# CHLORIDE FLUXES IN CELLS OF THE ISOLATED ROOT CORTEX OF *ZEA MAYS*

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*Abstract*

Isolated maize root cortical tissue is used to eliminate stelar complications in measuring fluxes. Comparison of isolated cortex and whole root segments shows that the cortex is not damaged by separation from the stele, that the initial influx estimate of the plasmalemma influx in cortical cells of whole roots (Cram and Laties 1971) is not affected by the presence of the stele, but that the quasi-steady influx to the vacuole of cortical cells may be overestimated at high external salt concentrations and with short loading times due to stelar contents. It is pointed out that tests of the validity and accuracy of the flux measurements depend, as in more complex analyses, on knowing the kinetics of exchange of the system. The validity of fitting three exponential components to the $^{36}Cl$ loss curve from isolated maize root cortex is confirmed by the equality of the exponential rate constants fitting the log content *v.* time and the log rate of loss *v.* time curves.

The initial influx, the influx to the vacuole, and the net chloride and net potassium influxes were all measured in the same tissue over a range of external KCl concentrations. From these, individual fluxes in the cell can be calculated.

The chloride influx across the plasmalemma rises more or less linearly with the external KCl concentration, and is mainly active up to 20 mM and has a large active component at higher concentrations. The passive permeability coefficient, $P_{Cl}$, is about $10^{-8}$ cm $s^{-1}$, and may increase with increasing external KCl concentration. At low external KCl concentrations the plasmalemma influx is the rate-limiting step in the influx to the vacuole. At higher concentrations the plasmalemma influx may be much greater than the influx to the vacuole, and is then no longer the rate-limiting step.

Net potassium and chloride influx at all KCl concentrations were equal within the experimental error, showing that movement of chloride into the vacuole is part of a net salt transport system in low-salt maize root cells. The influx to the vacuole and the plasmalemma chloride influx at low and high external KCl concentrations differ in their sensitivities to carbonyl cyanide *m*-chlorophenylhydrazone, temperature changes, and the nature of the accompanying cation. Accumulation of KCl leads to a reduction in chloride influx to the vacuole but not in plasmalemma influx. This reduction is not due to an increased internal hydrostatic pressure, since increasing the external osmotic pressure does not stimulate potassium or chloride influx in fresh or KCl-loaded tissue. The concentration to which KCl is accumulated is not proportional to the external KCl concentration or to chloride influx in fresh tissue, suggesting a homeostatic regulation of chloride transport.

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## I. Introduction

In a previous paper (Cram and Laties 1971) a simple, semiquantitative method of measuring the plasmalemma influx in plant cells was developed. It remained to be shown that stelar contents were not a complicating factor in measurements on root segments. The simplest way of doing this is to examine isolated root cortical tissue, and the results of such an examination, presented in this paper, confirm the validity of the simple method of measuring the plasmalemma influx. The method is then used to measure chloride fluxes over a range of chloride concentrations in the external medium and under various other conditions.

The relationship of influx to external concentration has long been the subject of investigation on the assumption that the relationship will show something of the nature of the transport process. This approach suffers from three drawbacks: (1) the concentration gradient is not the only passive driving force on ion movement, and the electrical potential gradient has generally not been measured; (2) individual fluxes have until recently not been distinguished; and (3) the interpretation of an influx isotherm is difficult. While the interpretation of the relationship between net flux or the ratio of two one-way fluxes and the electrochemical potential gradient under one set of conditions has an established thermodynamic basis (e.g. Katchalsky and Curran 1965), the interpretation of *changes* in fluxes in response to *changes* in passive driving forces depends also on the kinetics of the system, and there is little that can be generalized about the kinetics of active or passive transport processes (e.g. Goldman 1943; Eisenman 1968; Szabo *et al.* 1969). Thus conclusions about transport processes based on isotherms are inherently uncertain.

In this paper a thermodynamic approach is used to distinguish active from passive components of chloride fluxes at each external chloride concentration, and some qualitative differences between transport processes are established. The relationship between chloride transport and external chloride concentration is examined from the point of view of how the plant responds to changes in the external solution concentration. The response of root cells will be the first step in the response of the whole plant to variation, natural or otherwise, in the activity of mineral ions in the medium. In at least one cell system—barley roots—an initial change in ion fluxes in response to a change in the external solution concentration does not result in a proportional change in internal concentration. This implies some regulation of specific transport processes by internal factors (Pitman 1969). To what extent this also occurs in maize is also briefly examined.

## II. Materials and Methods

Maize (*Zea mays* L.) seedlings were grown with roots in aerated solutions of 0·5 mM $CaSO_4$, or were grown in vermiculite. The steles could be most easily removed from roots grown in vermiculite. The root tissue used was either 0·5–1-cm root segments from the region 1–6 cm behind the tip, or segments of isolated cortex. The latter were prepared by snapping the cortex 1 cm from the tip, pulling off the tip to expose a short length of stele, and pulling the stele out sideways (Yu and Kramer 1967). The isolated cortex was sliced in half along the break left by removing the stele, and cut into 0·5–1-cm segments. The stele breaks from the cortex at the endodermis (Ginsburg and Ginzburg 1970*a*), so this preparation consists of cortical and epidermal cells only.

After loading the tissue in various ways in each experiment, extracellular chloride, sodium, and potassium (including sodium and potassium in the cell walls) were washed out in 10 mM $CaSO_4$

over a period of 10–40 min. The tissue was then blotted, weighed, and dried down on a planchet if $^{36}Cl$ activity in it was to be measured. To prevent loss of HCl, the tissue was made alkaline, with $Ca(OH)_2$ when sodium and potassium were to be estimated. After measuring the $^{36}Cl$ activity, the tissue was washed off the planchet with distilled water, made up to a known volume, and aliquots used for estimating concentrations of sodium and potassium (by flame photometry) and chloride (by automatic titration with $AgNO_3$).

Influx of $^{36}Cl$ was measured as previously described (Cram and Laties 1971). Throughout the paper the best estimate of the plasmalemma influx ($M_{oc}$) is calculated from the $^{36}Cl$ activity after a 7·5-min load in $^{36}Cl$-labelled solution, followed by a 7·5-min wash in inactive solution. The influx to the vacuole ($M_{ov}$) is calculated from the $^{36}Cl$ activity after 7·5–60-min load in $^{36}Cl$-labelled solution, followed by a 30–60-min wash. These measurements are justified in the previous paper (Cram and Laties 1971) and the present one.

Concomitant net influx was estimated from the increase in content over the pretreatment, radioactive loading, and inactive washing periods. The increase in content was measured as the difference between initial and final samples.

The kinetics of efflux were examined as previously described (Cram 1968*a*) by measuring the loss of $^{36}Cl$ from loaded tissue to successive aliquots of inactive solution, and adding these to the final $^{36}Cl$ activity of the tissue. Some early results had to be discarded due to the slow exchange of chloride in the glue used in the tissue containers.

Raffinose was used as an impermeant osmoticum, as the reflection coefficient $\sigma$ for raffinose in *Valonia utricularis* is 1 (Zimmerman and Steudle 1970). A 30 min pretreatment is sufficient for the tissue to equilibrate after adding raffinose to the external solution (Glinka and Reinhold 1971).

Unless otherwise indicated, all results are expressed as mean$\pm$standard error of the mean (S.E.M.) for three replicates. Where more than three replicates were used, or the standard deviation (S.D.) is quoted, this is indicated in the text.

## III. Results

### (*a*) *Comparison of Activity of Isolated Cortices with Whole Roots and Whole Root Segments*

In isolated cortical tissue chloride influx ($M_{ov}$) to 0·1–0·2-g samples gave values of $100\pm15\%$ (6) (S.D.), when the mean influx (0·72 $\mu$mole $g^{-1}$ $hr^{-1}$ from 1·5 mM KCl) equalled 100%. The chloride influx to 0·3-g samples gave values of $100\pm3\%$ (3) (S.D.). Samples weighing 0·3–0·7 g were therefore used throughout, since it appears that the variability would be little further reduced by increasing the sample size.

The influx of chloride in freshly prepared cortex was measured. Two other portions of the same batch of material were placed in aerated 10 mM glucose or distilled water for 25 hr, both with 50 $\mu$g/ml chloramphenicol and 50 $\mu$g/ml streptomycin to reduce bacterial growth. $M_{ov}$ was then measured in the presence and absence of 10 mM glucose respectively. Chloride influx, in $\mu$mole $g^{-1}$ $hr^{-1}$, from 1·5 mM chloride, was $0·77\pm0·11$ in fresh tissue, $0·42\pm0·03$ after aging in the presence of glucose, and $0·47\pm0·04$ after aging in water. Thus there is some drift in the activity of the tissue, but this is not the same as in excised storage tissue where the tissue initially leaks and subsequently chloride influx increases by an order of magnitude. The drift in isolated maize cortex is probably not due to depletion of respiratory substrate, since external glucose has no significant effect.

Table 1 shows that chloride influx in isolated cortical tissue is 1·2–2 times greater than in whole roots or root segments prepared from the same batch of roots (except at high external solution concentrations when the steady influx to the whole

root tissue is complicated by the stele, as will be discussed subsequently). The response of the fluxes to changes in external solution concentration is the same in the two preparations.

TABLE 1

COMPARISON OF PLASMALEMMA INFLUX ($M_{oc}$) AND INFLUX TO THE VACUOLE ($M_{ov}$) IN ISOLATED CORTICES AND WHOLE ROOT SEGMENTS OR WHOLE ROOTS

Tissue for each experiment was from a single batch of roots

| Expt. | Tissue | KCl concn. (mM)* | $M_{oc}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | $M_{ov}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|---|
| A | Cortex | 0·1 | 0·76 | 0·72 |
| | | 25 | 16·2 | 5·6 |
| | Root segment | 0·1 | 0·62 | 0·56 |
| | | 25 | 14·4 | 7·1 |
| B† | Cortex | 1 | 1·77±0·15 | 1·78±0·13 |
| | | 50 | 18·0±0·2 | 5·6±0·2 |
| | Root segment | 1 | 0·95±0·01 | 0·89±0·02 |
| | | 50 | 7·7±0·8 | 5·1±0·4 |
| C‡ | Cortex | 50 | 14·6±0·3 (4) | 5·5±0·1 (4) |
| | Whole root | 50 | 12·2±0·15 (10) | 10·2±0·1 (10) |

* All solutions also contained 0·5 mM $CaSO_4$.
† Each measurement made on three replicates.
‡ No. of replicates given in parenthesis.

Figure 1 shows the time course of accumulation of chloride from 0·1 and 50 mM KCl by cortical tissue. A steady state is reached after about 50 hr. In other experiments (e.g. in the tissue used in the experiment of Table 5) it was found that an

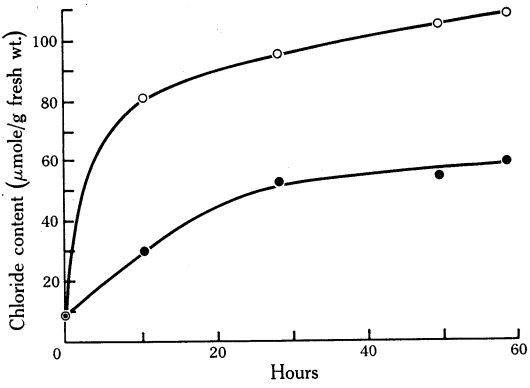



Fig. 1.—Accumulation of chloride by isolated maize root cortices. Tissue was washed in water for 19 hr, transferred to 0·1 mM KCl (●) or 50 mM KCl (○), both with 0·5 mM $CaSO_4$, and single samples removed and chloride content measured at the times indicated.

increase in the tissue potassium content parallels the increase in chloride content, and the same is true for whole root segments. KCl is therefore being actively transported into the tissue for at least 60 hr after isolation of the cortex.

These results show that the influx and accumulation of chloride in isolated cortices is the same as in whole root segments. Accumulation by whole root segments

must be primarily due to the activity of cortical cells since these make up 90% of the tissue volume, and therefore it is concluded that the activity of the cortex is essentially unaltered by separation from the stele.

### (*b*) *Kinetics of Exchange of Chloride in Isolated Cortices and Comparison with Exchange in Whole Roots*

#### (i) *Efflux*

The time course of loss of $^{36}Cl$ to inactive solutions was measured in fresh maize cortex tissue and in tissue which had accumulated KCl to a steady state in 0·1 and 50 mM KCl (the last points of Fig. 1).

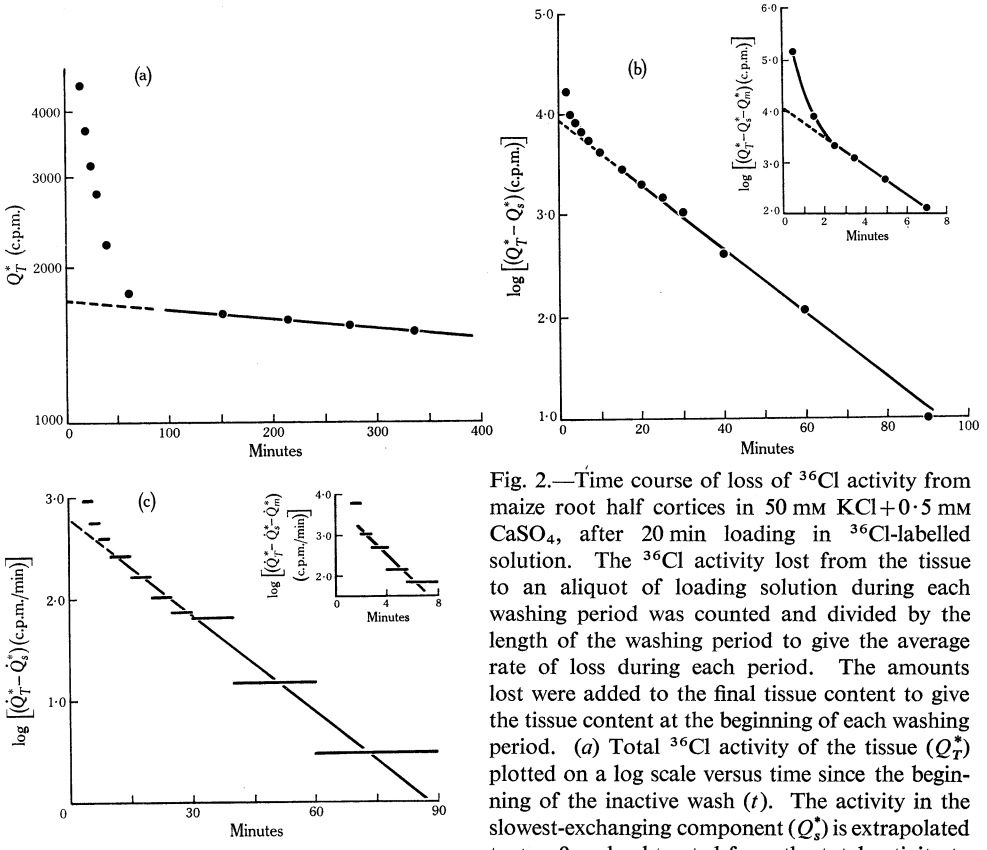



Fig. 2.—Time course of loss of $^{36}Cl$ activity from maize root half cortices in 50 mM KCl+0·5 mM $CaSO_4$, after 20 min loading in $^{36}Cl$-labelled solution. The $^{36}Cl$ activity lost from the tissue to an aliquot of loading solution during each washing period was counted and divided by the length of the washing period to give the average rate of loss during each period. The amounts lost were added to the final tissue content to give the tissue content at the beginning of each washing period. (*a*) Total $^{36}Cl$ activity of the tissue ($Q_T^*$) plotted on a log scale versus time since the beginning of the inactive wash ($t$). The activity in the slowest-exchanging component ($Q_s^*$) is extrapolated to $t = 0$ and subtracted from the total activity to give the activity in the faster-exchanging components. (*b*) Log ($Q_T^*-Q_s^*$) plotted against $t$. The activity in the mid-component of loss of $^{36}Cl$ activity ($Q_m^*$) was extrapolated to $t = 0$ and subtracted from the total. Inset: log($Q_T^*-Q_s^*-Q_m^*$) plotted against $t$. (*c*) Log($\dot{Q}_T^*$), i.e. log (rate of loss of activity during each washing period), was plotted against $t$, and the slowest component extrapolated and subtracted to give the rate of loss of activity in the faster phases. Log ($\dot{Q}_T^*-\dot{Q}_s^*$) is plotted against $t$. Inset: log ($\dot{Q}_T^*-\dot{Q}_s^*-\dot{Q}_m^*$) obtained as in (*b*) plotted against $t$.

Figure 2 shows the $^{36}Cl$ wash-out curve in tissue at a steady state with regard to total chloride content in 50 mM KCl. The logarithm of activity in the tissue is plotted against time in Figure 2(*a*). The slowest component is extrapolated towards

$t = 0$, its content during the first minutes of the wash-out subtracted from the total in the tissue, and the difference plotted on a semi-log graph in Figure 2(*b*). It is apparent that the curve is closely fitted by a term falling slowly and linearly with time, plus two terms falling faster and exponentially with time. The rate of loss in each successive washing period was also plotted against time on a semi-log graph, and the curve analysed graphically in the same way. Figure 2(*c*) shows the faster components of the rate versus time plot. These are fitted by exponential terms having the same rate constants as those fitting the content versus time plot. This agreement shows that exponential terms are a good fit to the data.

The three rate constants describing the loss of chloride are taken to describe the rates of exchange of extracellular spaces, cytoplasm, and vacuole (cf. Pitman 1963; Cram 1968*a*). The sizes of the intercepts do not in general equal the contents of the compartments.

TABLE 2

VALUES OF KINETIC PARAMETERS OF WASH-OUT CURVES FOR $^{36}Cl$ IN ISOLATED HALVES OF MAIZE ROOT CORTEX

Experiment A, fresh tissue, $^{36}Cl$ loading time 70 min; experiment B, tissue at a steady state (Fig. 1), $^{36}Cl$ loading time 20 min

| Parameter* | Experiment A: External solution 0·5 mM $CaSO_4$ plus | |
|---|---|---|
| | 1 mM KCl | 25 mM KCl |
| $t_{\frac{1}{2}f}$ (min) | 1·1±0·2 (3) | 1·0 |
| $t_{\frac{1}{2}c}$ (min) | 17·8±1·9 (3) | 16·8 |
| $t_{\frac{1}{2}v}$ (hr) | 15·8±1·4 (3) | 5·5 |

| Parameter* | Experiment B: External solution 0·5 mM $CaSO_4$ plus | | |
|---|---|---|---|
| | 0·1 mM KCl | 3 mM KCl | 50 mM KCl |
| $I_f$ (%) | 14, 8 | 8, 5 | 5, 4 |
| $I_c$ ($\mu$mole $g^{-1}$) | 0·005, 0·004 | 0·09, 0·08 | 2·1, 2·3 |
| $I_v$ ($\mu$mole $g^{-1}$) | 0·17, 0·14 | 0·43, 0·47 | 0·42, 0·37 |
| $t_{\frac{1}{2}f}$ (min) | 0·5, 0·8 | 1·8, 1·4 | 1·3, 1·1 |
| $t_{\frac{1}{2}c}$ (min) | 5·1, 6·1 | 9·2, 8·6 | 9·4, 9·1 |
| $t_{\frac{1}{2}v}$ (hr) | 61, 65 | 39, 49 | 27, 24 |
| $Q_v$ ($\mu$mole $g^{-1}$) | 62, 58 | 59, 58 | 107, 108 |

* Subscripts *f*, *c*, and *v* refer to fastest, "cytoplasmic", and "vacuolar" components of the wash-out curve; $t_{\frac{1}{2}}$ is the half-time for exchange of a component; *I*, the intercept at $t = 0$ of a component, obtained by extrapolation on a semi-logarithmic plot; and $Q_v$, the vacuolar chloride content—total tissue content is measured and cytoplasmic chloride is assumed to be negligible.

Table 2 gives values of two sets of kinetic parameters describing the exchange of $^{36}Cl$ in fresh cortical tissue, and in cortical tissue at a steady state in KCl (the last samples of Fig. 1).

As in other tissues the fastest component is present in dead tissue, and the two slower components are absent, confirming that the fastest component is extracellular in origin. Diffusion into this space must be rapid, so that the exchange must be primarily with the external solution, and the intercept of this component at $t = 0$ will be nearly equal to the content.

If the fastest-exchanging component is extracellular then the half-time for exchange should be constant with internal and external concentration (or apparent volume filled should be constant with external concentration). The fastest component can be least accurately measured. It varies considerably, but not in any systematic way with external or internal KCl concentration. The mean value for the half-times in Table 2 is $1 \cdot 1 \pm 0 \cdot 4$ min (10) (S.D.). The apparent volume occupied varies 2–3-fold, but the external solution concentration varies 500-fold, and therefore the fastest component must be considered to fit fairly well its expected behaviour as extracellular space having content proportional to external solution concentration.

In fresh tissue the half-time for turnover is slightly longer than in the tissue at a steady state, as in carrot tissue (Cram 1967). There is no large variation with external solution concentration. The values found are similar to those in barley roots (Cram and Laties 1971; Pitman 1971).

The data of Table 2 show that a washing time of 7·5 min would be sufficient to remove over 99% of extracellular chloride. The amount of extracellular chloride then remaining after loading in 50 mM KCl, and taking the extracellular spaces as 10% of the tissue, would be $0 \cdot 05$ μmole $g^{-1}$. After a 7·5-min load followed by a 7·5-min wash in 50 mM KCl the chloride content of the cell would be at least 1 μmole $g^{-1}$ (e.g. Table 2), so that the chloride in the extracellular spaces is negligible in comparison. At lower external concentrations extracellular chloride is an even smaller fraction of the total.

Table 2 also shows that washing for 1 hr will remove over 90% of the cytoplasmic component, but little of the vacuolar content as this exchanges so slowly. The vacuolar content, therefore, is obtained from the tissue content after a 60-min wash.

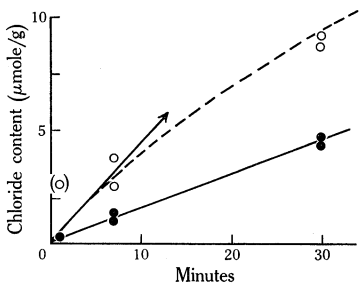



Fig. 3.—Time course of entry of chloride to halves of maize root cortices. Pretreated in 0·5 mM $CaSO_4$ for 4 hr. Transferred to 50 mM KCl + 0·5 mM $CaSO_4$ labelled with $^{36}Cl$, and samples taken at intervals and washed in inactive solution for 7½ min (○) or 50 min (●). $^{36}Cl$ activity in the tissue was then measured. The straight line was fitted visually. The dashed line is the sum of the component rising linearly and a component rising as $[1 - \exp(kt)]$ with $0 \cdot 7/k$ ($= t_{\frac{1}{2}}$) = 17 min.

(ii) *Influx*

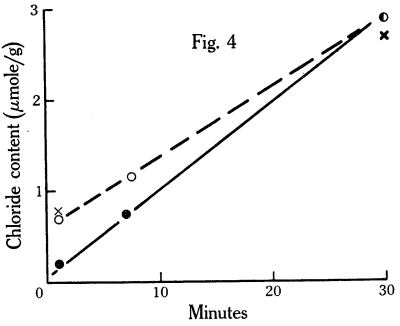
Figure 3 shows the time course of the increase in vacuolar content (after a 50-min wash in inactive solution), and of increase in total cell content (after a 7·5-min wash, which removes all extracellular chloride, but also some of the cytoplasmic chloride). The vacuolar content rises linearly with time, as expected whether cytoplasmic and vacuolar compartments are arranged in series or parallel. The line drawn through the

rise in the total chloride in the cells is the sum of the linear vacuolar term and an exponential term having a half-time of 17 min. The points are in reasonable agreement with this curve, showing that influx kinetics are consistent with efflux kinetics. The values after 1 min of loading are thought to deviate from the other points because of assymetry in diffusion through extracellular spaces, which will be the limiting factor over such short periods.

The initial influx to the cell must be equal to the plasmalemma influx ($M_{oc}$). Figure 3 shows that a fair estimate of the initial influx can be calculated from the cell content after a 7·5-min load followed by a 7·5-min wash. The influx to the vacuole ($M_{ov}$), which rises linearly with time, can be calculated from the content at any time after a long wash.

(iii) *Comparison of Whole Roots and Isolated Cortices*

Figure 4 shows the time course of influx to whole roots in comparison with influx to isolated cortices. Isolated cortices fill linearly with time, as a single (vacuolar) compartment. Whole roots fill with a linear component plus another smaller component which fills rapidly and does not lose its content during washing. The presence of the stele in whole roots, the only structural difference, could therefore be providing an extra phase, or preventing loss from an existing one in the cortex (the cytoplasm, for instance).



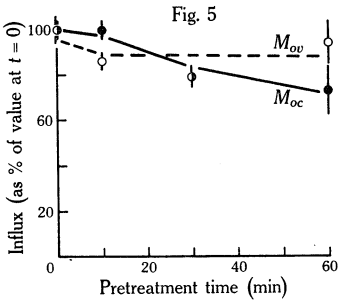




Fig. 4.—Time course of entry of chloride to halves of maize root cortices (●) and whole maize root sections (○, ×) in separate experiments. Details as in Figure 3, except that the washing period was 50 min in all cases, and some whole root segments (×) were washed at 0°C. For ease of comparison the values for isolated cortices (means of Fig. 3) were reduced so that the 30-min value equalled that of the whole root sample washed at room temperature. Standard errors of the means for the whole root samples fall within the symbols.

Fig. 5.—Effect of pretreatment in inactive solution on the plasmalemma influx ($M_{oc}$) and on influx to the vacuole ($M_{ov}$) in 25 mM KCl+0·5 mM $CaSO_4$. Tissue was pretreated in inactive solution for the indicated time, transferred to solution labelled with $^{36}Cl$, and samples removed and washed in inactive solution for 7½ min ($M_{oc}$) or 100 min ($M_{ov}$). Absolute values were not measured, but $M_{oc}$ was nine times $M_{ov}$ after zero pretreatment. Vertical bars show ±1 S.E.M.

Table 1 shows that the initial influx is the same in the whole root and in the cortex at 50 or 0·1 mM KCl, so the difference between the two is not due to an extra stelar compartment in whole roots. The decrease in the loss of chloride after a short loading period in the whole roots is therefore most probably due to retention in

the stele of chloride which in isolated cortices is lost from the cytoplasm to the solution. Most of the chloride in the cytoplasm after a short load therefore appears to pass to the stele from which it is lost only slowly, rather than out of the root across the epidermis. In experiment C of Table 1, 62% of the cell contents after a 7·5-min load plus a 7·5-min wash is lost from isolated cortices during the next 48 min, compared with 16·5% from whole roots. Therefore it appears that at least 45·5% of the chloride in the cortical cells moves to the stele. Of the chloride lost from the cells, 75% moves to the stele, and 25% moves to the external solution across the epidermis. This agrees with previous, more direct, estimates in maize and barley roots (Greenway 1967; Pitman 1971; Weigl 1971). It is possible, alternatively, that a larger fraction of chloride in the cortex moves to the stele and some then leaks back across the epidermis, although this seems unlikely (Jarvis and House 1970).

Figure 4 also shows that the vacuolar content is the same after washing at 20°C or at 0°C. The influx to the vacuole measured by different authors using different washing temperatures can therefore be compared.

Figure 5 shows the effect on the initial influx ($M_{oc}$) and the influx to the vacuole ($M_{ov}$) of pretreatment in unlabelled solution for up to 60 min. The influx to the vacuole is not significantly affected, which is a necessary consequence of the linear rise in vacuolar content with time in Figure 3. The plasmalemma chloride influx decreases by not more than 10–20%. As Laties (personal communication) has pointed out, if part of the influx across the plasmalemma is by one for one chloride–chloride exchange (as in carrot, Cram 1968*a*; and possibly also in maize, Weigl 1968), then increasing the cytoplasmic chloride concentration would increase the influx across the plasmalemma. However, this is not observed over a period when the cytoplasm would have had time to increase its internal concentration in response to the external solution concentration change.

The near constancy of these two fluxes for at least an hour shows that the high initial influx is not a transient property of the tissue after transfer to a new solution, and therefore that initial influx measured over short periods can be compared with net fluxes measured over longer periods.

These results and those of Figure 1 also show that there is no induction of chloride transport in maize roots grown under these conditions.

### (*c*) *Influx and Net Flux in Relation to External Solution Concentration*

The initial influx (plasmalemma influx, $M_{oc}$) and the steady influx to the vacuole were measured in isolated half cortices where stelar complications are eliminated. Chloride fluxes from 1 to 90 mM KCl + 0·5 mM $CaSO_4$ are shown in Figure 6. The values shown are from three experiments separated by up to 4 months. The variability, particularly in $M_{oc}$, is high, and the results were combined to give a representiative drift in fluxes with external KCl concentration. As can be seen in Figure 7, the variability in some other experiments was much less. Linear regression lines are drawn through the points from 10 or 20 to 90 mM KCl. The slope of $M_{oc}$ against concentration (0·47 $\mu$mole $g^{-1}$ $hr^{-1}$ $mM^{-1}$) is significantly higher than the slope of $M_{ov}$ against concentration (0·034 $\mu$mole $g^{-1}$ $hr^{-1}$ $mM^{-1}$) ($P < 0·001$). There is no indication of any decrease in the slope of $M_{oc}$ versus concentration at higher external concentrations, nor of any major discontinuity in the line. In conjunction with results

in various tables, it is concluded that below 1 mM KCl influx across the plasmalemma is nearly equal to the influx to the vacuole, both fluxes changing in parallel when the external KCl concentration is changed. Above 1 mM KCl the plasmalemma influx continues to rise, more or less linearly, whereas influx to the vacuole follows a "saturating" curve, increasing only very slowly above 10–20 mM KCl.

In other batches of tissue the influx to the vacuole was up to twice that in Figure 6. The plasmalemma influx was always more variable within individual experiments, and in different batches varied from 2 to 7 times the value of the influx to the vacuole at 50 mM.

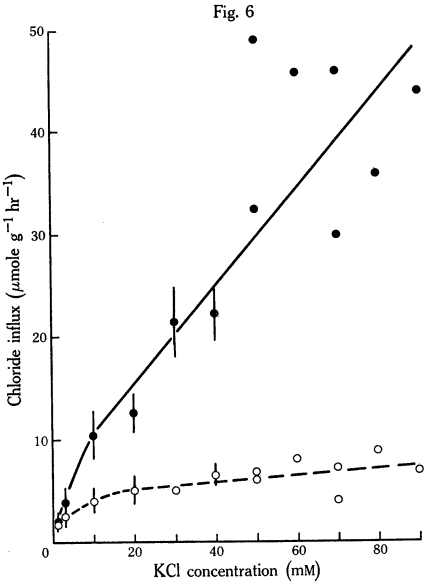
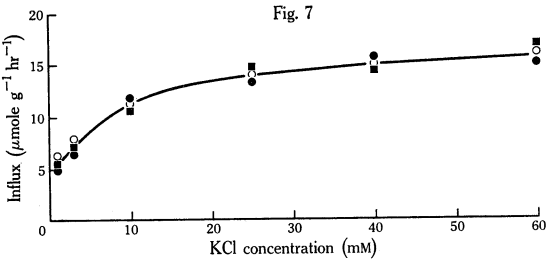



Fig. 6.—Plasmalemma influx (●) and influx to the vacuole (○) in maize root cortical cells from 1 to 90 mM KCl+0·5 mM $CaSO_4$. Values from three independent experiments are combined. Vertical bars show ±1 S.E.M. Lines of best fit are drawn from 10 or 20 to 90 mM KCl.

Fig. 7.—Chloride influx to the vacuole, $M_{ov}$ (●), net chloride influx (○), and net potassium influx (■) to maize root half cortices from 1 to 60 mM KCl. The means of the results in Table 3 are plotted against KCl concentration.




In several experiments the influx of chloride and the net flux of chloride were compared, and in some experiments the net potassium flux was also measured. Table 3 gives values (±90% confidence limits) of chloride influx to the vacuole, and chloride and potassium net fluxes from 1 to 60 mM KCl. All fluxes were measured on the same samples. The confidence limits for net potassium influx are fairly large because the flux is measured as a small difference between two large values. Fractional variation in individual potassium content was no larger than in chloride content. The means are plotted in Figure 7. Chloride influx and net chloride and potassium fluxes are not significantly different at any concentration. Since they follow the same trend with external KCl concentration, it would appear that they are nearly equal from 1 to 60 mM KCl. There is no significant discontinuity in the relationship of chloride influx to external KCl concentration. A rectangular hyperbola can be fitted and none of the points differs significantly from the least-squares best fit ($M_{ov} = 16{\cdot}7[\mathrm{KCl}]/\{[\mathrm{KCl}]+4{\cdot}8\}$ μmole $g^{-1}$ $hr^{-1}$).

In other experiments to check the equality of influx and net flux (Table 4) the variability was further reduced with larger samples and more replicates. The difference between net flux and influx in "low-salt" maize roots is small. Only the

TABLE 3

CHLORIDE INFLUX TO THE VACUOLE ($M_{ov}$) AND NET CHLORIDE AND POTASSIUM INFLUX TO MAIZE ROOT HALF CORTICES OVER A RANGE OF POTASSIUM CHLORIDE CONCENTRATIONS

| KCl concn. (mM)* | Influx ($\mu$mole $g^{-1}$ $hr^{-1}$) | | |
|---|---|---|---|
| | Chloride† | Net chloride‡ | Net potassium‡ |
| 1 | 4·9±0·1 | 6·1±0·8 | 5·4±3·3 |
| 3 | 6·4±0·2 | 7·8±1·0 | 6·8±3·5 |
| 10 | 11·6±0·2 | 11·3±0·8 | 11·1±3·4 |
| 25 | 13·6±0·2 | 14·1±1·2 | 14·5±3·4 |
| 40 | 15·4±0·4 | 15·1±0·9 | 14·9±3·3 |
| 60 | 15·2±0·2 | 16·2±0·7 | 16·9±3·4 |

* All solutions also contained 0·2 mM $CaSO_4$.

† Values shown as mean±standard error of the mean of three replicates.

‡ Values shown as difference of mean of three replicates from initial content±interval to 90% confidence limits for the increase in content. Initial chloride content: 4·9±0·3 (5) (S.E.M.) $\mu$mole $g^{-1}$; initial potassium content: 33·8±1·1 (6) (S.E.M.) $\mu$mole $g^{-1}$.

value at 3 mM KCl was significantly different from zero. Taken together, however, the results suggest that the difference between net flux and influx is constant at all external concentrations. The mean value of the difference is 0·29 $\mu$mole $g^{-1}$ $hr^{-1}$.

TABLE 4

CHLORIDE INFLUX ($M_{ov}$) AND NET FLUX ($M_{net}$) IN MAIZE ROOT CORTEX HALVES

| KCl concn. (mM)* | $M_{ov}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | $M_{ov}-M_{net}$ ±95% confidence limits ($\mu$mole $g^{-1}$ $hr^{-1}$) | $M_{net}$ as % of $M_{ov}$ |
|---|---|---|---|
| 0·1 | 1·06±0·02 | 0·22±0·27 | 79 |
| 1·0 | 1·41±0·03 | 0·36±0·54 | 74 |
| 3 | 2·70±0·01 | 0·41±0·26** | 85 |
| 5 | 2·69±0·08 | 0·17±0·36 | 94 |
| 50 | 6·42±0·47 | 0·21±0·91 | 97 |
| 50 | 5·78±0·15 | 0·37±0·38 | 94 |

* Solutions also contained 0·05–0·5 mM $CaSO_4$.

** Significantly different from 0, $P < 0·01$.

This would mean that the net flux is a smaller fraction of influx at lower external KCl concentrations, and consequently that net flux changes more in relation to external concentration than influx does.

The increased influx of ions at high external salt concentrations is not due to the increased osmotic pressure, as shown by the effects of 150 mM raffinose on potassium and chloride influx (Table 5). In *Valonia utricularis*, raffinose has a reflection coefficient of 1 (Zimmermann and Steudle 1970). The osmotic coefficient of

TABLE 5

EFFECT OF INCREASING THE OSMOTIC PRESSURE OF THE EXTERNAL SOLUTION ON THE FLUX OF CHLORIDE TO, AND OF THE FINAL CONCENTRATION OF POTASSIUM AND CHLORIDE IN, MAIZE ROOT TISSUE

In experiment A, chloride influxes $M_{oc}$ and $M_{ov}$ from 1 mM KCl solution to freshly excised root segments were measured. In experiment B these chloride influxes from 0·7 mM KCl solution to root cortex halves which had been loaded for 40 hr in 50 mM KCl were measured; the final potassium and chloride concentrations were 102 and 84 $\mu$mole $g^{-1}$ respectively. In experiment C final potassium and chloride concentrations in root segments were measured after an influx period of 5·5 hr from 10 mM KCl. The tissue initially contained 44·3±1·8 $\mu$mole $g^{-1}$ potassium and 14·7±0·9 $\mu$mole $g^{-1}$ chloride. In all experiments raffinose concentration was 150 mM; in experiment A, pretreatment time in raffinose was 30 min

| Expt. | $M_{oc}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | | $M_{ov}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | |
|---|---|---|---|---|
| | Control | +Raffinose | Control | +Raffinose |
| A | 1·5, 1·6 | 1·5, 1·6 | 1·2, 1·2 | 1·1, 1·2 |
| B | 1·64±0·03 | 1·07±0·05 | 0·39±0·09 | 0·38±0·02 |
| | Final potassium concn. ($\mu$mole $g^{-1}$) | | Final chloride concn. ($\mu$mole $g^{-1}$) | |
| | Control | +Raffinose | Control | +Raffinose |
| C | 57·1±0·8 | 60·1±2·8 | 30·6±1·2 | 33·7±0·8 |

raffinose at 150 mM is about 1·05, and that of KCl is 0·92 (Handbook of Chemistry and Physics 1967), so that 150 mM raffinose has an osmotic pressure equal to that of 86 mM KCl. 150 mM raffinose has no significant effect on chloride influx in fresh

TABLE 6

EFFECT OF LOADING WITH POTASSIUM CHLORIDE ON CHLORIDE INFLUX TO MAIZE ROOT CORTICES

| External solution | | Final chloride concn. ($\mu$mole $g^{-1}$)* | $M_{oc}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | | $M_{ov}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | | Ratio B/A (%) |
|---|---|---|---|---|---|---|---|
| KCl concn. (mM) | $CaSO_4$ concn. (mM) | | Fresh tissue | Loaded tissue | Fresh tissue (A) | Loaded tissue (B) | |
| 0·1 | 0·05 | 60±1 | — | 0·81±0·004 | 1·06±0·02 | 0·7±0·01 | 66‡ |
| 3 | 0·1 | 75±2 | — | 4·6±0·2 | 2·7±0·01 | 1·3±0·07 | 48‡ |
| 50 | 0·5 | 84±1 | 43±5† | 62±6† | 6·4±0·5 | 1·6±0·2 | 24‡ |

* Initial chloride concentration 4·7±0·2 $\mu$mole $g^{-1}$; loading period 40 hr.
† No significant difference. ‡ Inhibition significant at the 0·1% level.

or KCl-loaded maize root tissue, except for a 30% inhibition of the plasmalemma influx in KCl-loaded tissue, and also has no significant effect on net chloride and potassium influx. Both potassium and chloride fluxes in maize root tissue therefore appear to be independent of external osmotic pressure and internal hydrostatic pressure.

### (*d*) *Differential Effects on Plasmalemma Influx and Influx to the Vacuole*

#### (i) *Effects of Internal KCl Concentration*

The plasmalemma influx and the influx to the vacuole were measured in low-salt root tissue and in tissue which had accumulated KCl. Four sets of measurements were made, and the results of one set are shown in Table 6. The plasmalemma influx from 0·1 or 50 mM KCl was not significantly affected by loading with KCl in any experiment. The variability in $M_{oc}$ is high, but a 70% reduction in $M_{oc}$ in KCl-loaded tissue, as is found in barley roots (Cram and Laties 1971), would have been detected.

Chloride influx to the vacuole was reduced by loading the tissue with KCl at high but not at low external KCl concentrations. This result was also obtained in three other experiments.

If the influx to the vacuole is mainly by a straight-through influx, then these results show that this process is more sensitive to KCl loading at high external KCl concentrations than at low external KCl concentrations.

If the cell behaves as plasmalemma and tonoplast in series, then the results are not inconsistent with a 70–80% inhibition of the tonoplast influx by high vacuolar KCl concentration at all external KCl concentrations. This conclusion is reached from values of fluxes calculated as described in the discussion.

#### (ii) *Effects of an Inhibitor*

Figure 8 shows the effect of carbonyl cyanide *m*-chlorophenylhydrazone (Cl-CCP) on chloride influx. Sensitivity to Cl-CCP varies. In two other batches of tissue $10^{-7}$M Cl-CCP had no significant effect. In another experiment it was also found that $10^{-6}$M Cl-CCP inhibited the plasmalemma influx and the influx to the vacuole equally at 0·1 mM KCl.

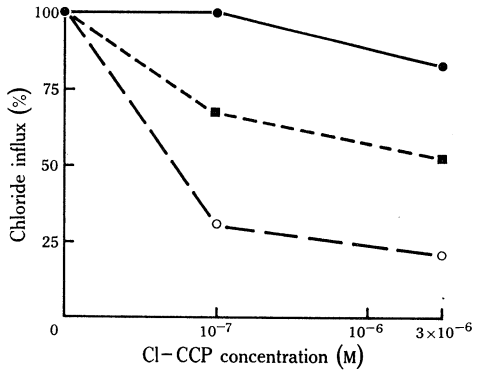



Fig. 8.—The effect of Cl-CCP on chloride influx to whole maize root segments. Tissue was pretreated in water±Cl-CCP for 80 min, and the fluxes then measured in 50 or 0·1 mM KCl±Cl-CCP. Control values:
● $M_{oc}$, 50 mM KCl = 21·2 μmole $g^{-1}$ $hr^{-1}$.
■ $M_{ov}$, 50 mM KCl = 8·8 μmole $g^{-1}$ $hr^{-1}$.
○ $M_{ov}$, 0·1 mM KCl = 0·26 μmole $g^{-1}$ $hr^{-1}$.

The influx to the vacuole is limited by $M_{oc}$ at 0·1 mM KCl, and, in agreement, $M_{ov}$ and $M_{oc}$ are equally highly inhibited by Cl-CCP. At 50 mM KCl a smaller fraction of $M_{oc}$ and of $M_{ov}$ are inhibited by Cl-CCP, as though a Cl-CCP-insensitive fraction of chloride influx increases more than a Cl-CCP-sensitive fraction as the external KCl concentration is raised. The differential effect of $10^{-7}$M Cl-CCP at 50 mM KCl ($M_{ov}$ inhibited by 33%; $M_{oc}$ not apparently inhibited) shows that at least 33% of the chloride influx to the vacuole is by a process independent of the influx across the plasmalemma, i.e. as though moving from bulk cytoplasm across

the tonoplast. Any "straight-through" transport from external solution to vacuole is less sensitive to Cl-CCP than is the tonoplast influx.

(iii) *Effect of Temperature Changes*

At 0·1 mM KCl the plasmalemma influx and the influx to the vacuole are again equally sensitive to temperature changes, both chloride fluxes increasing, with a $Q_{10}$ of 2·8, between 10 and 30°C. At 50 mM KCl the plasmalemma influx is not significantly altered by changing the temperature from 10 to 30°C ($Q_{10} = 0·9$), whereas the influx to the vacuole has a $Q_{10}$ of 1·9.

This again suggests that the plasmalemma influx at 50 mM KCl differs in nature from the plasmalemma influx at lower external KCl concentrations, and from the influx to the vacuole. The results would not exclude the possibility that the plasmalemma influx at high external KCl concentrations has a small temperature-sensitive component.

(iv) *Effect of Different Univalent Cations*

Table 7 shows that chloride influx from 50 mM Cl is very nearly the same from sodium and potassium salts. At 0·1 mM Cl, in contrast, chloride influx is nearly three times higher from NaCl than KCl. The plasmalemma influx again differs in its response at high and low chloride concentrations. The influx to the vacuole has the same sensitivity to the nature of the univalent cation as the plasmalemma influx at the same external chloride concentration.

TABLE 7

EFFECT OF SODIUM OR POTASSIUM ON CHLORIDE INFLUX IN BARLEY ROOTS

| Chloride concn. (mM) | Accompanying cation | $M_{oc}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) | $M_{ov}$ ($\mu$mole $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|
| 50 | $K^+$ | 16·9±0·2 | 3·4±0·2 |
| | $Na^+$ | 19·4±0·4 | 3·5±0·1 |
| 0·1 | $K^+$ | 0·31±0·03 | 0·18±0·02 |
| | $Na^+$ | 0·85±0·02 | 0·51±0·03 |

## IV. DISCUSSION

### (*a*) *Measurement of Chloride Fluxes in Maize Root Cortical Cells*

(i) *Plasmalemma Influx*, $M_{oc}$

In a previous paper (Cram and Laties 1971) it was argued that the initial influx to barley root segments estimated from the tissue contents after a short radioactive load followed by a short non-radioactive wash was a valid estimate of the plasmalemma influx. The primary concern was to show that at these short times the chloride in the tissue in excess of that expected from the linearly rising, slowly exchanging, fraction was in the cytoplasm and not in the extracellular spaces or in the stele.

In maize, as in barley, loss of extracellular chloride from the root is rapid ($t_{\frac{1}{2}} = 1·1$ min in maize) and would have been completed in the 7½-min washing period. This conclusion depends on the identification and characterization of the extracellular chloride loss, and therefore on the validity of the extrapolation of the more slowly exchanging components of the wash-out curve to $t = 0$. Other work

confirms that extracellular loss of ions in barley roots is complete within 7 min (Epstein *et al.* 1963). Therefore the chloride in the tissue after a 7½-min wash in excess of the linearly rising fraction is not in the extracellular spaces.

In this paper no essential difference was found between isolated cortices and whole root segments, which shows that none of the chloride in the tissue after short loading and washing times was in the stele (Table 1). This confirms the results of Laties and Budd (1964) and Hall *et al.* (1971), and agrees with Ginsburg and Ginzburg's observation (1970*b*) that isolated cortical tissue will actively transport ions. Separation of the cortex from the stele does not therefore appear to alter transport in cortical cells significantly. If leaks through plasmodesmata are a consequence of separation, it must be supposed that they close up rapidly.

The only remaining possibility appears to be that the chloride in the tissue after a short load and wash in excess of the linearly rising fraction is in the cytoplasm. Figure 3 shows that to a first approximation one can kinetically identify the extra chloride with the contents of the middle component of the wash-out curve [Fig. 2(*b*)], which also very probably corresponds to the cytoplasm. The plasmalemma influx is therefore not overestimated due to inclusion of extracellular contents. It may, however, be underestimated due to losses from the cytoplasm during the loading and washing periods (Cram 1969*b*). This error depends on the relation of the loading and washing times to the cytoplasmic exchange half-time which can be obtained from the mid-component of the wash-out curve. An idea of the size of the error in $M_{oc}$ therefore depends on the validity of fitting exponentials to the wash-out curve. In the present case $M_{oc}$ is calculated as previously (Cram 1969*b*) to be underestimated by about one-third. Since the cytoplasmic half-time is nearly constant over a wide range of external KCl concentrations (Table 2), the percentage error will be nearly constant over this range. Therefore, even allowing for this error, the form of the relationship between the plasmalemma influx and external KCl concentration shown in Figure 6 will be correct.

(ii) *Dependency of the Initial Influx Estimate on the Validity of the Exchange Kinetics*

The initial influx estimate of the plasmalemma influx depends on the validity of the identification of extracellular and cytoplasmic exchange as exponentially falling components of the wash-out curve. The justification for this has been discussed previously (Cram 1968*a*). Perhaps the most conclusive basis for an identification is the comparison of living and dead tissue. The loss from extracellular spaces would be expected to be similar to loss as by diffusion, and to be nearly the same as loss from dead tissue. Both of these are true of the fastest component of loss of chloride from living maize root tissue (e.g. Fig. 2, cf. Cram 1968*a*). The results shown in Figure 2 also add a confirmation of the goodness of fit of exponentials to the wash-out curve. This is the identity of the exponential rate constants fitting the two types of plot of the wash-out data. If a curve is of the form

$$\sum_i Q_{io} \exp(-k_i t),$$

then the slope will be

$$-\sum k_i Q_{io} \exp(-k_i t).$$

In a plot of slope versus time the rate constants will be the same as in the original curve, but the size of each component of the curve will be increased in proportion to its own rate constant, and not by a constant fraction. The components of the curve will therefore change relative to each other. A curve of four exponential components could well be fitted fortuitously by three rate constants, but it follows from the above that the plot of the slope of the curve would be unlikely to be *equally* well fitted by the *same* three rate constants. The equally good fit to the two types of plot of chloride loss from maize root cortices [Figs. 2(*a*)–2(*c*)] shows that the three exponentials are a good fit to the data.

There is therefore some justification for the curve-fitting and extrapolation used to find the washing period necessary to remove extracellular chloride and to find the error in the estimate of the plasmalemma influx.

(iii) *The Quasi-steady Influx to the Vacuole,* $M_{ov}$

The influx to the vacuole can be measured accurately when it is constant with time. On the other hand it cannot be interpreted easily, since it is the result of several transport systems. This flux has been discussed previously (Cram 1969*b*).

The observed non-linearity of content versus loading time after a 30-min wash in whole root segments (Fig. 4) is apparently due to the fact that labelled chloride enters the cytoplasm from the radioactive bathing solution, but moves out of the cytoplasm mainly to the stele during the non-radioactive wash; and the amount transferred to the stele is a significant fraction of the total chloride in the root under some conditions. In barley roots at low external concentrations the root content rises linearly with time (Epstein *et al.* 1963), and therefore stelar contents appear to be negligible. However, the estimate of the quasi-steady influx to the vacuole must be made with caution, particularly at short loading times and at high external solution concentrations when the stelar contents are more likely to be a significant fraction of the total in the roots, as in Figure 4 and Table 1, experiment C.

(iv) *Calculation of other Fluxes in the Cell*

In the present paper three fluxes have been measured: $M_{oc}$, $M_{ov}$, and the net influx, $M_{net}$. $M_{oc}$ is nearly constant for more than an hour after first transferring to salt (Fig. 5), and can therefore be used in conjunction with $M_{ov}$ and $M_{net}$, which are also constant over an hour or more (Figs. 3, 4, 5, and 7).

The efflux from the cell, $M_{co}$, is given by

$$M_{co} = M_{oc} - M_{net}.$$

In intact roots 75% of the chloride moves to the stele, and only 25% moves out across the membrane that chloride moves in across (Greenway 1967; Pitman 1971; Weigl 1971; present paper), and the same may be true of the isolated cortex (Ginsburg and Ginzburg 1970*b*). This means that the influx across the same membrane as influx may be only 25% of the total efflux from the cortical cells ($M_{co}$).

If the only flux into the vacuole is a "straight-through" influx, then $M_{ov}$ will equal this flux, and the influx across the plasmalemma will equal $M_{ov}$ plus the influx from the medium to the cytoplasm. At the other extreme, if the plasmalemma and

tonoplast are in series, then at a quasi-steady state (when the cytoplasmic content is not changing significantly with time)

$$M_{ov} = M_{oc}.M_{cv}/(M_{co}+M_{cv}),$$

and hence

$$M_{cv} = M_{ov}.M_{co}/(M_{oc}-M_{ov}).$$

Also

$$M_{vc} = M_{cv}-M_{\text{net}}.$$

### (*b*) *Characterization of Chloride Fluxes in Maize Root Cortical Cells*

#### (i) *Plasmalemma Fluxes*

Chloride fluxes in maize root cortical cells were calculated as above from the data in Figure 6 and Table 4. It was assumed that there is no straight-through influx to the vacuole, and that efflux is across the same membrane as influx. The lines passing through the values are shown in Figure 9.

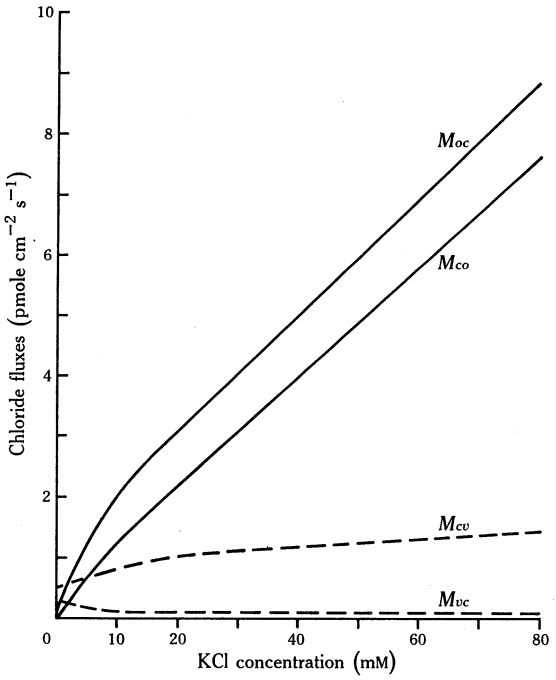



Fig. 9.—Chloride fluxes at the plasmalemma and tonoplast in maize root cortical cells over a range of external KCl concentrations. Calculated as in the text from the data of Figure 6 and Table 4.

The electrochemical potential on the two sides of the plasmalemma can only be obtained indirectly. The electrical potential difference between the external solution and the vacuole is assumed to be located mainly at the plasmalemma (Etherton and Higinbotham 1960; Greenham 1966). The chloride activity on the outside of the plasmalemma is assumed to be the same as in the bathing solution. The concentration in the cytoplasm is taken to be similar to the concentration in maize root tips which have equilibrated with the external solution (cf. Cram 1973*a*). Under the

conditions in which $M_{co}$ is estimated the cytoplasm would have reached a steady state after transfer to the salt solution. Chloride activity coefficients in bean root tips are nearly the same as in free solutions of the same concentration (Gerson and Poole 1972).

Table 8 gives these values for a range of external KCl concentrations. There is a net inwards chloride movement up its electrochemical potential gradient at all concentrations. One can calculate what proportion of the chloride influx is likely to be active by assuming all the efflux is passive and calculating the ratio of independent passive fluxes expected with this electrochemical potential gradient (Teorell 1949; Ussing 1949). This ratio is also shown in Table 8, together with values of

TABLE 8

COMPONENTS OF PLASMALEMMA CHLORIDE FLUXES, $M_{co}$, $M_{oc}$, AND CHLORIDE PASSIVE PERMEABILITY COEFFICIENT, $P_{Cl}$

| External chloride concn. (mM) | Cytoplasmic chloride concn. (mM)* | $(E_i)$† (mV) | $f(E_i)$‡ | $M_{co}$§ | $M_{oc}$‖ (passive) (pmole cm$^{-2}$ s$^{-1}$) | $M_{oc}$# (active) (pmole cm$^{-2}$ s$^{-1}$) | $10^8 \times P_{Cl}$ (cm s$^{-1}$)¶ |
|---|---|---|---|---|---|---|---|
| 0·1 | 13 | −95 | $1{\cdot}7\times10^{-4}$ | 0·10 | 0 | 0·18 | 0·2 |
| 1 | 19 | −75 | $2{\cdot}6\times10^{-3}$ | 0·16 | 0 | 0·44 | 0·3 |
| 3 | 20 | −61 | $1{\cdot}3\times10^{-2}$ | 0·32 | 0 | 0·76 | 0·6 |
| 20 | 26 | −34 | 0·20 | 2·1 | 0·42 | 2·7 | 4·4 |
| 50 | 40 | −20 | 0·57 | 4·8 | 2·7 | 2·2 | 8·2 |

* Steady-state chloride concentration in maize root tips.

† From Dunlop and Bowling (1970).

‡ $f(E_i) = (c_o/c_i)\exp(-zFE_i/RT)$.

§ From Figure 9, assuming efflux is entirely across a single membrane, and using a factor to convert $\mu$mole g$^{-1}$ hr$^{-1}$ to pmole cm$^{-2}$ s$^{-1}$ obtained from measurement of cell size.

‖ $M_{co}$ × ratio in column 4.

# $M_{oc}$ from Figure 9 minus the passive component of $M_{oc}$ calculated in column 6.

¶ Calculated from the constant-field equation of Goldman (1943)—see Cram and Laties (1971, p. 643).

efflux and expected passive influx. It appears that up to 20 mM KCl at least 90% of $M_{oc}$ is active, and probably 50% or more is active at higher external solution concentrations. This is a minimum estimate of the active component, since the efflux across the same membrane as influx may be only a fraction of the total efflux, depending on the fraction transferred to the stelar side of the tissue. Possibly a fraction of these tracer chloride fluxes is also due to exchange diffusion (cf. Weigl 1968; Cram 1968*a*; Cram and Laties 1971), though Figure 5 gives no evidence of exchange diffusion. It is therefore not certain how the active component of chloride influx changes with external chloride concentration at higher external chloride concentrations. The plasmalemma influx above 50 mM appears to be relatively large, but in fact is less than 10 pmoles cm$^{-2}$ s$^{-1}$, which is 2–3 times higher than maximum chloride fluxes in Characeae in pond water, but small compared with some active fluxes in marine algae (MacRobbie 1970).

Values of the chloride permeability coefficient calculated from $M_{co}$ and using the Goldman constant-field equation (Goldman 1943) systematically increase from $0{\cdot}2\times10^{-8}$ to $8\times10^{-8}$ cm s$^{-1}$ with increasing external KCl concentration. The passive permeability coefficient, $P_{Cl}$, may therefore not be constant at different KCl concentrations (possibly via an effect of ionic strength, cf. Szabo *et al.* 1969), or there may be an error in the value of the electrochemical potential or in regarding the efflux as an independent passive movement. A simple modification would be that an exchange diffusion component increases with external KCl concentration and constitutes an appreciable fraction of $M_{co}$ above 3 mM external KCl. It appears probable, however, that $P_{Cl}$ is around $1\times10^{-8}$ cm s$^{-1}$ in maize root cortical cells.

(ii) *Other Fluxes in the Cell*

Figure 7 shows that the quasi-steady influx to the cell is accompanied by an exactly equal amount of potassium. This component of chloride influx, at least, is therefore involved in net salt transport into the cell.

The tonoplast fluxes as calculated change relatively little with external concentration. They are not extrapolated to zero at zero external solution concentration since there is no way in which the tonoplast fluxes can be envisaged as depending on the external chloride concentration rather than the cytoplasmic concentration, and one cannot imagine that the cytoplasmic chloride concentration falls to zero at zero external chloride concentration. Tonoplast fluxes can be analysed in the same way as plasmalemma fluxes. If the cytoplasmic chloride concentration does rise as in root tips, and if the electrical potential difference across the tonoplast is small, then it appears that the tonoplast influx is not a simple passive movement. This would be consistent with its sensitivity to uncouplers (Fig. 9; see also Arisz 1958; Cram 1969*a*) and to internal chloride concentration (Table 6; see also Cram 1968*b*). The numerous uncertainties in the values make such a thermodynamic analysis of the tonoplast fluxes only marginally profitable.

(iii) *The Cytoplasmic Concentration*

The cytoplasmic content ($Q_c$) can be calculated from the relationship

$$Q_c = (M_{co}+M_{cv})/k_c.$$

Values of $k_c$ from Table 2 and of fluxes from Figure 9 can be used to calculate several values of $Q_c$. At 0·1 and 3 mM external KCl concentration in KCl-loaded tissue, and at 1 mM in fresh tissue the cytoplasmic content is about 1 $\mu$mole g$^{-1}$. If the cytoplasm occupies about 2% of the tissue volume the cytoplasmic concentration would be 50 mM, which is within the range measured directly in the streaming cytoplasm of the Characeae (MacRobbie 1970). At 25 and 50 mM external KCl concentration the cytoplasmic chloride content is 7·3 and 11·5 $\mu$mole g$^{-1}$ respectively. In 2% of the tissue volume the concentration would be about 500 mM. This is much higher than in root tips in the same solutions. It is also higher than the steady-state concentration in the vacuole (Fig. 1), and this raises the problem of an unequal osmotic pressure in cytoplasm and vacuole. One possibility is that the cytoplasm swells and the chloride concentration in it remains low at high external KCl con-

centrations. In a preliminary electron-microscopical examination of barley root cells, where the same problem arises (Cram and Laties 1971), no significant cytoplasmic swelling was observed in 50 mM KCl, though a 10-fold increase in volume would have been expected to have been apparent. In root tips the chloride concentration increases threefold over the same range of external KCl concentrations, and therefore only a threefold increase in the volume of the cytoplasm may have occurred, and this would possibly not have been detected. These high values of the chloride concentration calculated to be in the cytoplasm are a major problem; and one cannot tell whether they imply an error in flux estimates, exchange kinetics, or the volume of the cytoplasm.

(iv) *Qualitative Differences between Various Fluxes*

The plasmalemma chloride influx is less sensitive to temperature changes, Cl-CCP, and the nature of the accompanying cation at high external chloride concentrations. More extensive measurements are needed to show if the smaller sensitivity is due simply to a fraction of the total influx being passive at high external chloride concentrations.

The influx across the plasmalemma and the influx to the vacuole cannot be distinguished qualitatively at low external chloride concentrations, as would be expected since the plasmalemma influx is the rate-limiting step in the influx of chloride to the vacuole. At high external chloride concentrations the plasmalemma influx is less sensitive than the influx to the vacuole to temperature changes, Cl-CCP, and the nature of the accompanying cation. The effect of Cl-CCP agrees with previous results (Arisz 1958; Torii and Laties 1966; Cram 1969*a*) in showing the influx to the vacuole to be more sensitive to respiratory uncouplers than the influx across the plasmalemma.

The plasmalemma influx and the influx to the vacuole differ in their responses to a raised internal KCl concentration. The plasmalemma influx is not altered at any external chloride concentration, and in this regard maize differs from barley (Cram and Laties 1971). The influx to the vacuole is reduced by a high internal KCl concentration at high external KCl concentrations (as in carrot and barley—Cram 1968*b*; Cram and Laties 1971). A reduction at low external chloride concentrations may not be apparent because the plasmalemma influx, which is insensitive to internal KCl concentration, is the rate-limiting step. Venrick and Smith (1967) also found no effect of internal KCl concentration on rubidium influx from 0·1 mM RbCl in maize root cells.

### (*c*) *General*

(i) *Flux Measurement*

The method of calculating fluxes in this paper is based on the same concepts as in previous methods (Pitman 1963). The main difference in the method is the use of the initial influx estimate of the plasmalemma influx. As pointed out above, tests of the validity and accuracy of this estimate still depend on knowing the kinetics of exchange of the system.

The initial influx estimate of the plasmalemma influx has the advantage that it can be obtained rapidly and under non-steady-state conditions, but has the dis-

advantage that it is only semi-quantitative. It can be used cautiously as a guide to suggest trends or order-of-magnitude changes. The only quantitative measure of the plasmalemma influx is that derived from the analysis of exchange kinetics (Pitman 1963; Cram 1968*a*). This estimate of the plasmalemma influx is fairly unambiguous since it does not depend on the assumptions about the arrangement of compartments necessary for calculating other fluxes. The accurate estimate of the plasmalemma influx has the disadvantage that it is a lengthy measurement and can only be made at a quasi-steady state.

(ii) *Implications for Interpreting Influx Isotherms*

The results of Figure 9 show that in maize tissue the influx to the vacuole is limited by the influx across the plasmalemma at low external solution concentrations, but by the influx across the tonoplast at high external concentrations. This conclusion would be unaltered if there were a straight-through pathway to the vacuole. The influx to the vacuole therefore "saturates" at high external concentrations because the tonoplast influx or the straight-through influx saturates, while the plasmalemma influx continues to increase. This agrees with measurements on carrot tissue (Cram 1968*a*), barley root tissue (Cram and Laties 1971), potato tissue (Lannoye 1970), and beet tissue (Pitman 1963); and with the inference of Torii and Laties (1966) drawn from a comparison of root tip and vacuolate maize root cells. This conclusion concerning the *kinetics* of the system is not, of course, affected by conclusions about the *mechanisms* of any particular transport process.

The suggestion that the plasmalemma influx at high external concentrations is mainly passive (Torii and Laties 1966) does not appear to be supported by the results shown in Table 8 or by those of Gerson and Poole (1972), but this does not alter the conclusions about the kinetics of the system.

The isotherm of influx to the vacuole shown in Figure 7 can be closely fitted by a rectangular hyperbola. However, Figure 9 shows that none of the individual fluxes has a rectangular hyperbolic relationship to external concentration, and the fit of the vacuolar influx isotherm by a rectangular hyperbola is therefore purely fortuitous. The parameters of the fitted rectangular hyperbola bear no relation to characteristics of any individual transport process. To describe this complex of processes as a "system", particularly if values of parameters are ascribed to it, seems inappropriate. Figure 7 also shows that the vacuolar influx isotherm does not depart significantly from a monotonically rising curve when the variability of the points is established. Apparent discontinuities in vacuolar influx isotherms in other tissues (e.g. Elzam *et al.* 1964; Epstein and Rains 1965; Nissen 1971) have not been shown not to be due to random variation.

(iii) *Implications in Cell Function*

It is observed in root tips and in vacuolate cells that the steady state internal concentration increases less than proportionately to $M_{ov}$ and considerably less than proportionately to $M_{oc}$, or the active component of $M_{oc}$, in "low-salt" maize roots (Fig. 1; Table 8; and Cram, unpublished data). Two sets of results in this paper offer suggestions as to how the internal concentration may thus apparently be regulated independently of the external concentration. The first is the increase in $P_{Cl}$

calculated from $M_{oc}$ at higher external concentrations; the second is the reduction in influx to the vacuole in KCl-loaded tissue, which is a greater proportion at higher external solution concentrations (Table 6). The first would give some regulation of the cytoplasmic concentration in relation to the external concentration. The second would give some extra control of the vacuolar concentration, as discussed by Pitman (1969). A more quantitative discussion is not warranted by the accuracy of the present data.

The lack of effect of external osmotic pressure on chloride or potassium influx shows that the chloride influx does not decrease during accumulation as a response to the increase in internal hydrostatic pressure. In carrot and barley tissue there is a similar negative feedback of internal content on chloride influx, the decreased influx being in response to the increasing internal ($Cl+NO_3$) concentration, and not to an increased internal hydrostatic pressure or other factor (Cram 1973*b*).

Thus in barley, maize, and carrot root tissue the only regulation of chloride transport and accumulation appears to be in maintaining the internal chloride concentration less variable than the external chloride concentration. If the internal chloride concentration is homeostatically controlled, then chloride accumulation cannot at the same time be concerned in the homeostatic control of internal osmotic or hydrostatic pressure.

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