started. As shoot growth occurred, there was a decrease in percentage total nitrogen, followed by an increase in early fall. Total nitrogen content was closely associated with bud activity in tanweed rhizomes. Protein nitrogen in rhizome extracts was the only other character studied that was positively correlated with bud activity.

Peroxidase and polyphenoloxidase activities were closely correlated with one another and were highest during the period of greatest bud dormancy. Indoleacetic acid oxidase activity fluctuated considerably during the sampling period, but was lowest during July.

Bud activity, percentage total nitrogen, and protein nitrogen all were negatively correlated with peroxidase activity, polyphenoloxidase activity, and temperature. Activities of peroxidase, polyphenoloxidase, and indoleacetic acid oxidase were positively correlated with temperature.

Acknowledgments

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Literature Cited


Absorption of Fluoride and Chloride by Barley Roots

P. Venkateswarlu, W. D. Armstrong, and Leon Singer

Department of Biochemistry, College of Medical Sciences, University of Minnesota, Minneapolis, Minnesota

The tissues of some plants contain unusual amounts of fluoride. The leaves of ornamental camellias were found by Zimmerman, Hitchcock, and Gwirtsman (16) to contain 790 to 3060 mg fluoride per kg dry weight. These workers found commercial tea to have a fluoride content of 72 to 115 mg per kg and 1530 mg fluoride per kg was found in 1 sample of leaves of tea grown in a greenhouse. Our analyses of commercial tea gave results of 140 to 300 mg fluoride per kg dry weight. Some samples of gladiolus, tomato, rose and pine leaves were found (11) to contain 230 to 949 mg fluoride per kg of dry weight. Tissues of most other plants contain 0.1 to 10 mg fluoride per kg dry weight (3). Fluoride is present in soil in concentrations ranging between 37 and 1460 mg per kg (13). However, the fluoride content of the soil in which plants grow does not determine that of plants. Venkateswarlu (14) found tea leaves to contain 836 to 1300 mg fluoride per kg of dry leaves while the leaves of 16 other kinds of plants grown in the same soil contained only 3.3 to 20 mg fluoride per kg.

Differences between plants in their ability to assimilate fluoride could be associated with variations of the mechanisms of absorption of ions by the roots. This paper describes marked quantitative differences in chloride and fluoride absorption by barley roots and evaluates the mechanisms of absorption of these halogen ions by physical diffusion, exchange and me-
Elzam, Rains, and Epstein (4) investigated chloride absorption by barley roots. The absorption kinetics observed in 20-minute absorption periods with solutions of low chloride concentration (0.005–0.2 mM) were markedly different from those obtained with 0.5 to 50 mM chloride solutions. The results indicate carrier sites in the roots of high chloride affinity which operate at low chloride concentrations and that other sites, of lower affinity, come into play at the higher chloride concentrations. Bőszeoményi and Cseh (1), by use of chemical analytical methods, investigated the uptake of fluoride, bromide, chloride and iodide by roots and shoots of wheat seedlings from solutions which had single halide ion concentrations of 50 meq per liter. The rates of uptake in a 24-hour period were in the order: Br>Cl>I>F in both parts of the plants. Chloride uptake by the roots exceeded fluoride accumulation by 8.1 times. In the work reported here the difficulties and inaccuracies of chemical determination of small quantities of fluoride have been avoided by use of radioactive fluoride.

Materials and Methods

The methods used were essentially those described by Epstein and Hagen (8) except that distilled water instead of calcium sulfate solution was used in germination of the barley seeds (Hordeum vulgare L.) to eliminate chemical interaction between calcium and fluoride ions. Fresh excised roots in 0.5 g amounts were incubated at 27°C to 29°C in 20 ml volumes of solution containing known and equal concentrations of sodium chloride and sodium fluoride. The chloride was labeled with radiochloride (Cl36) and the fluoride with radiofluoride (F18). In some experiments the solvent was labeled with tritium as THO. The solutions were continuously aerated, except when anaerobic conditions were imposed, when nitrogen was used.

To follow the time course of the absorption of the ions the roots were removed from the solutions at time intervals up to 4 hours and immediately blotted between absorbent cotton sponges. Desorption of the ions was effected by placing the blotted roots, after a 3-hour absorption period, in 20 ml volumes of (a) distilled water, or (b) a solution which was 0.1 meq per liter with respect to nonradioactive chloride and fluoride. The desorption media were stirred with air and desorption was interrupted at intervals from 2 minutes to 2 hours. Particulars as to the time periods and ionic concentrations of the solutions are given in the figures and in the table.

The radioactivity measurements for Cl36 and F18 in the blotted roots from the desorption experiments were corrected for the determined radioactivity present in the desorption solution by subtraction of the following quantity: cpm/ml desorption solution times outer space volume (taken to be 0.25 ml/g fresh root, vide infra). This correction in the case of chloride was trivial and in the F18 measurements was only 0.6% of the F18 count of the roots. In the desorption experiments with the labeled water and metabolic inhibitors the roots were washed in a large volume of solution which was continuously renewed.

The effects of 10–3 M 2,4-dinitrophenol and 10–3 M sodium azide on the separate processes of absorption and desorption of chloride were examined in similar experiments. Absorption of chloride from 1.0 mM NaCl solutions in the presence of a metabolic inhibitor was determined at 2, 3 and 5 hours. These results are reported (table 1) as Cl36 content of the roots and are compared to the labeled chloride uptake, at the same time periods, from 1.0 mM NaCl solutions not containing an inhibitor. The roots used in the desorption studies with inhibitors had absorbed chloride from a 1.0 mM saline solution in the absence of a metabolic inhibitor. One set of the roots was blotted and examined without further treatment to give the quantity of absorbed Cl36 per unit weight of roots. Additional sets of roots, containing absorbed chloride, were washed for 0.5 or 1.0 hours with 1.0 mM NaCl solution or with 1.0 mM NaCl solution which was 10–3 M with respect to one of the metabolic inhibitors. The results of the experiments on desorption by solutions of metabolic inhibitors are given in table 1 and are compared with those obtained with saline desorption.

The standards of reference in the radioactivity

<table>
<thead>
<tr>
<th>Absorption time (hr)</th>
<th>Absorbed from 1.0 mM NaCl*</th>
<th>10–3 M Dinitrophenol in 1.0 mM NaCl</th>
<th>10–3 M Sodium azide in 1.0 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absorption experiments</td>
<td>Desorption experiments</td>
</tr>
<tr>
<td>2.0</td>
<td>750</td>
<td>670</td>
<td>720</td>
</tr>
<tr>
<td>3.0</td>
<td>2750</td>
<td>690</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>1160</td>
<td>360</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>2880</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>1050 (680)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>2100 (1610)**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 1.0 mM NaCl solutions of different Cl36 specific activity used in several experiments; this accounts for the variable Cl36 contents of the roots at the same absorption times. However, in a given experiment the labeled saline solutions, with or without inhibitor, had the same Cl36 specific activity.

** Indicates Cl36 content of the roots at several times. The percentage of absorption was calculated from the ratio of the counts of the blotted roots to the control roots.
measurements were aliquots of the initial solutions. Radiofluoride was measured with a well-type crystal scintillation counter and corrections for radioactive decay were made from the half-life of F$^{18}$ of 109.7 minutes (2). The F$^{18}$ counts of the roots were additionally adjusted for variations between samples in the measured height of the packed roots in the plastic counting tube. The correction to be applied was obtained from observations of the measured activity of a given amount of F$^{18}$ contained in different volumes of solution at measured heights in a plastic counting tube.

The moist weight of the blotted roots was obtained by weighing the tube plus root sample in a tared and stoppered plastic counting tube. After the F$^{18}$ count had been made the sample was dried at 110° to give the dry weight and it was ground to fine particles in an agate mortar. The powdered root samples were each mixed with a known weight of cellulose powder. Aliquots of the initial and ambient solutions from the absorption and desorption experiments were added to 5 ml of 4.5 % NaCl solution which was evaporated to dryness. Two hundred milligram amounts of the cellulose-root mixtures and of the NaCl powders were counted with a thin-window Geiger-Muller tube as a detector for Cl$^{36}$ content while contained in 1-inch diameter straight-walled counting dishes. This procedure made the factor of absorption of the beta-rays from Cl$^{36}$ uniform in all samples.

In the experiments involving tritium labeled water the blotted roots were immediately frozen in a small closed container. Falsely low results of the fraction of exchangeable root water were obtained when the roots were frozen in open containers due to condensation of water from the air. The container was attached to an inverted C-shaped tube of which it was a part. The apparatus was evacuated and closed while the roots were frozen. The roots were allowed to thaw and water was distilled at room temperature into the second limb of the apparatus by applying a dry-ice bath to the receiver. Tritium was measured by liquid scintillation counting.

**Results**

The root water reached a tritium content of 97 % of that of the ambient solution in 2 to 5 minutes and the quantity of exchangeable root water was not further increased in periods of time up to 3 hours. All of the labeled water was removed from the roots in 2 to 5 minutes by a stream of distilled water.

The results given on the ordinates of figures 1 to 3, indicating quantities of chloride and fluoride, were calculated from the radioactivity measurements for Cl$^{36}$ and F$^{18}$ in the roots and the fluoride and chloride specific activity of the initial solutions. These calculations require the assumption that the solution specific activities did not change during the course of the experiments. This assumption is justified by the circumstances that the seeds had been germinated for 5 days in distilled water. It would be expected that this treatment would have released the most minute traces of exchangeable fluoride and chloride from the root compartments. Furthermore, figure 5 demonstrates that equal quantities of chloride were desorbed by distilled water and by sodium chloride solutions. This observation is consistent with the conclusion that negligible quantities of bound chloride were available in the roots for exchange with Cl$^{36}$.

For the reason stated above the seeds were germinated in distilled water and calcium was not added to the ambient absorption solutions. Calcium is required by barley roots for normal cation absorption (7). The same roots absorbed bromide at an increased rate in the presence of 0.5 m$m$ CaSO$_4$ (15) but an increase of CaSO$_4$ concentration from 0.5 m$m$ to 10 m$m$ had no effect on chloride absorption by barley roots from solutions containing up to 10 m$m$ KCl (4). Foote and Hanson (10) found that marked depletion of soybean roots of calcium by pre-treatment with K-EDTA for 90 minutes reduced chloride accumulation and increased exchangeable chloride. Similar pretreatment for 30 minutes had no uniform effect on chloride transport by the roots.

Although the barley roots used in the experiments to be reported were not deliberately impoverished of calcium, the degree to which the quantitative aspects of chloride transport were affected by the circumstances of the experiments cannot be stated. Nevertheless, these circumstances did not prevent the exhibition of marked qualitative and quantitative differences between fluoride and chloride accumula-
Discussion

The results in figures 1 to 3 give the quantities of chloride absorbed by the roots and the chloride concentrations of the ambient solutions at the indicated time periods. Chloride was absorbed over extended time periods and against large concentration gradients. The ratios of concentration of chloride in root water to that in the ambient solutions at the 4-hour points were: 491 (fig 1), 217 (fig 2), and 11 (fig 3). The quantity of chloride transported increased with the chloride content of the solutions but not in a linear manner (fig 4). The decrease in rate of chloride absorption with time seen in figures 1 and 2 was probably a result of depletion of the solutions of chloride. If larger volumes of ambient solutions had been used depletion of the solutions of chloride, and the attendant effects on observed chloride absorption rates, would have been minimized (9).

The results obtained with fluoride transport were in marked contrast to those obtained with chloride. Fluoride absorption occurred quickly (fig 5) and did not increase significantly with time. Over 100 times as much chloride as fluoride was absorbed from solutions of initial 0.01 and 0.1 meq per liter halogen ion contents (fig 1, 2). The chloride uptake by the roots exposed to 1.0 meq per liter of the ions exceeded the fluoride uptake by 25 times (fig 3). There was a direct relationship between the fluoride contents of the root water and the solutions (fig 4) and fluoride was not absorbed against concentration gradients. The concentration ratios of fluoride in root water to solution water was of the order of 0.3 to 0.35.

The results reported in figures 1 to 3 suggest that processes requiring the expenditure of energy are required for absorption of chloride but not fluoride. Handley and Overstreet found (12) that chloride absorption by the vacuolated, but not the nonvacuolated, sections of roots of Zea mays to be metabolically determined. Figure 4 gives the results of chloride and fluoride absorption by barley roots from solutions of varied initial chloride and fluoride contents when the solutions were stirred with air or with nitrogen. Fluoride uptake was not affected by anaerobic conditions but the same condition markedly depressed the absorption of chloride. These results indicate that chloride transport in barley roots is associated with oxidative metabolic processes. The chloride absorbed in the experiments in which air was replaced by nitrogen can be considered to have resulted from (a) diffusion of chloride into the outer root space, and (b) chloride transported into the inner space to the extent permitted by the energy stores consequent upon the pre-experimental aerobic conditions of the roots.

Dinitrophenol uncouples oxidative phosphorylation and azide interfere with reoxidation of a cytochrome component of the electron transfer system in-
I. Involved mechanisms in involved further evidence on Dinitrophenol produced of chloride had exhaustion of over the roots containing dinitrophenol and Sodium azide. This observation supports inhibitor containing dinitrophenol roots alone.

The observation indicates that oxidative mechanisms are required for full retention of absorbed chloride in the root compartment into which chloride is transported by utilization of chemical energy.

There is evidence to indicate that fluoride and chloride are absorbed at different sites in barley roots. Elzam and Epstein (personal communication, O. E. Elzam and E. Epstein) found chloride absorption by barley roots from aerated solutions containing 0.05 M chloride and 0.5 M CaSO₄ at pH 5.4 and 30° not to be affected by the presence of up to 1.0 M fluoride or iodide. Bromide, under the same conditions, competitively inhibited chloride uptake by the roots and at a concentration of 1.0 M reduced chloride absorption to near zero. These results were interpreted by Elzam and Epstein to indicate that bromide and chloride are absorbed at common sites which have little affinity for fluoride and iodide ions. These recent observations of Elzam and Epstein and their interpretation are consistent with the findings reported in this paper of marked differences in quantitative aspects and of mechanisms of fluoride and chloride absorption by barley roots.

Some of the results can be interpreted in terms of outer and inner root spaces as described by Epstein (5, 6). Outer space is that volume of roots to which ions have free and ready access by diffusion while inner space is the region to which ions are transported by active mechanisms and from which they are not removed by diffusion or by exchange for ions in the ambient solution. Epstein evaluated (5) the outer space volume of barley roots through use of labeled SO₄⁻, SeO₃⁻, H₂PO₄⁻, and Ca⁺⁺ ions at 0.22 to 0.25 ml per g of fresh roots.

At the end of 2 hours only 1.4% of absorbed fluoride resisted desorption with water and 0.3% failed to be removed by saline solution (fig 5). These findings indicate that fluoride is essentially confined to the outer root space. The results for fluoride uptake in figure 4 permit the calculation, through the use of an equation given by Epstein (5), that the outer space volume is 0.34 ml per g fresh roots. The values on the ordinates of figures 1 to 3 for quantities of fluoride absorbed, when converted to units of μeq of fluoride per g of fresh roots, give by calculation an outer space of 0.23 to 0.28 ml per g fresh roots. On the basis of the usual premises 0.3% of absorbed fluoride was present in the inner space since this quantity of fluoride was not removed by desorption with water or NaCl-NaF solutions. The difference between the quantities of fluoride desorbed by water and by mineral ion solutions, i.e. 1.4% minus 0.3% = 1.1%, is an estimate of the fraction of absorbed fluoride which was present in the outer space in a bound but exchangeable form.

The findings in figure 5 with respect to chloride indicate that about 80% of absorbed chloride was not removed by desorption with water or NaCl-NaF solutions and there was no detectable difference between these desorption media in the amounts of chloride removed. These results would indicate, with the
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Fig. 5. Consecutive absorption and desorption of chloride (top) and fluoride (bottom) by barley roots. Halogen ions of absorption solutions labeled with radioisotopes; desorption solutions were unlabeled. Initial chloride and fluoride concentrations of absorption solutions and inert mineral desorption solutions was 0.1 meq per liter.

stated assumptions, that this fraction of absorbed chloride had been transported to the inner space by metabolic processes. There is, however, a perplexing point in reference to the acceptance of this inference if the 20% of absorbed chloride not allocated to inner space is present in the outer space. Outer space volumes calculated from figures 1 to 3 based on this quantity of desorbable chloride are considerably in excess of the entire root volume. A speculative explanation of this discrepancy is that 80% of absorbed chloride is an under-estimate of the amount of chloride present in the inner space as defined. It may be possible that some inner space chloride diffuses to the outer space when the chloride content of the latter is lowered and is in turn removed from the roots by diffusion or exchange into the desorption media. Deterioration with time of the oxidative mechanisms needed for retention of chloride in the inner space would allow retrograde movement of chloride from the inner space.

Summary

Barley roots discriminate markedly against fluoride and in favor of chloride absorption. A hundredfold difference in amounts of chloride and fluoride were found to be absorbed from solutions of initial equal fluoride and chloride concentrations in 3-hour periods.

Chloride was absorbed against apparent concentration gradients of up to 500 to 1 but fluoride was not absorbed against concentration gradients.

Anaerobic conditions and the metabolic inhibitors 2,4-dinitrophenol and sodium azide decreased the amounts of chloride absorbed. Anaerobic conditions had no effect on fluoride absorption which was in direct proportion to the fluoride content of the ambient solutions. Equilibrium conditions with respect to fluoride absorption were obtained in minutes but chloride absorption continued for longer time periods. These findings indicate that fluoride absorption by barley roots is a diffusion process and that oxidative metabolic mechanisms are required for the full extent of chloride absorption.

Absorbed fluoride was essentially completely desorbed from barley roots by water and inert salt solutions and 20% of absorbed chloride was removed by these desorption media under the same conditions.

Barley root water is 97% exchangeable.

Acknowledgment

We thank Dr. A. J. Linck and Dr. Allan H. Brown for assistance in planning the experiments and for discussions of their interpretations.

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Bioassay of Floral Stimulus in Xanthium

Norman E. Searle
Central Research Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Delaware

Estimation of the relative amounts of floral stimulus generated under various conditions is required for mechanism studies of floral initiation in Xanthium. A bioassay for floral stimulus has been developed from a previously described Xanthium system that exhibits unusual capacity for strong and reproducible flowering response (14).

Floral stimulus, here to be designated generically as florigen, can at present be estimated only by assays that relate intensity or quantity of stimulus to some scheme of floral development. A basic postulate is that the rate of early floral development is a function of the amount of florigen acting on the bud. Assessment of flowering response several days after initiation, however, necessarily registers contributions of ancillary processes which often obscure the quantitative relationships sought near the time of floral initiation. Rigorous control of environmental conditions and maximum efficiency of florigen production in the assay plant can be expected to minimize extraneous effects.

Several features of the new Xanthium assay method contribute to improved sensitivity and precision. A high level of florigen is achieved within a limited time period by exposure of selected donor leaf tissue to one 17-hour dark break in an otherwise continuous high-intensity light regimen. The amount of florigen produced and exported is regulated by cutting the donor leaf to a tab of definite size. Appropriate trimming of the plant channels the stimulus to a single receptor shoot of predetermined dimensions in the axil of the donor petiole. In this manner, the proportion of receptor tissue to maximally induced leaf tissue is fixed or otherwise controlled by the size of the donor tab. Separation of the floral development curves for 7- and 10-cm² leaf tabs clearly demonstrates that the differential stimulus from as little as 3 cm² of induced leaf tissue can be detected.

In the evaluation of treatments for regulation of flowering, 2 easily measured growth parameters provide clues for distinguishing between changes in floral response that may correlate specifically with reactions on the florigenic pathway and changes that are caused by shifts in pattern and vigor of vegetative development. The index of expansion of the donor leaf tab shows up abnormalities in the tissue generating the stimulus; the weight of the receptor shoot at dissection reveals growth aberrations at the site of differentiation. For example, 6-azauracil (6-AU) was found to arrest both growth of the receptor shoot and floral differentiation, an indication that it suppresses meristematic activity and is nonspecific. The herbicide 5-bromo-3-isopropyl-6-methyluracil (isocil) also inhibits flowering nonspecifically, but in contrast to 6-AU, it has no significant effect on the bud. It appears to act by blocking photosynthesis in the donor leaf.

Materials and Methods

Plants of the so-called continuous-light strain of Xanthium pensylvanicum were grown essentially as described previously, but without decotylizing the newly emerged seedlings (14). Light at 2200 ft-c,