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COMPARTMENTATION AND EXCHANGE OF CHLORIDE IN CARROT ROOT TISSUE

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SUMMARY

1. An attempt has been made to provide an experimental basis for interpreting the kinetics of the exchange of Cl^- in excised carrot tissue.

2. The fastest component of the efflux of tracer Cl^- from carrot tissue is shown to be extracellular in origin. The two slower components are shown to be subcellular in origin, and are equated with the cytoplasm and the vacuole.

3. When tissue is transferred from salt solution to water, the efflux of tracer Cl^- is much reduced. On transfer back to salt, there is an initial burst before the Cl^- efflux returns to the original level in salt. The initial burst is shown to represent loss of Cl^- from the cytoplasm. This indicates that Cl^- can move directly between the cytoplasm and the vacuole, and that the inhibition of Cl^- efflux in water is an effect mainly at the plasmalemma. Further quantitative considerations suggest that there is no direct connection between the vacuole and the external solution.

4. With the use of a model in which the cytoplasm and vacuole are arranged exclusively in series, the one-way fluxes at the plasmalemma and tonoplast and the cytoplasmic and vacuolar contents are estimated over a range of external Cl^- concentrations. In conjunction with electrical measurements, these results show that Cl^- is probably actively transported inwards across both plasmalemma and tonoplast, and that there is a 1-for-1 exchange diffusion component of the Cl^- fluxes at the plasmalemma. Anions, other than halide are much less effective than Cl^- in exchanging for internal $^{36}Cl^-$.

INTRODUCTION

The presence of cytoplasm and vacuole and of visible membranes bounding them suggests that ion movements and distribution within plant cells may be at least correspondingly complex.

Some algal coenocytes are so large that the cell wall, cytoplasm and vacuole can be physically separated[1,2] and the properties of these structural compartments investigated individually. In these plants the cytoplasm and vacuole have been shown to behave as kinetically distinct compartments which are arranged in series[1–3],

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although E.A.C. MACROBBIE (personal communication) has recently shown that this is not the complete picture.

Higher plant cells are too small to dissect, and consequently the only systematic method of investigating transport processes is to analyse the kinetics of movement of ions to or from at issue. Some other types of experiment have indicated that there are distinguishable movements of ions within higher plant cells[4,5], but these methods are not quantitative and cannot be used systematically.

PITMAN[6] was the first to analyse the kinetics of the loss of tracer from a higher plant tissue. He observed two exponential components, apart from an extracellular component, in the efflux of Br^-, K^+ and Na^+ from red beet tissue slices. These were shown not to originate in different parts of the tissue with different sized cells, and two K^+ phases within the tissue were shown to be connected. PITMAN interpreted the compartments to be cytoplasm and vacuole, and, assuming that they were arranged in series, calculated values for the plasmalemma and tonoplast fluxes and the cytoplasmic and vacuolar contents under several sets of conditions. Subsequently, several other authors have used this method of analysis[7–9].

The primary purpose of this paper is to establish a firmer experimental basis for this analysis. It will be shown that the kinetically defined compartments are subcellular in origin, and that they are arranged in series with regard to Cl^-. Other results indicate that there are active and exchange diffusion components of the Cl^- fluxes at defined boundaries within the cell.

METHODS

Discs, 1 mm thick and 1 cm in diameter, were cut from the xylem tissue of the storage root of the carrot (*Daucus carota* L.), and stirred with a stream of air in several changes of distilled water for 4 days at room temperature (20°). This brings the tissue to a state in which it will accumulate salt at a relatively high rate. In aerated distilled water the tissue remains in this state for at least another 4 days (ref. 7). The discs were transferred to the experimental solution several hours or days before the beginning of an experiment to allow the tissue to attain a nearly steady state in that solution.

Some discs were cut and aerated in water for the initial 4-day period under aseptic conditions. At the end of this period the aseptically prepared discs and the water in which they had been incubated were tested, at 25 and 37°, respectively, for the growth of organisms on Difco nutrient agar. No growth after 1 week was used as the criterion for a successful preparation of aseptic discs.

Solutions used in the experiments contained 5 mM Cl^-, and equal molarities of K^+, Na^+ and Ca^{2+}, except where otherwise stated.

For determining the time course of influx to the tissue, samples were immersed in a solution labelled with $^{36}Cl^-$, and at intervals removed, lightly blotted, counted under an end-window counter and returned to the solution.

For determining the time course of efflux, an apparatus very similar to that of MACROBBIE AND DAINTY[10] was used. Samples of discs were placed in glass tubes in a thermostatically controlled water bath. The solutions were stirred with a stream of air which also served to keep the discs circulating. About 3 g of discs were used per sample. With this weight of tissue the variability between samples is reduced to less than 10 %

(ref. 7). After loading the tissue for 1–2 hours in a radioactive solution, efflux was measured as the activity appearing in successive aliquots of inactive solution. The activity in each aliquot was found by precipitating the Cl^- as AgCl, filtering off the precipitate on a Millipore filter, and counting the activity in it under an end-window counter. When tested, this procedure gave 99.7 % recovery of $^{36}Cl^-$ activity from the solution. At the end of a wash-out, the remaining tissue Cl^- was extracted in 1 M HNO_3 at 100° for about 1 h. There was no detectable loss of Cl^- from a solution during this process. A small quantity of the extract was precipitated together with an amount of Cl^- equal to that in the washing aliquots, and also counted. Self absorption corrections were therefore not needed. For obtaining absolute values, small amounts of the extract and a standard solution were dried under alkaline conditions on planchettes, and counted. Total Cl^- was found by electrometric titration of a sample of the extract.

Movement of $^{36}Cl^-$ activity along strips of tissue, 1 mm thick, was measured after the strip had lain for several hours with one end in radioactive solution and the other in inactive solution. The strip was then cut into short lengths, and each analysed for radioactivity.

Electrical potential differences (p.d.) between the vacuole and the external solution were measured using microelectrodes having tip diameters less than 1 μ; resistance, about 10–20 MΩ; and tip potential in bathing solution less than 10 mV. Electrodes were connected to a Vibron electrometer.

Treatment of the primary data

The activity in the tissue at any time (t) when the bathing solution was changed during the washing-out period was found, and the logarithm of the activity in the tissue was plotted against time from the beginning of the wash-out. Using the technique of extrapolating the final linear component of the resulting plot to $t = 0$, subtracting this component from the total, and replotting the difference on another semi-logarithmic graph, two exponential components of the efflux of Cl^- from carrot tissue, with half-times of about 10 min and 300 h, could be distinguished, together with a fast, apparently diffusional component having a half-time for the exponential portion of about 1.4 min (Fig. 1).

For each component, the slope of the line, and hence the half-time for exchange ($t_{\frac{1}{2}}$) and the rate constant ($k = 0.693/t_{\frac{1}{2}}$), can be found; the value of the intercept at $t = 0$ (I) can also be determined. The other known parameters are the labelling time (t_{1n}) and the total final content of the cells (Q_T).

Most experiments reported here concern the effect of various treatments on the size of these parameters. Other types of experiment involve the effects of various treatments on the final, pseudo-steady state efflux.

RESULTS

The nature of the fastest exchanging component

The kinetics of exchange of the first component is as expected for diffusion from extracellular spaces. The half-time for the exponential portion is about that expected for diffusion from a phase within which movement is restricted to paths around the cells. The half-time is unaffected by the presence of inhibitors and changes in external

concentration, is only slightly affected by temperature changes, and is nearly proportional to the square of the thickness of the discs between 0.5 and 1 mm.

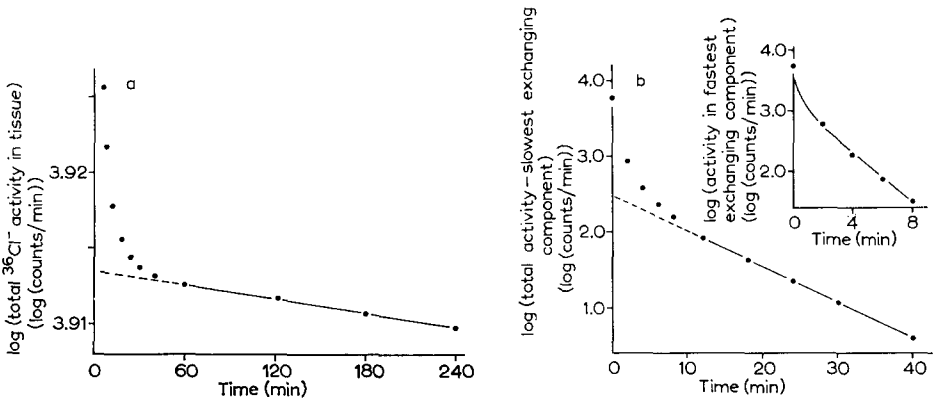

Fig. 1. Loss of $^{36}Cl^-$ activity to inactive solution after loading with $^{36}Cl^-$ for 1 h. The activity appearing in successive aliquots of inactive solution is added to the final activity in the tissue to give the total activity at any time during washing out. a. log (total activity in the tissue) is plotted against time. The slowest exchanging component is extrapolated back to $t = 0$ (---) and its estimated content subtracted from the total. b. log (total − slowest component activity) is plotted against time. Extrapolation and subtraction of the content of the intermediate component gives the content of the fastest exchanging component. Inset: log (activity in the fastest exchanging component) plotted against time.

The content of the phase is only affected by external concentration, and is equivalent to some 20 % of the tissue volume (about 5 % being intercellular spaces, and 15 % being cut cells at the surface of the discs).

In chloroform-killed tissue a component with a slightly faster half-time is present. Its content is equivalent to nearly 100 % of the tissue volume. This confirms that the diffusion component comes from the non-living part of the tissue. It also shows that cell walls so isolated are not an appreciable barrier to the diffusion of Cl^-.

This component of the efflux need only be considered further to ensure that diffusion within the discs does not limit the fluxes to and from the cells. It is simple to show (*e.g.* following BRIGGS AND ROBERTSON[11]) that in carrot tissue at 20° (assuming a relationship between flux and concentration similar to that found in barley roots[12] where the diffusion path is negligible) the concentration in the centre of a disc will become appreciably different from that in the external solution only when the discs are more than 1 mm thick, or the external concentration is less than about 1 mM. Full details of these experiments have been presented previously[7]. Extracellular Cl^- exchange in carrot is similar to that observed in beet[25].

The origin of the two slower exchanging components

By a process of elimination one can show that these components correspond to two subcellular compartments. (1) The two slower exchanging components are absent from the efflux from chloroform or ethyl acetate-killed tissue, and they therefore correspond to loss from living cells. (2) Changing the length of the washing periods does not alter the appearance of the efflux components, which are therefore not artifacts of the experimental procedure. (3) Table I shows results which exclude the

TABLE I

COMPARISON OF ASEPTICALLY AND NON-ASEPTICALLY PREPARED DISCS

Cores were cut from a root and sliced into 1-mm discs under aseptic conditions. Each sample (1 g) came from 1 core, and 1 or 2 cores came from each root. Samples were pretreated by shaking with a small volume of distilled water. Controls were cut and pretreated in the same way, but under non-aseptic conditions. Cl^- flux parameters were then measured as indicated in the text. After this type of pretreatment, the Cl^- fluxes were lower than normal. (Usually, with larger samples and greater randomisation of the discs during pretreatment, the variability was about one tenth of the variability in this experiment.) Measured values shown as mean ± S.E.M. None of the observed differences between the means is significant at the 10% level. The experiment was repeated with the same result.

Parameter	*Measured value*		
	Aseptically prepared	*Non-aseptically prepared*	*Student's t value*
I_v/t_{1n} (μmoles/g fresh wt. per h)	0.30 ± 0.10	0.61 ± 0.1	1.68
$k_c(h^{-1})$	3.4 ± 0.3	3.7 ± 0.6	0.38
$k_v \times 10^3$ (h^{-1})	7.4 ± 0.7	7.2 ± 1.4	1.07
I_c (μmoles/g fresh wt.)	0.032 ± 0.011	0.086 ± 0.037	1.12
Q_v (μmoles/g fresh wt.)	7.3 ± 1.9	9.6 ± 3.3	0.52

possibility that the faster, smaller component represents the exchange of Cl^- in bacteria. There is no significant difference between aseptically and non-aseptically prepared discs with regard to any of the measured parameters. Bacteria make no contribution to the exchange of Cl^- under these conditions (*cf.* ref. 13). (4) There is a range of cell sizes in the tissue, but this alone is not sufficient to account for the appearance of the two cellular components whose rate constants are so distinct and different[14,15]. (5) Anticipating the results of the next section, it can be shown that the compartments corresponding to these two efflux components are connected. If the compartments are different cell types, then the cells in the tissue must be connected. It can be estimated[7] from the relative size and rate of exchange of the components that, if this is the case, movement of tracer Cl^- along the tissue would be detectable, but in fact no such movement could be detected. Further, the electrical p.d. in adjacent cells of carrot tissue is not exactly the same, although similar, which shows that the cells cannot be freely connected to each other. The two cellular components, therefore, cannot originate in different cell types, and the only remaining possibility is that they correspond to two subcellular compartments. The interpretation that forces itself to one's attention is that these are the cytoplasm and vacuole, and they will subsequently be referred to as such.

The arrangement of the two cellular compartments

It has to be established whether the cytoplasm and vacuole are arranged in series with regard to ions, as their appearance under the microscope would suggest. The most general model is that the cytoplasm, the vacuole and the external solution are each connected to the other two. The analysis of tracer efflux defines compartments in terms of rate constants, but tells little of their arrangement.

Fig. 2 shows the sizes of the intercepts of the cytoplasm and vacuole after various periods of loading. The rate of increase of specific activity in both compartments is

consistent with their half-times for exchange during washing out. Since the cytoplasmic intercept rises with a different half-time from the vacuolar intercept, the specific activity in the cytoplasm cannot depend on that of the vacuole alone, *i.e.*, the cytoplasm must be connected to the external solution.

The demonstration of a direct connection between the cytoplasm and vacuole depends on the observation that on transfer from salt to water the efflux of tracer Cl^- drops to a much lower value. This could be an effect at the plasmalemma, at the tonoplast, or at a putative vacuolar–external solution connection. Consider the situation existing after the tissue has been loaded and washed in salt until the faster components have washed out. If the cytoplasm and vacuole are not connected,

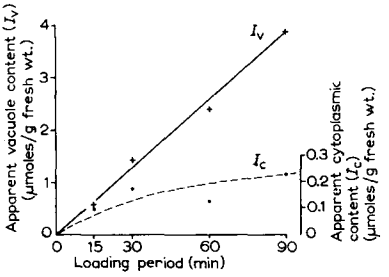

Fig. 2. The apparent contents of the cytoplasm (I_c) and of the vacuole (I_v) after various loading periods. Samples were loaded with $^{36}Cl^-$ for various periods and then washed out into inactive solution. Wash-out curves were constructed and the intercepts of the cytoplasmic and vacuolar components at $t = 0$ (I_c and I_v) were obtained from the graphs. (The exchange rate constant of the cytoplasmic or vacuolar component was the same after each loading period.) ---, exponential approach of the apparent cytoplasmic content to 100% with rate constant equal to that for exchange of the cytoplasm during washing out. Temp. 20°.

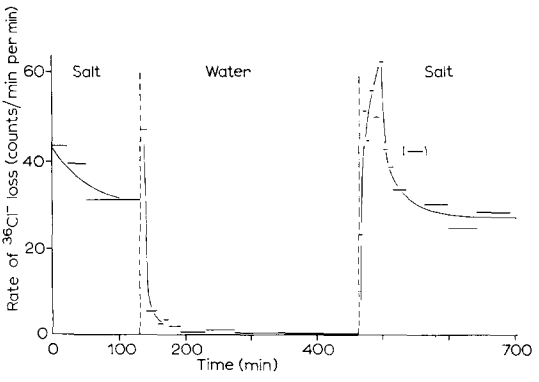

Fig. 3. Transient changes in rates of $^{36}Cl^-$ loss after transfer from salt to water and water to salt. Tissue was loaded with $^{36}Cl^-$ and washed in inactive solution overnight. During this time the cytoplasmic component of the efflux had fallen to zero. The tissue was then washed in several changes of inactive solution, distilled water, and inactive solution, as indicated; the rate of $^{36}Cl^-$ loss over successive periods was measured, and plotted against time. The fall during the first 2 h in inactive solution is an after-effect of being in a relatively small volume of solution overnight. The rate of loss of $^{36}Cl^-$ to inactive solutions in controls (not shown) remained steady after 2 h. One point (bracketed) is unaccountably high. Electrical p.d. in salt = −40 mV; in water after salt = −95 mV. Temp. 20°.

the cytoplasmic specific activity will be zero. If the cytoplasm and vacuole are connected, the specific activity of the cytoplasm will have reached a steady value lower than in the vacuole. Upon transfer to water, the extracellular spaces will empty and there will be no possibility of an indirect connection between the vacuole and the cytoplasm *via* the extracellular spaces. (It is simple to show[7] that the Cl^- concentration at the centre of a disc in water will rapidly fall to less than 1 μM, and that this is negligible.) In water the specific activity in the cytoplasm may rise, but *only* if it is directly connected to the vacuole, and if the inhibition of the Cl^- efflux in water is an effect mainly at the plasmalemma. If the cytoplasmic specific activity does rise in water, then upon transfer back to salt solution the cytoplasm will contribute to the efflux as it does when it is at a high specific activity immediately after loading. Fig. 3 shows that there is an initial transient burst of Cl^- efflux after transferring from salt to water and back to salt again.

Fig. 4 shows the kinetics of exchange of the initial burst component. There is an initial lag (which must be the time taken for diffusion through extracellular spaces) followed by an exponential fall with a half-time of about 10 min. This is within the range of half-times for the exchange of the cytoplasmic component observed in other experiments, and both the initial burst component and the cytoplasmic component are affected in the same way by changes in the vacuolar content. (When this is high,

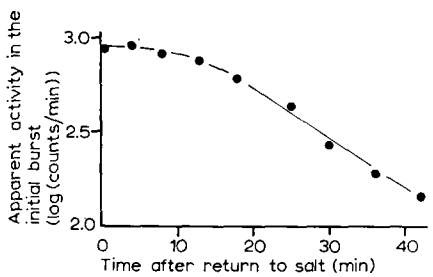

Fig. 4. Fall of activity in the initial burst of Cl^- loss after transfer from water to salt (*cf.* Fig. 3). log (activity in the tissue) was plotted against time for the period after transfer from water to salt, and the slowest exchanging component subtracted from the total (*cf.* Fig. 1) to give the activity apparently remaining in the initial burst at various times.

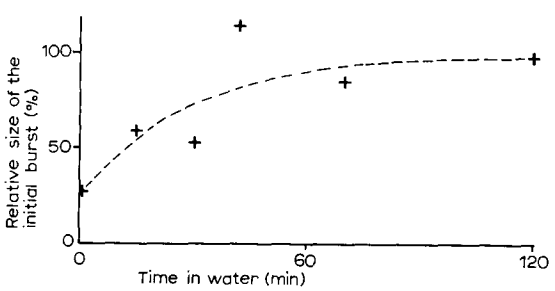

Fig. 5. The rise in the size of the initial burst of $^{36}Cl^-$ after longer periods in water. The total excess activity in the initial burst of Cl^- efflux was estimated graphically as in Fig. 4. The size of the initial burst is shown as a percent of the size after 120 min in water. The experiment was carried out at 8°, at which temperature there is no net Cl^- flux[7]. The control (t = 0) shows that frequent changing of the solution had a small effect on the steady Cl^- efflux in this experiment. One point ($t_{\frac{1}{2}}$ = 27 min and apparent content = 200 %) is omitted from the graph. Average $t_{\frac{1}{2}}$ for the initial burst component = 15 min. – – –, exponential approach to 100 % with $t_{\frac{1}{2}}$ = 20 min.

both components have half-times of about 10 min; when it is low, both components have half-times of about 30 min.) On these grounds one can equate the initial burst and cytoplasmic components. It follows that the cytoplasm and the vacuole must be directly connected, and that the inhibition of Cl^- efflux in water is mainly an effect at the plasmalemma.

The size of the initial burst upon returning to salt should rise to a maximum with a half-time which depends on the fluxes between cytoplasm and vacuole (and which will therefore be less than the half-time for washing out the cytoplasm in salt, which depends on the sum of the plasmalemma and tonoplast fluxes). Fig. 5 shows that, under conditions of no net Cl^- flux, the cytoplasmic specific activity rises in water about as fast, or slightly more slowly, than it falls in salt. This suggests that the tonoplast fluxes must be large compared with the plasmalemma fluxes under these conditions (low vacuolar content, 8°). This agrees with estimates of tonoplast fluxes, under similar conditions, made from independent measurements, and on the assumption that no parallel component of the fluxes to the vacuole is present (see next section). The qualitative agreement between these two independent estimates means that the assumptions in the second method of estimation must, at least to a first approximation, be correct; *i.e.*, there is no large parallel component of the fluxes between the vacuole and the external solution. It will be possible to set more precise limits to the size of any parallel flux to the vacuole by reducing the variability in these experiments. The large number of samples involved could not be dealt with at the time this series of experiments was in progress. As a working hypothesis, the cytoplasm and vacuole are assumed to be arranged exclusively in series.

Calculations from the measured parameters

Subscripts o, c and v refer to external solution, cytoplasm and vacuole, respectively.

It is required to relate the measured parameters to the cytoplasmic and vacuolar contents (Q_c and Q_v) and to the one-way fluxes at the plasmalemma and tonoplast (M_{oc}, M_{co}; M_{cv}, M_{vc}). The model used (*cf.* refs. 2 and 6) assumes, (1) that the cytoplasm and vacuole are in series, (2) that the cytoplasmic content is very much less than the vacuolar content (which is justified by osmotic considerations since their volumes are very different, at least in storage tissue of higher plants), and (3) that the tissue is in a steady state. (In preliminary experiments one can establish conditions when Q_T is constant, and the pseudo-steady efflux and influx are constant, which would suggest that all the fluxes are constant.)

Since the exchange rate constants of the two efflux components are so different, they can be treated as the rate constants for the exchange of individual compartments (although this is not generally true for a compartmental system[16,17]).

For the exchange of cytoplasmic tracer,
cytoplasmic rate constant (k_c) =

$$\frac{M_{co} + M_{cv}}{Q_c}$$

After loading for a time (t_{1n}) longer than $5 \times t_{\frac{1}{2}c}$, but short compared with $t_{\frac{1}{2}v}$, the cytoplasmic specific activity (s_c) will have risen to more than 97 % of its pseudo-steady value

$$\frac{M_{oc}}{M_{co} + M_{cv}}$$

(the specific activity of the external solution being taken as 1), while the vacuolar specific activity (s_v) will still be negligible. Subsequently during washing out, s_c will fall, with rate constant k_c, to

$$\frac{M_{vc}}{M_{co} + M_{cv}} s_v$$

which is negligible compared with

$$\frac{M_{oc}}{M_{co} + M_{cv}}$$

The fall in activity in the cytoplasm during washing out will therefore be

$$\frac{M_{oc}}{M_{co} + M_{cv}} Q_c$$

Of this,

$$\frac{M_{co}}{M_{co} + M_{cv}}$$

will move to the external solution, and

$$\frac{M_{cv}}{M_{co} + M_{cv}}$$

to the vacuole. (This point was overlooked by HOPE[18].)

Therefore,

content of cytoplasmic efflux component (I_c) =

$$\frac{M_{oc} \cdot M_{co}}{(M_{co} + M_{cv})^2} Q_c$$

The activity in the vacuole will rise as

$$\frac{M_{oc} \cdot M_{cv}}{M_{co} + M_{cv}} \left(t_{in} - \frac{1}{k_c}\right)$$

for loading times greater than about $5 \times t_{\frac{1}{2}c}$ and short compared with $t_{\frac{1}{2}v}$. During washing out, an amount

$$\frac{M_{oc}}{M_{co} + M_{cv}} \cdot \frac{M_{cv}}{M_{co} + M_{cv}} Q_c$$

will be transferred to the vacuole from the cytoplasm.

Therefore,

content of vacuolar efflux component (I_v) =

$$\frac{M_{oc} \cdot M_{cv}}{M_{co} + M_{cv}} t_{in}$$

For the exchange of vacuolar tracer,

vacuolar rate constant (k_v) =

$$\frac{M_{vc} \cdot M_{co}}{M_{co} + M_{cv}} \frac{1}{Q_v}$$

Q_T is used as an estimate of Q_v since it is assumed initially that Q_v is much greater than Q_c. Therefore,

$$\frac{I_v}{t_{in}} = \frac{M_{oc} \cdot M_{cv}}{M_{co} + M_{cv}}$$

$$k_c \cdot I_c = \frac{M_{oc} \cdot M_{co}}{M_{co} + M_{cv}}$$

$$k_v \cdot Q_v = \frac{M_{vc} \cdot M_{co}}{M_{co} + M_{cv}}$$

From these three equations and the equation for k_c, and with the initial steady-state condition reducing the number of independently variable one-way fluxes to three, one can obtain

$$M_{oc} = \frac{I_v}{t_{in}} + k_c \cdot I_c$$

$$M_{co} = k_v \cdot Q_v + k_c \cdot I_c$$

$$M_{cv} = (k_v \cdot Q_v + k_c \cdot I_c) \frac{I_v/t_{in}}{k_c \cdot I_c}$$

$$M_{vc} = (I_v/t_{in} + k_c \cdot I_c) \frac{Q_v \cdot k_v}{k_c \cdot I_c} \text{ or, } M_{vc} = M_{cv} - (M_{oc} - M_{co})$$

$$Q_c = \frac{M_{co} + M_{cv}}{k_c}$$

These equations can be rigorously derived from a general model of two compartments in series[19], with the simplifying conditions being included at the end. The equations can be applied even when there is a net flux of ions which is comparable with Q_c, provided that during the course of an experiment Q_v does not rise by more than a few percent, and assuming that the percent change in Q_c is not greater.

It will be noticed that in calculating the values of the tonoplast fluxes and Q_c, any errors (in measurement or in the model) will be multiplied. The estimates of the plasmalemma fluxes are therefore the most reliable. Further, since $k_c \cdot I_c$ is usually small compared with I_v/t_{in}, and the latter is the most accurate of the basic measurements[7], and as the estimate of M_{oc} does not depend on the assumption that Q_c is constant, M_{oc} is the most reliable of the estimates.

Further investigation of the effect of external Cl^- concentration on tracer Cl^- efflux

The relationships derived in the last section were used to calculate the sizes of the plasmalemma and tonoplast fluxes and the cytoplasmic content over a range of external Cl^- concentrations from 0.5 to 95 mM. These results are shown in Fig. 6. The primary data from which these results were calculated are given in Table II.

The net influx is nearly constant at Cl^- concentrations above 10 mM. At higher concentrations both plasmalemma influx and efflux continue to rise, but the tonoplast fluxes and Q_c remain nearly constant. It is surprising that up to 10 mM the tonoplast fluxes appear to fall, although the plasmalemma fluxes and the net influx are rising. The relation of electrical p.d. to external concentration will be discussed in a later publication.

Examination of storage tissue cells shows that the cytoplasm occupies about 5 % of the tissue volume[6], and the vacuole about 70 %. The concentration of Cl^- in the cytoplasm is therefore about 10 mM, and in the vacuole about 150 mM. If the electrical

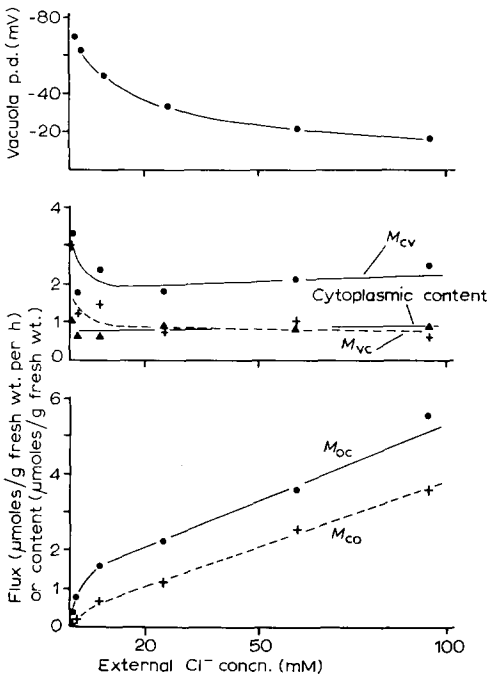

Fig. 6. Changes in the estimated plasmalemma and tonoplast fluxes and cytoplasmic content with changes in external Cl^- concentration. Solutions: 0.5 mM $CaSO_4$ + equal molarities of KCl and NaCl, Cl^- concentration as indicated on the abscissa. Temp. 20°. Average vacuolar content at the end of the experiment = 110 μmoles/g fresh weight. The experiment was repeated at a lower vacuolar content with qualitatively the same result[7]. Electrical p.d.'s were measured in a separate experiment. The primary data, from which these results were calculated as descirbed in the text, are given in Table II. M_{cv} = tonoplast influx; M_{vc} = tonoplast efflux; M_{oc} = plasmalemma influx; M_{co} = plasmalemma efflux.

TABLE II

PRIMARY DATA FOR THE RESULTS OF FIG. 6

The data were obtained from wash-out curves and final analysis of tissue Cl^- content, as described in the text. Extracellular Cl^- was removed from the tissue at the end of the experiment by washing briefly in water. Loading time, 66 min.

Parameter	*External Cl^- concn. (mM)*					
	0.5	*2*	*8*	*25*	*60*	*95*
I_v (μmoles·g^{-1})	0.38	0.69	1.17	1.37	1.67	2.25
I_c (μmoles·g^{-1})	0.0024	0.021	0.064	0.26	0.33	0.54
k_c (h^{-1})	3.5	3.3	5.1	3.4	6.0	6.0
$k_v \times 10^3$ (h^{-1})	0.61	0.97	3.1	2.7	5.1	3.3
Q_T (μmoles·g^{-1})	109	112	108	111	115	114

p.d. is located mainly at the plasmalemma (as in higher plant root cells[20] and in Nitella[21]), and if the Cl^- activity in the cytoplasm is not much less than 1, then these results indicate, particularly at low external concentrations, that Cl^- must be actively transported inwards at both the plasmalemma and tonoplast. It will be assumed that Cl^- efflux across the plasmalemma is driven by diffusional forces.

It will be seen that the plasmalemma Cl^- efflux increases nearly in proportion as the external Cl^- concentration is raised. Q_e and the electrical p.d. cannot be the physical factors causing this, and the only other physical driving force which changes is the external concentration. It is difficult to imagine how a diffusional plasmalemma efflux could be related directly to the external concentration. It seems more likely that it should be related to the influx which in turn is related to the external Cl^- concn. This suggests that a large fraction of tracer Cl^- efflux, and a smaller fraction of tracer Cl^- influx at the plasmalemma are linked. The facts that the fluxes at the plasmalemma rise together above about 8 mM and that the tonoplast fluxes and cytoplasmic content change relatively little over the same range of external concentration, strongly suggest that the main change in tracer fluxes at the plasmalemma above 8 mM is in a 1-for-1 exchange component.

TABLE III

EFFECT OF VARIOUS ANIONS IN STIMULATING PLASMALEMMA $^{36}Cl^-$ EFFLUX

The efflux of tracer at the peak of the initial burst is compared with the steady efflux in water. The values are recorded as a percentage of the Cl^- value. Each value is the mean of two experiments. Solutions had the same cation composition as the standard solution (see METHODS) with the various anions in place of Cl^-. Temp. 20°.

Anion	*Stimulation of $^{36}Cl^-$ efflux* (%)
Cl^-	100
Br^-	83
SCN^-	14
NO_3^-	9
SO_4^{2-}	4

The effects of other anions in stimulating the Cl^- efflux are shown in Table III. The maximum rate of Cl^- efflux during the initial burst after transferring from water to salt is used as a measure of the stimulated plasmalemma efflux, since the specific activities in the cytoplasm are initially the same. Br^- is almost as effective as Cl^- in stimulating $^{36}Cl^-$ efflux. The small stimulations by the other anions show that the plasmalemma exchange is not absolutely specific.

DISCUSSION

Two questions remain concerning the graphical analysis of the efflux. (1) Could any components of the exchange have been missed? (2) Is the fit to exponential functions justified?

The only component of any size that could have been missed is a compartment exchanging more slowly than the slowest of the compartments observed in these experiments. But no curvature in the final portion of the wash-out appeared even when it was followed for long periods in tissue that is nearly in a truly steady state; therefore, there can be no other major kinetically distinguishable compartment in the tissue.

Each of the efflux components has only 4–10 points on the line, and consequently expressions other than the exponential function could be fitted equally well. The

justification for choosing an exponential function is that, if there is a close enough fit to rule out the curves being better described by a diffusion equation, or some other even more curved line (*e.g.*, as discussed by LING[22]), then it seems intuitively most likely that a system exchanging tracer at or near the steady state should obey first-order kinetics (*i.e.*, that the loss of tracer is rate limited at the surface).

The kinetics of efflux of ions from a number of animal cells and tissues have been measured by various workers. A review of these results[7] suggests that first-order kinetics of exchange is found in cells with little internal structure (*e.g.* red blood cells, giant nerve axons and bacteria), whereas more complex exchange kinetics are found in cells having more internal structure (*e.g.* muscle, toad oocyte). (The conclusion that first-order loss from a cell can be correlated with a single visible bounding membrane can be drawn irrespective of whether one considers the deviations from first-order behaviour in other cells to be due to internal compartmentation[23], diffusion within the cell[22,21], or physical combination within the cell[22].) This correlation in animal cells gives some formal grounds for equating the two first-order efflux components in carrot cells with the whole of the cytoplasm and the whole of the vacuole, and for calling the boundaries of these kinetically defined compartments the plasmalemma and the tonoplast. This, of course, does not imply that the cytoplasmic Cl^- is homogeneous.

The properties of the extracellular Cl^- exchange in carrot are similar to those observed in beet[25].

It has been shown, with regard to Cl^- at least, that the cytoplasm is connected to the external solution, that the cytoplasm and the vacuole are connected, and that the fluxes between the vacuole and the external solution are, at most, a small fraction of the total fluxes to and from the vacuole. One further consideration strongly suggests that there is no parallel component of the flux from the vacuole to the external solution. Since the efflux drops nearly to zero in water, nearly all the loss from the vacuole must pass through an exchange system. It may be considered likely that the Cl^-–Cl^- exchange represents one system only, rather than that the plasmalemma efflux and the putative parallel component of the flux from the vacuole to the external solution (which would have to be very different in nature) should both have large exchange components. An exchange system has been shown to operate at the plasmalemma, and, if this is the only one, then all the efflux from the vacuole must pass through the cytoplasm, and there can be no parallel component of the flux from the vacuole.

E. A. C. MACROBBIE (personal communication) has shown that in *Nitella translucens* there appears to be a relatively small parallel component of the Cl^- (but not the K^+) flux into the vacuole. It is difficult at present to imagine what such a flux corresponds to in terms of structure within the cytoplasm. This flux makes little difference to calculations made on the series model, provided that the experiments are not of very short duration. Even if carrot in this way resembles Nitella, the calculated values shown in Fig. 6 and in subsequent publications will have only relatively small quantitative errors in them.

The estimation of the electrochemical potential gradient across the plasmalemma and tonoplast individually depends on several assumptions which it will be very difficult to test directly because of the thinness of the cytoplasmic layer (about 0.5 μ) and the toughness of the cell wall. Cl^- must be pumped into the vacuole. Carrot cells must be different from other plant and animal cells if they lack pumping activity at both the plasmalemma and tonoplast.

The results of Fig. 6 suggest that the plasmalemma Cl^- influx pump saturates at low concentrations, the increase in M_{oc} at higher concentrations being mainly in the exchange diffusion component. The maximum pump rate in carrot is then about 1 μmole per g fresh weight per h which is equivalent[26] to 0.18 pmoles/cm^2 per sec. This is about the same rate as that observed for *N. translucens* in the dark[2]. (In the light, the Cl^- influx in Nitella is more than 10 times higher[2].) In Nitella the tonoplast fluxes are about 100 times greater than in the carrot, even in the dark.

WEIGL[27,28] has shown that the efflux of ^{32}P from barley roots labelled with inorganic phosphate is stimulated by increasing the external phosphate (but not Cl^-) concentration, and that the efflux of $^{36}Cl^-$ is stimulated by increasing the external Cl^- concentration. The spatial location in the root of these stimulations was not investigated, but it would seem likely that the stimulation of Cl^- efflux is an effect at the plasmalemma of the root cells, as in the carrot.

The Cl^- exchange system in carrots is unlike that in the gastric mucosa[29] in its specificity to various anions. Since the exchange is not absolutely specific to halide ions, it may be that it is related to the Cl^- influx pump. For instance, a change in the internal specificity of a common 'carrier' would lead to changes in the net influx of Cl^-. This, of course, pictures the pump as being essentially an anion–anion exchange, rather than a neutral salt pump (as in the gall bladder[30]), or an electrogenic anion pump (as in the gastric mucosa[31]).

EPSTEIN[32] and several other authors have observed that the relation between the net tracer influx and external concentration in higher plant tissues can be described roughly as the sum of two Michaelis–Menten-type terms, one saturating at about 0.5 mM and the other at much higher external concentrations. It was originally proposed by EPSTEIN that this shows the presence of at least two transport processes, but it was not specified at what position in the cell these might operate. TORII AND LATIES[5] subsequently suggested that they might correspond to properties of the plasmalemma and the tonoplast. On this picture an active component of the influx at the plasmalemma would limit the overall influx to the vacuole at low external concentrations (less than 1 mM), and at higher concentrations the passive influx at the plasmalemma would rise approximately exponentially (as predicted by LATIES, MACDONALD AND DAINTY[33]) so that the transfer at the tonoplast would become rate-limiting for the overall influx to the vacuole. Individual fluxes in two higher plant tissues have been investigated[9] in order to determine whether the latter interpretation is correct. Unfortunately the authors' experimental procedure was unsatisfactory. Under the conditions of their experiments the equations used do not apply, or are only approximate. However, if their results for K^+ in corn roots are taken at their face value, they show that the tonoplast influx is the limiting factor at low external concentrations, and the plasmalemma influx increasingly limits at higher concentrations, which does not agree with either previous interpretation.

The results of Fig. 6, for Cl^- in the carrot at 20°, and those of PITMAN's[6] Fig. 4, for K^+ in the red beet at 2°, support the interpretation of TORII AND LATIES[5] of the duality in the relation of net tracer influx to external concentration. It can be seen in Fig. 6 that at 1 mM the net tracer influx to the vacuole is mainly limited by the influx at the plasmalemma, and that at higher concentrations, it is increasingly limited by the flux at the tonoplast.

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