Wounding Response in Relation to Polar Transport of Radiocalcium in Isolated Root Segments of Zea mays

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Summary. A perfusion bridge technique is described which permits the continuous collection of exudations from both ends of corn root segments. By exposing the central portion of the segments to radiocalcium, the amounts and rates of tracer movement in either direction may be determined. Typically, a peak in both acropetal and basipetal transport occurs at about 90 minutes after exposure to tracer. This transport peak is followed by a sharp decline to relatively low transport rates. Thereafter the 2 perfusates from opposite ends of a segment pair show significant differences. The acropetal increments decrease somewhat erratically to 0 at 10 to 12 hours, while the basipetal increments steadily increase to a steady-state value which remains constant from 8 to 24 hours. After a segment pair has reached steady-state polar transport, a fresh cut on the apical ends causes the resumption of acropetal transport. Such response suggests that polar transport in these root segments is at least partially a wound response. A possible explanation of the complex transport behavior is advanced.

Uptake and translocation of mineral salts in higher plants has long been known to be a complex event, its differing aspects having been described in terms of physical (passive) and of metabolic (active) processes. Moreover, the complex structural organization existing in roots suggests a catenary transport system involving separate absorption and translocation processes subject to tracer compartmental analysis. Recent theoretical developments would indicate that the distinction between metabolically active and passive transport may be somewhat arbitrary (21, 22) but the existence of separate absorption and translocation components of the transport system remains highly likely, regardless of their detailed molecular mechanism.

Transport relationships of the catenary type are especially amenable to compartmental analysis provided they can be separated into experimentally accessible compartments. The absorption of ions by roots and the subsequent release of a portion of these absorbed ions into the lumina of the xylem has been presumed to be such a catenary process (23). Recently the interdependent relationships of this process have been investigated by a compartmental approach for Cl⁻ ion in onion root (11, 13) and for Ca²⁺ ion in corn root (8). In the latter case and the present study, calcium was selected in spite of its complicating effects on ion absorption (7, 15) because this ion can reasonably be assumed mobile in xylem tissue (2) yet relatively immobile in phloem. In both approaches, the technique involved sealing excised roots between 2 or more compartments, after which the amounts of labeled ion appearing in the xylem exudate was measured as a function of time.

Both corn and onion root studies have indicated a) basipetal movement or polar transport of the labeled ion, b) initial lag time of 1 hour before label appeared at the cut surface, c) radial transport through the root symplast followed by a linear transport through xylem tissues. The present series of experiments extends earlier work in an attempt to elucidate these problems relating to the continuity of free space (6) and to the effect of liquid movement in xylem vessels on ion absorption (4, 14, 24). Previous techniques (8) have been modified so that label moved in either direction may be determined simultaneously for a given root segment and increments of transported label may be observed at short time intervals.

Materials and Methods

The primary roots of 4-day-old corn seedlings (Zea mays L. var Captan Peoria) were used as experimental material. The seedlings were dark-grown in 0.25 mM Ca(NO₃)₂ solution at 25 C. Roots were grown down glass tubes through which aerated culture solution was continuously circulated, a procedure which consistently provided straight roots. Only primary roots, 20 to 24 cm in length on the fourth day, were used as experimental material. The apical region, from which root seg-
Perfusate samples, collected directly into the counting vials, were evaporated to dryness, taken up in acidified toluene-ethanol liquid scintillant: toluene, 3080 ml; 100% ethanol, 920 ml; DPO (2,5-diphenyloxazole), 16 g and POPOP [2,2',p-phenylene-bis-(5-phenyloxazole)], 60 mg. Two drops of 3 N HCl were added to each 20-ml counting sample. Samples were counted in a Tricarb liquid scintillation spectrometer; maximum counting efficiency was 58% for $^{40}$Ca.

**Results**

*Polar Transport of Calcium.* In a series of single-label experiments, the amounts of $^{40}$Ca appearing in the perfusate from either end of a pair of root segments was determined. Typically, a peak in both acropetal and basipetal transport occurs during the 60 to 90 minute interval (fig 2A). The acropetal is usually less than the basipetal peak. Both peaks are followed by a sharp decline to relatively low half-hourly values. Thereafter the perfusate samples from opposite ends of a segment pair show pronounced differences. Basipetal transport increments increase while acropetal increments continue to decline until the segments become fully polarized 10 to 12 hours after excision. From 3 to 6 hours, basipetal transport rises linearly (fig 2B), and the tangent to this portion of the curve has a zero intercept of approximately one hour. As demonstrated previously (8), constant specific activity may be assumed; thus, at steady state transport (231 cpm/30 min, fig 2A) a basipetal rate for calcium may be established, amounting to 18 nmol per hour for a segment pair. Since the fresh weight of 210 to 65 mm segments is 64.1 mg, the basipetal calcium transport may be expressed alternatively as 0.29 mmole per hour per kilo F.W.

*Wound Reaction.* A perfusion bridge was modified to include an access port for insertion of a spring-loaded cutter. Segments 60 mm in length (cut at 10 and 70 mm from the apex) were sealed into this modified bridge and allowed to establish steady-state transport. Twelve hours after the initial sectionings, an additional 6 mm was excised from the apical ends only, leaving basal ends and the seals undisturbed. Immediately after this wounding there was an increase in acropetal transport with a corresponding decrease in basipetal transport. The sum of both transports continued the trend shown by basipetal transport prior to wounding (fig 3A). Occasionally, polarity was reversed for a temporary period (fig 3B). A repair process, apparent approximately 2 hours after wounding, is indicated by the fact that transport slowly returns to the normal polarized state. In this respect, wounding resembles the initial sectioning procedure. For example, if tracer is introduced 3 hours after sectioning, the rate of rise to steady-state transport is linear and corresponds to that
seen in figure 2B after the 3 hour minimum in basipetal transport (data not shown).

Transport Inhibition. Osmotic concentrations of 1 to 2 atmospheres in the external solution are known to prevent passive movement of ions probably by halting fluid flow through the xylem (3, 18). Mannitol at 0.12 M (3 atm) reduced the early transport peak by approximately 50%, but did not eliminate it. As table 1 shows, basipetal transport was significantly reduced during the time mannitol

Table I. Effects of Metabolic and Osmotic Inhibitors on Calcium-45 Transport by Paired Root Segments

<table>
<thead>
<tr>
<th>Inhibitor Present</th>
<th>Inhibitor Removed**</th>
<th>Inhibitor Present</th>
<th>Inhibitor Removed**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (pH 6.1)</td>
<td>18 183 ± 75* 90 7 ± 15* ... 337 ± 128* 90 177 ± 48* ...</td>
<td>4 156 90 193 271</td>
<td>4 75 60 154 243</td>
</tr>
<tr>
<td>Controls (pH 6.1)</td>
<td>6 276 90 5 5 441 90 216 231</td>
<td>4 34 63 1 3 53 60 23 125</td>
<td>4 24 60 1 3 90 60 40 30</td>
</tr>
<tr>
<td>Mannitol (0.12 M; pH 6.1)</td>
<td>6 139 90 4 1 156 90 193 271</td>
<td>4 75 60 154 243</td>
<td>4 34 63 1 3 53 60 23 125</td>
</tr>
<tr>
<td>KCN (0.1 M; pH 6.5)</td>
<td>4 75 60 ... ... 75 60 154 243</td>
<td>4 34 63 1 3 53 60 23 125</td>
<td>4 24 60 1 3 90 60 40 30</td>
</tr>
<tr>
<td>Dinitrophenol (0.05 M; pH 6.8)</td>
<td>4 24 60 1 3 90 60 40 30</td>
<td>4 34 63 1 3 53 60 23 125</td>
<td>4 24 60 1 3 90 60 40 30</td>
</tr>
<tr>
<td>Chloramphenicol (6 mm; pH 6.0)</td>
<td>4 24 60 1 3 90 60 40 30</td>
<td>4 34 63 1 3 53 60 23 125</td>
<td>4 24 60 1 3 90 60 40 30</td>
</tr>
</tbody>
</table>

* 99% Confidence limits.
** Two segment-pairs removed to inhibitor-free solution at 4 to 6 hours for remaining period.
Fig. 3. Alteration of transport behavior by wounding. Two experiments are shown. Polarity is temporarily reversed from 13 to 16 hours in B, heavy arrows at 12 hours denote times when 6 mm was excised from tip.

was present, and it was restored promptly after removal to mannitol-free solution.

Since external osmotic concentrations of 0.12 M mannitol failed to eliminate the early transport peak, several metabolic inhibitors were tried. Surfiffe found that chloramphenicol at concentration of 6 mM stopped sodium and potassium accumulation by beet and carrot slices (27). Hanson and Hodges observed inhibition of calcium accumulation in corn, using slightly lower concentrations of chloramphenicol. This inhibition was evidently due to uncoupling of oxidative phosphorylation (10). This compound at a concentration of 6 mM, pH 6.0, did not entirely eliminate the early transport peak (table 1). Basipetal transport was, however, reduced to a very low level thereafter with no subsequent recovery of the tissue after inhibitor removal at 6 hours.

Both 2,4-dinitrophenol (DNP) at 0.05 mM, pH 6.2, and 0.1 mM KCN, pH 6.5, were used as inhibitors in an attempt to test Arisz's suggestion that DNP inhibits vacuolar accumulation, whereas KCN prevents ionic penetration of the plasma membrane (1). Should Arisz's hypothesis be applicable to corn root tissue, KCN and not DNP might be expected to eliminate the early transport peak. Both of these inhibitors reduced the basipetal and acropetal peaks significantly (table 1). As with mannitol, there was immediate restoration of basipetal transport on removal of the roots to cyanide-

free solution at 4 hours: this recovery is shown in the table by the 8 hour value, 154 cpm, which is not significantly different from control values at 8 hours. DNP showed essentially irreversible inhibition. On removal of inhibitor at 4 hours, partial restoration occurred during 16 to 20 hours, as shown by the value of 125 cpm at 20 hours (table 1). Some evidence for differential action between KCN and DNP is indicated by the promptness of restoration following removal of KCN, but these findings can not be definitive.

In these inhibitor studies, a frequent but not invariable characteristic was advancement in time for the early peak from its normal position at 90 minutes to 60 minutes.

Water Transport. Since observed transport may depend on fluid movement within the vascular tissue, an attempt to observe such movement was made. Fifty-five mm segments were heat-sealed into 1 mm capillary tubing with 35 mm exposed to culture solution. Net water rates were determined from internal diameter of each capillary and change in height, over 24 hours, of a distilled water column covering the cut surface in the capillary. Both 0.25 mM Ca(NO₃)₂ and 2.5 mM CaCl₂, used as external culture solutions, yielded similar results (table II). An average of 0.024 ml of liquid was accumulated basipetally in a 24-hour period. Reverse segment orientation (acropetal movement) showed an average removal of 0.011 ml of liquid over the same period. This finding would indicate that in the bridge unit, fluid is moved in a segment pair both from the exposure chamber and from the acropetal arm of the bridge. Further tests of fluid transfer, in which 2.5 mM CaCl₂ solution was initially placed in the capillary tube, did not alter the volume of fluid moved basipetally.

Discussion

The demonstration of polarity (fig 2) confirms an earlier report for a simpler compartmental system (8). The peculiar shape of the calcium transport curves from 0 to 3 hours is, however, a new feature not detected by the earlier system, which raises important questions concerning the roles of metabolism and tissue compartmentation in the overall absorption-transport process.

Previous investigations with corn root (8) and with onion root (11) suggested that ionic transport occurs in vascular tissue, probably xylem. Metabolically maintained solute concentrations within the stele could cause fluid transport out the cut ends. Radiocalcium concentrations in the exudate were about 4 times those deduced from fluid volume transported and radioactivity concentration in the external culture solution (e.g., total activity on a fluid flow basis was calculated to be 2880 cpm; whereas, cumulated basipetal movement of label in the 24-hour period, was actually 10,549 cpm). The ob-
Table II. Water Transport by Single 35-mm Root Segments

<table>
<thead>
<tr>
<th>Segment lengths* and orientation</th>
<th>Conc in Ext (mm)</th>
<th>Conc in Soln** (mm)</th>
<th>Water level in capillary</th>
<th>Change in level</th>
<th>Volume moved* (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-55 ↓</td>
<td>0.25</td>
<td>32.1</td>
<td>59.2</td>
<td>27.1</td>
<td>0.0271</td>
</tr>
<tr>
<td>10-55 ↓</td>
<td>0.25</td>
<td>32.0</td>
<td>60.5</td>
<td>28.5</td>
<td>0.0285</td>
</tr>
<tr>
<td>10-55 ↓</td>
<td>2.5</td>
<td>29.2</td>
<td>48.4</td>
<td>19.2</td>
<td>0.0192</td>
</tr>
<tr>
<td>10-55 ↓</td>
<td>2.5</td>
<td>27.5</td>
<td>53.8</td>
<td>26.3</td>
<td>0.0263</td>
</tr>
<tr>
<td>10-55 ↓</td>
<td>2.5</td>
<td>23.2</td>
<td>51.0</td>
<td>22.8</td>
<td>0.0228</td>
</tr>
<tr>
<td>0-&lt;5 ↓</td>
<td>2.5</td>
<td>28.3</td>
<td>49.6</td>
<td>21.3</td>
<td>0.0213</td>
</tr>
<tr>
<td>10-55 ↑</td>
<td>2.5</td>
<td>28.5</td>
<td>16.9</td>
<td>-11.6</td>
<td>-0.0116</td>
</tr>
<tr>
<td>10-55 ↑</td>
<td>2.5</td>
<td>27.8</td>
<td>17.1</td>
<td>-10.7</td>
<td>-0.0107</td>
</tr>
</tbody>
</table>

* Arrow indicates direction of root apex; thus positive values in the change-in-level column indicate basipetal transport for ↓; acropetal transport for ↑; negative values indicate flow in opposite direction.

** 0.25 mm Ca(NO₃)₂; 2.5 mm CaCl₂

served transport curves (fig 2) differ radically, however, from the monotonic transport curve predicted for such a simple osmotic system.

Furthermore, inhibitor studies in both this and previous work indicated that the absorption-translocation process was metabolically mediated. For a compartmental system showing active transport, recent theoretical studies suggest that a conventional osmotic model for transport would be unrealistic (21, 22). Recent models (16) suggest that movement of non-ionic solutes, ions, and water in living tissue may be interrelated in a complex bioelectrical manner. The observation that metabolic inhibitors both failed to remove the initial transport peak entirely and tended to advance its occurrence in time again suggest that something more than a simple osmotic system is involved.

Movement of ions across the cortical symplast is thought to be in competition with vacuolar accumulation (19); thus, calcium transport rates should reflect a resultant including the rate at which vacuoles along the transport path reach turnover equilibrium. Comparison of the first few exudate samples with later steady-state samples suggests that the rate at which ions become available for transport by xylem is at first much less than the rate of movement once within the xylem. If root pressure normally exists in the xylem of intact corn roots, then exudation rates from both cut surfaces might be expected to be highest immediately after excision. If, furthermore, a rapid yet limited ionic movement into the vascular tissue is also possible by diffusional and/or metabolic processes, an early transport peak could result. A wound reaction, acting more rapidly in apical cells, might restrict exudation from both cut surfaces and ultimately plug the apical surface; thus duplicating the entire transport curve. Figure 4 schematically represents the presumed routes of ionic movement through the root segments as well as the presumed xylem exudation rate and sap concentration as a function of time. The product of the assumed rate and concentration curves produces a transport curve similar to that observed. Transport routes other than that indicated in figure 4 are, of course, possible. Longitudinal diffusion through the cortex is a possible route, although unlikely, because labyrinth factors should make such diffusion too slow to be competitive with a vascular route. Since the transported ion is calcium, the pathway through xylem shown seems the more likely.

Current literature indicates adequate evidence for the hypothesis advanced above. Ionic content of the cortical apoplast equilibrates rapidly with the external medium (17). Ion movement through the symplast has been shown to be relatively rapid (13, 20). Of equal importance are whether the proposed wound reaction exists and, if so, whether it can operate fast enough to produce the observed transport. Scott (25) reported healing of root surface by cutinization and suberization when cortical and epidermal cells are forced apart by developing lateral roots. Such healing reactions might also be expected at freshly excised surfaces. Also, contrary to classical opinion, the xylem vessel elements contain living protoplasm for considerable distances from the root apex (25). Engleman and Esau (5) reported wound responses in the phloem of Impatiens nultanii detectable within 5 minutes of wounding, with plugging of sieve plates complete within 30 minutes. Thus, at least in a living tissue, the wound response would appear to occur with
sufficient rapidity, and the observances of Scott (25) might permit extension to xylem tissue near the root apex. In figure 3, the observed return to normal polarity within approximately 2 hours after wounding may indicate time required for extensive plugging. The observed segment polarity would result from more rapid or efficient plugging at the apical cut. Such differences in cellular response are common in plant material, whether due to differential maturity between the cells at the segment ends or to polar movement of growth factors.

The zero intercept of 1 hour for the linearly rising basipetal transport of calcium was noted earlier in connection with figure 2B. This intercept suggests a minimum transit time along the complex transport path. Assuming rapid entry into and exchange with the cortical apoplast (17), the minimum transit time is probably controlled by movement through the plasma membrane and subsequent movement through the symplast to the xylem. Hodges and Vaadia (11) observed similar lag times for chloride ion, indicating that more chloride was transported than accumulated after an initial absorption period of 3 to 5 hours. Such behavior is entirely compatible with the presumed absorption-translocation sequence presented here.

The early transport behavior shown in this study may be a useful tool for discovering more about the movement of ions across the cortex and into the stele. Present observations are explained on the basis of demonstrated fluid flow coupled with an injury response, which may ultimately result in differential plugging of the segment pairs at the apical surface.

**Literature Cited**


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**Fig. 4.** Schematic model for absorption-translocation. Showing separate processes which would account for the observed transport-time relationship. Shaded areas suggest regions excluded by labyrinth factors.