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EFFECT OF CADMIUM INGESTION ON CADMIUM AND ZINC PROFILE IN MALE AND FEMALE RAT LIVER CYTOSOL

RAGAA EL-GAZZAR,* JAMES BOYLE, JOSEPH CORNICELLI and HAROLD G. PETERING†,‡

†Department of Environmental Health, Kettering Laboratory, University of Cincinnati, Cincinnati, OH 45267, U.S.A. and *High Institute of Public Health, 165 Horia Street Hadara, Alexandria, Egypt

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Abstract—A study of the changes in rat liver cytosol zinc and cadmium metalloprotein profiles obtained by G-75 Sephadex chromatography of male and female rats given oral cadmium chloride chronically at different low doses showed that the changes were dose related. Marked disturbances in the zinc-containing regions of the protein profile preceded any extensive formation of metallothionein, and the earliest signs of cadmium metalloprotein formation involved three equally prominent regions, a high molecular weight region (I), the metallothionein region (V) and a low molecular weight region (VI). The low molecular weight region (estimated at 3500 daltons) is of interest in that it is a newly recognized zinc-containing region even in the controls. Significant quantitative differences were found between males and females. Female livers consistently contained higher concentrations of cadmium and zinc. There also were important quantitative differences in the zinc and cadmium-containg cytosol fractions of the protein profiles of livers of males and females exposed to oral cadmium.

Cadmium, a toxic, ubiquitous, heavy metal, has been considered one of the most hazardous environmental contaminants resulting from modern industrialization [1], and consequently it has been the subject of many studies related to its human health effects [2]. In seeking the biochemical basis of cadmium toxicity, attention has been focused on a class of metal-binding proteins, metallothioneins (MT) or orthioneins, initially discovered by Kägi and Vallee [3, 4]. This class of proteins has recently been isolated from the cytosols of various organs of different species, including human kidneys [5–7]. It has been characterized by a low molecular weight, estimated at 6500–13,000 daltons depending on source and method of estimation, a high cysteine content, a virtually complete absence of tyrosine and tryptophan, and by its ability to chelate cadmium, zinc, mercury and, to a lesser extent copper [4, 7].

The biological role of MT has not been completely elucidated. Webb [8] has suggested that they may play a general role in the metabolism and detoxification of a number of heavy metals. Webb [9] and Leber *et al.* [10] have demonstrated that thionein can be induced by cadmium, mercury and zinc and it is, at least, two proteins with similar ultraviolet absorbances and metal-binding properties. Since cadmium has been shown to replace zinc in several metalloenzymes [11, 12] and since these two metals have somewhat similar characteristics, occupying the same group of the periodic table, the possibility has been suggested that cadmium toxicity may manifest itself when cadmium saturates MT and then competes with zinc for the active sites of several critical metalloenzymes; as a consequence of such action, Cd would alter normal zinc metabolism and thus offset normal cellular metabolism [13].

The data available in the literature show that cadmium interferes with the normal metabolism of zinc as well as of copper. Supplee [14] was one of the earliest investigators to report a definite antagonistic relationship between zinc and cadmium with respect to the growth of poultry. Cotzias *et al.* [15] also suggested that cadmium caused a marked perturbation of zinc metabolism in rabbits, and Petering *et al.* [16] demonstrated that some of the symptoms of cadmium toxicity in rats which resulted from a subacute oral exposure to the metal could be alleviated by increasing the amounts of ingested zinc.

Murthy *et al.* [17] have shown that cadmium absorption and retention are much greater in female rats than in males, when both sexes are maintained under identical dietary and environmental conditions. This could be the result of a better biosynthetic apparatus for thionein in females or of sex differences in the kinetics of absorption and retention of Cd. In this regard, Webb [8] in 1972 reported a second zinc-binding protein, also with a molecular weight of about 12,000 daltons, found in female control animals but absent in males and suggested this as a possible sex-linked characteristic.

It has not yet, however, been determined whether the ultimate biochemical role of MT is exclusively dependent on one protein or on a family of metalloproteins in which zinc may play a major role.

Since most earlier investigations were based on the acute induction of MT, this study was designed to investigate the effect of long-term oral exposure of male and female rats receiving a stock diet to different

‡ To whom reprint request should be sent.

levels of cadmium chloride on the formation of cadmium, zinc and other trace metal-binding proteins, especially with respect to sex-linked responses.

MATERIALS AND METHODS

Sprague–Dawley weanling rats with an average body weight of 50 g were fed a commercial stock diet (Purina Lab Chow) *ad lib.* Male or female rats were divided into four groups, and cadmium, as the chloride, was administered in the drinking water at 0.0, 4.3, 8.6, or 34.4 μg/ml for 200 days.

At death. the liver of each rat was excised, immediately frozen, and kept at −4°. When needed, the liver was thawed, minced and homogenized in enough cold potassium phosphate buffer (0.01 M, pH 7.8) to yield a 20% (w/v) homogenate. Our procedure then followed the method outlined by Winge [18]. The crude homogenate was centrifuged at 105,000 *g* for 1 hr in a Beckman centrifuge, model L2 65B. The supernatant fraction was subsequently heated to 60°, held there in a water bath for 15 min and centrifuged according to the method outlined in Fig. 1 to obtain a soluble extract. Protein contents of the

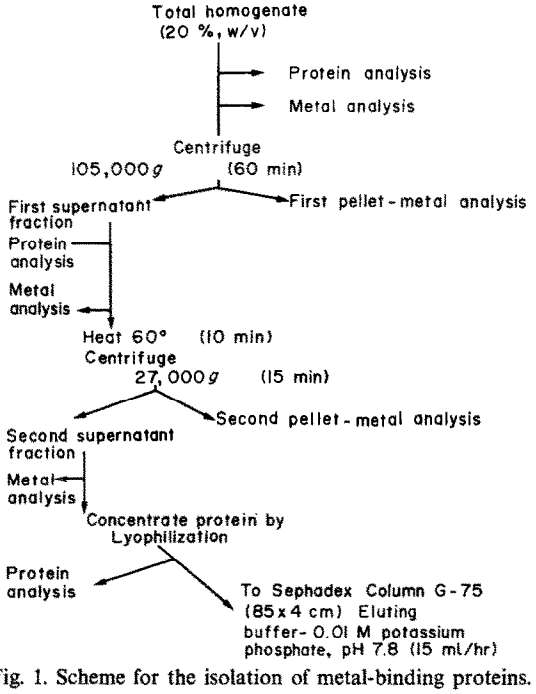

Fig. 1. Scheme for the isolation of metal-binding proteins.

subcellular fractions were determined by the microbiuret method of Goa [19]. Previous studies [8, 18] on cadmium metallothionein had demonstrated that soluble extracts obtained after heating retain a major percentage of hepatic cadmium. This extract was concentrated by lyophilization and chromatographed on a Sephadex G-75 column (85 × 2.5 cm) using 0.01 M potassium phosphate buffer at pH 7.8 as eluent. Fractions of 3 ml each were collected every 12 min. Optical densities of each fraction were monitored at 254 and 280 nm using a Gilford spectrophotometer 240 for their protein contents. Metal analysis for cadmium, zinc and copper was done directly on each fraction by atomic absorption spectrophotometry [20, 21]. Molecular weight estimations were done by calibrating the column with molecular weight reference standards of ovalbumin (45,000), chymotrypsinogen (25,000) and ribonuclease (13,700).

Metal concentrations in the crude liver homogenate supernatant fractions and pellets were also determined by atomic absorption spectrophotometry after wet acid digestion [20]. Fractions of the liver cytosol corresponding to estimated (or apparent) molecular weights of approximately 3000, 13,000, 27,000, 38,000 and 60,000 daltons were pooled separately and freeze dried. Electrophoretic analyses of the metallothionein fraction using standard equipment and methodology [22] were done on cellulose acetate paper strips.

RESULTS

Protein in subcellular fractions. In order to establish the consistency of the procedure outlined in Fig. 1, we determined the protein contents of the various fractions obtained from the seven female livers and nine male livers which were processed to produce cytosols suitable for Sephadex G-75 chromatography. The results for the crude homogenates and the two supernatant fractions are given in Table 1. These clearly show that there were no important differences in the amounts of protein in the supernatant fractions attributable to sex. They also indicate the consistency of the isolation of about 10 per cent of the homogenate protein as cytosol protein.

Distribution of zinc and cadmium in subcellular fractions. The details of zinc and cadmium distribution found in the various fractions of liver homogenates prepared according to the method outlined in Fig. 1 are presented in Tables 2 and 3 for females and males respectively. These data include means ± S. E. M. or individual values for the female 34.4 μg Cd/ml group and only representative values for the 4.3 and 8.6 μg Cd/ml groups, since not every fraction sample in these groups was analyzed.

Zinc. The amount of zinc in the 105,000 *g* first supernatant fractions accounted for 46–68 per cent of that in the female homogenates and 55–66 per cent of that in the male homogenates, which indicates the consistency of the fractionation. In addition, recoveries of zinc in the first supernatant fraction and pellet were 93–108 per cent for female samples and 94–110 per cent for male samples. This lack of sex difference in the per cent recovery of zinc was also evident in the values of the second supernatant fractions, which were the cytosol fractions analyzed chromatographically. In both female and male second supernatant cytosol perparations we found that the zinc values for the groups receiving 8.6 μg Cd/ml were the highest, a fact which was evident in the metalloprotein profiles to be discussed below.

It is of interest to note that the concentration of zinc in the crude homogenates of both males and females was increased by the level of dietary cadmium in a dose-related manner, the increase being greater in the female samples than in the male ones. It is also noteworthy that the loss of zinc in going from

Table 1. Protein content of subcellular fractions of rat liver homogenates*

	Protein content	
	Female (7)†	Male (9)†
Crude homogenate (g/sample)	1.54 ± 0.08	1.66 ± 0.07
First supernatant fraction (105,000 *g*) (% of crude homogenate)	30.3 ± 1.52	30.6 ± 1.90
Second supernatant fraction (heated) (% of crude homogenate)	8.9 ± 0.83	11.4 ± 0.99

* Values are expressed as means ± S. E. M.
† Number of samples.

Table 2. Zinc and cadmium in subcellular fractions of female rat liver homogenates

	Cadmium in drinking water (μg/ml)			
A. Zinc analysis	0	4.3	8.6	34.4
No. of samples	3	1	1	2
Crude homogenate (μg/g dry wt)	87 ± 7.50*	88	100	153 (150, 156)†
First supernatant fraction (% of crude homogenate)	46 ± 1.5*	54	68	55.5 (55, 56)†
Second supernatant fraction (% of crude homogenate)	24 ± 0.67*	26	46	27.5 (27, 28)†
B. Cadmium analysis				
crude homogenate (μg/g dry wt)	ND‡	11	20	102 (86, 118)†
First supernatant fraction (% of crude homogenate)	ND	77	82	78.5 (76, 81)†
Second supernatant fraction (% of crude homogenate)	ND	64	74	65 (65, 65)†
Dry weight applied to column (g)	0.24	0.29	0.27	0.29

* Mean ± S. E. M.
† Mean and actual values.
‡ not detectable.

Table 3. Zinc and cadmium in subcellular fraction of male rat liver homogenates

	Cadmium in drinking water (μg/ml)			
A. Zinc analysis	0	4.3	8.6	34.4
No. of samples	3	1	1	4
Crude homogenate (μg/g dry wt)	100.7 ± 5.9*	121	127	146.7 ± 7.6*
First supernatant fraction (% crude homogenate)	61 ± 3.2*	55	66	56 ± 2.2*
Second supernatant fraction (% crude homogenate)	25 ± 2.4*	32	44	32 ± 3.1*
B. Cadmium analysis				
Crude homogenate (μg/g dry wt)	ND†	7.0	17.0	58.0 ± 4.2*
First supernatant fraction (% crude homogenate)	ND	67	81	74 ± 1.4*
Second supernatant fraction (% of crude homogenate)	ND	58	76	68 ± 1.1*
Dry weight applied to column (g)	0.29	0.21	0.22	0.28

* Mean ± S. E. M.
† Not detectable.

the first supernatant fraction to the second one was substantial, being in the range of 16–28 per cent for females and 16–40 per cent for males, while, at the same time the protein losses averaged 21 and 19 per cent respectively. These results show that the zinc concentrations in the liver and its subcellular fractions were directly related to the level of dietary intake of cadmium.

Cadmium. Turning now to the cadmium concentrations found in the various subcellular fractions as shown in section B of Tables 2 and 3 it is evident that the recovery of cadmium was greater than that of zinc in the 105,000 *g* first supernatant fraction in both female and male samples, the range for females being 77–82 per cent and that for males being 67–81 per cent.

The losses of cadmium in the heated pellets were much less than those of zinc, being in the range of 4 to 8.5 per cent of that in the female liver homogenates and 2.8 to 8.7 per cent of that in the male liver homogenates, even though the amounts of protein removed were in the range of 18–21 per cent. The recovery of cadmium in the second supernatant fractions, which were used for metalloprotein G-75 Sephadex column chromatography, was 64–74 per cent in the case of female samples and 58–76 per cent for male samples. These results indicate that the method used was consistent and gave a high yield of cytosol cadmium, even though the concentration of cadmium in the original homogenate varied from 11 to 118 μg Cd/g in the female samples and from 7 to 58 μg Cd/g in the male samples.

The cadmium content of the homogenate was dose related, increasing as the dietary level of cadmium was increased, as was expected. The values also show that livers from females contained more cadmium than did those from male rats, it being about double at the highest dietary level of cadmium. This substantiates an earlier report from our laboratory [17].

One additional fact shown in Tables 2 and 3 is of importance, namely that the material applied to the G-75 Sephadex columns was similar in all cases, amounting to 8–11 per cent of the original homogenate proteins, being in the range of 0.21 to 0.29 g of total solids.

G-75 Sephadex metalloprotein profile. The protein profiles of G-75 Sephadex chromatography of cytosol preparations (second supernatant fractions), representative of the livers of rats receiving different amounts of cadmium, are given in Figs. 2 and 3, the former for female samples and the latter for male samples. The solid curve shows the protein fractions specified by 280-nm absorbancy of the column fractions, and the inserts show these curves as well as those of 254-nm absorbancy in the molecular weight region of approximately 20,000–5,000 daltons. This is done since 254-nm absorbancy is considered to be a good indicator of the Cd—S bond and so might indicate the appearance of cadmium thionein in the molecular weight region of 10,000 daltons.

Essentially, all of the 280 nm profiles show four protein peaks or bands, which may be designated I, III, pre-V and post VI, the roman numerals referring to Zn and/or Cd concentration regions in the fractions, as discussed below. There were some quantitative changes in the 280-nm profiles, but no qualitative ones. These can best be described by the ratios of the heights of peaks I/III, which seemed to change in relation to the cadmium intakes or cadmium contents of the livers. Thus, in Fig. 2, the ratios of I/III varied in the following manner: 2.0, 0.6, 0.9 and 1.6 for a, b, c and d cytosols respectively. Similarly, in Fig. 3, the ratios of I/III were 1.4, 1.3, 1.3 and 2.2 for a, b, c and d cytosols.

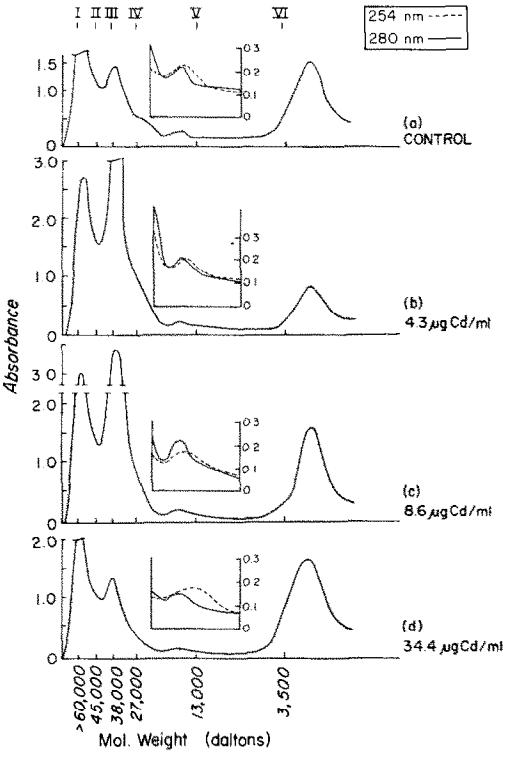

Fig. 2. Sephadex G-75 chromatography of female rat liver cytosol fed a semipurified diet with 0, 4.3, 8.6 and 34.4 ppm of cadmium in the drinking water. The final supernatant fraction from Fig. 1 was applied to a 4 × 85 cm column and eluted with 0.01 M K-phosphate buffer (pH 7.8) at a flow rate of 15 ml/hr. Each fraction was monitored at 254 and 280 nm for protein concentration. Approximate molecular weights were determined by chromatography of substances of known molecular weight.

The meaning of these changes is not clear, but they may indicate, as do the metal distributions among the chromatographic fractions, that cadmium ingestion causes more profound biochemical changes than we have heretofore realized.

The 254-nm absorbancy profiles shown in the inserts of Figs. 2 and 3 indicate that only when cadmium ingestion was at the highest level (d samples) was there a definite band in the 13,000 region of metallothionein, a shift which occurred in both male and female cytosol fractions. In the control samples and in the lower Cd-ingestion samples (a, b and c), the 254-nm region seemed to peak at about 15,000 daltons.

Figures 4 and 5 show profiles for zinc and cadmium bound to proteins in the eluates of the G-75 Sephadex columns from the same samples described in Figs.

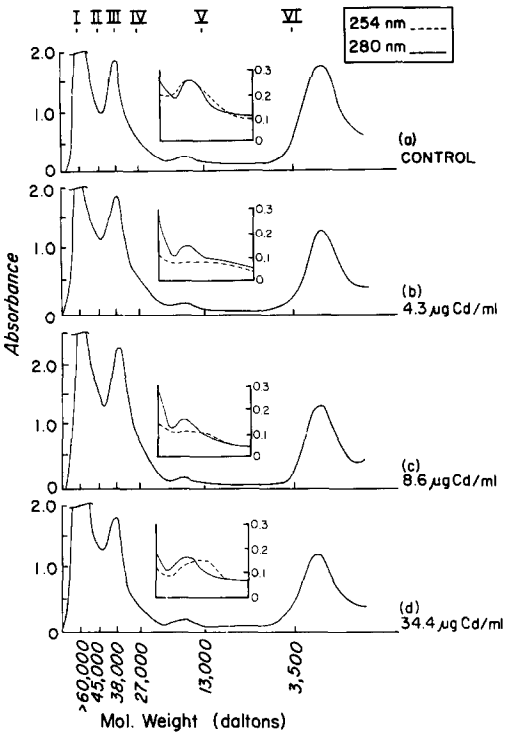

Fig. 3. Sephadex G-75 chromatography of male rat liver cytosol fed a semipurified diet with 0, 4.3, 8.6 and 34.4 ppm of cadmium in the drinking water. The procedure was as described in Fig. 2.

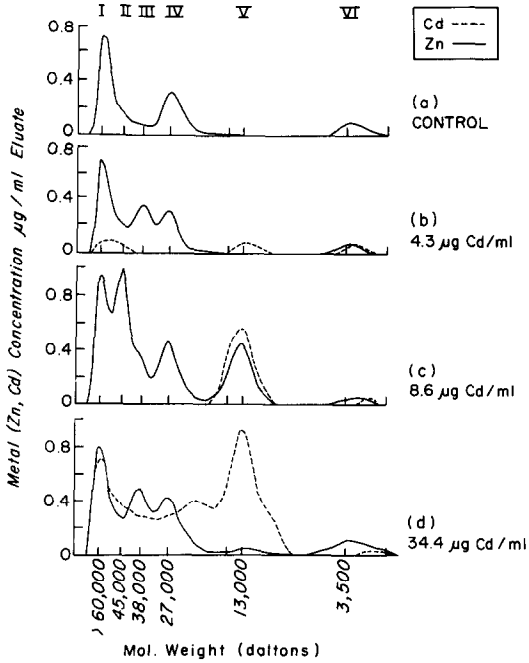

Fig. 4. Distribution of cadmium- and zinc-containg fractions from female rat liver cytosol by approximate molecular weight. The fractions collected in Fig. 2 were analyzed by atomic absorption spectrophotometry for cadmium and zinc, and the results plotted as a function of molecular weight. Cadmium concentrations in the drinking water were 0, 4.3, 8.6 and 34.4 ppm.

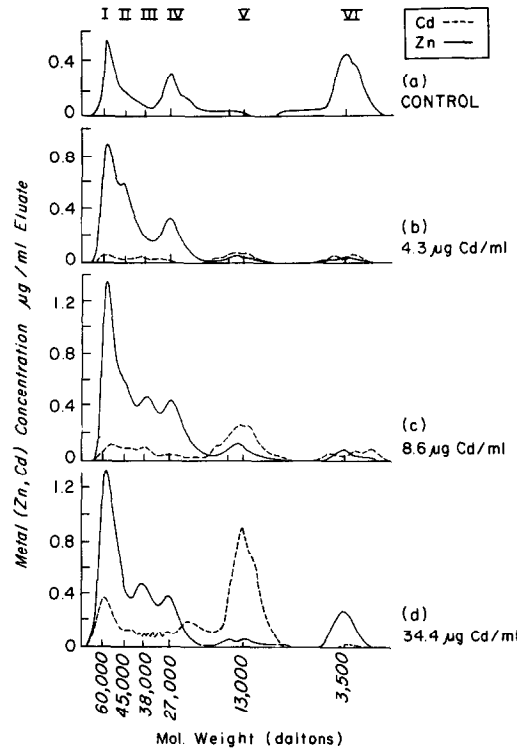

Fig. 5. Distribution of cadmium- and zinc-containing fractions from male rat liver cytosol by approximate molecular weight. The fractions collected in Fig. 3 were analyzed by atomic absorption spectrophotometry for cadmium and zinc, and the results plotted as a function of molecular weight. Cadmium concentrations in the drinking water were 0, 4.3, 8.6 and 34.4 ppm.

2 and 3. We have identified six distinctly different peaks or regions of concentration of zinc, cadmium or both in the various group cytosols which have been identified on the basis of estimated molecular weights. These are peak I containing proteins with molecular weights greater than 60,000 daltons, and peaks II, III, IV, V and VI containing proteins of about 48,000, 38,000, 27,000, 13,000 and 3500 daltons respectively.

Zinc distribution in protein fractions. In the control female liver cytosol (Fig. 4a) we find three distinct peak regions: i.e. I, IV and VI with evidence of shoulders at II and III. In the male control liver cytosol (Fig. 5a) the same three major peaks (I, IV and VI) were present, and in addition a definite shoulder at II and one beyond IV were present. The low molecular weight peak VI at about 3500 daltons is new, not having been previously reported. It is more marked in the male liver cytosol than in the female one.

When we examine the effects of oral cadmium administration on the zinc metalloprotein profiles, we note a series of dose-related changes (cf. Figs. 4b, c and d and 5b, c and d), which are different in male and female cytosol samples. Thus, when we look at Figs. 4b and 5b, profiles for the lowest cadmium intake (4.3 μg/ml of water), we see that a new peak, namely III, appears in the female sample, and two new ones, namely II and V, appear in the male sample. Peak V is the thionein region with apparent molecular weight of 12,000–13,000 daltons. In addition, in the

male cytosol there is a drastic reduction in peak VI, the lowest molecular weight region of zinc concentration.

In the zinc metalloprotein profiles from rats receiving 8.6 μg/ml of cadmium in the drinking water (Figs. 4c and 5c), further changes are noted. In the females (Fig. 4c) peak II now is the highest one, and peak III has become a shoulder with about the same amount of zinc as was present in Fig. 4b. Peak IV appears to be increased and peak V is now a very prominent one. No apparent change in peak VI is evident, as is also the case in Fig. 4b.

In the male zinc metalloprotein profile at the dosage of 8.6 μg Cd/ml (Fig. 5c), peak I is elevated above that in Fig. 5b, and as a result peak II has become a shoulder. Peak III is now prominent and there appears to be a small peak V in the MT region. We see in the male sample, as is evident in the female one, that there is a marked increase of total zinc in the cytosol fractions, and we note that distinctive quantitative differences between the sexes exist in the zinc profiles.

When the zinc profiles of the rats receiving 34.4 μg Cd/ml are examined, we find that some more drastic changes have occurred in comparison with the profiles found when the cadmium levels were lower. Thus, in the female cytosol (Fig. 4d) there is a marked reduction in the amount of protein-bound zinc. In addition, a loss of zinc peaks II and most of peak V has occurred, but peak VI now seems to have been increased to about the control value.

In the male profile (Fig. 5d) we see much less change between it and that found in Fig. 5c, which contrasts with the differences between female profiles (Fig. 4d and 4c). There is a loss of zinc in region II and some reduction in region V, but an increase of zinc in peak VI almost to the control value.

Cadmium distribution in protein fractions. There was no detectable cadmium in the control homogenates. When, however, we examined the cytosols from the lowest level of cadmium intake, i.e. 4.3 μg/ml of drinking water, three definite peaks in regions I, V and VI were found, with the female sample having more cadmium than the male one. As noted above, the exposure to this low level of cadmium (4.3 μg Cd/ml) caused a lowering peak VI zinc-binding protein and the appearance of other zinc-binding proteins in both sexes, with the thionein peak V of males having both zinc and cadmium. These data are important in that they do not show that there is a unique cadmium-binding protein region as the first result of chronic low level exposure but rather that there is the appearance of three protein regions in which cadmium is bound, a high molecular weight one (I), a very low molecular weight one (VI) and the low molecular weight region V, which we have identified as the metallothionein region.

When the cadmium intake was doubled to 8.6 μg/ml (Figs. 4c and 5c), peak V(MT) emerged as the major protein-bound cadmium region of the cytosols of livers from both sexes. It should be noted that, in the female sample at 8.6 μg Cd/ml (Fig. 4c), cadmium peak I was greatly reduced and peak VI was somewhat smaller. Peak V (MT) of females, however, also contained a large amount of zinc-binding protein, as noted above. In fact, the molar ratio of Cd/Zn in female peak V was $\frac{1}{2}$.

In the male profile, there was also a marked elevation of peak V, and it also contained an increased amount of zinc. The molar ratio of Cd/Zn in this peak was about 1/1 in contrast to female peak V. A considerable amount of cadmium was found in the high molecular weight region of the male profile, and an increased amount was also present in the lowest molecular weight region (peak VI). Thus, distinct qualitative and quantitative differences in the binding of cadmium to proteins of the liver cytosols of male and female rats were evident.

Finally, when we examine the profiles of the liver cytosols from rats receiving the highest dosage of cadmium, i.e. 34.4 μg/ml, we find that drastic changes have occurred. In the case of the female profile (Fig. 4d), cadmium in peak V (MT) was greatly increased with almost no zinc present in it. In region VI, cadmium was very small, but in this group it was found that cadmium was now present in large amounts in the protein fractions having molecular weights greater than 13,000 daltons. For example, region I now had a distinct cadmium peak, and there had emerged a new peak between peaks IV and V. It appears that there was as much cadmium in other higher molecular weight proteins, as there was in the metallothionein region of peak V. We also note that peak V has a definite shoulder in a region of about 8000 daltons, suggesting the formation of additional molecular species of cadmium-containing proteins.

In Fig. 5d, a similar situation is seen. Peak V is elevated and sharp, with very little zinc in it. The peak between IV and V noted above for the female profile is also evident. There is a great reduction in region VI cadmium bound to protein, and a considerable amount of cadmium in the higher molecular weight regions of the spectrum. In addition to the distinct peak between IV and V we found cadmium bound in the protein of region I. There was not the loss of zinc-binding proteins in the male profile that was seen in the female profile.

Thus, distinct changes were apparent which were associated with the increases in cadmium intake. These changes were manifested in the male and female profiles in different ways quantitatively but were quite similar qualitatively.

Preliminary electrophoretic separations of the metallothionein region (V) have shown two distinct bands, which differ only quantitatively between male and female animals. Further studies are needed to elucidate this point.

DISCUSSION

Although metallothionein in its cadmium and or zinc forms has been isolated from many sources and has been known for almost two decades, its function has not been fully established. It has been postulated to have a role in the detoxification mechanism of heavy metals, to be the agent producing the toxic effect of cadmium and to be an important protein involved in zinc homeostasis.

A difficulty in interpreting the role of metallothionein in cadmium toxicity has been the fact that very little effort has been made to determine the effect

of low oral or respiratory doses, which are the routes most likely to be experienced by humans. In addition, most studies have been concentrated on the direct effects of cadmium despite the fact that there are good data which show its ingestion has an effect on zinc and copper metabolism.

The data presented here are important in that they are based on long-term, low level oral administration of cadmium and thus give an insight into the effects of cadmium on metallothionein synthesis as well as on the distribution of zinc and cadmium in this and other proteins under conditions which are relevant to human exposure. In this way it has been possible to see the progression of steady state changes in the zinc and cadmium bound to proteins of the liver cytosols.

Our data show that the ingestion of cadmium in amounts greater than that found in the stock diet was necessary to cause the appearance of this element in detectable amounts in rat liver homogenates and cytosols. Therefore, when as little as 4.3 μg Cd/ml of drinking water (0.038 μM) was given, cadmium was found in the homogenates and cytosols of both sexes. Of interest is the fact that the cytosol cadmium at this low level of intake was found in three distinct fractions in both males and females, not just in fraction V, the metallothionein fraction. The other two fractions were a high molecular weight region (approx. 45,000 daltons) and a low molecular weight region of about 3500 daltons (peak VI).

As the level of intake of cadmium was increased, the bulk of the cadmium was found in peak V (MT molcular weight 12,000–13,000). When 8.6 μg/ml (0.075 μM) was given, peak V (MT) also contained a large amount of zinc, but when the high level of 34.4 μg Cd/ml was reached, peak V contained very little zinc and almost exclusively cadmium in both male and female cytosols. Thus, the Cd/Zn ratio of region V (MT) was dependent on the level of cadmium exposure and was not an inherent property of cadmium ingestion.

Furthermore, whereas the high molecular weight cadmium-binding proteins were small when 4.3 and 8.6 μg/ml of cadmium were given, they became of major importance when 34.4 μg/ml was given. This progression indicates that at 34.4 μg/ml the synthesis of cadmium metallothionein could not keep pace with the amount of cadmium being absorbed, and therefore cadmium was possibly being bound to proteins which normally bind zinc. This possibility is further indicated when we examine the zinc-binding protein regions I, II, III and IV. These regions are maximal with respect to zinc-binding protein when 8.6 μg Cd/ml was given with only a very small amount of cadmium being present, but they fall to the level of the 4.3 μg/ml cadmium groups when 34.4 μg/ml of cadmium is given. If one now adds the cadmium-binding and the zinc-binding proteins, one finds that the high molecular weight proteins approximate the profile found when 8.6 μg/ml of cadmium was given.

The finding that metallothionein was not the only cadmium-binding protein of rat liver cytosol when cadmium intake was low is an important finding, as is the fact that cadmium spilled over into normally zinc-binding protein regions when it was given in high amounts. Of equal and perhaps more significance is the observation that even at the lowest level of cadmium intake, when very little cadmium appeared in the cytosol, there was the appearance of zinc peak III, which was of greater magnitude than that of any or all of the three cadmium peaks. This disturbance of zinc-binding proteins progressed as cadmium intake rose, involved peak II as well, and was quantitatively different in males and females. Thus, it seems possible that some if not all of the low level chronic toxicity of cadmium may be due to a disturbance of zinc metabolism rather than to direct effects of cadmium metallothionein (Cd–MT).

In this regard, we found a significant quantitative difference in the responses of male and female liver tissue. There was always more cadmium in the female liver homogenates, particularly in the high molecular weight regions when the cadmium intake was high (Fig. 4d vs Fig. 5d).

The fact that female rats of the Sprague–Dawley strain accumulate more orally administered cadmium than do males in all organs examined is of considerable interest in view of the evidence of sex differences in the toxicity of this metal in this strain. Boscolo *et al.* [23] reported that systolic hypertension developed in male Sprague–Dawley rats given cadmium orally as the chloride in the same range as used here, whereas it did not develop in females. This is contrary to the observation of Perry *et al.* [24] with regard to the development of systolic hypertension in Long–Evans female rats. Pence *et al.* [25] have recently reported that intraperitoneal injection of cadmium chloride into Sprague–Dawley rats caused an increase in hexobarbital sleeping time as well as in hepatic microsomal metabolism in males, but not in females. These and other observations on sex differences in the toxicity of cadmium indicate that a careful examination of the mechanisms of these differences and their relationships to absorption, retention and excretion of this element is needed.

Although it has not been emphasized previously or even pointed out, careful examination of reports of other authors [5, 6, 26, 27] has also shown that a definite low molecular weight zinc-binding protein, similar to our peak VI, is a normal metalloprotein region of rat and human liver cytosol. This region needs to be characterized, as do the high molecular weight regions which are readily altered by chronic oral cadmium intake.

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REFERENCES

1. W. Fulkerson and H. E. Goeller, Eds., *Cadmium: The Dissipated Element*. Oak Ridge National Laboratory, ORNL NSF-EP-21 (1972).
2. L. Friberg, M. Piscator and G. F. Nordberg, *Cadmium in the Environment*. CRC Press. Cleveland, Ohio (1971).
3. J. H. R. Kägi and B. L. Vallee, *J. biol. Chem.* **235**, 3460 (1960).
4. J. H. R. Kägi and B. L. Vallee, *J. biol. Chem.* **236**, 2435 (1961).
5. T. L. M. Syversen, *Archs Envir. Hlth* **30**, 158 (1975).

6. Z. A. Shaikh and O. J. Lucis, *Archs Envir. Hlth* **24**, 419 (1972).
7. P. Pulido, J. H. R. Kägi and B. L. Vallee, *Biochemistry* **5**, 1768 (1966).
8. M. Webb, *Biochem. Pharmac.* **21**, 2751 (1972).
9. M. Webb, *Biochem. Soc. Trans.* **3**, 632 (1975).
10. A. P. Leber and T. S. Miya, *Toxic. appl. Pharmac.* **37**, 403 (1976).
11. B. L. Vallee and D. D. Ulmer, *A. Rev. Biochem.* **41**, 91 (1972).
12. J. E. Folk, E. C. Wolff and E. W. Schirmer, *J. biol. Chem.* **237**, 3100 (1962).
13. E. J. Underwood, *Trace Elements in Human and Animal Nutrition*. Academic Press, New York (1960).
14. W. C. Supplee, *Poult. Sci.* **41**, 827 (1961).
15. G. C. Cotzias, D. C. Borg and B. Selleck, *Am J. Physiol.* **201**, 297 (1971).
16. H. G. Petering, M. A. Johnson and K. L. Stemmer, *Archs Envir. Hlth* **23**, 93 (1971).
17. L. Murthy, D. P. Rice and H. G. Petering, in the Third International Symposium on Trace Element Metabolism in Man and Animals, July 25–29, Freising-Weihenstephan, West Germany (1977).
18. D. R. Winge, R. Premakumar and K. V. Rajagopalan, *Archs Biochem. Biophys.* **170**, 242 (1975).
19. J. Goa, *Scand. J. clin. Lab. Invest.* **5**, 218 (1953).
20. *Analytical Methods for Atomic Absorption Spectroscopy*, Perkin–Elmer Corp., Norwalk, Conn. (1971).
21. J. R. Sorenson, E. G. Melby, P. J. Nord and H. G. Petering, *Archs Envir. Hlth* **27**, 36 (1973).
22. *Gelman Clinical Electrophoresis Manual*, Gelman Instrument Co. Laboratory Products Division, Ann Arbor, Mich. (1974).
23. P. Boscolo, V. N. Finelli, H. Choudhury and H. G. Petering, paper presented at the International Symposium on Cadmium. Jena, East Germany, Aug. 2, 3 (1977).
24. H. M. Perry Jr., M. Erlanger and E. F. Perry, *Am. J. Physiol.* **232** (2) *H*114(1977).
25. D. H. Pence, T. S. Miya and R. C. Schnell, *Toxic. appl. Pharmac.* **39**, 89 (1977).
26. M. P. Richards and R. J. Cousins, *J. Nutr.* **106**, 1591.
27. R. W. Chen, P. P. Whanger and P. H. Weswig. *Biochem. Med.* **12**, 95 (1975).