

THE DIURNAL VARIATION IN THE TRANSLOCATION OF MINERALS ACROSS BEAN ROOTS

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Present knowledge about the mechanism whereby minerals are transferred from the nutrient medium to the root xylem is quite limited. The initial step involves an exchange of hydrogen, hydroxyl, or bicarbonate ions on the protoplasmic surface of the root for nutrient ions of the substrate (13). The amount of any ion gained by such exchange is a function of the rate at which metabolic energy is produced by the root, and, within limits, of the concentration of the ion in the substrate. Absorbed ions appear to enter into labile cytoplasmic complexes which are created or maintained by the expenditure of energy, and which seem to be specific for different ions or groups of related ions.

It is now generally held that the protoplasts of the root cells are interconnected by plasmodesmata forming a symplast of cytoplasm that extends from the root surface to the xylem elements (3, 5, 11, 20). Hence, ions entering the cytoplasmic phase by exchange have a pathway through living material to any of the three aqueous phases—external, vacuolar, or tracheal. The cytoplasm in some manner utilizes energy derived from aerobic respiration to speed the distribution of ions throughout the symplast, and to produce high vacuolar and tracheal concentrations of the ions.

There is no convincing evidence as to how movement of ions through the cytoplasm occurs, but several theories and speculations on the matter are current. LUNDEGARDH'S (11) theory of an inward anion migration as a countercurrent to an outward flow of electrons, and a cation migration as a series of exchanges for hydrogen ion, has received the most attention, but is now suspect for its failure to explain the action of dinitrophenol, which reduces ion absorption without decreasing respiration (15). OVERSTREET and JACOBSON (14) have published a review in which they fit the thermodynamic consequences of possible membrane properties to various theories of ion accumulation, and they find support for the view that ion transport through the cytoplasm occurs in conjunction with some ion-carrier, which is presumably formed by aerobic respiration and is removed by some metabolic process after transporting an ion to the inner phase. WIERSUM (20) believes that movement through the symplast may be by diffusion along concentration gradients in the intermicellar fluid of the cytoplasm, and by active, inwardly-directed, polar transport of ions bound to the colloidal

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phase. For those who hold that the active transport across the symplast is unidirectional, visualization of some such two-phase transport is essential to an explanation of the finding that ions can move from the stele outward to the external solution (12, 20).

The method by which ions are released from the cytoplasm to the vacuoles and xylem elements is as obscure as the means of transport. Conceivably, the release might require the expenditure of energy, and be properly termed secretion; on the other hand, the release may be spontaneous, the requisite energy expenditure for accumulation having been made when the ions were initially taken into the cytoplasm, or the energy may have been expended in the formation of some ion-carrier. The Dutch workers (1, 3, 20) seem largely to favor the secretion viewpoint, basing their opinion upon the relative impermeability of the tonoplast to ions resident in the vacuole, and upon the fact that vacuolar or xylem concentrations of ions may sometimes appear to exceed those of the cytoplasm. Most other opinions seem to favor passive release, or at least have the principle of spontaneous release incorporated into the postulated mechanism for absorption and transport. LUNDEGARDH (11) now pictures anion-respiration as maintaining a steady-state between active accumulation of salt from the external medium and a passive release to the xylem. He does not believe that it is necessary to visualize a symplast to account for this process; each cell accumulates salts on the outermost side and passively, but selectively, releases them on the inward side. The well-known exudation theory of CRAFTS and BROYER (7) incorporates the idea of passive release of ions to the xylem, and BROYER (6) also views the release of ions to the vacuole as a spontaneous process. He postulates that ions bound in cytoplasmic complexes at outer loci might migrate to inner phases where some change in metabolic rates or processes would favor release of the ions, increasing the activity of the ions in the intermicellar fluid, and resulting in a spontaneous movement of ions to the vacuole.

It appears to the authors that any distinction between secretion and passive release is rather academic. The cytoplasm cannot perform work beyond its boundaries, and the movement of ions into either vacuole or xylem element must be spontaneous. Regardless of mechanism, the thermodynamic consequences of accumulation require that the cytoplasm expend energy in raising the chemical potential of ions at the inner desorbing surface as compared with that at the outer adsorbing surface. Seemingly, if the mechanism expends energy directly or indirectly at the outer cytoplasmic surface in absorbing ions, the release is to be termed passive; if it expends energy at the inner surface in releasing bound ions, the release is to be termed secretion. Some confusion can be predicted if energy proves necessary for both absorption and release. In this report the term release will refer to the passage of the ions from the cytoplasmic to an aqueous phase and is not otherwise qualified.

These diverse opinions on one of the most fundamental of cytoplasmic mechanisms suggest that what is most needed at the present is more infor-

mation about variables which affect the process of transfer and release of ions to the vacuoles and xylem. It has occurred to the authors that useful information might be obtained from studies of the fate of tracer ions that have entered the root cytoplasm. Depending on the state of the cytoplasmic transfer and release mechanism at any instant, there should be certain probabilities that any one ion would remain with the protoplasm, pass into a vacuole, be deposited in the xylem, or even return to the external medium. If these probabilities can be altered by varying the temperature, oxygen tension, sugar supply to the root, functioning of enzyme systems, salt status, auxin supply, light, transpiration rate, or any other variable which past research has suggested might be concerned in the transfer and release mechanism, it is not unlikely that an extended estimate of the nature of the mechanism can be made.

It appears, however, that before any extensive investigations could be undertaken, some basic information was needed on the ability of the symplast to transport ions to the xylem. Studies of the diurnal fluctuation in exudation (17) indicate that there might be a diurnal variation in the capacity of the root symplast to absorb and/or translocate ions. If such is the case, all short-term experiments need to be planned for a specific time of day. HOAGLAND (10) and BROYER (5) have reported that low-salt, high-sugar barley roots initially transport a smaller proportion of absorbed potassium and bromide to the xylem than do high-salt, low-sugar roots. Presumably, in the more active low-salt roots a greater proportion of the ion being transported inward through the symplast is released to the vacuoles; as the ion concentration of the vacuoles increases, more and more of the absorbed ion reaches the xylem. EATON and JOHAM (8) found a positive correlation between the sugar content and the mineral content of cotton roots. Collectively, these findings indicate that the salt and sugar content of roots could have a large effect on the relative amounts of absorbed ion retained in the root, and should be taken into consideration in the culture of experimental plants. Accordingly, some rather simple experiments were performed to determine if there actually is a diurnal fluctuation in the amount of ion absorbed or translocated to the xylem, and the influence of sugar content and salt status on such variation. The 24-hour day was divided into short absorption periods, and by means of radioactive isotopes the distribution of absorbed ion between root and shoot at the end of each period was ascertained.

Methods and materials

Red Mexican bean (*Phaseolus vulgaris*) seeds were germinated under fluorescent lights, and selected seedlings were placed in notched covers over enameled pans containing six liters of a dilute Hoagland's solution (0.00167 M KNO_3 , 0.00167 M $\text{Ca}(\text{NO}_3)_2$, 0.00067 M MgSO_4 , 0.00017 M KH_2PO_4 , micronutrients and iron), six plants per pan. The solutions were aerated and the plants grown for 10 days under banks of fluorescent lamps on a 6:00 A.M. to 6:00 P.M. day, which was time-clock controlled. The light

intensity on the surface of the upper leaves was about 1000 foot-candles. A temperature of $73 \pm 1^\circ$ F and a relative humidity of $60 \pm 4\%$ were maintained in the growth room by means of an air-conditioning unit. The plants were always in complete darkness during the night period except for brief exposures to light from an incandescent bulb at the beginning and end of experimental absorption periods.

When low salt plants were desired no change of nutrient solution was made. High salt plants were obtained by placing the plants in full strength Hoagland's solution on the fourth day and by changing solutions on the seventh day. Previous study had indicated that both the growth and the salt requirement of the plants were less than for similar plants raised in full sunlight.

Radioactive isotopes of rubidium (Rb^{86}) and phosphorus (P^{32}) were used as tracers. Six liters of one twelfth strength Hoagland's solution were prepared for each absorption period of the experiment, this dilute solution being used in order that the absorption might be largely against a concentration gradient. For the phosphate translocation experiments, about one and one half microcuries of P^{32} per liter were added to the solution in which the phosphate concentration was 0.000083 M. In the rubidium translocation experiments, the nutrient solutions were made equimolar (0.005 M) with respect to rubidium and potassium by the addition of RbCl . About two microcuries of Rb^{86} per liter were used.

Analyses were made for radioactivity and total sugars. Harvested plants were cut into root and shoot, roots were blotted, and fresh weights taken. The roots were killed and thrice extracted in boiling 80% ethanol. The alcohol was evaporated from the combined extracts on a warming plate, the residue made to volume, and aliquots analyzed for radioactivity. Total sugars were estimated as glucose from the remaining extract by the method of FORSEE (9) with one modification; the extract was treated with invertase prior to clearing and decolorizing. It was found in testing the suitability of the method that if this was not done there was a loss of sucrose during the decolorizing step. Iodine tests on the extracted roots always failed to show the presence of starch. The root residue remaining after extraction was also analyzed for radioactivity in the experiments where P^{32} was used, for about one third of the total activity could not be extracted. In experiments using Rb^{86} , the extractions left so little radioactivity in the roots that it was scarcely discernible.

The dried shoots were wet-ashed in nitric acid and made to volume with dilute hydrochloric acid. Analysis for radioactivity was made with a Geiger-Muller counter on 2-ml. aliquots of solution, or extract, evaporated to dryness in porcelain capsules. All counting was at constant geometry. Conversion factors for expressing activities as micromoles of ion were obtained by determining the radioactivity of the nutrient solutions prepared for the experiments.

The amount of tracer ion found in the shoot at the termination of the experimental period represents that quantity of ion translocated across the

root, released to the xylem, and carried upward in the transpiration stream. It is not known how the tracer ion retained in the root is distributed. Portions of the total amount of ion must be resident in the protoplasm as part of the living system, or as ions in transit to the vacuoles and xylem; portions must be present in the cell sap, and there should be small amounts in the root xylem which have not yet been translocated upward to the shoot.

Experimental results

TIME COURSE OF RUBIDIUM TRANSLOCATION TO THE SHOOT DURING DAY AND NIGHT

This experiment was designed to determine how much time would elapse before detectable amounts of rubidium could be transported from the nutrient medium to the shoots. Low salt plants were cultured as it was believed that minimal translocation to the shoots would occur with this status. The solutions prepared for this experiment contained but 0.000075 molar RbCl. Starting at 10:00 A.M. on the day of the experiment, 12 plants were transferred to pans of the radioactive nutrient solution, replaced under the lights, and vigorously aerated. At one-, two-, and four-hour intervals, four individuals were harvested. At 2:00 A.M. of the following night the same procedure was carried out for 12 more plants. These latter plants were exposed

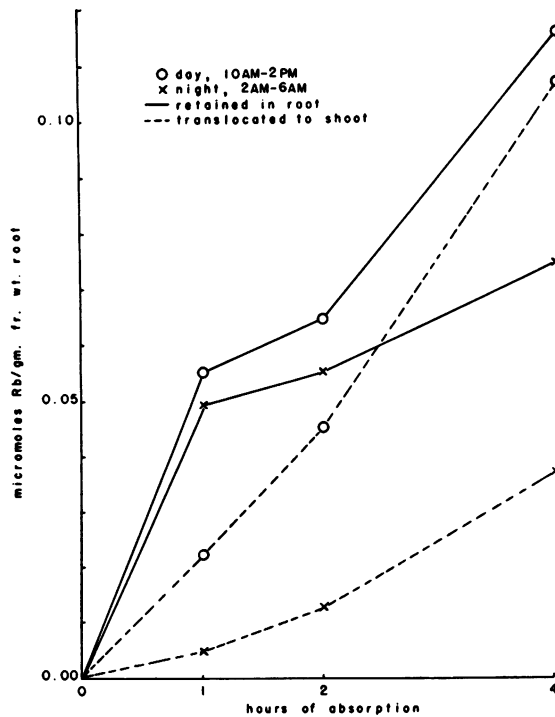


FIG. 1. Amounts of absorbed rubidium translocated to the shoots and retained in the roots of low salt bean plants during night and day absorption from dilute RbCl.

to indirect light from an incandescent bulb for a few minutes at the beginning of the experiment and again during each harvest.

The pertinent results of the experiment are depicted in figure 1. Each value represents the mean of four plants. Data on both the retention and translocation of rubidium are expressed per gram fresh weight of root. It is considered that since the root must perform the work essential to translocation of the ion to the xylem, such an expression is preferable to one based on the weight of the shoot.

The amount of rubidium retained by the roots in the first hour is much alike for both day and night absorption, and probably results from similar adsorption on the root surface by ion exchange. The rate at which rubidium is gained by the root after the first hour must more nearly reflect the capacity of the root to retain absorbed rubidium in cell sap or protoplasm; obviously, roots absorbing during the day had the greater capacity to accumulate rubidium. The most striking difference between night and day behavior occurred in the amounts of rubidium translocated to the shoot. Translocation was far greater during the day, whether expressed in absolute amounts or in per cent. of total ion absorbed and even during the first hour of absorption when the amounts of rubidium retained by the roots were similar. At the end of four hours nearly half of the rubidium absorbed during the day was in the shoot; at night, only one third had been translocated upwards. It was decided that in subsequent work a four-hour absorption period would be used in order to make certain that the amount of rubidium found in the root would reflect something of the metabolic status of the root, and not just ion exchange.

DIURNAL VARIATION IN THE AMOUNT OF ABSORBED RUBIDIUM TRANSLOCATED TO THE SHOOT

This experiment was designed to provide information on the diurnal variation in the amounts of rubidium absorbed, and on the relative distribution of the ion between root and shoot. Forty-eight plants were raised, 24 each of the low and high salt status. The experimental day was divided into six consecutive four-hour absorption periods, beginning at 6:00 P.M. when the lights were turned off. Six pans of the dilute Hoagland's solution plus RbCl and Rb^{86} were prepared, one for each absorption period. At the beginning of each period four low salt and four high salt plants were placed in a pan of the radioactive solution and replaced under the light canopy. The experimental solution was vigorously aerated to effect movement of the solution about the roots. At the end of four hours the plants were harvested.

Figure 2 presents the results of the experiment. It is evident that the plants were more active in absorbing, retaining and translocating rubidium during the day. Minimum root activity occurred about midnight, maximum about midday. As was indicated in the initial experiments, the ability of the root to translocate rubidium to the xylem was increased by the coming of day to a greater extent than was its ability to retain the ion.

The percentage data show that there was a significant increase in the

ability of the root cytoplasm to translocate rubidium from about midnight to early morning, but the greatest increase occurred with the coming of day. Low salt plants showed this increased translocation to a greater degree than did high salt plants, which is not in accord with the view that low salt plants tend to hold more of the absorbed ion in the root. Due to the acceleration of translocation with the coming of day, there was no consistent relationship between the percentage translocated and the total amount of rubidium absorbed.

No consistent correlation can be found between the total sugar content of the root and absorption, translocation, or retention. During the day some correlations might have appeared to exist, but they did not hold at night. If total sugar content of the root controlled the activity of the root, one

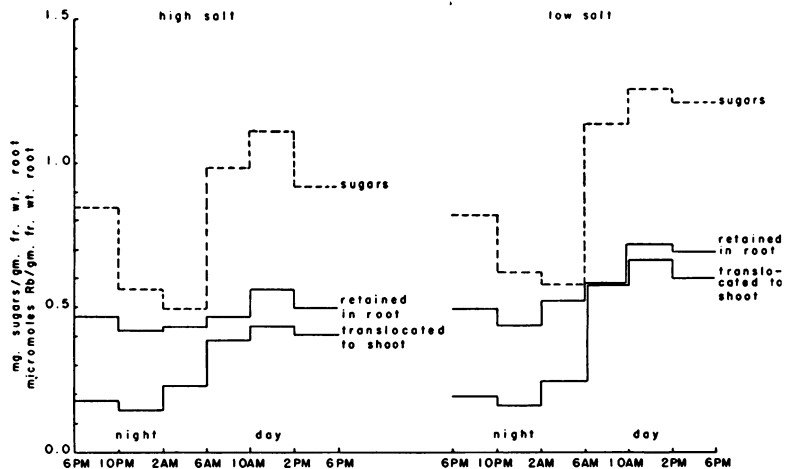


FIG. 2. Diurnal variation in the amounts of rubidium retained in the roots and translocated to the shoots of high and low salt bean plants during four-hour absorption periods.

would look for the closest correlation to occur at night when sugars might become limiting. As it is, the roots began increasing in activity during the last period of the night when the sugar content of the root was at a minimum.

The sugar content of the roots was quite low, which probably reflects their culture under low light intensities with favorable temperatures and oxygen supply. The fact that the low salt roots had but slightly higher sugar content than the high salt roots suggests that these plants did not represent the extremes of salt status usually associated with the terms. However, the capacities of the two types of plants to absorb rubidium were clearly different, and the difference in the per cent. of absorbed ion translocated during the day must also have resulted from the difference in salt-sugar status.

In general, the results are in agreement with the findings from exudation studies; that is, there exists a diurnal fluctuation in the activity of the root which is here reflected in rubidium absorption and translocation.

DIURNAL VARIATION IN THE AMOUNT OF ABSORBED PHOSPHATE
TRANSLOCATED TO THE SHOOT

This experiment was designed to provide the same information on the translocation of phosphate as was obtained for rubidium. It was performed in the same manner, except that no RbCl was present in the dilute Hoagland's solution used in the experimental periods. Figure 3 gives the results of the experiment. There were some similarities between the results for phosphate and rubidium. The time course of translocation to the shoot was

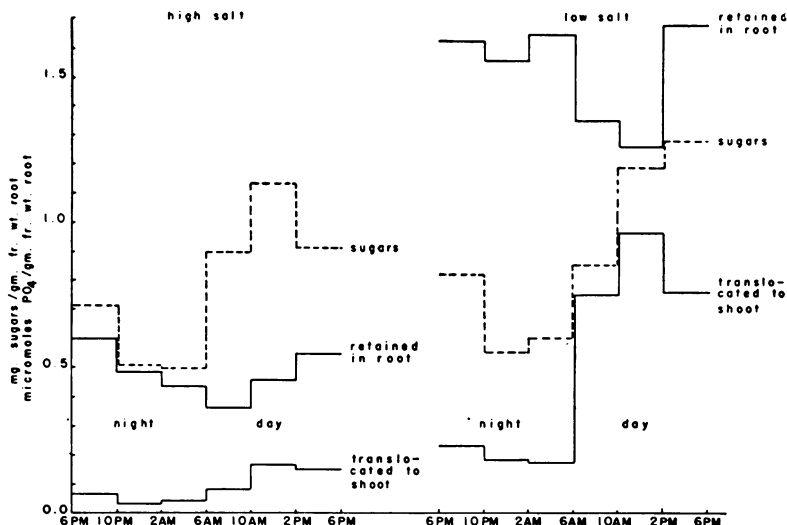


FIG. 3. Diurnal variation in the amounts of phosphate retained in the roots and translocated to the shoots of high and low salt bean plants during four-hour absorption periods.

of the same nature for both ions, with the minimum reached about midnight and the maximum about midday. Again, there was no consistent correlation between total sugar content and root activity in absorption, retention, or translocation. Unlike rubidium, however, phosphate was not retained in the roots in greater quantity during the day; rather, there was a significant decrease in phosphate retention. The data for high salt plants, in which there was less variability between the plants of any one period, clearly showed that the diurnal cycles for retention and translocation were out of phase, with the low point in retention being reached during the first period of the day. The corresponding cycles for rubidium were in phase, though differing in amplitude. In general, it appears that the increased daytime absorption of phosphate by bean plants was largely accounted for by the increased transfer of the ion to the shoot; whereas the increased daytime absorption of rubidium was due to increased capacity of the root to retain the ion as well as to increase transference to the shoot.

Although the percentages of absorbed ion translocated to the shoot were much higher during the day for both phosphate and rubidium, there were

striking differences shown for the two ions (fig. 4). A smaller percentage of phosphate was transferred to the shoot than was the case with rubidium. The increase in percentage of phosphate translocated was delayed until the first period of the day, while with rubidium there was a significant increase during the last period of the night. There was a significant decrease in the percentage of phosphate translocated during the last period of the day, while this was not true for rubidium. The diurnal change favoring rubidium transport across the root symplast began earlier and continued longer than was the case with phosphate transport.

Percentagewise, as well as in absolute amounts, low salt plants translocated more phosphate and rubidium to the shoot in a four-hour period than did high salt plants, but the salt status had a much greater effect in the case of phosphate. In general, the increased activity of the root brought about by the low salt status was much more apparent with phosphate than with rubidium.

THE EFFECT OF DARKNESS DURING THE NORMAL DAY PERIOD ON THE
TRANSLOCATION OF PHOSPHATE TO THE SHOOT

If, as suggested by the previous experiments, there is a change in the bean root symplast with the coming of day which tends to increase the proportion of absorbed ion passed into the xylem, there should be detectable increases in translocation during the day period even when the plants are kept in darkness. This experiment was designed to test this postulate. As low salt plants showed a tremendous surge in translocation of phosphate to the shoot in the first period of the day, it was decided to use this salt status and ion in order to gain the greatest sensitivity possible.

Procedures were those of the preceding experiment. The additional dark

TABLE I
TRANSLOCATION OF ABSORBED PHOSPHATE TO THE SHOOTS OF LOW SALT
BEAN PLANTS UNDER DAY CONDITIONS OF LIGHT AND DARKNESS.

Light condition	Period	Phosphate			Phosphate translocated to shoot	Sugar in root
		Retained in root	Translocated to shoot	Total absorbed		
		<i>micromoles/gm. fresh wt. root</i>			<i>%</i>	<i>mg./gm. fresh wt.</i>
Dark	6 P.M.-10 P.M.	1.056	0.081	1.137	7.2	0.90
	10 P.M.-2 A.M.	1.174	.079	1.253	6.2	.72
	2 A.M.-6 A.M.	1.179	.083	1.261	6.6	.56
Light	6 A.M.-10 A.M.	1.072	.486	1.558	31.3	1.02
	10 A.M.-2 P.M.	1.200	.715	1.915	37.4	1.42
	2 P.M.-6 P.M.	1.221	.470	1.690	27.2	1.41
Dark	6 A.M.-10 A.M.	1.011	.166	1.177	14.0	.30
	10 A.M.-2 P.M.	1.070	.172	1.242	13.7	.34
	2 P.M.-6 P.M.	.987	.095	1.082	8.9	.31
L.S.D. 5%*		0.262	0.126	0.404	3.3	0.16

*Least significant difference at the 5% confidence level.

treatment during the normal day period was given by placing 12 plants in a dark chamber constructed in the growth room. Results are given in table I. There was a great deal of variability in the individual plants of this experiment, as was always the case with low salt plants, and there are no significant changes in the amounts of absorbed phosphate retained in the roots; only the difference in phosphate retained by the roots of darkened and illuminated plants during the 2:00 P.M. to 6:00 P.M. period approached statistical significance. With phosphate translocation to the shoot, however, the response to light was large and unmistakable, as it was in the previous experiment. Those plants that were kept in the dark during the day period

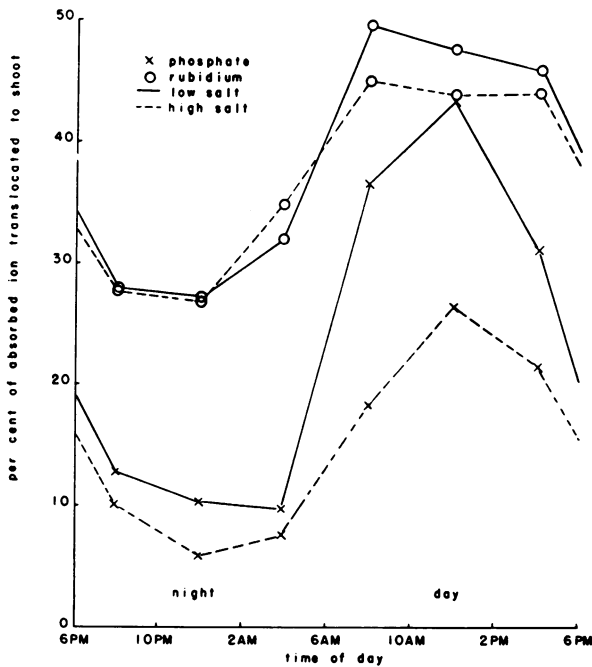


FIG. 4. Diurnal variation in the percentages of rubidium and phosphate absorbed in four-hour periods that are translocated to the shoots of high and low salt bean plants.

also showed an increase in translocation over the night level, but it missed significance. However, the per cent. of absorbed phosphate which was translocated to the shoot showed that the diurnal change in favor of transport across the symplast occurred with the normal coming of day even in darkened plants. Darkened plants absorbed about the same amount of phosphate during the day as they had during the previous night, but the proportion transported to the shoot was much larger.

It seems, then, that there exists a diurnal variation in the ability of the bean root symplast to transport phosphate to the xylem, but it is apparent that illumination of the plant serves to greatly augment this basic change. Again, most of the increased daytime absorption of phosphate is accounted for in the increased translocation of the ion to the shoot.

THE EFFECT OF ADDED SUCROSE ON THE TRANSLOCATION OF ABSORBED IONS BY DARKENED PLANTS

This experiment was performed to ascertain if adding sucrose to the nutrient medium would increase the rate of translocation of phosphate and rubidium to the shoots of darkened bean plants. Although there is some evidence that root-supplied sucrose is ineffective in increasing exudation (19), and it is stated that the influence of sugars on ion secretion into the xylem appears to be weak (2), incomplete experimentation in this laboratory shows that under some conditions (dilute sucrose in Hoagland's solution applied in the morning to roots of bean plants that have not been in the dark longer than overnight) applied sucrose will definitely increase the exudation of rubidium.

Forty-eight plants were raised for this experiment and were used in lots of 16. The first lot was used to test the effects of light and sucrose in maintaining the translocation rate of phosphate during the transition from day to night. Four plants were used in the absorption period from 2:00 P.M. to 6:00 P.M. to establish a basic rate for retention and translocation from which to measure deviations in the subsequent night period. The remaining plants were divided into three treatments for the 6:00 P.M. to 10:00 P.M. period: dark (control), continued light, and dark plus 0.005 molar sucrose in the experimental nutrient solution.

The two remaining lots of plants were used the following morning in a study of the effect of continued darkness and darkness plus sucrose on the acceleration of ion translocation that occurs as the plant passes from night to day. Phosphate was traced in one lot, rubidium in the other. Four plants from each lot were used in the 2:00 A.M. to 6:00 A.M. absorption period, again to establish a base from which to measure changes in the subsequent day period. There were three treatments in the 6:00 A.M. to 10:00 A.M. period: light (control), dark, and dark plus 0.005 molar sucrose in the nutrient solution.

Solutions and procedures were as previously described. The darkened plants were again held in the dark chamber in the growth room. The results of the experiment are displayed in table II. The evening experiment indicated that the phosphate retained by the root significantly increased if the plants were exposed to continued light in what is normally the first period of night. In darkness during the same period, there was no significant change in the amount of phosphate retained and the addition of sugar to the nutrient medium had no significant effect. Translocation of phosphate to the shoot was maintained in the day-to-night transition if the light continued, but fell if normal darkness followed the day. The percentage figures show the usual lower per cent. of translocation in the night under all treatments.

In the morning experiment the only significant change in phosphate retention with the passage from night to day was in the loss shown by plants kept in continued darkness. Light produced a large increase in translocation, but no significant change in retention. Applied sucrose increased the

retention of phosphate in the roots of darkened plants without producing any change in the amount of translocation. It is possible that the increased retention of phosphate in the root due to applied sucrose was not related to the problem studied here, but that it reflected the phosphorylation process associated with sucrose absorption (16, 18).

The data on percentage of absorbed phosphate translocated to the shoot again indicate that some diurnal change favoring transport across the root occurs with the normal coming of day. Some factor or factors associated with light accentuate this change. A comparison of the morning and evening

TABLE II
THE EFFECT OF SUCROSE APPLIED TO THE ROOTS OF DARKENED
BEAN PLANTS ON THE DISTRIBUTION OF PHOSPHATE AND
RUBIDIUM BETWEEN ROOT AND SHOOT.

Traced ion	Period	Light condition	Ion			Ion translocated to shoot
			Retained in root	Translocated to shoot	Total absorbed	
			<i>micromoles/gm. fresh wt. root</i>			%
PO ₄	2 P.M.- 6 P.M.	Light	1.005	0.073	1.078	6.7
	6 P.M.-10 P.M.	Dark	1.143	.020	1.163	1.8
	6 P.M.-10 P.M.	Light	1.716	.063	1.779	3.4
	6 P.M.-10 P.M.	Dark plus sucrose	1.283	.018	1.301	1.4
L.S.D. 5%*			.588	.035	.619	1.0
PO ₄	6 A.M.- 6 A.M.	Dark	1.229	.033	1.332	2.5
	6 A.M.-10 A.M.	Light	1.338	.132	1.470	8.9
	6 A.M.-10 A.M.	Dark	.932	.070	1.003	6.8
	6 A.M.-10 A.M.	Dark plus sucrose	1.482	.074	1.556	4.8
L.S.D. 5%*			.378	.040	.384	2.7
Rb	2 A.M.- 6 A.M.	Dark	.553	.197	.750	26.2
	6 A.M.-10 A.M.	Light	.654	.402	1.056	38.1
	6 A.M.-10 A.M.	Dark	.586	.222	.808	27.5
	6 A.M.-10 A.M.	Dark plus sucrose	.604	.246	.850	29.0
L.S.D. 5%*			0.040	0.030	0.062	1.6

*Least significant difference at the 5% confidence level.

transitions shows that light increases phosphate translocation in both cases but that the diurnal cycle is not to be denied. In the evening, continued light operates in opposition to the diurnal change; in the morning light operates with it. Increased sugar content may be the principal factor increasing translocation provided that it is supplied through the phloem, but when applied through the nutrient solution it is without effect.

The behavior of the plants with respect to rubidium was somewhat different than with phosphate. Some of these differences have been previously noted; a much greater percentage of rubidium was translocated to the shoot under all conditions, and the passage from night to day was accompanied

by large increases in both translocation and retention of the ion. Again, rubidium translocation was not accelerated by the coming of day to the same degree as was the case with phosphate. The change produced by applied sucrose was in the same direction as that produced by light, and since a linear relationship seems to exist between rubidium absorbed and rubidium translocated in the 6:00 A.M. to 10:00 A.M. period, it is unnecessary to postulate light factors other than photosynthesis to account for the acceleration of translocation induced by light. It should be noted that these low salt plants have translocated a much smaller percentage of phosphate than was the case in previous experiments. The reason for this is not known.

Discussion

This study shows that the probability that an ion entering the root symplast will be transported to the xylem depends on at least the following factors: (a) the time of day, (b) the species of ion concerned, (c) the salt status of the plant, and (d) light.

The reasons for a diurnal fluctuation in root activity are no more obvious in these experiments than they are in exudation studies to which one would expect them to be related if exudation is largely an osmotic phenomenon (4). For the present it must suffice to recognize that the root symplast possesses some inherent capacity to pass greater proportions of absorbed ion into the xylem during the day. This change is particularly true in the case of phosphate. With rubidium, the coming of day brings increases in both the amount of ion retained in the root and in the amounts translocated to the shoot, but the latter shows the greater increase. It is curious, too, that the change favoring rubidium translocation should begin before the coming of day and last throughout the light period, while increases in per cent. of phosphate translocated begin with the day and fall off in the afternoon. There is no obvious explanation for this difference in behavior.

The results obtained here are not in accord with the findings of HOAGLAND (10) and BROYER (5) that higher salt plants pass proportionately more ion to the xylem during the initial stages of absorption. It was in the low salt plants that the greatest percentage of absorbed ion was translocated to the shoot, particularly with phosphate during the day. It is conceivable that in the first hour or two of absorption the high salt bean roots might have passed proportionately more phosphate and rubidium to the xylem, but if this were the case the low salt roots must have had a very great acceleration of transport across the symplast in the latter portion of the four-hour absorption period in order to end the period with a greater amount translocated. It seems more likely that the low salt roots were more active in ion translocation from the beginning of the absorption period. Since Hoagland and Broyer did not actually compare plants cultured with different amounts of available salt, but rather low salt plants that had, and had not, been allowed to accumulate some salt in a previous absorption period, their experiments are not strictly comparable with these.

It is difficult to say with certainty that the effect of light on increasing ion translocation to the xylem is due solely to an increased supply of metabolite to the root. These experiments, however, suggest that increasing the root activity by increasing the root sugar content via the phloem serves to increase the percentage of absorbed ion translocated to the shoot. It is possible that light might have other entirely different effects tending to increase translocation, but this study is not adequate to disclose them.

Summary

A diurnal variation has been found to exist in the ability of Red Mexican bean roots to translocate absorbed rubidium and phosphate to the shoots. The maximum translocation occurs about midday, the minimum about midnight. The source of variation appears to reside in some obscure property of the root cytoplasm.

Superimposed upon the diurnal variation are effects due to the metabolic status of the roots. Within any one period of the day, those roots that absorb the most ion by reason of an adequate supply of metabolite, a low-salt, high-sugar nutritional status, or possibly some unknown factor associated with illumination, will also translocate a greater percentage of the ion to the shoot.

Rubidium and phosphate demonstrate quite different behavior in the bean plant. Both ions are absorbed in greater quantity during the day as compared to night, but the increase in phosphate absorption is largely accounted for by increased translocation to the shoot, while the increase in rubidium absorption is due to both increased translocation and accumulation within the root. Greater percentages of rubidium are translocated to the shoot than is the case with phosphate, and the daily increase in percentage of ion translocated begins earlier and continues longer with rubidium. The low salt nutritional status produces a much greater increase in the absorption and translocation of phosphate than it does of rubidium.

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LITERATURE CITED

1. ALBERDA, T. The influence of some external factors on growth and phosphate uptake of maize plants of different salt condition. *Rec. trav. botan. neerland.* **41**: 542-597. 1949.
2. VAN ANDEL, O. M., ARISZ, W. H., and HELDER, R. J. Influence of light and sugar on growth and salt intake by maize. *Proc. Kon. Ned. Akad. Wetensch. Amsterdam* **53**: 159-171. 1950.
3. ARISZ, W. H. Transport of organic compounds. *Ann. Rev. Plant Physiol.* **3**: 109-130. 1952.

4. ARISZ, W. H., HELDER, R. J., and VAN NIE, R. Analysis of the exudation process in tomato plants. *Jour. Exp. Bot.* **2**: 257-297. 1951.
5. BROYER, T. C. Further observations on the absorption and translocation of inorganic solutes using radioactive isotopes with plants. *Plant Physiol.* **25**: 267-376. 1950.
6. BROYER, T. C. The nature of the process of inorganic solute accumulation in roots. In: *Mineral Nutrition of Plants*. E. Truog, Editor. Univ. of Wisconsin Press, Madison. Pp. 187-249. 1951.
7. CRAFTS, A. S. and BROYER, T. C. Migration of salts and water into xylem of the roots of higher plants. *Amer. Jour. Bot.* **25**: 529-535. 1938.
8. EATON, F. M. and JOHAM, H. E. Sugar movement to the roots, mineral uptake, and the growth cycle of the cotton plant. *Plant Physiol.* **19**: 507-518. 1944.
9. FORSEE, W. T. Determination of sugars in plant materials. *Ind. and Eng. Chem., Anal. Ed.* **10**: 411-414. 1938.
10. HOAGLAND, D. R. Salt accumulation by plant cells, with special reference to metabolism and experiments with barley roots. *Cold Springs Harbor Symp. Quant. Biol.* **8**: 181-194. 1940.
11. LUNDEGARDH, H. The translocation of salt and water through wheat roots. *Physiol. Plant.* **3**: 103-151. 1950.
12. LUTTKUS, K. and BÖTTICHER, R. Über die ausscheidung von aschenstoffen durch die Wurzeln. *Planta* **29**: 325-341. 1939.
13. OVERSTREET, R. and DEAN, L. A. The availability of soil anions. In: *Mineral Nutrition of Plants*. E. Truog, Editor. Univ. of Wisconsin, Madison. Pp. 79-105. 1951.
14. OVERSTREET, R. and JACOBSON, L. Mechanisms of ion absorption by roots. *Ann. Rev. Plant Physiol.* **3**: 189-206. 1952.
15. ROBERTSON, R. W., WILKINS, M. J., and WEEKS, D. C. The effects of 2,4-dinitrophenol on salt accumulation and salt respiration. *Australian Jour. Sci. Res., Series B.* **4**: 248-264. 1951.
16. SÄID, H. Effect of phosphorus on the hydrolysis and absorption of sucrose by plant cells. *Nature* **162**: 496. 1948.
17. SKOOG, F., BROYER, T. C., and GROSSENBACHER, K. A. Effects of auxin on rates, periodicity, and osmotic relation in exudation. *Amer. Jour. Bot.* **25**: 749-759. 1938.
18. STREET, H. E. and LOWE, J. S. The carbohydrate nutrition of tomato roots. II. The mechanism of sucrose absorption by excised roots. *Ann. Bot. N.S.* **14**: 307-329. 1950.
19. WENT, F. W. and CARTER, M. Growth response of tomato plants to applied sucrose. *Amer. Jour. Bot.* **35**: 95-106. 1948.
20. WIERSUM, L. K. Transfer of solutes across the young root. *Rec. trav. botan. neerland.* **41**: 1-79. 1946-1947.