Active and Passive Transport of Potassium in Cells of Excised Pea Epicotyls

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ABSTRACT

The Ussing-Theorell equation, which provides a fundamental test for the independent passive movement of ions under conditions of nonequilibrium, has been used to assess the active and passive components of K+ uptake by segments of pea epicotyl (Pisum sativum L. cultivar Alaska), incubated for 24 hours in both 1-fold and 10-fold concentrations of a complete nutrient solution. Measurements of the rates at which 42K diffused out of the segments provided data from which were estimated the K+ content of, and the fluxes to and from, the nonfree space compartments, interpreted as being cytoplasm and vacuole. For this analysis the serial model of MacRobbie and Dainty and Pitman for the spatial arrangement of cell compartments was used. On the basis of these values, and measurements of electrical potential across the cell membranes, the vacuolar K+ concentration was found to be fairly close to that expected as a result of passive diffusion between the cytoplasm and vacuole provided that no potential exists across the tonoplast. Cytoplasmic K+ concentration, however, was much too high in both treatments to be accounted for in passive terms. It was concluded, therefore, that, on the basis of the model, the high ratio of influx to efflux was maintained in the cells by an active K+ pump located at the plasmalemma. There is some reason to question the applicability of this model for flux analysis to the conditions of high net influx as encountered here; nonetheless, it provides a first approach to an over-all flux analysis in pea stem tissue.

The most straightforward situation in which to assess the significance of cell electropotential, E (=PD), in determining the transmembrane concentration gradient of an ion is one in which the cells under study are at ionic equilibrium. In these circumstances the relationship between E and the concentration gradient of an ion resulting from passive diffusion is described by the Nernst equation:

\[ E = \frac{RT}{z_f F} \ln \left( \frac{C_j^e}{C_j^i} \right) \]

where E is the electrical potential difference between the inside and outside of the cell; \( z_f \) is the valence of ion \( j \); \( R \) is the gas constant; \( T \), the absolute temperature; and \( F \), the faraday. \( C_j^e \) and \( C_j^i \) are the external and internal concentrations of the ion \( j \).

In a study of the mineral ion contents and cell transmembrane electrical potentials of pea and oat seedlings, Higinbotham et al. (6) found little net change in the content of most ions during the incubation of excised root segments in the same nutrient solution as that in which the plants had been grown. Thus, for these tissues, a comparison between observed ionic concentration gradients and the gradients predicted by the Nernst equation, on the basis of observed PD, gave a good indication of how far passive diffusion accounted for the ionic distribution observed.

In contrast to root segments, segments of shoot tissue from these seedlings were found to be far removed from ionic equilibrium when immersed in the nutrient solution, there being considerable uptake of several ions. This is most notable for K+ content in pea epicotyl segments, the time course of which has been further examined by Macklon and Higinbotham (8). In an accumulating system such as this, the Nernst equation alone is not a sufficient criterion for evaluating the active or passive nature of ion transport. Where there is a net flux across a membrane, a better test for the independent passive movement of an ion is given by the following expression, deduced by both Ussing (16) and Theorell (15):

\[ \frac{J_j}{J^o} = \frac{C_j^o \exp \left( \frac{z_j FE}{RT} \right)}{\rho_j^o} = \frac{\rho_j^e}{\rho_j^o} \]

where \( J_j \) is the flux of the ion \( j \) from outside to inside the membrane and \( J^o \) is the flux from inside to outside the membrane; \( \rho_j^e \) is the electrochemical activity of ion \( j \) outside (where \( E \), by convention, is zero) and \( \rho_j^i \) \((= C_j^i \exp \left( \frac{z_j FE}{RT} \right)) \) is the electrochemical activity inside.

In order to test whether or not an ion moves in accord with the Ussing flux ratio equation in plant cells, it is necessary to have information on the fluxes, concentration gradients, and electropotential gradients across both the plasmalemma and tonoplast.

In this paper we present data from such an analysis of K+ in pea epicotyls in complete nutrient solutions. The compartmental analysis scheme of MacRobbie and Dainty (10), Pitman (11), MacRobbie (9), and Cram (1) was utilized. The results suggest that another model may be required for a system such as that encountered here in which high net uptake occurs.

MATERIALS AND METHODS

Pea seedlings (Pisum sativum L. cultivar Alaska) were grown, and samples of etiolated epicotyl segments 1 cm long (except as noted) were taken in the manner described in an earlier paper (8).

The nutrient solution used for growing the seedlings and incubating the segments, and for the efflux experiments, was of the following composition in mM: KCl, 1.0; Ca(NO3)2, 1.0; MgSO4, 1.0; NaH2PO4, 0.5; KH2PO4, 0.5; and sucrose, 11.0.

Received for publication June 24, 1969

1 This work was supported by National Science Foundation Grant GB 5117X and by funds provided for biological and medical research by the State of Washington Initiative Measure 171.

2 On leave of absence (September, 1966, to September, 1967) from the Macaulay Institute for Soil Research, Aberdeen, Scotland.
0.25; NaH₂PO₄, 0.904; Na₂HPO₄, 0.048; pH between 5.5 and 5.8. Used at this concentration, the solution is referred to as 1X. Experiments were also performed with nutrient solutions of 10 times this strength (10X).

Samples of segments, amounting to about 0.6 g, which were to be used for efflux measurements, were pretreated in nutrient solution for 12 hr and then transferred to a similar solution containing the radioisotope ⁴²K for a 12-hr loading period. Parallel samples were used to follow influx of labeled K⁺ at intervals up to 24 hr, and these samples were also used for chemical assay of K⁺. Details of the assay procedures are given in a previous paper (8). All experiments were carried out in a constant temperature room at 20°.

At the end of the 12-hr tracer-loading period, the samples to be used for efflux measurements were drained and placed in a cylindrical filter tube having a chamber 29 mm in diameter by 70 mm high, fitted with a drainage tap 8 mm in diameter. The tubes were mounted on a reciprocating shaker to simulate as closely as possible the conditions of incubation used in the uptake studies (8). A small polyethylene partition was inserted vertically in the neck of each tube to prevent escape of the segments during draining. Each sample was then immersed in 10-ml aliquots of the appropriate unlabeled nutrient solution for various periods of time permitting efflux of tracer. Each washing aliquot was collected in a beaker and then evaporated to dryness in a stainless steel planchet on a hotplate at 80° prior to counting. Initially solution changes were frequent, but thereafter the frequency was progressively reduced (see the time scale and experimental points in Figs. 1 to 3) to obtain adequate activity in each wash-out sample.

Efflux measurements were also made using freshly cut tissue from intact seedlings which had been preloaded for 16 hr with ⁴²K by flushing the vermiculite growing medium with labeled nutrient solution.

![Fig. 1](left). Time course of the reduction in the total amount of ⁴²K in a sample of segments during washing in unlabeled 1X solution. Data for tissue grown and incubated in 1X solution.

![Fig. 2](right). Time course of the reduction in the amount of ⁴²K estimated to be in cytoplasm, free space and cut cells of a sample during washing in unlabeled 1X solution.

**RESULTS AND DISCUSSION**

**Estimates of Compartments and Fluxes.** Efflux of labeled K⁺ from epicotyl segments was measured over a period of about 10 hr using duplicate samples of tissue grown and incubated in each strength of nutrient solution (1X and 10X). From counts of the activity remaining in the tissue at the end of the elution and from the activity in each of the washings an efflux curve for each sample was constructed, as in Figure 1. This can be considered as a compound curve, made up of first order rate losses from each of several compartments in the tissue; it is amenable to the method of analysis used by others (1, 2, 9-11). This yields a series of straight lines (from semilogarithmic plots) each characteristic of a particular phase. The contribution of the slowest compartment, assumed here to be the vacuole, to the efflux is indicated by the final, linear part of the curve in Figure 1. The line resulting has a slope which is the rate constant, kₚ (in cpm/time), for loss from the vacuole; the intercept at zero time (start of efflux) gives the amount of radioactivity in the vacuole. Subtraction of the vacuolar component from the activity of the total tissue at each time interval yields the efflux curve representing loss from compartments other than the vacuole, with the final linear phase being attributed to the cytoplasm (Fig. 2). Extrapolation of this line to zero time gives an intercept which is the amount of activity initially in the cytoplasm; its slope is the loss constant from the cytoplasm, kᵥ.
Further analysis in this manner reveals two additional phases (Fig. 3) which we consider to relate to the free space—possibly the Donnan space—in the tissue, and finally, the surface film of solution on the segments (perhaps including the water-free space).

The interpretation which is put on such flux data depends upon the spatial relationships which are considered to exist between the various cell compartments. They may be arranged in the series: wall, cytoplasm, and vacuole, so that ions moving between the outside solution and the vacuole necessarily traverse the cytoplasm. Alternatively, the cytoplasm and vacuole may each be accessible directly to the free space, in a parallel arrangement. The serial model is the one generally accepted for giant algal cells (2, 9, 10), and that this model is relevant to higher plant cells has been demonstrated convincingly by Pitman (11) with beet root tissue and Cram (1) with carrot root tissue. It is the serial model, therefore, which we have adopted as the basis for the interpretation of the present data.

From the graphical analysis of the results (Figs. 1–3) the efflux rate constant \( k \) and the half-time for exchange \( (t_{1/2}) \) can be estimated for each phase. In calculating the apparent amount of \( K^+ \) in each compartment at the start of the washing procedure, it is assumed that the cytoplasm and free space reach a steady state of specific activity well before the end of the 12-hr incubation in the labeling solution and that the “apparent” amount of \( K^+ \) in the compartment can in each case be taken as equal to the counts per minute at the intercept divided by the specific activity of the external solution. The amount of \( K^+ \) in the vacuole, \( Q_v \), is then represented by the difference between total \( K^+ \) estimated chemically on parallel samples and the sum of \( K^+ \) in the other compartments estimated by specific radioactivity. Finally, apparent influx, \( J_{v/w} \), across the tonoplast is estimated from the amount of tracer accumulated in the vacuole during the 12-hr loading period, in which net influx is linear (8). The experimental values for each compartment are given in Table I.

In general, the results shown in Figures 1 to 3 and Table I agree with similar wash-out data on other plants (10, 11) and are consistent with the concept that ions traverse compartments in the series: wall, cytoplasm, and vacuole. The rate constants for loss from each of these compartments show sharp differences from one another, thus providing a reasonable basis for such a graphical analysis.

The efflux and content values in Table I are apparent values, \( i.e., \) values not corrected for concurrent opposing fluxes. They considerably underestimate cytoplasmic content in particular, since, in basing them on data for the appearance of tracer in the washing medium, no account is taken of the concomitant transfer of ions between the cytoplasm and the vacuole. Nevertheless, Table I contains all the data required to estimate real values for the fluxes and for \( K^+ \) content of cytoplasm and vacuole. These are given in Table II. For the derivation of the equations permitting these calculations, the articles of MacRobbie (9), MacRobbie and Dainty (10), Cram (1), and Pitman (11) should be consulted. The equations are (9):

\[
\frac{dQ_v^*}{dt} = J_{oc} - \left( J_{oe} + J_{co} \right) - \frac{Q_v^*}{Q_c} + J_{ve} \cdot Q_c^* \cdot Q_v
\]

\[
Q_v \cdot \frac{dS_v}{dt} = J_{oc} - \left( J_{oe} + J_{co} \right) \cdot S_v + J_{ve} \cdot S_v
\]
where $Q^*$ is the amount of isotope in the compartment as designated by the subscript; likewise, $Q$ is the amount of the stable species; $a$, $c$, and $v$ designate, respectively, the outer solution, cytoplasm, and vacuole; $r$ is time; $J$ is the ion flux in the direction indicated by the subscripts; $S_v$ is the specific activity in the cytoplasm as a fraction of that outside; and $S_e$ is the specific activity in the vacuole as a fraction of that outside. Calculations of compartmental amounts and fluxes may be made using the relationships given as follows:

$$J_{in} \text{ (for cytoplasm)} = J_{oc} = k_v I_v + \frac{I_v}{t_{iso}}$$

where $k_v$ is the loss rate constant for the second slowest phase believed to be cytoplasmic; $I_v$ is the apparent content of this phase, i.e., the cpm at the intercept (at the start of efflux) divided by the specific activity of the external solution; $I_v/t_{iso}$ is the apparent influx (tracer-labeled) to the vacuole (which, of course, must pass through the cytoplasm).

$$J_{out} \text{ (of vacuole)} = J_{vc} = J_{oc} \frac{k_v Q_v}{k_v I_v}$$

$Q_v$, the amount of K$^+$ in the vacuole, is the amount determined for the total tissue less the amounts found in the cytoplasm and walls at the start of efflux.

$$J_{out} \text{ (of cytoplasm)} = J_{oc} = k_d I_d + k_v Q_v$$

where $k_d$ is the loss constant of the slowest phase, the vacuole, and the other notations are as above. Here it is assumed that the K$^+$ content is steady so that $J_{oc} = J_{co} = J_{oc} - J_{ve}$. Thus, $J_{in}$ (for vacuole) $= J_{ve} = J_{oc} - J_{eo} - J_{co}$. $Q_v = \frac{l_v(I_{eo} + I_{co})}{S_v I_v}$

$S_v$, the specific activity (as a fraction of $S_o$) of the cytoplasm at the start of efflux is $J_{oc}/(I_{eo} + J_{eo})$. The estimates of K$^+$ content found here for pea epicotyl cells (Table II) are reasonably close to those found previously for red beet root tissue (11) and for barley roots (12). However, estimates of K$^+$ concentration in the cytoplasm seem high relative to vacuolar concentration (Table V); they exceed the values reported for beet (11) and also the concentrations in pea root cells measured by Etheron (3) with a K$^+$ specific microelectrode.

**Efflux of Freshly Cut Tissues.** In a recent paper (8) we reported the occurrence of a lag phase in K$^+$ uptake by freshly excised pea epicotyl segments. One possible explanation would be that during the lag period K$^+$ efflux exceeds influx as reported for beet root tissue (14). Consequently an experiment was designed in which intact seedlings were allowed to take up $^{+}$K$^+$ from nutrient solution added to the vermiculite substrate and then epicotyl segments were excised for efflux measurements during the initial immersion period. The data are reported in Table III. Under these conditions the external concentration and specific activity in neither the substrate nor the wall space surrounding the absorbing protoplasts were known exactly; therefore, only the rate constant and half-times are given and amounts in each compartment and the fluxes were not estimated. In addition, in this experiment the effect of segment length, 1 cm vs. 2 cm long, was tested.

The efflux rate constants and half-times of exchange for vacuole and cytoplasm in freshly cut tissue (Table III) indicate that efflux from these compartments is a little higher than from tissue incubated 24 hr. Examination of the efflux curve for each sample showed that the rate constant for efflux from the vacuole changed about 5 hr after excision, and so two values (for zero time and 7 hr) are shown in Table III. However, even the initial efflux would be small compared with subsequent influx, and these data provide additional evidence for the view that the lag in net K$^+$ uptake by epicotyl segments, reported earlier (8), is the result of a delayed influx and not an initially high efflux. The effect of segment length on efflux is not impressively large. Although 1-cm segments have twice the area of cut surface per gram, the efflux rate constant does not show this magnitude of difference in loss rates from any compartment; in fact, the faster compartments show little difference due to segment length. It can be concluded that diffusion is not restricted to the cut surface area and that the length of segments—or distance of diffusion from innermost tissue—is not very significant in the isotope washout method of compartmental analysis. **Estimates of Compartment Volumes.** Although the data of Table II provide information on the amounts of K$^+$ in the cytoplasm and vacuole, they do not reveal concentrations without information on the volumes of these compartments. Measurements were made to provide some estimates of these volumes. From estimates of the relative volumes of cortex and vascular tissue it was concluded previously (8) that experimental results from epicotyl segments reflect, predominantly, the behavior of the cortical parenchyma. Further examination of fresh hand-cut sections of epicotyl segments was undertaken to establish the dimensions of an average cortical cell and the relative volumes of cytoplasm and vacuole. It proved to be difficult to distinguish the cytoplasm in these cells, which at this level in the epicotyl (more than 1.5 cm from the apex) are fully vacuolated and approaching the limits of their final extension. Even at a magnification of 1000X, the cytoplasm was of measurable thickness only where it enclosed the nucleus, which was in every instance appressed to the cell wall. There the cytoplasm appeared as a layer less than 1 μ thick. A minimum volume for the cytoplasm in fully vacuolated cells can be approximated by measuring the mean volume of cytoplasm in cells at the early stages of vacuolation (where the cytoplasm is

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**Table III. Analysis of Efflux from Epicotyl Segments Cut from $^{+}$K-labeled Seedlings**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compartiment</th>
<th>$k$</th>
<th>$t_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1-cm segments (1X)</td>
<td>Vacuole (zero time)</td>
<td>$10.44 \times 10^{-5}$</td>
<td>110.00</td>
</tr>
<tr>
<td></td>
<td>Vacuole (7 hr)</td>
<td>$6.85 \times 10^{-5}$</td>
<td>169.00</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm</td>
<td>$2.10 \times 10^{-2}$</td>
<td>33.00</td>
</tr>
<tr>
<td></td>
<td>Free space</td>
<td>$2.04 \times 10^{-1}$</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>Cut cells</td>
<td>2.34</td>
<td>0.30</td>
</tr>
<tr>
<td>2. 2-cm segments (1X)</td>
<td>Vacuole (zero time)</td>
<td>$6.36 \times 10^{-1}$</td>
<td>181.00</td>
</tr>
<tr>
<td></td>
<td>Vacuole (7 hr)</td>
<td>$4.51 \times 10^{-1}$</td>
<td>256.00</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm</td>
<td>$2.69 \times 10^{-2}$</td>
<td>26.00</td>
</tr>
<tr>
<td></td>
<td>Free space</td>
<td>$2.11 \times 10^{-1}$</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>Cut cells</td>
<td>1.86</td>
<td>0.37</td>
</tr>
<tr>
<td>3. 1-cm segments (10X)</td>
<td>Vacuole (zero time)</td>
<td>$3.99 \times 10^{-4}$</td>
<td>290.00</td>
</tr>
<tr>
<td></td>
<td>Vacuole (7 hr)</td>
<td>$2.57 \times 10^{-4}$</td>
<td>449.00</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm</td>
<td>$2.14 \times 10^{-2}$</td>
<td>32.00</td>
</tr>
<tr>
<td></td>
<td>Free space</td>
<td>$1.97 \times 10^{-1}$</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>Cut cells</td>
<td>2.17</td>
<td>0.32</td>
</tr>
<tr>
<td>4. 2-cm segments (10X)</td>
<td>Vacuole (zero time)</td>
<td>$3.19 \times 10^{-5}$</td>
<td>362.00</td>
</tr>
<tr>
<td></td>
<td>Vacuole (7 hr)</td>
<td>$1.93 \times 10^{-4}$</td>
<td>597.00</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm</td>
<td>$2.08 \times 10^{-2}$</td>
<td>33.00</td>
</tr>
<tr>
<td></td>
<td>Free space</td>
<td>$1.95 \times 10^{-1}$</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td>Cut cells</td>
<td>2.27</td>
<td>0.31</td>
</tr>
</tbody>
</table>
easily seen) and calculating the percentage of the cell volume that this represents after final enlargement (Table IV). This figure is 0.6% of the protoplast, and this would appear to set a lowermost limit for cytoplasm volume (Table IV). As an upper limit to the cytoplasmic volume, allowing for the probability of an increase in the absolute volume of the cytoplasm during cell expansion, a mean thickness of 0.5 μ can be taken; this gives a value of 3.4% of the total protoplasm. The limits arrived at in this way are shown in Table IV, in which other cell measurements are also presented. For the calculation of volumes, the cells were considered to approximate the form of a right cylinder; 1 g, fresh weight, was taken as 1 cm³; wall and air space were each estimated to constitute 3% of the volume and cut cells 5% of the volume. Thus 87% of the volume is occupied by protoplasm.

**Fit to Ussing Flux Ratio Equation.** Both in 1× and 10× solution, cortical cells of pea epicotyl exhibit a considerable electrochemical difference, of negative polarity, between the bathing medium and the vacuole. The available information in higher plants (4) indicates that most or all of this potential resides across the plasmalemma, and that there is presently no clear evidence that a significant potential difference occurs across the tonoplast. Thus, it is here assumed to be zero.

With the information on fluxes and amounts of ions in the cytoplasm and vacuole (Table II) and the estimates of compartment volumes, together with data for electropotentials from previous work (8), it is now possible to apply the Ussing flux ratio equation, equation 2, as a test for the independent passive movement of K⁺. Table V summarizes the results. The serial model used describes the ions as moving from the external solution across the plasmalemma to the cytoplasm, then across the tonoplast to the vacuole. As may be seen in Table V, for each sample, in both concentrations of external solution, the electrochemical activity ratio between the outer solution and the cytoplasm is much smaller than the flux ratio; thus, the influx of K⁺ to the cytoplasm is against a strong energy gradient suggesting an active inward transport at the plasmalemma. However, in the case of the cytoplasm to vacuole relationship the flux ratio approximates the electrochemical activity ratio as predicted by the Ussing equation for a passive diffusion process.

The cytoplasmic K⁺ concentrations in Table V are those obtained if the cytoplasmic volume is taken at its upper limit (3.4% of the protoplast). If the cytoplasmic volume were taken at its lower limit (0.6% of the protoplast, Table IV), much higher concentration values would be obtained, but the general conclusions would be unaltered. Active transport at the plasmalemma (against an even stronger energy gradient) would be implied, and passive diffusion would be sufficient to account for influx into the vacuole. The only additional indication would be the possibility of a K⁺ efflux pump at the tonoplast effecting transport from the vacuole to the cytoplasm.

**Permeability Coefficient.** The permeability coefficient, P, for K⁺ across the plasmalemma can be calculated from the data provided. Since K⁺ appears to be actively transported into the cytoplasm, it is assumed to be passively leaking outward. The unidirectional flux equation based on the Goldman (5) model is:

\[
J_w = P_K zFE C_{j,pl} \exp zFE/RT
\]

where \( C_{j,pl} \) is the estimated concentration of K⁺ in the cytoplasm and with other notations having the same meaning as given before. Here \( C_{j,pl} \) was multiplied by the activity coefficient. The average \( P_K \) of the two experiments at an external concentration of 1× was 1.02 \( \times 10^{-8} \) cm sec⁻¹, and of 10×, 1.01 \( \times 10^{-8} \) cm sec⁻¹. These values are about 20-fold less than those reported for broad bean roots by Scott et al. (13) based on a unidirectional flux inward. As noted by Scott et al., the flux of water through the roots could influence K⁺ influx; this would lead to an overestimate of \( P_K \).

**Critique of Results.** There are some reasons to question the accuracy of these initial attempts to test the flux ratio equation in higher plant cells undergoing rapid uptake. The primary difficulty lies in the fact that the method is best adapted to cases in which little or no net influx occurs, i.e., when only the specific activity in the compartments change with no net mass transfer. The amount of isotope removed in a typical wash-out experiment of 10-hr duration is about 3.7% of the amount taken up (with 12-hr loading). Of this the loss from the surface film and wall space amounts to about 2.1% and from the vacuole to approximately 6.6%.

### Table IV. Dimensions of Cortical Parenchyma Cells in Third Internode Segments of Etiolated Epicotyls

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Fully Vacuolated Cells (Mean of 20)</th>
<th>Newly Vacuolated Cells (Mean of 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell length, mm</td>
<td>0.318</td>
<td>0.016</td>
</tr>
<tr>
<td>Cell volume, mm³ (× 10⁴)</td>
<td>8.99</td>
<td>...</td>
</tr>
<tr>
<td>Protoplast volume, mm³ (× 10⁴)</td>
<td>8.46</td>
<td>0.09</td>
</tr>
<tr>
<td>Cytoplasm volume, mm³ (× 10⁴)</td>
<td>0.29</td>
<td>0.05</td>
</tr>
<tr>
<td>Cytoplasm as % of protoplast</td>
<td>3.4²</td>
<td>55</td>
</tr>
<tr>
<td>Cytoplasm of newly vacuolated cell as % of protoplast after enlargement</td>
<td>0.6</td>
<td>...</td>
</tr>
<tr>
<td>No of intact cells per g of tissue</td>
<td>32,000</td>
<td>...</td>
</tr>
</tbody>
</table>
| Surface area of plasmalemma, cm²/g tissue | 707                                | ... 

1 Segments, as used in experiments, taken 1.5 cm from apex of seedlings grown 7 days. Newly vacuolated cells were observed in sections taken from the apical hook.
2 Taking cytoplasm as a 0.5-μ layer lining the cell wall.

### Table V. Test of Ussing Flux Ratio Equation: Data from Experimentally Determined 4₂K Fluxes, Chemical Concentrations, and Electropotentials in Pea Epicotyl Segments

<table>
<thead>
<tr>
<th>Sample</th>
<th>External Solution</th>
<th>Cell Compartment</th>
<th>( J_w )</th>
<th>( K_{in} )</th>
<th>( E^\circ )</th>
<th>( \mu K_{in} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1X</td>
<td>Cytoplasm</td>
<td>14.0</td>
<td>145</td>
<td>-128</td>
<td>2.6³</td>
</tr>
<tr>
<td>2</td>
<td>1X</td>
<td>Vacuole</td>
<td>2.4</td>
<td>56</td>
<td>0</td>
<td>2.6³</td>
</tr>
<tr>
<td>3</td>
<td>10X</td>
<td>Cytoplasm</td>
<td>16.9</td>
<td>193</td>
<td>-128</td>
<td>4.0³</td>
</tr>
<tr>
<td>4</td>
<td>10X</td>
<td>Vacuole</td>
<td>2.1</td>
<td>54</td>
<td>0</td>
<td>2.3³</td>
</tr>
</tbody>
</table>

1 Assuming cytoplasm occupies 30% of total tissue volume (= 3.6% of protoplasm) and vacuole occupies 84% of total tissue volume (excluding air space).
2 From Macklon and Higinbotham (8).
3 The electrochemical activity ratio across the plasmalemma.
4 The electrochemical activity ratio across the tonoplast.
1.0%; the amount thought to come from the cytoplasm is only 0.6%. Obviously calculated larger errors net is (Fig. arbitrary considerations. As best function of such which presented analyses. is quite may be made It should be clear that the criticisms of the serial model compartmental analysis are not a denial of the series aspect and are restricted to questioning the degree of accuracy in the case in which a strong net influx occurs. In previous analyses on giant-celled algae the cells were in flux equilibrium (or nearly so) with the external solution (2, 9, 10) or subjected to low temperature, thus avoiding large net changes (11, 12). However, Cram (1) measured the Cl− fluxes of carrot root disks at 20°; he concluded that the influx to the cytoplasm was the most reliable of the several estimates. In his work it is not clear whether a strong net accumulation occurred during the experiments.

Acknowledgments—The authors thank Robert B. James, James S. Graves, and Karen M. Johnson for technical assistance; Dr. Enid A. C. MacRobie for aid in the mathematical analysis of results; and Dr. Alan Koch for suggestions on the manuscript.

LITERATURE CITED