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Control of Chromium Concentrations in Food Plants. 1. Absorption and Translocation of Chromium by Plants

Earle E. Cary,* W. H. Allaway, and Oscar E. Olson[1]

Plants accumulated Cr from nutrient solutions but retained most of this Cr in the roots. The barrier to translocation of Cr from roots to tops of plants was not circumvented by supplying ^{51}Cr in the form of organic acid complexes, Cr(III), Cr(VI), or by increasing the Cr(III) concentration in the nutrient solutions. Plants, or plant tissues, that tend to accumulate Fe also accumulate Cr. Attempts to increase the Cr concentration in certain leafy vegetables seem more promising than attempts to increase Cr in edible seed crops. Chromium concentration in some leafy vegetables grown with a continued supply of soluble Cr may be tenfold greater than those found in surveys of Cr in foods.

The primary objective of this series of experiments was to provide a basis for designing crop production practices that might increase the chromium (Cr) concentration in food and feed crops. Mertz et al. (1974) discussed the potential value of an increase in levels of dietary Cr in a nutritionally effective form. Mertz (1969) outlined some of the chemical properties of Cr that may affect the role of this element in biological systems.

Much early (Pratt, 1966) and recent (Turner and Rust, 1971) work on Cr in plants was concerned with Cr toxicity. All of this work indicates that Cr(VI) is more toxic to plants than Cr(III) and that plants showing visual symptoms of toxicity contain very little more Cr in their tops than did normal plants. Although there are reports of plant growth responses to addition of small amounts of Cr to soils or culture solutions, recent experiments using highly purified cultures and sensitive analytical techniques indicate that if Cr is essential to the tested species, the required levels (in terms of concentration in plant tissue) are lower than for any known essential nutrient (Huffman and Allaway, 1973a).

Lyon et al. (1969a), who studied the metabolism of Cr in the accumulator plant *Leptospermum scoparium*, found Cr trioxalate to be one of the Cr compounds present in roots and leaves. In another study, this group (Lyon et al., 1969b) found CrO_4^{2-} in extruded xylem sap of *L. scoparium* when $Na_2{}^{51}CrO_4$ was fed to the roots. However, chromate was not found in soluble fractions of leaves, stems, and roots.

Myttenaere and Mousny (1974) reported evidence of greater removal of Cr(III) and Cr(VI) than of CrEDTA from nutrient solutions by rice roots, but a higher percentage of the Cr from CrEDTA taken up by the roots was translocated to the rice tops than was the case for Cr(III) or Cr(VI). The chemical form of Cr in the roots, as measured by solubility in different extractants, seemed similar regardless of whether Cr(III) or Cr(VI) was added to the nutrient solution.

Huffman and Allaway (1973b) found that over 90% of the ^{51}Cr removed from solutions by beans and wheat was still in the roots 20 days after the last addition of ^{51}Cr to the solutions. Most of this Cr was in soluble fractions. Less than 0.1% of the total ^{51}Cr in the plant was in the seeds. The ^{51}Cr in the leaves was primarily in the form of a low molecular weight anionic complex.

Shewry and Peterson (1974) intensively studied the uptake and translocation of CrO_4^{2-} from nutrient solutions by barley seedlings. They suggest that most of the Cr retained in the roots is present in soluble form in vacuoles of root cells. Very little translocation of Cr from the roots to the tops took place except at concentrations of CrO_4^{2-} in the solution high enough to injure the roots.

EXPERIMENTAL SECTION

General Methods. Seeds were germinated in sand and grown with one-half strength Johnson's (Johnson et al., 1957) solution containing FeEDTA. Individual plants were then removed from the sand, the roots were washed, and the plants were placed in polystyrene holders so that the roots were suspended in continuously aerated treatment solution.

Reagent grade chemicals were used and ^{51}Cr was carrier free. Concentrations of stable Cr were determined by the method of Cary and Olson (1975) and ^{51}Cr activity was measured using γ-ray spectrometry (Nuclear-Chicago automatic γ well counting system). A large sample volume detector (Nuclear-Chicago, Tobor) was used for ^{51}Cr activity measurements of roots or tops from entire plants.

Organic Cr complexes were prepared by reacting ^{51}Cr(III) with the ligands at 80 °C for 20 h at a Cr:ligand ratio of 1:3. Cr(III) and Cr(VI) were verified by paper electrophoresis.

Uptake and Translocation of Different Forms of Cr by Various Plant Species. The objective of this group of exploratory experiments was to rapidly survey various crops and different sources of Cr to identify potential opportunities to increase the Cr concentration in the tops of edible plants.

Wheat (*Triticum aestivum* C.V. Chris), corn (*Zea mays*

U.S. Plant, Soil and Nutrition Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ithaca, New York 14853.

[1]Present address: Station Biochemistry, South Dakota State University, Brookings, S.D. 57006.

Table I. Sorption and Translocation of Different Cr Sources from Nutrient Solutions by Different Plants

Source of ^{51}Cr	Tomato		Wheat		Potato		Pea		Bean		Corn	
	% uptake[a]	Ratio[b]	% uptake	Ratio	% uptake	Ratio	% uptake	Ratio	% uptake	Ratio	% uptake	Ratio
$CrCl_3$	44	0.03	73	<0.01	52	<0.01	35	0.03	28	0.03	55	0.02
Na_2CrO_4	65	0.01	44	<0.01	50	<0.01	43	0.03	34	<0.01	32	0.03
Cr oxalate	40	0.01	62	<0.01	43	<0.01	83	>0.01	46	<0.01	30	0.03
Cr tartrate	11	0.03			16	<0.01	3	0.04	16	0.01	13	0.02
CrEDTA	2	0.50			1	0.78	1	3.57	1	9.00	5	0.52
Cr methionine					54	<0.01			45	<0.01		
Cr citrate									12	0.02		

[a] Percent uptake is the percent of the ^{51}Cr removed from 200 ml of solution by one plant in 48 h. [b] Ratio is the ratio of ^{51}Cr in the tops to ^{51}Cr in the roots after 48 h in one-half strength Johnson's solution, following 48-h exposure to solutions containing ^{51}Cr.

v. Royal Crest 69-37), potato (*Solanum tuberosum* v. Sebago), tomato (*Lycopersicon esculentum*), pea (*Pisum sativum*), and red kidney bean (*Phaseolus vulgaris*) were germinated and single plants were placed in one-half strength Johnson's solution (pH 6.5) to which the ^{51}Cr sources were added at a concentration of 0.05 ppm of Cr. The plants remained in the ^{51}Cr solutions for 48 h, with the solution pH readjusted to 6.0 with NaOH at 24 h. After ^{51}Cr exposure the plants were placed in one-half strength Johnson's solution without added Cr for an additional 48 h. The tops and roots were then harvested and ^{51}Cr activity in each was determined. The percent of the ^{51}Cr removed from solution was determined by measuring the ^{51}Cr in solution before and after the exposure period.

The results of these experiments are shown in Table I. Although the measurements shown in Table I involved single plants only, it is obvious that, with the exception of the Cr from the EDTA complex, there was very little translocation of any ^{51}Cr from any Cr source from the roots to the tops in any species. Even though ^{51}Cr from CrEDTA was apparently readily translocated from the roots to the tops, the roots removed very little Cr from this source from the nutrient solution. There were no consistent differences among the Cr(III), Cr(VI), Cr oxalate and Cr methionine treatments in removal from solution or in translocation to plant tops. Chromium from complexes of tartrate and citrate was apparently not sorbed by roots quite as well as some of the other Cr sources.

Very little ^{51}Cr was detected in the one-half strength Johnson's solution used during the 48-h period after exposure of the roots to ^{51}Cr. This indicates that the ^{51}Cr is held tightly in or on the roots and is not appreciably subject to exchange by ions present in Johnson's solution.

Uptake and Translocation of Cr(III) as Affected by Cr Concentration in Nutrient Solutions. The effect of increasing Cr concentration in solution on its uptake and translocation by plants was measured in order to ascertain if the mechanisms involved become saturated at high Cr concentrations. Cr(III) was used in this experiment since previous work indicated that it was less toxic to plants than Cr(VI). Wheat (14-days old) and tomato (17-days old) plants were placed in Johnson's solution containing various concentrations of $^{51}CrCl_3$. After 6 days the plants were removed and the Cr contents of tops and roots were measured. The results with wheat and tomato were similar. The data for tomato are plotted in Figure 1.

The results indicate that the uptake of Cr from solution tended to level off at about 50 ppm of solution Cr. The concentration of Cr in solution required to achieve saturation or near saturation of the uptake process in experiments of this type is probably dependent upon the ratio of root surface to volume of solution. It appears that saturation of the Cr uptake process would not take place at any Cr concentration that could be maintained in a soil solution.

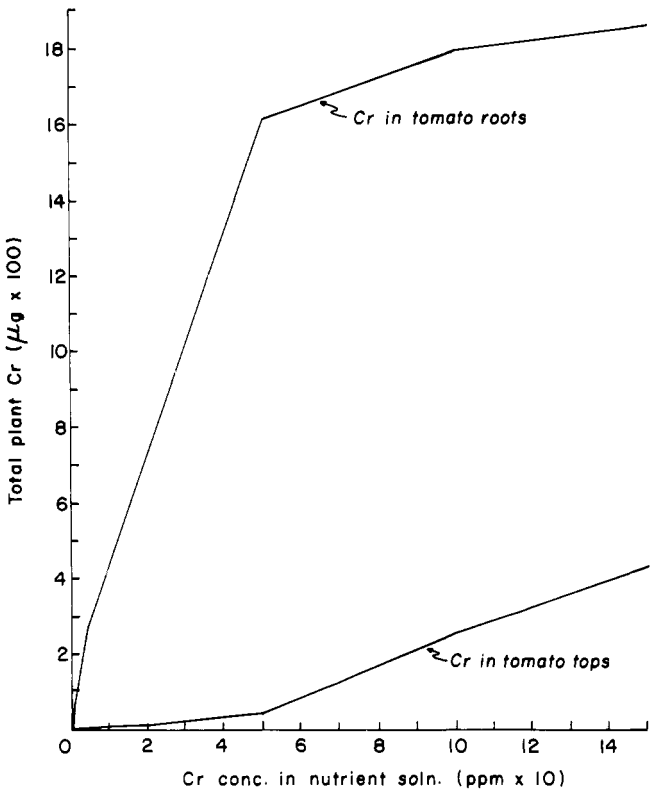

Figure 1. Effect of Cr(III) concentration in nutrient solution on the Cr content of tomato tops and roots.

There was evidence of a breakthrough of Cr from the roots to the tops of tomatoes when the roots were exposed to Cr concentrations in excess of 25 ppm.

In this experiment a floc of Cr hydroxide was apparent in the culture solutions containing 10 ppm of Cr or more. Therefore, at least part of the apparent accumulation of Cr by the plant roots may have been due to precipitation of oxides or hydroxides of Cr on the root surfaces.

Effect of pH on Uptake of ^{51}Cr from Solutions. The uptake of ^{51}Cr from $^{51}CrCl_3$, $Na_2{}^{51}CrO_4$, and [^{51}Cr]oxalate from solutions of pH 5, 6, 7, or 8 was measured using wheat plants 25 to 30 cm tall. The ^{51}Cr was in one-half strength Johnson's solution at a total Cr concentration of 0.05 ppm. The pH of these solutions was adjusted to the starting value at 6 h and again at 24 h with NaOH after the plants were placed in the solutions. The plants were removed from the solutions and ^{51}Cr activity determined after 48 h (Figure 2).

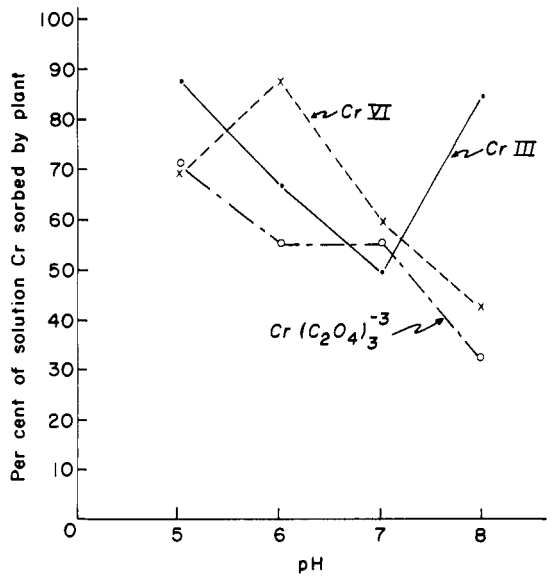

Figure 2. Effect of pH on the removal from nutrient solution of ^{51}Cr added in different chemical forms by wheat plants.

Chromium(VI) was sorbed to a slightly greater extent than either of the other Cr sources at pH 6 to 7. The high removal of $^{51}Cr(III)$ at pH 8 may have resulted from precipitation of chromium hydroxide on the plant roots, since the solubility of $Cr(OH)_3$ is at a minimum at this pH value. In all treatments, over 99% of the ^{51}Cr removed from the solution was still in the roots at the end of the experiment. The results with Cr(VI) are in general agreement with those of Shewry and Peterson (1974).

Uptake of Cr from Soil by Different Plant Species. The objective of this experiment was to evaluate differences among plant species in their ability to take up Cr from soil. To maximize the availability of Cr added to the soil, an alkaline soil (Lima silty clay loam, Glossoboric Hapludalf, pH 7.4) was treated periodically with Cr(VI) in solution while the plants were growing. The mixed soil was placed in two plastic-lined beds (88 × 120 cm, 30 cm deep) in a greenhouse. Each bed contained 187.5 kg of soil placed over a 3-cm base of washed sand. Barley (*Hordeum vulgare*, C.V. Erie), beet (*Beta vulgaris*, C.V. Ruby Queen), buckwheat (*Fagopyrum esculentum*, C.V. New York Common), potato, rutabaga (*Brassica napus*, C.V. York Sweda), snap beans (*Phaseolus* spp., C.V. Midos), spinach (*Spinacia oleracea*, C.V. High Pock), sweet corn, and swiss chard (*Beta cicla*) were seeded in each bed. One of the beds was treated with K_2CrO_4 in solution added in three equal portions, 15, 22, and 29 days after seeding for a total of 0.5 ppm of Cr added to the soil. The other bed was used as a control. Both beds received the same application of N, P, and K.

The potatoes and buckwheat were harvested 70 days after seeding and the other plants were harvested 97 days after seeding. Chromium concentration in these plants is shown in Table II. Pronounced differences in Cr accumulation from the added chromate are apparent in different plant species. Relative differences in Cr concentration among plants on the minus Cr bed were much less than differences among the same species on the Cr-treated bed. The two cereal crops were less effective in Cr accumulation in the leaves than the other species tested.

Chromium–Iron Interactions in Plants. In some of the exploratory studies, the rate of uptake and translocation of ^{51}Cr added to 0.01 M $Ca(NO_3)_2$ solutions was compared with that of ^{51}Cr added to one-half strength Johnson's solution. These comparisons showed more rapid

Table II. Concentration of Cr in Plant Parts as Affected by Addition of 0.5 ppm of Cr as K_2CrO_4 to the Lima Soil

		Cr concn, ppb dry wt	
Species	Tissue	Cr added	No Cr added
Corn	Leaf	418	272
	Stem	100	45
Table beet	Leaf	1400	418
	Stem	335	228
Barley	Stem and leaf	270	160
Swiss chard	Leaf	1080	232
Rutabaga	Leaf	2450	345
	Stem	750	105
Snap bean	Leaf and stem	1100	375
	Pod	198	135
	Seed	12	N.D.
Spinach	Leaf	1412	276
Potato	Leaf	1075	236
Buckwheat	Leaf and stem	3225	375
	Stem	1175	68
	Blossoms	2100	190
	Seed	475	7

Table III. Effect of Deletion of Fe and Other Nutrients from Johnson's Solution on Removal of ^{51}Cr by Wheat Seedlings and on Translocation of ^{51}Cr to the Tops

	% ^{51}Cr removed from solution			^{51}Cr in tops of plants, ppb of Cr
Culture solution[a]	1st day	2nd day	3rd day	
Complete solution	66	80	71	7
Minus P	63	71	69	20
Minus Fe	7	4	0	32
Minus all trace elements except boron	7	0	0	30

[a] Solutions prepared by eliminating nutrients as shown from Johnson's solution.

removal of ^{51}Cr from Johnson's solution but the translocation of ^{51}Cr from roots to tops was frequently increased in plants growing on 0.01 M $Ca(NO_3)_2$. Therefore, experiments were directed toward determining the effects of different nutrient ions on Cr uptake and translocation.

In the first experiment, four wheat seedlings, about 30 cm tall, were placed in each of the following: Johnson's solution, Johnson's solution minus P, Johnson's solution minus Fe, and Johnson's solution minus Fe, Cu, Zn, Mn, and Mo. In the two treatments in which Fe was present it was added as $FeSO_4$. After 3 days in these solutions, the solutions were renewed and 0.05 ppm of Cr as K_2CrO_4 labeled with ^{51}Cr was added to each. The amount of ^{51}Cr removed in 24 h was measured, and the solutions were renewed, including the ^{51}Cr addition for a second 24-h period, followed by a third renewal after another 24-h exposure period. The percent of the ^{51}Cr removed from the solution was again measured at the end of each of these later exposure periods. After the third exposure period, the solutions were renewed without Cr additions, and the plants were grown for an additional 3 days. ^{51}Cr concentrations in the tops and roots of the plants were then measured.

It is evident that removal of ^{51}Cr from the culture solution was greatly enhanced by the presence of $FeSO_4$ (Table III). Removal of trace elements in addition to iron did not change this effect from that of removal of Fe alone. More ^{51}Cr was translocated to the tops of plants growing in the Fe-deficient nutrient solutions, even though these plants had removed much less Cr from the cultures. There was also some enhanced translocation of ^{51}Cr to the tops of the plants growing in P-deficient solutions as contrasted

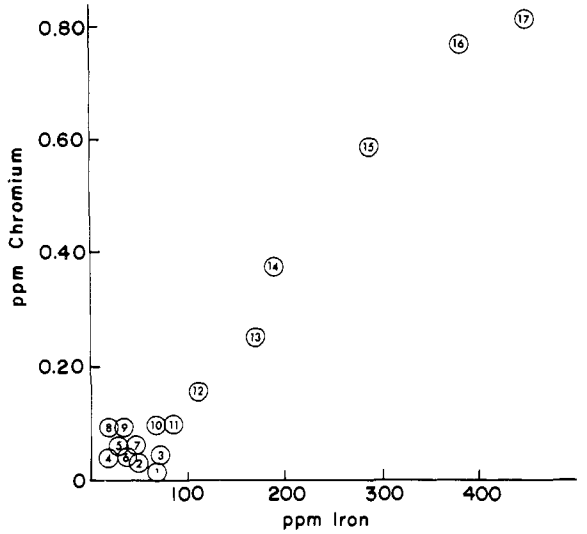

Figure 3. Relation of Fe concentration to Cr concentration in plants grown on adjacent plots of Riverhead fine sandy loam: (1) peas, green seed; (2) beans, dry seed; (3) peas, mature, dry seed; (4) potato, tubers; (5) sweet corn, seed; (6) cauliflower, florets; (7) tomato, fruits; (8) turnip, roots; (9) cabbage, heads; (10) snap bean, green pods; (11) lettuce, heads; (12) beet, roots; (13) carrot, roots; (14) beet, leaves; (15) spinach, mature leaves; (16) spinach, immature leaves; (17) turnip, leaves.

to those in complete solutions, but this may have been due to root injury since the roots of the plants in the P-deficient solutions turned brown after the 4th day. The tops of the plants did not show symptoms of Fe deficiency in any of the treatments.

Further experiments were conducted to measure the effect of different sources of Fe on the uptake and translocation of Cr(III) and Cr(VI) additions to the nutrient solution by Fe-deficient wheat (Sheridan variety, 3–4 weeks old). In these experiments removal of Cr from solutions was again greater when the solutions contained Fe(II) and Cr(VI) (53%) than when Fe(II) was not present (15%). The effects of Fe(II) on the removal of Cr(III) from solution were smaller (39%) than the effects of Fe(II) on the removal of Cr(VI). Iron present as FeEDTA had no measurable effect on the removal of either Cr(VI) or Cr(III) from solution. It therefore appears likely that at least part of the enhanced removal of Cr from solutions shown in Table III is due to reduction of Cr(VI) by ferrous ions and subsequent precipitation of Cr(III) on the roots. Coprecipitation of mixed Fe and Cr hydrous oxides may also be involved in Fe–Cr interactions on removal of Cr from solutions.

Analyses of plants grown side by side in the same field provide additional evidence of a relationship between Fe and Cr in the tops of plants. Chromium and Fe concentrations were measured in edible leaves, seeds, or fruit of a number of vegetable crop species grown in adjacent field plots on Long Island, New York. No Fe or Cr treatments were applied to these plots. The results are shown in Figure 3. A relationship between the concentration of Fe and Cr in different species and different tissues of the same species is evident. The leafy vegetables that accumulate Fe also accumulate Cr. Fleshy roots of beets and turnips had lower Cr concentrations than the leaves, but Cr/Fe ratios were similar. From this it appears that Cr and Fe are both translocated by similar or by related processes.

Concentration of Cr in Plants from Continuous Cr Supplementation. In order to evaluate the upper limits of Cr concentration that might occur in plants, lettuce, spinach, and buckwheat were grown for extended periods in Cr-supplemented nutrient solutions. Seedlings were transplanted to 3.3-l. pots (4 plants per pot) containing one-half strength Johnson's solution plus FeEDTA. The Cr treatments initiated at this time were: 0, 0.05, 0.1, and 0.15 ppm of Cr added to the nutrient solution as Na_2CrO_4. Nutrient solution and Cr treatments were renewed weekly. Lettuce was harvested and dried after 3 weeks. After 6 weeks, the second cutting of lettuce and the first cutting of spinach were taken. After 9 weeks lettuce was harvested for the third time and spinach was harvested for the second time. At this time the Cr treatments on the lettuce and spinach were doubled. These treatments were reapplied every third day over a 6-day interval and regrowth was harvested after 15 days. Buckwheat was harvested after the 14th Cr renewal. The seeds were separated from the remainder of the aerial portion. Chromium concentrations in these plants were determined by analyzing oven-dried, ground samples for total Cr. There were no signs of Cr toxicity.

Table IV. Chromium Concentration in Lettuce, Spinach, and Buckwheat as Affected by Cr(VI) Treatment in Solution Culture

Cr treatment, ppm	Cr concn, ppb of Cr						
	Lettuce cutting			Spinach cutting		Buckwheat	
	1st	2nd	3rd	1st	2nd	Seed	Leaf and stem
None	107	49	80	62	40	N.D.[a]	124
0.05	382	350	321	443	2000		1041
0.10	509	778	806	855	4500		1917
0.15	544	1176	894	1640	5700	391	3288
	4th cutting			3rd cutting			
None	141			187			
0.30	302			2670			
0.45	1750			*b*			
0.60	1950			7840			

[a] Not detectable. [b] Plants died from downy mildew infection.

The results (Table IV) indicate that Cr concentrations in certain plants can be increased to levels that would represent important increases in dietary Cr intake. Differences in Cr accumulation by different species are evident even at these very high Cr levels. The supply of available Cr necessary to ensure these concentrations appears to be higher than that likely to be available to field-grown plants. The Cr concentrations in buckwheat indicate that even though this plant is a Cr accumulator, accumulation of Cr in the seed was only about one-tenth that in the leaf and stem. Low seed concentrations are consistent with those found in seeds of plants grown in the field on Long Island from Figure 2. Edible seeds of peas, dried beans, and sweet corn ranged from 0.01 to 0.04 ppm of Cr. Similar low levels of Cr have been reported in the grain of hard red wheat (Welch and Cary, 1975).

DISCUSSION

Mechanisms involved in Cr uptake and translocation are not well defined. Further clarification may be difficult because of the uncertainty concerning the ionic species of Cr present in different systems. Cr(III) forms inner orbital complexes with many ligands and transformation from one species to another is generally very slow (Mertz, 1969). In addition, Cr(VI) appears to be reduced to Cr(III) during passage from culture solutions to plant leaves, but the site of this reduction is not known and may differ due to small differences in the physiological status of the plant (Lyon et al., 1969a). These factors militate, at this time, against

clear interpretation of many experiments on Cr uptake and translocation.

Even so, the experiments reported here, plus consideration of other research on Cr in plants, permit some useful generalizations concerning the possibility of increasing Cr concentrations in food plants. Plants readily take up several different chemical forms of Cr from culture solutions. Most of this Cr is retained in the roots. The barrier to translocation of Cr from the culture to the top of the plant is not circumvented by addition of any of the inorganic Cr or any of its organic complexes studied in these experiments. It seems unlikely that a Cr compound or complex will be identified that will be highly effective as a soil additive to increase Cr concentrations in food crops.

Even though the tendency to retain Cr in the roots is common to all plant species studied thus far by various workers, there are quantitative differences among plant species in this regard. Of the food crops studied, the leafy vegetables that tend to accumulate Fe (i.e., spinach, turnip leaves, etc.) appear to be the most effective in translocating Cr to the edible tops of the plant. The leafy vegetables such as head lettuce and cabbage that do not accumulate relatively high concentrations of Fe in their leaves are substantially less effective in translocation of Cr to the leaves. All measurements of Cr concentrations in seeds of beans, peas, corn, and wheat have shown very little indication of Cr transport into seeds. Attempts to increase Cr concentration in edible cereals and pulses do not appear to be promising. Under conditions where a supply of Cr is maintained in the nutrient solution, the Cr concentration in the leaves of spinach and lettuce may be an order of magnitude higher than concentrations reported in a survey of Cr in vegetable foodstuffs (Thomas et al., 1974). If the Cr in leafy vegetables is present even in part, in nutritionally effective forms, improvement of leafy vegetables as sources of dietary Cr appears to be promising. However, there is basis for some uncertainty over the nutritional value of the Cr in leafy vegetables. Huffman and Allaway (1973b) found very little absorption of Cr contained in bean leaves when the leaves were fed to rats. Toepfer et al. (1973) reported that Cr in leafy vegetables was not active in the potentiation of insulin unless the vegetables were subjected to acid hydrolysis. On the other hand, Chen et al. (1973) found that oxalate increased intestinal absorption of Cr compared to absorption of $CrCl_3$, an indication that Cr oxalate complexes, a probable form of Cr in plants (Lyon et al., 1969a), may be a nutritionally available source of Cr.

The identity of the form or forms of Cr required to correct impaired glucose metabolism in animals has not been definitely established. There is evidence that Cr(III) coordinated with two nicotinic acid molecules, plus other unidentified ligands, may be especially effective for potentiation of insulin (Mertz et al., 1974). Evaluation of the Cr in leafy vegetables must await a more definite characterization of the nutritionally effective Cr compounds and the development of sensitive assay procedures for these compounds.

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