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FURTHER OBSERVATIONS ON THE ABSORPTION AND TRANSLOCATION OF INORGANIC SOLUTES USING RADIOACTIVE ISOTOPES WITH PLANTS

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(WITH THREE FIGURES)

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Introduction

In earlier studies on the absorption of inorganic solutes by plants (**9**), the solute status of the system at the time of experimentation was shown to be of importance. Thus high-salt abscised roots were markedly restricted in further accumulation while with low-salt systems, rapid accumulation was subsequently evident. Where previously absorbed salt could move from the roots of high-salt plants to the shoots thereof, further inorganic solute could be readily absorbed from a nutrient medium. Thus it appeared that depletion of the previously accumulated salt in roots through translocation to the shoot allowed the continuance of the absorption process.

It has been shown also (**5**), with low-salt intact plants, that over short intervals of time the larger amount of salt absorbed was retained within the root system; presumably both in protoplasm and in vacuoles thereof, but particularly in the latter (cf. Exp. 2, here). Concurrent translocation to the shoot was limited, but took place more rapidly with time, following at least partial "saturation" of the roots relative to salt. A similar inference may be drawn from a study of decapitated plants, where the composition of exudates from the stumps indicates the movement of solute from root to shoot (**8**). Here, it was shown that salt movement to the upper portions of the plant was proportionally more rapid with previously treated (higher-salt) root systems. Again, for a period of several hours, a large portion of the absorbed salt, but decreasing with time, was retained within low-salt roots. With prolonged treatment, the exudates from both sets approached each other in solute composition indicating that after at least partial approach toward a maximum salt level within the cells of the root, a larger proportion of the inorganic solute currently absorbed, moved inward to the xylem and thence upward in the exudation stream.

Other exudation studies (**3**) on the transport of solutes, followed by water, to the xylem of roots suggest that unless solute is rapidly replenished from an external source, the flow of solution as bleeding or guttation under favorable conditions from shoots, soon becomes negligible. Such a situation would arise particularly where a large proportion of the solute has moved, during previous treatment, into vacuoles of the cells intervening between the root surface and the xylem elements. Salt so resident appears to be subject to retention, that is, it does not readily migrate outward therefrom across cytoplasm to the bathing medium of cells. This may be the result of an impermeability of limiting surfaces (or the cytoplasm as a whole) to salt species *per se,* or if capable of ready outward diffusion, due to rapid concurrent reaccumulation thereof by actively metabolizing cells (**2, 4**). It should be recognized, however, that protracted migration may occur from vacuoles of roots. Thus a marked depletion of previously absorbed salt of roots through movement to the shoot may occur where further solute is withheld from the external supply (**11**).

It has been further suggested, on theoretical grounds (**1, 7**) and from inferences drawn from experimental data (**12**), that solute can move from cell to cell more readily by passage along continuous strands of protoplasm, where such a symplast exists. Thus, though salt in vacuoles is not readily translocated across roots, a ready overall pathway is afforded by the interconnecting protoplasm.

It was of interest to confirm, with the use of radioactive isotopes, the effect of the previous salt status of roots on the nature of subsequent accumulation of inorganic solute by abscised roots. Further, with the use of radioisotopes, a way seemed possible for testing the postulate that salt moves along a symplast pathway (at least in part) from cells near the surface of roots to the xylem. These studies are here reported.

Cultural treatment of plants

Barley plants were germinated, transplanted and allowed to grow in a glasshouse by a standardized technique described earlier (**9**). The inorganic solute supply was such that at the end of approximately three weeks, the plants were vigorous, yet in a low-salt, high-sugar condition. No forced aeration was applied during growth, to conserve carbohydrate within the plant systems. Sets of plants (168 plants per set) were subjected to experimental treatment as indicated below.

Experimental results and discussion

EXPERIMENT I.—Barley plants were separated into two lots. One group of plants was pretreated by exposing the roots to a KBr solution (25 milliequivalents per set) for a period of 16 hours. The second group was placed into water during the comparable interval of time. The roots of each group (approximately 100 gm. fresh weight per set) were then abscised and immersed in 3 liters of 0.00025 normal KBr*. The cultures

were aerated continuously during the experimental period. One pair of each group was subjected to a temperature of 0.5° C. a second to 20° C. Samples of the culture solutions were taken at successive intervals of time and measurements of radioactivity made thereon. Results of these measurements are recorded in the graph of figure 1. The curves show the relative decreases in radioactivity of the culture media (*i.e.*, the absorption by the roots) with time.

It may be seen from the graph, that more rapid absorption occurred at the higher temperatures both with the pretreated (plants with higher initial KBr concentrations) and the control roots. At the higher temperatures, nearly all of the radioactive bromide moved into the roots over a

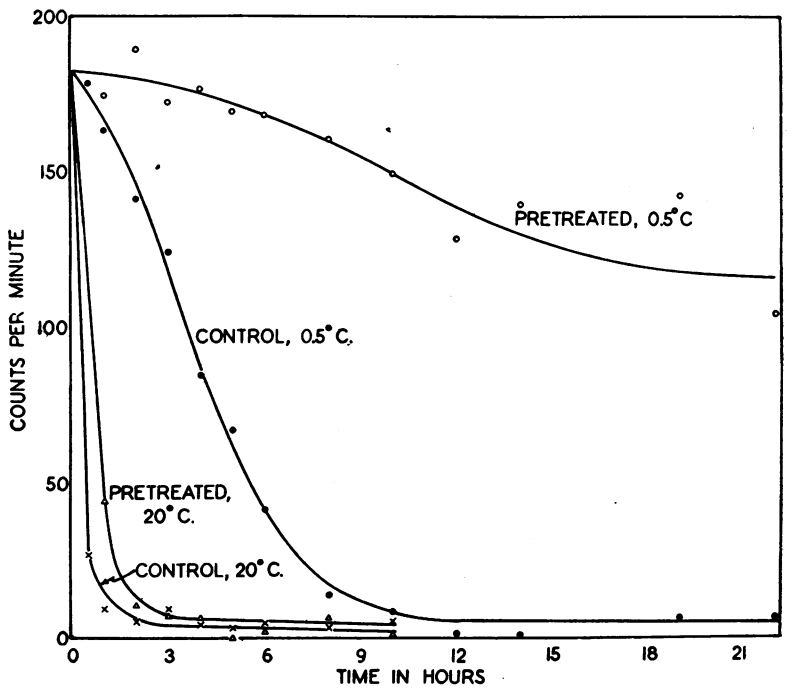

FIG. 1. Effects of pretreatment with inorganic solute and concurrent temperatures on the absorption of bromide by abscised barley roots.

short interval of time. No doubt this influx is related in large measure to metabolic accumulation, exchange adsorption playing a minor role. Regardless of the distribution of the absorbed solute in space within the roots, a very high accumulation ratio is evident.

The pretreated low temperature set shows definite absorption of radio bromide. This would seem to be most logically attributed to an exchange adsorption, particularly isotopic exchange between the protoplasm, together with cell surfaces, and the external medium. The control roots at the lower temperature display a greater accumulation relative to the pre-

treated group and less than either set at higher temperatures. This pronounced influx at lower temperature is probably related to a large extent to metabolic accumulation, exchange adsorption being of the order indicated for the low temperature, pretreated roots, or even less, since the possibility of isotopic exchange is excluded. These data are in accord with earlier evidence that metabolic accumulation can proceed, though more slowly, even at low temperatures (**6**), as well as with those indicating ready exchange under similar conditions (**6, 10**).

Another feature is evident from these curves. The movement of radioactive bromide is less rapid into the pretreated roots which have a higher initial salt level. Thus it becomes clear, that not only is the accumulation level affected by the previous salt status of abscised roots (**9**), but also the rate of influx is thus modified.

As a whole, this experiment delimits the course of exchange absorption and metabolic accumulation as modified by the previous status of roots and the concurrent temperature conditions.

EXPERIMENT II.—This experiment was designed to test the postulate that inorganic solute may move readily along a protoplasmic path between cells and particularly as a way in high-salt tissues and where labile salt is translocated from vacuoles to the xylem when the external source may become limiting. The experimental conditions are outlined in table I. The barley plants were decapitated such that exudate could be collected therefrom. The roots were bathed by aerated media during two intervals of time. During the first interval, part were subjected to KBr and part to $CaSO_4$, of equal normality. Exudate was collected from zero to three and three to six hours and composited within treatments. At the end of these periods, some roots from each group were harvested, washed, centrifuged and frozen for composite expressed sap analysis (**9**). Other sets of plants were transferred, after root washing, to radioactive bromide solutions and further exudate collected from zero to three and three to six hours, during a second interval of time. At the end of these periods the remainder of the roots from each group were harvested and treated similarly to those at the end of interval 1. Analytical results are presented in table I and particularly pertinent data are graphically shown in figures 2 and 3.

Certain general features may be pointed out before detailed discussion of the graphs. It has been pointed out in the introduction, from earlier study with exudates (**3**), that unless concurrent accumulation of labile solutes takes place, the rate of exudation decreases with time. This situation may be observed here as well, where $CaSO_4$ and KBr treated plants are compared. (cf. column 4, and 7 and 10). $CaSO_4$ is slowly accumulated while KBr rapidly migrates into root cells, and into the xylem where the solute concentration relative to the external medium is effective in causing exudation to tend to occur. With some species of plants, little or no exu-

TABLE I

Sets	Experimental conditions			Volumes	Br*		Specif. cond.	Bromide		K
	Interval 1	Interval 2	Sample	Sap or exudate per set ml. cor.[1]	c/m/ml.	tc/m²	Mhos × 10⁴	m.e./l	T m.e.	m.e./l
(Columns)	1	2	3	4	5	6	7	8	9	10
1	Control roots		Roots	79.	.....	.....	48.3	.....	.....	35.1
2	KBr 3 hrs.		Roots	68.	.....	.....	73.0	12.1	0.82	63.8
			Exudate	10.0	.....	.....	24.8	10.1	0.10	11.8
3	KBr 6 hrs.		Roots	72.	.....	.....	71.4	16.2	1.17	65.5
			Exudate	12.7	.....	.....	29.5	17.7	0.23	17.3
4	KBr 6 hrs.	KBr* 3 hrs.	Roots	78.	300.	23400.	79.3	22.2	1.73	72.8
			Exudate	13.9	650.	9025.	35.7	27.0	0.38	35.6
5	KBr 6 hrs.	KBr* 6 hrs.	Roots	75.	510.	38200.	91.7	31.6	2.37	82.9
			Exudate	13.8	896.	12350.	44.6	30.5	0.42	.....
6	$CaSO_4$ 3 hrs.		Roots	75.	.....	.....	51.0	.....	.....	36.6
			Exudate	4.4	.....	.....	19.7	.....	.....	5.9
7	$CaSO_4$ 6 hrs.		Roots	74.	.....	.....	49.7	.....	.....	37.2
			Exudate	4.8	.....	.....	13.6	.....	.....	3.7
8	$CaSO_4$ 6 hrs.	KBr* 3 hrs.	Roots	70.	638.	44700.	73.0	19.4	1.36	63.5
			Exudate	12.3	632.	7770.	27.5	17.5	0.22	17.0
9–10	$CaSO_4$ 6 hrs.	KBr* 6 hrs.	Roots	81.	860.	69600.	81.2	25.2	2.04	66.7
			Exudate	14.5	858.	12420.	38.9	25.4	0.37	23.7
	KBr solution							4.3	12.8	4.5
		KBr* solution			185.	555000.		4.8	14.5	5.83

[1] Per 167 plants, each set.
[2] Total counts per minute Br* per set (167 plants) per 3 hr. period.
All cultures approximately 0.005N; 3 liters per pan of 167 plants each.

date is obtained, even over short intervals of time, from low-salt plants under otherwise favorable conditions (**3**). Where the $CaSO_4$ medium is subsequently replaced by KBr*, the exudate concentration and rate of flow are concurrently increased. As shown earlier, marked accumulations of solutes arise in the xylem relative to certain dilute bathing media (cf. columns 7, 8 and 10, here). As noted elsewhere (**3**), there is a high indi-

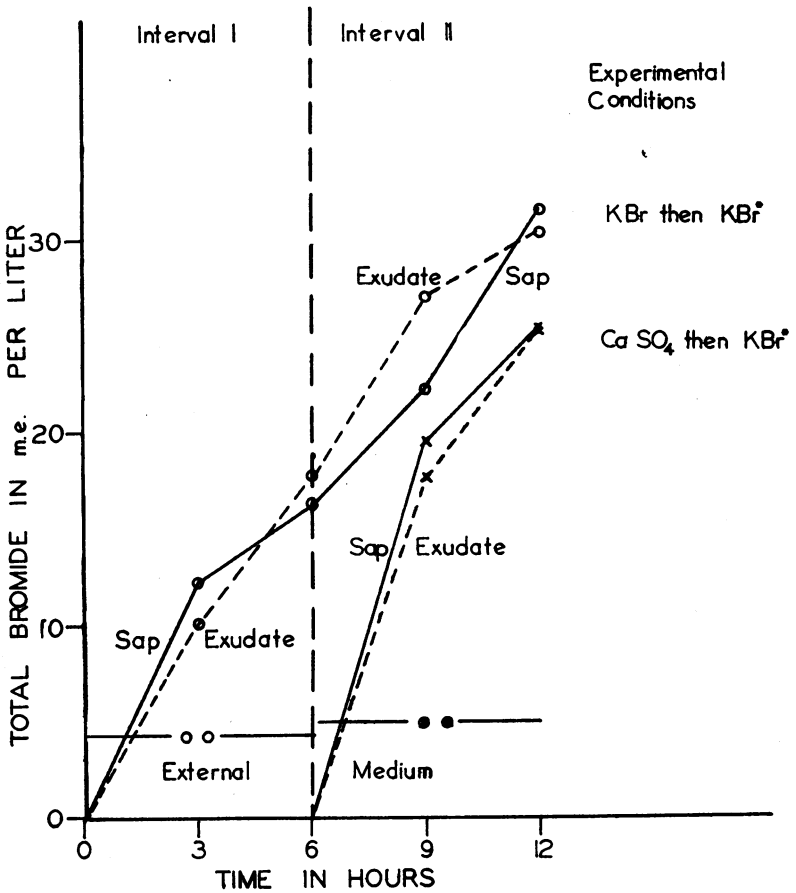

FIG. 2. Composition of root saps and exudates from decapitated barley plants with time; total (non-radioactive plus radioactive) bromide concentration in milliequivalents per liter of solution. For experimental conditions, see table I.

vidual variability in exudation rate (less in composition of sap) among a lot of plants of the same species, cultured similarly. In the current study, the numbers of plants are sufficiently great to make the noted differences of significance.

In figure 2, the total bromide concentrations in exudates and composite expressed saps of the roots are plotted against time. A large portion of the cells of the root are highly vacuolate and it is assumed that the com-

posite sap concentrations nearly equal those of the vacuoles in magnitude which seems reasonable if at any particular time there is a tendency toward constrained dynamic equilibrium for solute and water among the external and internal media and the interposed protoplasm. There is quite close agreement at each of the time periods between the total bromide concentrations in the exudate and in the root sap. The measured values are plotted at the end of the particular time period. The exudates

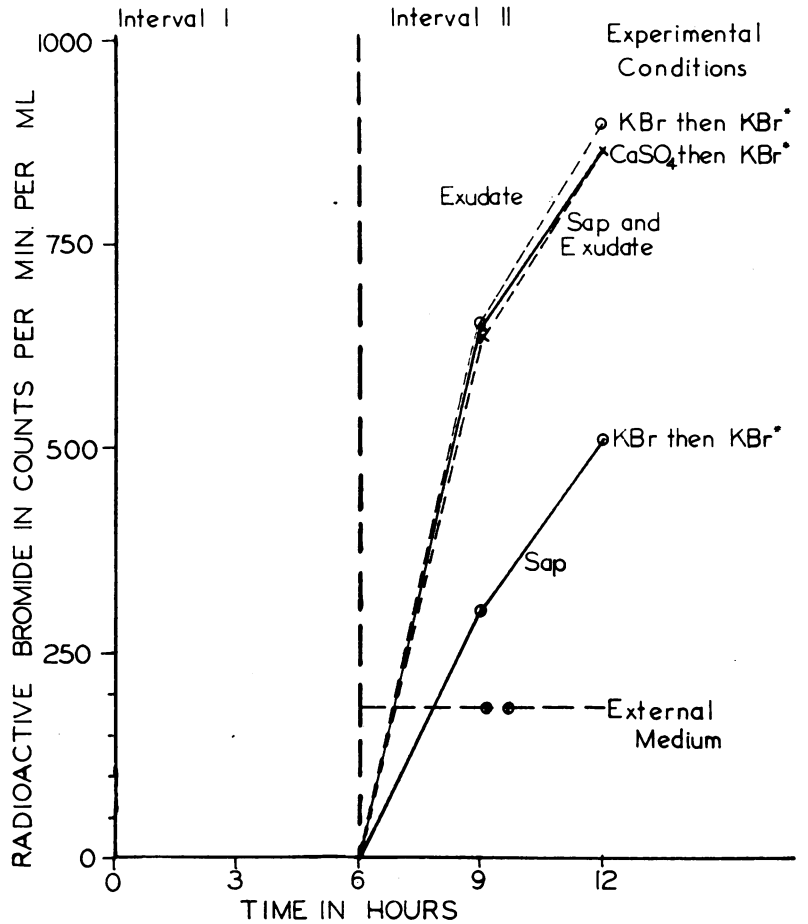

FIG. 3. Composition of root saps and exudates from decapitated barley plants with time; radioactive bromide only, in counts per minute per milliliter of solution. For experimental conditions, see table I.

were collected throughout the time period, therefore, the composition represents an average for the time involved, while the sap values represent those at the end of the corresponding period. For this reason, one might have expected the sap values to possibly exceed those of the exudates as plotted, however this is not significantly the case. It would seem that these close correspondences in magnitude would obtain if the inorganic

solute (accompanied by water, osmotically) on influx moves rather proportionally into lumena of both the vacuoles of low-salt root cells and the xylem. Although the relative distances to these two types of lumena may be considerably different, the permeabilities of the effectively interposed protoplasm and energy intensity gradients may be of similar orders of magnitude for low-salt roots. At all events, the data indicate movement of total bromide into the vacuolate cells and into the stelar vessels such that accumulation levels are quite parallel with time. For those plants pretreated with $CaSO_4$, the relative total bromide concentrations in sap and exudate (figure 2) agree well with the bromide measured by radioactivity (shown in figure 3).

The curves of figure 3 indicate the relative movements of radio-bromide supplied to two systems—now dissimilar because of the preconditioning during interval 1. One set is relatively high in bromide and total salt while the other is relatively low in total salt, being without bromide. With supply of bromide to low-salt tissues, as before, the radioactivity levels with time are similar in sap and in exudate. However, it is very strikingly shown that with the bromide pretreated roots a marked difference in radioactive bromide concentration obtains in these two solutions (fig. 3) although the totals are of similar order of magnitude (fig. 2). The inference which may be drawn from this apparent difference in distribution of radioisotope is that the vacuoles of the cells of these roots are already fairly high in non-radioactive bromide and this concentration increases less rapidly with time, for it is tending to approach, in being a closed system, a level characteristically maximum for this species under the imposed conditions. On the other hand, the xylem solution is subject to mass flow, with continued movement, to replenish the loss, of solute and water into the relatively open lumen. Thus a differential migration of radioisotope may be envisioned across and along the protoplasm in favor of the plasma continuum—the symplast. In other words, while a small proportion of the radioactive bromide is migrating into the vacuoles to build up the sap total bromide concentration, a larger proportion is moving along the protoplasmic pathway directly to the xylem lumena. These data, therefore, seem to support the postulate that solute may move directly along the protoplasm from cell to cell to the stele, by-passing, so to speak, the intervening vacuoles in large measure when the vacuoles are already relatively high in salt concentration. This idea has been schematically represented elsewhere, in a diagram of two possible plant systems for solute movement (1, fig. 1). That such relatively independent movement can take place seems clear from the present results.

Summary

With the use of abscised barley roots immersed in radioactive bromide solutions, it has been possible to more closely delimit the modes of migration of electrolyte into tissues at low and high temperatures as well as into

systems of previously high- or low-salt content. This study suggests several points. First, there is a rapid exchange of isotopes between the external medium and roots; second, under favorable temperature conditions isotopic or ion exchange is relatively less important than migration by metabolic accumulation; third, that with high-salt roots at low temperature, migration may be restricted almost exclusively to adsorption exchange; and fourth, that metabolic accumulation may be pronounced even with roots at low temperature, provided they are currently in a relatively low-salt condition.

With the use of decapitated barley plants and isotopes of bromide it has been shown that two pathways are afforded for the migration of inorganic solutes into roots. First, with low salt plants, salt may migrate across the cytoplasm of cells into vacuoles as well as to more remote lumena, *e.g.*, the xylem. Second, where the roots are initially in a relatively high-salt condition, subsequent migration takes place differentially along continuous protoplasmic paths (the symplast) to the xylem, circumventing, in a measure, movement into or through intervening vacuoles. Where depletion of vacuolar solute occurs through translocation, the probable pathway is likewise along the protoplasmic continuum.

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