Chemical Form of Technetium in Corn (Zea mays) and the Gastrointestinal
Absorption of Plant-Incorporated Tc by Laboratory Rats*

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ABSTRACT

The purpose of this study was to investigate the food chain availability of technetium incorporated into plant tissue, its chemical form in corn leaves, and the potential for gastrointestinal absorption of plant-incorporated technetium. Technetium-95m was biologically incorporated into corn leaves (Zea mays) via root uptake. Chemical fractionation of the ^{95m}Tc in corn leaves showed that approximately 60% was extractable with boiling ethanol and weak mineral acids. The remainder of the technetium was associated chiefly with the cell walls and was extractable only by harsh chemical treatment (boiling 2 M NaOH). Gel permeation chromatography (Sephadex G-25) of the corn leaf cytosol, obtained by centrifugation of homogenates, indicated that about 50% of the ^{95m}Tc co-chromatographed with anionic pertechnetate; however, it was impossible to distinguish if this was pure pertechnetate or technetium complexed with small organic molecules. Technetium-95m was administered to laboratory rats in a single dose in the following ways: (1) intravenous injection of pertechnetate, (2) pertechnetate mixed with a standard laboratory food, and (3) a meal containing ^{95m}Tc biologically incorporated into corn leaves. High concentrations of ^{95m}Tc were found in the thyroids, hair, kidneys, and liver of rats that were injected or fed the contaminated diets. Technetium rapidly disappeared from the liver, kidneys, and other tissues, but there was no loss from the thyroids or the hair for two weeks after administration. The urinary excretion of technetium decreased, and its fecal excretion increased when technetium was fed to rats as ^{95m}Tc incorporated into corn leaves. The percent of the administered dose absorbed into the rat thyroid gland and the kidneys was less when technetium was biologically incorporated into corn leaves than when pertechnetate simply was mixed with rat food. Biological incorporation of technetium into plants appears to reduce the potential of this radionuclide for food chain transfer by decreasing its availability for gastrointestinal absorption.

INTRODUCTION

Technetium-99, which undergoes radioactive decay by beta emission, has a radioactive half-life of approximately 213,000 years. This radionuclide has a high fission yield associated with the use of all types of reactor fuel, including plutonium, uranium, and thorium. Releases of Tc-99 may occur during reprocessing of spent reactor fuel, from the conversion of this reprocessed fuel to UF_6 , and from the enrichment of this recycled material. Releases may also occur at sites designated for the disposal of radioactive wastes generated from the preceding operations. Prior research has shown that Tc-99 has an extremely high potential for uptake via plant roots into the terrestrial food chain [1]. The mobility of technetium into plants from contaminated soils can be attributed to the high water solubility and the environmental stability of the pertechnetate anion. Therefore, under circumstances of environmental releases of Tc-99, plants may be an important source of technetium for food chain transfer to animals and humans [2].

In this work, we have used a short-lived (60-d half-life) gamma-emitting isotope, ^{95m}Tc , to make inferences about the behavior and fate of Tc-99 in the food chain. We have characterized the chemical form of technetium in corn leaves, an important constituent of livestock silage, and conducted feeding studies to determine the availability of plant-incorporated technetium when fed to rats.

MATERIALS AND METHODS

PLANT CULTURE

Corn plants (Zea mays) were grown under greenhouse conditions for six weeks in 4.5 L of nutrient solution. After germination, the technetium-95m, in pertechnetate form, was supplied to the plants in fresh nutrient solution. The nutrient solution was changed after the first three weeks of culture with the addition of ^{95m}Tc . The technetium concentration in the nutrient solution was maintained at approximately 60 - 80 μCi $^{95m}Tc/L$, with a calculated concentration of 0.313 μCi Tc-99/L (the latter served as a carrier). After six weeks of growth in the contaminated nutrient solution, the corn was harvested and separated into leaves, stalks, and roots and then frozen. Portions of the corn leaves were freeze-dried and used to prepare a contaminated diet for the rodent-feeding studies.

CHEMICAL EXTRACTIONS

Fresh and freeze-dried samples of corn leaves were successively extracted according to the methods of Bowen et al. [3]. Each extraction was replicated once. The plant material was first macerated in boiling 95% ethanol (5 min), followed by centrifugation to separate the residue from the supernatant. The residue was extracted twice more with boiling ethanol (2 min), and the combined supernatants were filtered to obtain a clear ethanol-soluble fraction. The residue was then successively extracted three times with 0.2-M hydrochloric acid (room temperature), three times with 0.5-M perchloric acid (80°C), and once with boiling 2-M NaOH (10 min). Centrifugation (12,000 g for 10 min), followed by filtration of the pooled supernatants, was used to obtain a clear extract following each chemical treatment. Equal volumes of acetone were added to the acid extracts to precipitate the "acetone-insoluble" fractions. The gelatinous precipitates were collected by centrifugation and washed twice with a small volume of 50% acetone. Technetium-95m in extracts, acetone insoluble fractions, and the residue was analyzed by gamma spectrometry.

After thawing, frozen corn leaves (chopped into 1-cm long pieces) were also extracted with demineralized water to determine the amount of ^{95m}Tc in the plant tissue that was removable by three successive overnight soakings at 4°C. Extracts were concentrated by evaporation and analyzed for ^{95m}Tc .

DIFFERENTIAL CENTRIFUGATION

Samples of fresh and freeze-dried corn leaves were homogenized at 4°C in 50 mL of 0.02-M tris-HCl buffer (pH 7.2) containing 0.5-M sucrose and 0.01-M magnesium chloride. Each homogenate was filtered through cheesecloth, and the filtrate was centrifuged first at 10,000 g for 20 min to collect cell debris, nuclei, plastids, and mitochondria and then at 100,000 g for 70 min to separate the microsomal pellet from the "postmicrosomal supernatant" or cell cytosol. Pellets and aliquots of the supernates were analyzed for ^{95m}Tc by gamma spectrometry.

CHEMICAL FORM IN THE POSTMICROSOMAL FRACTION

The postmicrosomal supernatants (5 mL) from the prior centrifugation of freeze-dried and fresh corn leaves were chromatographed using Sephadex G-50 (MW fractionation range 1,500 - 30,000) and G-25 (MW fractionation range 1,000 - 5,000). The column dimensions were 79 x 2.6 cm for the G-50 and 95 x 2.6 cm for the G-25. The columns were equilibrated with the eluant (0.02-M tris-HCl buffer, pH 7.2) and housed in a refrigerated room at 4°C. Quality of the column packing was evaluated, and the void volume was determined twice for each column by chromatographing blue dextran (MW 1,000,000). The flow rate was approximately 0.4 mL/min for all fractionations. Eluant from the columns was monitored continuously for UV absorbance at 280 nm (to detect proteins and polypeptides containing aromatic acids) and collected in 13-mL fractions by an automatic sample collector. A sample of ^{95m}Tc in the form of ammonium pertechnetate was chromatographed on the G-25 column for comparison with chromatographs of the cell cytosol from homogenized corn leaves. The amount of ^{95m}Tc in the various fractions was analyzed by gamma spectrometry.

ANIMAL FEEDING STUDIES

Adult, female, Wistar rats (R/cnb inbred strain; 200-g body weight) were used in the feeding trials. Rats each received one of the following treatments: 1) a single intravenous injection of a solution containing 2.6 μCi of ^{95}mTc in pertechnetate form (i.v. treatment); 2) a single overnight feeding of ^{95}mTc (pertechnetate) added to a pulverized standard laboratory ration (chow) to give a dose of 3.3 μCi per rat (Tc + chow treatment); 3) a single overnight feeding of ^{95}mTc contaminated corn leaves that were freeze dried, milled, and mixed in a ratio of 1-g powdered corn leaves to 10-g clean, pulverized chow to give a dose of 8.0 μCi per rat (Tc + corn treatment). The contaminated diets were prepared by adding approximately 30 mL of water to 30 g of the mixed dry food and drying at 40°C to form hardened pellets. The contaminated diets were also extracted according to the methods of Bowen et al. (explained above) to characterize the form of ^{95}mTc in the two types of food. The rat chow contained (RMH-B from Hope Farms) 25% protein, 6.2% fat, 58.4% carbohydrates, and 6.1% minerals.

The rats were housed in the laboratory at room temperature in cages with wire screen floors that permitted the collection of urine and feces. Rats were given access to the contaminated food in the evening. Uneaten food was removed from the cages and weighed the following morning to determine the administered dose. During the first seven days after injection or feeding, excreta were collected and analyzed for ^{95}mTc . There were 12 rats in each treatment group. Three rats from each of the experimental groups were killed on days 1, 3, 7, and 14 after administration of the ^{95}mTc . The rats were dissected, and 18 different body tissues were sampled, weighed, and analyzed to determine the concentration of ^{95}mTc . Blood was obtained by heart puncture. Hair and skin were sampled from a small area on the abdomen. The fat sampled was abdominal fat. The contents of the stomach and intestines were extruded and were not included with the intestinal tissue for analysis. Thyroids, kidneys, liver, reproductive system, lungs, spleen, pancreas, adrenals, heart, and brain were removed intact and analyzed whole.

RESULTS AND DISCUSSION

CHEMICAL FORM IN PLANTS

Table I shows the percentage of ^{95}mTc extracted from fresh and freeze-dried corn leaves by successive treatments with boiling ethanol, weak mineral acids, and boiling 2-M NaOH. The variation between replicate extractions was small with standard deviations typically being less than 20% of the mean. Boiling 95% ethanol, which extracts lipids, free amino acids, amino sugars, and pigments, removed an average 24% of the ^{95}mTc from dry corn leaves. A greater amount (40%) of technetium was extracted from fresh corn leaves with the ethanol treatment. This may be due to the water soluble ^{95}mTc present in homogenized fresh leaves being diluted into the ethanol fraction. In order to compare

fresh and dried leaves, it is better to consider the first two extracts together instead of independently. The two successive treatments with weak mineral acids, which both extract ionic forms, removed an average of 35% of the technetium from the dry corn leaves. Very little of the technetium in the leaves was coprecipitated with proteins, pectates, and nucleic acids from the acid fractions by acetone. In both fresh and dry leaves, almost one-third or more of the ^{95m}Tc was associated with proteins and polysaccharides extractable with boiling 2-M NaOH. For the most part, these are not free proteins in the cell cytosol. The harsh treatment required for the removal of proteins and polysaccharides indicates that technetium in this fraction was chemically bound in the corn leaves.

The interpretation of the previous extractions is complicated by the possibility that chemical changes in the plant material early in the procedure may lead to later artifacts in the extractions. This problem is minimal in the simplified extraction of fresh chopped corn leaves by three successive overnight treatments with demineralized water at 4°C. Most of the ^{95m}Tc that could be extracted was removed from the chopped corn leaves by a single overnight soaking. The percentages of the ^{95m}Tc removed in the first, second, and third extractions with water were 46, 8, and 4, respectively. Approximately 42% of the ^{95m}Tc could not be removed from the leaves by soaking in water. This unextractable portion corresponds approximately to the amount of ^{95m}Tc that is bound in dry corn leaves (i.e., the 40% extractable by boiling 2-M NaOH, plus that present in the final residue [Table I]).

Differential centrifugation studies showed that a high percentage of ^{95m}Tc in freeze-dried corn leaves was present in the postmicrosomal supernatant (cell cytosol). The extracted leaves following homogenation in buffer retained 33% of the ^{95m}Tc in the residue while the filtrate contained 67%. In the filtrate alone, 80% of the ^{95m}Tc was found in the clear cell cytosol obtained by centrifugation at 100,000 g, while only 20% was present in the cell debris and organelles. The distribution was only slightly different in the fresh corn leaves: 29% in the residue, 71% in the filtrate; and in the filtrate, 67% of the ^{95m}Tc was in the cell cytosol while 33% was associated with cell debris and organelles. Therefore, approximately 50% of the ^{95m}Tc in corn leaves was present in ionic form or bound to soluble proteins or smaller molecules in the cell cytosol.

CHEMICAL FORM IN THE CELL CYTOSOL

Gel permeation chromatography of the corn leaf postmicrosomal fraction on a Sephadex G-50 column resulted in more than 60% of the sample radioactivity being eluted in a single peak at an elution volume to void volume ratio (V_e/V_o) ranging from 2.9 to 3.1. This indicated that most of the ^{95m}Tc in the cell cytosol was associated with molecules $< 1,500$ MW and the need for use of a column more suited to this size of molecule. Approximately 40% of the ^{95m}Tc in the postmicrosomal fraction chromatographed diffusely on the G-50 column with a range in the V_e/V_o ratio of 1.0 to 2.8, which indicated some association of technetium with larger organic molecules in a MW range of 1,500-30,000 Daltons. Total recovery of the sample radioactivity applied to the G-50 column was about 98%, and there were no apparent differences between the chromatographs of fresh and freeze-dried corn leaves.

When samples of the postmicrosomal supernatant were chromatographed on Sephadex G-25, the total recovery of radioactivity from the column was 82% of that applied. A chromatograph of the postmicrosomal supernatant from freeze-dried corn leaves on the G-25 column showed that 50% of the sample radioactivity eluted from the column in a single peak with a K_{av} value of 1.5, while 49% eluted diffusely between K_{av} values of 0 and 1, indicating that some ^{95m}Tc was associated with molecules in the MW range of 1,000-5,000 (Fig. 1). The partition coefficient, $K_{av} = V_e - V_o / V_t - V_o$ (where V_t is $3 \times V_o$), defines the solute behavior on the gel independently of the bed dimensions and column packing. When ^{95m}Tc as ammonium pertechnetate was chromatographed on the G-25 column, it eluted in a single peak with a K_{av} value of 1.5 and 90% recovery (Fig. 1). Based on the similar K_{av} values, it could be concluded that part of the ^{95m}Tc in the corn leaf cytosol was present as pertechnetate anion. However, in corn leaves the peak at $K_{av} = 1.5$ exhibited appreciable UV absorbance at 280 nm; this, however, was not the case in the chromatograph of pertechnetate alone. The UV absorbance associated with the chromatography peak at $K_{av} = 1.5$ in corn leaves indicated that this peak could be ^{95m}Tc bound to small organic molecules (peptides), in addition to some free pertechnetate anion. Also, some heterocyclic or homocyclic aromatic compounds, including some peptides, are known to interact with Sephadex G-25 gel, resulting in K_{av} values > 1.0 . In

any case, the ^{95m}Tc in the corn leaf cytosol that eluted at $K_{av} = 1.5$ is probably not entirely free pertechnetate anion alone.

EXPERIMENTS WITH LABORATORY RATS

The tissue distribution of ^{95m}Tc in laboratory rats after intravenous injection with pertechnetate was similar to the distribution found in rats that were administered contaminated diets. Tissue concentrations in rats at three and seven days following intravenous injection of pertechnetate are shown in Fig. 2. In every case, the most highly contaminated tissues were rat thyroids, hair, kidneys, liver, and skin. Although samples of rat hair were collected by shaving the abdomen, the high ^{95m}Tc concentrations in hair apparently were not due entirely to surface contamination by excreta because washing specimens of the hair twice with water (5 min each) removed a total of only 22% of the radioactivity. The high concentration of ^{95m}Tc in the skin, as well as in the hair, indicated that technetium was being biologically incorporated into hair. Although rats were elevated above their excreta by a wire screen, the possibility of contamination of the abdominal hair by urine or feces on the screen cannot be ruled out.

The elimination of ^{95m}Tc from the four most contaminated tissues in rats injected with pertechnetate is shown in Table II. There was no significant loss of ^{95m}Tc from rat thyroids or hair over the two-week period of the experiment, as determined by analysis of variance. Concentrations of ^{95m}Tc in kidneys and liver decreased by approximately 50% between one day and three days after injection (Table II) and continued to decline in concentration through 14 days after injection. By 14 days following administration of the ^{95m}Tc to rats via injection or via contaminated food, the concentrations in many tissues (excluding thyroids, hair, and kidneys) had fallen below or near our detection limit of approximately 10 pCi/g for one g of fresh tissue.

The chemical form of technetium in freeze-dried corn leaves mixed with the rat chow was different from the chemical form of ^{95m}Tc added to pulverized laboratory rat chow (Table III). The extractability of ^{95m}Tc from both types of contaminated food was different from that observed for ^{95m}Tc incorporated into corn leaves alone (Table I). Mixing ^{95m}Tc , either as pertechnetate or as technetium incorporated

into corn leaves, with the rat chow apparently influenced the extractability of ^{95m}Tc from the two types of food. The main difference between the two diets was the greater percentage of ^{95m}Tc found in bound forms (i.e., proteins and polysaccharides extractable by boiling 2-M NaOH, and cellulose and lignin) in the food made from contaminated corn plus rat chow (Table III). Even though ^{95m}Tc was added to rat chow as pertechnetate anion to make one of the contaminated foods, most of the ^{95m}Tc was not extracted in the weak mineral acid fraction. Based on the extraction data in Table III, technetium simply mixed with the rat chow apparently interacted with the protein and lipophilic components already present in this food. The rat chow contained 24.9 protein, and 16 different vitamins.

The cumulative excretion of ^{95m}Tc in urine and feces combined during the first seven days after administration of technetium by the various treatments was as follows: i.v. treatment - 91%, Tc + chow - 82%, and Tc + corn - 74%. The distribution of ^{95m}Tc between urinary and fecal excretion varied depending upon the route of administration (Fig. 3). For pertechnetate injected into rats, excretion via the urine was greater than that via the feces. Technetium found in the feces of injected rats may have been due to the wetting of feces by contaminated urine or the movement of ^{95m}Tc from the body into the intestine by way of the bile. Cumulative urinary excretion was nearly equal to fecal excretion in rats eating ^{95m}Tc mixed with their rat chow, but urinary excretion was greatly decreased in rats fed ^{95m}Tc incorporated into the corn leaves. The decreased urinary excretion of ^{95m}Tc in rats fed Tc + corn as compared to rats fed Tc + chow indicated that technetium was made less available for gastrointestinal absorption by biological incorporation into corn leaves.

The percent of the administered dose present in rat thyroids, kidneys, liver, and carcass (i.e., whole body minus 18 organs in Fig. 2) as a result of the three methods of ^{95m}Tc administration to rats also indicated a lowered gastrointestinal absorption for technetium biologically incorporated into corn leaves (Fig. 4). There was less incorporation of ^{95m}Tc into thyroids, kidneys, liver, and carcasses three days following the administration of the isotope in rats fed the Tc + corn diet as compared to the Tc + chow diet. Analysis of variance showed that the difference between the percent dose absorbed from the two

diets was statistically significant ($P < 0.05$) for thyroids and kidneys. The carcass contained a greater amount of ^{95m}Tc than any of the body tissues, probably because of the high concentration of ^{95m}Tc present in the rat hair (most of the hair remained with the rat carcass). The percent-administered dose found in the hair of rats from the various treatment groups followed the same pattern as that in the carcass. However, the amount of ^{95m}Tc in the hair was extremely variable between rats, as reflected by the standard deviations being 75 to 100% of the mean.

CONCLUSION

Based on the results of the chemical extractions, almost 40% of the ^{95m}Tc biologically incorporated into corn leaves via root uptake is present in the plant tissue in forms that are not readily extractable, except by boiling 2-M NaOH. These "bound" forms of ^{95m}Tc in the leaves appear to be mostly Tc complexes with proteins and polysaccharides of the plant cell walls. Roucoux [4] found that about 38% of the Tc-99 incorporated in soybean leaves was associated with the NaOH extractable fraction and its residue.

Differential centrifugation studies showed that about half of the ^{95m}Tc in the corn leaves was present in ionic form or associated with soluble protein and smaller organic molecules. Chromatography of the cell cytosol demonstrated that ^{95m}Tc was associated with molecules in the MW range of peptides (1,500 to 5,000 Daltons), as well as with smaller molecules and some larger molecules. About half of the ^{95m}Tc in the cell cytosol co-chromatographed with ^{95m}Tc pertechnetate, but UV absorbance data indicated that the ^{95m}Tc smaller than 1000 MW was not present as pertechnetate anion alone. Roucoux [4] also found that Tc-99 in soybean leaves was associated principally with small molecules less than a MW of 10,000 Daltons.

Once assimilated into the body, technetium was rapidly excreted from most tissues except thyroid and hair. The present study confirms the result of Sullivan et al. [5], which showed that incorporation of ^{95m}Tc into plant tissues (soybeans) resulted in decreased gastrointestinal absorption by rats and guinea pigs relative to the absorption of inorganic ^{95m}Tc . Associations with organic molecules in plants are

perhaps partly responsible for the lowered availability of technetium from the GI tract when it is incorporated into vegetation. The diminished availability of ^{95m}Tc incorporated into corn leaves may also be due to the poor digestibility of this particular diet in the rat intestine. Nonetheless, biological incorporation of technetium into plants appears to reduce the potential food chain transfer of this radionuclide through decreased availability for gastrointestinal absorption.

REFERENCES

1. R. E. Wildung, K. M. McFadden, and T. R. Garland, "Technetium Sources and Behavior in the Environment," J. Environ. Qual. 8, 156-161 (1979).
2. J. E. Till, F. O. Hoffman, and D. E. Dunning, Jr., "A New Look at Tc-99 Releases to the Atmosphere," Health Phys. 36, 21-30 (1979).
3. H. J. M. Bowen, P. A. Cawse, and J. Thick, "The Distribution of Some Inorganic Elements in Plant Tissue Extracts," J. Exp. Bot. 13, 257-267 (1962).
4. P. Roucoux, "Etude du Comportement du Technetium (Polluant Radioactif) chez le Soja en Milieux Contamine ou non en Cadmium (Polluant Conventionnel)," Ph. D. thesis, Faculte des Sciences Agronomiques, Universite Catholique de Louvain, Louvain-la-Nueve, Belgium, 1980.
5. M. F. Sullivan, T. R. Garland, D. A. Cataldo, and R. G. Schreckhise, "Absorption of Plant-Incorporated Nuclear Fuel Cycle Elements from the Gastro-Intestinal Tract," IAEA-SM-237/58, pp. 447-457 in Biological Implications of Radionuclides Released from Nuclear Industries, Vol. I, International Atomic Energy Agency, Vienna, Austria, 1979.

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- Table III. Extractability of ^{95m}Tc from two different diets fed to laboratory rats. Diet "Tc + chow" is ^{95m}Tc (pertechnetate) freshly added to uncontaminated standard laboratory rat chow. Diet "Tc + corn" is 1 g of dry, powdered corn leaves (containing ^{95m}Tc biologically incorporated in the plant tissue via root uptake) mixed with 10 g of uncontaminated rat chow.

Table I. Fractionation of ^{95m}Tc incorporated in corn leaves (*Zea mays*) by root uptake from a contaminated nutrient solution.

Extractant --	Contents of fraction	% of total in leaves ^a	
		Fresh	Freeze-dried
95% boiling ethanol --			
	Lipids, amino sugars, free amino acids, lipophilic pigments	40 (1)	24 (4)
0.2-M hydrochloric acid --			
Supernatant	Ionic forms, including salts of organic acids, phosphates, carbonates	22 (2)	28 (1)
Precipitate with acetone	Proteins and pectates	1 (0.3)	1 (0.6)
0.5-M perchloric acid (80°C) --			
Supernatant	Remaining ionic forms	6 (0.1)	7 (2)
Precipitate with acetone	Nucleic acids and some proteins	<1	<1
Boiling 2-M sodium hydroxide --			
	Most proteins and some polysaccharides	29 (1)	37 (4)
Residue	Cell wall material (cellulose and lignin)	2 (1)	3 (0.5)

^aEach value is the mean of two replicates and the standard deviation is in parenthesis.

Table II. Elimination of ^{95m}Tc from tissues in rats injected with pertechnetate expressed as mean percent dose in each tissue at day 1, 3, 7, and 14 after injection. Numbers in the same row sharing the same alphabetic superscript are not significantly different, as determined by analysis of variance and Scheffe's multiple comparison test.

Tissue	Percent dose at day				F-value (df = 3,8)	Prob.
	1	3	7	14		
Hair	0.16 (a)	14.6 (a)	7.40 (a)	8.40 (a)	3.80	NS
Thyroid	0.12 (a)	0.15 (a)	0.10 (a)	0.12 (a)	0.85	NS
Kidney	0.92 (a)	0.51 (b)	0.23 (b)	0.14 (b)	21.6	<0.001
Liver	0.66 (a)	0.32 (b)	0.07 (b)	0.02 (b)	20.4	<0.001

NS = no significant difference between the various days.

Table III. Extractability of ^{95m}Tc from two different diets fed to laboratory rats. Diet "Tc + chow" is ^{95m}Tc (pertechnetate) freshly added to uncontaminated standard laboratory rat chow. Diet "Tc + corn" is 1 g of dry, powdered corn leaves (containing ^{95m}Tc biologically incorporated in the plant tissue via root uptake) mixed with 10 g of uncontaminated rat chow.

		<u>% of total Tc-95m in food</u>	
Extractant --	Fraction containing	Tc + chow	Tc + corn
95% boiling ethanol --			
	Lipids, amino sugars, free amino acids, lipophilic pigments	42	12
Weak mineral acids (a) --			
	Ionic forms, including salts of organic acids, phosphates, and carbonates	36	35
Boiling 2-M NaOH and residue --			
	Most proteins and polysaccharides, as well as cellulose and lignin	22	53

^a0.2 M hydrochloric acid (room temperature) followed by 0.5 M perchloric acid at 80°C.

FIGURE 4

ORNL-DWG 83-16320

SEPHADEX G-25

FIGURE 2.

DECREASED GI ABSORPTION OF BIOLOGICALLY INCORPORATED Tc-95m

FEEDING TRIAL WITH RATS

FIGURE 4.