Ion Absorption by Shoot Tissue: Technique and First Findings with Excised Leaf Tissue of Corn[1, 2]

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Roots are the principal organs by which terrestrial higher plants initially absorb inorganic solutes from their substrates. Root tissues have therefore been the favorite experimental objects for the study of cellular mechanisms of ion absorption, and many important points have been established concerning this process: dependence on respiratory metabolism, achievement of high accumulation ratios, selectivity, characteristic concentration isotherms, and others.

Nevertheless, the acquisition of exogenous ions by root tissue is only the first step in the chain of events which are summed up in the term plant nutrition. Ions having traversed the cortex eventually are delivered into the nonliving conducting system of the xylem and carried therein upwards to the shoot. Before these ions can participate in essential metabolic processes and biochemical reactions within the cells of leaves they must be absorbed by these cells from the solution bathing them. The solution delivered by the xylem into the extracellular fluid of the leaf becomes the inorganic nutrient substrate of the leaf mesophyll cells—the equivalent of the soil solution and its extension, the solution in the outer space, for the cells of roots.

There is no body of information on the processes of ion absorption by the cells of the shoot, and especially the cells of leaves of terrestrial higher plants, comparable to that existing for root and tuberous tissues. Reference is made, however, to the studies of Arisz and his associates of ion transport in leaves of the aquatic plant, Vallisneria (1), and to the work of Kylin on absorption of sulfate by leaf tissue of several terrestrial plant species (6). Do the metabolic processes of ion absorption by shoot cells resemble those familiar from the study of excised roots? Is the selectivity of the plant a function of the selective mechanisms of root cells only, or do cells of shoot tissue exhibit similar (or different) patterns of selectivity? It is to these and related questions that the present investigation addresses itself.

Materials and Methods

Culture of Plants. Seeds of corn *Zea mays* 'DeKalb 805' were placed in a 1-liter flask in which they were thoroughly rinsed with repeated changes of demineralized water. The flask was then filled with water and placed in a dark cabinet at room temperature (about 24°), with aeration. After 19 hours the seeds were repeatedly rinsed and replaced in water. After 5 more hours the seeds were again rinsed and then planted on cheesecloth supported over 0.2 mM $CaSO_4$ solution and replaced in the dark cabinet. Two days after planting the germinating seeds were rinsed and the $CaSO_4$ solution replaced with fresh solution. Four days after planting the seedlings were individually transplanted to 4-liter containers with nutrient solution consisting of 1.0 mM $Ca(NO_3)_2$, 0.5 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 4.0 μM $FeSO_4$, and micronutrients (5). In addition, each plant received 4 μmoles of $FeSO_4$ twice weekly and K as described below. Water in the containers was brought up to level twice weekly. Immediately after transplanting, the seedlings were transferred to a growth chamber where they grew until they were used at the age of 22 days. The photoperiod was 14 hours and the light intensity about 3000 ft-c. Day and night temperatures were 25° and 15°, respectively.

In this type of study it is customary to use a tissue which has a low content of the ion being studied. In the experiments presented here, Rb was the substrate ion investigated. It was surmised that this element would behave much as an analog of K in leaf tissue as it is known to do in root tissue. For this reason, it was considered desirable that the K content of the tissue be as low as possible. The production of leaf tissue low in K was managed by the addition of small amounts of KNO_3 at frequent intervals to the culture solution. The total KNO_3 added throughout the growth period of 22 days was 550 μmoles.

Experiments. The leaf tissue used for experiments came from the 3 youngest leaves of each plant. The tissue was prepared by cutting it into either discs or slices which were immediately floated on demineralized water. Experimental samples always consisted of a definite number of pieces, selected at random from the total supply. The number of pieces per sample was such that the fresh weight of the sample was on the order of 100 mg. The total elapsed time between cutting and beginning of the experiment was 3 hours during which time the tissue was constantly in water. During this time the discs or slices for each sample were counted out and then rinsed. Discs were rinsed by transferring them to

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a perforated glass basket in which they were dipped repeatedly in changes of water followed by 1 minute of rinsing in running water. They were then transferred for 1 hour to a glass tray of water at the temperature of the experiment. Slices of tissue were enclosed in cheesecloth bags as described earlier in connection with work on excised roots (4), except that the cheesecloth was of a finer weave. They were washed by dipping in changes of water, then immersed in aerated water at the experimental temperature for one hour.

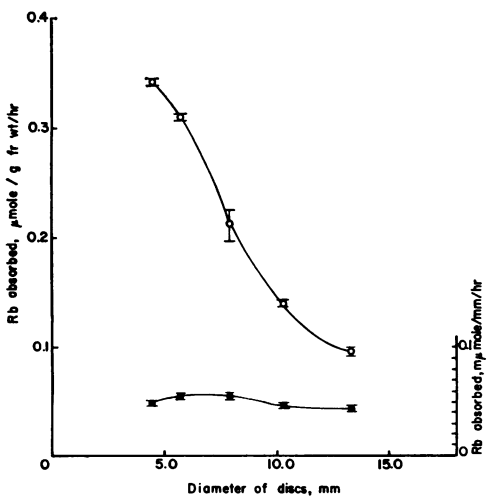
The experimental procedure consisted of transferring each sample to an aerated experimental solution for a predetermined time. All experimental solutions contained RbCl at 0.02 mM, labeled with Rb^{86}, and $CaCl_2$ at 0.5 mM. The pH, unbuffered, lay between 5.2 and 5.8. Differences in pH in this range were without effect on the results obtained. Except where otherwise noted, the experimental temperature was 30°. At the conclusion of the absorption period, each sample was removed from the solution, rinsed for 1 minute in 3 changes of washing solution, and then transferred to an aerated desorbing solution for 30 minutes in order to remove reversibly adsorbed Rb ions and those in the outer space. Washing and desorbing solutions contained $CaCl_2$ at 0.5 mM concentration, and KCl. The KCl concentration of the desorbing solution was 2.0 mM for the experiments with discs, and 5.0 mM when slices were used. Temperature of the washing and desorbing solutions was 20° for discs, 10° for slices. (These differences in the concentration and temperature of the desorbing solutions were found to be without effect on the results obtained.) After desorption the samples were rinsed for 1 minute in distilled water at room temperature to remove excess salt of the desorbing solution. The samples were then transferred to planchets and ashed at 550°. The ash was moistened, treated with a spreading solution, and dried. Samples were counted with a thin-window G-M gas-flow tube at least 3 times to a minimum of 1280 counts each time. Replicate samples were blotted and weighed.

Results

Leaf Discs. When leaf discs of different sizes were used, the uptake of Rb on a fresh weight basis varied inversely with the diameter of the discs as shown in figure 1. If the uptake by the same discs was plotted on the basis of unit length of exposed edge, then the various sizes of discs showed approximately the same rates of uptake. Thus it appeared that the entry of ions was limited by the length of exposed edge.

Circular Bands. In order to increase the ratio, length of edge to amount of tissue, circular bands of tissue were prepared by cutting discs and then cutting out the center portion of each disc using a smaller cork borer. Various size combinations of cork borers were used to vary the width of the bands. The rate of uptake was again inversely related to the width of the band and thus limited by the length of the cut edge of the tissue.

Slices of Tissue. Practical mechanical difficulties in cutting circular bands of tissue narrower than 1.7 mm led to abandoning this method of preparing tissue in favor of cutting thin slices. Long strips of leaf tissue, 12 mm wide, were mounted in a hand microtome and oriented so that the slices would be cut at right angles to the length of the leaf. Thus the slices would be 12 mm long, and their width could be determined by the setting of the advance mechanism of the microtome. When slices whose widths varied from



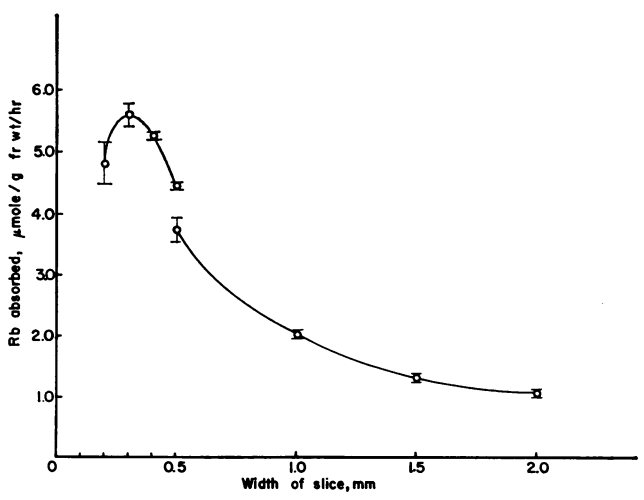


FIG. 1. Absorption of Rb by corn leaf discs of varying diameters. Open circles, absorption on a unit weight basis (left ordinate); black dots, absorption on a basis of unit length of edge (right ordinate). Circles and dots are averages of duplicates indicated by upper and lower ends of vertical lines. Experimental conditions, 1-hr period, 0.02 mM Rb*Cl, 0.5 mM $CaCl_2$, volume, 500 ml, 30°, aerated. Note difference in units on left and right ordinates.

FIG. 2. Absorption of Rb by slices of corn leaf tissue of varying widths. Data from 2 experiments, each indicated by a separate line. Symbols and conditions as for figure 1.

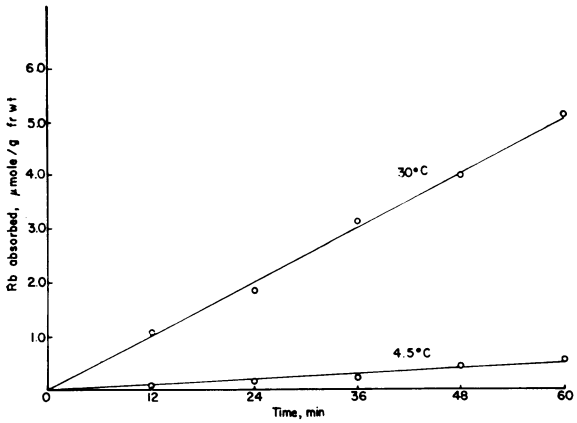


FIG. 3. Absorption of Rb by slices of corn leaf tissue 300 μ wide. Except for time, the temperature of the cold run, and the volume of solution (1000 ml in this experiment), conditions as for figure 1. Data reported represent unreplicated samples.

2000 μ down to 200 μ were used, the uptake on a weight basis reached a maximum near 300 μ as can be seen in figure 2. On the basis of these results, slices of leaf tissue 300 μ wide have been selected as experimental objects in experiments with corn.

Time Course of Absorption at Different Temperatures. Figure 3 shows the time course of absorption of Rb by slices of leaf tissue 300 μ wide. As can be seen from this graph the process is linear with time both at 30° and at 4.5°. If one assumes that 1 g of tissue is equivalent to 1 ml of water, the accumulation ratio attained in 1 hour at 30° is 250: 1. The rate of absorption is sharply decreased by reduced temperature. The rate at 4.5° was 8.6 % of the rate at 30°.

Discussion

The larger the size of the disc or tissue slice, the lower is the rate of absorption when expressed on the basis of unit weight of tissue (fig 1, 2). This indicates that in the larger pieces of tissue a progressively greater percentage of the cells fails to participate in absorption; only those cells near the cut edges absorb ions from the ambient solution. This conclusion is borne out by the finding that when expressed on the basis of unit length of cut edge, absorption varies little in discs or slices of different sizes.

The relationship discussed above between the size of the tissue slices and the rate of absorption parallels that found by Steward and Harrison for absorption of Rb and Br by potato discs (7). These workers concluded from experiments with discs of varying thickness that active absorption of the ions was confined to the layers of cells near the cut surfaces of the discs.

It was desired to use slices so narrow that all cells capable of ion absorption would in fact be participating in absorption. This condition would prevail when the width of the slices had been reduced to the point where absorption, expressed on a unit weight basis, no longer increased with decreasing width of the slices. Figure 2 shows that this condition was approximated at a width of 300 μ. Further reduction of the width of the slices caused the absorption per unit weight of tissue to decline. The reason for this is thought to be that in such narrow slices a significant proportion of the cells present is cut or otherwise damaged.

The characteristics of Rb absorption by corn leaf tissue as revealed in the experiment shown in figure 3 resemble those of absorption of this ion by barley roots (2, fig 1). Absorption is a linear function of time, extrapolating to zero absorption at zero time. Trivial, nonmetabolic uptake by superficial cation exchange does not contribute to the absorption measured in the experiment because of the presence of excess Ca in the experimental solutions and the desorption of any readily exchangeable Rb* ions by K after the absorption period (2, 4). The rate of absorption at 4.5° was 8.6 % of that measured at 30°. The corresponding value for Rb absorption by barley roots was 10 % (2). Even actual rates of absorption, on a basis of unit weight of tissue, are very similar in excised corn leaf tissue and excised barley roots. The rate of absorption in the experiment shown in figure 3 was 5.0 μmole Rb absorbed per gram fresh weight per hour, at 30°. The corresponding rate of absorption by barley roots from a solution of the same concentration (0.02 mM) was 5.8 (3, fig 2).

There has been much interest recently in foliar application of nutrients (8). In this technique nutrients are applied directly to the leaves of plants, by spraying the leaves with appropriate solutions. Penetration of the solutes through the epidermis and its cuticular covering is a matter of foremost interest in experiments on foliar application. Penetration through the surface layer of the leaf does not appreciably contribute to absorption in the experiments reported here. The experiments showing the dependence of the rate of absorption per unit weight of tissue on the length of the cut edge demonstrate that the initial port of entry of the ions into the leaf tissue is almost exclusively the cut edge. What is measured here, then, is cellular absorption from the solution bathing the leaf cells, much as happens under natural conditions when ions delivered by the ascending xylem stream into the extracellular (cell wall) spaces of the leaf are absorbed by the cells of the leaf.

Summary

The significance of ion absorption by the cells of leaves in the over-all process of plant nutrition is discussed. A technique has been worked out for experiments on ion absorption by leaf tissue of corn. When leaf tissue is cut into discs it fails to absorb at its potential maximal rate because only those cells near the cut edge participate in absorption. When the tissue is cut into slices 300 μ wide this limitation is avoided.

It was found that the process of cellular absorption of Rb in corn leaf tissue resembles that in excised roots of barley in the following respects: absorption at the concentration used is a linear function of time for at least 1 hour; the accumulation ratio reached in 1 hour, with an external concentration of 0.02 mM Rb, is over 250: 1; the rate of absorption at 4.5° is 10 % or less of the rate at 30°.

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Transport and Distribution of Auxin during Tropistic Response
II. The Lateral Migration of Auxin in Phototropism of Coleoptiles [1, 2]

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Since the pioneering experiments of N. G. Cholodny and F. W. Went (11, 12, 27; also see 28) it has been known that phototropic curvature in the asymmetrically illuminated oat coleoptile is associated with a difference between the amounts of auxin passing through the lighted and shaded sides. This was at first ascribed to lateral migration of auxin across the plant. No obvious decrease in the basipetal transport of applied auxin could be found. Another explanation was that some auxin is photolytically destroyed on the lighted side. This was supported by the frequent observation that total yields of endogenous auxin from phototropically stimulated plants were less than from dark controls (26, 27, 28) and also by much work on model systems (14, 23). Nevertheless, 3 lines of evidence point away from this interpretation.

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Firstly, at least in corn coleoptile tips, light causes no decrease in the total yield of auxin obtained in agar receiving blocks (7, 8). This was shown both for the "first positive" and "second positive" curvature (see 26), and discrepant results of other workers could be explained by the observation (6) that brief exposure of corn or oat coleoptiles to phototropically ineffective red light decreases the subsequent auxin production by the tips. Since most experimenters had not excluded red from their safe-lights, the decreased auxin yields which they reported do not necessarily constitute evidence for a role of auxin destruction in phototropism.

Secondly, calculations made by Bünning (9) and by Thimann and Curry (26) show that an incident photon causing the "first positive" curvature of Avena would have to be capable of destroying at least several hundred IAA molecules in order to account for the observed asymmetry. Such a high quantum yield certainly rules out a direct photolytic effect.

Thirdly, von Guttenberg (19) showed that although decapitated oat coleoptiles give only a very slight response to 3 hours' stimulation by unilateral