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Effects of Dietary Zinc Depletion and Food Restriction on Intestinal Transport of Cadmium in the Rat[1] (42179)

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Abstract. The effects of Zn depletion and short-term fasting on intestinal transport of Cd were examined in perfused rat small intestines. The small intestine was isolated with its vascular network intact, then simultaneously perfused from the luminal and vascular sides. A Zn-depleted state that results in marked hypozincemia was produced in some rats by feeding a Zn-deficient diet for 4 days. Uptake of Cd from the luminal perfusate was greater in the Zn-depleted rats, whereas transport of Cd to the vascular perfusate was not affected. Fasting overnight prior to perfusion did not influence Cd transport nor alter the effect of Zn depletion on Cd uptake. The Cd concentration in the soluble fraction of intestinal mucosa from perfused intestines was not different between Zn-depleted and Zn-adequate rats. Gel filtration chromatography of the soluble fraction showed a shift in the distribution of Cd from metallothionein to high molecular weight ligands in intestines from Zn-depleted rats. The decrease in amount of metallothionein-associated Cd corresponded to a decrease of total intestinal metallothionein as measured by the Cd-binding assay. The results suggest association of Cd with intestinal metallothionein did not influence the absorption of Cd under these conditions.

Cadmium is avidly taken up and retained by the gastrointestinal tract (1–4). Absorption of Cd from the intestinal tract, however, is restricted, being generally reported as less than 5% of an oral dose [reviewed in (5)]. Given that Cd is not known to be an essential element, is not under homeostatic control (6), and shows only limited absorption, it is unlikely that unique transport mechanisms specific for transport of Cd in the intestine exist. On the other hand, there is evidence that the homeostatic transport mechanisms of some essential metals can accommodate intestinal Cd transport. Stimulation of iron transport mechanisms by dietary iron restriction will increase Cd absorption (7, 8). Likewise, dietary Ca restriction results in enhanced intestinal Cd absorption (9).

The purposes of this study were to examine the effects of zinc-depletion and short-term fasting on the intestinal transport of Cd. Other investigators have previously reported that zinc depletion has no effect on intestinal Cd transport (10, 11). For those studies, Cd absorption in animals that had been fasted overnight prior to the experiments was determined by whole-body Cd retention following an oral dose (10), or by perfusion of a 10-cm segment of proximal intestine (11). Since food restriction is a factor which can increase intestinal absorption of metals (12), this effect could possibly obscure the effects of zinc depletion on Cd transport. Moreover, the intestinal metallothionein (MT) level may be a factor in the regulation of Cd absorption (13). Since hepatic MT levels are responsive to both zinc depletion and fasting (14), we were interested in whether these factors also influence intestinal MT levels and have resultant effects on intestinal Cd transport.

The present study has employed a two-factor design which included overnight fasting and dietary zinc level as variables. Intestinal transport of Cd was studied using simultaneous vascular and luminal perfusions of the isolated rat small intestine (15). Considering the limited intestinal absorption for Cd, the double intestinal perfusion system has the advantages of providing a direct measure of Cd absorption and utilizing the entire length of the small intestine.

Materials and Methods. *Animals and diets.* Male CD strain rats (Charles River Breeding Laboratories, Wilmington, Mass.), weighing

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200–300 g were housed in suspended stainless-steel cages. They were fed a commercial diet (Purina Rat Chow, Ralston Purina Co., St. Louis, Mo.) and tap water *ad libitum.* A 12 hr light–dark cycle (0700–1900 and 1900–0700 hr, respectively) was used. Commencing 4 days prior to the experiments, rats were individually housed and provided with a modified AIN-76 diet (Dyets Inc., Bethlehem, Pa.) and glass-distilled, deionized water *ad libitum.* For one group the diet was deficient in zinc, (<1 mg Zn/kg) (16), and the other group was fed the same diet supplemented with 32 mg Zn/kg. Food was withdrawn from one-half of the rats in each of the two dietary groups at 1700 hr of the fourth day. The nonfasted rats of the zinc-adequate dietary group were pair-fed to the nonfasted rats of the zinc-deficient dietary group on the fourth night in order to ensure that equal amounts of diet were consumed. For the first three days equal amounts of diet were consumed by all groups.

Intestinal perfusion. The technique for simultaneous vascular and luminal perfusions of the rat small intestine has been previously described (15, 16). Briefly, the small intestinal vasculature was isolated by ligation of the celiac and colic arteries, the pancreatic vein, and the inferior and superior mesenteric veins at the cecum. An inflow cannula was positioned through the abdominal aorta into the superior mesenteric artery, and an outflow cannula was positioned in the portal vein. The small intestinal vasculature was perfused without recirculation at approximately 3 ml/min and 50 mm Hg pressure at the inflow cannula. The vascular perfusate contained 46.2 g high molecular weight dextran (United States Biochemical Co., Cleveland, Ohio), 1 g glucose, 3 mg dexamethasone (Sigma, St. Louis, Mo.), and 50 ml horse serum (GIBCO, Grand Island, NY) per 1 liter of bicarbonate buffered Krebs–Ringer solution. The vascular perfusate was maintained at 37°C and continuously gassed with 5% CO_2/95% O_2.

The intestinal lumen was perfused from just distal to the pylorus, to just proximal to the ileocecal junction. The nonrecirculating luminal perfusion was initiated with infusion of a 5-ml bolus of luminal perfusate followed by perfusion at 0.2 ml/min over a 45-min period. The composition of the M199 culture medium (GIBCO) based luminal perfusate was as described by Steel and Cousins (16), except that it was buffered at pH 6.5 and was not supplemented with Zn. The luminal perfusate included 20 μM $CdCl_2$ and 0.5 μCi ^{109}Cd/ml.

Intestinal uptake of Cd was measured as the disappearance of ^{109}Cd from the luminal perfusate. Absorption of Cd was measured as the appearance of ^{109}Cd in the vascular perfusate effluent at the portal vein cannula.

In vivo Cd absorption. Six rats were randomly divided into two groups of three each. One group was fed the Zn-deficient diet, the other group was fed the Zn-adequate diet. Both groups were provided diet and glass-distilled deionized water *ad libitum* for 5 days, thereafter food consumption of both groups was restricted to 80% of Zn-adequate *ad libitum* food consumption. Beginning on Day 4 of the dietary regimen, both groups received 25 ppm Cd, as $CdCl_2$, in their drinking water. On Day 10, animals were sacrificed by decapitation. Tissue Cd concentration of the liver, kidney, and intestinal mucosa and serum Zn concentration were measured by atomic absorption spectrophotometry (AAS).

Gel filtration chromatography of mucosal cytosol. Small intestines were flushed with 50 ml of cold 0.9% saline, excised, slit lengthwise and the mucosal layer was scraped from underlying tissue with glass slides. The mucosa was homogenized in 2 vol (w/v) of cold buffer (250 m*M* sucrose, 0.1% NaN_3, and 10 m*M* Tris–acetic acid, pH 8.6) with 10 strokes using a glass-Teflon homogenizer. The mucosal homogenates were immediately centrifuged at 40,000*g*, 4°C, for 60 min. Aliquots of the 40,000*g* supernatants were immediately chromatographed in an ascending fashion using 2.5 × 55-cm columns of Sephadex G-75, equilibrated and eluted with 10 m*M* Tris–acetic acid, pH 8.6, 0.1% NaN_3 at 4°C. Eluted fractions were assayed for absorbance at 280 nm and either ^{109}Cd or zinc content.

Measurements. Zinc concentration of serum and mucosal supernatants was measured by AAS (14). To determine tissue levels of Cd, tissues were dried overnight at 100°C and then dry ashed at 450°C for 36 hr. The ash was then dissolved in HNO_3 and Cd content determined by AAS (17). ^{109}Cd was measured using a γ-ray spectrometer. Cd content of radioactive samples was calculated from the specific activity of ^{109}Cd in the initial solutions.

Tissue MT levels were measured using a Cd-binding assay (18). MT concentrations were calculated from the amount of ^{109}Cd bound in the supernatant solutions.

Protein concentration of mucosal supernatants was determined by the dye binding method of Bradford (19).

Statistics. The effects of dietary zinc level and fasting were assessed by two-way analysis of variance (ANOVA) with the SAS ANOVA procedure (SAS Institute, Inc., Cary, N.C.). Student's t test was used for comparison of means in the *in vivo* experiment.

Results. *In vitro Cd transport in perfused small intestines.* Following the 4 days of dietary conditioning, serum zinc concentrations were significantly depressed ($P < 0.05$) in the groups fed the zinc-deficient diet. Serum zinc concentrations in nonfasted rats were 1.5 ± 0.1 and 0.8 ± 0.3 μg Zn/ml for Zn-adequate and Zn-depleted groups, respectively. In fasted rats, these concentrations were 1.4 ± 0.2 and 1.0 ± 0.2 μg Zn/ml for the Zn-adequate and Zn-deficient groups, respectively. Uptake of Cd by the perfused small intestine, as measured by the disappearance of Cd from the luminal perfusate, was significantly ($P < 0.05$) greater in the Zn-depleted groups than in the Zn-adequate groups (Table I). Cadmium absorption in the Zn-depleted groups, as measured by Cd appearance in the vascular effluent, tended to be greater than that in the Zn-adequate groups (Table I). However, the influence of dietary zinc status on Cd absorption was not a significant effect ($P > 0.05$). An overnight fast influenced neither Cd uptake nor absorption. Absorption amounted to only 1.2–1.5% of the Cd taken up by the intestine, and the percentage of intestinal Cd which was transported to the vascular perfusate was independent of both dietary zinc status and fasting.

Cadmium content of the post perfusion 40,000g mucosal supernatants was not different among the four groups (Table I). Gel filtration chromatography of the supernatants resulted in two Cd containing peaks (Fig. 1), a high molecular weight peak (HMW) and a second lower molecular weight peak, which, based upon previous experience, is metallothionein (20, 21). Zinc-depletion resulted in both an increase ($P < 0.01$) in the amount of Cd associated with HMW peak and a corresponding decrease ($P < 0.05$) in the amount of Cd associated with the MT peak (Table I). A comparable effect was observed with mucosa from both fasted and fed rats.

Intestinal zinc and metallothionein. Zinc concentrations of the 40,000g mucosal supernatants (Table II) from nonperfused intestines and the distribution of Zn between the two major Zn peaks isolated by gel filtration chromatography (Fig. 2) were not influenced by dietary zinc status or fasting. Total intestinal MT as measured by the Cd binding assay was

TABLE I. EFFECT OF ZINC STATUS AND FASTING ON Cd UPTAKE AND ABSORPTION AND THE DISTRIBUTION OF Cd BETWEEN HIGH MOLECULAR WEIGHT LIGANDS AND METALLOTHIONEIN IN MUCOSAL SUPERNATANT OF SMALL INTESTINE

	Nonfasted		Fasted	
	Zinc-adequate diet	Zinc-deficient diet	Zinc-adequate diet	Zinc-deficient diet
Cd uptake[a,b]	35.3 ± 7.1	43.2 ± 3.9*	36.4 ± 1.2	43.6 ± 2.4*
Cd absorption[a,b]	0.43 ± 0.13	0.53 ± 0.09	0.43 ± 0.24	0.66 ± 0.08
% absorption/uptake[a]	1.2 ± 0.4	1.2 ± 0.2	1.2 ± 0.6	1.5 ± 0.2
Supernatant Cd[a,c]	487 ± 149	505 ± 83	441 ± 69	570 ± 58
HMW Cd[a,c]	149 ± 34	241 ± 14**	109 ± 8	253 ± 36**
MT Cd[a,c]	249 ± 89	160 ± 33*	296 ± 57	161 ± 41*
HMW/MT Cd[a]	0.62 ± 0.11	1.54 ± 0.25**	0.38 ± 0.08	1.61 ± 0.27**

[a] Means ± SD, $n = 3$.

[b] Nanomoles Cd/g mucosa/45 min.

[c] Picomoles Cd/mg supernatant protein.

* Significant effect ($P < 0.05$) of diet, determined by ANOVA.

** Significant effect ($P < 0.01$) of diet, determined by ANOVA.

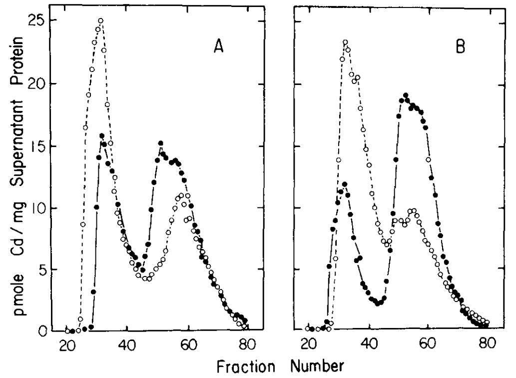

FIG. 1. Distribution of Cd in supernatants from mucosa of perfused rat intestine following perfusion of the intestinal lumen with a luminal perfusate containing 20 μM Cd (and ^{109}Cd). The 40,000g supernatant of mucosal homogenates were fractionated using 2.5 × 55-cm columns of Sephadex G-75 as described under Materials and Methods. Rats were fed either the zinc-adequate diet (—) or the zinc-deficient diet (---). The rats were either fed (A) or fasted (B) prior to the perfusion experiments. Each of the four profiles is a composite derived from three separate fractionations.

significantly ($P < 0.01$) diminished by zinc depletion and significantly elevated by fasting (Table II).

Cd transport in vivo. To determine if the effects of Zn depletion on *in vitro* intestinal uptake and distribution of Cd influence *in vivo* absorption or retention of Cd, rats fed the Zn-deficient or Zn-adequate diet were exposed to Cd via drinking water for 1 week. Diet had no effect on Cd retention in liver, kidney, or small intestine (Table III).

Discussion. The 4-day duration of dietary conditioning with the zinc-deficient diet was sufficient to produce a Zn-depleted state as indicated by serum zinc level, as has been shown previously (14, 21). The extent of Zn depletion did not markedly depress food consumption nor affect mucosal Zn level (Table II). The 4-day dietary regimen has previously been shown to produce near maximal stimulation of intestinal zinc absorption (15). This stimulation has been observed as an induction of apparent carrier-mediated transport processes for both zinc uptake by rat intestinal brush border membrane vesicles (22), and zinc absorption in the simultaneous luminal and vascular intestinal perfusion system (16).

In contrast to the previous reports (10, 11), we observed Cd uptake by the perfused small intestines of zinc-depleted rats to be approximately 20% greater than that of Zn-adequate rats. Cd absorption also tended to increase with zinc depletion, but the effect of dietary treatment was not significant. Absorption did, however, remain a constant percentage of Cd uptake (Table I). This indicates that a process mediating Cd uptake is responsive to zinc depletion, whereas the processes responsible for translocation of Cd beyond the luminal mucosal membrane are not. A Zn-depletion-induced mechanism mediating uptake of Zn at the intestinal brush border membrane, which was observed by Menard and Cousins (22), could be responsible for the increased Cd uptake in Zn-depleted rats.

While Zn depletion increased Cd uptake from the lumen, the level of Cd in the soluble fraction of the mucosa and the percentage of mucosal Cd translocated to the vascular perfusate (Table I) were not affected by Zn depletion. As such, it could be expected that the

TABLE II. EFFECT OF ZINC STATUS AND FASTING ON THE ZINC AND TOTAL METALLOTHIONEIN CONCENTRATIONS OF THE MUCOSAL SUPERNATANT FROM NONPERFUSED SMALL INTESTINE

	Nonfasted		Fasted	
	Zinc-adequate diet	Zinc-deficient diet	Zinc-adequate diet	Zinc-deficient diet
Supernatant Zn^{a,b}	0.24 ± 0.02	0.22 ± 0.02	0.24 ± 0.02	0.24 ± 0.03
Total metallothionein^{a,c}	84 ± 26	66 ± 18*	139 ± 28**	81 ± 33*,**

[a] Means ± SD, $n = 6$.

[b] Microgram Zn/mg protein.

[c] Picomoles metallothionein/mg protein.

* Significant effect ($P < 0.01$) of diet, determined by ANOVA.

** Significant effect ($P < 0.01$) of fasting, determined by ANOVA.

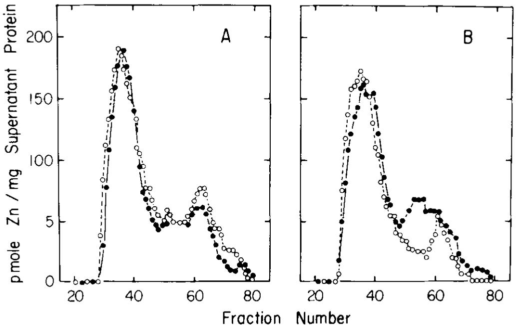

FIG. 2. Distribution of Zn in supernatant from mucosa of nonperfused rat intestine. The 40,000*g* supernatant of mucosal homogenates were fractionated using 2.5 × 55-cm columns of Sephadex G-75 as described under Materials and Methods. Rats were fed either the zinc-adequate diet (—) or the zinc-deficient diet (---). The rats were either fed (A) or fasted (B) prior to the experiments. Each of the four profiles is the result of chromatography of supernatants pooled from six intestines.

site of the effect of Zn depletion was restricted to the brush border membrane. Preliminary data showed more particulate-bound ^{109}Cd was present in mucosa from intestines of the zinc-depleted groups where uptake was greater than in control groups. Zn depletion also clearly influenced Cd distribution in the soluble fraction of the mucosa (Fig. 1). There was a decrease in the amount of Cd associated with MT and a corresponding increase in that associated with the HMW ligands in cytosol from Zn-depleted rats (Table I). Since the zinc content and its distribution between the HMW and MT fractions of the mucosal supernatant were not influenced by Zn depletion (Fig. 2), the altered Cd distribution cannot then be attributed to changes in zinc binding resulting from a change in Zn status.

Levels of MT in the mucosal supernatant of nonperfused intestine were lower in Zn-depleted groups than in Zn-adequate groups (Table II). This is in agreement with our previous findings that dietary zinc status is a controlling factor for MT gene expression in intestine (21) and liver (20). The reduced level of intestinal MT in Zn-depleted groups may also be related to an increased rate of MT degradation as reported for hepatic MT of Zn-deficient rats (23). Intestinal MT levels were increased following an overnight fast (Table II). Fasting has previously been shown to increase hepatic MT level (14). While intestinal MT levels were influenced by Zn depletion and fasting, total Cd content of the postperfusion mucosal supernatant was not different between the treatment groups (Table I), and the molar ratio of MT-bound Cd (Table I) to MT (Table II) was relatively constant among all treatment groups (2–3 moles Cd/mole MT). Furthermore, the treatment group with the greatest MT level (fasted, Zn-adequate) corresponded to the group with the greatest amount of Cd associated with MT. Therefore, the shift of Cd from MT to HMW ligands in the intestinal mucosa of the Zn-depleted rats appears to be directly related to a decrease in intestinal MT levels. It has been previously proposed that intestinal MT might sequester large acute oral doses of Cd in mucosal cells hindering subsequent transfer to the systemic circulation (13). Kello *et al.* (24), however, have shown Cd absorption was not diminished by elevated intestinal MT levels. Similarly, in the present experiments, Cd absorption was observed to be independent of Zn-depletion-mediated decreases of intestinal MT (Table II) and MT-bound Cd (Table I). As neither elevated nor diminished intestinal MT levels affect Cd absorption, intestinal MT does not appear to be a determinant of Cd absorption. Based upon these data, however, we cannot rule out an effect of intestinal MT on Cd absorption when pharmacological levels of the metal enter the intestine and induce synthesis of the protein.

Under present conditions Cd uptake by perfused rat intestines was greater in Zn-depleted than in Zn-adequate controls. In contrast, Zn status was reported by Hahn and Evans (10) and Foulkes and Voner (11) to have no effect on Cd uptake. Fasting was initially

TABLE III. EFFECT OF ZINC STATUS ON Cd RETENTION AFTER A 1-WEEK EXPOSURE TO Cd IN THE DRINKING WATER

	Zinc adequate	Zinc deficient
Liver[a,b]	11.1 ± 1.2	10.0 ± 0.1
Whole kidney[a,b]	2.7 ± 0.2	2.5 ± 0.1
Intestinal mucosa[a,b]	8.2 ± 0.7	7.8 ± 0.6

[a] Means ± SD, $n = 3$.

[b] Nanomoles Cd/g tissue.

considered as a factor which could influence Cd transport. The results of perfusion experiments show overnight fasting was not a determinant of Cd transport (Table I), and therefore did not clarify the disagreement on the effects of Zn depletion on Cd uptake. Dexamethasone which stimulates uptake of Zn in the rat intestine (25) and was present in the perfusate of the present studies is not likely to be related to the difference, although this glucocorticoid was used in all perfusions. In addition, the rate of Cd uptake observed for Zn-adequate rats in the present experiment, 35 nmole Cd $(g \cdot 45\ min)^{-1}$ or approximately 12% of the perfused dose (Table I) is in agreement with previously reported Cd uptake values for *in vivo* perfused rat intestines without dexamethasone. Kello *et al.* reported Cd uptake by rat jejunum perfused with 20 μM Cd amounted to approximately 9% of the perfused Cd (24). Foulkes (26) reported that Cd uptake by perfused rat jejunum obeys saturation kinetics with apparent K_m of 0.1–0.2 mM and V_{max} of 0.01 μmole $(g\ min)^{-1}$. Using these kinetic parameters, Cd uptake in the present experiments is predicted to be 40–75 nmole $(g \cdot 45\ min)^{-1}$. Consequently, there is no evidence to suggest that use of dexamethasone in the perfusate had an acute influence on Cd uptake.

The duration of dietary Zn restriction may also have influenced the effect of Zn depletion on Cd uptake. In the present experiments, the Zn-depleted groups were fed a Zn-deficient diet for 4 days, as the maximal stimulation of Zn transport is achieved at that time (15), whereas, in the previous studies (10, 11) rats had been maintained on Zn-deficient diets for 2 weeks. Overt signs of deficiency occur within that period which could affect many transport and other processes. The discrepancy in effect of Zn depletion on Cd uptake may also reflect the intestinal region in which Cd uptake is responsive to Zn depletion. In the present study, an effect of Zn depletion was observed when the entire length of the small intestine was perfused. In previous studies, no effect was found when Cd uptake was measured in the proximal 15 cm of small intestine (10) or in the proximal 10 cm of jejunum (11). While there is no clear consensus regarding the site where most Zn transport occurs, many investigators have reported Zn transport to be greater in distal than in proximal regions of the small intestine [reviewed in (27)]. We have also observed the percentage of inhibition of Cd uptake by Zn to be greater in distal regions than in proximal regions of the rat intestine (unpublished). As such, it is possible that the effect of Zn depletion on Cd uptake occurs in the distal small intestine. Since consumption of Cd-containing H_2O for 1 week did not influence Cd retention by the liver, kidney, or intestine the effect of Zn depletion on Cd uptake observed in the perfused intestine does not contribute to a measurable difference in absorption of this toxic metal *in vivo.*

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