UPTAKE AND TRANSPORT OF IONS IN BARLEY SEEDLINGS

II.* EVIDENCE FOR TWO ACTIVE STAGES IN TRANSPORT TO THE SHOOT

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Abstract

For some purposes it is adequate to explain the exudation of salt from roots, and the transport from root to shoot, as the result of a single active transport at the outer surface of the cells of the root. This paper shows that it is also necessary to have a second, active transport prior to entry to the xylem. Support for this model is based on a variety of observations. Tracer uptake interpreted on a symplast model is investigated experimentally and tested by comparison with simulated properties of the system. The effects of CCCP on uptake and transport are related to this model and compared with effects of low salt content on transport and accumulation. The behaviour of excised roots is also compared with the behaviour of whole plants.

I. Introduction

There are two aspects of ion uptake by plants that appear to be related to active transport in the roots. First, the root is remarkably efficient at taking up ions from soil or solution, and there is general acceptance that this process is due to active transport at the outer surface of root cells. This uptake may also be regarded as entry to a symplast in the root.

Second, the root secretes ions into the xylem and as a result there can be exudation of solution from cut roots that is commonly at much higher concentration than the external solution. In barley seedlings, comparison of potassium and sodium content of the shoot with the proportions of these ions in the exudate from the root shows that the root can regulate the amounts transported to the shoot (Pitman 1965*a*). Another aspect of this regulation is shown by certain halophytes. The xylem sap of the mangrove *Rhizophora mucronata* can be 50–100 times less concentrated than the seawater around the roots. These roots can exclude NaCl but take up potassium and allow transport of water to the shoot (Scholander *et al.* 1966).

Both these aspects of ion uptake by the plant can be accounted for by a model with a single active transport process which builds up levels of ions in the root cells. Leakage from these cells into the stele then leads to exudation. This simple "one-pump" hypothesis is suitable for many studies of ion transport. Anderson, Aikman, and Meiri (1970) found it adequate to use one active process in a model relating ion flow and water flow through excised roots. Estimation of fluxes and transport through roots can be based on a symplast model without specifying the nature of the flux between symplast and xylem (Weigl 1969; Pitman 1971, Fig. 6).

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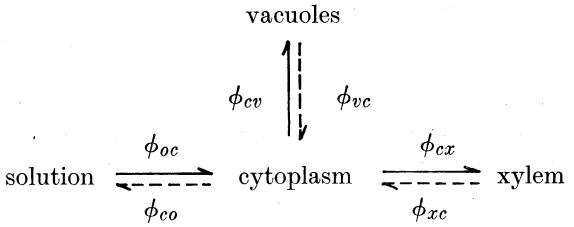
Laties (1969) reviewing work on the relation between absorption of ions into the root and transport from root to shoot tended to favour the one-pump model. He suggested that there was no unequivocable evidence for the alternative hypothesis, that transport to the shoot involved active uptake both at entry to the symplast, and during passage from symplast to the xylem. This model will be referred to as the "two-pump" hypothesis. More recently Dunlop and Bowling (1971*a*, 1971*b*, 1971*c*) have suggested there is evidence that movements of potassium and chloride into the xylem are both passive processes and not active. Their suggestion was based on measurements of potential differences between cells and external solution, and between xylem exudate and solution.

Despite the convenience of the one-pump hypothesis for some studies, this model does not account for interactions between ion uptake and water flow found in barley and mustard seedlings. The relationship between potassium and sodium uptake to barley seedlings and to mustard seedlings is better explained by involvement of two active processes (Pitman 1965*b*, 1966). In both plants, the ratio of potassium to sodium in the shoot appears to be proportional to that in the root (Pitman 1966, Fig. 3), but not to that in the solution. Rate of transpiration did not affect the ratio of potassium to sodium in the root, but when external concentration was above about 20 mM, the selectivity to the shoot was reduced without altering total potassium plus sodium uptake. For example, in a solution containing 15 mM K+45 mM Na:

	Barley		Mustard	
Transpiration	High	Low	High	Low
Roots—K/Na	$2\cdot9\pm0\cdot1$	$3\cdot1\pm0\cdot1$	$2\cdot5\ \pm0\cdot1$	$2\cdot4\pm0\cdot1$
Shoots—K/Na	$2\cdot5\pm0\cdot1$	$3\cdot6\pm0\cdot25$	$0\cdot73\pm0\cdot07$	$2\cdot6\pm0\cdot1$

The uptake of potassium plus sodium to barley shoots was 60 ± 4 and 62 ± 3 μ-equiv per plant at high and low transpiration; for mustard the shoots contained $3\cdot20\pm0\cdot1$ and $3\cdot25\pm0\cdot5$ μ-equiv mg^{-1} respectively. This combination of altered selectivity but constant total uptake cannot be explained on the one-pump hypothesis.

The purpose of this paper is to present evidence for two mechanisms of transport to the shoot, using methods of measuring transport through excised roots described previously (Pitman 1971). Figure 6 of that paper showed the fluxes used to interpret transport through the root, but the system can be expressed more simply as:



where solid arrows show suggested active processes (see p. 246 for symbols). The problem is to determine whether the transport into the xylem from the symplast (ϕ_{cx}) involves an active step. On the one-pump hypothesis ϕ_{oc} is active, but not ϕ_{cx}; on the two-pump hypothesis both ϕ_{oc} and ϕ_{cx} are active.

Active transport of chloride in barley root cells is strongly inhibited by the inhibitor CCCP (carbonyl cyanide *m*-chlorophenylhydrazone). The extent of inhibition depends on salt concentration in the solution, on whether excised roots or whole plants are used and, of course, on the concentration of the inhibitor (Greenway 1965; Lüttge and Laties 1967). It is shown in the present paper that CCCP is a suitable inhibitor of the processes of uptake and transport, and it is then used to switch off these processes. From comparison of effects of CCCP when tracer is either in the external solution or in the cell vacuoles, it is suggested that ϕ_{cx} must be treated as an active process and that the two-pump hypothesis best describes uptake and transport of KCl in barley roots.

II. Material and Methods

(*a*) *Plant Material*

Seedlings of *Hordeum vulgare* cv. Cape were germinated and grown on $CaSO_4$ solution as described in the previous paper (Pitman 1971). After 6 days, low-salt roots were transferred to a 10 mM KCl solution for 24 hr to bring them to high-salt status; for use in efflux experiments roots were brought to high-salt status on 10 mM KCl labelled with ^{36}Cl.

(*b*) *Solutions*

All solutions contained 0·5 mM $CaSO_4$ in addition to KCl or NaCl. The inhibitor CCCP was dissolved in 0·2 mM NaOH and diluted to give a stock solution containing 200 μM CCCP. Fresh stock solution was prepared for each experiment, and diluted as required.

(*c*) *Measurement of Transport of Chloride and* ^{36}Cl

The apparatus used to measure transport* has been described in the previous paper [Pitman 1971, Fig. 2(*a*)]. Briefly, excised roots were set up with the cut end in a collecting chamber and the main length of the root in a chamber containing labelled KCl. These two chambers were separated by a guard chamber to eliminate leakage of tracer into the collecting chamber. Total chloride transport was measured by replacing KCl in the collecting chamber with KNO_3 of the same concentration.

(*d*) *Measurement of Efflux*

Excised high-salt roots containing labelled chloride were set up with the cut end in one chamber and the rost of the root in another chamber [Pitman 1971, Fig. 2(*b*)]. In this way, efflux from the stele could be calculated separately from that from the rest of the root. The tissue was rinsed in unlabelled KCl for 2·5 hr before starting measurements. This treatment removed ^{36}Cl from free space and cytoplasm so that tracer was predominantly in the vacuoles. The efflux was calculated from the specific activity of the chloride in the roots, allowing for unlabelled chloride in free space and cytoplasm.

(*e*) *Measurement of Tracer Uptake*

Uptake to the roots was taken as the sum of tracer transported through the roots plus the tracer retained in the roots (accumulation). The amount of transport was measured as described earlier in this section. Accumulation was measured from the amount of tracer in the roots after a certain period in labelled solution, followed by a rinse of 40 s in ice-cold water. This rinse eliminated all but 0·2 μ-equiv g^{-1} of the free space content (see Fig. 5 q.v.).

* Accumulation, transport, and uptake are used as defined in the previous paper (Pitman 1971).

(*f*) *Simulation Procedure*

Uptake of tracer and transport of tracer through the root was computed using the system set out in Figure 6 of the previous paper (Pitman 1971). In this system there were fluxes ϕ_{oc}, ϕ_{co} into and out of the cytoplasmic phase, which was equivalent to the symplast. From the cytoplasmic phase there were fluxes ϕ_{cv} and ϕ_{vc} into and out of the vacuole and fluxes ϕ_{cx} and ϕ_{xc} into and out of the xylem. Specific activities were S_o, S_c, S_v, and S_x in solution, symplast, vacuoles, and xylem respectively. Since $S_x = S_c$, $(\phi_{cx}.S_c - \phi_{xc}.S_x)$ can be written more conveniently as $R'.S_c$, where $R' = \phi_{cx} - \phi_{xc}$.

For the cytoplasmic phase, taking $S_o = 1 \cdot 0$, the change in amount of tracer, Q_c^*, is

$$dQ_c^*/dt = \phi_{oc} - (\phi_{co} + \phi_{cv} + R').S_c + \phi_{vc}.S_v,$$

and in the vacuole, Q_v^* changes as

$$dQ_v^*/dt = \phi_{cv}.S_c - \phi_{vc}.S_v.$$

Transport to the stele is

$$dQ_x^*/dt_x = R'.S_c.$$

Uptake to each phase was calculated by assuming specific activity was constant for a short time increment $\Delta t = 0 \cdot 01$ min. At the end of each period specific activities were recalculated from the new values of Q_c^* and Q_v^* and these new values used for the subsequent period. A lag was introduced in calculating transport from stele to solution from the summation of $R'.S_c$. The specific activity in the xylem was assumed to equal S_c.

III. Results and Discussion

The exchange of tracer with plant roots is more complex than the exchange with cells as the root has a net transport of ions through the cortex and the stele. Both the amount retained in the root (accumulation) and the amount transported need to be determined to estimate the total entering the root, i.e. the uptake.

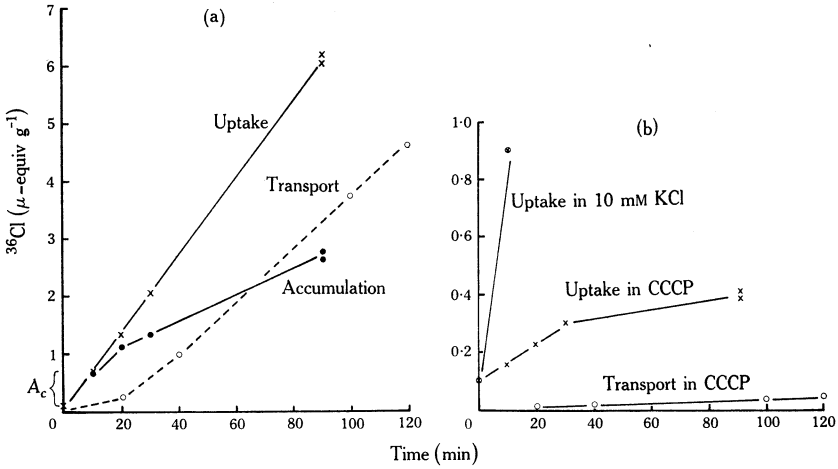


Fig. 1(*a*).—Uptake, accumulation, and transport of ^{36}Cl from labelled 10 mM KCl by excised high-salt roots. A_c is the extrapolate of accumulation to $t = 0$ less free space content. (*b*) Effect of 10 μM CCCP on uptake and transport of ^{36}Cl from labelled 10 mM KCl. Note difference in scale. Uptake from 10 mM KCl, as in Figure 1(*a*), is given for comparison.

The time courses of uptake, accumulation and transport for excised high-salt roots in 10 mM KCl are shown in Figure 1(*a*). Uptake was linear over the 2 hr shown.

TABLE 1

COMPUTED UPTAKE, ACCUMULATION, AND TRANSPORT OF TRACER USING THE MODEL OF FIGURE 6*

	Variation in ϕ_{oc}, ϕ_{co}				Vary Q_c			Effect of inhibition			Data from efflux analysis*		
Set No.:	1	2	3	4	5	6	7	8	9	10	11	12	13
Fluxes (μ-equiv g^{-1} hr^{-1})													
ϕ_{oc}	10·0	6·0	5·0	4·2	10·0	5·0	5·0	0·5	3·5	5·0	7·8	4·6	4·9
ϕ_{co}	6·0	2·0	1·0	0·2	6·0	1·0	1·0	2·0	5·0	1·0	3·2	1·0	1·2
ϕ_{cv}	2·0	2·0	2·0	2·0	2·0	2·0	2·0	0·2	0·2	0·2	2·9	1·5	1·7
ϕ_{vc}	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·9	1·5	1·7
ϕ_{cx}	4·0	4·0	4·0	4·0	4·0	4·0	4·0	0·3	0·3	4·0	4·6	3·6	3·7
Content, Q_c (μ-equiv g^{-1})	2·0	2·0	2·0	2·0	0·5	1·0	0·5	2·0	2·0	2·0	2·5	2·0	1·7
Uptake rates (μ-equiv g^{-1} hr^{-1})													
0–10 min	8·16	5·60	4·83	4·17	6·23	4·71	4·56	0·46	2·87	4·82	7·13	4·44	4·67
30–90 min	5·02	4·54	4·32	4·07	4·98	4·28	4·28	0·22	0·59	4·13	5·5	3·89	4·04
Accumulation rates (μ-equiv g^{-1} hr^{-1})													
0–10 min	7·81	5·37	4·64	4·01	5·28	4·36	3·98	0·46	2·86	4·63	6·87	4·28	4·47
30–90 min	1·72	1·67	1·63	1·58	1·63	1·43	1·40	0·18	0·42	0·75	2·27	1·41	1·42
Transport rates (μ-equiv g^{-1} hr^{-1})													
40–100 min	3·33	2·94	2·77	2·59	3·35	2·87	2·88	0·04	0·18	3·55	3·32	2·58	2·69
A_c (μ-equiv g^{-1})	1·70	1·35	1·21	1·06	0·67	0·78	0·53	0·19	1·09	1·86	1·62	1·25	1·14
Shoulder	2·00	1·60	1·30	1·20	0·80	0·80	0·70	0·20	1·00	1·50	1·90	1·30	1·30

* From Pitman (1971).

As expected from previous experiments the rate of transport developed to a maximum over 30–60 min and there was a corresponding decrease in the rate of accumulation.

This complementarity of accumulation and transport would support the view that ϕ_{oc} is limiting entry to the root, as expected from the one-pump hypothesis. On the two-pump model the same result would require ϕ_{oc} to be determined by the joint demands of net uptake to the stele and net uptake to the vacuoles. It has been shown elsewhere that accumulation measured after a long rinse in unlabelled salt solution is linear with time and does not show a "shoulder" as in Figure 1(*a*) (Pitman, Courtice, and Lee 1968). The shoulder is partly due to uptake to the cytoplasmic phase [$Q_c.S_c$ in Figure 6 of the previous paper (Pitman 1971)] and partly due to tracer in the transport pool or process but not yet secreted from the stele.

The results of Figure 1(*a*) can be related to the fluxes into and out of each phase and the amounts of chloride in this phase. The initial uptake approximates to $\phi_{oc}.S_o$, whereas the later rate of uptake is more nearly ($\phi_{oc}.S_o - \phi_{co}.S_c$). It was observed that the initial rate of uptake was nearly the same as that over the later period (30–90 min) so the term $\phi_{co}.S_c$ must be small. The ratio of rate of transport to rate of uptake gives an estimate of S_c which from Figure 1(*a*) would be $0 \cdot 7$; ϕ_{co} must therefore be small.

The effect of variation in fluxes and other parameters was investigated by computing tracer uptake as described above. The results are shown in Table 1. Values of fluxes ϕ_{oc}, ϕ_{co} were selected to cover the expected range and to show the effect of variation in ϕ_{co} both with the same net transport ($\phi_{oc} - \phi_{co} = 4 \cdot 0$ μ-equiv g^{-1} hr^{-1}). Fluxes into and out of the vacuole were set at $2 \cdot 0$ μ-equiv g^{-1} hr^{-1} since roots showed no net accumulation and as this value was consistent with tracer measurements. Results of computation give rates of accumulation and uptake from 0 to 10 min (initial rate) and between 30 and 90 min (later rate); transport rate is given over 40 to 100 min. Sets 1–4 show that initial and later rates of uptake are the same only when ϕ_{oc} is small—as already suggested, otherwise there is a marked shoulder in the time course of uptake as well as in accumulation. Reduction in Q_c (sets 5–7) also leads to less difference in initial and later rates of uptake but only if less than $0 \cdot 5$ μ-equiv g^{-1}; the observed values were between $1 \cdot 5$ and $2 \cdot 5$ μ-equiv g^{-1}. Sets 11–13 show computed uptake for fluxes estimated by efflux analysis using data from a previous paper (Pitman 1971). Sets 12 and 13 agree very well with the results of Figure 1(*a*).

Though the "shoulder" and A_c are difficult to interpret, values were calculated and shown in Table 1 for comparison with observed results (see also Table 2). The long-term rate of accumulation of tracer by roots in 10 mM KCl has been interpreted previously as a measure of the flux ϕ_{cv} (Pitman *et al.* 1968; Pitman 1969; Cram and Laties 1971). Table 1 shows that this view is supported by comparison between observed and computed rates of accumulation using fluxes determined by efflux analysis. It should be noted that the long-term of accumulation underestimates ϕ_{cv} by about 20%.

The effect of 10 μM CCCP on the components of uptake is shown in Figure 1(*b*). There was a strong inhibition of accumulation and transport and reduction in A_c. In this experiment tissue was pretreated for 30 min before addition of tracer but even when CCCP was added at the same time as the tracer there was clear and rapid inhibition of both processes. Rates of uptake, accumulation, and transport from these

and other experiments are collected in Table 2. The rate of uptake was estimated over the initial 10–20 min allowing 0·1 μ-equiv g^{-1} for free space content. Accumulation was taken as the rate between 30 and 90 min and transport between 40 and 100 min.

The rapid action of CCCP and the lack of any large shoulder in the time course of accumulation are taken to show that CCCP has inhibited ϕ_{oc}. Other evidence shows that ϕ_{cv} was inhibited too. Thus roots in 5 μM CCCP+10 mM KCl lost 0·9 μ-equiv g^{-1} hr^{-1} chloride, but net loss was zero from roots in KCl alone. Table 1 gives computed uptake for inhibition of ϕ_{oc}, and ϕ_{cv}, which is consistent with the time courses of uptake in Figure 1(*b*).

TABLE 2

EFFECT OF CCCP CONCENTRATION ON ^{36}Cl UPTAKE FROM 10 mM KCl SOLUTION

CCCP (μM)	Accumulation (μ-equiv g^{-1} hr^{-1})	Transport (μ-equiv g^{-1} hr^{-1})	Uptake (μ-equiv g^{-1} hr^{-1})		A_c (μ-equiv g^{-1})	Shoulder (μ-equiv g^{-1})
			30–90 min	0–10 min		
Nil	1·0	0·8	1·8	1·7	0·4	0·8
4	0·6	0·08	0·7	0·9	<0·1	0·1
4*	0·4	0·10	0·5	0·7	<0·1	0·1
10	0·35	0·10	0·45	0·7	<0·1	0·1
10*	0·20	0·05	0·25	0·45	<0·1	0·1
Nil†	1·4	2·8	4·2	4·0	0·6	1·0
4*	0·15	0·07	0·22	0·6	0·1	0·2
10*	0·10	0·02	0·12	0·4	0·1	0·2
Nil	0·7	—	—	2·4	0·5	1·1
10*	0·15	—	—	0·6	0·1	0·2
Nil	2·0	1·2	3·2	3·3	0·4	0·9

* Pretreated 30 min in CCCP. † Data of Figures 1(*a*) and 1(*b*).

Measurements of total and tracer chloride transport were made by the method referred to above (p. 245) and are set out in the following tabulation:

	10 mM KCl	10 mM KCl +10 μM CCCP	Inhibition (%)
Total chloride transport (μ-equiv g^{-1} hr^{-1})	4·7±0·2	<0·2	>96
^{36}Cl transport	4·1±0·2	<0·05	>98
^{36}Cl accumulation	2·1±0·2	0·2±0·02	91

The results show strong inhibition of total chloride transport and of tracer chloride transport and accumulation. A limitation of the method of measuring net transport is that its accuracy depends upon the level of chloride that can be measured from the collecting chamber. In these experiments the experimental limit of chloride was 0·2 μ-equiv g^{-1} hr^{-1}, but this was sufficient to show strong inhibition of total transport of chloride.

Addition of CCCP at the same time as tracer, or even beforehand, does not test the speed of action on the transport process since there is a lag in establishment of a new rate of tracer transport due to changes in specific activity within the root. A better test of the effectiveness of CCCP is to add the inhibitor to roots in which a

steady rate of tracer transport has already been established. Figure 2 shows the results of such an experiment. Roots were set up in the apparatus described above and measurements made after 60 min to determine the steady, uninhibited rate of tracer transport. After 2 hr CCCP was added to bring the concentration to 10 μM in the labelling chamber. Although inhibition of uptake was instantaneous [Fig. 1(*b*)], there was a lag of about 30 min before tracer transport fell to a low level, about 10–15% of the uninhibited rate. Separate measurements using roots from the same batch showed that accumulation was inhibited from 2·6 to 0·15 μ-equiv g^{-1} hr^{-1}.

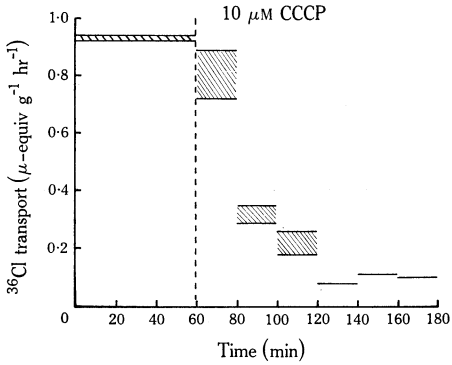


Fig. 2.—Effect of 10 μM CCCP on rate of transport of ^{36}Cl from labelled 10 mM KCl solution. Note lag before full inhibition is achieved. Duplicate samples.

The amount of tracer exuding from the root after addition of CCCP can be estimated from Figure 2 as 0·3 μ-equiv g^{-1} hr^{-1}. Part of this tracer must have been that already in the xylem when CCCP was added. In a previous paper (Pitman 1971) it was found that the apparent rate of flow was 18 cm hr^{-1}; for roots 4 cm long in which tracer transport was 1·0 μ-equiv g^{-1} hr^{-1}, the amount in the xylem can be estimated at 0·2 μ-equiv g^{-1}. It can be concluded that most, if not all, of the tracer exuding from the root after addition of CCCP was already in the xylem, and that CCCP acted rapidly on transport.

An estimate of this amount is important in comparing the two models. On the one-pump model, transport is due to the high electrochemical activity of ions in the symplast, allowing the ions to diffuse into the xylem at a lower electrochemical activity. This high electrochemical activity in the symplast is maintained by uptake at the outside of the root and is buffered by the large capacity of the vacuoles. The small lag measured in Figure 2 implies that only a small amount may be lost from the symplast before the electrochemical activity falls to such a level that transport is inhibited. The explanation of the small lag on the two-pump model is simpler, as both pumps would be stopped by CCCP, and rapidity of action depends on diffusion of CCCP to the site of action.

It is clear from comparison of total and tracer chloride transport that much of the chloride is derived from the vacuoles, being replaced by chloride taken up from solution. The contribution of the vacuoles to the transport process can be studied directly by using roots with labelled chloride predominantly in the vacuoles.

Roots were brought to high-salt status using 10 mM KCl labelled with ^{36}Cl and were then put in frequent changes of unlabelled 10 mM KCl for a total of 2·4 hr. As a

result of this pretreatment, the specific activity was high in the vacuoles but low in the free space and cytoplasmic phase (Pitman 1971). These roots were then set up in the apparatus to measure efflux from the cut end separate from that from the surface of the root as referred to above.

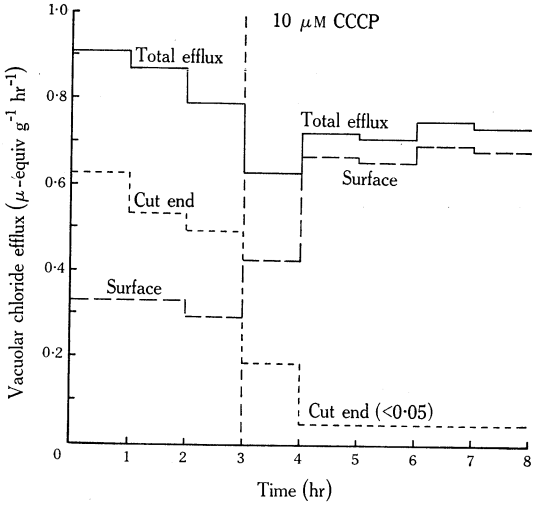


Fig. 3.—Efflux of ^{36}Cl from high-salt barley roots and effect of 10 μM CCCP. Total efflux, efflux from cut end, and efflux from surface are shown.

Figure 3 shows that addition of 10 μM CCCP had little effect on the loss of tracer from the root as a whole. This lack of action of CCCP on a total efflux was also found in other experiments, and for potassium and sodium as well as for chloride. For roots in a solution of 7·5 mM KCl+2·5 mM KCl the results were:

Ion	Temperature (°C)	Efflux (μ-equiv g^{-1} hr^{-1})	Efflux in CCCP (μ-equiv g^{-1} hr^{-1})
Cl	9·0	0·7	0·75
	25·0	1·6	1·85
	12·5	0·8	0·8
K	20·0	1·8	2·1
Na	20·0	0·25	0·25

Tracer efflux from the root cells is not ϕ_{vc}, but $\phi_{vc}(\phi_{co}+R')/(\phi_{co}+\phi_{cv}+R')$. Similarly tracer accumulation is not ϕ_{cv}, but $\phi_{cv}(\phi_{co}+R')/(\phi_{co}+\phi_{cv}+R')$. It appears that tracer accumulation underestimates ϕ_{cv} by about 20% (Table 1), so the tracer efflux underestimates ϕ_{vc} by the same amount. An increase in tracer efflux of about 20% could be found if CCCP inhibited ϕ_{cv} and R', though in practice any increase between 0 and 20% could be accounted for in this way since the inhibition may not be complete.

Though not affecting loss of ions from the cells, CCCP strongly inhibited transport from vacuoles to the stele by at least 90–95% and there was a complementary rise in diffusion of tracer through the surface of the root to the solution (Fig. 3). The action of CCCP blocked the pathway through the stele and switched movement of tracer into the alternative pathway through the cortex.

Such a result would be explained simply if there were two pumps involved. The explanation on the one-pump hypothesis is more complicated. It would be necessary for inhibition of entry to the symplast to inhibit further transport out of the symplast and hence block that pathway for tracer from the cell vacuoles.

Another way of reducing chloride entry to the root is to reduce the external concentration to a very low value. For example, in 10 mM KCl the rate of uptake was $2 \cdot 6$ μ-equiv g^{-1} hr^{-1}; in $0 \cdot 2$ mM KCl it was $1 \cdot 1$ μ-equiv g^{-1} hr^{-1}. The rate of uptake in $0 \cdot 5$ mM $CaSO_4$ alone was difficult to estimate but the maximum chloride concentration in the solution due to loss from the roots was not more than 5 μM. At this concentration separate measurements showed the rate of uptake was $0 \cdot 25$ μ-equiv g^{-1} hr^{-1}.

Previous experiments showed that net transport of chloride is reduced by transfer from 10 to $0 \cdot 5$ mM KCl (Pitman 1971). Figure 4 shows the effect on tracer

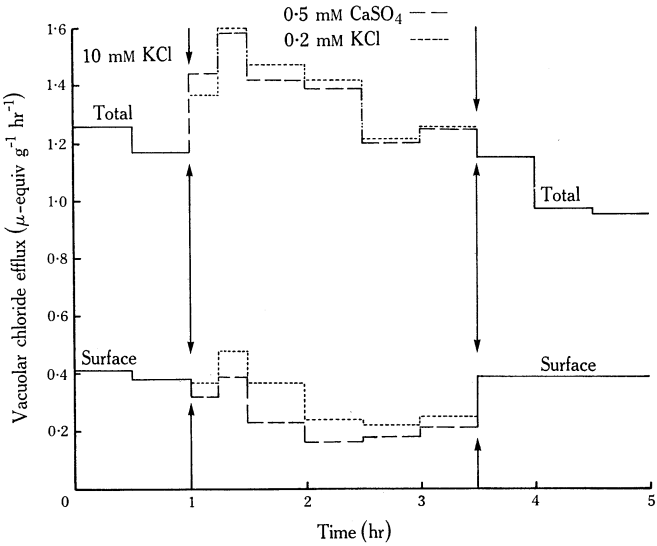


Fig. 4.—Effect of changing solution from 10 mM KCl to $0 \cdot 2$ mM KCl or $0 \cdot 5$ mM $CaSO_4$ alone (zero KCl) on efflux of ^{36}Cl. The efflux from the cut end is the difference between total efflux and that from the surface.

efflux of changing the external solution from 10 to $0 \cdot 2$ mM KCl or to $0 \cdot 5$ mM $CaSO_4$ alone. There was a small increase in total efflux which appeared to be a transient effect, but no inhibition of tracer transport from the stele despite reduction in uptake. The percentage of vacuolar tracer passing to solution through the cortex fell from 33% in 10 mM KCl to 15% in $0 \cdot 5$ mM $CaSO_4$ (zero KCl) and 19% in $0 \cdot 2$ mM KCl. The efflux persisted for at least $2 \cdot 5$ hr during which at least $3 \cdot 0$ μ-equiv g^{-1} were transported from the stele. In contrast, transport of tracer was reduced to 10% within 60 min in CCCP and only $0 \cdot 1$ μ-equiv g^{-1} transported after the inhibitor was added (Fig. 2).

If there were only one step involved in passage across the root, i.e. at the outer surface of the symplasm, inhibition of uptake to the cells should have the same effect

on transport from the stele irrespective of how it were brought about. It would be expected that transport would continue until the level in the root had decreased so much that its electrochemical potential could no longer drive transport into the stele, and that there would be the same "lag" in reduction in transport. The difference in behaviour in low-salt and in CCCP is taken to show the need for a second active step in the transport process.

It might be argued that in solutions of very low chloride concentration there is no inhibition of uptake, but that chloride diffusing into the free space is reabsorbed before it can pass into the solutions. However, in CCCP, inhibition of uptake prevents reabsorption and tracer can then diffuse out to the solution. To test this possible criticism, measurements were made of free space exchange at low temperature (2°C) when uptake was low, and at 25°C when uptake was about 10 times larger. Figure 5

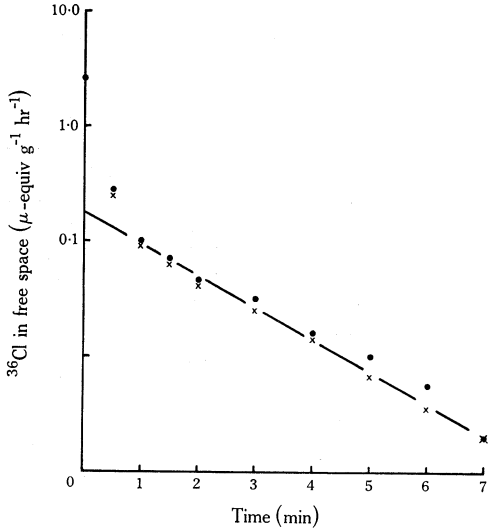


Fig. 5.—Exchange of ^{36}Cl from labelled roots with 10 mM KCl at 25°C (●) and 2°C (×). The cytoplasmic component has been deducted. Total free space content was 2·8 μ-equiv g^{-1}.

shows that there was no detectable difference in the free space exchange. If reabsorption were important there should have been less tracer diffusing from the slower component of the free space at the higher temperature than at the lower temperature.

These experiments have all been carried out using excised roots, and differ from reported experiments using whole plants in the degree of inhibition of uptake. Thus Lüttge and Laties (1967) showed that while tracer transport was inhibited almost completely at low concentrations of KCl (0·2 mM), inhibition by 1·0 μM CCCP in solutions of 20–40 mM was only 30%. Greenway (1965) has also shown that there is a relatively large component of uptake to whole barley seedlings that was insensitive to CCCP and dinitrophenol. This component was also found to be independent of transpiration over a wide range, but at higher rates of water transport than found in the present experiments.

Table 3 shows investigations of the effect of CCCP on chloride uptake to whole plants, in order to show the extent to which the present experiments with excised roots were comparable with the behaviour of the whole plant.

Low-salt plants were transferred to 10 mM KCl+0·5 mM $CaSO_4$; after 5·5 hr some plants were sampled and the rest transferred to solutions of the same salt concentration but varied CCCP concentration. Plants were sampled again 17 hr later and chloride content measured. At the first harvest the shoots contained 1·20 μ-equiv per plant and the roots 1·85 μ-equiv per plant. The plants weighed 150 mg and shoot to root ratio was 3·0.

Net uptake to the plants fell to zero between 9 and 6 μM CCCP but transport to the shoot fell to only 30% of the uninhibited rate and was not completely inhibited at any level of CCCP. As a result of inhibition of accumulation there was a net loss from the roots in 6 μM CCCP. Much of the chloride lost from the root cells was transported to the shoot.

TABLE 3

EFFECT OF CCCP ON UPTAKE, TRANSPORT, AND ACCUMULATION OF CHLORIDE AND ^{36}Cl

All rates expressed as μmoles g^{-1} root hr^{-1}

CCCP (μM)	Net chloride			Tracer ^{36}Cl		
	Uptake	Transport	Accumulation	Uptake	Transport	Accumulation
0	4·7	3·4	1·4	3·8	1·7	2·1
1	5·1	3·9	1·2	2·4	1·3	1·1
3	3·5	2·8	0·7	1·8	1·0	0·8
6	0·5	1·0	−0·5	0·9	0·6	0·3
9	−0·2	1·0	−1·2	0·6	0·5	0·1
12	—	—	—	0·6	0·5	0·1

At the end of the uptake period some plants were put into solutions labelled with ^{36}Cl and tracer uptake, transport and accumulation determined over a 3-hr period. Accumulation and transport are not linear processes so the average over 3 hr will underestimate the rate of transport and overestimate accumulation but only by about 10% (see Fig. 1). Despite this limitation this procedure gives a useful measure of the effect of CCCP.

Accumulation of ^{36}Cl was inhibited nearly 50% in 1 μM CCCP and 90–95% in 9 μM CCCP. Transport of ^{36}Cl was not as strongly inhibited and even in 9 μM CCCP the inhibition was only 70%.

This comparison of the effect of CCCP on whole plants and excised roots shows that transport can be strongly inhibited in excised roots but not in whole plants. A major difference between whole plants and excised roots is the rate of water flow. Each whole plant transpired at about 30 mg hr^{-1} (2·1 mg cm^{-1} root hr^{-1}) or 6 mg hr^{-1} per root; in CCCP this rate was reduced by about 50%, but was still 3·0 mg hr^{-1} per root. Exudation from each excised root was measured at 0·35 mg hr^{-1}, falling to less than 0·1 mg hr^{-1} in CCCP, i.e. less than 3% of the rate of water movement in the whole plant.

Net chloride transport from the roots of whole plants was about 3·6 μ-equiv g^{-1} hr^{-1} falling to about 1·0 μ-equiv g^{-1} hr^{-1}. These rates are equivalent to 0·13 and

0·037 μ-equiv hr^{-1} per plant respectively and the concentrations of chloride in the xylem were 4·3 and 2·5 mM. Concentrations in the xylem of excised roots are about 10 times larger and represent the osmotic pressure needed to draw water through the roots. When exudation per plant was 1·8 mg hr^{-1} the chloride transport was 0·095 μ-equiv hr^{-1}, equivalent to a concentration of 52 mM. When active transport into the stele is inhibited by CCCP the rate of exudation falls as the concentration can no longer be maintained. It is suggested that there is an appreciable passive flux of chloride into the xylem (about 1·0 μ-equiv g^{-1} hr^{-1}) and when transpiration provides a water flow this passive flux becomes equivalent to the net flux into the xylem. When water flux is low, the internal high concentration leads to an appreciable efflux so that net chloride flux is now small. This difference in behaviour between whole plants and excised roots is considered not to affect the arguments presented in favour of the two-pump hypothesis.

IV. Conclusions

For many purposes it is adequate to treat transport of ions across the root as a one-pump hypothesis, but it is considered from the results presented in this paper that there is evidence that transport to the xylem involves a second active step. The arguments put forward may be summarized as follows:

(1) The inhibitor CCCP strongly reduces the fluxes ϕ_{oc} and ϕ_{cv}. This view is supported by other measurements on ion uptake to barley roots (Pitman, Courtice, and Lee 1968; Cram and Laties 1971).

(2) Though inhibiting ϕ_{oc} and ϕ_{cv}, CCCP does not reduce total tracer efflux from the root cells, supporting the view that ϕ_{vc} and ϕ_{co} are passive fluxes, at least for chloride, and that CCCP has no drastic effect on the membrane permeability. It would be unlikely for the fluxes of both cations and anions to be so little changed if the lack of effect were due to changes in cell potential compensating exactly for a change in membrane permeability.

(3) CCCP inhibits ϕ_{cx} rapidly and effectively. The apparent lag in effect of CCCP (Fig. 2) is largely due to the need to empty the xylem vessels before any inhibition is seen. The rate of loss of tracer would be expected to fall exponentially, as the rate of exudation appears to be determined by concentration in the xylem and the water conductivity of the roots (Anderson, Aikman, and Meiri 1970).

(4) Reduction in ϕ_{oc} when roots are in $CaSO_4$ alone does not inhibit loss of tracer from the root *via* the stele. A large and continuing net transport from the root took place after transfer of roots from 10 to 0·2 mM KCl or to $CaSO_4$ alone.

(5) The switch in diffusion path from stele to cortex when roots were put into CCCP (Fig. 3) cannot be reconciled with the continuing transport from the stele in solutions of low concentration (Fig. 4). This behaviour could be explained simply on the two-pump model, when ϕ_{cx} would be active and so inhibited by CCCP.

(6) Diffusion in the free space does not appear to be a major barrier to movement of ions from the root (Fig. 5). The rate constant for exchange of the slower free space component was $k = 34\ s^{-1}$ compared with $k = 19\ s^{-1}$ found for sodium and potassium (Pitman 1965*c*).

In discussion of these results a model has been used of a "symplast". In the original symplast theory* entry occurred at the outer surface of the root and transport occurred across the root in the symplast. Measurements of free space exchange show that the root cells in the cortex are relatively accessible to ions by diffusion in the cell walls (Pitman 1965*c*). In some circumstances, especially when external concentration is high, the outer boundary of the symplast may be the collective surface area of the cells of the cortex. When solution concentration is very low, the effective area of entry may have to be at the surface of the root. For this reason symplast has been used loosely to refer to the protoplasmic continuum in a set of cortical and stelar cells of indeterminate number acting as a pathway for entry to the xylem. In some circumstances this set may include all the cells in a sector of the root; in other cases it may be restricted to cells at the endodermis and in the stele, or even to stelar parenchyma alone. The set may be a linear file along a radius, or it may be a meandering pathway as seen in movements of dye across the root.

The strongest evidence against active transport being involved in passage from symplast to stele comes from the potential measurements of Dunlop and Bowling (1971*a*, 1971*b*, 1971*c*). Their results show that both potassium and chloride move to lower electrochemical activity in passing into the xylem, and that there is no earlier stage at which one ion appears to be actively transported apart from the outer cell membrane. However, potential measurements are only useful as an indication of active transport if the membrane is relatively impermeable to the ion transported and if there are large concentration differences involved. In some situations active transport may not result in a large contribution of potential, and under these conditions effects of inhibitors may be a more sensitive test of a metabolically dependent transport. Thus large inhibition of transport of potassium and chloride in *Chaetomorpha darwinii* can be brought about by CCCP, but with little effect on cell potential (Findlay *et al.* 1971).

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