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COMPARATIVE TISSUE DISTRIBUTION OF CADMIUM IN MICE DOSED WITH PARTIALLY PURIFIEO EXTRACTS OF OYSTER

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ABSTRACT

Two experiments were conducted to -examine the relative tissue distribution of ingested cadmium (Cd) from edible oyster tissue. In the first experiment, oysters containing high or low, concentrations of Cd were extracted in physiological buffer and the intact oyster, supernatant, or pellet were incorporated into diets at levels that would give equal Cd, concentrations to each diet. Cadmium chloride was added to low Cd oyster diets to increase the Cd level to that found in the high Cd oyster diets. All diets were balanced for several constituents, including metals, and fed to mice for 7 days. Cadmium deposition in liver, kidney, duodenum, jejunum-ileum, and femur were similar for all treatments. Excretion of Cd was lower via the feces and higher via the urine in mice fed Cd occurring in the oyster pellet compared to mice fed intact oyster or oyster supernatant fractions. In the second experiment, the major soluble chemical form of Cd occurring in the oyster tissue was partially purified by a combination of centrifugation, gel permeation and ion exchange chromatography, and ultrafiltration. techniques. Metals were measured in the extract by atomic absorption spectrophotometry or anodic stripping voltammetry. Amino acids were identified in the/extract by reverse phase HPLC and thin layer chromatography. Mice were gavaged with the extract and compared to mice gavaged with combinations of chemically pure sources of the metals and amino acids.. Mice dosed with intrinsic oyster Cd retained greater than 4 times more Cd in their livers and greater than 3 times more Cd in their kidneys than any other treatment. Mice dosed with $CdCl_2$ and L-taurine retained more Cd in jejunum-ileum than the other treatments. Duodenal retention of Cd was similar in all treatments+ The results suggest that the major soluble form of Cd in dietary oyster has a high affinity for tissues critical to Cd toxicity.

INTRODUCTION

Cadmium is a toxic heavy metal often found in various foodstuffs (Jelinek and Gunderson, 1978). Researchers have measured the biological availability of intrinsically bound Cd. Generally, the total retained oral dose of intrinsic Cd was similar to or lower than that of extrinsic Cd, although differences in tissue distribution occurred in the consuming animal. Fox et al. (1978) compared the bioavailability of Cd occurring in eastern oysters (Crassostrea virinica), calico scallops (Argopecten ibbus), canine liver, and c spinach to CdC12 fed to Japanese quail. Consumotion of each of these food sources of Cd resulted in lower deposition of Cd in the jejunum-ileum compared to inorganic Cd. Chaney et al. (1978) compared the liver and kidney deposition of Cd in mice fed lettuce grown on soils amended with sewage sludges from various sources. Mice fed lettuce grown on certain sludges accumulated significantly higher renal and hepatic concentrations of Cd compared to mice fed lettuce grown. on unamended soil. Welch and House (1980) determined that the whole-body retention of -Cd in rats fed lettuce leaves labeled intrinsically and extrinsically with 109 Cd was similar. Lagally et al. (1980 and 1980a) compared the retention and tissue distribution of Cd from diets containing 4 to 8 ppm Cd as that occurring in calico scallop tissue or as $CdSO_4$. Lower retention of Cd was observed in kidneys of rats fed calico scallop whereas retention of Cd in liver, brain, testes, and femur were the same from either dietary Cd source. When mice were fed 1.8 ppm total dietary Cd for 28 days (Siewicki et. al., 1983), kidney, liver, and small intestine concentrations of Cd were lower in mice fed oyster (C. virginica) bound Cd compared to mice fed $CdC1_2$. Sullivan et.al. (1984) compared the bioavailability of intrinsic oyster Cd to $CdC1_2$ fed to mice. Mice fed 0.4 ppm total dietary Cd retained similar amounts from either source in the whole body. However, significantly greater amounts of oyster Cd were retained in the kidney and duodenum. When quail were fed 0.1 or 0.2 ppm total Cd as either intrinsic oyster Cd or CdC12, similar depositions of Cd occurred in liver, kidney, or duodenal tissue. Only jejunum-ileum deposition was lower in quail fed oyster Cd (Tao et al., 1984) . When mice were fed 0.2 to 1.0 ppm Cd as intrinsic oyster Cd or as $CdC1_2$, liver, duodenum, and jejunumileum deposition of Cd was similarj but mice fed oyster Cd retained more Cd in the kidneys and less in femur than mice fed $CdC1_2$ (Siewicki et al., 1984).

The causes of the different tissue depositions of Cd from various food sources are unknown.' Although many minerals known to interact with Cd during their concurrent metabolism were balanced in several of the studies cited above, the tissue distribution differences were still observed. Cherian et al. (1978) first examined the tissue distribution of a purified, organic fraction- of. Cd extracted from a tissue and found that Cd-metallothionein from rat liver had a much higher affinity for kidney deposition than $CdC1_2$. Since metallothionein is heat stable (Cherian, 1974), it was assumed that this source of Cd may be a major form absorbed from the human diet. Cherian (1979) later demonstrated that dietary Cd-metallothionein is transported to the kidney intact.

The experiments described herein were conducted to determine if specific chemical forms of Cd occurring in commercially available oysters cause the enhanced. renal uptake of dietary oyster Cd compared to inorganic Cd. Comparison of the ingestion of both supernatant and pellet fractions of oyster containing Cd resulted in identical tissue distribution in the first experiment. However, in the second experiment, when the major soluble chemical form of Cd in oyster tissue was further purified and gavaged into mice, this form resulted in greatly enhanced hepatic and renal Cd deposition.

METHODS

Oyster Exposure and Preparation. Oysters (Crassostrea virginica) were acquired from the Massachusetts coast and were divided into two croups. One group was dosed with 10 ppb supplemental Cd in the water (including a tracer level of ^{109}Cd) for 14 days by the methods of Hardy et al. (1984). Approximately 60% of the Cd in the water of the Cd dosed oysters was bound . to the diatom Thallasiosira seudonanna, to mimic natural sources and exposure routes of Cd (Hardy et w The resulting material is hereafter referred to as intrinsically radiolabeled oyster Cd. The remaining group was maintained similarly but without supplemental Cd. The oysters were frozen live, shucked,. and lyophilized to maintain the chemical integrity of the Cd complexes. Lyophilized material was ground in a stainles steel hammer mill (Weber Bros. and White Metal Works, Inc., Hamilton, MI) blended, and frozen (-20°C) until used.

Oyster Extract Preparation. Twenty g of low (1.16 ppm) or intrinsically fm) Cd lyophil i zed oysters were combined with 400 ml of cold 10 mM phosphate buffer- (pH 6.0) and homogenized with a Polytron homogenizer (Model PCU 1, Brinkman Instruments, Inc., Westbury, NY) for 2 min for experiment 1. Homogenates were centrifuged 60 min at 10,000 x g. Pellets were rinsed twice with 300 ml of buffer. Supernatants were combined and lyophilized as were the pellets. Supernatants were diluted to 35 ml with distilled water, subsampled for metal analyses, and stored at -4O°C until used. Pellets were ground in a glass mortar and pestle and stored at -20°c. The intact oyster, supernatants, and pellets were measured for Cd, Zn, Cu, Fe, Mg, and Mn.

Three batches of extract were prepared and combined for use in experiment 2. Five g of lyophilized, intrinsically labeled oyster containing a

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moderate concentration of Cd (2.15 ppm) were homogenized in 30 ml of cold 100 mM phosphate buffer (pH 6.0) containing 200 uM phenylmethylsulfonyl fluoride (a-protease inhibitor) in an ice bath. The homogenates were centrifuged 60 min at 27,000 x g. Pellets were washed once with 20 ml of buffer. The combined supernatants were filtered through a 2.5.cm diameter column containing 16 ml of Bio-Rad P-6 DG gel (Bio-Rad Laboratories, Richmond, CA) and washed with 30 ml of buffer. The filtrate was ultra-filtered. at 4°C in an Amicon model 402 filter apparatus (Amicon Corp., Lexington, MA) 200 ml chamber under nitrogen gas at 60 PSI through an Amicon YM30 (30,000 MW cut-off) membrane. The filtrate (less than 30,000 MW) was then applied to a 2.6 X 21.4 cm chilled (2-6°C) column containing DEAE A-25 anion exchange resin. The column was eluted with 100 ml of 10 mM phosphate buffer (pH 6.0) followed by a linear 500 ml gradient of 0.5 M NaCl in 10 mM phosphate buffer (pH 6.0). Ten ml ractions were collected and counted. The-tubes containing the greatest Cd activity were pooled and lyophi-lized. The lyophilized extract was diluted to approximately 5 ml with water and desalted on a 1.6 x 40 cm chilled. (2-6°C) co n containing Sephadex G--15 resin. The tubes containing the greatest Cd activity from the desalting step of each of the three batches were pooled, lyophilized, and diluted with a minimum of distilled water. Concentrations of Cd, Zn, Mg, Fe, Cu, and Mn were measured in the oyster and the resulting extract.

pure Exposure. Young (18-20 g) male, ICR mice were acquired from ar an- Prague-Dawley (Indianapolis, IN) and fed ad libitum the AIN-76™ diet (American Institute of Nutrition, 1977) until commencing their exposure period for both experiments. In experiment 1, the mice were allotted to treatment groups to yield an equal weight distribution in each treatment 7 days after arrival and then restricted-fed thTM appropriate diets, 4.0 g per day, for 7 days. Modifications of the AIN 76 diet were made in which the extracts replaced equal amounts of sucrose and casein on a dry weight basis. Diets containing both high and low Cd oyster products were composed of 2.30% intact oyster, 8.47% supernatant, or 1.08% pellet resulting in a total of 6 diets. Distilled water was added to the diets not containing the super-natants to balance the moisture content of all diets. All diets were approximately isocaloric and isonitrogenous due to the substitutions of oyster products for sucrose and casein. All diets contained 0.20 ppm Cd as either intrinsic radi109 beled Cd in the high Cd oyster extract diets or extrinsic CdC12 plus CdC12 in the low Cd oyster extract diets. Copper, Fe, Mg, and Mn concentrations were balanced in all diets (table 4) to the nutritional requirement levels for mice and rats (National Research Council,, 1972). The Zn concentration was balanced in all diets to 43 ppm rather than the requirement level of 12 ppm due to the high contribution from oyster. Chemical forms of each supplemented trace element were identical to that normally used in the AIN 76™ diet. Mice were housed in individual stainless steel metabolism cages in which urine, feces, and refused diet were collected and weighed daily. Mice were weighed then sacrificed with CO_2 gas. Tissues were carefully excised with surgical-grade stainless steel instruments to avoid cross contamination. Duodena were designated from the pyloris to 14 cm distal; jejunum-ileum from that point to the cecum.

Extraneous tissue, e.g., renal capsule, was removed from all tissues. Intestinal samples w e thoroughly rinsed inside and out with 0.9% saline then blotted. The Cd activity was measured in the tissues. The percent of dose was calculated as [CPM tissue + CPM diet] x 100.

In experiment 2, mice were fed the basal AIN 76™ diet for 6 to 20 days before use. Twenty-four hours before dosing; diet and water bottles were removed from the mice. Mice were dosed-with 250 u1 of oyster extract or with one of the following treatments: CdC12 alone or CdC12 combined with Zn, Mg,Fe, Ca, P, homarine, or taurine. Treatments were also prepared to examine the combined effects of: (1) Zn, Mg, and Fe; (2) Ca, P, As, glycine, valine, and leucine; and (3) Ca., P, As, threonine, lysine, and proline. Control mice were dosed. with CdC12 only. All minerals were provided at the same concentrations as occurred in the extract (table 3); amino acids were provided at 5 mg/ml. All treatments receiving minerals or amino acids; alone or in combination, also included CdCl2 (radiolabeled) at 1.87 ppm Cd (equal to the oyster extract). All minerals were added as the chloride salt except the following: As203 for As; 'and, equimolar KH2P04 and Na2HP04 (to mimic the phosphorus sources used in the phosphate extraction buffer) for P. Nine nf4 HCl was added to all treatments to insure solubility of the metals.. Cadmium, Zn., and Fe salts were obtained from Ricca ChemicalCo. (Arlington,. TX); MgC12 from Sigma Chemical Co. (St. Louis, MO.); Homarine-HCl from Aldrich Chemical Co. (Milwaukee, WI); L-taurine from Becton Dickinson (Rutherford, NJ); L-lysine from General Biochemicals Lab Park (Chagrin Falls, OH); CaC12 from J. T. Baker Chemical Co. (Phillipsburg, NJ); As_2O_3 from Fisher Scientific Co. (Fairlawn, NJ); L-glycine, L-valine, L-leucine, L-threonine, and L-proline from Grand Island B ogical Co. (Grand Island, NY). Sol ons contained approximately 2 uCi ^{184}Cd per ml of accelerator produced $^{164}CdC12$ (New England Nuclear, Boston, MA). Mice were weighed and sacrificed with CO2 gas exactly 6 days later (+ 5 min); tissues were obtained as described for experiment 1.

Chemical Analyses. Zinc, Mg, Fe, Ca, Cu, and Mn were measured in oysters, diets, and extracts by flame atomic absorption (IL-751, Instrumentation Laboratory, Wilmington, MA) following dry ashing (Siewicki et al., 1983). Cadmium in oysters and diets was measured by flame atomic absorption and Cd in oyster extracts was measured by differential pulse anodic stripping voltammetry (Jones et al., 1977). Arsenic and Se were measured by hydride generation flame atomic absorption (Varian-Techtron Ltd., Melbourne, Australia). Phosphorus was measured by the calorimetric method of Fiske and Subba Row (1925);

Amino acids in the extract of experiment 2 were identified and their approximate concentrations were estimated by two methods: (1) the HPLC elution time and relative absorbance at 280 nm of dansyl derivatized extract, and (2) the migration of derivatized and underivatized extract on thin layer chromatography (TLC,) plates. Dansyl derivatives .of 100 u1 of extract or 5. mg of amino acid in 100 u1 were made by adding 100 u1 of 0.2 M sodium borate and 200 u1 of 5% dansyl chloride in acetone and heating at 50°C for 30 min.

Excess dansyl chloride was- destroyed by adding 40 u1 of 6 M ammonium hydroxide. Derivatized,samples were then lyophilized and taken to 500 u1 with 3:l 0.01 N sodium acetate:acetonitrile. Ten u1 of sample were applied to a Varian MCH-10 $_{18}$C reverse phase column in a Varian 5000 high performance liquid chromatograph (Varian Instruments, Palo Alto, CA). Samples were eluted (0.7 ml per min) with a linear gradient of 0 to 45% acetonitrile in a buffer of 0.01 M sodium acetate (pH 4.5). Dansylated amino acid standards were obtained from Sigma Chemical Co. (St. Louis, MO).

Hydrolyzed oyster extract (1 ml) from experiment 2 was prepared by refluxing in 100 ml of 6 N HCl for 16 hr followed by drying in a Rotovap (Buchler Instruments), rinsing twice with 10 ml distilled water, drying, and-reconstituting to 10 ml with distilled water. Various concentrations of the extract, the derivatized extract, and the hydrolyzed extract were applied to silica gel analytical TLC plates or silica gel analytical TLC' plates with fluorescent indicator' (J. T. Baker Chemical Co., Phillipsburg, NJ) and developed in 5:4:1 n-butanol: distilled water: glacial acetic acid. One ug of the derivatized or underivatized amino acid standards were chromatographed simultaneously. Homarine and dansyl-amino acid derivatives were observed by fluorescence under, UV254 light. All amino acids other than homarine were also identified by spraying the plate with ninhydrin (Krebs et al., 1969). The presence of homarine was further supported by comparing the UV scan (Cary 219, Varian Instruments, Palo Alto, CA) of the extract before and after acidification to pH.2.0 (Siewicki et al., 1983a).

Tissues, diets and extracts were homogenized in 2 ml of concentrated HN03 overnight at room temperature. Samples were taken to a final volume of 7 ml (10 ml for livers) with water then counted in a Beckman. Gamma 8000 (Beckman Instruments, Inc., Fullerton, CA) at 15 to 400 KeV for a minimum of 35,000 counts.

Statistical Analyses. One way analysis of variance and Student-Newman-Keul's multiple-range test were used to test for significance ($P < 0.05$ or 0.01) in both experiments (Steel and Torrie, 1960).

RESULTS

Oyster Extractions

Results of the mineral analyses of oyster extracts used in experiment 1 and 2 are presented in tables 1 and 2, respectively. Cadmium, Zn, Cu, and Fe concentrations were higher in the pellet than in the supernatant or the original oyster tissue. Manganese concentrations were approximately the same in the dried pellet as in the dried intact oyster and total Mg was present more in the liquid supernatant than the pellet, Upon partial purification of the low molecular weight high Cd fraction containing high levels of

Cd in oysters, substantial amounts of Zn, Mg, Fe, and Ca were associated with the fraction containing Cd (table 3). Identification of, amino acids present in the dansylderivatized low MW fraction by reverse phase HPLC is illustrated in figure I. Peak I with a retention time of approximately 27 min coeluted with glycine; peak II (40 min) coeluted with taurine, homarine, lysine, and threonine;. and; peak III coeluted with proline. Thin layer chromatography separation of amino acids in the dansyl derivatized low MW fraction indicated glycine, threonine, lysine, taurine,.proline, and homarine were present. The fast moving spot with the highest Rf value co-chromatographed with phenylalanine and leucine, but in the ninhydrin-stained-underivatized low MW fraction, both amino acids occurred in low concentration. Valine was not present in the dansyl derivatized fraction but co-chromatographed with a minor spot in the ninhydrin-stained underivatized low MW fraction; The presence of homarine wasfurther confirmed by UV spectrophotometry since it had a maximum absorbance at 272 nm which did not shift upon acidification. The low molecular weight fraction did not appear to contain any small peptides since the thin layer chromatographed spots present in the hydrolyzed extract were identical with those present in the unhydrolyzed extract.

Experiment 1 Mice. Table 5 indicates the tissue distribution of cadmium as dietary high Cd oyster fractions compared to CdC12 in combination with control oyster fractions. The largest concentration ((approximately 3% of dose) was retained in the duodenum. Only about 0.15% was retained in liver and 0.13% retained in kidneys. No differences were observed in tissue retention of Cd by any treatments. In contrast, mice fed intrinsic oyster pellet Cd excreted less. Cd in the feces than the intrinsic intact oyster or intrinsic oyster supernatant Cd fed mice and excreted more in. the urine than the intact oyster plus CdC12 or oyster supernatant plus CdC12 fed mice. When comparing only the extrinsic Cd treatments, the oyster pellet plus CdC12 fed mice.excreted more Cd in the urine than the intact or supernatant oyster plus CdC12 fed mice. The differences between treatments in total Cd recovered may reflect differences in retention of Cd by the residual carcasses that were not analyzed (table 5).

Experiment 2 Mice. Mice dosed with semipurified oyster extract containing intrinsic Cd retained greater than 4 times more Cd in liver and greater than 3 times more Cd in kidneys than any other treatment tested. Mice fed 1.87 ppm Cd as CdC12 plus 5 mg taurine per ml retained more Cd in the jejunum-ileum than mice dosed with any other treatment.- No differences were observed in duodenal retention of Cd (see table 6).

Comparison of Experiment 1 and 2 Mice. Mice fed diets containing 0.2 ppm Cd in experiment 1 for 7 days before sacrifice consumed approximately 5.6 pg of total Cd, whereas mice in experiment 2 were dqsed once with 0.47 pg of Cd then killed 6 days later. Liver, kidney, and jejunum-ileum retentions of Cd (as a percent of dose) of the single dosed mice were much higher than in the mice fed for 7 days, whereas duodenal concentrations were lower.

DISCUSSION

The chemical binding of Cd in the eastern oyster has been identified in the soluble fraction, of highly dosed oysters- (Casterline and Yip, 1975; Ridlington and Fowler, 1,979). Most of the Cd was associated with a 10,000 MW cytoplasmic protein. This protein is inducible in oysters with extreme environmental Cd concentrations (Frazier, 1979; Siewicki et al., 1983a). Howard and Nickless (1977) observed that Zn and Cu were bound to very low molecular weight complexes in oysters and that both metals were associated with taurine (copper was also bound to homarine). Our early work (Siewicki et al., 1983a) indicated that Cd was associated with taurine and homarine and that Zn and Cu were also associated with this complex. Results reported in the present study indicate that Zn, Mg, Fe, and Ca were present in large amounts in a less purified fraction- (due to the large quantity required) than that examined in the earlier work. Further additional amino acids other than taurine and homarine were detected in this fraction compared to the earlier work. Table 1 indicates that most of the Mg was in the soluble, fraction. By comparing the data in tables 1 and 3.it -is apparent that further purification steps removed large amounts of Zn and Cu, whereas, the relative concentrations of Fe and Mg were not appreciably changed. Apparently, Zn and Cu are enriched in other fractions of the soluble material.

Cherian (1979) showed, that orally dosed Cd-metallothionein is not metabolized in the liver and is transported to the kidney intact. The same phenomenon does not seem to occur when oyster Cd is ingested. We have shown (Siewicki et al., 1983) that the Cd in oyster is degraded to a very small complex(es) in the gut before absorption. The present findings indicate, however, that a factor within oyster tissue either modifies the transport process, the hepatic metabolism, or the renal deposition of Cd. We propose that the major effect of oyster constituents on Cd tissue retention is an altered hepatic metabolism that allows more Cd to be deposited in the liver and more to be transported via the portal system, through the liver, and on to the kidney where additional deposition occurs. Chen and Ganther (1975) showed that the kidney normally has a higher binding capacity for Cd than the liver (78 nmol/g vs 38 nmol/g). If the renal binding capacity of Cd were low, the effect of altered hepatic metabolism would probably have little effect on the renal deposition of Cd. However, our results indicate that the presence of oyster in the-diet along with Cd causes more Cd to be deposited into both the liver and the kidney. Additional research is necessary to determine if changes in chemical form of Cd occurs in transport under different circumstances. If only one form of Cd occurs in transport 'during normal circumstances of low Cd intake, then it would seem even more likely that the effects of dietary oyster are to modify hepatic metabolism.

The effects of metals and amino. acids associated with the partially purified oyster extract were investigated in an attempt to further identify the component in oyster responsible for altering Cd metabolism. Zinc, Mg, Fe, Ca, P, and As were each investigated in experiment 2. Copper, Mn, and

Se were found in only very low concentrations in the oyster extract. Thus, it was reasonable that the latter three minerals did not cause the high renal and hepatic Cd uptake, of the mice dosed with the -oyster extract. Taurine and homarine have been identified in the high Cd fractions of eastern oysters (Siewicki et al., 1983a) and in the high Zn and Cu fractions of the European flat oyster (Coombs, 1974). Other amino acids identified in the extract were also investigated.

Several of the constituents that were investigated for their effect on tissue deposition of Cd are known to influence Cd metabolism. Zinc, iron and protein generally reduce the toxicity of Cd to animals when fed in excess of nutritional requirements -(Fox, 1974). Deficiencies of these same nutrients as well as Ca will enhance Cd toxicity.

Zinc was dosed in experiment 2 at the same level as it occurred In the extract (8.25 pg/g or 2.06 total pg). Rats were fed diets containing 30, 300, or 1000 ppm Zn for nine weeks (Campbell et al., 1978). Higher Zn intake caused slightly lower renal Cd depositlon. However, little is known. about the short term effects of concomitantlow level exposure to Cd and Zn.

Taurine was the only amino acid investigated that caused a significant difference in the tissue distribution of ingested Cd. Jejunum-ileum concentrations of Cd were higher in mice dosed with 5 mg/ml L-taurine plus 1.87 ppm Cd as $CdCl_2$ compared to the other treatments. Experiments are ongoing. in our laboratory to further quantify the levels of taurine and other organic fractions in oysters. Additional studies are underway to determine if higher levels of amino acids and different combinations of amino acids and metals will influence the renal and hepatic metabolism of ingested Cd.

The present results support the results of feeding studies (Sullivan et al. 1984; Siewicki et al., 1984) in which dietary oyster Cd was retained in critical tissues such as kidney to a greater extent than inorganic Cd. Additional research is necessary to determine if one or more constituents of oyster. cause this effect and whether the effect is caused by chemical binding to Cd before absorption or simultaneously (perhaps competitively) with absorption.'

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TABLE 1

Concentrations (ppm) of Minerals in Oysters and Oyster Extracts Used in Experiment 1.

	Low Cadmium Oyster			High Cadmium Oyster		
	Intact	Supernatant	Pellet	Intact	Supernatant	Pellet
Cadmium	1.16	0.571	1.70	8.71	2.36	18.5
Zinc	1290	250	1970	1870	464	1980
Copper	17.9	5.38	47.5	28.8	5.96	50.4
Iron	421	20.2	553	186	25.4	414
Magnesium	2360	1020	625	6000	2860	883
Manganese	10.5	1.90	14.7	4.14	0.751	2.75

TABLE 2

Concentrations of Minerals in Lyophilized Oyster Used to Prepare Extract for Experiment 2.

Mineral	ppm + SEM
Cadmium	2.15 ± .181
Zinc	2130 ± 102
Magnesium	6150 ± 329
Iron	169 ± 8.1
Copper	28.7 ± .52
Manganese	5.13 ± .380

TABLE -3

Concentrations of Minerals in Oyster Extract Used in Experiment 2

Mineral	ppm + SEM
Cadmium	1.87 + .123
Zinc	8.25 + .162
Magnesium	2040 + 27
Iron	12.5 + 3.87
Calcium	481' + 5.0
Phosphorus	22,700 + 480
Arsenic	1.68 + .055
Copper	0.51 + .055
Manganese	0.14 + .060
Selenium	<0.2

TABLE 4

Concentrations of Minerals in Diets Used in Experiment 1

TABLES

Comparison of Percent of Cadmium Dose in Tissues and Excreta of Mice in Experiment 1[a]

	Low Cadmium Oyster + $CdCl_2$			High Cadmium Oyster		
	Intact	Supernatant	Pellet	Intact	Supernatant	Pellet
Liver	0.152 ± .0102	0.155 ± .0063	0.146 ± .0101	0.170 ± .0063	0.153 ± .0118	0.147 ± .0134
Kidneys	0.134 ± .0193	0.120 ± .0098	0.138 ± .0243	0.114 ± .0066	0.120 ± .0114	0.111 ± .0117
Duodenum	2.986 ± .3854	3.206 ± .6812	2.934 ± .5701	2.652 ± .5238	2.281 ± .2340	3.847 ± 1.134
Jejunum-ileum	0.436 ± .0448	0.416 ± .0431	0.454 ± .0292	0.803 ± .3411	0.360 ± .0705	0.483 ± .0559
Femur (10^3)	0.720 ± .0725	0.693 ± .0470	0.716 ± .0615	0.714 ± .0403	0.693 ± .0643	0.691 ± .0809
Feces	83.1 ± 1.96^{xy}	83.1 ± 2.01^{xy}	80.8 ± 1.40^{xy}	88.4 ± 0.88^{x}	86.2 ± 0.87^{x}	75.5 ± 5.50^{y}
Urine (X10)	0.231 ± .0325^{y}	0.231 ± .0432^{y}	0.438 ± .1727^{x}	0.408 ± .0585^{x}	0.442 ± .0950^{x}	0.719 ± .1362^{x}
Total Recovery[c]	86.9	87.0	84.5	92.1	89.1	80.8

[a] Mice (8 per treatment) were pair-fed diets containing 0.2 ppm Cd as that occurring in intact high cadmium oyster, or the soluble or insoluble fractions. Similarly prepared low cadmium oyster fractions plus $CdCl_2$ were compared. Mice were sacrificed after 7 days feeding.

[x,y] Mean ± SEM. Means in a row sharing a common superscript are not significantly different, $P > 0.05$, as indicated by one-way analysis of variance and Student-Newman-Keul's multiple-range test.

[b] Does not include residual carcass.

TABLE 6

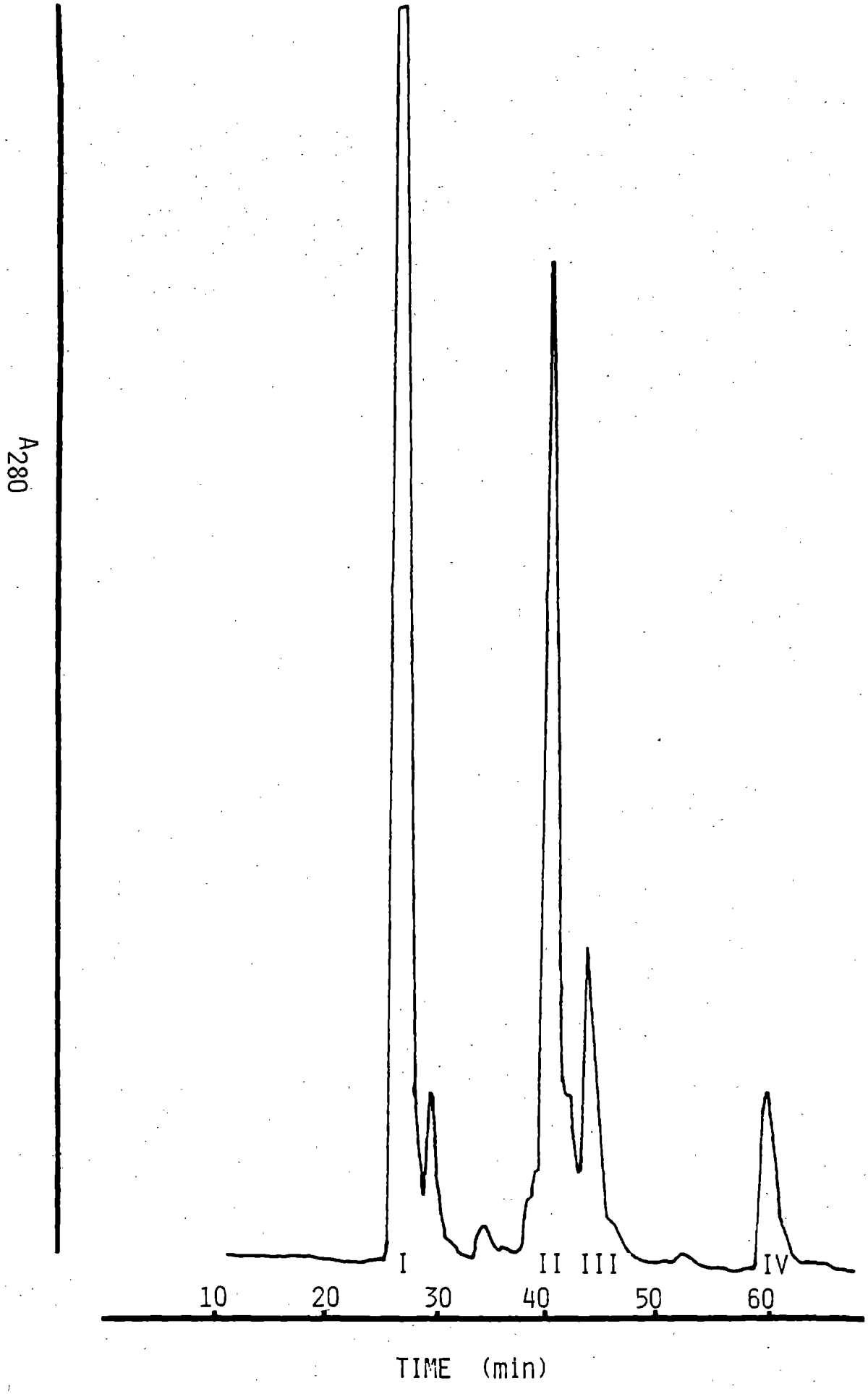
Comparison of Percent of Cadtmium Dose Retained by Tissues of Mice Gavaged with Oyster Extract Cadmium or Cadmium Chloride in Combination with Variers Metals or Amino Acids in Experiment 2"

	Oyster Extract	$CdCl_2$	$CdCl_2$ + Zn	$CdCl_2$ + Mg	$CdCl_2$ + Fe	$CdCl_2$ + Zn/Mg/Fe
Liver	1.683 ± .2366^{x}	0.372 ± .0532^{y}	0.655 ± .0491^{y}	0.391 ± .0635^{y}	0.563 ± .1175^{y}	0.438 ± .0748^{y}
Kidney	0.572 ± .0848^{x}	0.163 ± .0211^{y}	0.285 ± .0268^{y}	0.158 ± .0263^{y}	0.199 ± .0413^{y}	0.142 ± .0151^{y}
Duodenum	0.202 ± .0155	0.173 ± .0272	0.198 ± .0169	0.152 ± .0237	0.181 ± .0385	0.171 ± .0253
Jejunum-ileum (10)	0.719 ± .1066^{xy}	0.483 ± 0813^{y}	0.386 ± . 176^{y}	0.385 ± .0604^{y}	0.396 ± .0789^{y}	0.395 ± .0517^{y}

	$CdCl_2$ + Homarine	$CdCl_2$ + L-Taurine	$CdCl_2$ + Ca/P	$CdCl_2$ + P	$CdCl_2$ + Ca/P/As/ Gly/Val/Leu	$CdCl_2$ + Ca/P/As/ Thr/Lys/Pro
Liver	0.639 ± .0789^{y}	0.698 ± .1192^{y}	0.222 ± .0267^{y}	0.259 ± .0375^{y}	0.271 ± .0366^{y}	0.242 ± .0319^{y}
Kidney	0.232 ± .0338^{y}	0.273 ± .0523^{y}	0.125 ± .0181^{y}	0.203 ± .0221^{y}	0.168 ± .0157^{y}	0.152 ± .0146^{y}
Duodenum	0.187 ± .0014	0.249 ± .0268	0.174 ± .0334	0.193 ± .0277	0.166 ± .0277	0.161 ± .0231
Jejunum-ileum (10)	0.557 ± .0054^{y}	0.953 ± .0295^{x}	0.393 ± .0522^{y}	0.563 ± .1021^{y}	0.509 ± .0591^{y}	0.454 ± .0686^{y}

[a] Mice ($n \geq 8$) were dosed with 0.25 ml containing intrinsic or extrinsic ^{109}Cd and sacrificed exactly 6 days later. Metals supplied at the same concentration as occurred in the oyster extract; amino acids supplied at 5 mg per ml.

[x,y] Mean ± SEM. Means in a row sharing a common superscript are not significantly different, $P > 0.01$, as indicated by one-way analysis of variance and Student-Newman-Keul's multiple-range-test. Comparisons are made between all twelve treatments for a given tissue.



Fractionation of dansylated low MW oyster-Cd extract by reverse phase! Peaks were identified, using dansyl-amino acid standards: peak I, glycine; peak II, taurine, homarine, threonine, and lysine; peak III, proline; peak IV, phenylalanine.