THE USE OF SHORT-TERM AND QUASI-STEADY INFLUX IN ESTIMATING PLASMALEMMA AND TONOPLAST INFLUX IN BARLEY ROOT CELLS AT VARIOUS EXTERNAL AND INTERNAL CHLORIDE CONCENTRATIONS

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Abstract

Chloride influx measured with ^{36}Cl over a 10 min loading period followed by a 5 min wash in inactive solution is used as an estimate of influx across the plasmalemma in barley roots. Chloride influx measured over a 60 min loading period followed by a 30 min wash is used as an estimate of the quasi-steady influx from the external solution to the vacuole.

The 10 min load, 5 min wash flux estimate is shown not to be in error due to inclusion of extracellular contents in the root cortex or in the stele. It therefore appears to be a good estimate of the influx across the plasmalemma of the cortical cells.

At external chloride concentrations below about 5 mM the plasmalemma influx and the influx to the vacuole are approximately equal, and therefore the influx across the plasmalemma appears to be the rate-limiting step in the influx of chloride to the vacuole. At external chloride concentrations above about 10 mM the plasmalemma influx increases nearly linearly with external chloride concentration to very high values at 80 mM, whereas the influx to the vacuole does not increase above 20–30 mM. The "saturation" in influx to the vacuole at high external chloride concentrations therefore appears to be determined by the saturation of transport across the tonoplast or of a transport process directly through the cytoplasm to the vacuole, in confirmation of the conclusion of Torii and Laties (1966).

Some observations suggest that part of the fluxes across the plasmalemma are mediated by exchange diffusion.

In salt-loaded tissue the plasmalemma influx and the influx to the vacuole are both reduced. This apparent negative feedback relationship between chloride content and influx of chloride is briefly discussed.

I. Introduction

The initial influx of radioactivity to a plant cell must be equal to the influx across the outermost boundary of the cell. The requirements and inherent difficulties in measuring the true initial influx to a plant cell were discussed in a previous paper (Cram 1969). The requirements are that the influx must be measured over a period

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short compared with the turnover time of any detectable cellular compartment, and that any subsequent rinsing after the loading period must be negligible compared with this turnover time. The difficulty is that the washing time has to be long enough to remove all the extracellular contents from the tissue so that the cell contents can be measured. During the time taken to wash out the extracellular contents some of the intracellular contents will have been lost, and the initial influx to the cell, presumably across the plasmalemma, will consequently be underestimated. The error due to loss from a rapidly exchanging intracellular compartment may be considerable, as in carrot for instance, where the half-time for cytoplasmic turnover is around 10 min, and the time necessary to wash out 97% of the extracellular spaces is 7 min (Cram 1968*a*). The error will be small if the cytoplasm exchanges much more slowly than the extracellular spaces, as in beet at low temperatures (Briggs, Hope, and Pitman 1958; Pitman 1963).

The first requirement in such experiments is to know the time taken to wash out the bulk of the extracellular contents. This can be found from an examination of the time course of the loss of tracer from the tissue. One can also make some rational estimate of the error in the initial influx estimate from a knowledge of the cytoplasmic turnover half-time. This can also be found from examination of the wash out of tracer from living tissue, provided that extracellular, cytoplasmic, and vacuolar components of the efflux can be distinguished and identified.

The flux that has usually been measured in investigations of the ionic relations of higher plant cells is the steady influx to the cell. After the loading period in radioactive solution, it has been usual to wash the tissue with inactive solution for a period of 30 min or more. During this period any rapidly exchanging compartment will have lost the tracer in it, and consequently any initial faster influx will have been missed, even when very short loading times were used (Cram 1969). This steady (in fact quasi-steady) influx must be supposed to be the rate of movement from the external solution to the vacuole. For brevity this flux will be called the "influx to the vacuole". It is the result of several transport processes and must not be confused with the influx from the cytoplasm to the vacuole across the tonoplast.

If it is possible to estimate the size of the initial influx to the cell, then this can be compared with the influx to the vacuole. Despite the expected drawbacks, it seemed worth while to use the method as a rapid and fairly unambiguous, although only semiquantitative, way of seeing whether the plasmalemma influx is greater than the influx to the vacuole, and thus checking some of the results obtained by other methods (Torii and Laties 1966; Cram 1968*a*, 1968*b*).

The only method of making routine quantitative estimates of fluxes within plant cells under physiological conditions seems likely to be the critical use of compartmental analysis of tracer exchange kinetics. This method must be used with caution since errors in graphical analysis can easily be made (e.g. Van Liew 1962) and the model to which such results are fitted is at best only likely to be a good approximation to the actual system (Cram 1968*b*; MacRobbie 1969). The method of measuring the initial influx to a cell and comparing this with the influx to the vacuole does not suffer from the uncertainties in the interpretation of more complex tracer exchange kinetics, although both are based on the same principles. It suffers from some uncertainties of its own and can be considered only semiquantitative, as indicated above.

In the first part of this paper it is attempted to establish that the short-term influx is a reliable estimate of the initial influx to the cell and, in particular, that it is not an artefact due to including extracellular contents in the estimate of cell contents after a short loading period. In the second part of the paper the method is used to estimate the sizes of the plasmalemma influx and the influx to the vacuole in relation to the concentration in the external solution in freshly excised, low-salt tissue and in tissue which has accumulated salt.

II. Materials and Methods

Barley (*Hordeum vulgare* L.) seedlings were grown on cheesecloth on stainless steel screens in 0·5 mM $CaSO_4$ with 50 mg chloramphenicol per litre + 50 mg streptomycin per litre in the solution to reduce bacterial growth. The seedlings grew in the dark and the solution was aerated. Six-day-old seedlings were harvested and their roots cut into approximately 1-cm segments and placed in an aerated solution of 0·5 mM $CaSO_4$ until used in an experiment. For measuring influx, samples of root segments were placed in aerated solutions of the required concentration labelled with ^{36}Cl in a thermostatically controlled water-bath, usually held at 25°C. After the loading period the root segments were transferred to non-labelled solutions of the same concentration. Following the washing period the root segments were lifted out, blotted, and weighed in scintillation counting vials, and dried down under alkaline conditions (not sufficiently alkaline to interfere with subsequent scintillation counting). The ions were then extracted in 1 ml of water, scintillation fluid added, and the whole stirred and counted. Standards were treated similarly. Self-absorption corrections were made as necessary. Volumes of solution were such that there was only a small percentage change in their concentration during the period of immersion of the root samples. Influx is calculated as tissue content (counts per minute per gram fresh weight of tissue/counts per minute per μmole of chloride in the external solution) divided by the loading time.

For measuring efflux, root segments were loaded for about 90 min with chloride in solutions containing ^{36}Cl as tracer, and then transferred to a washing-out apparatus similar to that used in earlier work (MacRobbie and Dainty 1958; Cram 1968*a*). The washing-out procedure was as described in those papers.

III. Results

(*a*) *Loading and Washing Times for Estimating Initial Influx of Chloride to Barley Roots*

For estimating the initial influx the washing time must be just sufficient to remove the major fraction of the extracellular contents. If the washing time is shorter than this the extracellular contents will be a significant fraction of the total in the tissue, especially after a short loading time, and tissue content divided by loading time will not be a measure of the initial influx to the cells. On the other hand the best estimate of initial influx is with the shortest possible washing time.

The time taken to wash out the extracellular contents was estimated from the kinetics of loss of tracer from barley roots. Tissue samples were loaded with ^{36}Cl in solutions of 0·1 mM, 1·0 mM, and 25 mM KCl for about 90 min, and then washed in successive aliquots of inactive solution. The wash-out curves were similar to those obtained in experiments on several other plant tissues by various authors, and could be divided graphically into three exponential components. The separation of the two faster exchanging phases was not completely satisfactory, probably mainly due to the fact that there was a net accumulation of salt by the cells during the course of the experiment, resulting in non-first-order exchange kinetics. The best fit to the

two slower exponential components was with the half-times shown in Table 1. The uncertainty in fitting the slope of the intermediate component by eye is not more than ±20%.

TABLE 1

HALF-TIMES FOR THE TURNOVER OF THE TWO SLOWER EXCHANGING COMPARTMENTS OF THE WASH OUT OF ^{36}Cl FROM BARLEY ROOTS

KCl concentration (mM):	0·1	1·0	25
Half-time for cytoplasmic turnover (min):	10	9	9
Half-time for vacuolar turnover (hr):	19·3	16·9	11·4

The half-time for the exponential part of the fastest exchanging component lay between 0·4 and 1·1 min at all three concentrations. The apparent content of this component was proportional to the external concentration within a factor of 3, being equivalent to between 3 and 8% of the tissue volume. Since the external concentration varied 250-fold, this agrees fairly well with the fastest exchanging component having a content proportional to the external concentration, as expected of extracellular chloride.

On the basis of these results the initial influx has been estimated as the content of the tissue after 10 min radioactive loading followed by 5 min wash in inactive solution, divided by $\frac{1}{6}$ hr. For simplicity this will frequently be called the plasmalemma influx. The influx to the vacuole from the external solution has been estimated as the content of the tissue after 60 min radioactive loading followed by 30 min non-radioactive wash. The influx to the vacuole was the same when estimated from a 10 min load followed by a long wash as when estimated from a 60 min load followed by a long wash, as has previously been observed by many authors.

(*b*) *Values of Plasmalemma and Tonoplast Influxes over a Range of External Solution Concentrations*

The values of the plasmalemma influx (10 min load–5 min wash) and of influx to the vacuole (60 min load–30 min wash) in freshly excised 6-day-old barley root segments over a range of external chloride concentrations are shown in Figure 1(*a*). At low external chloride concentrations the plasmalemma influx is nearly equal to the influx to the vacuole. In another experiment it was found that the plasmalemma influx is about equal to the influx to the vacuole over the range 0·02–1·0 mM. It must be supposed that only the plasmalemma influx changes as a direct consequence of changes in the external solution concentration, and that any change in the influx to the vacuole is the result of a change in the plasmalemma influx. Since the influx to the vacuole changes by the same amount as the plasmalemma influx, it appears that the influx to the vacuole from the external solution is closely controlled by the influx across the plasmalemma at low external concentrations.

Above 10 mM chloride the influx to the vacuole does not continue to increase as the external solution concentration increases, but saturates at 20–30 mM, as found previously by many authors. The plasmalemma influx, however, continues to rise more or less linearly until it is more than 10 times greater than the influx to the vacuole at 80 mM. The same result has been obtained in several experiments on both barley and maize roots. The results shown in Figure 1(*a*) are quoted as they are from the same batch of tissue as the results of Figure 1(*b*).

These results show that the influx to the vacuole in these tissues is not limited by the influx across the plasmalemma at high external concentration, and must therefore be limited by a transport process within the cell, presumably at the tonoplast. Alternatively, if chloride moves from the external solution to the vacuole only via a direct path through the cytoplasm (cf. *Nitella*, MacRobbie 1969), then this straight-through path saturates at 20–30 mM, and there is no movement inwards from the bulk of the cytoplasm to the vacuole across the tonoplast. However the chloride arrives at the vacuole, it is a transport process within the cell and not at the plasmalemma which is saturated at high external solution concentrations, in confirmation of the conclusions of Torii and Laties (1966) and Cram (1968*a*).

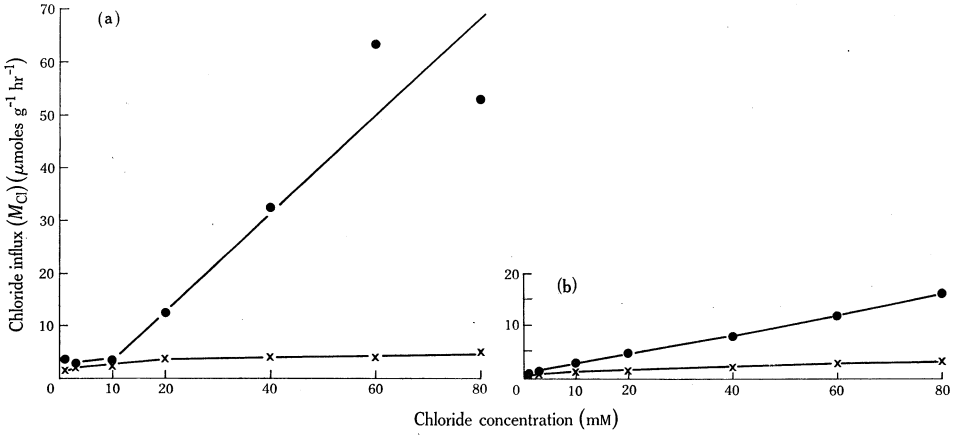

Fig. 1.—Plasmalemma influx and influx to the vacuole of ^{36}Cl in low-salt and high-salt barley roots. Plasmalemma influx (●): 10 min ^{36}Cl uptake followed by 5 min wash in inactive solution. Influx to the vacuole (×): 60 min ^{36}Cl uptake followed by 30 min wash in inactive solution. Results obtained from two halves of a single batch of barley root segments. (*a*) ^{36}Cl fluxes in freshly excised low-salt root segments. (*b*) ^{36}Cl fluxes in root segments which had accumulated salt from 25 mM KCl + 5 mM $CaCl_2$ for 22 hr.

Figure 1(*b*) shows the same measurements on samples of tissue from the same batch as Figure 1(*a*), which had accumulated salt from a solution of 25 mM KCl + 5 mM $CaCl_2$ for 22 hr. The data of Pitman, Courtice, and Lee (1968) show that after this treatment the roots would probably have contained 90–100 μ-equiv. g^{-1} of chloride. Fresh tissue contains about 5 μ-equiv. g^{-1} of chloride. The sugar content of the salt-loaded tissue would have fallen (Pitman and Nair, quoted in Pitman 1969*b*).

After the tissue has accumulated salt, the plasmalemma influx and influx to the vacuole are lower than in the fresh tissue. At all concentrations the plasmalemma influx is reduced by about 70% after loading with salt. The influx to the vacuole is reduced by only 35% at high concentrations, but the reduction increases at lower concentrations until at 1 mM it is also reduced by about 70% after loading the tissue with salt.

At high external chloride concentrations, although the plasmalemma influx is reduced by over 70% it is still several times greater than the influx to the vacuole in the fresh tissue. Therefore the reduction in the influx to the vacuole after loading

with salt cannot be the result of the reduction in the plasmalemma influx, but must be a direct effect on a flux within the cell, possibly at the tonoplast. At lower concentrations the plasmalemma influx increasingly limits the influx to the vacuole, and the greater reduction in the influx to the vacuole can reasonably be accounted for as a consequence of the reduction in the plasmalemma influx.

These results show that the plasmalemma influx is directly reduced as a consequence of salt having been accumulated in the vacuole; and that the influx to the vacuole is additionally and independently reduced, possibly due to a reduction in the influx from the cytoplasm to the vacuole across the tonoplast. The results also suggest that at low external concentrations the greater reduction in influx to the vacuole can be accounted for as a consequence of the reduction in the plasmalemma influx. The mechanism by which the two fluxes are reduced, and the relation of these reductions to the control of cellular contents (cf. Cram 1968*b*; Pitman 1969*b*) will be considered in Section IV.

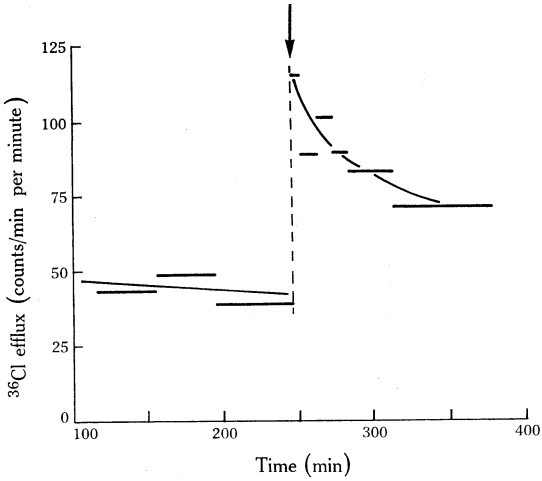

Fig. 2.—Effect of external chloride concentration on the efflux of ^{36}Cl from barley roots. Tissue segments were loaded in ^{36}Cl-labelled 0·1 mM KCl+0·5 mM $CaSO_4$, and washed in inactive solution of the same composition until the faster components had been lost. They were then transferred (arrow) to 25 mM KCl+0·5 mM $CaSO_4$. The time scale is from the beginning of washing out after the end of the ^{36}Cl loading period.

(*c*) *Possibility of Exchange Diffusion of Chloride across the Plasmalemma at High External Chloride Concentrations*

Figure 2 shows the effect of increasing the external chloride concentration from 0·1 to 25 mM on the efflux of ^{36}Cl from barley roots. The roots were loaded in a solution of 0·1 mM KCl+0·5 mM $CaSO_4$ labelled with ^{36}Cl, washed in a non-labelled solution of the same composition until the faster-exchanging components of chloride efflux had been lost, and then transferred to 25 mM KCl+0·5 mM $CaSO_4$. There is an initial sharp rise in ^{36}Cl efflux, followed by a slower fall to a new steady level about twice that in 0·1 mM KCl (cf. the half-times for vacuolar turnover at 0·1 and 25 mM chloride in Table 1. Figure 2 was obtained from the 0·1 mM sample of Table 1). This change of chloride efflux on transfer from 0·1 to 25 mM chloride is similar to that observed in carrot (Cram 1968*a*), except that in the barley root experiments there was a net chloride influx at the time of the experiment, whereas there was no net flux in the carrot experiments. This initial burst in chloride efflux on transfer from low to high external chloride concentration must be given the same interpre-

tation as the initial burst in carrot tissue. It was argued in a previous paper (Cram 1968*a*) that this initial chloride burst can only be accounted for if there is an immediate external chloride-stimulated chloride efflux across the plasmalemma, followed by a slower change in ^{36}Cl efflux as the concentration and specific activity in the cytoplasm changes. The observation also implies that there is a direct movement of chloride from the vacuole to the cytoplasmic phase from which chloride is lost to the external solution. The external chloride-dependent chloride efflux is most simply pictured as one-for-one coupled fluxes of chloride in and out across the plasmalemma.

IV. Discussion

(*a*) *Validity of the Estimate of Plasmalemma Influx*

As the quasi-steady influx to the tissue (after a long wash) is constant with time and absent in dead tissue, it is difficult to escape the conclusion that it represents tracer accumulation in the large vacuoles of the root cortical cells. In interpreting the fact that the 10 min load–5 min wash flux estimate is greater than the 60 min load–30 min wash flux estimate, it is necessary to account for the extra chloride in the tissue after the short load–short wash treatment in excess of that expected from the long load–long wash flux estimate. The extra chloride could (1) have diffused into the cortical extracellular spaces from the outside of the root, and/or (2) diffused down the stele from the cut ends of the root segment. It could also (3) have been transported directly into the stele after by-passing the cortical cells. In these three cases the short load–short wash influx estimate would be greater than, and in no way related to, the plasmalemma influx. The extra chloride could also (4) be in the stele after having passed through the "symplasm" of the cortical cells. And lastly (5) the extra chloride could be in the cytoplasm of the cortical cells. In the fourth and fifth possibilities the inclusion of the extra chloride in the plasmalemma influx estimate would be correct. No definitive experiment for distinguishing alternatives (3) and (4), in as far as they apply to the transport of chloride to the stele, has yet been devised.

(1) The primary objection to the 10 min load–5 min wash flux estimate is that after only 5 min washing some extracellular contents may have remained in the tissue. As already established, the phase of fastest loss from living tissue, which has all the characteristics of being from extracellular spaces within the root cortex, is almost completely removed in 5 min. However, (2) the living root has a stelar conduit open to the solution at either end. A rough calculation shows that chloride diffusion into such a pipe 1 cm long (the average length of the segments) would approach half completion in 3–4 hr. After a 10 min loading period chloride would have diffused into the stele from either end. During washing out this chloride would only partly be lost immediately from the ends of the stele, the rest initially diffusing further down the stele. To contain sufficient chloride to account for the difference between the initial higher influx estimate and the lower quasi-steady influx estimate, it is estimated that the xylem vessels would have to occupy more than 40% of the cross-sectional area of the root, which is much greater than the percentage observed (e.g. Anderson and House 1967). Thus diffusion down the stele does not seem a possible way of accounting for the difference between the short load–short wash and the long load–

long wash flux estimates. In addition it is probable that the root segments are exuding solution from both ends (e.g. Rosene 1941) and that this bulk flow would prevent diffusion of salt into the open ends of the stele.

Direct evidence that the stelar contents do not complicate the measured fluxes [and therefore eliminating possibilities (2), (3), and (4)] comes from a comparison of the fluxes in whole maize roots and in isolated maize root cortices sliced longitudinally in half (Cram, unpublished data). There is no significant difference between either of the flux estimates in the two preparations. Therefore the stele is not a complicating factor in interpreting such measurements in maize and presumably also in barley.

A comparison of Figures 1(*a*) and 1(*b*) shows that the 10 min load–5 min wash flux estimate represents a true cell flux and is not in error due to overlooking some other factor. It must be supposed that any error due to non-cellular contributions being included in the short load–short wash flux estimate would be the same in salt-loaded and fresh (low-salt) tissue from the same batch of roots. This error cannot be greater than the extra chloride in the 10 min loaded–5 min washed high-salt tissue above that expected from the 60 min load–30 min wash flux estimate in high-salt tissue. At 80 mM, for instance, the high-salt tissue content after 10 min load plus 5 min wash is $2\cdot7$ μmoles g^{-1}. Of this $(3\cdot6/6)$ μmole g^{-1} must be the flux into the vacuole, and therefore the error cannot be greater than $2\cdot7-0\cdot6 = 2\cdot1$ μmoles g^{-1}. In fresh tissue the content after 10 min load plus 5 min wash is $11\cdot1$ μmoles g^{-1}. The error in this cannot be greater than $2\cdot1$ μmoles g^{-1}, i.e. at least $11\cdot1-2\cdot1 = 9\cdot0$ μmoles g^{-1} must be due to the cells' activities. Thus the error in the initial influx estimate in fresh root tissue cannot be greater than $(2\cdot1/11\cdot1) = 20\%$. For reasons given above the error in the initial influx due to possibilites (1) and (2) is thought to be much less than this. Alternative (5) is the only remaining possibility, i.e. the extra chloride remaining after the short load–short wash treatment is in the cytoplasm of the root cells. It therefore appears that the influx estimated after a 10 min load followed by a 5 min wash is a true estimate of the influx across the plasmalemma of the root cortical cells.

From knowledge of the cytoplasmic turnover half-times at various concentrations (Table 1) one can make a rational estimate of the error in the plasmalemma influx so obtained. The cytoplasmic turnover half-time is about 10 min from $0\cdot1$ to 25 mM chloride, and it will be assumed to be about the same up to 80 mM for the purposes of calculation. From Figure 1 of Cram (1969) it can be seen that after a loading period equal to one cytoplasmic turnover half-time the difference between the true plasmalemma influx and the influx to the vacuole will be underestimated by 28%. During a washing period of 5 min (half of one cytoplasmic half-time) about 30% of the cytoplasmic contents will have been lost, and the difference between the two fluxes will have been further underestimated by this amount. Therefore after a 10 min load plus 5 min wash the difference between the plasmalemma influx and the influx to the vacuole will be $0\cdot7\times0\cdot7 = 0\cdot5$ of the true value. This factor depends on the cytoplasmic turnover half-time in comparison with the loading and washing times only, and therefore is the same at all concentrations in these experiments. At 80 mM, for instance, the difference between plasmalemma influx and influx to the vacuole is $67-5 = 62$ μmoles g^{-1} hr^{-1}. The true plasmalemma influx is therefore estimated as $(62/0\cdot5)+5 = 129$ μmoles g^{-1} hr^{-1}.

Whatever the correctness of these extrapolations, the conclusion remains that without ambiguity one can detect by this method a plasmalemma chloride influx considerably higher than the influx to the vacuole at high external chloride concentrations.

(*b*) *Location and Magnitude of Individual Fluxes*

It was argued above that the short load–short wash influx estimate is of the influx across the plasmalemma of the root cortical cells. The quantitative interpretation of the quasi-steady influx in terms of other fluxes is in general impossible without other data, but with the assumption that there is a net influx as in maize (Cram, unpublished data) at all concentrations (which cannot be higher than the influx to the vacuole, since the specific activity of the chloride arriving in the vacuole cannot be greater than the specific activity of the external solution) one can set limits on the sizes of some other fluxes within the cell.

(i) *Efflux from the Cell*

The plasmalemma influx was not measured strictly at a steady state, but it will be assumed that it remains approximately constant over the first hour that the root is in salt. During this time the amount of chloride arriving in the vacuole from the external solution (as measured with ^{36}Cl) is very much less than that entering the cell, and therefore there must be a large efflux from the cell at high external concentrations. Greenway (1967), Weigl (1969, 1971), and Pitman (1971) have measured the loss of chloride to the external solution and from the cut end of roots, and found a significant and sometimes a major fraction to be lost via the stele. Hence in the present experiments it is probable that some of the chloride moves through the symplasm and into the stele across the plasmalemma of the stelar parenchyma, rather than moving directly out across the plasmalemma of the cortical cells. On the other hand, Weigl (1969) found that the external chloride-stimulated ^{36}Cl efflux from maize roots was to the external solution only, as would be expected of a chloride–chloride exchange. The relative sizes of these two pathways of efflux from the cortical cells in the present experiments are unknown.

(ii) *Influx to the Vacuole*

The two extreme alternative pathways are shown in Figure 3. Except when the chloride contained in the straight-through path is a significant fraction of the total in the cell (Pallaghy, Lüttge, and Willert 1970; but cf. Weigl 1971) the time course of exchange of the whole cell in both cases is the sum of two exponential components. Methods of distinguishing the two alternatives in higher plants have not been devised. Therefore both extremes will be considered.

In case A the "plasmalemma influx" will be a measure of $M_{oc}+M_{ov}$, and the influx to the vacuole will be a measure of the straight-through flux, M_{ov}. The saturation in influx to the vacuole would then correspond to a saturation of M_{ov}. There would be no movement of chloride directly from the bulk of the cytoplasm to the vacuole.

In case B the "plasmalemma influx" will be an estimate of the influx to the cytoplasm, M_{oc}. The influx to the vacuole will be equal to $M_{oc}.M_{cv}/(M_{co}+M_{cv})$, (where

M_{co} will include loss to the external solution as well as to the stele). The fraction $M_{oc}/(M_{co}+M_{cv})$ is equal to the specific activity in the cytoplasm relative to that in the external solution at a quasi-steady state. At high external concentrations, where M_{oc} is large compared with the influx to the vacuole, and hence M_{co} is also nearly as large, the fraction $M_{oc}/(M_{co}+M_{cv})$ will tend to 1, and the influx to the vacuole will be very nearly equal to the tonoplast influx, M_{cv}. At lower external concentrations one can draw no conclusions unless further data are available. If, however, the net influx is nearly equal to the influx to the vacuole (i.e. the efflux from the vacuole to the cytoplasm is small) then since the influx to the cytoplasm (M_{oc}) will be equal to the loss from the cytoplasm ($M_{co}+M_{cv}$), the fraction $M_{oc}/(M_{co}+M_{cv})$ will be equal to 1, and the influx to the vacuole will still be equal to the tonoplast influx. Otherwise if M_{vc} is comparable to M_{oc} the fraction $M_{oc}/(M_{co}+M_{cv})$ will be less than 1, and the influx to the vacuole will be an underestimate of the tonoplast influx.

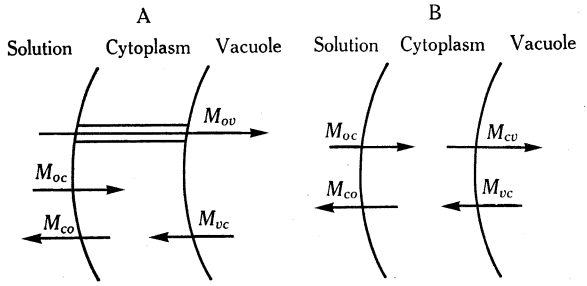

Fig. 3.—Two pathways by which chloride might move to the vacuole of a plant cell. Individual transport processes represented by arrows, and labelled to indicate the compartments between which the movement takes place. M is the rate of movement, or flux, in units of μmoles g^{-1} hr^{-1}.

Therefore, in both case A and case B, it appears that the transport process which saturates at high external chloride concentrations is within the cell. In case A, it would be the straight-through flux M_{ov} and in case B the tonoplast influx, M_{cv}.

The quasi-steady influx to plant cells has been found by many authors to show some complexity in relation to the external concentration (reviews: Epstein 1966; Laties 1969). From a comparison of vacuolate and predominantly non-vacuolate maize root cells, Torii and Laties (1966) concluded that the influx to the vacuole at low external concentrations (below 1 mM) is limited by the plasmalemma influx, but that at higher external concentrations (above 5–10 mM) the observed "saturation" of influx to the vacuole is determined by the saturation of the tonoplast transport process. This implies that the plasmalemma influx rises to large values at high concentrations, although showing some properties of saturation between about 0·5 and 1 mM (cf. Elzam, Rains, and Epstein 1964). These somewhat surprising postulated properties of the plasmalemma influx in maize can be explained in terms of the considerations of Laties, MacDonald, and Dainty (1964) of the fluxes of chloride in potato tissue, i.e. that the plasmalemma influx is made up of a passive component which increases more than proportionately to external chloride concentration, plus an active component which saturates below 1 mM. Torii and Laties' results are open

to the objection that the difference between vacuolate and non-vacuolate cells may not be due to the presence or absence of the tonoplast, but to the different properties of the transport across the plasmalemma in the two cell types.

As discussed above, the results of Figure 1(*a*) support Torii and Laties' hypothesis that it is the influx within the cell, and not across the plasmalemma, which saturates at high external concentrations.

(*c*) *Nature of the Transport Processes at Individual Membranes*

The influx across the plasmalemma shows no saturation at high external concentrations. Table 2 shows the values of the passive permeability coefficient, P_{Cl}, calculated from the constant-field equation (Goldman 1943; Hodgkin and Katz 1949)

$$M_{oc} = (zFE_i/RT).P_{Cl}.[Cl]_o/[1-\exp(zFE_i/RT)]$$

using values of M_{oc} in Figure 1(*a*), together with the factor to convert μmole g^{-1} hr^{-1} to pmole cm^{-2} sec^{-1} given by Pitman (1969*a*); values of E_i from Pitman *et al.* (1971), extrapolated to the high concentration values by comparison with the results of Etherton and Higinbotham (1960), Macklon and MacDonald (1966), and Poole (1966); and a value of RT/F of 25 mV.

TABLE 2

ESTIMATED PLASMALEMMA CHLORIDE PERMEABILITY COEFFICIENT, P_{Cl}, IN LOW-SALT BARLEY ROOT CORTICAL CELLS

External Chloride Concn. (mM)	Electrical Potential Difference (mV)*	Plasmalemma Influx (pmole cm^{-2} sec^{-1})†	$10^7 \times P_{Cl}$ (cm sec^{-1})
1	89	0·74	69·0
3	71	0·56	10·4
10	52	0·72	2·4
20	41	2·5	3·0
40	(30)	6·5	3·1
60	(23)	10·0	2·8
80	(19)	13·4	2·6

* From Pitman *et al.* (1971). Values for 40–80 mM by extrapolation.

† Calc. from Figure 1, the values at 60 and 80 mM being interpolated.

There is no need to postulate active transport to account for the relationship between chloride influx across the plasmalemma and the external solution concentration above 10 mM, since in this range P_{Cl} is constant at around 3×10^{-7} cm sec^{-1}. The apparent rise in P_{Cl} below 10 mM would be expected if an active component of the influx were a larger fraction of the total influx at lower concentrations. There does not appear to be any discontinuity in the properties of the plasmalemma in contrast to *Nitella* (Spanswick, quoted in Laties 1969).

The chloride permeability calculated by Pitman *et al.* (1971) to account for the electrical potential difference in barley root cells in terms of the constant-field equation (Goldman 1943; Hodgkin and Katz 1949) was 1×10^{-8} cm sec^{-1}. The chloride permeability calculated from the results of Figure 1(*a*) would be reconciled with this

value if about 90% of the plasmalemma influx at high concentrations is non-conducting, i.e. is exchange diffusion, and therefore should not be included in the value of the influx used to calculate P_{Cl}. A one-for-one chloride–chloride exchange would not alter the internal chloride concentration, and therefore would have no physiological significance. It is therefore possible that only a small fraction of the flux measured is involved in the control of intracellular contents, and that this small fraction has a relationship to external chloride concentration different from that of the total chloride influx.

The question of whether there is active transport at the tonoplast depends on knowing values of flux across the tonoplast, cytoplasmic concentration, and electrical potential difference across the tonoplast. None of these are known. Etherton and Higinbotham (1960) found that the tonoplast potential difference in incipient root hair cells was very small. If there is no large straight-through influx to the vacuole, then Figure 1(*b*) indicates that the tonoplast influx saturates at high external concentrations. The cytoplasmic chloride concentration at any external concentration is unknown, but since the potential difference across the plasmalemma is falling at high external concentrations, and the influx is rising, one is lead to suspect that the cytoplasmic chloride concentration continues to rise after the tonoplast influx has stopped rising, and this would bring one to the conclusion that chloride does not cross the tonoplast by passive independent diffusion.

The control of the final steady state level of accumulation of ions in plant cells is a basic question in the study of plant cell ion relations. The problem has been discussed recently (Cram 1968*b*; Pitman 1969*b*) and the conclusion reached that an apparent negative feedback relationship must exist between vacuolar ion content and influx to the vacuole, i.e. influx across the tonoplast or influx via a straight-through pathway, though the second alternative was only briefly referred to in the first of these two papers. In the experiments reported in the present paper the influx to the vacuole is reduced in the high-salt root cells, as in carrot root cells (Cram 1968*b*), and the plasmalemma influx is also reduced, which is unlike the situation in carrot. An increase in the potential difference across the plasmalemma, of the order of magnitude of that observed by Pitman *et al.* (1971) in partly salt-loaded barley roots, would account for the observed decrease in the plasmalemma influx on the constant-field membrane model. If exchange diffusion is a major fraction of the plasmalemma influx it is difficult to know whether this explanation for the decrease in influx is a possibility.

The negative feedback relationship between chloride content and chloride influx to the vacuole is an interesting and obviously important phenomenon. Whatever the pathway by which chloride (and presumably also its accompanying cation) reaches the vacuole, the simplest imaginable system of controlling the final vacuolar level is one with a constant inwards pump(s) and an increasing passive outwards leak(s). This system does not seem to be operating in carrot, even taking into account the various membranes that might be involved [Cram 1968*b*; cf. the situation in the red blood cell (Post, Albright, and Dayani 1967)]. In teleological terms a negative feedback relationship in this situation is the best combination of rapidity and economy of energy output. With a pump and leak system a particular level would be more rapidly reached with large active and passive fluxes, but at the cost of a greater continuous output of energy to maintain the final level. With a

lower output of energy, and consequently lower active fluxes, the passive flux has also to be lower and rapidity in reaching the new level is sacrificed. With a negative feedback relationship the fluxes and necessary energy output are only large during the approach to the final level, which is in consequence relatively rapid. As the final level is reached, active fluxes are reduced so that influx equals the small efflux, and a smaller continuous output of energy is needed to maintain the steady level.

Whether the supply of energy to active transport is ever limiting in a normal non-photosynthetic plant cell, and whether this may be involved in the apparent negative feedback relationship (Pitman, Mowat, and Nair, unpublished data) or whether there is a more subtle feedback signal (cf. Gutknecht 1968) are questions that remain to be answered.

A negative feedback from vacuolar content to the influx across the tonoplast is of interest also as it must then be a controlling factor in the swelling and shrinking of the cytoplasm, as discussed by Dainty (1968).

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VI. References

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