e.g., as in water or the 0 Ca series.

Table I

Influence of 10 meq/l $CaCl_2$ on K Accumulation, Loss in Water, Exchange & Total Uptake at Various KCl Concentrations

Treatments	K in Microequivalents per g fr wt			
	Accumulation 4 hr	Loss in H_2O 1 hr	Loss in KCl solution 1 hr	Total uptake
Upper cm *segments. No calcium.*				
KCl meq/l				
0.4	1.77	0.181	0.179	2.130
0.8	2.85	0.321	0.346	2.517
1.2	3.36	0.492	0.479	4.322
1.6	3.74	0.489	0.567	4.796
2.0	4.23	0.591	0.799	5.620
$CaCl_2$, 10 meq/l. KCl meq/l.				
0.4	0.37	0.075	0.045	0.490
0.8	0.69	0.094	0.034	0.818
1.2	0.87	0.141	0.054	1.065
1.6	1.20	0.190	0.087	1.477
2.0	1.43	0.245	0.084	1.759
2nd cm *Segments. No calcium.*				
KCl meq/l				
0.4	1.30	0.319	0.196	1.815
0.8	2.23	0.433	0.420	3.083
1.2	2.55	0.527	0.610	3.687
1.6	2.74	0.641	0.865	4.246
2.0	3.22	0.744	1.000	4.964
$CaCl_2$, 10 meq/l. KCl meq/l.				
0.4	0.41	0.079	0.037	0.526
0.8	0.65	0.169	0.043	0.862
1.2	0.81	0.224	0.052	1.086
1.6	1.06	0.321	0.078	1.459
2.0	1.22	0.292	0.079	1.591

Uptake period 4 hours, H_2O treatment 1 hour, exchange period 1 hour.

With respect to the age of tissue (tables I & II) the uppermost and 2nd cm segments show differences. Uptake by the upper segment characteristically exceeds that for the 2nd cm. For this reason, further study of the gradient along the stem was made with respect to K uptake, effect of auxin on K uptake, and total K and Ca content.

Table II

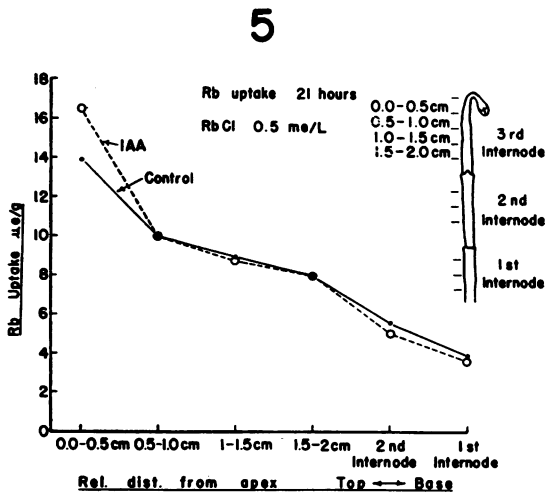
Loss of K From Segments With 1 Hour Immersions in H_2O Followed by 1 Hour Immersion in KCl Solutions*

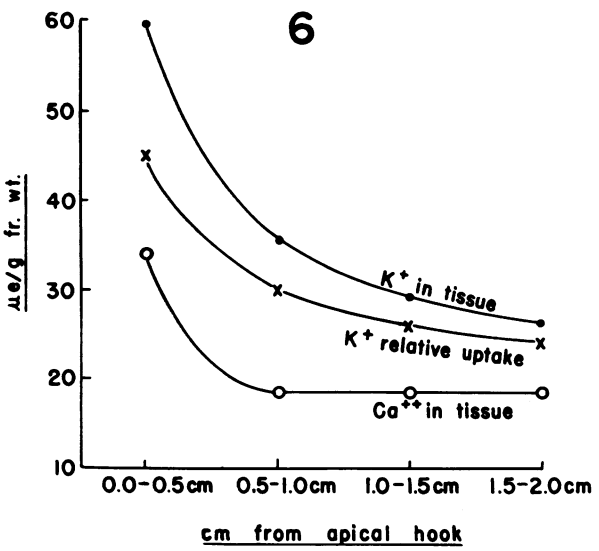
Treatments	% of Total K uptake	
	Mean loss to H_2O	Mean loss in KCl solution
1*st* cm *Segments*		
Ca 0	10	11
Ca 10	13	6
2*nd* cm *Segments*		
Ca 0	15	16
Ca 10	19	5

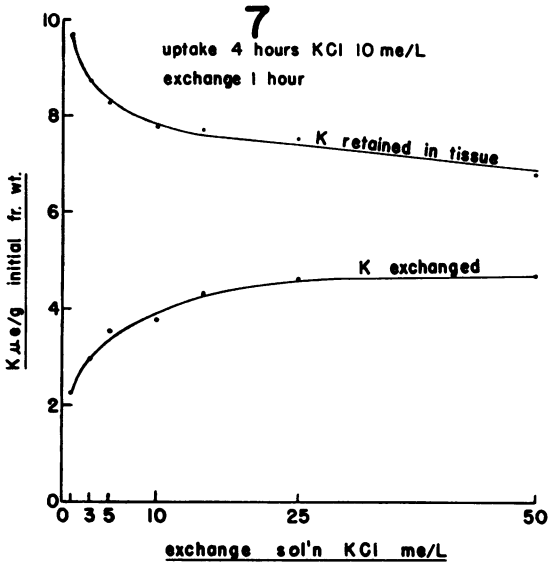
* The figures are averages of data from the various KCl concentrations as given in table I.

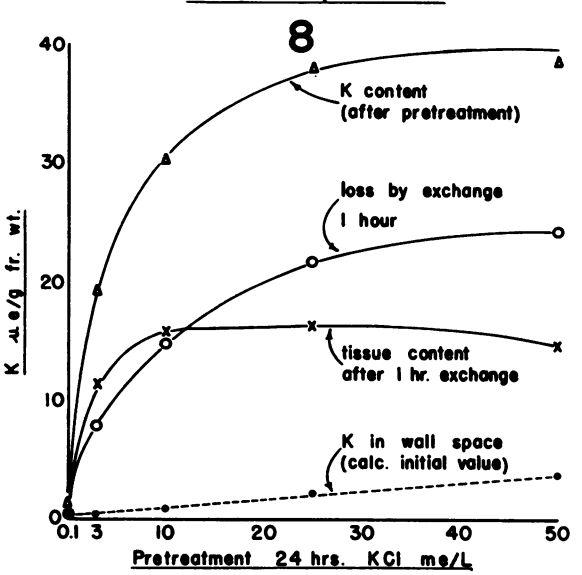
► Relationship of Tissue Age to K Uptake, Effect of Auxin, & K & Ca Content of Tissue: The fact that etiolated pea epicotyl shows a gradient along its axis with respect to capacity to growth response of segments has previously been reported (20). Experiments in this study within the limits of similarity of the tests are in agreement. Segments of 0.5 cm length excised from the third internode and from the second and first internodes had differing capacities for growth, Rb uptake, and response to auxin. The relationship of Rb uptake (as measured by Rb^{86}-labeled RbCl solution) to position along the axis is shown in figure 5; this figure also shows that IAA (1 ppm) chiefly affects the upper 0.5 cm segment; the second 0.5 cm segments were not influenced by IAA in Rb uptake rate. From the uppermost segment downward there was a decrease in rate of Rb absorption. The growth curve (not shown) was similar excepting that increases in fresh weight induced by IAA were noted in both the upper segments.

From the existence of the gradient of Rb uptake capacity it could be suggested that the initial total K









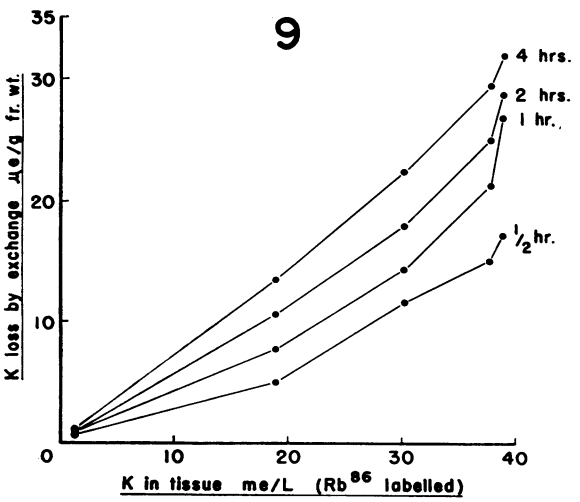


Fig. 5. The uptake of Rb by excised segments as related to distance from the apical hook. Rb uptake is expressed as amount of labeled external solute taken up per gram initial fresh weight.

Fig. 6. The relationship of K and Ca content per gram fresh weight and of labeled K uptake by segments cut different distances from the epicotyl apical hook.

Fig. 7. The influence of external KCl concentration on exchange (loss of Rb^{86} labeled K) for 1 hour.

Fig. 8. Loss by exchange of K as influenced by internal concentration. The segments were pretreated for 24 hours in a series of labeled KCl solutions (as shown on the ordinate), then allowed to exchange in unlabeled KCl solution at 10 meq/l. The curve showing K in the wall space was estimated as the amount in 10 % of the tissue weight.

Fig. 9. Loss at various time intervals by exchange as related to internal labeled K content. Data from the same experiment as figure 8.

content of the segments might differ and that the differences in Rb uptake rate might be rather directly related to internal K concentration. Assay of the upper four 0.5 cm segments showed this to be the case (fig 6). In addition, Ca assays showed a similar distribution of this cation (fig 6).

The above data appear to be in accord with the concept that a Donnan system may be in operation in the pea stem segments. In such a system most Rb or K would be retained against loss to water but would be exchanged for ions of similar charge. Accordingly, two experiments were designed to test this relationship: one in which the internal K content of several tissue samples was equal and labeled with $Rb^{86}Cl$ solution (10 meq/l) for a 4-hour pretreatment, then allowed to exchange for 1 hour in a range of external KCl concentrations (fig 7); in the second experiment tissue samples were pretreated 24 hours in a range of labeled KCl concentrations, then allowed to exchange ½, 1, 2, and 4 hours in 10 meq/l KCl solution (fig 8).

When the internal K concentration was high relative to the external concentration, relatively little loss from the tissue occurred (fig 7). However, at high external concentration, e.g., 50 meq/l which about equals internal concentration (fig 8), much larger amounts of exchange were noted. It is quite apparent that high concentration gradients of K have much less effect on exchange than the total amounts of labeled cation available for exchange. This was true also in the case in which the amount of internal labeling was caused to vary between tissue samples (fig 8) and the external concentration for exchange was constant at 10 meq/l. Greater amounts of labeled K in the tissue were related to greater amounts of exchange. The amount of labeled K remaining in the tissue after exchange was the accumulated fraction; this was reasonably constant over the range of 10 to 50 meq/l KCl in the pretreatment solutions. The latter result appears to be in accord with the concept of a carrier which became saturated at 10 meq/l such that higher external (pretreatment) concentrations failed to give increased accumulations. On the other hand, some experiments have shown higher accumulation values in this range (e.g., see fig 2); thus, another possible explanation is that KCl in the absence of other ions may increase permeability and decrease retention capacity. This idea is supported by the additional data on the relationship of K exchange to the tissue K content (as shown by Rb^{86} labeling) at the several times of exchange (fig 9). Here at higher concentrations K exchange is greater than would be expected if the relationship were simply proportional.

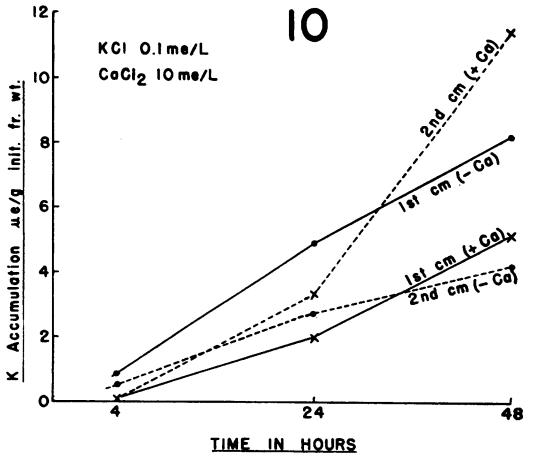
► Effect of Ca on K Uptake in 24 to 48 Hour Periods of Treatment: Since the Viets effect was not shown in short (4 hr) periods, longer experiments were tried. In view of the axial gradient, experiments were performed with tissue samples in which the uppermost cm segments and second cm segments were kept in separate series. The influence of Ca on K accumulation is shown in figure 10. Both kinds of segments show an early depressing effect of Ca on K accumulation, but by the end of 48 hours the second cm segments show enhanced K absorption. In the first cm segments Ca depressed K accumulation throughout the treatment period. Thus, in the response of stem segments to Ca-enhanced K accumulation, there is a distinct effect of differentiation or age.

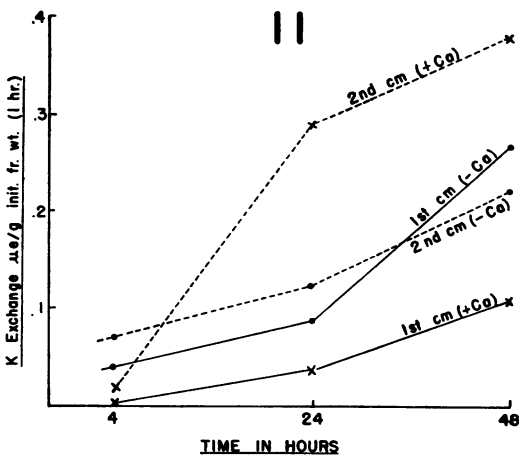
The K exchange fraction curves (fig 11) in general resemble those for accumulation.

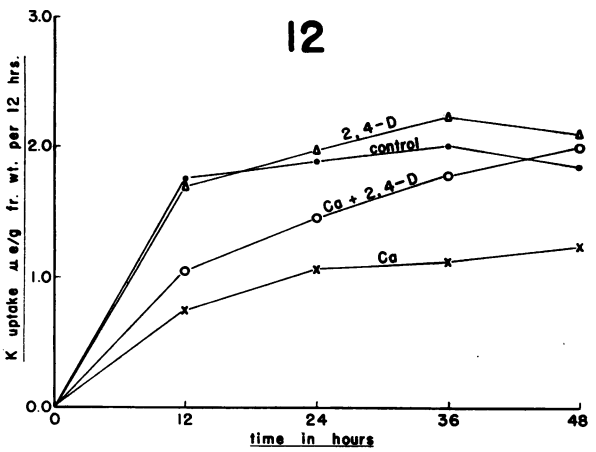
► Influence of Auxins & Ca on K Uptake: The fact that auxin may enhance K uptake has been reported previously (3, 11). Thus, it was of interest to ascertain whether or not the Ca and auxin effects are additive. The influence of each alone and combined was tested on both first and second cm segments. Since IAA is destroyed by pea tissue, for these tests 2,4-dichlorophenoxy acetic acid (2,4-D) was used. As expected, in first cm segments Ca-depressed and 2,4-D-enhanced K uptake rates (fig 12) and total (cumulative) uptake (fig 13). However, the combination of 2,4-D and Ca gave steadily increased rates of K uptake after the first 12 hours, and at the end of 48 hours the rate exceeded that of the controls (fig 12). This suggests that one effect of auxin here on these rapidly growing segments is to hasten a differentiation process which then allows the tissue to show the Viets effect. The Ca + 2,4-D treatment in these segments invariably showed a marked enhancement of K uptake over that of Ca alone.

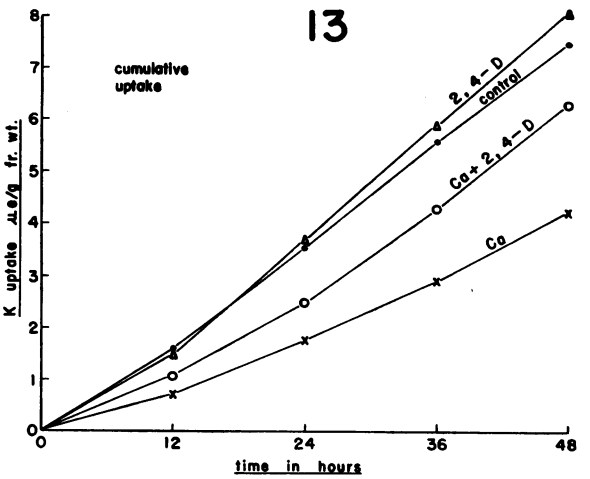
In contrast with the response of upper 1st cm segments the 2nd cm segments showed a marked increase in K uptake rate in the presence of Ca alone (without 2,4-D) (fig 14). As in the 1st cm pieces this effect was not immediate but, rather, required 24 to 48 hours to develop. The final cumulative uptake of K in the presence of Ca did significantly exceed that of the controls, and in three such experiments the accumulation values (total cumulative K uptake less the K exchange fraction) exceeded those of each of the other treatments (table III). In these lower segments 2,4-D appeared to have little or no effect in the absence of Ca, but in the presence of Ca it depressed K uptake relative to Ca alone (i.e. minus 2,4-D). [The apparent enhancement of K uptake by 2,4-D shown in figure 15 in this case was largely attributable to the exchange fraction rather than accumulation (see table III).]

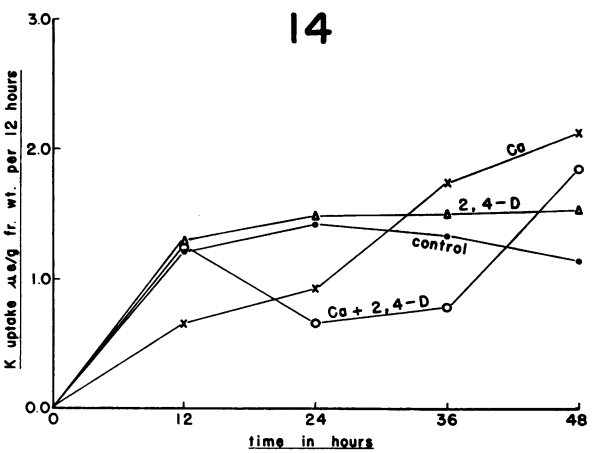
Analysis of the data of final tissue exchange and accumulation from three 48-hour experiments shows somewhat more clearly the relationship of Ca and 2,4-D effects on growth and ion uptake (table III). In the upper cm segments Ca depressed growth and both K exchange and accumulation; the latter effect might be expected if there is a non-specific component of K absorption, and if Ca were acting as an interfering (or competing) ion. Auxin stimulated growth but failed to stimulate ion uptake relative to the control; however, it did overcome the interfering effect











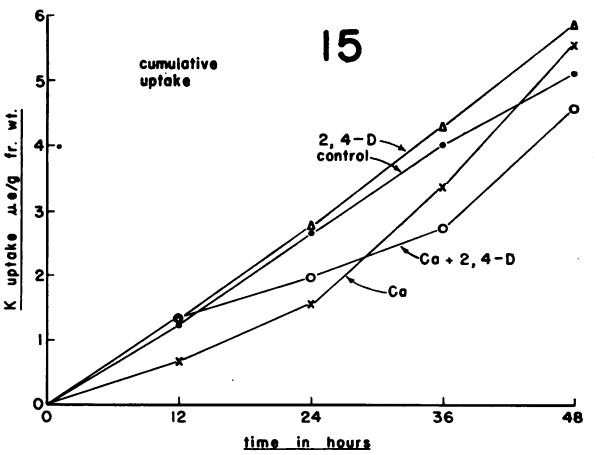


Fig. 10. The time course of K accumulation by 1st (*upper*) cm segments and 2nd cm segments as influenced by Ca.

Fig. 11. Exchange of K in the foregoing experiment.

Fig. 12. The uptake (accumulation plus exchange) of K in 12-hour intervals by 1st cm segments as influenced by Ca and 2,4-D, KCl 0.1 meq/l, $CaCl_2$ 10 meq/l, 2,4-D 1 ppm.

Fig. 13. Cumulative uptake of the foregoing test.

Fig. 14. The uptake of K in 12-hour intervals by 2nd cm segments. (Otherwise as in fig 12).

Fig. 15. Cumulative uptake by 2nd cm segments of foregoing test.

Table III

Influence of Ca & 2,4-D on Fresh Weight K Exchange & K Accumulation*

	Fr. wt.		K Exchange			K Accumulation		
	mg		μeq	μeq/g	μeq/g	μeq	μeq/g	μeq/g
	Initial	Final		Initial fr. wt.	Final fr. wt.		Initial fr. wt.	Final fr. wt.
1st cm								
Control	672	1049	0.133	0.199	0.128	4.24	5.99	4.07
Ca	668	918	0.037	0.062	0.044	3.00	4.23	3.44
2,4-D	640	1300	0.159	0.256	0.133	4.14	5.92	3.53
Ca + 2,4-D	667	1174	0.062	0.103	0.059	4.01	5.67	3.67
2nd cm								
Control	975	1086	0.228	0.243	0.220	3.62	3.53	3.34
Ca	963	1016	0.085	0.109	0.104	5.23	5.09	5.07
2,4-D	956	1292	0.240	0.270	0.203	3.92	3.87	3.04
Ca + 2,4-D	954	1102	0.062	0.073	0.063	3.85	3.78	3.43

* Means of three experiments, each of 48 hours duration. Ca concentration 10 meq/l, K 0.1 meq/l, 2,4-D 1 ppm.

of Ca on K uptake in absolute amounts and in μeq per unit initial fresh weight. On the basis of final fresh weight the controls exceeded each of the three treatments; on the other hand 2,4-D and Ca plus 2,4-D treatments showed enhanced growth and also allowed ion uptake to almost keep pace with this growth. It can be suggested from the data that any auxin stimulation of K uptake is dependent on the Ca status of the tissue as well as its capacity to grow.

The 2nd cm segments showed (table III) a striking enhancing effect of Ca on K uptake; this was true of absolute amounts and also of the amount of K per unit initial or final fresh weight. In these segments 2,4-D, if anything, depressed K uptake relative to the Ca alone treatment.

The effect of Ca on the K exchange fraction is also shown in table III. It is clear in these tests, as in previous ones, that the exchange fraction is not purely non-specific and a function of the external solution; in this case a ratio of 100/1 eq of Ca to K should have lowered the K fraction much more than was the case. In the upper cm 2,4-D had relatively little effect (as a percentage of total uptake) except perhaps in conjunction with Ca in which case the exchange was increased somewhat. It is clear, however, that the exchange fraction was too small to account for the 2,4-D-induced ion uptake in the presence of Ca. In the 2nd cm pieces 2,4-D showed a reverse effect, since the 2,4-D plus Ca treatment gave a lower exchange than Ca alone; this exchange, however, seems to be directly related to the higher accumulation value; the same was true in the Ca and Ca plus 2,4-D treatments of the 1st cm pieces.

► Pretreatment in Ca & 2,4-D: Several tests were performed to see whether or not pretreating segments in Ca, 2,4-D, or a combination of the two would influence the subsequent performance of the tissue with respect to K uptake. Such pretreatments, although having some influence, failed to replace the effect of the presence of Ca or 2,4-D directly in the medium during the K uptake period.

► Relationship of Ca Concentration to K Uptake: Several experiments were performed to ascertain the relationship of Ca concentration at a given level of K on K uptake. Some of the tests were conducted during the summer when it was discovered that at higher temperatures (27–32 C) the excised segments showed less growth and lower K uptake than at the

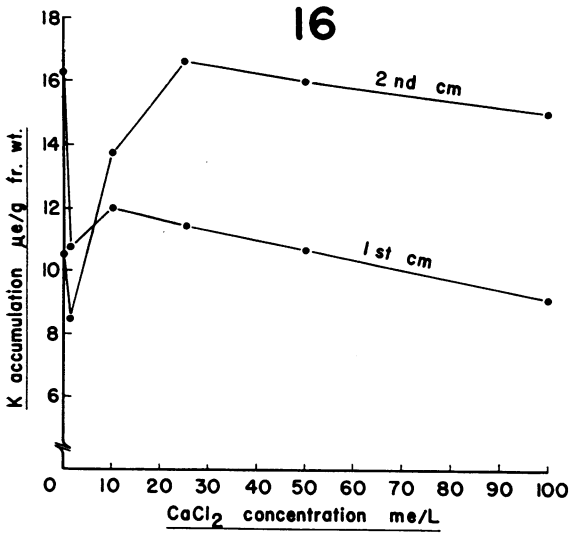
Fig. 16. The influence of $CaCl_2$ concentration on K accumulation by 1st and 2nd cm segments. KCl 1 meq/l. Duration of treatment 44 hours. Sucrose added at 0.1 % concentration and solution changed at 21 hours.

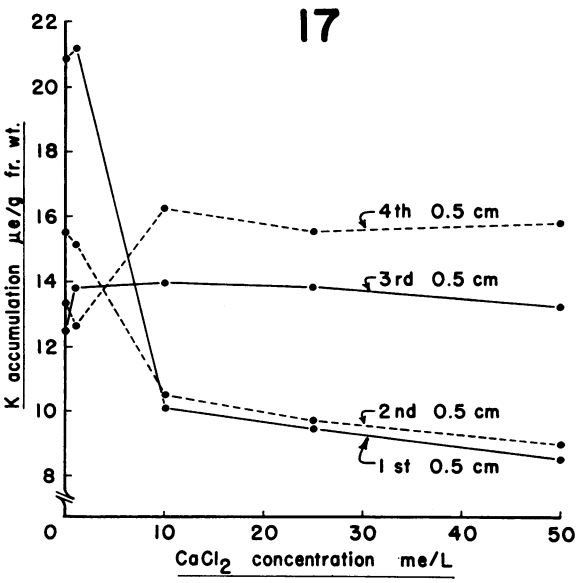
Fig. 17. The influence of $CaCl_2$ concentration on K accumulation by 0.5 cm segments along the epicotyl axis (*from top down*). KCl concentration 1 meq/l. Duration 43 hours, no sucrose present.

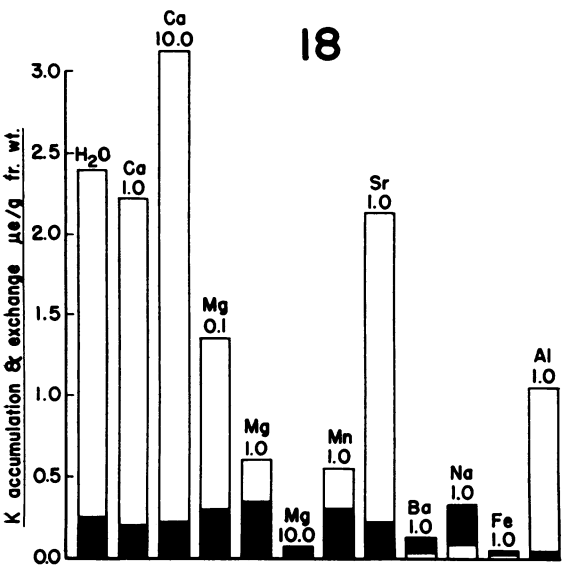
Fig. 18. The effect of various cations as shown (as chloride salts) on K accumulation (*unshaded bars*) and on exchange (*black bars*). KCl concentration 0.1 meq/l. Duration of test 40 hours. 2nd cm segments.

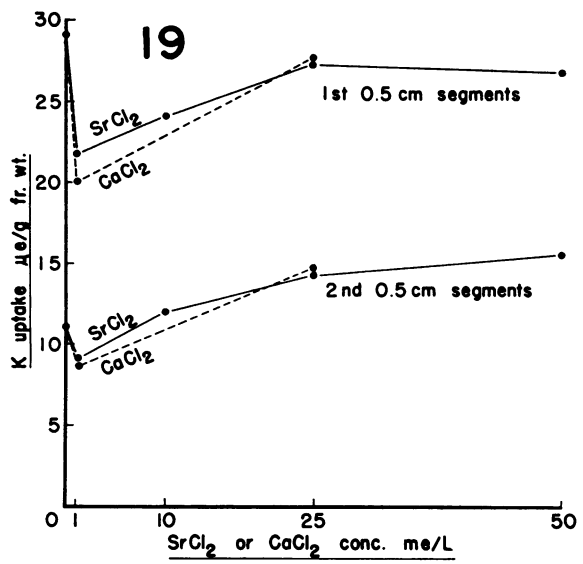
Fig. 19. The influence of Sr as compared to that of Ca on K uptake by the uppermost 0.5 cm segment (*1st in graph*) and 0.5 cm segments excised 1.5 cm below the apical hook (*2nd 0.5 cm in graph*). KCl concentration 1.0 meq/l, sucrose 0.5 %. Duration of treatment 42 hours.

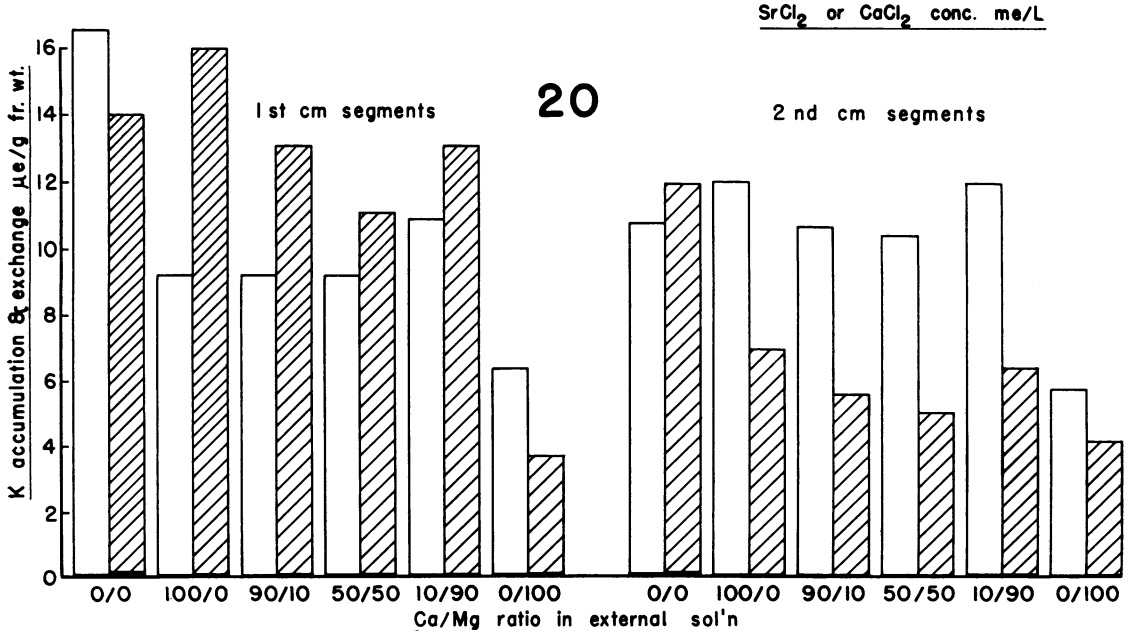
Fig. 20. The effect of 2,4-D in the presence of various Ca/Mg ratios on K accumulation and exchange by 1st and 2nd cm segments. Minus 2,4-D treatments are represented by unshaded bars, 2,4-D treated by bars with diagonal lines; exchange represented by solid black bars. KCl concentration 1 meq/l, total Ca+MgCl was 0 in the controls and in all others 25 meq/l.


16
2 nd cm
1 st cm
K accumulation μe/g fr. wt.
CaCl2 concentration me/L


17
4th 0.5 cm
3rd 0.5 cm
2nd 0.5 cm
1 st 0.5 cm
K accumulation μe/g fr. wt.
CaCl2 concentration me/L


18
H2O
Ca 1.0
Ca 10.0
Mg 0.1
Mg 1.0
Mg 10.0
Mn 1.0
Sr 1.0
Ba 1.0
Na 1.0
Fe 1.0
Al 1.0
K accumulation & exchange μe/g fr. wt.


19
1st 0.5 cm segments
2nd 0.5 cm segments
SrCl2
CaCl2
K uptake μe/g fr. wt.
SrCl2 or CaCl2 conc. me/L


20
1 st cm segments
2 nd cm segments
K accumulation & exchange μe/g fr. wt.
0/0
100/0
90/10
50/50
10/90
0/100
Ca/Mg ratio in external sol'n

usual laboratory temperatures (22–25 C). This higher temperature effect was believed to result from the development of a carbohydrate deficiency since addition of sucrose restored the capacity to respond as at lower temperature. When sucrose was used the solutions were changed at about 12 hour intervals to avoid contamination by microorganisms. Under these conditions three of four experiments with a range of Ca concentration showed three distinctive effects on K uptake (fig 16). At low concentration —1 meq Ca—Ca interfered with K uptake. Higher Ca concentration gave a reversal of this effect and in 2nd cm segments 10 meq/1 Ca or more gave K accumulation in greater amounts than the control. A similar trend may be noted in the 1st cm segments; however, the effect of Ca in promoting K absorption was less, and at no Ca level did K accumulation exceed that of the control (with no Ca). At still higher concentrations Ca depressed K uptake.

In figure 17 is shown the results of a Ca concentration test on the upper four 0.5 cm segments of the pea epicotyl. In this case sucrose was not added and the upper segments failed at higher Ca concentration to show the partial enhancement of K uptake noted in figure 16. With respect to the Viets effect, there were clear differences related to the age and differentiation of epicotyl tissue.

► Influence of Other Cations on K Uptake: Viets (25) has reported that polyvalent cations other than Ca accelerated K (& Br) accumulation in excised barley roots. Accordingly, the effect of the following cations, Mg, Sr, Ba, Fe, Al, and Na as Cl salt solutions at an initial pH of 5.6 on K uptake by pea segments was tested. From the results (fig 18) it can be suggested that of these cations only Sr appeared to simulate Ca in enhancing K accumulation. Similar results were obtained with tris-buffered solutions at pH 7.1. An additional experiment comparing Ca with Sr at various concentrations (fig 19) confirmed this interpretation.

In view of the special importance of Mg as an essential element and one required in many respiratory enzymatic reactions an experiment was performed to evaluate the relationship of Ca Mg ratios on K uptake in the absence and presence of 2,4-D. The results shown in figure 20 again indicate the enhancing effects of 2,4-D on K accumulation by 1st cm segments in the presence of Ca; in the absence of Ca, i.e., with Mg only, 2,4-D markedly depressed K accumulation. The ratio of Ca to Mg appeared to have little effect over a wide range but with no Ca, Mg markedly depressed accumulation. The response of 2nd cm segments differed sharply from that of the 1st cm. In general, treatments including some Ca but no 2,4-D had higher K accumulation. In the presence of Ca, 2,4-D depressed K uptake, and Mg alone depressed uptake.

Discussion

Etiolated pea stem segments, as might be expected, show a rather well defined capacity for ion accumulation and exchange and one which compares favorably with other excised tissue segments. In addition, apparently for the first time, the results show that, as in roots, K accumulation by excised stem tissue may be enhanced by the presence of Ca. This effect is dependent upon the age, or differentiation, of the tissue, since only the 2nd cm segments gave this response with Ca alone. This effect is also dependent on Ca concentration and requires about 24 hours or longer to develop. In the upper (1st) cm segments Ca depressed K accumulation, but addition of auxin reversed this effect. The finding that Ca must be present in the external solution—pretreatments in Ca solutions having little effect—is in agreement with other work (24).

It seems quite evident that Ca and auxin each have certain functions which are independent of one another. On the other hand, with respect to ion accumulation the acceleration of K influx in 1st cm segments by auxin seems clearly to be dependent upon the presence of Ca in the external solution. A reverse situation appears to exist in the 2nd cm segments in which Ca alone usually caused acceleration of K influx whereas addition of 2,4-D with Ca depressed K uptake; here again it was noted in these older segments that 2,4-D alone enhanced K influx. In this case one interpretation might be that the exogenous source of auxin gave a higher than optimal supply of auxin but that again both auxin and Ca are operative. Another possible interpretation is that auxin has a role in growth and differentiation and once certain steps are completed then any exogenous supply is likely to be inhibitory.

The suggestions have been made that auxin increases the permeability of cells (21) and that one effect of auxin is to increase the cation exchange rate (8). The exchange fraction of K as measured in this study is believed to represent primarily what has been referred to as apparent free space (AFS) (1, 17). It comprises the amount in the wall space in the water (WFS) and an amount bound to wall matter (DFS). This exchange fraction was too small to account for the K influx acceleration caused by Ca or auxin in this study, although it might account for the auxin effect in the absence of Ca. Thus the auxin effect must involve the exchange of protoplasmic K by influencing either permeability or a carrier system.

From recent studies on the mechanism of cell enlargement it can be suggested that auxin may induce plasticity and subsequent cell enlargement by increasing the rate of formation of pectic methyl ester groups (14). Ca stiffens cell walls reducing growth and K softens them increasing growth; and these ions appear to be bound exchangeably to wall pectic carboxyl groups. The fact that Ca has a remarkable

effect on K accumulation seems a notable coincidence despite the fact that one would expect no relationship between wall material and cation absorption. However, equilibria at the juncture of the protoplast and the wall could be involved in the K influx process, and wall metabolism during growth and differentiation may be correlated with, if not directly involved in, the establishment of internal-external ionic equilibria or homeostasis. In the absence of better evidence it seems more likely that wall pectic materials are not closely linked with ion accumulation, particularly in view of the evidence showing that IAA does not act by inducing a loss of Ca from the wall (2).

Another possible relationship involved is the development in growing cells of mitochondria which appear to require both Ca (5) and auxin (7, 16). Under appropriate conditions the increase in respiration induced by auxin may apparently be related to an increased Rb influx rate (11). In general, the auxin effect seems to be relatively rapid requiring only an hour or two (11) whereas the Ca effect (on enhancement of Rb or K uptake) requires a longer period (24 to 48 hr). It, therefore, would appear from the time required that mitochondria formation is not a result of the auxin in the present data although it could be an effect of Ca treatment.

Ca has been known for years to influence permeability and in the absence of more conclusive data to the contrary it seems more logical to view the Ca effect in the more or less classical terms of decreasing membrane permeability and thus of increasing net influx by increasing retention. In terms of present theories of ion transport by carriers Ca could be important as a membrane structural agent or it might be directly involved in carrier action, or both. The evidence seems clear that Ca has more than one effect, namely, either depressing or enhancing K uptake depending on the concentration of Ca. K in the absence of Ca may then tend to increase permeability by replacing Ca from rather specific sites in the membrane; K, and other univalent cations, e.g., H and Li, may drastically influence ionic permeability by displacing Ca from important structural positions in the plasmalemma at or near the protoplasmic surface. The toxic effect of single salt solutions could then be viewed as the result of alterations of protoplasmic structure. Ca seems to play a relatively unique role normally as an ion antagonist and presumably it may act by occupying specific sites at the protoplasmic surface to give the protoplast greater stability and greater selectivity as indicated in recent reports (4, 13).

Another possible effect of Ca and of Mg should be considered. Mg may play an important role for many enzymatic reactions in aerobic respiration and other metabolic sequences (19). Ca appears not to be effective in most enzymatic reactions in the glycolytic pathway, and in some cases may actually depress certain important metabolic reactions such as adenosinetriphosphate action possibly linked to ion transport (22). The ratio of Ca to Mg in the nutrient solution has been shown to influence adenosinetriphosphatase activity in Helianthus plants, low ratios yielding less activity (6). Some evidence has been adduced that ATP may be involved in K or Rb uptake by pea segments (9) and thus it is quite possible that one effect of the presence of Mg in the external solution is related to adenosinetriphosphate activity. In the presence of Ca, Mg tended to reverse the acceleration of K uptake induced by auxin.

Summary

A study has been made of uptake of labeled K by excised segments of the third internode of etiolated pea seedlings. Both accumulation and exchange were measured; these fractions are believed to approximate the amounts, respectively, in the protoplasm and in the wall spaces. In addition, the study encompassed the influence on K uptake from KCl solution of age or differentiation of epicotyl segments, of Ca, of auxin and the interaction of these factors. The results led to the following conclusions:

► K Uptake in the presence of Ca is enhanced in the 2nd cm stem segments. In the 1st (upper) cm segments Ca depressed K uptake but the addition of an auxin, 2,4-dichlorophenoxyacetic acid, with Ca enabled these segments to absorb K more rapidly. Thus, the stimulation by auxin of K (or Rb) absorption appears to be dependent upon the presence of Ca in the external solution or, perhaps, the Ca status of the tissue.

► The effect of Ca in accelerating K uptake required 24 to 48 hours to develop and in upper segments, at least at higher temperatures (approximately 28–30 C), required an exogenous supply of sucrose.

► At a given concentration of KCl there was an optimal concentration of $CaCl_2$ with respect to K uptake; lower and higher concentrations tended to depress K uptake.

► Of the following ions: Sr, Ba, Mg, Mn, Fe, Al, and Na, only Sr appeared to enhance K uptake; the others markedly depressed K uptake.

► Ca/Mg ratios had little effect except a depression of K uptake at 0 % Ca/100 % Mg. Auxin in the presence of Mg without Ca tended to sharply depress K uptake in both 1st and 2nd cm segments. The presence of some Mg with Ca and auxin depressed K uptake relative to Ca auxin but did not completely overcome the auxin effect.

► Some possible explanations of these effects are discussed but evidence is not adequate at present to make definite conclusions.

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