Chromium in Plants: Distribution in Tissues, Organelles, and Extracts and Availability of Bean Leaf Cr to Animals

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Chromium-51-labeled Cr(III) or Cr(VI) was added in seven increments to plant nutrient solutions, to a final level of 0.044 ppm. The Cr was added over a 14-day period at flowering stage in bean and wheat plants. At maturity bean plants contained about 55% and wheat had removed 81% of the added ^{51}Cr. Bean roots contained about 92% and wheat roots 95% of the total plant ^{51}Cr. Seeds contained only 0.02 to 0.1% of the total plant ^{51}Cr. Uptake and translocation of ^{51}Cr differed only slightly between the Cr(III) and Cr(VI) sources. Most of the ^{51}Cr in fresh bean leaves was present as a soluble low molecular weight anionic complex that was not associated with any subcellular organelle. Rats fed the ^{51}Cr-labeled bean leaves retained less than 0.5% of the dose after 48 hr.

Recent evidence has shown that Cr is essential in animal and human nutrition (Mertz, 1969; Schroeder, 1968; Underwood, 1971). Although there is a lack of information on effective sources of dietary Cr, plants may be a major source of Cr for animals and man. This work was undertaken to determine how much Cr is taken up by plants from low Cr nutrient solutions, to identify forms of Cr in plants, and to determine the availability of plant leaf Cr to rats.

Although beneficial effects of Cr on plants have been reported, the essentiality of Cr for plants has not been established. Chromium generally appears to accumulate in plant roots and is poorly translocated to the tops (Lyon *et al.*, 1969b; Tiffin, 1972; Turner and Rust, 1971). Lyon *et al.* (1969a,b) reported that in the Cr-accumulator plant *Leptospermum scoparium*, Cr was translocated in the xylem sap as chromate and was present in roots and leaves as three anionic Cr complexes, one of which was a trioxalatochromium(III) complex.

Mertz and Roginski (1971) reported that an unidentified Cr-containing compound called glucose tolerance factor (GTF) was more readily absorbed by animals than inorganic Cr and was more effective than inorganic Cr as a cofactor for insulin in glucose metabolism. *In vitro* oxidation of glucose by rat epididymal fat tissue as a measure of GTF showed brewers' yeast, liver, and wheat grain to be good sources of GTF (Toepfer *et al.*, 1973). Spinach, potatoes, and carrots contained no detectable GTF.

MATERIALS AND METHODS

Plant Culture and Labeling. Wheat (*Triticum aestivum*) and beans (*Phaseolus vulgaris*) were grown in purified nutrient solution (<0.02 ppb of Cr) in 3.2-l. polyethylene containers as described by Huffman and Allaway (1973). At flowering stage in the bean and boot stage in the wheat, the nutrient solutions were replaced and radiochromium treatments were initiated.

A stock radiochromium solution of known specific activity was prepared by adding carrier-free $^{51}CrCl_3$ (obtained from New England Nuclear, Boston, Mass.) to 2.5 ml of a 200 μg of Cr/ml of $CrCl_3$ solution and oxidizing the mixture according to the method of Urone and Anders (1950). The solution was diluted to a final concentration of 10 μg of Cr/ml. The Cr(VI) treatment was made by adding 2 ml of the stock solution and 0.8 ml of 0.1 *N* acetic acid to the nutrient solution. The Cr(III) treatment was prepared by mixing 2 ml of the stock solution and 0.8 ml of 0.1 *N* acetic acid in a vial and reducing the Cr with 0.6 ml of 0.001 *M* ascorbic acid. This solution was then added to the nutrient solution.

Treatments were added every other day, for a total of seven additions. On day 22 (9 days after final treatment), the solution in the culture containers was made to volume and sampled. The nutrient solutions were then changed and no additional Cr was added. The wheat was grown to maturity (40 days) and the bean was grown until several dry mature pods were present (50 days). The plants were harvested and separated into seed, chaff or pods, leaves, stems, and roots. The tops of the wheat plants and the bean seeds were air dry when harvested. The other plant tissues were freeze-dried. After drying, roots were pulverized in plastic bags and the other plant tissues were ground in a Wiley mill to pass a 20-mesh chrome-plated screen.

Samples of each of the plant tissues were weighed and assayed for radioactivity in a γ spectrophotometer.

Extraction Procedure. Samples of the dried plant tissues were extracted successively (Lyon *et al.*, 1969b) with boiling 80% ethanol, boiling water, 0.2 *N* HCl, 0.5 *N* $HClO_4$ at 80°, and boiling 2 *N* NaOH. Acetone was added to the 0.2 *N* HCl and 0.5 *N* $HClO_4$ extracts to give acetone precipitate from HCl-soluble and acetone precipitate from $HClO_4$-soluble fractions, respectively. The fractions were concentrated by evaporation and assayed for radioactivity.

Organelle Separation. Several weeks after radiochromium treatment, 5-g (fresh wt) samples of leaves were taken and homogenized at 0° in 25 ml of 10 m*M* potassium phosphate buffer (pH 7) containing 0.5 *M* sucrose, 2 m*M* cysteine, 2 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, and 0.1% bovine serum albumin. Each homogenate was filtered through cheesecloth, and differential centrifugation was used to separate the various organelle fractions. The nuclei and debris pellet was collected at 1500 × *g* for 2 min, the mitochondrial pellet at 15,000 × *g* for 15 min, and the microsomal pellet at 100,000 × *g* for 60 min. All pellets were washed once with buffer but the washings were not recombined with the supernatant. The pellets and an aliquot of the supernatant were assayed for radioactivity.

Forms of Cr in Leaf Supernatant. The supernatant from the differential centrifugation of leaf tissue was subjected to several physical and chemical separation techniques, including dialysis, 100% saturation $(NH_4)_2SO_4$ precipitation, 10% cold trichloroacetic acid precipitation, membrane filtration (Centriflow-CF50A, Diaflow UM-2 and UM-10), gel permeation chromatog-

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raphy (Sephadex G-75 and G-10), high voltage electrophoresis (Whatman No. 1 paper, 1.5 kV, 9 mA, 10 m*M* potassium phosphate [pH 7] buffer), and paper chromatography (descending on Whatman No. 1 paper with 3:1:1 methanol–butanol–water irrigant). The sheets from electrophoresis and paper chromatography were cut into 1- or 2-cm strips and the strips were assayed for radioactivity.

Preparation of ^{51}Cr-Labeled Compounds. Three ^{51}Cr-labeled organic acid complexes were prepared. Chromium oxalate was prepared by combining 0.077 μmol of the Cr(VI) stock solution with 0.48 μmol of oxalic acid. The solution was made acid with HCl and heated at 90° overnight. Chromium citrate was prepared by combining 0.192 μmol of ^{51}Cr-labeled $CrCl_3$ with 0.58 μmol of citric acid and heating the mixture overnight at 90°. Chromium aconitate was prepared by combining 0.1 μmol of the Cr(VI) stock solution with 4 μmol of *trans*-aconitic acid and heating the mixture overnight at 90°. The chromate standard was prepared by diluting the Cr(VI) stock solution 1:50 with water.

Rat Feeding Experiment. Eight male Sprague–Dawley rats (48 days old) raised from weaning on a commercial dog food diet (containing 2.5 μg of Cr/g) were obtained from a colony maintained at this laboratory. The rats were placed in metabolism cages. Food was withdrawn for 18 hr before the rats were treated with radiochromium from either the plant or inorganic Cr sources. Leaves from beans grown on a Cr(VI) treatment and on a O-Cr treatment were harvested, boiled in a small amount of water to destroy proteolytic inhibitors, and then freeze-dried. The plant source radiochromium treatment was prepared by mixing 0.32 g of the pulverized freeze-dried leaves containing ^{51}Cr with 1.6 g of pulverized diet. The inorganic Cr treatment was prepared by adding sufficient ^{51}Cr(VI) stock solution to 0.32 g of O-Cr bean tissue to give approximately the same number of counts as the plant source treatment and mixing this with 1.6 g of pulverized diet. The rats were paired by weight; one of each pair was fed the plant Cr source and the other the inorganic Cr source. After 4 hr, the labeled feed was removed and the rats were assayed for radioactivity in a whole body counter. They were subsequently counted at 12, 24, 48, and 72 hr. Urine and feces were collected at 12, 24, 48, and 72 hr, concentrated by evaporation, and then assayed for radioactivity. After 72 hr, the rats were killed and samples of blood, muscle, liver, kidney, spleen, pancreas, and epididymal fat were taken and assayed for radioactivity.

Total Cr in the rat diet and in the plant tissues was determined by the method of Cary and Allaway (1971).

RESULTS AND DISCUSSION

Uptake of Cr. Table I shows the amount of ^{51}Cr remaining in the nutrient solution immediately before the final ^{51}Cr addition and 9 days after the final treatment with radiochromium. Wheat appeared to be more efficient in removing Cr from solution than was bean. Within species, there was little difference between the uptake of ^{51}Cr from the Cr(VI) or Cr(III) source. The chemical form of Cr in the nutrient solutions after addition of the Cr treatment is not known. Since the pH of the nutrient solution was slightly acid, Cr(VI) may have been reduced by reducing substances in the nutrient solution. Thermodynamic calculations, using the standard free energies of formation values summarized by Garrels and Christ (1965), suggest that under sufficiently oxidizing conditions Cr(VI) would predominantly exist as the $HCrO_4^-$ and CrO_4^{2-} ions. Chromium(III) in the nutrient solution was probably complexed by the various anions present.

The Cr concentration used in the nutrient solution was selected to be nontoxic. Above 0.05 μg of Cr/l. of solution, decreases in plant growth have been reported (Haas and Brusca, 1961). In other work at this laboratory, the distribution of Cr in bean and wheat plants that were supplied Cr throughout their growing period was similar to that found in this study with the 22-day uptake period. The Cr was added in the seven alternate-day additions to maintain low nutrient solution concentration to prevent extraneous precipitation or other reactions as a result of adding a single large dose.

Table I. Percent of ^{51}Cr Remaining in Nutrient Solution at Final Treatment and 9 Days after Final Treatment with ^{51}Cr-Labeled Cr(III) or Cr(VI)

Plant	Treatment	% ^{51}Cr remaining before final treatment	% ^{51}Cr remaining after 9 days
Bean	Cr(VI)	41	28
	Cr(III)	44	21
Wheat	Cr(VI)	4	6
	Cr(III)	4	5

If uptake is calculated on the basis of total Cr in the plants at harvest, the bean plants contained an average of 55% of the total ^{51}Cr added and the wheat plants contained an average of 81% of the added ^{51}Cr. This Cr must be tightly bound by the plant roots since the culture solution was replaced with a low Cr nutrient solution four times after the radiochromium treatment. The 15 to 20% difference between the data for removal of ^{51}Cr from solution and the final plant content of ^{51}Cr was probably a result of absorption of Cr by the culture container and removal by subsequent Cr-free nutrient solutions.

Distribution of Cr in Plant Tissues. Table II shows the concentration of Cr and percent of total plant Cr in the various plant tissues after harvest. The Cr concentrations shown were calculated from radiochromium activity in the plants and the known specific activity of the stock radiochromium solution. Chemical analysis of the bean tissues gave values that generally agreed within 20 ng of Cr/g of the value calculated from the radiochromium measurements. Analysis of the wheat tops gave values considerably higher than those computed from radiochromium measurements. It is suspected that this represents surface Cr contamination of the wheat tissue.

In the plant tops the Cr concentration was highest in leaves and lowest in seeds. The apparent exclusion of Cr from seeds may result from lack of a direct connection of vascular tissue to developing seeds (Esau, 1965). Nutrients must cross cell membranes to reach the developing seed and these membranes may restrict the movement of Cr into the embryo.

Other investigators have reported that Cr is readily absorbed by plant roots but little is translocated to the tops unless very high concentrations of Cr are present in the rooting media (DeKock, 1956; Lyon *et al.*, 1969b; Tiffin, 1972; Turner and Rust, 1971). However, Hunter and Vergnano (1953) reported that even in cases of Cr toxicity, high concentrations of Cr were not necessarily found in plant tops. Lyon *et al.* (1969b) reported root-to-top ratios of Cr concentration greater than 100 for the Cr-accumulator plant *L. scoparium* after a 24-hr absorption period.

Extraction of Cr in Plants. Table III shows the percentage of ^{51}Cr extracted by the various extractions of Cr(VI)-treated plant tissues. The percentages of Cr extracted from tissues of plants grown on the Cr(III) source were similar to those for Cr(VI)-treated plants. Because of the low ^{51}Cr activity in seeds and the difficulty of extracting seeds with water, only the values for ethanol extractions are reported.

Within species, the extractability of Cr differed little between Cr(VI)- or Cr(III)-treated plants. In general, the distribution of Cr in all plant tops was similar. Most of the Cr in all of the plant tops (excluding seeds) was in the water- and acid-soluble fractions. These data suggest that

Table II. Distribution of ^{51}Cr in Wheat and Beans Grown in Solution Culture with ^{51}Cr-Labeled Cr(III) and Cr(VI)

	Bean				Wheat			
	Cr(VI)		Cr(III)		Cr(VI)		Cr(III)	
Plant tissue	ng Cr/g	% total Cr	ng Cr/g	% total Cr	ng Cr/g	% total Cr	ng Cr/g	% total Cr
Seed	3	0.03	2	0.02	1	0.1	1	0.1
Chaff (pods)	32	0.5	50	0.9	18	0.9	16	1.1
Stems	26	1.1	37	1.5	21	3.0	15	1.7
Leaves	144	5.1	166	6.5	74	1.1	64	1.4
Roots	3791	93.2	3096	91.5	5378	94.9	3982	95.7

Table III. Percent of ^{51}Cr Extracted by Various Extractions of Wheat and Beans Grown in Solution Culture with ^{51}Cr-Labeled Cr(VI)

Plant	80% ethanol	Ether, %	Water, %	HCl, %	Acetone precipitate from HCl-soluble, %	$HClO_4$, %	Acetone precipitate from $HClO_4$-soluble, %	NaOH, %	Residue, %
Wheat									
Grain	49								
Chaff	9	2	24	31	2	18	3	5	6
Stems	16	0	67	5	3	1	2	3	4
Leaves	4	0	47	29	1	12	0	5	2
Roots	3	0	9	58	1	9	1	14	4
Bean									
Grain	41								
Pods	10	1	42	17	4	10	1	6	9
Stems	22	2	28	17	4	18	1	4	4
Leaves	16	0	39	25	1	12	0	4	3
Roots	5	1	6	2	0	13	1	37	34

Cr from plant tops would be soluble in the stomach of a monogastric animal. However, a difference was noted between the extractability of Cr in wheat and bean roots. In wheat roots, most of the Cr was present in the HCl-soluble fraction whereas in the bean roots, most of the Cr was in the NaOH-soluble and residue fractions. As noted above, wheat roots were more efficient than bean roots in removing Cr from the nutrient solution. These data suggest that wheat roots may accumulate Cr in soluble forms, possibly in the vacuole, whereas bean roots may immobilize substantial amounts of Cr by nonspecific absorption on cell walls or by precipitation in or on the roots. Lyon *et al.* (1969b) reported that in *L. scoparium* roots, which were allowed to absorb radiochromium for 24 hr, 32% of the root Cr was ethanol-soluble, 27% water- and HCl-soluble, 16% $HClO_4$-soluble, 15% NaOH-soluble, and 7% remained in the residue. Although the method of labeling differed somewhat from the experiment presented here, a comparison of their results with ours suggests that incorporation of Cr into roots varies considerably among species.

Toepfer *et al.* (1973) reported that Cr in wheat grain appears to be present as GTF and the level of alcohol-soluble Cr in grain correlates positively with GTF activity, as measured by the epididymal fat test. They suggest that plant tissues other than grain do not contain biologically active Cr even though they may contain high levels of ethanol-extractable Cr.

Bowen *et al.* (1962) determined the distribution of several elements in successive extractants of tomato leaves. Although there are some similarities, none of the elements tested (Ca, Co, Cu, Fe, Mn, Mo, K, Na, W, Zn) appear to have a distribution identical with that of Cr in wheat and bean leaves.

The observation that only small amounts of Cr are present in protein (acetone precipitate from HCl-soluble and NaOH-soluble) or nucleic acid (acetone precipitate from $HClO_4$-soluble) fractions agrees with the data of Lyon *et al.* (1969b) for *L. scoparium*. However, this does not agree with the conclusion of Bourque *et al.* (1966), who reported that Cr in wheat roots was predominantly bound to protein.

Results of an extraction procedure such as used here must be used cautiously. Chemical changes early in the extraction procedure may cause artifacts in subsequent extractions.

Organelle Separation. Table IV shows the distribution of Cr among the various subcellular fractions after differential centrifugation of leaf homogenates. Regardless of Cr source or species, most of the ^{51}Cr in the leaf was present in the final supernatant. The leaf Cr was also soluble in the final supernatant from homogenates obtained using a 10 m*M* morpholinopropane sulfonic acid buffer (pH 5.5).

The data from the successive extraction of dried leaves and the data from the organelle separation showing the high extractability of Cr from fresh leaves with phosphate buffer do not appear to be compatible. However, if one considers that the phosphate buffer would likely extract the ethanol-, water-, and acid-soluble Cr fractions, the discrepancy is lessened. The extraction data represent a different type of chemical removal than the organelle separation. When boiled, freeze-dried bean leaf tissue was suspended in phosphate buffer and centrifuged, most of the ^{51}Cr was in the supernatant.

Mathur and Doisy (1972) determined the distribution of ^{51}Cr in liver fractions of rats after an intravenous injection of $CrCl_3$. They found only 12 to 18% of the Cr in the supernatant and the rest was distributed between the nuclear, mitochondrial, and microsomal fractions. The fact that their results differ from ours suggest that Cr is distributed differently in animal cells than in plant cells.

Forms of Cr in the Supernatant. Cold TCA precipitated less than 2% of the ^{51}Cr in the supernatant from the wheat and beans grown on the Cr(VI) treatment, but precipitated 14% from the wheat and beans grown on the Cr(III) treatment. The reason for this difference between the Cr(III) and Cr(VI) treatment, in light of the other observed similarities, is not known. In either case, the data

Table IV. Distribution of ^{51}Cr in Various Subcellular Fractions after Differential Centrifugation of Leaf Homogenates

Plant	Treatment	% ^{51}Cr in fraction[a]			
		Nuclei and debris	Mitochondria	Microsome	Supernatant
Wheat	Cr(III)	3	2	1	89
	Cr(VI)	4	2	1	87
Bean	Cr(III)	4	4	2	79
	Cr(VI)	4	3	1	86

[a] Values do not add to 100% because pellet washings were not recombined.

suggest that only a fraction of the total Cr is associated with protein or nucleic acid.

The ^{51}Cr content of the supernatant was not changed by passing it through a Centriflow filter with a 50,000 molecular weight cutoff. From 60 to 90% of the Cr was retained by ultrafilters with a 10,000 or 1000 molecular weight cutoff. However, passing the supernatants through a G-75 Sephadex column suggested a molecular weight of less than 3000. Labeled Cr oxalate and chromate solutions were then tested with the 10,000 and 1000 molecular weight cutoff Diaflow membranes, and 84 to 89% of the ^{51}Cr was retained by the membranes. Other low molecular weight anions (*e.g.*, VO_3^-) do not interact with the Diaflow membranes. The authors have no explanation of why chromate and the anionic Cr(III) complexes do interact with the membranes.

The chemical form of Cr in leaves was further investigated using the final supernatant from Cr(VI)-treated bean leaves. All of the Cr in the supernatant was dialyzable against phosphate buffer.

Gel permeation chromatography using Sephadex G-10 gel showed that the ^{51}Cr in the final supernatant was eluted in one peak and in the same volume with Cr citrate but before Cr oxalate, Cr aconitate, or chromate. Labeled $CrCl_3$ solution could not be completely eluted from the column with phosphate buffer.

Figure 1 shows a normalized and combined electrophoresis pattern for the supernatant and various synthesized Cr complexes. The ^{51}Cr in the supernatant appears as a single peak which does not run identically with any of the compounds tested.

Paper chromatography of the supernatant and the various Cr compounds did not give a clear separation in the system used. The ^{51}Cr activity in all samples streaked from the origin for a distance of several centimeters.

Further investigation is needed to conclusively identify the form of Cr in the bean leaf supernatant. In all the experiments described above, the leaf Cr appeared as a single compound that behaved similarly but not identically to the synthesized anionic Cr complexes. It is apparent from this preliminary work that Cr is predominantly present in bean leaf tissue as a single anionic complex of low molecular weight.

Absorption of Plant Cr by Rats. Table V summarizes the data on the retention of ^{51}Cr by rats fed radiochromium from either an inorganic or bean leaf source. After 48 hr, less than 0.5% of the Cr from either source remained in the rats. Over 95% of the initial activity was present in the 24-hr fecal sample. The organ and tissue samples contained less than 0.2% of the initial dose. The average urinary excretion of Cr from rats fed the plant source Cr was 0.5% of the initial dose and in rats fed the inorganic Cr source it was 0.3% of the dose.

The absorption of ^{51}Cr from both sources was surprisingly low. MacKenzie *et al.* (1959) reported that fasted rats fed $Na_2{}^{51}CrO_4$ excreted about 6% of the dose in the urine, whereas nonfasted rats excreted about 3% in the urine. All but a fraction of a percent of the remainder of

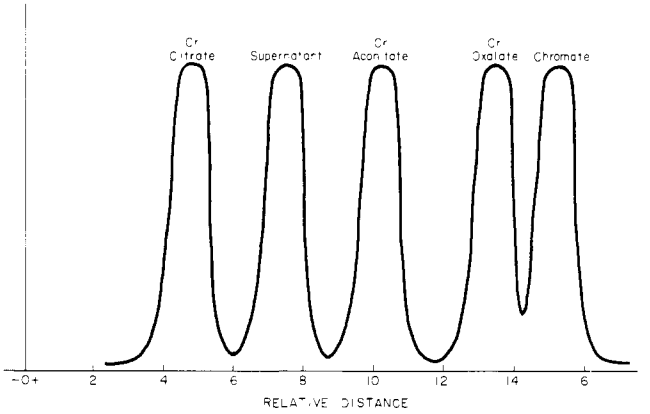

Figure 1. Normalized electrophoresis pattern of ^{51}Cr from bean leaf supernatant and various Cr compounds.

Table V. Chromium Remaining in Rats at Various Times after Feeding Bean Leaf Tissue with Incorporated ^{51}Cr or Inorganic Cr

Treatment[a]	% ^{51}Cr remaining in rat				
	4 hr[b]	12 hr	24 hr	48 hr	72 hr
^{51}Cr bean	100	55.6	1.9	<0.5	<0.5
CrO_2^{2-}	100	41.5	1.1	<0.5	<0.5

[a] Average of four rats. [b] Radioactivity in rat 4 hr after feeding was taken as 100% dose.

the dose was excreted in the feces. Visek *et al.* (1953) reported that less than 0.5% of an oral dose of $^{51}CrCl_3$ was absorbed from the gastrointestinal tract of a rat. Donaldson and Barreras (1966) concluded that if chromate can be protected from reduction in the stomach it is highly absorbed by the intestine, but if it is reduced to trivalent Cr, it is poorly absorbed. It would seem likely then that the Cr(VI) added to the diet was reduced to Cr(III) in the diet or in the stomach and for this reason was very poorly absorbed.

Rollinson (1966) suggested that the poor absorption of Cr(III) from the intestine is due to the olation and insolubility of Cr at neutral pH's. He postulated that complexing agents that will stabilize Cr in solution at these pH's may enhance the absorption of Cr. Using everted jejunum segments, Mertz and Roginski (1971) found that complexes of Cr with several amino acids were absorbed more readily than inorganic Cr. The Cr in the bean leaf was soluble in a pH 7 phosphate buffer, but absorption of the leaf Cr by the rat was not increased. It is possible that the bean leaf complex was destroyed in the rat's stomach.

Mertz *et al.* (1965) and Hopkins and Schwarz (1964) showed that the absorption of $CrCl_3$ by rats is a fairly constant percentage of the dose, regardless of the size of the dose or Cr status of the animal. Thus, it is not likely that the low absorption of Cr found in this experiment can be attributed to the Cr status of the rats.

CONCLUSION

This work indicates that plants may restrict the movement of nutritionally effective forms of Cr from the soil into human and animal diets. Most of the Cr taken up from soil would remain in the plant roots. Of the Cr translocated to the tops, most is present in leaves and this Cr is not utilized by animals. Based on the work by Toepfer *et al.* (1973), Cr might be expected to be present in nutritionally effective forms only in the seeds of plants, and only very little of the Cr taken up from the soil by plants is translocated to the seeds.

A search for food plant species that accumulate nutritionally effective forms of Cr in edible portions of plants may be worthwhile.

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Cucurbit Seeds. II. Nutritive Value of Storage Protein Isolated from *Cucurbita foetidissima* (Buffalo Gourd)

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The storage globulin of *Cucurbita foetidissima* H.B.K. seed was isolated and evaluated nutritionally. Arginine, aspartic acid, and glutamic acid were the most abundant amino acids. Ratios of the content of each essential amino acid to the content of total essential amino acids indicated that lysine, cysteine, and tryptophan were low and methionine and isoleucine were borderline. With respect to total protein nitrogen furnished by essential amino acids, the globulin was between soybean protein and proteins of cottonseed, sunflower, and peanut. The corrected protein efficiency ratio was 1.66 compared with casein at 2.50 g of weight gain of weanling rats per g of protein intake. In these respects, *C. foetidissima* globulin resembled globulins of other oilseeds.

In view of the increasingly large demand for protein to support a burgeoning world population, oilseeds are being considered as primary nutritional and economic sources of edible protein for foodstuffs (Altschul, 1962). In this regard, *Cucurbita foetidissima* H.B.K. should be investigated as a valuable source of oilseed protein. *C. foetidissima* (Buffalo Gourd) is a wild xerophilous species that potentially could yield 3000 lb of seeds/acre in desert regions (Curtis, 1946). Decorticated seeds, which comprise about 70% of the weight of whole seeds, contain about 50% oil and 35% protein. (For a review of cucurbit seed composition, see Jacks *et al.* (1972).) Since the oil is edible (Bolley *et al.*, 1952; Shahani *et al.*, 1951) and since large scale isolation of purified oilseed protein is readily accomplished by current technology (Meyer, 1971), examination of the nutritional aspects of *C. foetidissima* seed protein was of interest. Amino acid composition, amount of each essential amino acid relative to the total amount of essential amino acids (A:E ratios), amount of protein nitrogen supplied by essential amino acids (E:T ratio), amount of weight gain of weanling rats per unit of protein intake (PER), and digestibility of crystallized *C. foetidissima* globulin are reported here.

MATERIALS AND METHODS

C. foetidissima seeds, obtained from W. P. Bemis, University of Arizona, Tucson, Ariz., were dehulled in a Bauer mill. Hulls were removed with a Bates laboratory aspirator and then by hand-picking. Decorticated seeds were pulverized with a pin mill, and 960 g of powder was extracted with 2.5 l. of hexane–acetone (3:2, v/v) three times by methods described previously (Jacks *et al.*, 1970). Globular protein was isolated from dried meal by the procedure of Vickery *et al.* (1952). The yield from 535 g of oil-free meal, prepared from 960 g of powdered whole seed, was 205 g of crystallized globulin. This globulin con-

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