UPTAKE AND TRANSPORT OF IONS IN BARLEY SEEDLINGS

I. ESTIMATION OF CHLORIDE FLUXES IN CELLS OF EXCISED ROOTS

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Abstract

A comparison is made of transport of chloride through roots of barley seedlings, and the fluxes of chloride into the cells of the root estimated from efflux analysis. About 75% of tracer diffusing out of the cells in the root passes to the solution through the stele, but despite this complication it is considered that fluxes across plasmalemma and tonoplast could be estimated. It is shown that a model based on the symplasm theory can relate the specific activity of chloride transported to the shoot with the specific activity of a cytoplasmic phase calculated from the fluxes in the root cells.

I. INTRODUCTION

Excised roots of barley seedlings have been used in various ways to study processes of salt transport at the cellular level. Kinetic studies of low-salt roots have been used as the basis for the hypothesis that there are two mechanisms of uptake, one dominant at low concentrations (below 0·5 mM) and the other at higher concentrations (e.g. 10 mM) (Epstein 1966). High-salt roots have been used to estimate fluxes at the plasmalemma and tonoplast, and for measurements of tracer uptake. From these kinds of experiments it has been suggested that there is selective transport of potassium relative to sodium at the plasmalemma, and that there is active transport of both potassium and chloride at the tonoplast (Pitman and Saddler 1967; Pitman, Courtice, and Lee 1968). Bean roots and bean root segments have also been used for flux measurements based on tracer exchange (Scott, Gulline, and Pallaghy 1968; Pallaghy and Scott 1969).

However, the root is more than a collection of cells. In many ways it behaves as an organ secreting salt to the shoot. In contrast to the continuing rise in rate of uptake with concentration found in studies with low-salt roots, transport to the shoots of barley seedlings growing in culture solution is remarkably unaffected by external potassium chloride [or (K+Na)Cl] concentrations (Pitman 1965*a*; Johansen, Edwards, and Loneragan 1968). Instead, transport in high-salt plants (i.e. net increments of ions) is limited mainly by the metabolic status of the plant, and is correlated with relative growth rate. It is severely reduced by a period in the dark or by the addition of respiratory inhibitors (Greenway 1965; Pitman 1965*a*).

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A large transport of chloride or potassium through excised roots to the xylem vessels could be a potential source of error or misinterpretation (cf. Greenway 1967). For many cases—as in studies of uptake to low-salt roots—this criticism probably is not relevant. However, estimation of fluxes in root cells from tracer exchange of excised roots could include serious errors if transport is ignored.

One purpose of this paper is to investigate the effect of the transport process on flux estimation. Another is to attempt to relate fluxes estimated from tracer exchange with transport to the shoot. In this way some of the work with excised roots can be related to transport in the whole plant. Two levels of potassium chloride were selected for study; a low concentration (0·5 mM) in which mechanism I should be dominant, and a higher concentration in which mechanism II is operating (10 mM).

II. Materials and Methods

(a) *Terms Used*

To avoid confusion over terms, "transport" is used to mean the amount passing from root to shoot (or from the cut end of the root back into solution when using excised roots). "Accumulation" means the amount retained in root cells, but excluding free space content. "Uptake" is defined as the sum of transport and accumulation. All these quantities are expressed relative to root weight.

(b) *Plant Material*

Seeds of barley (*Hordeum vulgare* cv. Cape) were germinated on blotting paper and then planted on stainless steel gauze in 0·5 mM $CaSO_4$ solution and grown in the dark at 25°C. When used after 7 days from germination the major root was about 10 cm long and the other roots about 6 cm. No lateral roots had emerged. Plants and excised roots were pretreated for 24 hr in 10 mM KCl. Figure 1 shows the change in chloride levels in low-salt plants when put into 10 mM

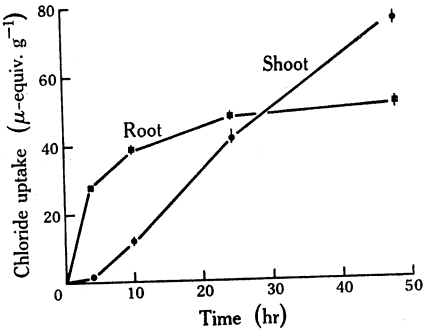

Fig. 1.—Time course of uptake of chloride to roots and shoots of 7-day-old, low-salt, barley seedlings.

KCl. After 24 hr in solution there was zero net accumulation, but steady rate of transport from the roots. This was a convenient stage for measurement of transport and for estimation of fluxes. An added advantage of starting with low-salt roots in this way was that transport and accumulation took place at the expense of the high levels of sugar in root and shoot at the start of the experiments. Unlike high-salt plants, salt uptake by and transport in these plants were not dependent on relative growth rate.

(c) *Solutions*

All solutions contained 0·5 mM $CaSO_4$ in addition to KCl at the concentration given.

(d) *Transport Experiments*

The apparatus of Figure 2(*a*) was used to measure transport of chloride and ^{36}Cl through excised roots. Roots pretreated for 24 hr in 10 mM KCl (as in Fig. 1) were set up horizontally in the

apparatus with the cut ends in the collecting chamber (7·5 ml) and the tips in the labelling chamber (25 ml). Between the labelling solution and the collecting chamber was a guard chamber 3 mm wide (2·5 ml). The roots were sealed into the partitions with a silicone grease. The concentration of tracer was measured in the guard chamber at the end of the experiment to establish the adequacy of the seal. Solutions were stirred with a stream of air passed in through hypodermic needles.

Measurements of ^{36}Cl transport were made by sampling all the solution in the chamber and replacing it with unlabelled solution. The sample of solution was dried on a 5-cm planchet. For measurements of chloride transport the potassium chloride solution in the collecting and guard chambers was replaced with potassium nitrate of the same concentration. Again, the guard chamber was sampled at the end of the experiment to test that no chloride diffused through the seal. After measuring the ^{36}Cl on the planchet. chloride was taken up with distilled water and estimated by coulombic titration. Recovery was effectively 100% and in this way both labelled and unlabelled chloride were measured in the same sample.

Amounts of ^{36}Cl are expressed as microequivalents and estimated by dividing the total number of counts by the specific activity (counts per microequivalent) of the labelling solution.

The rates of transport of tracer and of unlabelled chloride relative to root weight were calculated from the amount of ^{36}Cl (Q^*) and chloride (Q) appearing in the collecting chamber. Transport of tracer was calculated relative to the weight of root in the labelled solution (a) and was taken as Q^*/a.

Calculation of the rate of chloride transport was complicated because part of the root was in KCl (a) and part in KNO_3 (b). Transport of chloride from roots in KCl is made up of part derived from the solution and part derived from the vacuole. Tracer transport as measured in this experiment tends to measure the chloride transport from the solution. Transport of chloride from roots in KNO_3 is restricted to that from the vacuoles. Tests showed that tracer lost from the vacuoles was the same to KCl as to KNO_3 solution. The amount Q is therefore made up of transport from vacuoles from a weight b, and transport from solution plus vacuoles from weight a. Rate of loss was calculated relative to root weight as $Q+Q^*(b/a)/(a+b)$, Q^*/a being an estimate of the transport from the solution.

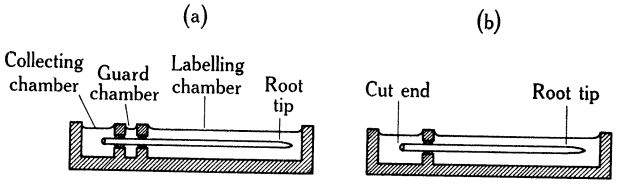

Fig. 2.—Apparatus used for transport (a) and efflux (b) measurements. See text for description.

(e) Flux Determinations

The apparatus in Figure 2(b) was used to measure tracer loss from roots to unlabelled solutions. The excised roots were prepared as for transport studies, but in labelled KCl. At the end of 24 hr the roots differed from those in unlabelled KCl only with respect to the labelling. As chloride in the roots was low initially the specific activity was effectively the same throughout the root as in the labelling solution.

In experiments at concentrations other than 10 mM, the roots were pretreated for 1 hr in a solution at the same specific activity as the labelling solution but of appropriate concentration. This procedure was intended to allow free space and fluxes into the cell to equilibrate with the new concentration, but without changing the specific activity. The roots were then blotted lightly and set up with the cut end in the smaller chamber (7·5 ml). The volume in the other chamber was about 25 ml and barrier was sealed as before. Both chambers were stirred by aeration.

At intervals the solution in each chamber was sampled and replaced by unlabelled solution. The whole sample was dried on a 5-cm planchet for counting. At the end of the experiment the roots were cut along the boundary and each part weighed. The two samples were then combined and extracted with boiling distilled water for chloride and ^{36}Cl determination. All experiments were at room temperature which was constant during an experiment. Room temperature was between 19 and 21°C.

An estimate of rate of loss from the surface was obtained from tracer diffusing into the other chamber. Tracer diffusing into the other chamber came from both surface and cut end; using the estimate of loss from the surface, the loss from the cut end was calculated.

Tracer loss from the roots is expressed relative to the total weight of root. The values used in flux calculation were extrapolates to the start of the elution when all chloride was at the same high specific activity.

(*f*) *Experiments with Intact Plants*

Rates of transport from roots of intact plants were estimated from changes in content of the shoots over a period of about 24 hr (see Fig. 1). Rates of tracer accumulation and transport were estimated from the amount of ^{36}Cl in roots or shoots after 60–90 min in labelled solution. Allowance was made for the initial lag in uptake to the shoot (see Fig. 4). The relative growth rates were low (about 0·06 day^{-1}) and rates of water flow were about 5 mg cm^{-2} hr^{-1} relative to the root surface.

III. Results

(*a*) *Transport Measurements*

Rates of net chloride and ^{36}Cl transport from excised roots were determined using the apparatus of Figure 2(*a*). After tracer was added to the labelling compartment there was a lag before it could be detected in the solution, but then the rate of tracer transport increased to a maximum that stayed steady for at least 3 hr [Fig. 3(*a*)].

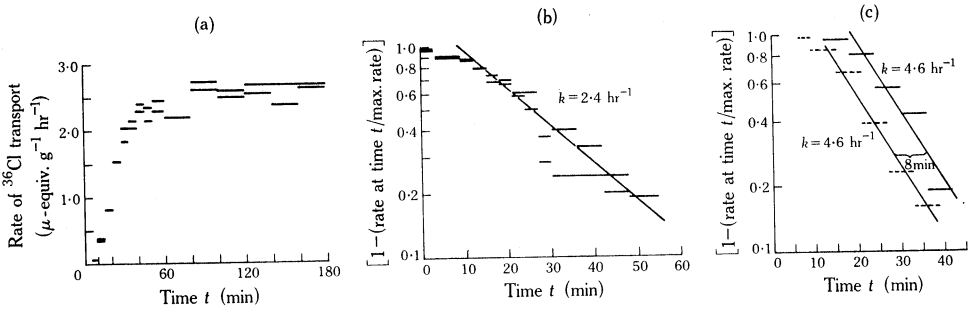

Fig. 3.—(*a*) Rate of ^{36}Cl transport through excised high-salt roots. Label was added at $t = 0$. (*b*) Rate of ^{36}Cl transport from roots 3 cm long plotted logarithmically. Duplicate samples. Different experiment from (*a*). (*c*) Logarithmic plot of rate of ^{36}Cl transport as in (*b*). Roots 7 cm long labelled either over terminal 75% (– – –) or terminal 40% (——). See text for details.

The establishment of the steady rate of transport increased as a function of $(1-e^{-kt})$. Figure 3(*b*) shows the rate of transport from roots 3 cm long plotted in the form $\log_{10}$[1−(rate at time t/maximum rate)]. After the initial lag of 9 min the graph became linear; the slope of the line gave an estimate of k as 2·4 hr^{-1} (half-time = 17 min).

Figure 3(*c*) shows similar graphs for roots 7 cm long labelled over different lengths of the root; two sets were labelled over the tip 2·8 cm (40% of the root), the other two sets were labelled over 75% of the root (the rest of the root was in unlabelled KCl). As might be expected the lag was larger when tracer had to travel 2·4 cm further to the collecting chamber. The slope of each line gave $k = 4·6$ hr^{-1}

(half-time = 9 min). The lag between the curves was equivalent to a velocity of flow of 18 cm hr^{-1} (2·4 cm per 8 min). It is suggested that the rate constant measured in these experiments is determined by the rate constant for tracer exchange in the cytoplasm, but this point will be considered again later.

Values for the steady rates of ^{36}Cl and of net chloride transport after 24 hr in salt solution are given in Table 1(*a*). Values for roots that had been in salt solution 72 hr are given in Table 1(*b*).

In comparison with these values, the rate of chloride transport from roots of intact plants that had been in salt solution 24 hr ranged from 3·5 to 4·3 μ-equiv. g^{-1} hr^{-1} with an average of 4·0 μ-equiv. g^{-1} hr^{-1} when the concentration was 10 mM KCl. Transport from plants in 0·5 mM KCl ranged from 1·5 to 3·0 μ-equiv. g^{-1} hr^{-1} with an average value of 2·5 μ-equiv. g^{-1} hr^{-1}. Where comparisons were made at the same time on whole plants and excised roots the transport from the excised root was usually about 20% larger than from roots of whole plants.

TABLE 1

STEADY RATES OF TRANSPORT OF ^{36}Cl AND OF CHLORIDE FROM THE CUT END OF EXCISED ROOTS*

In (*a*), each value is the mean ± the standard error of the mean of a number of separate determinations (given in parenthesis). The roots had been in 10 mM KCl for 24 hr before measurements were made. In (*b*), duplicate values are given for measurements made after roots had been in 10 mM KCl for 72 hr. The rate of tracer accumulation is also given

Concn. of KCl Solution (mM)	Transport from Cut End (μ-equiv. g^{-1} hr^{-1})		Tracer Accumulation (μ-equiv. g^{-1} hr^{-1})
	^{36}Cl	Total Chloride	
	(*a*) Roots in salt solution 24 hr		
0·5	1·6±0·1 (6)	2·6±0·2 (6)	0·9±0·1 (6)
10·0	2·7±0·1 (4)	4·9±0·1 (4)	1·9±0·2 (4)
	(*b*) Roots in salt solution 72 hr		
0·5	0·15, 0·25	0·7, 0·8	0·5, 0·6
10·0	0·5, 0·9	1·3, 1·9	1·5, 1·6

* See Figure 2(*a*).

The difference in rate of transport from excised roots and in whole plants is suggested to be due to differences in rate of transport into the xylem along the length of the root. The excised roots were only the terminal 3 cm of roots 6–10 cm long, and the 3-cm lengths were more active (relative to weight) than 6-cm lengths. For example, the rate of ^{36}Cl accumulation to the terminal 3 cm of root in Figure 3(*c*) was 2·8 μ-equiv. g^{-1} hr^{-1} but to the next 2·4 cm it was only 1·4 μ-equiv. g^{-1} hr^{-1}, giving an average for the 5·4 cm length of root of 2·2 μ-equiv. g^{-1} hr^{-1}. In another experiment rates of both accumulation and transport of tracer by the terminal 3 cm were 40–60% larger than by the next 3 cm of root.

Figure 4 shows the time course of tracer accumulation and transport in intact plants. Accumulation was linear but there was a lag of about 15 min in establishment of transport. In general, the rate of tracer transport was 40–60% of the net chloride transport. Accumulation of tracer in roots of intact plants was not found to be different from that in excised roots.

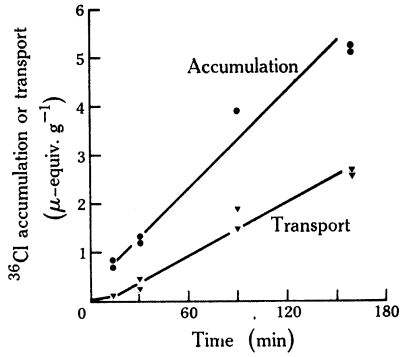

Fig. 4.—Tracer accumulation in roots and transport to shoot in high-salt plants put into labelled solution after 24 hr in 10 mM KCl. The net chloride transport was 3·4 μ-equiv. g^{-1} hr^{-1}. Tracer accumulation was 2·0 μ-equiv. g^{-1} hr^{-1} and tracer transport 1·10 μ-equiv. g^{-1} hr^{-1}.

Table 2 gives values for rates of tracer transport and accumulation in intact plants after different periods in salt solution. When low-salt plants are first put in KCl solution the rate of transport is small (cf. Fig. 1) due to competition between the processes of accumulation and transport. In the experiments described in this paper the plants had very low relative growth rates (0·06 day^{-1}) and after 48 hr in salt solution the high endogenous levels of sugar of the low-salt plants had been depleted.

TABLE 2

RATES OF UPTAKE, ACCUMULATION, AND TRANSPORT OF ^{36}Cl IN WHOLE PLANTS AFTER DIFFERENT PERIODS IN POTASSIUM CHLORIDE SOLUTION

Plants were of low-salt status at zero time, and were immersed in 10 mM KCl solution for the periods shown. The total chloride transport at 20 hr was 2·5 μ-equiv. g^{-1} hr^{-1} (relative to roots)

Time in Solution (hr)	Chloride Levels (μ-equiv. g^{-1})		^{36}Cl Rates (μ-equiv. g^{-1} hr^{-1})		
	Shoot	Root	Uptake	Transport	Accumulation
0	0·7	0·2	6·7	0·2	6·5
20	38	49	5·0	1·8	3·2
44	113	61	3·5	0·8	2·7

The low rates of chloride uptake and transport after 24 hr in salt solution are then reflections of the low metabolic status of the plants. For the same reason rates of transport were lower for excised roots from plants treated 72 hr [Table 1(*b*)] than from plants treated only 24 hr in salt solution [Table 1(*a*)].

The ratio of ^{36}Cl to total chloride transported from excised roots was 0·61 in 0·5 mM and 0·55 in 10 mM KCl. Table 3 gives a number of estimates of ^{36}Cl and total

chloride transport made with intact plants. The specific activity of chloride transport was generally lower than from excised roots. This difference is again probably due to non-uniformity along the root.

In all these comparisons the difference between tracer and total chloride transport must be due to chloride derived from root cells, or more particularly from the vacuoles of the cells. The vacuolar contribution can be estimated in another way by loading the roots with labelled chloride and following the rate at which tracer is lost from the cut end of the root, using the apparatus of Figure 2(*b*). This approach can also be used to estimate fluxes into and out of the root cells.

TABLE 3

NET CHLORIDE TRANSPORT AND RATE OF TRACER TRANSPORT IN WHOLE PLANTS AND RATE OF TRACER ACCUMULATION

Concn. of KCl Solution (mM)	Total Chloride Transport (μ-equiv. g^{-1} hr^{-1})	^{36}Cl Transport (μ-equiv. g^{-1} hr^{-1})	^{36}Cl Accumulation (μ-equiv. g^{-1} hr^{-1})	Specific Activity of Transport
0·5	1·6	0·4	1·0	0·25
	2·9	0·7	1·7	0·24
10·0	1·8	0·7	2·1	0·39
	2·6	1·8	3·2	0·70
	2·8	1·35	1·7	0·48
	3·3	1·2	1·9	0·36
	3·4	1·1	2·0	0·32
	4·2	1·3	3·1	0·31

(*b*) *Calculation of Fluxes in Root Cells*

Fluxes can be calculated from the kinetics of tracer exchange with plant cells. This approach has been used for giant algal cells (MacRobbie and Dainty 1958; Hope 1963; Dodd, Pitman, and West 1966); for homogeneous plant tissues such as beet (Pitman 1963) and carrot (Cram 1968); and for some roots (Pitman and Saddler 1967; Pallaghy and Scott 1969). It is assumed in calculating fluxes in this way that the cells can be treated as three phases in series: free space, cytoplasm, and vacuole. In the above papers the procedure is described for estimating fluxes from analysis of the tracer efflux.

If this procedure were to be followed for excised barley roots the efflux of tracer from the root as a whole in Figure 5(*a*) would be divided into the following compartments on the basis of their rate constants:

(1) Free space 2·2 μ-equiv. g^{-1}; rate constant $k_f = 18{\cdot}1$ hr^{-1} (half-time = 2·3 min).

(2) Cytoplasmic component 1·2 μ-equiv. g^{-1}; rate constant $k_c = 3{\cdot}6$ hr^{-1} (half-time = 11·5 min). Initial flux out = 4·3 μ-equiv. g^{-1} hr^{-1}.

(3) Vacuolar component 65 μ-equiv. g^{-1}; rate constant $k_v = 0{\cdot}02$ hr^{-1}. Steady efflux = 1·3 μ-equiv. g^{-1} hr^{-1}.

In the present experiments calculation of the cytoplasmic content Q_c, and the fluxes ϕ_{oc}, ϕ_{co}, ϕ_{cv}, ϕ_{vc} (subscripts o, c, and v refer to solution, cytoplasmic phase, and vacuoles respectively) is simplified by two factors. First, the specific activity was uniform in the tissue at the start of the experiment and the same as in the labelling solution ($s_c = s_v = s_o = 1$). Second, net uptake was zero, so $\phi_{oc} = \phi_{co}$ and $\phi_{vc} = \phi_{cv}$; this condition could be tested by showing that net tracer accumulation was the same as net tracer efflux from the vacuoles of labelled roots.

Under these conditions the slow efflux extrapolated to zero time was $\phi_{vc}.\phi_{co}/(\phi_{co}+\phi_{cv})$. The total efflux from the non-free space at zero time was ϕ_{co}, which could be estimated as the sum of the slow efflux and the efflux at zero time from the cytoplasmic component [see Figs. 5(a) and 5(b)]. From these two values $\phi_{cv}(=\phi_{vc})$ could be caluclated. The slope of the exchange of the cytoplasmic component gave the rate constant $k_c = (\phi_{co}+\phi_{cv})/Q_c$, from which Q_c could be calculated.

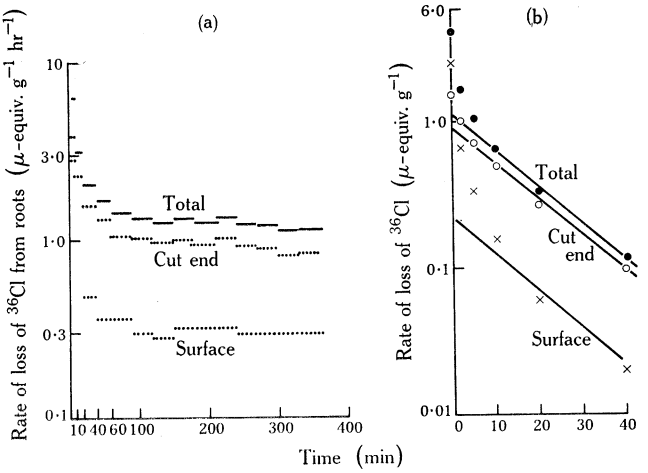

Fig. 5.—(a) Rate of loss of ^{36}Cl from the root to unlabelled 10 mM KCl solution. The amount lost through the cut end and through the surface was estimated as described in the text. (b) Rate of loss of tracer from the cytoplasmic phase in (a) shown as the contribution from the cut end and from the surface of the root to the total loss to the solution.

Using the results of Figure 5(a) it can be calculated that $\phi_{oc} = \phi_{co} = 4{\cdot}3+1{\cdot}3 = 5{\cdot}6$ μ-equiv. g^{-1} hr^{-1}. Also $[\phi_{vc}.\phi_{co}/(\phi_{co}+\phi_{vc})] = 1{\cdot}13$ μ-equiv. g^{-1} hr^{-1}. Therefore ϕ_{vc} $(= \phi_{cv}) = 1{\cdot}7$ μ-equiv. g^{-1} hr^{-1} and $Q_c = 1{\cdot}2\times(7{\cdot}3/5{\cdot}6)\times(5{\cdot}6/4{\cdot}3) = 2{\cdot}1$ μ-equiv. g^{-1} hr^{-1}. These values predict a tracer uptake of $1{\cdot}3$ μ-equiv. g^{-1} hr^{-1}; parallel measurements in unlabelled, salt-saturated tissue gave $1{\cdot}4\pm0{\cdot}05$ μ-equiv. g^{-1} hr^{-1}.

The estimation of fluxes from these measurements of tracer exchange assumes that tracer lost from the cells passes rapidly to the solution. Although this assumption is valid for diffusion in the free space, Figures 3(a), 3(b), and 3(c) show that there was a lag of 5–10 min before tracer lost from the cells in the root was transported to the solution. Figures 5(a) and 5(b) show that the tracer passing to the solution from the cut end, i.e. through the xylem, is a very high proportion of the total flux from the

roots—about 75–80%. This distribution is true both for cytoplasmic and vacuolar components. To what extent does the transport in the xylem invalidate the flux calculations?

It can be seen intuitively that one effect of the lag in transport from the xylem will be overestimation of ϕ_{co}, which is based on extrapolation of cytoplasmic content back to $t = 0$ using a graph such as Figure 5(*b*). A better estimate of ϕ_{co} could be obtained by extrapolation to the end of the lag period. If it is assumed that cells along the xylem have a specific activity that falls exponentially with time and that the xylem sap flows with a uniform velocity, then the specific activity collected in the xylem sap is an average over the period taken for sap to flow from one end of the root to the other. In this case the specific activity can be expected to fall exponentially with the same rate constant as the cells along the xylem but with a lag equal to $\frac{1}{2}$(velocity of flow/length of root). Using the rate of flow determined from the data of Figure 3(*c*), the lag is estimated to be about 5 min.

From the data of Figures 5(*a*) and 5(*b*), ϕ_{co} towards the free space can be estimated as $1{\cdot}2$ μ-equiv. g^{-1} hr^{-1} and taking the extrapolate to 5 min instead of zero, the flux to the xylem is $3{\cdot}7$ μ-equiv. g^{-1} hr^{-1}. The total flux out of the cytoplasm is therefore $4{\cdot}9$ instead of $5{\cdot}6$ μ-equiv. g^{-1} hr^{-1}, an overestimate of about 15%. More important than the overestimation of ϕ_{co} is the interpretation of the fluxes, which should be applied to a model like that in Figure 6 rather than the conventional three-phase system.

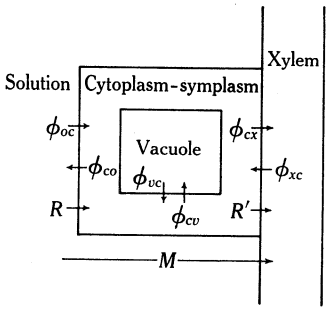

Fig. 6.—Suggested model relating fluxes into root cells and the transport process in the root. For details see text.

According to Figure 6 transport to the shoot can take place either through the cytoplasmic phase (R') or through the free space (M). Hence

$$\text{net transport} = R' + M, \tag{1}$$

and

$$\text{tracer transport} = R' . s_c + M . s_o. \tag{2}$$

As will be seen later, M appears to be very small in the present experiments. When net accumulation is zero,

$$R = \phi_{oc} - \phi_{co} = \phi_{cx} - \phi_{xc} = R'.$$

Tracer exchange of the cytoplasmic phase is formally like that of the conventional three-phase model. Thus

$$\text{tracer accumulation} = \phi_{cv} . (s_c - s_v), \tag{3}$$

and

$$\text{tracer uptake} = (\phi_{oc} . s_o - \phi_{co} . s_c) + M . s_o. \tag{4}$$

In the quasi-steady state, when s_c and s_v are changing only slowly,

$$s_c = (\phi_{oc}.s_o+\phi_{vc}.s_v)/(\phi_{co}+R'+\phi_{cv})$$
$$= (\phi_{oc}.s_o+\phi_{vc}.s_v)/(\phi_{oc}+\phi_{cv}). \quad (5)$$

In this equation R', and not ϕ_{cx}, is used because the specific activity of the xylem is determined by the transport process and not by s_o. In the present experiments s_x is effectively the same as s_c. For this reason loss from the cut end measures R' and not ϕ_{cx}. Loss of tracer from the surface measures ϕ_{oc} and the sum of $R'+\phi_{co}$ is equal to ϕ_{oc}. The fluxes ϕ_{cv} (= ϕ_{vc}) can be calulated from the total loss from the roots as already described. Using the previous values, $R' = 3{\cdot}7$, $\phi_{co} = 1{\cdot}2$, and $\phi_{oc} = 4{\cdot}9$.

TABLE 4

FLUXES OUT OF THE CYTOPLASMIC PHASE TO SOLUTION, VACUOLE, AND SURFACE FOR ROOTS IN POTASSIUM CHLORIDE SOLUTION

Values are the mean of duplicates which differed by less than 15%. Also given are the rate constants for exchange of the cytoplasmic phase. The influx (ϕ_{oc}) is the sum of ϕ_{co} and R'

Expt. No.	Concn. of KCl Solution (mM)	Fluxes (μ-equiv. g^{-1} hr^{-1})				k_c (hr^{-1})
		ϕ_{co}	R'	ϕ_{cv}	ϕ_{oc}	
1	0·5	0·7	2·2	1·6	2·9	3·8
2	0·5	0·9	2·7	1·6	3·6	3·0
3	10·0	1·2	3·7	1·7	4·9	3·75
4	10·0	1·0	3·6	1·5	4·6	3·0
5	10·0	3·2	4·6	2·9	7·8	4·2

Table 4 gives several estimates of fluxes for roots in 0·5 and 10 mM KCl. Comparison of values in experiments 4 and 5 shows how variable different sets of tissue can be even though grown under similar culture conditions. Thus ϕ_{oc} ranged from 4·6 to 7·8 μ-equiv. g^{-1} hr^{-1} for roots in 10 mM KCl. The total influx at the plasmalemma (ϕ_{oc}) was appreciably larger than the flux to the vacuole (ϕ_{cv}) for roots in 10 mM KCl. Consequently rates of tracer accumulation will tend to be limited by the flux to the vacuole. These properties are in agreement with chloride fluxes estimated on the basis of the conventional three-phase model (Pitman 1969). The present results differ from previous suggestions in showing that transport through the root can be a large proportion of the influx to the cell.

Table 4 also gives the rate constants for cytoplasmic exchange, which correspond to times for half-exchange of 11–15 min. The value from experiment 4 (3·0 hr^{-1}) should be compared with the rate constant for establishment of transport in Figure 3(*b*) (2·4 hr^{-1}) as the same set of tissue was used.

Table 5 gives further determinations of the proportion of the vacuolar efflux passing through the xylem. These results were obtained from the rate of tracer loss from cut end and surface as shown in Figure 5(*a*). The values are extrapolates of the slow component back to $t = 0$. The proportion passing out of the cut end is high over the whole of the concentration range, and is perhaps greater at low concentrations.

The total rate of loss is also independent of concentration, as already found for ϕ_{cv} (Table 4).

Comparison of tracer exchange at different concentrations showed another problem of this method of estimating fluxes. In elution of roots labelled in 0·1 mM KCl the tracer lost in the first 5 min was 0·6–0·7 μ-equiv. g^{-1} hr^{-1}. This rapid loss from roots in solution of low concentration represents a percentage free space of several hundred, which is untenable. The expected value is about 25%. This discrepancy can be explained as being due to the contents of the xylem vessels which would be at 20 to 30 mM, even in solutions of this low concentration (Pitman 1965*a*).

TABLE 5

COMPARISON OF TRACER LOST FROM VACUOLES THROUGH THE CUT END WITH THAT LOST THROUGH THE FREE SPACE IN EXCISED ROOTS

Concn. of KCl Solution (mM)	Transport from Root Surface (μ-equiv. g^{-1} hr^{-1})	Transport from Cut End (μ-equiv. g^{-1} hr^{-1})	Total Transport (μ-equiv. g^{-1} hr^{-1})	Ratio of Surface to Total Transport
10·0	0·30	1·0	1·3	0·23
	0·27	0·66	0·9	0·29
	0·40	0·80	1·2	0·33
	0·70	1·40	2·1	0·33
1·0	0·14	0·77	0·9	0·16
0·5	0·35	0·75	1·1	0·32
	0·14	1·15	1·3	0·13
0·1	0·22	1·0	1·2	0·18
	0·18	1·2	1·4	0·13

(*c*) *Relationship between Transport and Fluxes*

The model shown in Figure 6 can be tested by comparing different estimates of the rate of transport from excised roots. These are:

(1) Direct estimation of chloride lost from the cut end [cf. Fig. 2(*a*)]. In terms of the model this gives $R'+M$.

(2) Tracer transport through the root is equal to $R'.s_c+M.s_o$. As $s_v = 0$ and $s_o = 1$, $s_c = \phi_{oc}/(\phi_{oc}+\phi_{cv})$, which can be calculated from the fluxes.

(3) Tracer loss from the cut end of labelled roots at the start of an elution [$t = 0$ in Figure 5(*a*)] gives R' as $s_c = 1$ at this stage.

(4) Tracer loss from the cut end falls to a steady value which extrapolates at $t = 0$ ($s_v = 1$ and $s_o = 0$) to $R'.\phi_{cv}/(\phi_{oc}+\phi_{cv})$, and again R' can be estimated.

A comparison of rate of transport estimated in these four ways is given in the following tabulation (fiducial limit = 0·3 μ-equiv. g^{-1} hr^{-1}):

KCl Concn. (mM)	Rate of Transport (μ-equiv. g^{-1} hr^{-1})			
	Method 1	Method 2	Method 3	Method 4
0·5	2·5	2·2	2·2	2·1
10·0	4·8	3·6	3·6	3·5

In 0·5 mM KCl there was reasonable agreement between the different estimates. In 10 mM KCl the rate of transport determined directly was about 20% greater than that calculated from tracer measurements. This difference between net chloride and tracer chloride transport was a general one. Table 1 showed that the ratio of tracer to total chloride transport was 0·55 in 10 mM KCl. By contrast, the specific activity of the cytoplasm calculated from the fluxes was about 0·75.

This inconsistency was not due to the term M for direct transport across the root. A large value of M would give a greater value of ratio of tracer to net chloride transport than that calculated from the fluxes. The agreement between the estimates based on tracer measurements suggests that M is small and can be ignored in the present experiments.

Table 3 showed that the specific activity of chloride transported from excised roots was appreciably greater than from root to shoot of whole plants. There will be an even greater difference between estimates based on fluxes and observations using whole plants.

IV. Discussion

It has been shown in this paper that excised high-salt roots retain many of the properties of intact roots and can be used to study the properties of salt transport in the whole plant. Rates of transport from excised roots were high and within 20% of transport in whole plants. The absolute rate of transport of potassium and chloride from roots of barley seedlings is related to relative growth rate (Greenway 1965; Pitman, unpublished data). In the present experiments the relative growth rate was small ($< 0{\cdot}06$ day^{-1}) and transport depended on endogenous reserves. Roots of low-salt plants contain high levels of sugar (60 mM) which are consumed as salt is taken up. As the sugar level falls so does the rate of transport and accumulation. Tables 1 and 2 showed that both excised roots and whole plants had reduced rates of uptake and transport after several days in salt solution.

It was part of the purpose of this paper to examine the relation between transport and fluxes into the root cells. One result has been to show that most tracer diffusing out of cells in high-salt roots passes into the transport process and not directly to the solution. Consequently the use of tracer exchange from excised roots is a doubtful way of estimating fluxes from cells as the properties of the exchange are largely due to the transport process. For example, high loss of potassium from excised roots may be due to the transport process and not to cell fluxes as suggested by Pitman and Saddler (1967).

An important result was that the rate constant for establishment of the steady rate of tracer transport [Figs. 3(a)–3(c)] was effectively the same as the rate constant for exchange of the cytoplasmic phase [Figs. 5(a), 5(b); Table 4]. This similarity was not due to the large involvement of the transport process in efflux from excised roots, as the rate constant was the same for loss from the surface as from the cut end. These results show the involvement of the cytoplasmic phase in transport to the shoot, and imply that the rate of transport is related to fluxes into and out of the cytoplasmic phase since the capacity of the cytoplasmic phase is limited (about 2 μ-equiv. g^{-1}).

There was satisfactory agreement between rates of transport calculated in different ways (see tabulation, p. 417). Both these results justify the use of the model

in Figure 6 as a basis for studying the transport process in relation to uptake. A weakness of the model was the discrepancy between rates of tracer transport in excised roots and in whole plants. Table 3 showed that there were good grounds for thinking that tracer transport in whole plants was only 50% of that expected from net chloride transport, and from the specific activity calculated from the fluxes.

In this model root cells were assumed to act as a symplasm. There is some evidence for this view as the rate of movement of ions across the root can be more rapid than expected for diffusion in a free space (Pitman 1965*a*). A similar model was used by Weigl (1969) to discuss fluxes in relation to transport. If mixing across the root were slow, or if the rate of tracer exchange of cells in the outer cells differed from that in the inner cells of the cortex, then gradients of specific activity would be set up while tracer was being taken up to unlabelled roots. Gradients in s_c are also likely along the length of the root, as fluxes are not constant along the root, and these gradients too could reduce tracer transport to the shoot. This problem of gradients in the root is more important to tracer movement than to net chloride transport. It is likely that the extent to which gradients develop is related to the water flux through the root.

The rate of transport of some ions has been found to depend on water flow, but these observations have been mainly based on tracer transport, or using low concentrations. For example, Crossett (1968) found that rate of tracer phosphate transport in barley was reduced in the dark (when transpiration falls). Greenway (1965) found that there was reduced transport of tracer Cl^- when transpiration was low and Greenway, Klepper, and Hughes (1968) also showed that fluxes were related to water potential. Experiments of the kind described in this paper to measure transport of ^{86}Rb from 10 mM KCl when transpiration was varied showed that transport of ^{86}Rb was reduced by 30% when transpiration was reduced by 70%.

In view of the difference between tracer and net transport the effect of transpiration could be on specific activity and not on net transport. Total transport of univalent cations was not affected by water flow (Pitman 1965*b*), even though the ratio of K/Na was reduced at higher transpiration rates. In the present experiments, water flow was small and tracer tended to be at the base of the shoot rather than in the leaf blade. Higher rates of transpiration may have led to greater tracer transport, but clearly further experiments are needed on this important point.

Values of ϕ_{oc}, ϕ_{cv}, and ϕ_{vc} estimated from this model were only 10–20% different from fluxes calculated from the conventional model. The main difference was in the flux ϕ_{co}, which was much smaller due to the large net influx at the plasmalemma. Figures 5(*a*) and 5(*b*) show that the efflux estimated from excised roots can be controlled mainly by the transport process as well as by ϕ_{cv}.

There were too few different concentrations used to warrant comparison of transport and uptake at different levels of operation of mechanisms I and II as done by Hodges and Vaadia (1964). Using the same set of tissue ϕ_{oc} was found to be 3·6 μ-equiv. g^{-1} hr^{-1} in 0·5 mM KCl and 4·9 μ-equiv. g^{-1} hr^{-1} in 10 mM KCl. If ϕ_{oc} is interpreted as mechanism I at 0·5 mM and mechanisms I and II at 10 mM (Epstein 1966) then, for mechanism I, $\phi_{oc} = 3{\cdot}6$ μ-equiv. g^{-1} hr^{-1} and, for mechanism II, $\phi_{oc} = 1{\cdot}3$ μ-equiv. g^{-1} hr^{-1}. Values estimated for low-salt roots at 25°C were 4·0 μ-equiv. g^{-1} hr^{-1} (mechanism I) and 4–6 μ-equiv. g^{-1} hr^{-1} (mechanism II)

(Pitman 1969). These results support more clearly than previously the suggestion (Pitman, Courtice, and Lee 1968; Pitman 1969) that these components of uptake do not have the same significance in high-salt roots as in low-salt roots. The flux at the tonoplast was very nearly independent of concentration as found for beet (Pitman 1963) and carrot (Cram 1968). The lack of response of rate of transport to concentration (Table 5) agrees with observations on K^+ transport made by Johansen, Edwards, and Loneragan (1968).

A model of this kind is clearly only an approximation to reality. The organization of the closely packed stele with thick cell walls, dense cytoplasm, and lack of air spaces is very different from the organization of the cortex, where cells are thin-walled, with large air spaces between them. The root acts both as a secreting organ and as an organ efficient at uptake from soil and solution. In such a system the symplast may involve all cells in the cortex or only a few along lines of easier transport. The fluxes estimated on a root weight basis may refer only to a limited part of the root. Despite these shortcomings the model has some value in relating the transport and uptake processes through the cytoplasmic phase of the root cells.

A current problem in understanding uptake of ions by roots and their transport to the shoot is the location of the active transport. In the present model either ϕ_{oc} or ϕ_{cx} or both fluxes could be responsible. No attempt has been made in this paper to consider this problem, but the techniques and present data provide a useful basis for determining the location of the transport process.

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