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# Electrochemical Gradients and $K^+$ and $Cl^-$ Fluxes in Excised Corn Roots[1,2]

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## ABSTRACT

**The compartmental analysis method was used to estimate the $K^+$ and $Cl^-$ fluxes for cells of excised roots of *Zea mays* L. cv. Golden Bantam. When the measured fluxes are compared to those calculated with the Ussing-Teorell flux-ratio equation, an active inward transport of $Cl^-$ across the plasmalemma is indicated; the plasmalemma $K^+$ fluxes are not far different from those predicted for passive diffusion, although an active inward transport cannot be precluded. Whether fluxes across the tonoplast are active or passive depends upon the vacuolar potential which is unknown. Assuming no electropotential gradient, the tracer flux ratios are fairly close to those predicted for passive movement. However, if the vacuole is positive by about 10 millivolts relative to the cytoplasm, the data suggest active inward transport for $K^+$ and outward transport for $Cl^-$.**

**Fluxes to the xylem exudate were found to be more accurately estimated from the specific radioactivity of the cytoplasm (symplasm) than from the external solution specific radioactivity. The electrochemical gradients for $K^+$ and $Cl^-$ between the xylem vessels and the surrounding stelar parenchyma indicate active $K^+$ and passive $Cl^-$ movement into the vessels. The data are interpreted as being in accord with radial transport through the symplast into living vessels.**

The involvement of active transport systems at some stage in the delivery of ions to the xylem vessels of roots is not disputed. However, the location of the active transport sites within the root is uncertain. One of the major theories for ion transport to the vessels, that of Crafts and Broyer (5), postulates that ions are actively accumulated across the plasmalemma of epidermal cells and pass thereafter by diffusion through the symplasm (3) to the stelar parenchyma, from which they are delivered to the vessels by passive leakage. According to another leading theory, ions destined for the xylem impinge upon the endodermis through the free space of the cortex. Subsequently the ions are actively transported either through the endodermis to the stele (35) or secreted into the vessels by cells within the stele (23). Recently, an older theory (20) has been revived which assigns transport into the vessel exudate to cytoplasm within the vessels (1, 2, 8, 18).

The present study was undertaken to characterize further the mechanism for delivery of ions to the xylem vessels. The basic approach was an analysis of the electrochemical gradients and ionic fluxes between the cortical cells and the external medium and those between the exudate and the stelar parenchyma cells. If, as recent evidence indicates (23, 30), the stelar parenchyma is functional in delivery of ions to the vessels, then the electrochemical gradients between the stelar parenchyma and the vessels would be important. Such knowledge should give an indication of the sites of active or passive radial ion transport into the vessels.

Use has been made of the Ussing-Teorell flux-ratio equation, equation 1, as a test for active or passive ion transport.

$$J_i/J_o = (C_o/C_i)\exp(-z_jFE/RT) \quad (1)$$

In this equation, $J_i/J_o$ is the ratio of influx to efflux; $C_o$ and $C_i$ are the ion concentrations outside and inside a membrane, respectively; $E$ is the electrical potential difference on one side of the membrane with respect to the other side; $z_j$ is the algebraic valency; $F$ is the Faraday; $R$ is the gas constant; and $T$ is the absolute temperature. Close agreement between flux ratios calculated with this equation and those experimentally determined constitutes evidence for passive ion transport; discrepancies suggest, but do not in every case prove, active transport.

## MATERIALS AND METHODS

Excised roots of *Zea mays* L. cv. Golden Bantam were treated as previously described (11). Briefly, seeds were placed in vermiculite moistened with nutrient solution for 3 days, then the seedlings were placed in Plexiglas supports and suspended over aerated nutrient solution for 2 days; usually 10 seedlings were suspended over several liters of solution with all but the basal 0.5 cm (approximately) of root in solution. Roots used for experiments were 13 to 15 cm long and showed few or no branch primordia. After excision under solution, polyethylene exudate collection tubes were sealed to about 2 mm of the basal root end with a paraffin-lanolin mixture (11). The nutrient solution used throughout had in mM: 1 KCl, 1 $Ca(NO_3)_2$, 0.25 $MgSO_4$, 0.904 $NaH_2PO_4$, and 0.048 $Na_2HPO_4$; the solution pH was 5.5 to 5.7.

**Electrical Potential Measurements.** Measurements of the electrical PD[4] between the xylem exudate and the external solution were made as previously described (11). PD measurements were made at about 1-hr intervals on the roots used for the tracer flux determinations; the electrodes were brought into contact with the exudate and the external solution only for the several minutes necessary for PD measurement; this minimized effects due to KCl leakage from the capillary electrodes. Values of cortical and stelar cell PD values were taken from a previous paper (9).

**Analyses of Root and Exudate Ion Content.** For determination of ionic content, roots were handled with the same procedure as in the ion flux experiments (see below) but in the absence of

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[4] PD: potential difference.

tracers. At the prescribed times, after the initiation of an experiment, five roots were cut into 1-cm segments and placed in deionized $H_2O$ for 30 min to free the tissue of any freely diffusible (free space) ions. Segments were then taken from the water, gently blotted, and weighed; $K^+$ and $Cl^-$ were extracted in hot dilute acid or base, respectively. The $K^+$ in the extract was determined by flame emission photometry and $Cl^-$ by amperometric titration. Ion concentrations in the xylary sap were assayed in a similar manner after taking samples from the exudate collection tube with a microsyringe (11).

**Basic Procedure for Flux Determinations.** For determination of ion flux (transport) to the exudate, duplicate samples of five roots, each held in Plexiglas holders, were suspended over 400 ml of aerated nutrient solution labeled with $^{42}K^{36}Cl$. This arrangement permitted determination of $^{42}K$ and $^{36}Cl$ transport to the exudate, both while loading roots for elution and during the elution period (see below). Samples of exudate were collected with a microsyringe from the exudate collection tubes and transferred to planchets for counting. The exudate samples collected from each of the five roots held in a single Plexiglas support were pooled for counting. The $K^+$ and $Cl^-$ in each planchet were removed by rinsing thoroughly with hot base and assayed for total content by the methods described above. After an 8-hr tracer loading period, the roots in each holder were transferred as a group at the prescribed times to a series of elution solutions which were identical to the uptake solution but without tracers; these solutions were vigorously stirred by aeration. Elution periods were initially 30 min followed by 1-hr periods (Figs. 2 to 5). For assay of radioactivity in the elution samples, each was evaporated to dryness in planchets and counted with a gas flow counter. Volatilization of $^{36}Cl$ during evaporation was prevented by addition of tris. Data presented for the elution experiments are the averages for two identical runs made on the same day.

**Tracer Elution.** Estimates of the amounts of tracer in the cytoplasm and vacuole, the various membrane fluxes, and the cytoplasmic specific radioactivity were determined by elution experiments following essentially the theory and methods of Cram (6), Pitman (29), and Poole (32). Efflux curves were constructed from the cpm remaining in the tissue at the end of elution together with the cpm from each of the washings and the cpm appearing in the exudate. (In this paper, data shown for method I represent calculations which did not take into account loss of tracer to the exudate; alternatively, with method II tracer loss to the exudate was taken into account.) The amount of tracer remaining in the tissue at any time during elution was determined by subtracting the sum of cpm lost to the external solution and to the exudate from the previous cpm in the tissue. The logarithm of the cpm was then plotted for each time from the beginning of elution (Figs. 2 and 4). By extrapolating the linear component of the resulting plot to time zero, subtracting this component from the total, and replotting the difference as another semilogarithmic graph (Figs. 3 and 5), each of two exponential compartments for the efflux of $^{42}K$ and $^{36}Cl$ were distinguished. For each compartment, the slope of the line is the rate constant. The extrapolated values of the linear portions of the elution curves to time zero give the tracer content of the vacuole and cytoplasm at the onset of elution. One component (Figs. 2 and 4), the slow exchanging phase, is thought to represent vacuolar exchange, while the more rapidly exchanging component (Figs. 3 and 5) represents exchange from the cytoplasmic and wall phases. The amount of tracer in the cell wall may be determined by taking the difference between the total activity of the tissue and the activities in the cytoplasm and vacuole.

The graphical analyses detailed above and the other known experimental parameters give the following quantities: $t$, period of tracer uptake (hr); $Q_v$, ionic content of vacuole estimated from the total tissue content at end of elution ($\mu$eq $g^{-1}$)—the assumption was made that the tissue volume is 90% vacuole and that the $H_2O$ content of the tissue is 90% of the fresh weight; $Q_x$, ionic content of xylem exudate or total ion in exudate ($\mu$eq $g^{-1}$); $I_c$, apparent tracer content of the cytoplasm as estimated from the time zero extrapolate of the linear portion of Figures 3 and 5 (cpm $g^{-1}$); $I_v$, apparent tracer content of the vacuole as estimated from the time zero extrapolate of the linear portions of Figures 2 and 4 (cpm $g^{-1}$); $k_c$, rate constant for exchange of cytoplasmic compartment as estimated from slope of lines in Figures 3 and 5 ($hr^{-1}$); $k_v$, rate constant for exchange of vacuolar compartment as estimated from slope of lines in Figures 2 and 4 ($hr^{-1}$); $s_o$, specific radioactivity of the external solution (cpm $\mu eq^{-1}$); $s_v$, vacuolar specific radioactivity at end of experiment calculated as $I_v/Q_v$ (cpm $\mu eq^{-1}$); $J_{nx}$, net flux to exudate as determined from $\mu$eq appearing in exudate, $Q_x$, and time for accumulation of exudate ($\mu$eq $g^{-1}$ $hr^{-1}$).

From these parameters and from equations 9 to 13, the following quantities can be determined: $J_{oc}$, flux from external solution to cytoplasm ($\mu$eq $g^{-1}$ $hr^{-1}$).; $J_{co}$, flux from cytoplasm to external solution ($\mu$eq $g^{-1}$ $hr^{-1}$); $J_{cv}$, flux from cytoplasm to vacuole ($\mu$eq $g^{-1}$ $hr^{-1}$); $J_{vc}$, flux from vacuole to cytoplasm ($\mu$eq $g^{-1}$ $hr^{-1}$); $Q_c$, ionic content of the cytoplasm estimated with equation 13 ($\mu$eq $g^{-1}$); $s_c$, cytoplasm (or symplasm) specific radioactivity (cpm $\mu eq^{-1}$); calculated from $s_c = I_c/Q_c$ (method I), or $s_c = J_{oc}/(J_{co} + J_{cv})$ (method II).

The equations to calculate the unknown quantities listed above from the known parameters are essentially those outlined by Cram (6) and Poole (32). The tracer content of the cytoplasm, $Q_c^*$ changes by influx from the outside, influx from the vacuole, and loss of tracer from the cytoplasm to the outside and vacuole (loss to the exudate is considered later). The change in amount of tracer for the cytoplasm is:

$$dQ_c^*/dt = J_{oc} - (J_{co} + J_{cv}) \cdot Q_c^*/Q_c + J_{vc} \cdot Q_v^*/Q_v \quad (2)$$

and the tracer content of the vacuole, $Q_v^*$, changes as

$$dQ_v^*/dt = J_{cv} \cdot Q_v^*/Q_c - J_{vc} \cdot Q_v^*/Q_v \quad (3)$$

With constant $Q_c$ and $Q_v$ during the experiment and $s_o = 1$, the equations are equivalent to

$$Q_c \cdot ds_c/dt = J_{oc} - (J_{co} + J_{cv}) \cdot s_c + J_{vc} \cdot s_v \quad (2a)$$

and

$$Q_v ds_v/dt = J_{cv} - J_{vc} \cdot s_v \quad (3a)$$

The assumptions are made that: (*a*) the cytoplasm and vacuole are compartments in series; (*b*) the specific radioactivity is uniform throughout each compartment; (*c*) the tissue is in a steady state as regards compartmental content and fluxes. The net flux is, therefore,

$$J_n = J_{oc} - J_{co} = J_{cv} - J_{vc} \quad (4)$$

In Figures 2 to 5, the rate constants for the cytoplasm and vacuole are seen to be very different, and they can be considered to be the rate constants for the exchange of the two compartments. The rate constant for cytoplasmic exchange is given by

$$k_c = (J_{co} + J_{cv})/Q_c \quad (5)$$

At the end of tracer uptake and again at the end of elution, the cytoplasmic tracer is considered to be in a steady state with the amounts of tracer entering and leaving the compartment being equal. Taking this into account and from equations 2 to 5, the following relationships can be derived.

The vacuolar tracer content at the end of elution is

$$I_v = (J_{oc} \cdot s_o \cdot t)\, J_{cv}/(J_{co} + J_{cv}) \quad (6)$$

During tracer uptake the steady state cytoplasmic tracer content is $J_{oc} \cdot s_o/k_c$. The apparent content of tracer in the cytoplasm on transfer to elution solution is

$$I_c = (J_{co} \cdot s_o/k_c) J_{co}/(J_{co} + J_{cv}) \quad (7)$$

At the end of elution, the exchange of the vacuolar content is

$$k_v \cdot Q_v \cdot s_v = (J_{vc} \cdot s_v) J_{co}/(J_{co} + J_{cv}) \quad (8)$$

It, therefore, follows from equations 4 to 8 that:

$$J_{oc} = (I_v/t + I_c \cdot k_c)/s_o \quad (9)$$

$$J_{co} = I_c \cdot k_c/s_o + k_v \cdot Q_v \quad (10)$$

$$J_{cv} = J_{co}(I_v/t)/(I_c \cdot k_c) \quad (11)$$

$$J_{vc} = J_{cv} + J_{co} - J_{oc} \quad (12)$$

$$Q_c = (J_{co} + J_{cv})/k_c \quad (13)$$

The conventional three-phase system used to estimate fluxes, outlined above, does not permit a proper interpretation of the fluxes with exuding roots: transport to the xylem vessels is not taken into account. Therefore, a model similar to that of Pitman (30) is followed and is given in Figure 1. Following Pitman (30), and as shown in Figure 1, transport to the vessels may be considered to be either through the symplasm $J_{nx}$ ($=J_{cx} - J_{xc}$) or through the free space, $J_{fs}$. Thus, net transport to the vessels is equal to $J_{nx} + J_{fs}$ and tracer transport is equal to $J_{cx} \cdot s_c + J_{fs} \cdot s_o$. Pitman (30) demonstrated that $J_{fs}$ is very small and may be neglected. The data presented here corroborate this (Table VI). In accordance with the reasoning for the conventional three-phase model, and taking loss from the cytoplasm to the vessels into account, equation 2a become

$$Q_c \cdot ds_c/dt = J_{oc} - (J_{co} + J_{cv} + J_{cx}) s_c + J_{vc} \cdot s_c \quad (14)$$

Transport to the stele is

$$dQ_x^*/dt = J_{cx} \cdot s_c \quad (15)$$

For the present paper, net transport to the vessels, $J_{nx}$, is the total (chemical) ion flux to the vessels while the tracer flux is $J_{cx}$, and according to Pitman (30)

$$J_{nx} = J_{oc} - J_{co} = J_{cx} - J_{xc} \quad (16)$$

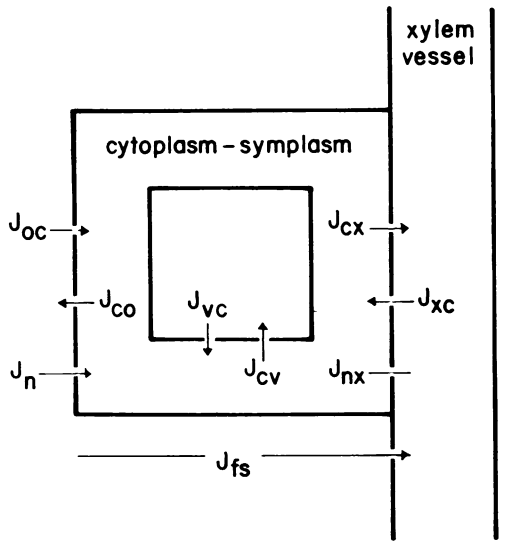


FIG. 1. Components of the fluxes into and out of each root compartment. $J$ is the flux in the direction indicated by the arrow and the subscripts; solution ($o$), cytoplasm-symplast ($c$), vacuole ($v$), xylem vessels ($x$). $J_n$ is net flux into the root and $J_{nx}$ net flux to the vessels. $J_{fs}$ is flux to the vessels through the free space. (After Pitman [31])

## RESULTS

From Table I it may be seen that the roots used in this study were in a steady state as regards both tissue content and exudate concentration. From 24 to 48 hr the tissue content for $K^+$ and $Cl^-$ remained steady but began to decline around 48 hr. Preliminary unpublished results indicated that there is a similar time course for tissue content of the roots of intact plants except that there is no decline after 48 hr as with excised roots. The roots used in this study which had been treated for 2 days in nutrient solution before excision were assumed to have reached a constant tissue content. Table I also shows changes in exudate $K^+$ and $Cl^-$ concentration for roots which had been treated in nutrient solution for 24 hr before detopping. Concentrations of both ions in the exudate are relatively steady between 8 and 30 hr after detopping. For the present study, roots were allowed to exude for 10 hr after excision and before tracer loading, making the tracer uptake and elution fall within the time period of steady transport to the exudate. The precipitous drop in ionic concentration of the exudate shown in Table I for the 8 hr following excision probably occurred because excess accumulated ions were washed out of the xylem vessels by the flow of water through them. This is logical since ions accumulate to high levels in the vessels of slowly transpiring plants. The drop in exudate concentration of $K^+$ and $Cl^-$ at about 30 hr after excision is likely the result of metabolic substrate depletion due to shoot removal (25).

**Tracer Fluxes.** Table II gives values of $Q_v$ and $Q_x$ and the parameters determined from the elution curves (Figs. 2 to 5). These quantities and equations 9 to 13 were then used to

Table I. *Potassium and Chloride Tissue Content and Xylem Exudate Concentration after Various Times in 1 mM KCl Nutrient Solution*

| Time in Solution | Tissue Content | | Exudate Concn | |
|---|---|---|---|---|
| | $K^+$ | $Cl^-$ | $K^+$ | $Cl^-$ |
| *hr* | *μeq g⁻¹* [a] | | *μeq ml⁻¹* [b] | |
| 0 | 34 ± 4.6 | 7.2 ± 1.4 | 26.1 | 5.32 |
| 8 | 45.2 ± 13 | 12.7 ± 0 | 17.6 | 4.81 |
| 12 | 50.6 ± 6.3 | 14.7 ± 2.5 | 16 | 4.65 |
| 18 | 60.3 ± 8.9 | 18.6 ± 3.1 | 17.3 | 4.72 |
| 24 | 67.2 ± 2.6 | 20.1 ± 5.9 | 17.8 | 4.89 |
| 30 | | | 16.5 | 4.36 |
| 48 | 65.4 ± 5 | 20 ± 2.5 | 12 | 2.71 |

[a] Values represent means ± SD for triplicate 0.5-g samples of 1-cm segments. Bathing solutions were changed every 8 hr. Time zero content was measured immediately after removal from vermiculite.

[b] Assays were made on exudate which had accumulated in the collection tube over a period of 1 hr. Concentrations shown are averages from the assay of the pooled exudate of two groups of five plants each. Roots were treated in nutrient solution with 1 mM KCl 24 hr before excision. The first exudate samples were collected during the 1 hr immediately after excision.

Table II. *Exudate and Tissue Content*

The values were determined by chemical assay and quantities determined from $^{42}K^{36}Cl$ elution experiment (Figs. 2 to 5).

| | $Q_v$ | $Q_x$ | $10^{-5} I_v$ | $10^{-5} I_c$ | $10^{-3} k_v$ | $k_c$ | $t_{\frac{1}{2}v}$ | $t_{\frac{1}{2}x}$ |
|---|---|---|---|---|---|---|---|---|
| | *μeq g⁻¹* | | *cpm g⁻¹* | | *hr⁻¹* | | *min* | *hr* |
| Method I | | | | | | | | |
| $K^+$ | 66.5 | 17.2 | 9.8 | 2.16 | 5.8 | 0.78 | 54 | $1.2 \cdot 10^4$ |
| $Cl^-$ | 18 | 4.9 | 0.960 | 0.148 | 13.8 | 1.13 | 53 | $5 \cdot 10^1$ |
| Method II | | | | | | | | |
| $K^+$ | 66.5 | 17.2 | 11.3 | 1.47 | 6.91 | 0.94 | 44 | $1.0 \cdot 10^4$ |
| $Cl^-$ | 18 | 4.9 | 1.11 | 0.126 | 17.5 | 1.20 | 35 | $3.9 \cdot 10^1$ |

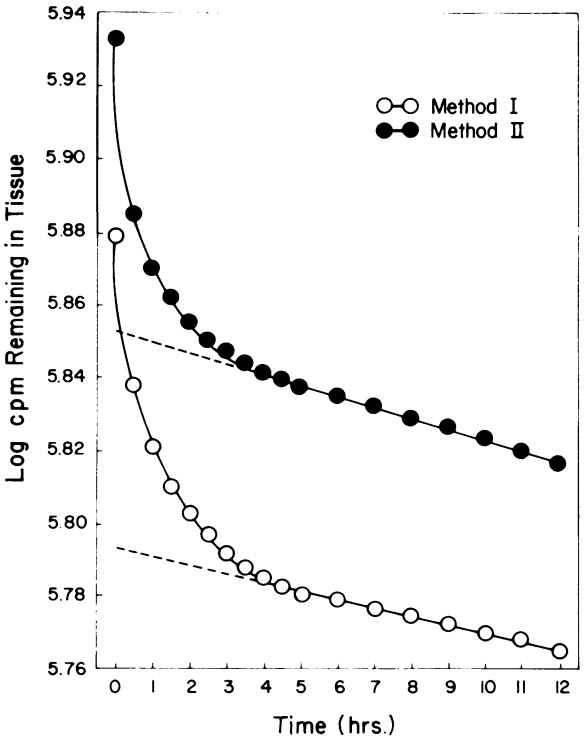


FIG. 2. Loss of $^{42}K$ from excised corn roots to unlabeled solution, plotted as the logarithm of the cpm remaining in the tissue. Extrapolation of the line to zero time gives the cpm initially present in the vacuole. With method I (○——○) tracer loss to the exudate was not taken into account; with method II (●——●) tracer loss to the exudate was accounted for.

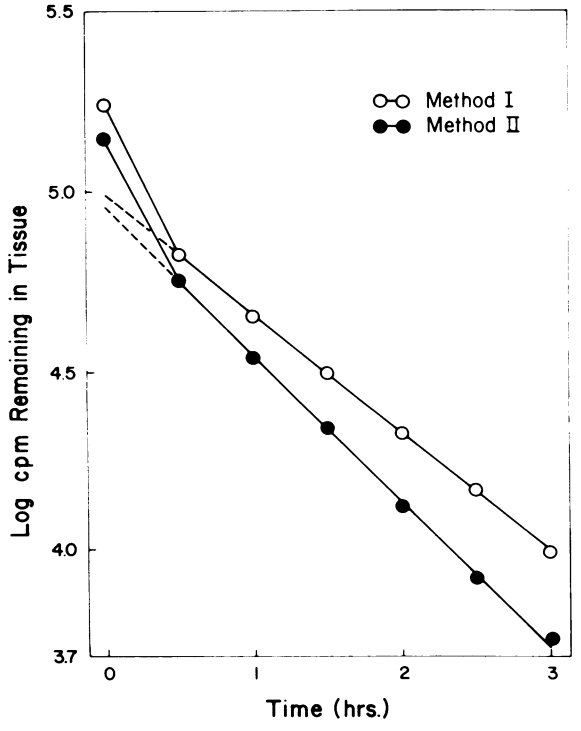


FIG. 3. Loss of $^{42}K$ after subtraction of the vacuolar phase (Fig. 2). Loss from 0.5 to 3 hr represents loss from the cytoplasm. The intercept gives the apparent tracer content of the cytoplasm at the start of elution.

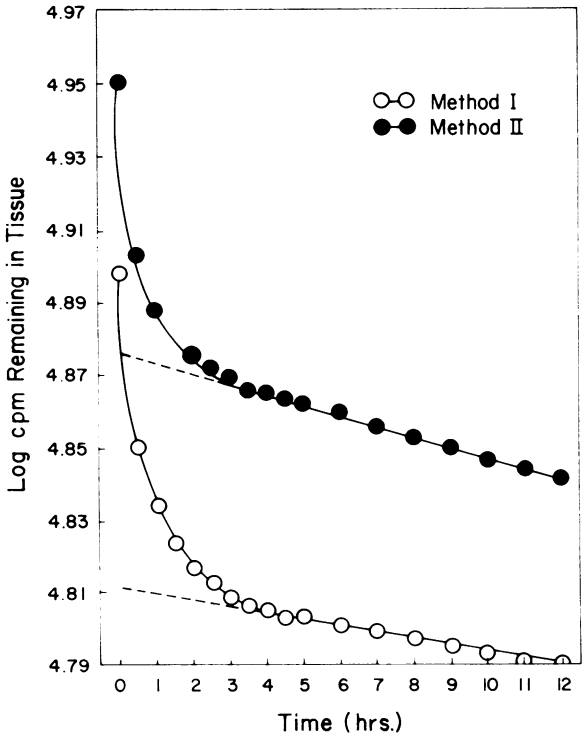


FIG. 4. Loss of $^{36}Cl$ from excised corn roots to unlabeled solution plotted as the logarithm of the cpm remaining in the tissue. Other conditions and the symbols are the same as for Fig. 2.

calculate the tracer fluxes and $Q_c$ for $K^+$ and $Cl^-$ shown in Table III. Values for the specific radioactivities, $s_m$, $s_c$, $s_v$, and $s_x$, estimated from the fluxes and other quantities in Tables II and III are listed in Table IV. It may be seen that $s_c$, $s_v$, and $s_x$ after the 8-hr loading period had not risen to the level of $s_o$. Estimates of $s_c$ as shown were made by two methods. When $s_c$ is calculated from $s_c = J_{oc}/(J_{co} + J_{cv})\, s_o$ (method II), the per cent equilibration is 66.6 for $K^+$ and 65.8 for $Cl^-$. With $s_c = I_c/Q_c$ (method I), the per cent equilibration is 33.8 for $K^+$ and 34.2 for $Cl^-$.

Transport of $^{42}K$ and $^{36}Cl$ to the xylem vessels, $J_{cx}$, and the total or net vessel transport, $J_{nx}$, are shown in Table V. $J_{cx}$ as given in this table has been calculated both from $s_o$ and the two types of estimates of $s_c$. In the case of $J_{cx}$ calculated with $s_o$, the transport rates for both $K^+$ and $Cl^-$ are lower than the corresponding rates for $J_{nx}$. When, on the other hand, $J_{cx}$, is calculated with either type of $s_c$ estimate, rates closer to $J_{nx}$ are obtained. This is to be expected if ions are transported to the vessels via the symplasm, as is widely believed (19, 21), and $s_c$ has not approached the level of $s_o$. Use of $J_{oc}/(J_{co} + J_{cv})s_o$ appears to overestimate $s_c$ and, therefore, underestimate $J_{cx}$. With $s_c = I_c/Q_c$, values of $J_{cx}$ are more realistic when compared with $J_{nx}$. Reasons for the discrepancies between $s_c = J_{oc}/(J_{co} + J_{cv})s_o$ and $s_c = I_c/Q_c$ are not apparent, but use of the latter appears to give the more reliable estimates of $J_{cx}$; therefore, calculations or considerations of $J_{cx}$ which follow will be from estimates made utilizing $s_c = I_c/Q_c$.

The values of $J_{cx}$ and $J_{nx}$ shown in Table V were calculated from exudate samples which accumulated in the collection tubes during the 30 min immediately prior to the beginning of the elution experiments. Thus, $J_{cx}$ was estimated from $s_c$ and assumed to be essentially at the same level as at the beginning of elution.

The lack of agreement between $J_{cx}$ estimated from $s_c = J_{oc}/(J_{co} + J_{cv})s_o$ and $J_{nx}$ shown in Table V is probably not due to

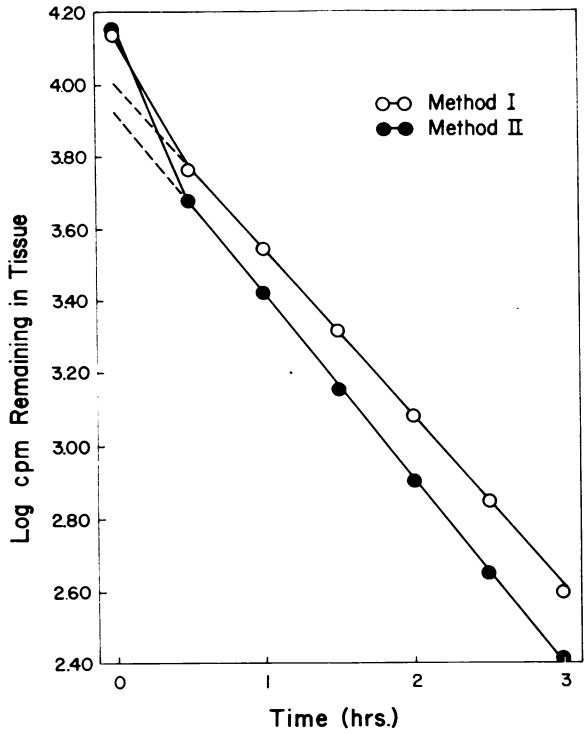


FIG. 5. Loss of $^{36}Cl$ after subtraction of the vacuolar phase (Fig. 4). Loss from 0.5 to 3 hr represents loss from the cytoplasm. Intercepts give initial amounts in the cytoplasm. Symbols are as defined for Fig. 2.

Table III. *Tracer Fluxes and Cytoplasmic Content*

These quantities were calculated from the data in Table II and equations 9 to 13.

| | $J_{oc}$[a] | $J_{co}$ | $J_{cx}$ | $J_{vc}$ | $Q_c$[b] | |
|---|---|---|---|---|---|---|
| | $\mu eq\ g^{-1}\ hr^{-1}$ | | | | $\mu eq\ g^{-1}$ | mM[c] |
| Method I | | | | | | |
| $K^+$ | 2.32 | 1.71 | 1.24 | 0.63 | 3.78 | 108 |
| $Cl^-$ | 1.14 | 0.91 | 0.654 | 0.424 | 1.65 | 47 |
| Method II | | | | | | |
| $K^+$ | 2.18 | 1.54 | 1.74 | 1.10 | 3.48 | 99.4 |
| $Cl^-$ | 1.16 | 0.92 | 0.84 | 0.60 | 1.47 | 42 |

[a] These values for $J_{oc}$ were calculated from equation 9. For $J_{oc} = J_{cx} + J_{co}$, see Table VII.

[b] Calculated from equation 13.

[c] Calculated on the assumption that the cytoplasm constitutes 3.5% of the tissue volume and that $H_2O$ content is 90% of the tissue weight as determined by oven drying at 95 C.

Table IV. *Specific Radioactivities for External Solution, Cytoplasm (Estimated by Two Methods), Vacuole, and Exudate*

| | $s_o$ | $s_c$[a] | $s_c$[b] | $s_v$[c] | $s_x$[d] |
|---|---|---|---|---|---|
| | $10^{-8}$ cpm $\mu eq^{-1}$ | | | | |
| Method I | | | | | |
| $K^+$ | 1.25 | 0.572 | 0.986 | 0.147 | 0.391 |
| $Cl^-$ | 0.251 | 0.0892 | 0.184 | 0.0533 | 0.166 |
| Method II | | | | | |
| $K^+$ | 1.25 | 0.422 | 0.830 | 0.170 | 0.391 |
| $Cl^-$ | 0.251 | 0.0857 | 0.165 | 0.0617 | 0.166 |

[a] Estimated as $I_c/Q_c$.

[b] Estimated from $J_{oc}/[(J_{co} + j_{cv}) \cdot s_o]$.

[c] Estimated as $I_v/Q_v$.

[d] Estimated from cpm $ml^{-1}$ appearing in exudate $\cdot/\cdot$ $\mu eq\ ml^{-1}$ in exudate just prior to elution experiment.

transport through the free space, $J_{fs}$. This is demonstrated in Table VI by the constancy of appearance of radioactivity in the vessel exudate after removal of tracers from the bathing solution. In addition, Figures 3 and 5 indicate that most of the wall and surface $^{42}K$ and $^{36}Cl$ should wash out in less than 30 min. Since the cytoplasmic half-time for exchange is 44 min for $^{42}K$ and 35 min for $^{36}Cl$ (method II), some of the tracer appearing in the exudate after about 1.5 hr of elution undoubtedly comes from the vacuole.

The small decrease in radioactivity appearing in the exudate after removal from tracer solution does not appear to be due to slow turnover rates of the fluid in the vessels. The fluid turnover rate was estimated from the vessel volume and the exudation rate. Estimates of vessel volume were made by averaging the diameters of vessels made at successive 1-cm increments along roots of about 14 cm length. Assuming the vessels to be right cylinders, the mean volume for five roots ± standard deviation was 0.24 ± 0.4 $\mu$l/cm root length. On the basis of an average root length of 14.4 cm, the total vessel volume would be 3.46 $\mu$l. With an average exudation rate of 8 $\mu l\ hr^{-1}$ plant$^{-1}$ (11), there would be 2.35 turnovers of vessel fluid per hr, assuming water movement to the vessels to be uniform along all portions of the root. This should be sufficient to flush the tracer from the vessels and cause a decrease in cpm well before that shown in Table VI.

With exuding roots, tracer is lost from the cytoplasm during elution, not only to the bathing solution but also to the xylem vessels. Thus, loss of tracer to the wash-out solution measures only $J_{co}$ and does not account for $J_{cx}$. The total tracer entering the cytoplasm should, therefore, be $J_{oc} = J_{co} + J_{cx}$ (30). When $J_{oc}$ is calculated in this manner from the values of $J_{co}$ and $J_{cx}$ given in Tables III and V, respectively, the following quantities are obtained: for $K^+ J_{oc}$ = 2.45 $\mu eq\ g^{-1}\ hr^{-1}$ (method I) and 2.47

Table V. *Transport of $K^+$ and $Cl^-$ to Xylem Vessels*

$J_{cx}$ was calculated by utilizing different specific radioactivities. Also shown is the total $K^+$ and $Cl^-$ transport (net transport).

| | $J_{cx}$ Calculated from | | | Total $K^+$ or $Cl^-$ $J_{nx}$ |
|---|---|---|---|---|
| | $s_o$ | $s_c = I_c/Q_c$ | $s_c = J_{oc}/[(J_{co} + J_{cv}) \cdot s_o]$ | |
| | $\mu eq\ g^{-1}\ hr^{-1}$ | | | $\mu eq\ g^{-1}\ hr^{-1}$ |
| Method I | | | | |
| $K^+$ | 0.20 | 0.74 | 0.40 | 0.98 |
| $CL^-$ | 0.13 | 0.41 | 0.20 | 0.46 |
| Method II | | | | |
| $K^+$ | 0.20 | 0.93 | 0.47 | 0.98 |
| $Cl^-$ | 0.13 | 0.43 | 0.22 | 0.46 |

Table VI. *Radioactivity Appearing in Xylem Vessel Exudate Just before, and at Various Times after, Removal of Roots to Elution Solutions*

Exudate taken from roots used for elution experiment. Zero time represents the cpm appearing in the exudate 30 min immediately prior to the elution experiment.

| Time in Elution Solution | Flux | |
|---|---|---|
| | $K^+$ ($\times 10^{-4}$) | $Cl^-$ ($\times 10^{-3}$) |
| hr | cpm $g^{-1}\ hr^{-1}$ | |
| 0 | 3.94 | 3.36 |
| 1 | 3.92 | 3.34 |
| 2 | 3.78 | 2.20 |
| 3 | 3.29 | 1.41 |
| 4 | 2.14 | 0.859 |

$\mu$eq g$^{-1}$ hr$^{-1}$ (method II); for Cl$^-$ $J_{oc}$ = 1.31 $\mu$eq g$^{-1}$ hr$^{-1}$ (method I) and 1.35 $\mu$eq g$^{-1}$ hr$^{-1}$ (method II).

**Flux Ratios.** Experimentally determined and predicted flux ratios (with equation 1) for the plasmalemma and tonoplast are given in Table VII. For experimental $J_{oc}/J_{co}$ ratios, the corrected values of $J_{oc}$ given above were used; $J_{co}$, $J_{cv}$, and $J_{vc}$ were taken from Table III. The predicted flux ratios were calculated from equation 1 utilizing ionic contents, $Q_c$ and $Q_v$, as given in Tables II and III. The electrical potential used for both the plasmalemma and tonoplast of 105 mv, inside negative, was taken from a previous study (9); the assumption that the PD across the tonoplast is zero seems justified from studies by Davis (10) and Etherton and Higinbotham (14) which demonstrated that the major potential drop is across the plasmalemma. Table VII shows that the experimental plasmalemma flux ratios for both $^{42}$K and $^{36}$Cl are different from, and indicate net fluxes in directions opposite to, those predicted for passive driving forces by Equation 1. In the case of $^{36}$Cl, the discrepancy between the experimental and predicted ratios is great and indicative of active Cl$^-$ influx across the plasmalemma. The discrepancy with $^{42}$K is not nearly so great, and the influx of K$^+$ across the plasmalemma may well be passive, although active transport cannot be excluded. Across the tonoplast, assuming an electropotential gradient of zero, the transport of both K$^+$ and Cl$^-$ may be passive, judging by the Ussing-Teorell equation; the predicted and experimental flux ratios are quite close (Table VII). If we assumed that the vacuole is +10 mv relative to the cytoplasm, these ratios indicate fairly strongly that K$^+$ is pumped inward against the electrochemical gradient; the reverse is true for Cl$^-$ transport (Table VII). There are data on barley roots showing that the vacuole is about 9 to 35 mv positive to the cytoplasm (25); the greater error in these measurements is likely to be the fluxes.

It is not possible to compare experimental and predicted flux ratios for the xylem vessels since $J_{cx}$ and $J_{xc}$ are not distinguishable by experiment. Also, from $J_{nx} = J_{oc} - J_{co} = J_{cx} - J_{xc}$, negative values of $J_{xc}$ are obtained since $J_{nx}$ is larger than $J_{cx}$. It is possible to compare the experimental $J_{nx}$ with that calculated ($J_{nxc}$) from the Goldman net flux equation (16) shown below.

$$J_{nxc} = -P_j \cdot \frac{z_jFE/RT}{1 - \exp z_jFE/RT} \cdot (C_j^c - C_j^x \exp z_jFE/RT) \quad (17)$$

Here, $C_j^c$ and $C_j^x$ are the ionic concentrations in the cytoplasm of parenchyma cells and the xylem exudate, respectively; $P_j$ is the permeability coefficient. The other symbols have the usual significance. The electrical potential, $E$, is the average potential of the exudate of the roots measured at hourly intervals during uptake and elution, −35 mv, with respect to that of the xylem parenchyma cells, −105 mv (9); the exudate is, therefore, +70 mv relative to the xylem parenchyma. For these calculations the assumption is made that $C_j^c$ for the stelar parenchyma is the same as $C_j^c$ for cortical cells. This assumption seems reasonable in view of the work of Dunlop and Bowling (12) who found K$^+$ to be at essentially the same concentration in the cortical and stelar parenchyma of corn roots.

In order to use equation 17 for the calculation of $J_{nxc}$, a value of $P_j$ is needed. This can be calculated for the plasmalemma from the Goldman efflux equation (16).

$$J_{co} = P_j \cdot \frac{z_jFE}{RT} \cdot \frac{C_j^c \exp z_jFE/RT}{1 - \exp z_jFE/RT} \quad (18)$$

The Goldman efflux equation is used here because it is considered that $J_{co}$ for both K$^+$ and Cl$^-$ is passive. The calculation of $P_j$ with equation 18 utilizes a cell PD of −105 mv (9). Values for $P_j$ and $J_{nxc}$ are given in Table VIII, and the latter should be compared with $J_{nx}$ in Table V. The values of $P_j$ in Table VIII are similar to those from other studies with higher plant tissue (28). $J_{nxc}$ for K$^+$ in Table VIII is negative which indicates that the electrochemical gradient would cause a net flux of K$^+$ from the xylem vessels to the xylem parenchyma. This, of course, is not likely and active transport is implied. With Cl$^-$, $J_{nxc}$ is positive and indicates that the electrochemical forces move Cl$^-$ passively into the vessels. Table VIII also presents $J_{cx}/J_{xc}$ ratios calculated with the Ussing-Teorell equation. The calculated flux ratios in this case cannot be compared with experimental $J_{cx}/J_{xc}$ ratios since there are no values for $J_{xc}$. Even so, the calculated ratios indicate that the electrochemical driving forces should cause a net flux of K$^+$ from the vessels to the parenchyma cells, but the reverse is true, suggesting active inward transport. For Cl$^-$, the ratio is such that there should be a net passive flux of Cl$^-$ from the xylem parenchyma to the vessels. The tracer flux ratios shown in Table VIII tend to corroborate the conclusions arrived at with the $J_{nxc}$ values, calculated using equation 17.

## DISCUSSION

In the present paper the over-all nature of the elution curves and the parameters derived from them are similar to those of other studies with higher plant tissue (6, 26–32). The discontinuity or "shoulder" after 2 to 3 hr of elution shown in the curves of Pallaghy *et al.* (27) with corn roots and Pallaghy and Scott (26) with bean roots was not found in this study.

Considerable differences are revealed in a comparison of the plots in Figures 2 to 5 in which tracer loss to the exudate has not been taken into account (method I) with those in which it has been accounted for (method II). Not only is the total tissue tracer content greater at the outset of elution with method II,

Table VII. *Predicted and Experimental Flux Ratios for Root Cells*

| | Cytoplasm ($J_{oc}/J_{co}$) | | Vacuole ($J_{cv}/J_{vc}$) | | |
|---|---|---|---|---|---|
| | Predicted[a] | Experimental[b] | Predicted $E$ = 0 mv | Predicted $E$ = +10 mv | Experimental[b] |
| Method I | | | | | |
| K$^+$ | 0.79 | 1.43 | 1.52 | 1.04 | 1.97 |
| Cl$^-$ | 0.00040 | 1.44 | 2.40 | 3.64 | 1.54 |
| Method II | | | | | |
| K$^+$ | 0.84 | 1.60 | 1.52 | 1.02 | 1.58 |
| Cl$^-$ | 0.00044 | 1.47 | 2.20 | 3.60 | 1.40 |

[a] Calculated from equation 1 and $Q_c$ and $Q_v$ from Tables II and III. External KCl concentration 1 mM, a PD of −105 mv, cytoplasm to outside, and assuming no PD across the tonoplast, or a PD of +10 mv.

[b] Calculated from the unidirectional fluxes given in Tables III and corrected values for $J_{oc}$ given in the text.

Table VIII. *Permeability Coefficients for Plasmalemma of Corn Root Cells, $P_j$, Predicted Net Fluxes to Vessels, $J_{nxc}$, and Calculated Vessel Flux Ratios*

| Ion | $P_j$ | $J_{nxc}$ | $J_{cx}/J_{xc}$[c] |
|---|---|---|---|
| | $10^8$ cm·sec$^{-1}$ [a] | $\mu$eq·g$^{-1}$·hr$^{-1}$ [b] | |
| K$^+$ | 5.65 | −6.95 | 0.38 |
| Cl$^-$ | 0.132 | 0.645 | 129 |

[a] Calculated from the Goldman efflux equation (equation 18) on the basis that there is 1.11 × $10^3$ cm$^2$ of cell surface per gram of tissue (29). Values of $C_j^c$ and $J_{co}$ are taken from Table III and $E$ = −105 mv (see text).

[b] Calculated from $P_j$ values in this table, $C_j^x$ from Table I, $C_j^c$ from Table III, and $E$ = +70 mv (see text), utilizing the Goldman net flux equation (equation 17).

[c] Calculated from the Ussing equation (equation 1), $E$ = +70 mv, $C_j^x$ from Table I and $C_j^c$ from Table III.

but also the rate constants, $k_c$ and $k_v$, are larger. Method II also gives lower values of $s_c$, and consequently greater estimates of $J_{cx}$, than method I (Tables IV and V). Contrasted to method I, method II for the plasmalemma fluxes gives decreased $J_{oc}$ and $J_{co}$ for $K^+$ with effectively no changes in $J_{oc}$ or $J_{co}$ for $Cl^-$. Regarding the tonoplast fluxes, method II gave greater $J_{cv}$ and $J_{vc}$ for both $K^+$ and $Cl^-$ than method I.

The total influx to the tissue, on the assumption that transport to the vessels is throught the symplast (19,21,30), has been considered to be $J_{oc} = J_{co} + J_{cx}$. Contrary to the findings of Pitman (30) for $Cl^-$ in barley roots, the influx of tracer from the cytoplasm to the vessels is less than flux from the cytoplasm to either the root exterior or the vacuole.

Both Davis (8) and Pitman (30) have suggested that $s_c$ rather than $s_o$ should be used to calculate $J_{cx}$. This is corroborated in this study by the demonstration that $J_{cx}$ estimated from $s_c$ gives rates which more nearly approach the rates for total transport, $J_{nx}$, to the vessels than do estimates made from $s_o$ (Table V). It is not presently understood why the two methods for estimating $s_c$ should give such vastly different values. However, of the two, estimates made with $s_c = I_c/Q_c$ give the more reliable estimates of $J_{cx}$ since the values obtained are closer to $J_{nx}$. It is not reasonable that $J_{cx}$ should be so much less than $J_{nx}$ when, as a general rule, in tracer flux studies the tracer influx is greater than the net (chemical) flux by an amount equal to the efflux. It must be concluded that tracer flux to the vessels of high salt roots, as used here, and determined from $s_c$, is underestimated but not to as great an extent as when estimated from $s_o$. This is apparently not the case with roots initially of low salt status since, according to Pitman (30), $s_o = s_c = s_v = s_x$ after treatment of low salt barley roots for about 24 hr in labeling solution.

The interpretation of the electrochemical gradients and the fluxes to the xylem vessels in this paper are based on the assumptions that: (*a*) the root cells exterior to the xylem vessels do not act as a single, homogeneous membrane separating the vessels from the ambient solution; and (*b*) the stelar cells are not leaky as required by the Crafts-Broyer hypothesis and, in fact, they may actively participate in the lateral transport of ions into the vessels. Regarding the former, it is obvious that the concentrically arranged tissues outside the vessels are of a complex nature and cannot be considered as a single membrane. With regard to the latter assumption, several lines of evidence from entirely different experimental approaches demonstrate that the stelar parenchyma are not leaky and are capable of accumulating ions (4, 7, 12, 23, 33, 34, 36). The evidence for ion-accumulation capacity of steles is at variance with the conclusions of Laties and co-workers (21, 22, 24) who believe that freshly isolated root steles are leaky and do not accumulate ions and are physiologically similar to steles *in situ*. The reasons for the discrepancies in the work of Laties and co-workers and that of other investigators are not readily apparent, but the evidence now seems to be in favor of nonleaky stelar cells which have ion-accumulation capacity.

The data from this and two previous studies (9, 11), taken together, demonstrate that the electrochemical gradients for $K^+$ and $Cl^-$ between the vessels and xylem parenchyma are large enough to affect transport of these ions into the vessels. For a xylem exudate PD of $-30$ to $-50$ mv (11) and cell membrane PD values of about $-105$ mv (9), the algebraic difference of PD between stelar parenchymatous cells and the exudate is 54 to 74 mv (exudate positive). The difference in concentrations, in mM, between the cytoplasm of parenchyma and the exudate is 82 for $K^+$ and 37 for $Cl^-$ (Tables II and III). Therefore, using the Ussing-Teorell flux-ratio equation (Equation 1) as a test, the data in Table VIII indicate active transport of $K^+$ from the symplasm to the vessels; for $Cl^-$ passive transport is indicated.

For the cortex and epidermal cells, the results shown in Table VII using the flux-ratio equation suggest that there is an active process moving $Cl^-$ inward across the plasmalemma; the discrepancies in ratios for $K^+$ transport are quite small so that the movement may well be passive. Also, as seen in Table VII, assuming no electropotential gradient across the tonoplast, the experimental and predicted flux ratios are nearly enough alike to suggest passive movement. However, if the vacuole is positive by 9 to 35 mv, as indicated in other tissues (17, 25), the flux ratios (Table VII) indicate that $K^+$ is pumped inward and $Cl^-$ outward. This result for $K^+$ would be similar to that reported for *Avena* coleoptile cells (28). The greater error in the data used in these calculations is likely to lie in the flux measurements which represent an average for the root. Differences of severalfold in fluxes through zones along the root are well known (1); consequently, an error in flux measurements of 2- to 3-fold could well occur. It is not at all likely that the data showing active $Cl^-$ transport into the cytoplasm is in error. In contrast to the present findings and, as well, to those of Pierce and Higinbotham (28), Pallaghy and Scott (26) believe that $K^+$ is moving passively into the cortical cells of broad bean roots.

While the data in this study suggest active $K^+$ transport from the symplasm into the vessels, the exact location of the active pump is not presently clear. However, the demonstration of membrane-bound cytoplasm in vessels up to a distance of about 10 cm from the root tip (1, 8, 18, 33), raises the question: Are the xylem vessels themselves in any way active in ion transport into the xylary stream? The studies thus far do not indicate whether or not the cytoplasm is intact through the entire vessel length. It is conceivable that even if cytoplasm is not intact throughout the entire vessel, it may be functional in ion delivery to the exudate. Such an idea was first set forth by Hylmö (20) in his "test tube" theory.

Higinbotham *et al.* (18) have recently presented both anatomical and physiological evidence which they consider to be consistent with Hylmö's hypothesis. In their interpretation of the "test tube" theory, Higinbotham *et al.* envisage extension of the symplasm into the vessel cytoplasm. They believe that ions are delivered to the vessel cytoplasm via the symplast and, thereafter, are pumped by the tonoplast into the vessel lumen, much as the tonoplast pumps ions into the vacuole of parenchyma cells. Plasmodesmata have been demonstrated in the radial walls of differentiating vessels of primary and secondary xylem of *Vicia faba* (33). Physiological evidence for extension of the symplasm into the vessels comes from the work of Dunlop and Bowling (13), who found that depolarization of the PD for epidermal cells and for the exudate under the influence of increased external KCl follow a similar time course. Their work is, thus, suggestive of plasmodesmata in vessel walls, *i.e.*, any significant electrical coupling between the stelar parenchyma with their high resistance plasmalemmas and the vessels would require some type of cytoplasmic connections.

Higinbotham *et al.* (18) interpret the exudate PD as that of a long cell which is short circuited after the membranes disrupt. Compared with intact cells, they view the lower PD of the exudate as constituting a measure of the cell wall pathway resistance. They attribute diffusion of $K^+$ outward from the cell wall Donnan phase as augmenting the exudate PD. The interpretations of Higinbotham *et al.* regarding the extension of the symplast directly into the vessels or of the nature of the exudate PD in no way contradict the ideas set forth in the present paper concerning the importance of electrochemical gradients between the vessels and the stelar parenchyma. In fact, it seems attractive to postulate that influx to the vessels is controlled by electrochemical forces or pumps across the vessel tonoplast. In keeping with this line of reasoning, ions coming to the stele through the free space could be pumped across the vessel membranes in the normal fashion.

In view of the above discussion and the data from this study, the following scheme for the radial transport of $K^+$ and $Cl^-$ into

the xylem vessels seems plausible. Both $K^+$ and $Cl^-$ are actively pumped across the plasmalemma of epidermal and cortical cells. Subsequently, the ions move via the symplasm to the stele where plasmodesmata extend the symplasm into the vessels. Once in the vessel, cytoplasm $K^+$ is actively pumped across the tonoplast into the open-ended vessel vacuole (vessel lumen). Movement of $Cl^-$ across the vessel tonoplast is passive.

The suggestion of two active stages in radial $K^+$ transport set forth here agrees in concept with Pitman (31). Pitman, however, assigns two-stage pumping to $Cl^-$ in barley roots, which is at variance with the single pump for $Cl^-$ postulated here. Pitman bases his conclusions primarily on the inhibition of both $J_w$ and $J_{c,x}$ fluxes of $Cl^-$ by the inhibitor carbonyl cyanide *m*-chlorophenylhydrazone. Pitman does not, consider the necessary eletrochemical gradients or the possibility of active $J_{c,x}$ transport of $K^+$ with $Cl^-$ moving along passively to maintain charge neutrality. Dunlop and Bowling (12, 13) have considered the electrochemical gradients between the exudate and the exterior of corn roots and have concluded that active pumping of $K^+$ and $Cl^-$ is at the outer root surface and that movement from the symplasm to the vessels is passive. Ginsburg and Ginzburg (15), in studies on corn roots with the stele removed, have demonstrated that $Cl^-$ movement across the hollow cortex cylinder is active and that $K^+$ is passive. Their results are only suggestive of active uptake into the symplasm and do not give any indication of the stelar mechanism for transport of ions into the vessels.

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