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Function of Bean Roots and Stems in Sodium Retention [1, 2]

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Sodium is excluded from the tops of many plants (1–4, 7, 8), and hence accumulation of this ion in the leaves of such plants which apparently are rather sensitive to it (2) is avoided. The barrier to sodium transport is effective at moderate external sodium concentrations. At high external concentrations (2, 7, 8), and as a result of prolonged growth in a saline medium (1), sodium contents are found to increase in the stems and sometimes also in the leaves of so-called Na nonaccumulator plants. Gauch and Wadleigh (4) assumed that sodium movement might be restricted by the membranes of certain extrastelar tissue, excluding it from the vascular system. Huffaker and Wallace (8) suggested that the term Na nonaccumulator plant should be considered as a quantitative rather than a qualitative expression. Bernstein et al. (1) found comparable levels of sodium accumulation in the roots, wood, and bark of young apricot trees grown for one year in saline plots; only the leaves and twigs of these plants exhibited restricted sodium accumulation. The authors supposed that sodium had entered the vascular tissue of the trees and that its translocation into the leaves via the transpiration stream had been restricted by retention in the living wood parenchyma cells. During the third year of these experiments sodium levels increased also in the leaves of various stone-fruit trees. Bernstein et al. (1) consider this increase to be due to the development of heart wood during the third year of growth, release of sodium by the senescent parenchyma and its subsequent translocation into the leaves via the transpiration stream.

No direct proof has been brought forward for any of the hypotheses regarding the location of the barrier to sodium transport and the mechanism of its rupture at high external concentrations. The interpretation proposed by Bernstein et al. (1) for sodium leakage to the leaves during the third year of their experiment would not be adequate to explain the gradual advance of sodium in the stem with increasing external concentrations as found in brief tracer experiments (7, 8). The present work is an attempt to supply more data about sodium transport and distribution in bean plants—a Na nonaccumulating species—in order to provide evidence for the elucidation of these phenomena.

Materials and Methods

Brittle wax bean seeds (*Phaseolus vulgaris* L.) were germinated in vermiculite at 25° and grown at this temperature in a light chamber in an aerated 0.1 Hoagland solution—1 (5). Plants with primary leaves fully expanded were then transferred for 24 hours to various experimental solutions. Sodium transport was studied in both intact and derooted plants. For the latter experiments the roots were detached under water before the plants were transferred to the experimental solutions. Except for the composition of the external solution, experiments were carried out under the same conditions as previous cultivation. The plants were hermetically fitted into beakers, dipping only with part of their roots or, in the case of derooted plants, with the basal centimeter of their stems, in the experimental solution. Water loss thus occurred only through the plant and was measured whenever necessary by weight difference, with an accuracy of $\pm$ 0.025 g.

Experimental solutions contained 2×10^{-4} M $CaSO_4$ and NaCl at desired concentrations labeled with 0.2 μc per ml of Na^{22} and/or Cl^{36}. Na^{22} was supplied by the Radiochemical Centre, Amersham, England, as NaCl and was diluted with stable NaCl to the required activity. Chlorine-36 was procured

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from the Oak Ridge National Laboratory, as HCl; this was neutralized with NaOH and also diluted with stable NaCl to the required activity.

At the end of the experimental periods the roots or stem base, respectively, were rinsed in water and 0.05 M NaCl, and the plants were then immediately prepared for autoradiography or counting.

In preparation for autoradiography the plants were blotted dry, placed between 2 sheets of lens paper, then between 2 additional sheets of filter paper and ironed dry with a hot electric iron as recommended by Wybenga (10). The dry plants were autoradiographed using Kodak X-ray film which was placed on the lens paper; an exposure time of 24 hours was adequate.

For more accurate assay of sodium distribution, plants were dissected into various segments: roots were separated from the plant in the case of whole plants, the basal 10 cm of the stem were cut into 1.0-cm segments and the leaves were detached from the rest of the stem. The various plant parts were oven dried and weighed. The dry plant material was transferred to 3.5-cm diameter beakers. In order to assure uniform geometry the tissue was covered with 5 ml 0.1 M HCl and left overnight before counting Na^{22} disintegrations with a scintillation detector. For Na^{22} assay in aqueous solutions aliquots were dried or diluted to 5 ml and counted in the same kind of vessels. During the reported experiments Cl^{36} had to be determined only in solution; appropriate aliquots were spread and dried on planchets and counted with an end-window Geiger counter. Absolute amounts of Na or Cl were calculated by comparison with standard samples with known specific activity. When NaCl was labeled simultaneously with Na^{22} and Cl^{36} 1 ml aliquots of solution were prepared as for Cl^{36} estimation and counted with both the scintillation and Geiger counters. Previously the ratio of the integrations from a certain amount of Na^{22} counted by each counter was established. According to the counts obtained from the Na^{22} and Cl^{36} labeled sample with the scintillation counter its Na content was calculated; and using the above ratio the Geiger counts of the sample due to Na^{22} were also estimated. By substracting this estimate from the total Geiger counts those due to Cl^{36} were obtained (Cl^{36} irradiation is not detected by the scintillation counter while part of Na^{22} irradiation is detected by the Geiger counter).

Results

Distribution of Na^{22} and Cl^{36}. Autoradiographs of bean plants grown with their roots in either Na^{22} or Cl^{36} labeled 10^{-3} M NaCl (fig 1; A, B) showed that chlorine was distributed in the whole plant while almost all of the accumulated sodium was retained in the root and none was visible above the most basal part of the stem. When only the distal centimeter of the root tip was dipped during the experiment in the Na^{22} labeled NaCl solution, radioactivity was still uniformly distributed in the entire root system (fig 1, C). This result is interpreted to indicate free sodium movement in the roots. Such movement could have occurred in the roots' free space and can thus not be regarded as final proof for the absence of a barrier to sodium transport into the stele.

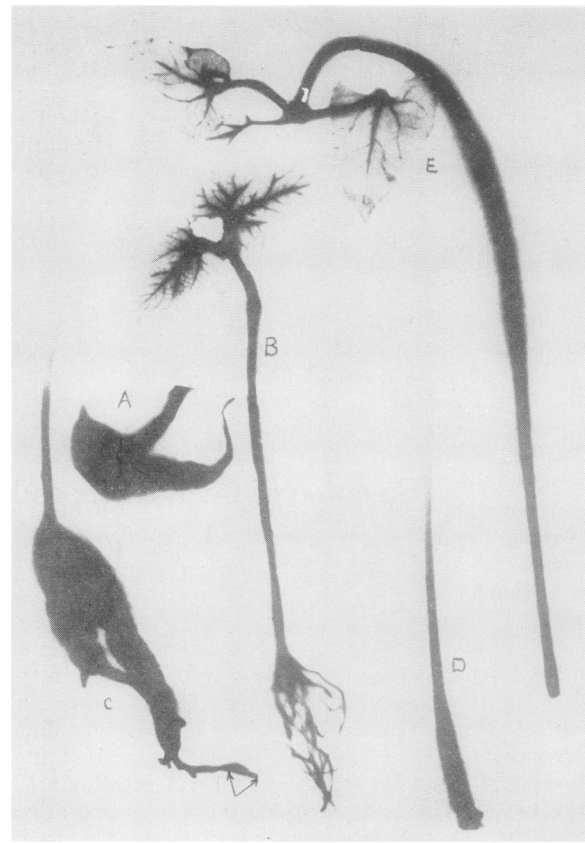


FIG. 1. Autoradiographs of bean plants grown for 24 hours in labeled 10^{-3} M NaCl. A: Intact plant, Na^{22}-labeled. B: Intact plant, Cl^{36}-labeled. C: Intact plant dipped in the solution only with root tip between arrows, Na^{22}-labeled. D: Derooted plant Na^{22}-labeled. E: Derooted plant Cl^{36}-labeled.

Experiments with derooted plants provide more evidence. Autoradiographs of such bean plants whose stems had been dipped in either Na^{22} or Cl^{36} labeled NaCl did not differ in the main from those of intact plants (fig 1; D, E). Chlorine was again distributed throughout the whole plant while sodium was retained in the lower part of the stem.

To determine whether sodium and chloride uptake from the external solution by derooted plants proceed at equal rates, stem-bases were kept in 7 ml of 10^{-3} M NaCl labeled with both Na^{22} and Cl^{36}. During the experiment about 3 ml of water were lost by transpiration. Due to the relatively large decrease in volume it should have been possible to detect differences in the rates of uptake by determinations of external Na^{22} and Cl^{36} concentrations. No significant change in concentrations was found during the experiment (table I). It seems that sodium and chloride ions enter the stem of derooted plants with the trans-

Table I

Na^{22} and Cl^{36} Concentration in NaCl Solution Labeled with Both Isotopes, before and after 24 Hours in Contact with Roots of Bean Plants

Measurement	cpm × 10^{-2}/ml solution	
	Na^{22}	Cl^{36}
Before contact	201	146
After 24 hours	202 ± 3	133 ± 24

piration stream and that sodium is then bound by the surrounding tissue.

More precise information about the distribution of sodium in the basal parts of bean plants was obtained from intact plants placed with their roots in Na^{22} labeled 10^{-3} M NaCl solutions when the plants were dissected at the end of the experiment and Na^{22} concentrations determined in the various sections. A linear decrease of the logarithm of labeled sodium concentration in the stem with distance from its base was found. The relation is expressed by the regression line $\log y = 1.33 - (0.42 \pm 0.05)x$ (y = Na concentration in μeq per gram dry tissue; x = distance from stem base in cm). A similar experiment with derooted plants resulted in the regression line $\log y = 2.60 - (0.35 \pm 0.02)x$. Each of these regressions was calculated using the combined data from 3 plants. The mode of sodium distribution as revealed in these experiments induced the supposition that upward leakage of sodium in plants grown at rather high sodium concentrations might be due to a gradual sodium saturation of some sites in the root and stem tissues. This assumption was tested in further experiments.

Salt Tolerance of the Bean Plants. Plants remained turgid and apparently healthy during one week in NaCl solutions with concentrations up to 6 × 10^{-2} M although growth was greatly impaired. At 7 × 10^{-2} M NaCl plants wilted severely within 24 to 48 hours. Gradual accommodation of plants to salt did not alter this limiting value of external NaCl concentration. According to these results 5 × 10^{-2} M was the highest concentration used in the following experiments.

Sodium Distribution at Various External Concentrations. Intact plants were transferred to 10^{-3}, 2 × 10^{-2} and 5 × 10^{-2} M Na^{22} labeled NaCl solutions and dissected at the end of the 24-hour experimental period. The pattern of sodium distribution in the stems at the higher concentrations differed from the logarithmic relation found at 10^{-3} M NaCl in the medium (fig 2). At 10^{-2} M NaCl the rate of the decrease of log sodium concentration in the stem increased with distance from the stem base. The regression approached linearity only from the seventh centimeter onward. The decrease of sodium concentration with distance from the stem base was found to be still more gradual at an external NaCl concentration of 5 × 10^{-2} M. Similar experiments were carried out with derooted plants; the external NaCl concentrations chosen were 10^{-3}, 10^{-2} and 5 × 10^{-2} M. The sodium front ascended much higher in the stems of these plants with rising NaCl concentration (fig 3) than in the stems of intact plants. In fact at an external NaCl concentration of 5 × 10^{-2} M sodium was found to be uniformly distributed throughout the whole stem. At both 5 × 10^{-2} and 10^{-2} M NaCl in the medium sodium leaked to the leaves, in the 5 × 10^{-2} M NaCl treatment this constituted 27 percent of the total sodium absorbed from the solution (table II).

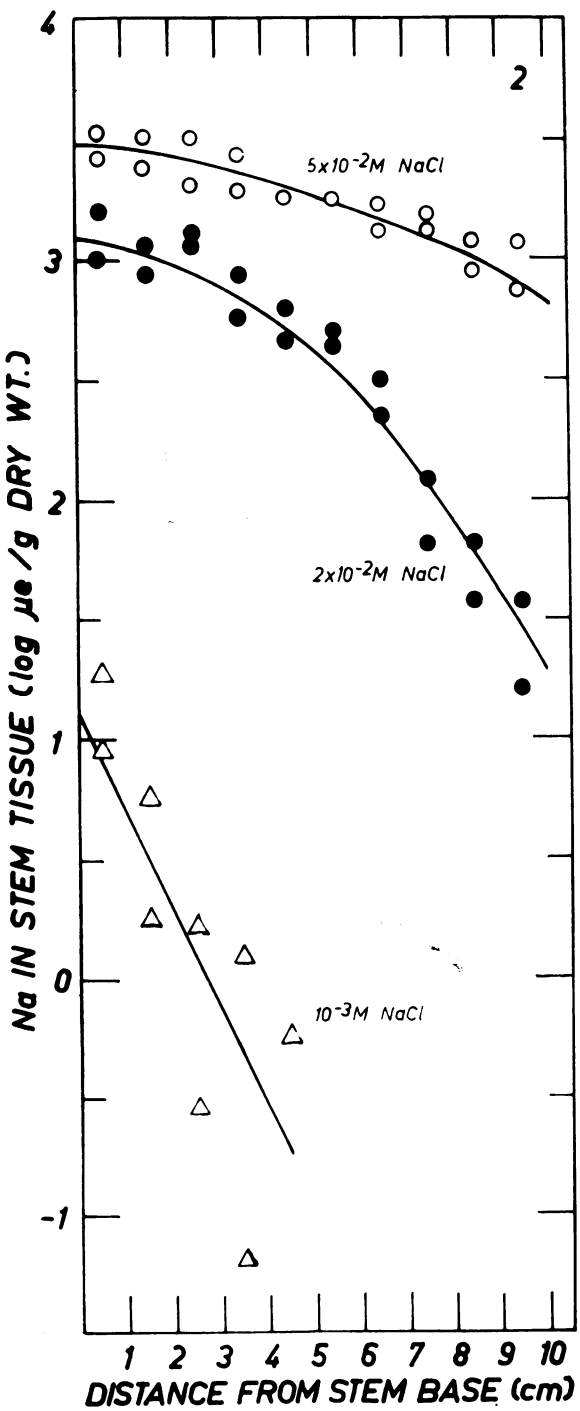
In an additional experiment the distribution of Na^{22} labeled NaCl in plants previously grown in a medium containing NaCl at a high concentration was compared to the distribution in nonpretreated plants: one group of plants was grown for a week in a 0.1 Hoagland solution which was changed daily. A second group was grown in the same basal nutrient medium but with the addition of increasing amounts of NaCl. The saline medium was started with a NaCl concentration of 5 × 10^{-3} M; this was increased to 10^{-2} M after one day and then by daily increments of 10^{-2} M to a final concentration of 5 × 10^{-2} M. Each group of plants was then transferred to Na^{22} labeled 10^{-3} M NaCl and after 24 hours the plants were dissected and counted. In NaCl pretreated plants the additional labeled sodium was found to be retained less in the roots and the basal part of the stem than in non-pretreated ones (table III). The sodium front accordingly also ascended much higher in the stems of pretreated than of non-pretreated plants.

Discussion

Free sodium movement in the roots of bean plant was demonstrated by the uniform sodium distribution in the roots of plants when only the root tip was in contact with the labeled medium. Such movement could have occurred in the free space, and therefore more direct evidence for the nonexistence of a specific barrier to sodium transport into the vascular tissue was obtained with derooted plants. In such plants sodium was retained in the stem base in spite of direct contact between the solution and the vascular system. A parallel experiment with Cl^{36} showed that only the sodium from NaCl is retained in the stems of derooted plants as it is in intact ones. The results presented thus appear to refute the assumption that sodium retention is due to its exclusion from the vascular tissue (7). Sodium accumulation from the vessels by the surrounding living tissue as suggested by Bernstein et al. (1) seems to be indicated.

An inverse logarithmic relation between sodium content of the stem and distance from its base was found at low external NaCl concentrations. This relation suits Horwitz's (6) model for flow through a pipe with irreversible loss through the walls. When external sodium concentrations were increased a divergence from the logarithmic relation was obtained. This situation is displayed particularly in experiments with derooted plants, where sodium was found to be distributed uniformly in the basal five centimeters of the stem at an external NaCl concentration of

10^{-2} M, and in the whole stem at 5×10^{-2} M. Apparently sodium-binding sites in root and stem tissue approached a saturated state, hence the vascular sap was not depleted of sodium ions and these ascended with the transpiration stream. Such gradual saturation of binding sites seems to explain the often recorded sodium leakage to the tops of nonaccumulator plants at high external concentrations (2, 7, 8). This may also be one reason for sodium accumulation in the leaves of stone-fruit trees after prolonged



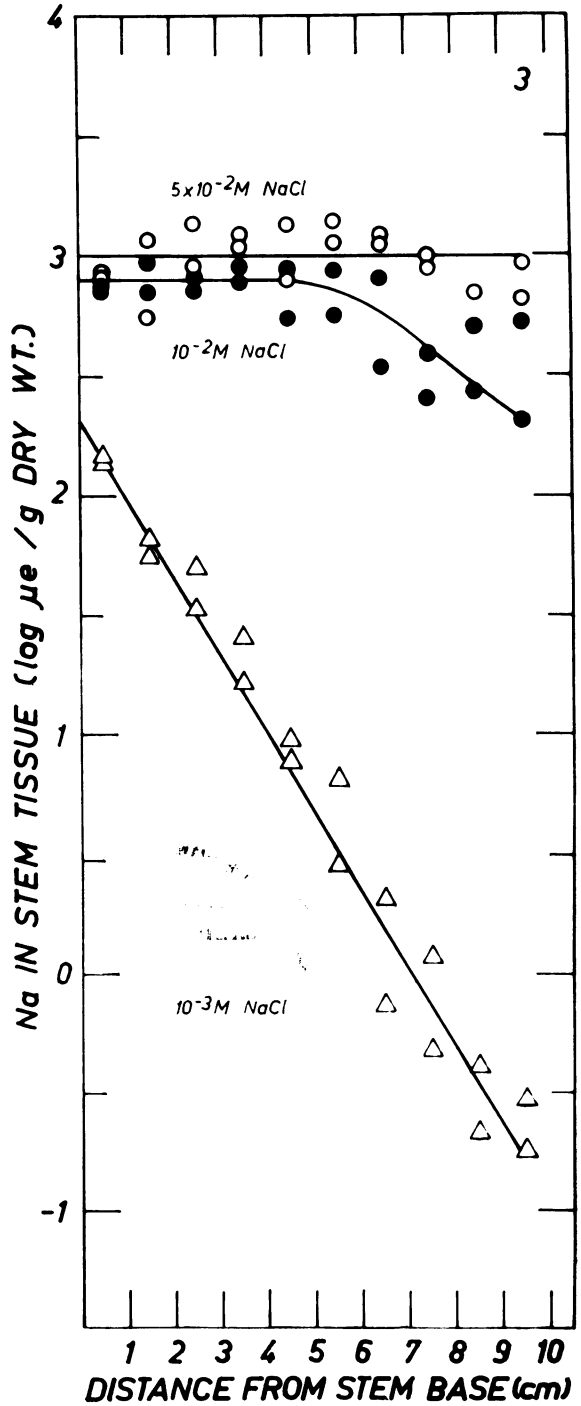


FIG. 2 (*left*). Sodium distribution along stems of intact bean plants grown for 24 hours with their roots in contact with 10^{-3} M, 2×10^{-2} M and 5×10^{-2} M NaCl.

FIG. 3 (*right*). Sodium distribution along stems of derooted bean plants grown for 24 hours with their stem bases in 10^{-3} M, 10^{-2} M and 5×10^{-2} M NaCl.

Table II

Distribution of Na^{22}-labeled Sodium in Derooted Bean Plants Grown for 24 Hours with Their Stem Bases in Na^{22}-labeled NaCl Solutions of Various Concentrations

Plant part	μeq labeled Na			% of total uptake		
	10^{-3} M NaCl	10^{-2} M NaCl	5×10^{-2} M NaCl	10^{-3} M NaCl	10^{-2} M NaCl	5×10^{-2} M NaCl
Basal 10 cm of stem	4.70	31.1	71.5	99.6	80.5	31.7
Rest of stem	0.02	7.4	92.5	0.4	19.2	41.2
Leaves	...	0.1	61.0	...	0.3	27.1

Table III

Distribution of Na^{22}-labeled Sodium in Bean Plants

The bean plants were grown for 24 hours with their roots in Na^{22}-labeled 10^{-3} M NaCl and previously grown for one week in 0.1 Hoagland solution with (NaCl pretreated) or without (controls) unlabeled NaCl.

Plant part	μeq labeled Na		% of total uptake	
	NaCl pretreated	Controls	NaCl pretreated	Controls
Roots	2.15	4.12	61.6	98.7
Root-stem transition zone (1 cm)	0.46	0.04	13.4	0.8
Stem 2nd cm	0.39	0.02	11.3	0.4
Stem 3rd–5th cm	0.43	< 0.01	12.6	0.1
Rest of stem	0.04	...	1.1	...

growth in a saline medium as reported by Bernstein et al. (1).

Although the roots of bean plants seemingly do not contain any specific barrier to sodium movement, they fulfill an important function in sodium retention. This conclusion is borne out by a comparison of sodium distribution in intact and derooted plants. The same may be concluded from the relative Na^{22} distribution in plants pretreated with unlabeled NaCl and in non-pretreated ones. Sodium retention by bean roots appears to be a result of competition with sodium transport to the stele rather than of its prevention. This competition is due to sodium binding by sites bordering its path to and in the stele. No similar retention of K, Ca or Mg in Na-nonaccumulator plants has been reported (3, 9); thus the binding of sodium ions in the basal parts of such plants seems to be Na-specific.

Summary

Autoradiographs of bean plants (*Phaseolus vulgaris* L.) show that Na^{22} is retained in the basal parts of the plant. This is found in intact plants with only their roots in contact with the labeled solution, as well as in derooted plants whose stem bases are dipping in the radioactive solution.

At low external NaCl concentrations the logarithm of sodium content in the stems is inversely related to distance from the stem base. With increasing external NaCl concentrations a gradual sodium saturation of the stem tissue seems to occur and sodium distribution in the plant approaches uniformity.

It is concluded that sodium retention in bean plants is due to competition of root and stem tissue with sodium transport to and in the stele rather than to its prevention.

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