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Dietary Protein Effects on Cadmium and Metallothionein Accumulation in the Liver and Kidney of Rats

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The relationship of dietary protein to cadmium absorption and tissue deposition was studied in male Sprague-Dawley rats exposed to different levels of cadmium in the drinking water. In animals fed a high-protein or low-protein diet and drinking water containing 25 or 50 ppm cadmium, liver and kidney cadmium and metallothionein were both significantly higher in rats fed the high-protein diet for 2 to 4 months. These differences may possibly be explained by the concentration of cysteine observed between these two diets. When cysteine was added to the low-protein diet to the level observed in the high-protein diet and fed to rats receiving 25 ppm cadmium in the drinking water, significant dietary differences in liver and kidney cadmium and metallothionein were not observed. The importance of dietary protein to cadmium-induced toxicity was also assessed in these studies. The activity of catechol-*o*-methyltransferase was used as a measure of cadmium-induced toxicity. The activity of this enzyme in the lung, liver and heart was significantly lower in rats fed a low-protein diet than those fed the high-protein diet and 50 ppm cadmium. Metallothionein concentration in the lung and liver from low-protein-fed rats was approximately half the level observed in rats fed the high-protein diet, which suggests a relationship between cadmium-induced toxicity and metallothionein concentrations. These results illustrate the importance of considering dietary protein (and possibly cysteine) when studying cadmium metabolism in experimental animals.

Introduction

Several investigators have reported that dietary protein may influence the intestinal absorption of cadmium. Fitzhugh and Meiller (*1*) were the first to report that the toxicity of cadmium was increased by a low-protein diet. Since then, abnormalities of the bone, liver, and blood have been observed in animals given cadmium (orally or by injections) and fed a low-protein diet (*2,3*). These abnormalities were not observed when the level of protein in the diet was relatively high.

In short-term studies (i.e., days), Suzuki et al. (*4*) compared the effect of low- and high-protein diets on the accumulation of cadmium in several tissues and observed higher levels of cadmium in the liver, kidney, and whole body of mice fed low protein diets for 24 hr before and after an oral dose of ^{115}Cd. In long-term studies, rats fed low- or high-protein diets and drinking water containing 50 ppm cadmium for 3 months were found to have significantly higher concentrations of cadmium and metallothionein (MT) in the liver and kidney than those rats fed the high-protein diet (*5*). These results suggest that the effect of dietary protein may be associated with the length of exposure.

Although Itokawa et al. (*2*) observed abnormalities of bone in rats fed cadmium and low-protein diets for 30 days, bone cadmium was higher in rats fed the high-protein diet. Thus, in relatively long-term studies, the tissue level of cadmium may be higher in animals fed a high-protein diet, but the toxicity of cadmium may be greater in animals fed a low-protein diet. These conflicting effects may be associated with the tissue level of MT. For example, MT synthesis may be reduced in animals fed a low-protein diet (*5*), which would allow cadmium to bind to other macromolecules (i.e., enzymes), thus increasing the tissue toxicity of this element.

The present studies were performed to determine, in rats exposed to drinking water containing different levels of cadmium, the importance of dietary protein and exposure time on the tissue

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accumulation of cadmium and MT. The relationship of the tissue level of MT to cadmium-induced toxicity was also assessed. Since cysteine has been suggested as a factor explaining the effect of dietary protein, studies were performed to determine its effect on cadmium absorption and tissue accumulation.

Methods

Male rats of the Sprague-Dawley strain, approximately 3 months old, and weighing an average of 150 g, were used. They were randomly divided into several groups and fed (*ad libitum*) drinking water and purified diets as follows. Group A, the control group, was fed various control diets [high-protein or low-protein (Table 1)] and deionized water. Groups B and C were fed, respectively, a low-protein diet or high-protein diet and drinking water containing 5, 25, or 50 ppm cadmium. Group D was fed a low-protein diet with 400 μg of L-cysteine/g diet added and drinking water containing 25 ppm cadmium. The rats were exposed to these diets and drinking water for a total period of 4 months and at 1-month intervals; 96 rats from each experimental group (i.e., 4 for each level of cadmium/group/time point) and 32 controls (i.e., 4 for each dietary level) were killed by decapitation. The urine (collected directly from the bladder), heart, kidney, and liver were then removed, and blocks of these tissues and urine were analyzed as follows.

Tissue and Urinary Cadmium

The concentration of cadmium was determined in the urine, lungs, heart, kidney, and liver as previously described (*5*). Briefly, tissue was lyophilized and digested in concentrated nitric acid, and cadmium was determined on diluted samples by flameless atomic absorption using a Perkin-Elmer 603 spectrophotometer equipped with a deuterium ARC for background correction.

Tissue Concentration of Metallothionein

The concentration of MT was determined in the lung, heart, kidney, and liver as described by Piotrowski et al. (*6*) and Kotsonis and Klaassen (*7*). In brief, these tissues were homogenized in 1.15% KCl (10 mL/g) and 100 μg of ^{203}Hg (1.57 Ci/mole) was added to the homogenate (1 mL). This mixture was allowed to react for 12 min at room temperature before the addition of 300 μL of 10% TCA, and then was centrifuged at 1000*g* for 10 min. A 250-μL aliquot of the supernatant was added to 5 mL of saline, and the radioactivity of this mixture was determined with a Searle Auto-Gamma scintillation spectrophotometer. The amount of MT per gram of tissue was determined by measuring the amount of ^{203}Hg in the TCA supernatant.

Table 1. Chemical composition of the various diets.

Ingredient	Ingredients in diet, %		
	Low protein plus cysteine	Low protein	High protein
Casein (vitamin free)	5.5	5.5	67.5
Sucrose	27.56	27.6	9
Corn oil	5	5	5
Lard	5	5	5
L-Cysteine	0.04	0	0
Dextrin	46.55	46.55	3.15
Methionine	0.15	0.15	0.15
RP vitamin mixture[a]	2.0	2.0	2.0
Choline chloride	0.20	0.20	0.20
Mineral mixture[b]	5.0	5.0	5.0
Nonnutritive fiber (Sokka-floc)	3.0	3.0	3.0

[a]Each diet contained the following vitamins/kg of diet: thiamine HCl, 20 mg; riboflavin, 20 mg, niacin, 90 mg; pyridoxine HCl, 20 mg; D-calcium pantothenate, 60 mg; folic acid, 4 mg; D-biotin, 0.4 mg; inositol, 200 mg; metadione sodium bisulfite, 20 mg; vitamin A acetate, 22 IU/g; vitamin D_3, 2.2 IU/g and DL-α-tocopherol acetate, 50 IU/kg.

[b]Diets were adjusted to contain the following mineral nutrients: calcium, 0.75%; phosphorus, 0.45%; potassium, 0.46%; sodium, 0.29%; magnesium, 0.065%; manganese, 65 mg/kg; iron, 60 mg/kg; zinc, 20 mg/kg; copper, 15 mg/kg; fluoride, 5 mg/kg/; cobalt, 3.2 mg/kg; chromium, 3 mg/kg; iodine, 0.6 mg/kg; molybdenum, 0.8 mg/kg; and selenium, 0.2 mg/kg. The phosphorus content was 0.42% in the low-protein diet and 0.48% in the high-protein diet. Cadmium in the diets was less than 0.04 μg/g dry weight.

Catechol-O-Methyltransferase Activity

Catechol-*O*-methyltransferase (COMT) was measured in the heart, liver, and lung by a modification of McCaman's procedure (*8*). In a 10-mL culture tube, the following were added: potassium phosphate, 0.08 M (pH 7.8); $MgCl_2$, 5×10^{-3} M; 3,4-hydroxybenzoic acid, 1×10^{-4} M; *S*-adenosyl-L-(methyl-^{14}C)-methionine, 1 nmole; and 5–80 μg protein of a 78,000*g* supernatant. This reaction mixture was incubated at 38°C for 30 min, and the reaction was stopped by the addition of 3 μL of 3 N HCl. Ethyl acetate (100 μL) was added and thoroughly mixed to extract the 3-methoxy-4-dihydroxybenzoic acid. After a brief (3 min) centrifugation to separate the phases, a 50-μL portion of the ethyl acetate was removed and added to a toluene-based phosphor solution (10 mL); radioactivity was measured in a scintilla-

tion spectrophotometer. Since the amount of *S*-adenosyl-L-(methyl-^{14}C)-methionine extracted was less than 0.2% by this procedure, correction was made for this blank value by incubating the radioactive substrate with boiled enzyme.

Short-Term Absorption Studies

In these studies, rats were fed a high, low, or low protein with L-cysteine (400 μg/g) diet for 30 days prior to an oral dose of cadmium. Each group was given a 1 mL solution by intubation containing $^{109}CdCl_2$ (0.40 mg/rat) in a slurry of the respective diets. In each group, four rats were killed at 24, 48, and 72 hr after administration of this isotope. Sections of selected organs were removed, post-mortem lyophilized to constant weight, and radioassayed using a Searle well-type gamma scintillation counter with a sodium iodide thallium-activated crystal. Counting error was reduced to less than 5% at 95% confidence level by counting all samples to 10,000 counts. All counts recorded were 5 to 10 times greater than background.

In experiments for which statistical analysis was performed, results were analyzed by the Student's *t*-test. The expressions $p<0.05$, $p<0.01$ and $p<0.001$ are used to indicate significance at the 5, 1 and 0.1% levels, respectively.

Results

The controls (i.e., rats fed a low- or high-protein diet and deionized water) were grouped together because significant differences were not observed for the measurement described below. Food and water intake was monitored on a weekly basis over the 4-month experimental period. The mean daily food intake during this period for the various groups was: group A, 25.9 ± 1.6; group B, 29.3 ± 0.8; group C, 24.6 ± 0.7; and group D, 27.6 ± 0.6 g. The mean daily intake of water for those rats given 25 and 50 ppm cadmium was slightly lower than that for the controls (controls, 26.6 ± 1.8 mL; 5 ppm cadmium, 27.3 ± 0.9 mL; 25 and 50 ppm cadmium, 24.7 ± 1.1 mL).

Differences between initial and final body weights in the various groups were only observed between the low- and high-protein diets (Table 2). Cadmium treatment did not cause a significant change in body weight. Furthermore, significant differences in wet weight of the kidney, liver, lung, and heart, following 4 months of treatment were not observed between the low- and high-protein diets, irrespective of the level of cadmium in the drinking water.

The tissue concentration of cadmium in the liver and kidney of rats fed the various diets and drinking waters containing cadmium are shown in Figures 1–4. These figures only describe the levels of cadmium observed in groups B, C, and D. The concentration of cadmium in the liver and kidney of group A (controls) did not exceed 2.9 ± 0.8 μ g/g dry weight over the 4-month experimental period. As shown in these figures, the concentration of cadmium in both the liver and kidney appeared to increase with dose at all time intervals. At levels of 25 and 50 ppm cadmium, the tissue concentration of this element after two months of exposure was significantly higher when rats were fed the high-protein diet (Figs. 1 and 2). Although the level of cadmium in the liver and kidney was higher in rats fed the high-protein diet and 5 ppm cadmium, significant differences between these diets were only observed in the kidney after 4 months of exposure (Fig. 3).

In an attempt to define the dietary factor re-

Table 2. Effect of dietary protein and drinking water cadmium on body weight at the end of 4 months of treatment.

Treatment group	Body weight, g[a]		
	Initial	Final	Difference
Controls			
Low-protein	166 ± 4	215 ± 9	49
High-protein	153 ± 3	323 ± 8	170
Low-protein + 400 μg cysteine	159 ± 4	233 ± 5	73
Experimental			
5 ppm Cd, low-protein	148 ± 4	200 ± 6	52
High-protein	152 ± 3	315 ± 11	167
25 ppm Cd, low-protein	153 ± 2	205 ± 8	52
High-protein	160 ± 6	328 ± 15	168
50 ppm Cd, low-protein	155 ± 1	195 ± 5	40
High-protein	158 ± 3	316 ± 10	158
25 ppm low-protein + 400 g cysteine	151 ± 2	229 ± 8	78

[a]Mean ± SEM for four rats per experimental group.

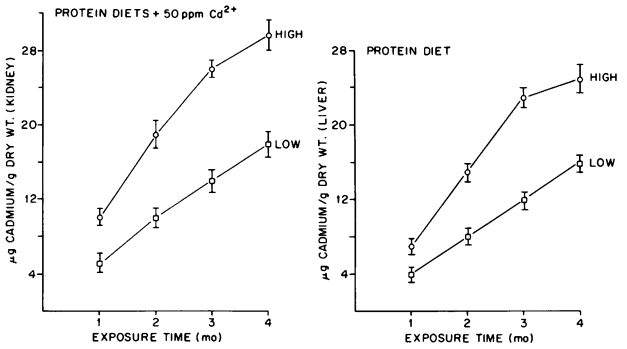

FIGURE 1. Rats were fed a high- or low-protein diet and drinking water containing 50 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

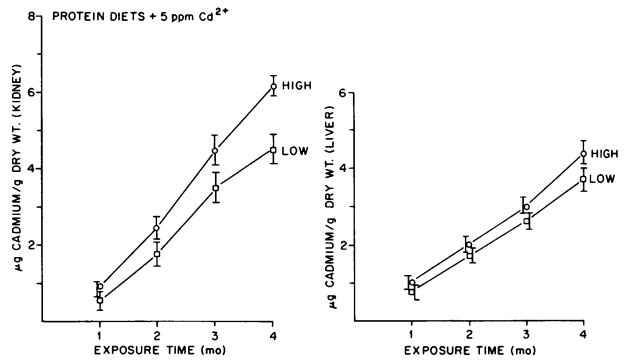

FIGURE 3. Rats were fed a high- or low-protein diet and drinking water containing 5 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

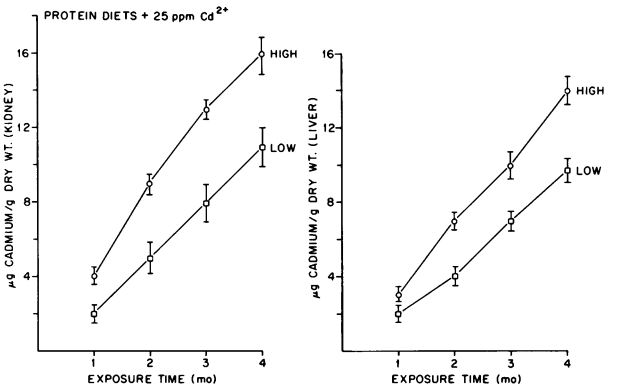

FIGURE 2. Rats were fed a high- or low-protein diet and drinking water containing 25 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

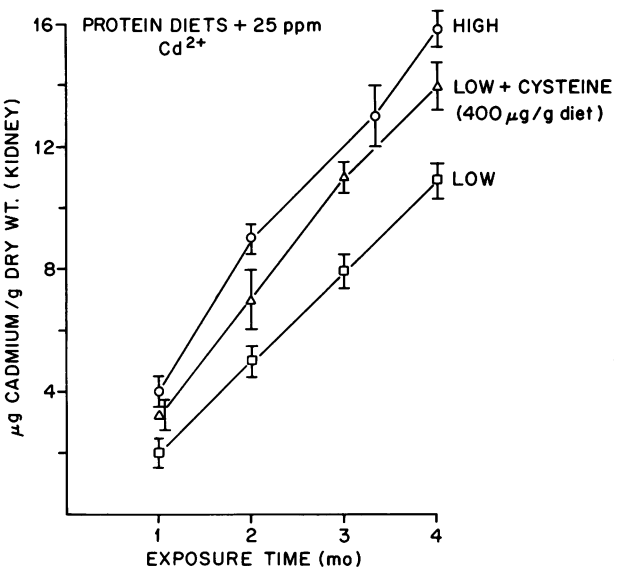

FIGURE 4. Rats were fed the low-protein diet with L-cysteine added (400 ± g/g of diet) and drinking water containing 25 ppm cadmium. At each time point, four rats from each group were killed and the concentration of cadmium was determined in the kidney. Results are expressed as means ± SEM. Data from Figure 2 were included as a comparison.

sponsible for these differences, cysteine was added to the low-protein diet to a level that was present in the high-protein diet (i.e., high-protein 52 mg/100 g, low-protein 10 mg/100 g of diet). In rats fed this supplemental diet and drinking water containing 25 ppm cadmium for 4 months, the concentration of cadmium in the liver and kidney increased to levels similar to that observed in rats exposed to the high-protein diet and 25 ppm cadmium (Fig. 4).

The concentration of MT in the liver and kidney increased with the dose of cadmium (Figs. 5 and 6). As observed for tissue cadmium, the concentration of MT was significantly higher in both tissues in rats fed the high-protein diet following 3 to 4 months of treatment. In rats exposed to 5 ppm cadmium, significant differences between diets were not observed. MT levels in the liver and kidney from rats fed the low-protein diet with cysteine added and 25 ppm cadmium were 390 ± 60 and 365 ± 73 μg MT/g, respectively, after 4 months of exposure. These levels at 4 months are higher than observed in rats given 25 ppm cadmium and fed the low protein diet (i.e., 270 ± 35 and 250 ± 23 μg/g, respectively) and lower than

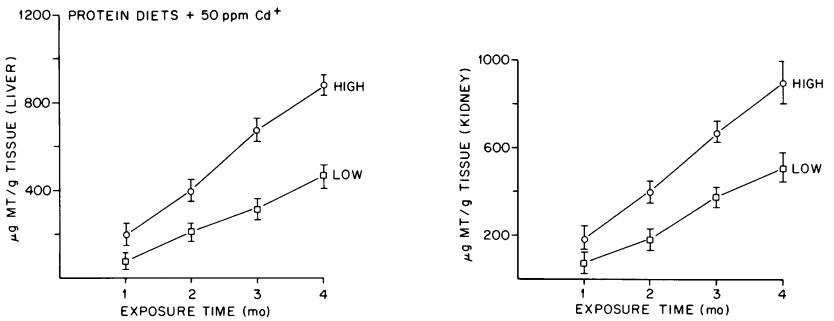

FIGURE 5. Metallothionein (MT) was determined in the liver and kidney (as discussed in method section) from rats fed the high or low protein diet and drinking water containing 25 ppm cadmium. Results are expressed as means ± SEM.

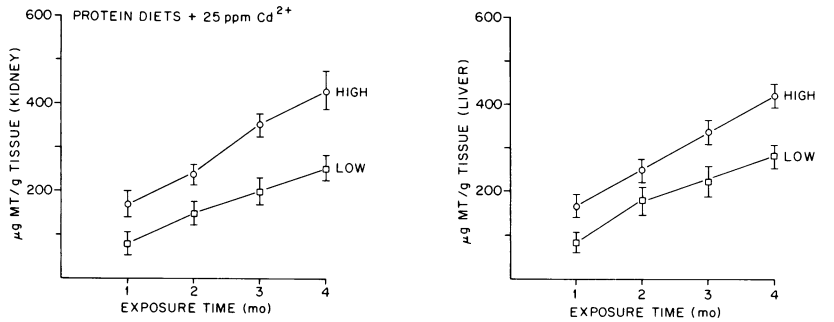

FIGURE 6. Metallothionein (MT) was determined in the liver and kidney (as discussed in text) from rats fed the high- or low-protein diet and drinking water containing 50 ppm cadmium. Results are expressed as means ± SEM.

Table 3. Long-term effect of dietary protein and drinking water cadmium on the urinary excretion of cadmium at various time intervals.

Treatment group	Cd, ppm	Cd, μg/g creatinine[a]			
		1 month	2 months	3 months	4 months
Controls		1.3 ± 0.3	1.8 ± 0.6	1.6 ± 0.4	2.3 ± .08
Experimental					
Low-protein	5	1.2 ± 0.4	2.6 ± 0.7	2.9 ± 0.5	3.2 ± 0.8
High-protein	5	1.8 ± 0.4	3.2 ± 0.6	3.0 ± 0.9	3.4 ± 0.4
Low-protein	25	7.9 ± 1	13.8 ± 2.6	17.4 ± 2.5	25.8 ± 6
High-protein	25	12.8 ± 2	14.1 ± 4	20.9 ± 1.5	39.9 ± 4
Low-protein	50	16.2 ± 2	23.9 ± 3	39.9 ± 8	66.7 ± 10
High-protein	50	18.6 ± 4	35.4 ± 1	55.4 ± 7	89.9 ± 12
Low-protein (plus cysteine)	25	10.8 ± 2	18.9 ± 3	26.2 ± 0.9	31.9 ± 3

[a]Mean ± SEM for four rats per experimental per time point.

observed in rats fed the high-protein diet (i.e., 430 ± 66 and 460 ± 39 μg/g, respectively).

The urinary excretion of cadmium is shown in Table 3. The level of cadmium in the urine increased with dose and exposure time. However, significant differences in the excretion of cadmium between the diets were only observed following 3 and 4 months of treatment. Nevertheless, the level in rats fed the high-protein diet and 25 or 50 ppm cadmium was higher over the experimental period.

Table 4. Effect of cadmium on catechol-*O*-methyltransferase (COMT) and metallothionein (MT) levels in the lung, heart and liver from rats given for 4 months, drinking water containing 50 ppm cadmium and a high- or low-protein diet.

		MT, μg/g wet weight[a]		COMT, μmole/mg protein/hr[a]	
Treatment group	Tissue	2 months exposure	4 months exposure	2 months exposure	4 months exposure
Controls					
Low-protein	Lung	0.09 ± 0.07	0.13 ± 0.07	15 ± 2	17 ± 3
	Liver	7 ± 2	20 ± 5	13 ± 1.6	15 ± 1.9
	Heart	—	—	2.4 ± 0.4	3.0 ± 0.2
High-protein	Lung	0.10 ± 0.08	0.17 ± 0.10	14 ± 4	18 ± 3
	Liver	10 ± 1.6	35 ± 8	14 ± 2	15 ± 1.9
	Heart	—	—	2.6 ± 0.3	3.1 ± 0.6
Experimental (50 ppm Cd)					
Low-protein	Lung	0.19 ± 0.07†	0.29 ± 0.10*	8.1 ± 0.9*	5.2 ± 1.0*
	Liver	155 ± 48†	369 ± 35‡	9.3 ± 1.3	8.7 ± 2*
	Heart	—	—	1.29 ± 0.3*	0.73 ± 0.15†
High-protein	Lung	0.66 ± 0.13†	0.89 ± 0.17†	10 ± 2	12 ± 3
	Liver	385 ± 29‡	806 ± 79‡	13 ± 4	11 ± 2
	Heart	—	—	2.0 ± 0.4	2.1 ± 0.5

[a]Means ± SE for four rats/group/time point.
*$p < 0.05$.
†$p < 0.01$.
‡‡$p < 0.001$.

A relationship has been suggested between MT concentration and cadmium-induced toxicity of tissues (*9,10*). In some organs, the toxicity of cadmium shows an apparent decrease as the concentration of MT increases. To determine if dietary protein affected this relationship, MT and COMT were both determined in the lung, liver and heart of rats fed a low- or high-protein diet and given 50 ppm cadmium in the drinking water. COMT was used as a measurement of cadmium-induced toxicity, (*11*). MT was not detected in the heart of the controls or the experimental group (Table 4). As compared to respective controls, MT levels in the lung and liver increased significantly following the exposure to 50 ppm cadmium and these diets. However, this increase was substantially greater in rats fed the high-protein than in rats fed the low-protein diet. COMT activity was significantly lower in the lung, liver and heart of rats fed the low-protein diet and 50 ppm cadmium. In rats fed the high-protein diet, significant decreases in the activity of this enzyme were not observed.

The tissue retention and urinary excretion of cadmium were determined in the three dietary groups (i.e., groups B, C and D). Differences in the retention of ^{109}Cd in the liver, kidney and gastrointestinal tract were observed between the various diets (Table 5). At each time point, for the liver and kidney, the retention of ^{109}Cd appeared higher in rats fed the low-protein rather than the high-protein diet. With the exception of the kid-

Table 5. Retention of ^{109}Cd in various tissues expressed as a percentage of initial dose.

		^{109}Cd retention, % of initial dose/g dryweight[a]		
Experimental group	Tissue	24 hr exposure	48 hr exposure	72 hr exposure
High-protein diet	Liver	0.48 ± 0.14	0.48 ± 0.08	0.44 ± 0.11
	Kidney	0.98 ± 0.19	0.88 ± 0.13	0.40 ± 0.09
	GI tract	96 ± 1.5	95 ± 2.3	95 ± 1.1
Low-protein diet	Liver	0.69 ± 0.15	0.70 ± 0.14	0.68 ± 0.19
	Kidney	1.39 ± 0.21	1.20 ± 0.17	0.90 ± 0.10
	GI tract	97 ± 2.1	97 ± 1.8	97 ± 1.0
Low-protein diet plus cysteine	Liver	0.66 ± 0.13	0.60 ± 0.19	0.53 ± 0.17
	Kidney	1.63 ± 0.26	1.0 ± 0.16	0.73 ± 0.15
	GI tract	94 ± 2.3	94 ± 1.0	93 ± 1.7

[a]Rats were previously fed the respective diets for 30 days prior to intubating a solution containing ^{109}Cd and the respective diets. At the various time points, rats were killed and the liver, kidneys and gastrointestinal tract (including the esophogus, stomach and intestine, contents included) were lyophilized, weighed and radioactively determined. The value shown at each time point represent an average from six rats. The time points given are from the administration of initial dose. Values are the means ± SEM.

Table 6. Urinary excretion of ^{109}Cd as a percentage of the initial dose in rats previously fed diets for 30 days with different levels of protein.

Treatment group	^{109}Cd excreted, % of initial dose[a]		
	24 hr after exposure	48 hr after exposure	72 hr after exposure
Low-protein	0.03 ± 0.0009	0.05 ± 0.019	0.02 ± 0.010
High-protein	0.05 ± 0.010*	0.05 ± 0.007	0.03 ± 0.013
Low-protein plus cysteine	0.07 ± 0.010†	0.03 ± 0.016	0.03 ± 0.015

[a]Mean ± SEM for six rats.
*$p < 0.05$.
†$p < 0.01$.

ney at 48 and 72 hr, similar observations were made in rats fed the low-protein diet with cysteine added.

The urinary excretion of cadmium, as a percentage of the initial dose, is shown in Table 6. Although the pattern of excretion was different among these groups, significant differences between groups B and C and between C and D were observed only at 24 hr.

Discussion

The present studies support previous reports on the long- and short-term effect of dietary protein on the accumulation of cadmium in the liver and kidney. As previously observed (*5*), in relatively long-term exposures high-protein dietary feeding was associated (when compared to feeding a low-protein diet) with significantly high concentrations of cadmium in the liver and kidney. Results described above support and extend this observation by data which show that the effect of the high-protein diet is independent of the dose of cadmium, whereas the magnitude of difference is dependent on the dose. We have also observed in short-term feeding studies high levels of liver and kidney cadmium in rats fed the low-protein diet similar to that reported by Suzuki et al. (*4*). Thus, in long-term exposures, the high-protein diet is associated with high tissue levels of cadmium whereas the reverse is observed in short-term exposure. These contrasting results may be explained by decreased excretion and/or increased tissue retention.

These factors were studied by determining the amount of cadmium excreted in the urine during short- and long-term exposures and by measuring MT. The urinary excretion of cadmium during the first 24 hr following an oral dose of ^{109}Cd was significantly greater in rats fed the high-protein diet. Similar results were observed in the long-term studies. However, significant differences were only observed after 90 days of exposure to 25- or 50-ppm cadmium. These results suggest that the observed lower concentrations of cadmium may occur in the kidney because of the initial increase in the urinary excretion of this element. Results also suggest that the relationship of urinary cadmium to the kidney level of this element change as the exposure is continued. This change may be associated with the induced synthesis of MT (*12,13*). Thus, for example, the synthesis of this protein may be regulated by both dietary protein and the tissue level of cadmium. Optimal conditions for protein synthesis would be expected in rats fed the high-protein diet (*14,15*). As shown above, MT levels in both the kidney and liver were significantly higher in rats fed the high-protein diet and 25 or 50 ppm cadmium. Thus, the lack of a significant change in the excretion of cadmium in rats fed the high-protein diet (i.e., long-term exposures) may be associated with an increase in the tissue retention of cadmium due to the increase in MT synthesis.

Marked increases in the urinary excretion of cadmium have been associated with renal damage (*16*). In a previous report, we have observed renal damage in rats fed a high-protein diet and drinking water containing 50 ppm cadmium for 90 days (*5*). Thus, renal damage may explain the marked increase in urinary cadmium following 90 to 120 days of exposure to the high-protein diet and drinking water containing 25 or 50 ppm cadmium.

Cadmium is known to form complexes with the thiol group of cysteine and reduced gluthathione (*17,18*). Since the concentration of cysteine in the high-protein diet was fourfold higher than found in the low-protein diet, experiments were performed to determine the importance of cysteine in explaining these dietary differences. Results showed that when cysteine was added to the low-protein diet to the level present in the high-protein diet, significant dietary differences in

liver and kidney cadmium and MT were not observed.

In the long-term studies, the level of cadmium observed in the urine from animals fed the low-protein diet with cysteine added was similar to the levels observed in rats fed the high-protein diet. However, in the short-term studies, the cadmium level in the urine 24 hr after treatment was higher in rats given the low protein plus cysteine than the high- or low-protein diet groups. Furthermore, of the three groups studied, the tissue levels and the amount of cadmium absorbed were highest in the group fed the low protein with cysteine added. These short-term results suggest that cysteine affects both the absorption and tissue retention level of cadmium. This suggestion is in part supported by the studies of Kennedy in which cysteine was shown to increase the kidney level and the nephrotoxicity of cadmium (*19,20*). An important factor in the effect of cysteine was the ratio of this amino acid to cadmium. These investigators reported marked renal lesions and kidney cadmium concentrations when the dose of cysteine was 250 times the dose of cadmium or greater. In the present study, the ratio of cysteine to cadmium was determined from the daily food and water consumption. For rats fed these diets and drinking water containing 5, 25 or 50 ppm cadmium the ratios were, respectively: low protein, 21.5, 4.3 and 2.3; high protein, 88, 17.6 and 9.5; and low protein plus cysteine, 104, 20.7 and 11.2. These ratios suggest that dietary cysteine would have a greater effect on the accumulation of cadmium in the kidney when the exposed level to cadmium was relatively low. Thus, the dose of cadmium and this ratio may both be considered important factors in cadmium metabolism.

Several investigators have suggested that the intracellular level of MT may protect the cell against cadmium-induced toxicity (*10*). For example, cadmium toxicity in rats is reduced if they are pretreated with zinc (*21*). Zinc stimulates the synthesis of MT. In an attempt to determine the importance of dietary protein on MT synthesis and cadmium toxicity, long-term studies were performed. The toxicity of cadmium was determined by measuring the activity of COMT. The activity of this enzyme has been previously shown to be sensitive to cadmium by this investigator and others (*22*). Results from these studies show that dietary protein affects both the activity of this enzyme and MT levels. The activity of COMT and MT in the lung and heart was significantly lower in rats fed the low-protein diet than those fed the high-protein diet and 50 ppm cadmium. These results provide additional support to the importance of MT in protecting the cell against cadmium-induced toxicity.

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