METABOLIC EFFECTS ON ION FLUXES IN *NITELLA TRANSLUCENS*

II. TONOPLAST FLUXES

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Summary

By separation and analysis of the cytoplasm and vacuole after uptake of radioactive tracer by the cells of *Nitella translucens*, the ion fluxes at both membranes of the cell have been estimated.

In summer material, the rate of transfer of both potassium and chloride from the cytoplasm to the vacuole was extremely high. The fluxes of both potassium and chloride at the tonoplast were affected by light, temperature, and external chloride concentration to an extent which would not be expected of passive fluxes. The transfer of both these ions across the tonoplast appeared to be correlated with the influx of chloride to the cytoplasm. It was suggested that two separate metabolic processes were involved in salt accumulation—an active uptake of chloride in the cytoplasm and a subsequent transfer of salt to the vacuole.

In winter material, both the influx of potassium and the transfer of potassium to the vacuole were abnormally low; both fluxes could be stimulated by the addition of phosphate to the solution and both were inhibited by carbonyl cyanide *m*-chlorophenyl hydrazone. Thus cells which had very little active uptake of potassium also transferred very little potassium from cytoplasm to vacuole; by contrast the tonoplast fluxes of both sodium and chloride were very high.

In spite of this big seasonal difference in cation discrimination, there was no evidence of any basic difference in the anion uptake in the two types of material; in both there appeared to be the same general relation between the influx of chloride to the cell and the rate constant for exchange of cytoplasmic chloride with the vacuole.

Puromycin inhibited the influx of chloride to the cell, and also decreased the chloride flux at the tonoplast; this may argue for a link with protein turnover.

I. INTRODUCTION

It is possible to estimate the fluxes of ions at both membranes of *Nitella translucens* by the separation and analysis of cytoplasm and vacuole after the uptake of radioactive tracer to the cell. There are two ways in which tonoplast fluxes may be estimated in such an experiment:

(1) By allowing the cell to reach the quasi-steady state in which the activity entering the cell is almost balanced by that leaving the cytoplasm to the vacuole or to the outside solution, and the two phases rise in parallel, and by determining the steady difference in specific activity between cytoplasm and vacuole in this state (MacRobbie 1964);

(2) By determining the proportion of activity reaching the vacuole in short times of uptake, during the early stages of the approach to the quasi-steady state.

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The second method has the advantages of involving fewer separate determinations on each cell, and of a shorter uptake time in which there is less chance of any change in ion fluxes.

Potassium and chloride fluxes at the tonoplast have previously been measured using the first method. The fluxes were found to be very high and were much more dependent on external conditions than one would have expected of passive fluxes. A metabolic transfer of salt from cytoplasm to vacuole was suggested from these results.

This paper describes further measurements of ion fluxes at the tonoplast, using the second method. It includes a preliminary experiment in which sodium fluxes were also measured. Again there is evidence that the transfer of ions from cytoplasm to vacuole is a metabolic process.

II. Methods

General methods have been described in Part I (MacRobbie 1966). A number of different artificial pond waters were used in the experiments to be described, and the chemical composition of the solutions used is given in Table 1.

Table 1

ION CONCENTRATIONS IN THE SOLUTIONS USED

Solution	Na^+ (mM)	K^+ (mM)	Rb^+ (mM)	Ca^{2+} (mM)	Cl^- (mM)	SO_4^{2-} (mM)
A	1·0	0·1	—	0·1	1·3	—
AB	1·0	0·1	—	1·1	1·3	1·0
B	0·2	0·1	—	1·0	0·3	1·0
RB	0·1	0·1	0·1	1·0	0·3	1·0
N	0·05	0·1	—	2·1	0·35	2·0

(*a*) *Separation of Samples*

The cell was removed from the solution and blotted to remove adhering liquid. One end of the cell was cut and the open end threaded on to a fine pipette; the other end was then cut and the fluid contents blown out into liquid paraffin, while the chloroplast layer remained attached to the cell wall. The nature of this fraction has been discussed previously and it is believed (MacRobbie 1964) that this cytoplasmic fraction is not contaminated with cell sap. The method provides samples of cell sap, and of cell wall plus cytoplasm, for separate analyses for radioactivity and chemical composition (by flame-photometry for sodium and potassium, by electrometric titration for chloride). The contribution of chloride in the cell wall to this fraction of wall plus cytoplasm is small, since it is present only in the water free space of the cell; the error is negligible in long-term uptakes, but in some short-term experiments a rinse of a few seconds was given before cutting the cell, to remove ions from the water free space without allowing time for any significant exchange of ions between cytoplasm and vacuole. In solutions in which potassium is present in much higher amount, in the Donnan free space, its contribution was estimated separately and subtracted from the total. In later experiments the amount of calcium in the solution was

increased (solutions B, AB, and N) so that monovalent cations were present only in low amounts in the cell walls; it was then considered that, given a few seconds rinse, the contribution of ions in the wall could be neglected.

(*b*) *Possible Contamination of Cytoplasmic Sample with Cell Sap*

In the course of these experiments some results were obtained which can be used to set limits to the extent of sap contamination of the sample of cytoplasm, and vice versa, and it seems worth while to quote these here.

At the shortest times of uptake used, the percentage of the total radioactivity taken up by the cell which was found in the chloroplast layer was as great as 96%, and there was no indication that this extrapolated back to anything less than 100% at zero time. This was tested for both potassium and chloride, and seems to rule out any significant contamination of the sap sample with cytoplasm.

The strongest evidence ruling out significant contamination of the sample of chloroplast layer with cell sap comes from an experiment in which cells were labelled with both ^{42}K and ^{36}Cl in the same experiment. Under the conditions of this particular uptake, the distribution of label between cytoplasm and vacuole were very different for potassium and chloride; thus only 4% of the ^{42}K had reached the vacuole, but 80–90% of the ^{36}Cl was found in the vacuole. The concentration of labelled potassium in the vacuole was only 0·007 mM, whereas labelled chloride had reached 0·7 mM (both values the mean of seven cells).

The chloroplast layer contained (per square centimetre) chloride equivalent to 1·2 μl of sap, labelled chloride equivalent to 3·6 μl of sap, potassium equivalent to 1·8 μl of sap. If one takes the lowest of these values, putting the chloride content of the layer at zero, one can calculate the amount of tracer potassium associated with this volume of sap; this would amount to only 0·3% of the tracer potassium found in the layer, and hence one must assume that more than 99% of the potassium content estimated for this sample is indeed cytoplasmic. This in turn implies that the volume of contaminating sap is not more than 0·3% of 1·8 μl or 0·005 μl, and its contribution to the total chloride in the layer cannot be more than 0·5% of the amount estimated. Since this value is derived from zero chloride in the layer it is a serious overestimate of the true amount, but there seems to be no virtue in approaching a more realistic value through successive approximations, as the contamination error is already negligible.

This experiment is the extreme case, but two others in which, respectively, 37 and 54% of the total ^{42}K were in the vacuole, while 87 and 91% of the ^{36}Cl had reached the vacuole, gave equally firm conclusions.

(*c*) *Estimation of Fluxes*

The cell is considered as two intracellular compartments—the cytoplasm and the vacuole—in series with one another, and it is assumed that the specific activity in the chloroplast layer isolated is a fair estimate of that in the cytoplasm as a whole. Justification for these assumptions was given previously (MacRobbie 1964) and some further justification is given below. It was previously shown that there is a lag in the

rise of vacuolar activity of the length predicted, that a fairly constant difference in specific activity between cytoplasm and vacuole is reached, and that in cells labelled over half their length only, the activity in the cytoplasmic layer isolated seems to be a fair estimate of the activity in the phase feeding the vacuole. The specific activities in the cytoplasm and in the vacuole are given by the equations:

$$Q_c(\mathrm{d}S_c/\mathrm{d}t) = [M_{oc}+M_{vc}S_v-(M_{co}+M_{cv})S_c], \tag{1}$$

$$Q_v(\mathrm{d}S_v/\mathrm{d}t) = (M_{cv}S_c-M_{vc}S_v), \tag{2}$$

where M_{oc} = flux from outside to cytoplasm,
M_{co} = flux from cytoplasm to outside,
M_{cv} = flux from cytoplasm to vacuole,
M_{vc} = flux from vacuole to cytoplasm,
S_c = cytoplasmic specific activity as a fraction of that outside,
S_v = vacuolar specific activity as a fraction of that outside,
Q_c = amount of ion in the cytoplasm, per unit area of cell,
Q_v = amount of ion in the vacuole, per unit area of cell.

There are two experimental conditions in which it is possible to use these equations to measure ion fluxes at both membranes. The first is that used earlier (MacRobbie 1964) in which the uptake time is long enough for the cytoplasmic specific activity to have reached a quasi-steady level; in this case there is an almost constant difference of specific activity between cytoplasm and vacuole as the two phases rise in parallel. If it is assumed that the cell is in a steady state then we can put M_1 for the flux at the plasmalemma ($M_1 = M_{oc} = M_{co}$), and M_2 for the flux at the tonoplast ($M_2 = M_{cv} = M_{vc}$). We then have, in this quasi-steady state, for long times of uptake

$$M_2 = (Q_vS_v/t) \,.\, 1/(S_c-S_v),$$

and can estimate M_2 from measurements of total vacuolar activity and specific activities in cytoplasm and vacuole.

The effect of the assumptions that the cell is in flux equilibrium was discussed previously (MacRobbie 1964). Since the cytoplasm is a small phase in contact with a very large vacuolar phase, with the ion content of the cytoplasm much less than the amount crossing the tonoplast per hour, a new flux equilibrium at the tonoplast must be set up very rapidly if conditions change; over the long experimental periods, for which this method is used it seems valid to put $M_{co} = M_{vc}$. As the specific activity in the cytoplasm is generally less than 10% of that outside during any of the experimental periods used, the assumption that $M_{oc} = M_{co}$ affects the calculation of M_1 only very little. It is therefore assumed safe to use these equations.

The second method is by the determination of the proportion of activity reaching the vacuole in a short time of uptake, during the early stages of the approach to the quasi-steady state. During this time $S_v \ll S_c$, and we may put $S_v = 0$ in equation (1) above. The cytoplasmic activity will rise with a time course given by

$$S_cQ_c = (M_{oc}/k)(1-\mathrm{e}^{-kt}),$$

where k, the rate constant for exchange in the cytoplasm, is equal to $(M_{co}+M_{cv})/Q_c$.

The total entry to the cell in this time may be taken as $M_{oc}t$, since S_c is small, and we therefore have, for the proportion of activity in the cytoplasm, the relation

$$\frac{\text{activity in the cytoplasm}}{\text{total activity in the cell}} = \frac{1-e^{-kt}}{kt}.$$

A graph was constructed of $(1-e^{-kt})/kt$ against kt, from which it was possible to read off kt for any given proportion of activity in the cytoplasm.

This method yields a value for the rate constant for cytoplasmic exchange $k = (M_{co}+M_{cv})/Q_c$, and the sum of the fluxes out of the cytoplasm may be calculated from this by multiplying by the cytoplasmic content. The error in the determination of cytoplasmic content (due to variability in the sample obtained and possible loss of cytoplasm) is much greater than the error in the determination of k, and for that reason the results have been primarily quoted in this form; in some cases a value is

TABLE 2

INFLUX TO THE CELL, AND RATE CONSTANT FOR CYTOPLASMIC EXCHANGE, DETERMINED BY UPTAKE, OR BY UPTAKE FOLLOWED BY WASHING OUT

Results quoted are means ± standard error of the mean

Treatment	No. of Cells Measured	Influx of Potassium (pmoles cm^{-2} sec^{-1}	Rate Constant, k (hr^{-1})
Uptake 2–4 hr	10	1·2±0·1	0·058±0·005
Uptake 3–3½ hr, equal washing-out period	6	1·0±0·1	0·062±0·006

also quoted for the sum of the effluxes from the cytoplasm, kQ_c. For chloride, the efflux of chloride to the outside solution is always a negligible fraction of this sum, but there are some conditions and some cells in which both potassium effluxes from the cytoplasm may be significant in determining the rate constants for cytoplasmic exchange. But in general the rate constant is determined by the flux from cytoplasm to vacuole, and can be used to determine this flux and changes in it.

It is necessary to consider the time limits for which this analysis is valid—for which the assumption $S_v \ll S_c$ is tenable. With the relative contents of cytoplasm and vacuole in the cells, an arbitrary limit of $S_v = 0{\cdot}1\ S_c$ is reached with about 30–40% of the total activity in the cytoplasm for chloride, or 40–50% for potassium The method was therefore only used with cells in which more than 40% of the total activity was in the cytoplasm; that is, it was used only up to $kt = 2{\cdot}2$. In general the proportion of cytoplasmic activity was much higher than this, and S_v was therefore a smaller fraction of S_c.

A variant of this experiment is to label the cells for time t, and then to leave them for an equal time in an inactive washing-out solution. After this combination of treatments the proportion of activity in the cytoplasm is $(1-e^{-kt})(e^{-kt}/kt)$, and kt can again be estimated.

This serves as a useful check on the model of two phases in series with one another, and it is worth recording here that the values of k obtained by uptake measurements are consistent with those obtained on comparable cells by uptake followed by washing-out. This is shown in Table 2, for potassium.

Some further justification for the use of the model comes from the fact that the two methods, the analysis after short times of uptake and the steady-state analysis, appear to give the same results (see Fig. 4).

III. Results

(a) *General*

As the present results can only be considered together with those obtained previously, the main conclusions will be summarized here. In previous work (on "summer" *Nitella*, using the steady-state method for tonoplast fluxes) very high values were obtained for the fluxes of both potassium and chloride at the tonoplast. The fluxes of both potassium and chloride from the cytoplasm to the vacuole were affected by light, temperature, and external chloride concentration to an extent that would

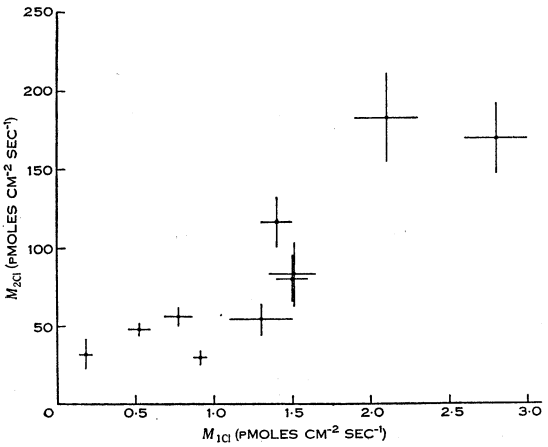

Fig. 1.—Flux of chloride from cytoplasm to vacuole, M_{2Cl}, plotted against the influx of chloride to the cell, M_{1Cl}. Points represent means of at least 10 cells in experiments under different conditions of light, temperature, and external chloride concentration; the standard errors of the means are shown.

not be expected of passive fluxes. In addition the transfer of both these ions from cytoplasm to vacuole under different conditions appeared to be correlated with the initial uptake of chloride into the cytoplasm. These correlations are shown in Figures 1 and 2, in which, respectively, the fluxes of chloride and potassium at the tonoplast are plotted against the influx of chloride to the cell. In these cells there was no correlation between the influx of potassium to the cell and the flux of potassium at the tonoplast.

The present results deal with *Nitella* in its winter state—a period from about December when the influx of potassium is very low, and it appears that the transfer to the vacuole is also much lower. In some earlier experiments in December, when only a few cells were in this state, it was noticed that these were abnormally low both in respect of M_{1K} and M_{2K}. The present cells were all in this state and both M_{1K} and M_{2K} were low.

The most striking result is the very different behaviour, in these cells, of potassium compared with chloride or sodium. This is brought out in Table 3, which

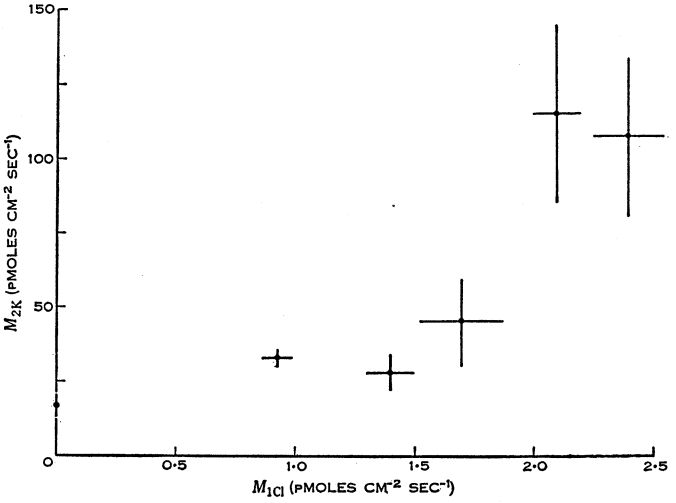

Fig. 2.—Flux of potassium from cytoplasm to vacuole, M_{2K}, plotted against the influx of chloride to the cell, M_{1Cl}. Points represent the means of at least 10 cells; the standard errors of the means are shown.

shows the percentage of the total activity which is found in the vacuole after given times of uptake. After long times of uptake the specific activities in the vacuole and

TABLE 3

PERCENTAGE OF ACTIVITY FOUND IN THE VACUOLE

Time of Uptake (hr)	Solution	No. of Cells	Average Percentage of Total Activity Found in Vacuole		
			K^+	Cl^-	Na^+
0·4–1·3	A	9	3–7		
2–4	AB	10	5–10		
3–3·5*	AB	6	20–30		
0·25–1·3	B	7		15–50	
0·7–1·3	AB	9		34–51	
0·8–1·4	AB	8		31–46	
7–9	B plus 0·1 mM phosphate	8	37	87	
15–17	RB plus 0·1 mM phosphate	7	54	91	
1–2	N	10			> 90

* Plus equal washing period.

in the cytoplasm are very nearly equal, and thus the percentage of the total activity found in the vacuole tends to the percentage of the total cell content—about 90% of the total chemical content for potassium and 94–95% for chloride.

It is clear that in these "low potassium influx" cells the rate of transfer of both sodium and chloride to the vacuole is very high, whereas that of potassium is relatively low. This contrasts with previous behaviour of *Nitella* in its "normal" state of higher potassium influx, in which the rate of potassium transfer from cytoplasm to vacuole was also extremely high.

Smith (1965) found that in these winter cells the influxes of both chloride and potassium could be stimulated by the addition of low concentrations of inorganic phosphate to the artificial pond water. After overnight treatment the potassium flux rose to a value approaching the normal 1 pmole cm^{-2} sec^{-1}, and in the results below, partial stimulation was achieved by the addition of phosphate along with the radioactive solution in short-term uptake experiments.

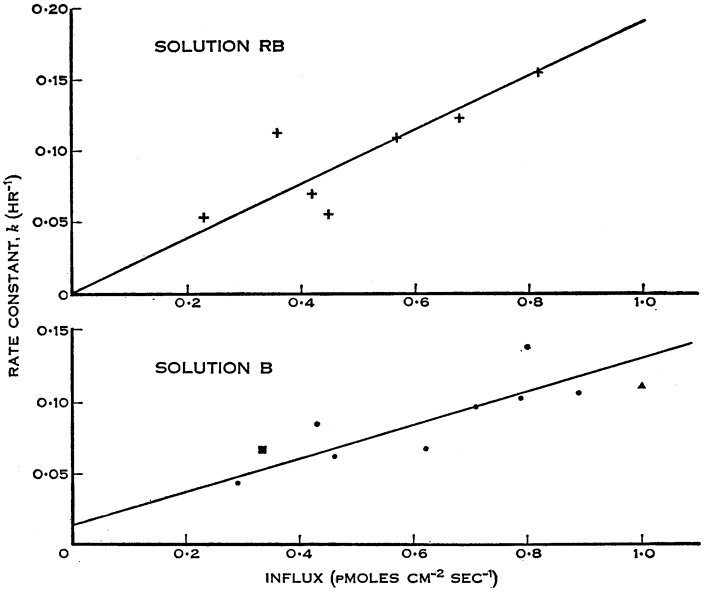

Fig. 3.—Rate constant for exchange of potassium in the cytoplasm, k, plotted against influx of potassium to the cell in winter (i.e. phosphate-deficient) cells. For the two experiments shown 0·1 mM phosphate has been added to the two basic solutions used. ■ Mean of eight such cells treated with 5×10^{-6}M CCCP. ▲ Mean of eight cells rather later in the season, when the potassium flux has increased.

In these phosphate-stimulation experiments, in which 0·1 mM disodium hydrogen phosphate was added to the solution, the association of low potassium influx with low transfer of potassium to the vacuole is also shown. In Figure 3, the rate constant for exchange from the cytoplasm into the vacuole (k) is plotted against influx of potassium to the cell (M_{1K}), in two experiments with different solutions. In each case there is a significant correlation between k and the influx to the cell, and the calculated relations are given below:

8 cells in solution B for 7–9 hr: $k = 0{\cdot}11\ M_{1K} + 0{\cdot}015$ $(P < 0{\cdot}015)$

7 cells in solution RB for 15–17 hr: $k = 0{\cdot}19\ M_{1K}$ $(P < 0{\cdot}02)$

Also shown in Figure 3 is the mean of eight similar cells treated with 5×10^{-6}M carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), and a point for early spring cells in which the potassium influx has risen to a more normal value, although the potassium transfer to the vacuole remains lower than in summer material. It is

TABLE 4

EFFECT OF CCCP (5×10^{-6}M) ON POTASSIUM FLUXES

Solution	Time (hr)	No. of Cells	Influx of Potassium to Cell, M_{1K} (pmoles cm^{-2} sec^{-1})	Rate Const., k (hr^{-1})	Potassium Content of Cytoplasmic Phase, K_c (nmoles cm^{-2})	kK_c (pmoles cm^{-2} sec^{-1})
A	16–17	8	0·44±0·09	>0·15		
A+CCCP	5–6	7	0·14±0·01	0·020±0·0035	117±10	0·65
B+phosphate	7–9	8	0·62±0·06	0·086±0·010	109±10	2·6
B+CCCP	1·0–1·7	8	0·33±0·02	0·05 ±0·02	107± 9	1·6
B	0·4–1·3	8	1·0 ±0·2	0·11 ±0·01	147±16	4·5
B+CCCP	0·7–1·2	7	0·11±0·02	0·07 ±0·01	134±15	2·6

interesting that these two points fall so close to the calculated relation at the upper and lower ends of the range respectively.

Thus it appears that in winter cells both the potassium influx to the cell and the transfer of potassium from cytoplasm to vacuole are stimulated by the addition of

TABLE 5

EFFECTS OF PUROMYCIN ON CHLORIDE FLUXES

The basic solution used was AB

Concentration of Puromycin (mM)	Period of Pretreatment with Inhibitor (hr)	Period of Uptake (hr)	No. of Cells	Chloride Influx to Cell, M_{1Cl} (pmoles cm^{-2} sec^{-1})	Rate Constant, k (hr^{-1})
0	—	0·7–1·3	9	1·47±0·19	1·28±0·16
0·25	0·5	1·7–2·3	8	0·79±0·06	0·74±0·11
0	—	0·8–1·4	8	1·26±0·09	1·0 ±0·15
0·3	0·5	2–3	6	0·74±0·11	0·67±0·09
0·3	—	17–18	10	0·75±0·08	—
0·3	17	3	8	0·76±0·11	—

low concentrations of phosphate to the solution, and that this effect is abolished by 5×10^{-6}M CCCP.

Later in the season the potassium flux is higher and most of the results which follow refer to these "spring" cells.

(*b*) *Effect of CCCP*

The results on potassium fluxes of treatment with 5×10^{-6}M CCCP (in the light) are given in Table 4. Spanswick (personal communication) has found that the potential becomes more negative for an hour or more after the addition of CCCP; it then decreases over several hours to a low value. In three of the four experiments in Table 4, the uptake times were short, to cover the initial period during which the potential was still high, and in which the decrease in potassium influx could not be attributed to a decrease in passive influx. Again an inhibition is seen in both influx of potassium and transfer to the vacuole.

CCCP appeared to have very little effect on chloride fluxes at either membrane, but as the uptake times were badly chosen for the chloride kinetics, this conclusion about the tonoplast flux needs to be confirmed.

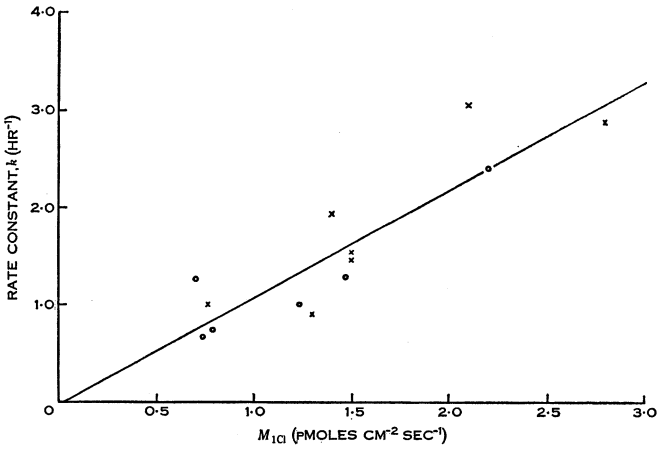

Fig. 4.—Collected results on chloride fluxes. The rate constant for chloride exchange in the cytoplasm is plotted against influx of chloride to the cell. × Steady-state specific activity measurements. ○ Measurements of percentage cytoplasmic activity after short uptake times. The calculated regression line is drawn for which $r = 0{\cdot}88$, significant at $P < 0{\cdot}01$.

(*c*) *Effects of Puromycin*

Smith (1965) found that puromycin inhibited the influxes of potassium, chloride, and phosphate to the cell, while leaving CO_2 fixation or glucose uptake unaffected. His results gave qualitative indications that there was also an inhibition of tonoplast fluxes. In the present results puromycin inhibited both chloride influx to the cell and chloride transfer to the vacuole as shown in Table 5. The degree of inhibition is less striking than that found by Smith, who found a reduction to 10% of the control value in an uptake of about 16 hr. However, no increase in the percentage inhibition was found by increasing the time of treatment or pretreatment with the inhibitor.

IV. Discussion

A comparison of the present results with those obtained previously is interesting. The chloride influxes and the rate of chloride transfer to the vacuole are somewhat lower, but all the results can be incorporated into the general relationship shown in Figure 4, and there is no suggestion of any important difference in kind between the two sets. Estimates of tonoplast flux on the present material by the two methods gave consistent results.

On the other hand, the rate of potassium exchange between cytoplasm and vacuole is very different in the present material; estimates of k, the rate constant, on the previous cells are in the range 0·4–3·0 hr^{-1} in different conditions (half-times 0·2–1·8 hr), while the present values are in the range 0·06–0·15 hr^{-1} (half-times 5–12 hr). The present values are, however, consistent with the small number of cells with low influxes found among the winter experiments in the previous set, in which the transfer to the vacuole was equally low. Thus the difference appears to be a genuine seasonal difference in cell behaviour, and there is clear indication that the two methods of estimating tonoplast fluxes give consistent results.

As most of the results have been quoted in the form of rate constants for cytoplasmic exchange it is worth giving rough values for the fluxes calculated from these. Potassium fluxes were in the range 2–6 pmoles cm^{-2} sec^{-1} in these experiments; chloride fluxes in the range 40–130 pmoles cm^{-2} sec^{-1}. The exchange of sodium from cytoplasm from vacuole was too rapid for the uptake time chosen in the preliminary experiment to yield an accurate value for k; from the very high percentage of activity found in the vacuole after an uptake of only 1–2 hr it can be deduced that in these cells sodium flux at the tonoplast is comparable with the chloride flux.

By comparison, in one experiment with *Chara australis* a value of 1·0 hr^{-1} for k was found, corresponding to a tonoplast sodium flux of about 20 pmoles cm^{-2} sec^{-1}, for 10 cells in solution B (MacRobbie, unpublished data).

It is not clear why the rate of potassium movement to the vacuole should be so low in this condition. The indications are that the ratio of K/Na in the tonoplast traffic has decreased, but further experiments are needed to establish this as a definite conclusion. Two interpretations are possible of the phosphate stimulation of both potassium influx and potassium transfer at the tonoplast, and of the effects of CCCP on this. One could argue from this that the transfer of potassium across the tonoplast was a process requiring ATP (as is the potassium influx) and that in winter cells the rate of supply of ATP was rate-limiting for both processes, and could be stimulated by the addition of phosphate; M_{1K} and M_{2K} would then appear to be correlated. One must then argue that in summer cells the supply of phosphate is not rate-limiting, at least for the influx, and there is then no correlation between M_{1K} and M_{2K}. Alternatively one could argue that in order to be transported to the vacuole, potassium must first be accumulated by an active mechanism in some particular region of the cytoplasm, and that very little of the potassium taken up passively is available for transport to the vacuole. This argument is difficult to sustain, and the results with cells which are pumping potassium do not suggest that a part of the influx is into a region from which there is only slow transfer to the vacuole; one would then expect

to find much more activity in the cytoplasm than is in fact found. One could, however, argue that potassium which has entered the cell passively has access by an ATP-dependent process either to the vacuole or to a special region in the cytoplasm from which transfer occurs to the vacuole.

The evidence remains that in normal cells there is no correlation between potassium influx and potassium flux at the tonoplast, but that, rather, the transfer of potassium from cytoplasm to vacuole is linked with chloride movements. This suggested that the transfer from cytoplasm to vacuole may be as salt rather than as individual ions. The relationship shown in Figure 4 suggests there is no basic difference between the two types of material in respect of anion uptake, in spite of the big difference in their abilities for cation discrimination, both at the plasmalemma and at the tonoplast. It is clear that a more complete study of the movements of all three ions, sodium, potassium, and chloride, in both seasonal states of the cell, is needed before it is possible to make any firm interpretation of the results.

In the phosphate-starved cells the correlation between M_{1K} and M_{2K} could be interpreted in terms of a mutual dependence of potassium influx and potassium movement at the tonoplast on supplies of ATP. It may be that in high potassium influx cells, in which the potassium transfer at the tonoplast is correlated with chloride influx but not with potassium influx, that the correlation is similarly an indirect one. The chloride influx seems to be related directly to electron flow rather than to the associated phosphorylations (MacRobbie 1965, 1966), but it is then possible that the strict dependence of phosphorylation on electron flow might be reflected in apparent correlations between processes dependent on electron flow and ATP consumption respectively.

The effects of puromycin are interesting in view of the fact that, as yet, the only known effect of the substance is as an inhibitor of protein synthesis. Sutcliffe (1960) interpreted the inhibition of net salt uptake in beetroot slices by chloramphenicol in terms of a connection between ion transport and protein synthesis. This has been questioned by others, on the grounds that chloramphenicol has effects on other processes (Hanson and Hodges 1960; Hanson, Stoner, and Hodges 1963) and that the isomer of chloramphenicol which does not inhibit protein synthesis in bacteria will nevertheless inhibit salt uptake (Ellis 1963). The results with puromycin are not open to these objections, and may indeed imply a link with protein turnover. It is perhaps surprising that the effect is on the influxes of all three ions to the cell, potassium, chloride, and phosphate, and also on chloride transfer to the vacuole, with a suggestion from Smith's results that the potassium flux at the tonoplast is also reduced. These effects should repay further study.

V. Acknowledgments

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