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Chromium Uptake and Transport in Barley Seedlings (*Hordeum vulgare* L.)

R.A. Skeffington*, P.R. Shewry**, and P.J. Peterson
Department of Botany and Biochemistry, Westfield College (University of London), Kidderpore Avenue, London NW3 7ST, U.K.

Summary. Potassium chromate is more toxic to the growth of barley in solution culture than chromic chloride, though apparent uptake of the latter is much faster. Inhibitor studies indicate that CrO_4^{2-} uptake is "active" whereas Cr^{3+} uptake is passive, demonstrating that the two forms do not share a common uptake mechanism. Studies on the form of Cr inside root cells show that in plants fed CrO_4^{2-} the Cr remains largely unchanged whereas in plants fed Cr^{3+} a little CrO_4^{2-} (0.5 per cent) is produced. This conversion is dependent on the presence of living material and is probably enzymatic. Chromate uptake follows Michaelis-Menten kinetics at low concentration and is competitively inhibited by sulphate. Transport of chromium up the root is very slow, accounting for the low levels of Cr in the shoots. Chromate is transported better than Cr^{3+} though still to a very limited extent. These experiments provide a physiological basis for previous observations.

Introduction

Normal soils contain between 5 and 3,000 μg/g Cr, but the chemical form of the element in soils and the amount which is available to plants is difficult to assess (Shewry and Peterson, 1976). There is some evidence for the presence in some soils of the trivalent chromium ion (Cr^{3+}) and the hexavalent chromate ion (CrO_4^{2-}) (Soane and Saunder, 1959; Shewry and Peterson, 1976), and these are also thought to be the predominant forms of Cr in seawater (Fukai, 1967). Chromate is in pH-dependent equilibrium with other forms of Cr(VI) such as $HCrO_4^-$ and dichromate ($Cr_2O_7^{2-}$) with CrO_4^{2-} being the predominant form at pH > 6. Dichromate in soil will tend to be reduced to Cr^{3+} by organic matter, making interconversion between the various forms of soil chromium a possibility.

There are conflicting views on the uptake and metabolism of these ions by plant roots. Bourque et al. (1967), working with wheat, concluded that CrO_4^{2-} but not Cr^{3+} was absorbed by the roots, but their experiments were performed by reducing CrO_4^{2-} to Cr^{3+} with sodium ascorbate and studying uptake in the presence of the ascorbate solution. Blincoe (1974) claimed that Eckert and Blincoe (1970) had shown that CrO_4^{2-} was absorbed rapidly but Cr^{3+} was absorbed to a limited extent. Examination of the latter paper reveals no evidence which supports this conclusion. Myttenaere and Mousny (1974) working with rice have concluded the opposite, namely that Cr^{3+} is taken up more rapidly than CrO_4^{2-}. They also suggest that CrO_4^{2-} may be reduced to Cr^{3+} before entering the cell.

The ionic form of chromium inside cells is also the subject of debate. Bourque et al. (1967) and Myttenaere and Mousny (1974) considered that it was predominantly bound to proteins but when the distribution of chromium in tissues was investigated by solvent extraction techniques (Lyon et al., 1969; Huffman and Allaway, 1973; Shewry and Peterson, 1974) this was found not to be the case. One of the chromium compounds in *Leptospermum scoparium* root cells was identified as trioxalatochromium (III) ion by Lyon et al. (1969) while Blincoe (1974) has reported the occurrence of anionic complexes of low molecular weight in lucerne shoots.

We have recently reported some factors controlling uptake and transport of $^{51}CrO_4^{2-}$ by barley seedlings grown in solution culture (Shewry and Peterson, 1974). In this paper we provide evidence on the kinetics of Cr uptake by barley seedlings, the form of

* *Present address*: Department of Botany, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX. U.K.
** *Present address*: Department of Biochemistry, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ. U.K.

chromium within the root, and discuss the apparent block in Cr transport from roots to shoots.

Materials and Methods

Isotope Administration

Barley (*Hordeum vulgare* L. cv. Julia) was grown under controlled conditions as described previously (Shewry and Peterson, 1974). After 6 d growth in culture solution the roots of whole plants were rinsed briefly in deionized water and placed in 1 l of solution containing 250 μM $CaCl_2$, radioisotope (Radiochemical Centre, Amersham) and carrier where appropriate. Plants were incubated with vigorous aeration at 22° C for various periods of time under various desorption conditions, were divided into roots and shoots and dried at 80° C prior to ^{51}Cr determination in a Beckman Biogamma solid scintillation counter, or ^{32}P determination by measurement of Cerenkov radiation in a Beckman LS 150 liquid scintillation counter, quenching in the latter case being corrected for by the channels ratio method.

Solvent Extraction and High Voltage Paper Electrophoresis

Roots were ground with mortar and pestle with 80% v/v aqueous ethanol. The residue was removed by centrifugation at 1,000 × g and re-extracted twice with 80% ethanol and twice with deionised water. The bulked ethanol extracts were reduced to dryness in a rotary film evaporator and phase separated between water and diethyl ether. Ten μl samples of the aqueous phase were subjected to high voltage paper electrophoresis on the Miles Hivolt Electrophoresis unit at 300 mA for 15 min in pyridine-acetic acid buffer, pH 5.3 (Lyon et al., 1969). The electrophoretogram was cut into 1 cm strips at right angles to the direction of ion migration and the ^{51}Cr content of each determined as described in the previous paragraph.

Determination of Total Chromium

Plant material was dried, ashed at 450° C, taken up in 2N HCl and the Cr concentration measured on a Varian Techtron AA6 atomic absorption spectrophotometer.

Results and Discussion

In this paper, the term "apparent uptake" is used to indicate the amount of Cr observed in the roots when free Cr has been washed off with water, and the term "uptake" is restricted to values obtained when Cr has beeen desorbed from the Donnan free space by a wash in non-radioactive Cr.

Figure 1 illustrates that, in long term experiments, CrO_4^{2-} inhibits the growth of root and shoot of barley to a greater extent than Cr^{3+}, in agreement with earlier work (Hewitt, 1953). In spite of this, apparent uptake of Cr^{3+} was greater than that of CrO_4^{2-} in the roots, but more Cr appeared in the shoots when the plants was fed CrO_4^{2-}. These data were supported by short-term experiments using ^{51}Cr

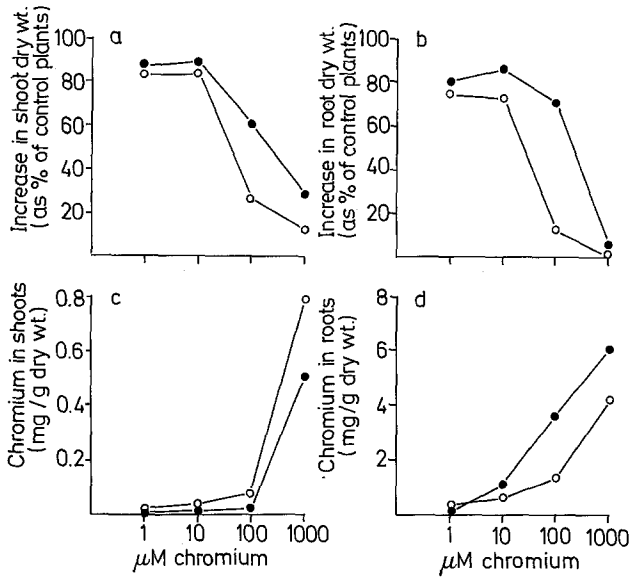

Fig. 1a–d. The effect of chromium concentration on chromium uptake and transport and on the growth of barley seedlings. Four-day old seedlings were transferred to nutrient solution containing K_2CrO_4 (o–o) or $CrCl_3$(●–●) and grown as described in Materials and Methods for 9 days. Growth was measured as the increase in dry weight in shoots **a** and roots **b** and expressed as a percentage of that in controls without added chromium. Shoots **c** and roots **d** were also analysed for chromium. All results are the mean of two replicate samples of 8 seedlings

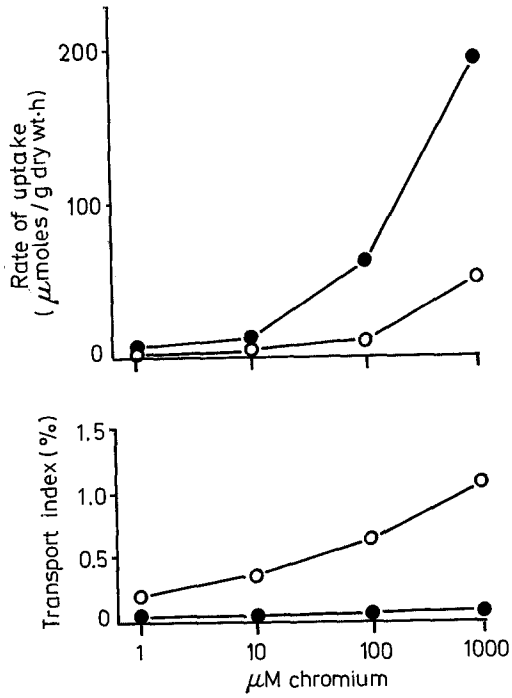

Fig. 2. The effect of chromium concentration on the rate of apparent uptake and transport of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$. $^{51}Cr^{3+}$ (●–●) or $^{51}CrO_4^{2-}$ (o–o) diluted with $CrCl_3$ or K_2CrO_4 respectively were supplied to barley seedlings for 1 h in the presence of 250 μM $CaCl_2$. The roots were then desorbed in 1 l deionised water for 15 min, and the ^{51}Cr content determined. Transport is expressed as a transport index (Cr in shoots × 100/total Cr in plant). Results are the mean of 2 replicate samples of 10 seedlings

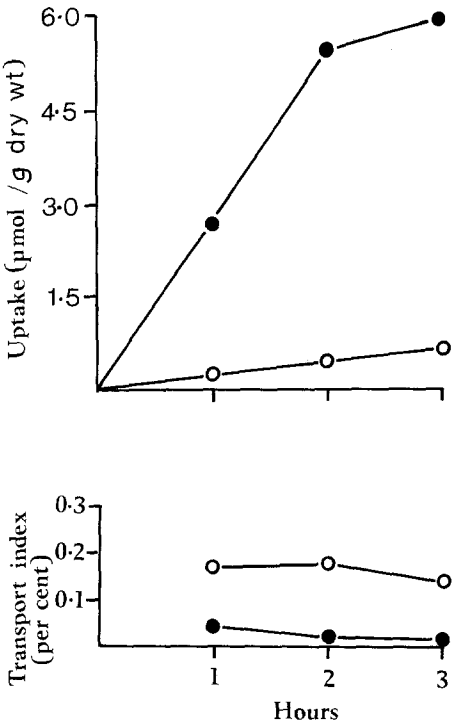

Fig. 3. Time course of apparent uptake and transport of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ by barley seedlings. $^{51}Cr^{3+}$ (●–●) or $^{51}CrO_4^{2-}$ (○–○) diluted with carrier $CrCl_3$ or K_2CrO_4 to a final concentration of 1 μM were administered in 1 l 250 μM $CaCl_2$. At hourly intervals samples of the roots were blotted and desorbed for 15 min in 1 l deionised water. Each point represents the mean of 2 replicate samples of 10 seedlings

Table 1. The effect of inhibitors on the uptake of chromic, chromate and phosphate ions

Treatment	Amount of Ion taken up (μmoles/g dry weight root tissue/h)		
	Chromic	Chromate	Phosphate
No addition	1.74 ± 0.37	0.66 ± 0.12	1.21 ± 0.24
10^{-4} M NaCl	1.42 ± 0.31	0.42 ± 0.04	1.25 ± 0.24
10^{-4} M Na.azide	1.49 ± 0.42	0.15 ± 0.02	0.57 ± 0.18
10^{-5} M DNP	2.20 ± 0.49	0.22 ± 0.03	0.54 ± 0.02

Inhibitors were predissolved and administered to the plants for 15 min prior to the addition of isotope. Each ion was fed at 10 μm, as $^{51}CrCl_3$, $K_2{}^{51}CrO_4$ or $KH_2{}^{32}PO_4$ as appropriate, in 1 l of 250 μM $CaCl_2$. Each pot contained 50 μCi of the radioactive species. After 4 h, each set of plants was desorbed for 30 min in the non radioactive ion at 20 μM. Each value given is the mean uptake into the roots of 10 plants ± the standard error

Table 2. The distribution of ^{51}Cr in solvents after sequential extraction of barley roots

Fraction	Distribution (per cent)	
	$^{51}Cr^{3+}$	$^{51}CrO_4^{2-}$
80% (v/v) aqueous ethanol	7.0	62.0
(of which Diethyl ether)	(0.2)	(1.2)
Water	8.2	22.2
Insoluble Residue	84.8	15.8

Carrier-free (2.8 nM) $K_2{}^{51}CrO_4$ or $^{51}CrCl_3$ were fed to barley seedlings for 24 h in 250 μM $CaCl_2$, after which the roots were washed in deionised water and extracted as described in Materials and Methods. Results are the mean of 3 replicate samples of 5 seedlings

as is shown in Figure 2. The transport index (i.e. percentage of Cr absorbed by the plant that is found in the shoots) increased with increasing concentration of CrO_4^{2-} but remained substantially the same for Cr^{3+}. Apparent uptake of both ionic forms remained linear for at least 2 h and the transport index did not substantially alter with time (Fig. 3). The fall in the apparent rate of Cr^{3+} uptake after 2 h is probably due to depletion of Cr^{3+} in the feeding solution as 70% of it had been removed by the roots by this time.

Bourque et al. (1967) suggested that only CrO_4^{2-} could enter plant cells, whereas Myttenaere and Mousny (1974) considered that CrO_4^{2-} had to be reduced to Cr^{3+} before entry. Evidence for independent uptake mechanisms for Cr^{3+} and CrO_4^{2-} by barley is shown in Table 1. The metabolic inhibitors sodium azide and dinitrophenol (DNP) substantially reduced uptake of CrO_4^{2-} but uptake of Cr^{3+} was not affected. Values for orthophosphate, an anion known to be taken up actively by barley (Loughman, 1966) are included for comparison.

Thus at 10 μM concentration, CrO_4^{2-} uptake depends on metabolic energy whereas Cr^{3+} uptake does not. Any interconversion of the two forms before uptake must therefore be limited in extent, if it occurs at all.

The multiplicity of cation binding sites in cells and cell walls will adsorb Cr^{3+}, which may explain why Cr^{3+} is less toxic and not transported as well as CrO_4^{2-}, though apparently taken up more readily. This interpretation is supported by data on solvent extraction of plants supplied with $^{51}CrO_4^{2-}$ and Cr^{3+} (Table 2). Most of the absorbed CrO_4^{2-} exists in the soluble fraction of the roots, while most of the Cr^{3+} is in the insoluble residue.

Shewry and Peterson (1974) showed that sulphate would inhibit CrO_4^{2-} uptake at various concentrations of both ions. A more detailed kinetic investigation of this effect is shown in Figure 4 which is a Lineweaver-Burk plot of data showing the rate of CrO_4^{2-} uptake as a function of concentration in the range 10–160 μM with and without 10 μM potassium sulphate. Chromate uptake follows Michaelis-Menten

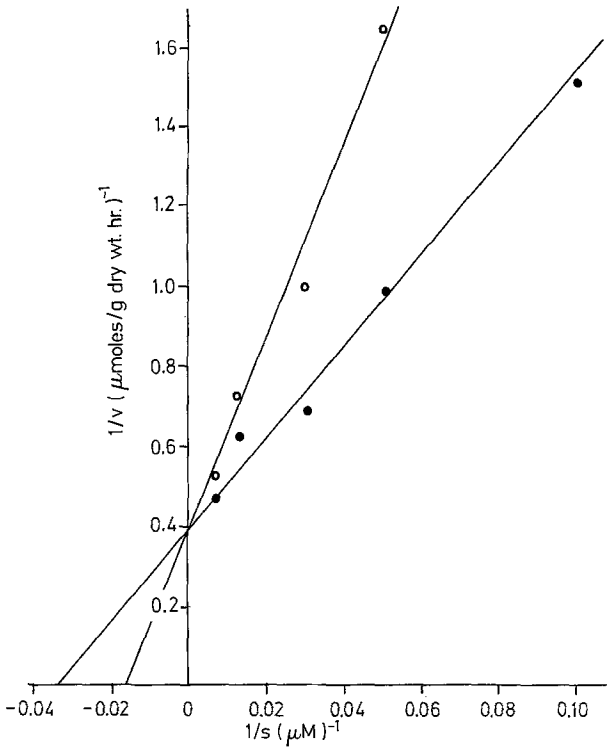

Fig. 4. The effect of K_2SO_4 on uptake of $^{51}CrO_4^{2-}$ by barley seedlings (Linewaver-Burk plot). Barley seedlings grown as described in Materials and Methods were incubated in 250 μM $CaCl_2$ containing 10, 20, 40, 80 or 160 μM K_2CrO_4 with an appropriate amount of $^{51}CrO_4^{2-}$ added, both with and without 10 μM K_2SO_4 at each concentration. After 3 h, the roots of the plants were rinsed and desorbed for 30 min in an excess of non radioactive K_2CrO_4, dried and counted. Each point represents the mean uptake of CrO_4^{2-} into the roots of 10 plants at a given concentration [(○), +10 μM K_2SO_4; (●), no addition], where V is the rate of CrO_4^{2-} uptake, and S is the chromate concentration supplied

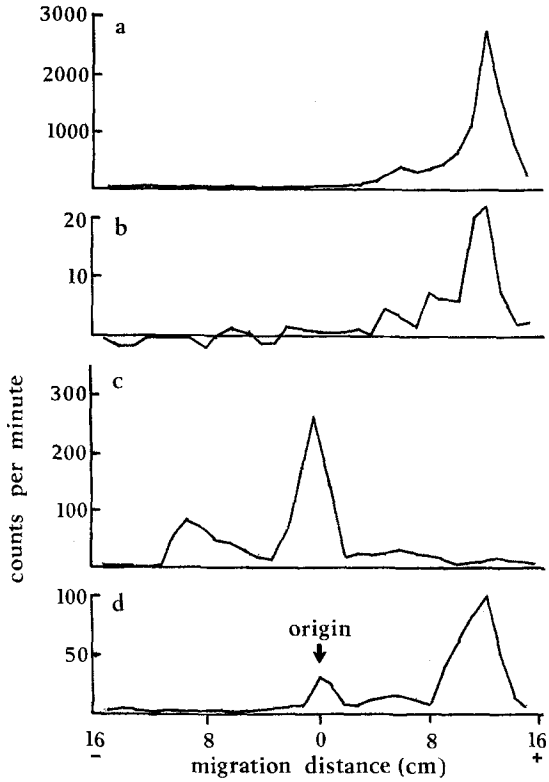

Fig. 5a–d. The distribution of ^{51}Cr in electrophoretograms of the alcohol-soluble fractions of $^{51}CrO_4^{2-}$ and $^{51}Cr^{3+}$-fed barley roots. Seedlings were fed 2.8 nM $K_2{}^{51}CrO_4$ or $^{51}CrCl_3$ for 24 h in 1 l 250 μM $CaCl_2$, the roots rinsed in deionised water for 30 min, and extracted and subjected to high voltage paper electrophoresis as described in Materials and Methods. The diagram shows counts due to ^{51}Cr on the electrophoretogram: **a** CrO_4^{2-} standard; **b** seedlings fed $^{51}Cr^{3+}$; **c** Cr^{3+} standard; **d** seedlings fed $^{51}CrO_4^{2-}$

kinetics in this concentration range, which is within the range conventionally called System I, though Nissen (1974) postulates that there may be more than one absorption isotherm in this region. This form of kinetics is usually interpreted as meaning that the ion in question has a specific "carrier" to transport it across the plasmalemma. Sulphate appears to inhibit CrO_4^{2-} uptake competitively (i.e. SO_4^{2-} affects the K_m for CrO_4^{2-} but not the V_{max}). This implies that CrO_4^{2-} is travelling via the "carrier" normally used to transport SO_4^{2-}. Kinetic parameters deduced from Figure 4 are: K_m for CrO_4^{2-}, 29.4 μM; V_{max}, 2.5 μmoles/g D.W./h; K_i for SO_4^{2-}, 9.65 μM. As the K_m for SO_4^{2-} uptake by barley roots in this concentration range is about 12 μM (Nissen, 1971, mean of 4 values given), CrO_4^{2-} appears to have a lower affinity for the "carrier" than SO_4^{2-}, which is consistent with the above model. The V_{max} for CrO_4^{2-} uptake is comparable to those observed by Bowen (1969) for uptake of the micro-nutrients Cu^{2+}, Zn^{2+} and Mn^{2+} by sugar cane leaf tissue.

It is of interest to establish whether interconversion between Cr^{3+} and CrO_4^{2-} takes place inside the plant tissues and whether any complex formation takes place. Figure 5 shows that in barley plants fed $^{51}CrO_4^{2-}$ for 24 h, the only Cr species extractable from the roots was CrO_4^{2-}. When plants were fed $^{51}Cr^{3+}$ under the same conditions, however, CrO_4^{2-} was again the only species detected. Further experiments showed that this effect occurred independently of Cr^{3+} concentration, nor was the feeding solution the source of the CrO_4^{2-} as none could be detected in it. These results indicate that some Cr^{3+} can be converted to CrO_4^{2-} after entering the tissues. However, when roots from plants not previously supplied with Cr were ground in the presence of Cr^{3+} and/or CrO_4^{2-} (Fig. 6) and the aqueous ethanol fraction subjected to electrophoresis, the Cr^{3+} again could not be detected, presumably as it was adsorbed onto the residue, whereas CrO_4^{2-} was unaffected. This strongly suggests that the apparent absence of Cr^{3+} in the Cr^{3+}-

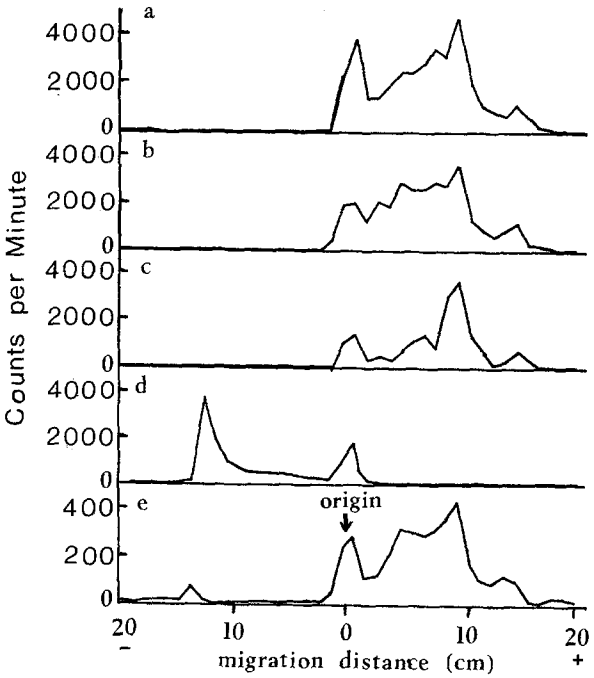

Fig. 6a–e. Absorption of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ by barley roots on extraction. 10 ml of 0.5 μM $^{51}CrCl_3$, $K_2{}^{51}CrO_4$, or a mixture of the two, each containing 50 μCi of ^{51}Cr, were ground with 0.5 g of washed unlabelled barley roots grown under the standard conditions. The alcohol-soluble fraction of each homogenate was subjected to high voltage paper electrophoresis as described in Materials and Methods. Results shown are representative of replicate runs. The treatments were: **a** added $^{51}CrO_4^{2-}$; **b** $^{51}CrO_4^{2-}$ standard; **c** mixture of $^{51}CrO_4^{2-}$ and $^{51}Cr^{3+}$ added; **d** Cr^{3+} standard; **e** $^{51}Cr^{3+}$ added

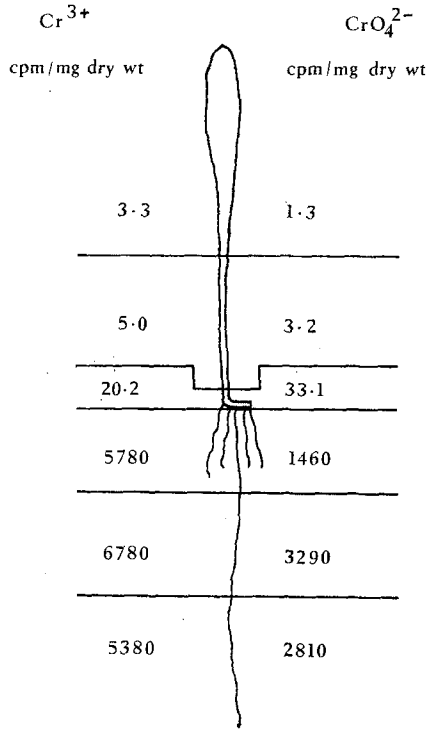

Fig. 7. Transport of Cr in barley seedlings. Barley seedlings were fed $^{51}Cr^{3+}$ or $^{51}CrO_4^{2-}$ for 4 h at 10 μM under identical conditions to those described in Table 1. After drying, the plants were cut into sections, as shown in the diagram above, and the ^{51}Cr content of each section determined separately. Values shown represent the mean Cr uptake of 4 plants per treatment, expressed as cpm/mg D.W.

Table 3. Transport of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ in roots and shoots

	Total Cr absorbed (n moles)	% of total radioactivity in segments					
		Part fed	0–1 cm	1–2 cm	2–3 cm	3–4 cm	rest
a) *Isotope fed to distal half of root*							
Cr^{3+}	60.8	99.5	0.1	0.1	0.1	0.0	0.1
CrO_4^{2-}	35.2	96.6	1.8	1.0	0.3	0.2	0.1
b) *Isotope fed to proximal half of root*							
Cr^{3+}	132	99.94	0.04	0.01	0.00	0.00	0.00
CrO_4^{2-}	70.7	99.8	0.1	0.0	0.0	0.0	0.1
c) *Isotope fed to base of shoot*							
Cr^{3+}	13.82	76.5	3.3	2.1	1.4	1.3	15.4
CrO_4^{2-}	5.73	46.8	7.3	6.4	4.9	2.5	32.0

10 μM K_2CrO_4 or $CrCl_3$ containing 100 μCi ^{51}Cr/l was fed in 250 μM $CaCl_2$ to either the proximal or the distal half of a seminal root of a barley seedling. The rest of the root was bathed in 250 μM $CaCl_2$ alone and feeding continued for 4 h. The roots were then cut at the boundary of the part fed isotope, washed, and desorbed in 100 mM K_2CrO_4 or $CrCl_3$ as appropriate for 20 min, dried, and the part of the root not fed isotope was cut into 1 cm segments leading away from the part fed. Each segment was analysed for ^{51}Cr. Shoots were cut off from the roots just above the hypocotyl and immediately the lowest 5 mm were immersed in feeding solutions identical to those used for roots. After 4 h, the shoots were treated as for roots. Results are expressed as the % age of the total chromium absorbed contained in a particular segment. Each treatment is the mean of 6 plants

supplied plants may be an artefact of the preparation procedure.

It is notable in Figure 6 that some CrO_4^{2-} was produced when Cr^{3+} was macerated with barley roots. This reaction required the presence of live roots before it would take place. No CrO_4^{2-} was formed from Cr^{3+} in the absence of plant material or when boiled roots were used. Presumably the reaction is an enzymatic one. Nevertheless only a very small proportion of the Cr^{3+} absorbed appeared as CrO_4^{2-} on the electrophoretogram: about 0.5 per cent for the data in Figure 5.

Figures 1 and 2 show that more ^{51}Cr was transported from root to shoot when CrO_4^{2-} rather than Cr^{3+} was supplied to the plant. Nevertheless, in terms of the total ^{51}Cr in the plant this proportion is extremely low. This blockage in transport has been previously noted by several workers (see Pratt, 1966; Shewry and Peterson, 1974). Figure 7 shows the distribution of ^{51}Cr in barley seedlings fed $^{51}Cr^{3+}$ or $^{51}CrO_4^{2-}$ for 4 h. There was a one hundred-fold drop in concentration across the hypocotyl when either form of Cr was supplied. The anatomy of the barley hypocotyl contains no obvious block to Cr transport, so we propose that Cr is not penetrating the root to the vascular tissue to any extent and cannot be translocated longitudinally in the cortex. To test this, $^{51}Cr^{3+}$ or $^{51}CrO_4^{2-}$ was suppled to the bases but not the tops of barley roots and vice-versa, and also to the base of the cut stems. The results (Table 3) show that only 0.5 per cent of the Cr^{3+} absorbed by the basal part of the root had moved upwards after 4 h, while 3.4 per cent of absorbed CrO_4^{2-} moved under the same conditions. Transport down the roots was even slower: only 0.06 per cent of the Cr^{3+} and 0.2 per cent of the CrO_4^{2-} moved down the root when the upper parts of the root were fed ^{51}Cr. These results suggest that Cr is transported largely by the xylem. However. the ions are quite mobile when fed to the base of cut stems; 24.2 per cent of the Cr^{3+} and 53.2 per cent of the CrO_4^{2-} absorbed are transported beyond the basal 0.5 cm. Evidently Cr^{3+} and CrO_4^{2-} enter the vascular tissue with difficulty but once there can be transported readily. Once in the xylem, CrO_4^{2-} moves more readily than Cr^{3+} presumably because the latter is held up by ion exchange on the vessel walls, as happens for Ca^{2+} (Bell and Biddulph, 1963). This would also explain why CrEDTA moves faster than Cr^{3+} into shoots (Myttenaere and Mousny, 1974) as CrEDTA is not retarded by ion exchange. An analogous effect has been noted for CaEDTA (Isermann, 1971). These low rates of transport can quantitatively explain the low levels of Cr in shoots compared to roots.

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