BUTTERWORTH HEINEMANN

Adaptation responses in rats to long-term feeding of high-zinc diets: emphasis on intestinal metallothionein

Philip G. Reeves

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND USA*

When faced with an abundance of heavy metals in the diet, higher animals produce metallothionein (MT) in the intestinal mucosa to sequester the metals in an attempt to reduce their absorption into the body. This paper shows that this strategy may only be used for short-term exposure, and a more effective, sustainable strategy is adopted during long-term exposure. The concentration of dietary zinc was abruptly elevated and offered to rats continually for a specified period. The results showed that an abrupt change in dietary zinc caused an immediate elevation of intestinal MT concentration, which remained elevated for about 2 weeks and then began to decline. After about 5 weeks, MT concentrations in the intestines of rats fed high-zinc diets were not different from controls fed normal-zinc diets for the entire period. The concentrations of zinc in serum, liver, and kidney followed the same course as mucosal MT, elevated during the initial phase of feeding high-zinc and near control values during the latter phase of feeding. Although the rats were consuming diets with zinc concentrations about 7 fold higher than controls, and intestinal MT concentrations were not elevated, the serum and tissue concentrations of zinc were near control values. This suggests that the induction of intestinal MT may be an immediate and short-term strategy for coping with high intakes of certain metals, and that some other, more efficient mechanism is adopted during long-term exposure. (J. Nutr. Biochem. 6:48–54, 1995.)

Keywords: zinc; copper; metallothionein; intestine; absorption; adaptation; rat

Introduction

All living organisms strive to maintain a steady state. They have developed various mechanisms to resist change and to preserve physiological conditions that remain relatively constant throughout life.[1] When acted upon by various physical properties of the environment, including changes in diet or food supply, organisms adopt various strategies in order to cope.[2] One of the strategies that some organisms use to cope with potentially toxic heavy metals in their food supply is to increase the production of metallothionein (MT). MT are small proteins that contain sulfhydryl groups that bind heavy metals. In higher animals, the organ of initial response is the intestinal mucosa. When exposed to heavy metals, there is a rapid up-regulation of the MT genes, and a few hours later, large amounts of MT proteins are produced. If the concentrations of metals are relatively small, they are effectively bound up by MT, and possible toxic effects are averted.

Intestinal MT is induced also by essential minerals, such as zinc, and binds essential minerals such as zinc and copper. If mucosal concentrations of MT are high enough, normal utilization of these minerals may be altered. Under these circumstances, the organism might regard MT as harmful and attempt to adopt some defensive mechanism to reduce its effects. In this regard, in one of our studies to determine the effect of zinc-induced MT on the cobinding of copper in intestinal mucosa cells of rats, we observed that the magnitude of MT induction with chronic feeding of high-zinc diets appeared to become less with the duration of feeding. Rats fed high-zinc diets for 1 week had 2½ times more MT in the mucosa as those rats receiving the diet for 2 weeks.[3]

To follow up this observation, we designed experiments

---

*The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

Address reprint requests to Dr. P.G. Reeves at the USDA, ARS, Grand Forks Human Nutrition Research Center, 2420 2nd Avenue North, Grand Forks, ND 58202-9034 USA.

Received April 13, 1994; accepted August 17, 1994.

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(94)00008-A

to determine to what extent intestinal mucosa MT would adapt when rats were fed high-zinc diets for an extended period. Results of these studies are given in detail in this paper. Similar results were obtained from two different studies; only one is reported here.

Materials and methods

Animals and diet

This study was approved by the Animal Use Committee of the USDA, ARS, Grand Forks Human Nutrition Research Center and was in accordance with the guidelines of the National Institutes of Health on the experimental use of laboratory animals.[4]

Two hundred and fifteen, 8-week-old Sprague–Dawley rats were purchased from Sasco, Inc. (Omaha, NE USA†). Upon arrival at the laboratory, they were fed a diet similar to AIN-93G[5] except that it was modified to contain 50 mg of zinc and 4 mg of copper/kg. Five days later, the rats were randomly divided into various experimental groups. The first study was designed to determine whether intestinal mucosa MT could adapt to chronic feeding of a high-zinc diet. It was made up of two groups of rats. One group contained 75 rats that continued to receive the modified AIN-93G diet. Another group of 70 rats began to receive the AIN-93G formulation that was modified further to contain 350 mg of zinc and 4 mg of copper/kg. This study was run for 42 days. On days, 3, 7, 14, 21, 28, 35, and 42, 10 rats from each group were killed and their tissues prepared for analyses. Five rats were killed at time 0.

The second study was designed to determine whether acclimation of rats to high-zinc diets by periodic elevation of dietary zinc would affect the induction of MT in the intestinal mucosa. This study consisted of two groups of rats. One group was the same as the first group in the study described above. Rats in the other group were acclimated to a high-zinc diet by weekly incremental increases in dietary zinc concentration. Briefly, all rats in this group were first fed the diet with 50 mg of zinc/kg for 1 week, and 10 rats were killed. The remaining rats were then switched to a diet containing 100 mg of zinc/kg for another 7 days, and 10 more rats were killed. This scheme of increasing dietary zinc by 50 mg/kg at weekly intervals continued until the last group of 10 rats had received all preceding concentrations of zinc plus 350 mg of zinc/kg for the final week. The dietary concentrations of zinc were 50, 100, 150, 200, 250, 300, and 350 mg/kg. At each 7-day interval, 10 rats from each of the two groups were killed and their tissues prepared for analyses.

Analytical procedures

To preserve maximal concentrations of intestinal MT, the rats were not fasted before they were killed. Beginning at 0900 hr, on the day of each kill, each rat was anesthetized with 50 mg of pentobarbital sodium/kg of body weight. The abdominal cavity was opened and blood was withdrawn from the abdominal artery into Monovette tubes (Sarstedt, Newton, NC USA) for the collection of serum. Blood samples were allowed to clot at room temperature for 5 min and the tubes were placed in crushed ice.

After blood was withdrawn, the liver and kidneys of each animal were perfused via the vena cava with 50 ml of ice-cold saline (145 mmol NaCl/L of deionized water). Liver and kidneys were immediately removed and divided into various portions and frozen in liquid nitrogen. The tissues were stored at −80°C until analysis.

A 20 cm segment of the intestine, beginning at the pylorus, was excised and the contents were washed out with ice-cold saline. The segment was slit open and the mucosal layer was gently scraped off with the edge of a glass slide. The scrapings were stored at −80°C until they were analyzed for MT, zinc, and copper within 2 weeks after collection.

Blood samples were centrifuged at 4°C for 20 min at 1500*g*. Serum was collected and immediately frozen at −20°C. Proteins were precipitated from 0.5 mL of serum with 1.0 mL of deionized water and 0.5mL of 0.45 mol 5-sulfosalicylic acid/L of deionized water. The mixture was allowed to sit at room temperature overnight and was then centrifuged at 2000*g* for 20 min. The resulting supernatant was analyzed for zinc and copper by flame atomic absorption spectroscopy (AAS).

Samples of intestinal mucosa, liver, and kidney were analyzed for zinc and copper by AAS. Briefly, the tissues were weighed, lyophilized to constant weight, and charred in a muffle furnace at 450°C for 12 hr. Each of the charred samples was suspended in 2 ml of aqua regia and heated to dryness on a hotplate. The samples were returned to the oven and heated at 450°C for another 12 hr. The mineral residue was dissolved in 1 ml of aqua regia and diluted appropriately with deionized water. Liver standard reference material (1577b, National Institute of Standards and Technology, Gaithersburg, MD USA) was analyzed with each batch of tissue samples for quality control. All analyzed standards were within the specified range for each mineral.

A portion of the frozen intestinal scrapings was homogenized in buffer containing 50 mmol Tris-HCl/L and 1 mmol 2-mercaptoethanol/L, pH 7.4. One milliliter of the homogenate was heated for 10 min at 95°C and centrifuged for 5 min at 10,000*g*. Aliquots of the supernatant were analyzed for MT by the method of Eaton and Cherian.[6]

Statistical analysis and graphics

Data are expressed as means ± standard error of the mean (SEM). Because a different set of rats was killed at weekly intervals, we used the simple Student's *t* statistic to determine differences between groups fed normal and high dietary zinc at each week. A calculated *P* value of 0.05 or less was considered significant. The graphs were produced by the SigmaPlot Computer Graphing Program (Jandel Scientific, San Rafael, CA USA; Windows Version 1.01). The curves in each graph were generated by using the curve fitting program in the SigmaPlot program.

Results

During each of the two study periods, rats gained approximately 5g/day. There were no significant effects of zinc treatment on weight gain (data not shown).

Figure 1A shows the adaptation response of MT in rats chronically fed a diet containing 350 mg of zinc/kg. Clearly, the initial response of rats receiving the high-zinc diet was a marked increase in the concentration of intestinal MT. After reaching a peak between days 3 and 14 it began to decline. By day 21, MT concentrations were one-half the values for the previous week. MT continued to decline and reached near-control values by the end of the experiment.

A different pattern emerged when rats were acclimated to consuming a high-zinc diet *(Figure 1B)*. When the rats were given increasing amounts of dietary zinc each week,

†Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

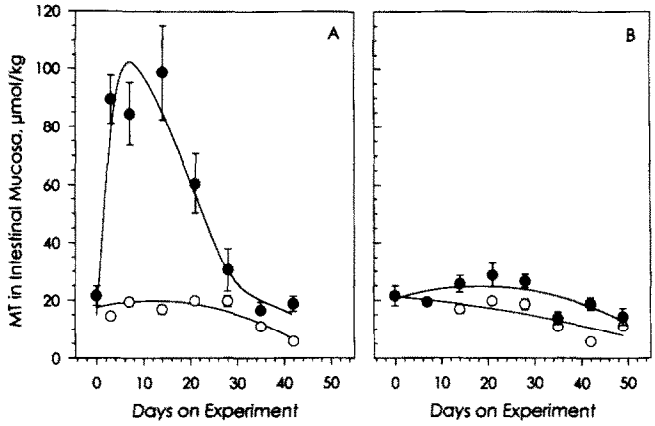

Figure 1 **(A)** Metallothionein (MT) concentrations in the intestinal mucosa of rats chronically fed 50 mg of zinc/kg of diet (open symbols) and 350 mg of zinc/kg (closed symbols). The points represent means ± SEM of 10 rats at each period. The differences between diet groups at days 3 through 21 were highly significant ($P < 0.001$). **(B)** Metallothionein (MT) concentrations in the intestinal mucosa of rats acclimated to increasing concentrations of dietary zinc. Open symbols represent values from rats fed 50 mg of zinc/kg of diet continually. Closed symbols represent values from rats fed diets in which the concentration of zinc was increased by 50 mg/kg each week beginning at day 7. The points represent means ± SEM of 10 rats at each period. Only at days 14, 28, and 42 were there significant differences between diet groups ($P < 0.02$).

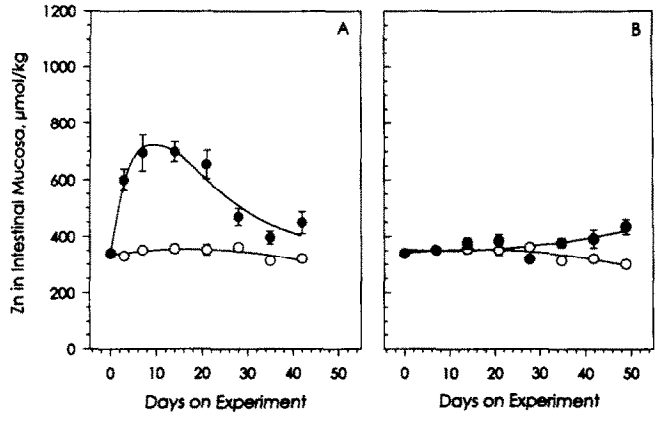

Figure 2 **(A)** Zinc concentrations in the intestinal mucosa of rats chronically fed 50 mg of zinc/kg of diet (open symbols) and 350 mg of zinc/kg (closed symbols). The points represent means ± SEM of 10 rats at each period. The differences between means at days 3 through 21 were significant ($P < 0.001$). At days 28 through 42, the differences were significant, but less than at previous periods ($P < 0.02$). **(B)** Zinc concentrations in the intestinal mucosa of rats acclimated to increasing concentrations of dietary zinc. Open symbols represent values from rats fed 50 mg of zinc/kg of diet continually. Closed symbols represent values from rats fed diets in which the concentration of zinc was increased by 50 mg/kg each week beginning at day 7. The points represent means ± SEM of 10 rats at each period. The differences between diet groups at days 35 through 49 were significant ($P < 0.01$).

the differences between the amounts of MT in the intestinal mucosa of rats fed 50 and 350 mg of zinc/kg was significantly different ($P < 0.05$) between groups only at days 14, 28, and 42. Even though during the last week of the experiment rats were consuming diets containing 350 mg of zinc/kg, mucosal MT did not increase as it had in the initial phase of the first experiment.

Similar results were observed for mucosal zinc *(Figures 2A* and *2B)*. There was an initial increase and then a decline to near-control values *(Figure 2A)*; however, the decline did not begin until after day 21. During the acclimation phase *(Figure 2B)*, the concentration of mucosal zinc did not begin to differ between groups until day 35, and only during the last three periods was it significantly ($P < 0.01$) higher in rats fed 350 mg of zinc/kg than in those fed 50 mg/kg.

Serum zinc concentration showed a similar pattern to intestinal MT and zinc, with a peak concentration at day 7 *(Figure 3A)*. By the end of the experiment, it was near the values found in control rats. During the acclimation study *(Figure 3B)*, the serum zinc concentration was significantly ($P < 0.03$) elevated in those rats receiving the higher amount of dietary zinc. Differences occurred at periods where rats had received 100, 150, 200, or 300 mg of zinc/kg diet for 1 week. The pattern of serum zinc values closely mimicked those of intestinal MT.

Although the magnitude of the difference in peak response of liver and kidney zinc between rats fed 50 and 350 mg of zinc/kg was only one-half that for intestinal and serum zinc, the patterns of responses were similar *(Figures 4A* and *5A)*. In the group fed 50 mg of zinc/kg, there was a tendency for liver zinc to increase as the study progressed. In the acclimation study *(Figure 4B)*, the amount of zinc in liver was generally higher ($P < 0.02$) in those rats fed the high-zinc diet than in those fed the control diet; the difference between groups was less for kidney *(Figure 5B)* than for liver. Nevertheless, the animals had acclimated to the slow increases in dietary zinc so that no exaggerated elevation of liver or kidney zinc was observed.

Under some conditions where high-zinc diets are fed, there is a decrease in copper status of the animal. *Tables 1* and *2* show the concentrations of copper in intestinal mucosa, serum, liver, and kidney. In this study, there were no indications that feeding 350 mg of zinc/kg of diet continually for 42 days or during acclimation to high-zinc diets significantly affected the copper status of rats. However, there was a significant increase ($P < 0.01$) in kidney copper concentration with time, but it was not related to kidney concentrations of zinc, a possible reflection of MT concentration.[3]

Discussion

When animals are faced with extraordinary conditions in the environment, including a change in diet, they adapt by altering biochemical and physiological mechanisms to maintain homeostasis. When animals consume diets containing high concentrations of some heavy metals, including zinc, they adapt by producing large amounts of metallothionein proteins (MT) in the intestinal mucosa. MT subsequently binds the metals and limits their uptake into the body, thus reducing the possibility of harmful effects. This could be considered a type of defense mechanism, but the present study showed that prolonged feeding of diets high in zinc content caused a further adaptation response that lowered

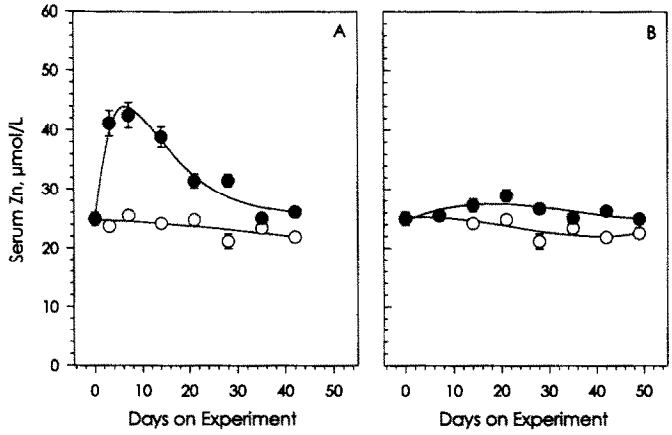

Figure 3 **(A)** Zinc concentrations in the serum of rats chronically fed 50 mg of zinc/kg of diet (open symbols) and 350 mg of zinc/kg (closed symbols). The points represent means ± SEM of 10 rats at each period. The differences between means at days 3 through 28 were significant ($P < 0.001$). The difference was significant between treatments at day 42, but less than at previous periods ($P < 0.02$). **(B)** Zinc concentrations in the serum of rats acclimated to increasing concentrations of dietary zinc. Open symbols represent values from rats fed 50 mg of zinc/kg of diet continually. Closed symbols represent values from rats fed diets in which the concentration of zinc was increased by 50 mg/kg each week beginning at day 7. The points represent means ± SEM of 10 rats at each period. Only at days 14, 21, 28, and 42 were there significant differences between diet groups ($P < 0.05$).

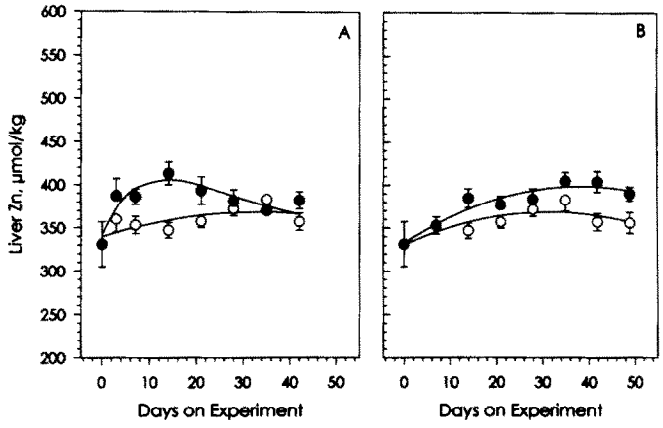

Figure 4 **(A)** Zinc concentrations in the liver of rats chronically fed 50 mg of zinc/kg of diet (open symbols) and 350 mg of zinc (closed symbols). The points represent means ± SEM of 10 rats at each period. Only at days 7 and 14 were there significant differences between groups ($P < 0.03$ and $P < 0.001$, respectively). **(B)** Zinc concentrations in the liver of rats acclimated to increasing concentrations of dietary zinc. Open symbols represent values from rats fed 50 mg of zinc/kg of diet continuously. Closed symbols represent values from rats fed diets in which the concentration of zinc was increased by 50 mg/kg each week beginning at day 7. The points represent means ± SEM of 10 rats at each period. At days 14, 42, and 49 there were significant differences between treatments ($P < 0.03$).

the concentration of MT in the intestinal mucosa. This suggests that MT induction may act only as an initial defense and a more efficient and sustained mechanism is adopted when high-zinc intake is prolonged.

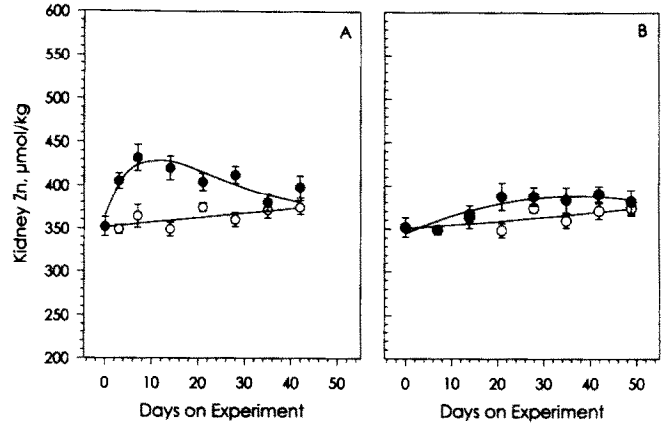

Figure 5 **(A)** Zinc concentrations in the kidney of rats chronically fed 50 mg of zinc/kg of diet (open symbols) and 350 mg of zinc/kg (closed symbols). The points represent means ± SEM of 10 rats at each period. Significant differences occurred at days 3, 7, 14, 21, and 28 ($P < 0.01$ to $P < 0.05$). **(B)** Zinc concentrations in the kidney of rats acclimated to increasing concentrations of dietary zinc. Open symbols represent values from rats fed 50 mg of zinc/kg of diet continually. Closed symbols represent values from rats fed diets in which the concentration of zinc was increased by 50 mg/kg each week beginning at day 7. The points represent means ± SEM of 10 rats at each period. There were no significant differences between groups at any period.

The first experiment showed that the initial response of rats to consuming diets containing high concentrations of zinc was to produce large amounts of MT in the intestinal mucosa. This response was maintained for about 14 days and was accompanied by increased concentrations of mucosal zinc, and high concentrations of zinc in serum, liver, and kidney. The rats subsequently adapted to the high dietary zinc intake, and by days 28 to 42, MT and zinc concentrations in intestinal mucosa were markedly reduced compared with the initial response. Even though the rats continued to consume high-zinc diets, and mucosal MT was only slightly elevated over controls; serum zinc and liver and kidney zinc were near control values. Intestinal MT has been touted as one of the regulators of zinc absorption.[7] However, under the extreme conditions of high-zinc feeding, it appears to have little influence.

The rats also adapted to incremental increases in dietary zinc concentration by maintaining a constant concentration of MT in the intestinal mucosa that was near control values. In the second experiment, the zinc concentration in the diet of rats was increased by 50 mg/kg each week for 42 weeks. There were no exaggerated increases of intestinal MT after each increase in dietary zinc. However, we only measured MT 7 days after dietary zinc was increased. There may have been an immediate response at day one, but MT concentration had returned to control values by day 7. In addition, serum, liver, and kidney zinc concentrations were not elevated at any period of change in dietary zinc concentration.

Studies by Hoadley et al.[7] suggested a role for MT in the absorption of zinc during states of zinc depletion and fasting. The present study, however, suggests that during prolonged consumption of high zinc, rats may adopt alternative mechanisms to reduce the amount of zinc in the body that do not involve MT. The nature of these mechanisms is

Table 1 Copper concentrations in the intestinal mucosa, serum, liver and kidney of rats adapted to dietary intakes of 50 and 350 mg of zinc/kg of diet*

	Diet Zn (mg/kg)†							
	Serum		Intestinal mucosa		Liver		Kidney	
Days	50	350	50	350	50	350	50	350
0	15.1 ± 1.1	15.1 ± 1.1	32.3 ± 2.0	32.3 ± 2.0	47.1 ± 2.7	47.1 ± 2.7	88.9 ± 9.2	88.9 ± 9.2
3	14.0 ± 0.6	15.0 ± 0.5	31.1 ± 1.4	35.1 ± 2.0	58.8 ± 1.7	57.4 ± 3.1	85.4 ± 5.1	95.6 ± 6.8
7	13.9 ± 0.6	14.1 ± 0.3	35.5 ± 1.3	35.6 ± 1.3	57.0 ± 2.4	51.6 ± 1.8	105.1 ± 12.2	92.9 ± 6.1
14	14.2 ± 0.5	14.1 ± 0.4	32.9 ± 2.0	35.8 ± 0.8	53.7 ± 1.6	59.2 ± 1.2	102.6 ± 9.2	117.7 ± 9.2
21	15.4 ± 0.7	14.5 ± 0.8	33.1 ± 1.6	37.9 ± 2.9	51.4 ± 0.6	53.3 ± 2.2	110.2 ± 7.1	97.9 ± 6.9
28	15.5 ± 0.9	16.1 ± 0.6	31.4 ± 1.0	32.9 ± 0.6	54.8 ± 1.7	54.9 ± 2.0	126.3 ± 8.5	123.4 ± 7.0
35	16.4 ± 0.9	15.2 ± 0.4	29.9 ± 1.7	29.7 ± 1.4	57.1 ± 1.6	54.9 ± 1.1	123.3 ± 6.5	124.8 ± 7.9
42	16.7 ± 0.6	15.6 ± 0.5	30.1 ± 2.0	30.6 ± 1.3	55.1 ± 1.6	57.9 ± 1.5	129.8 ± 9.6	120.6 ± 9.1

*Values are means ± SEM of 10 replicates. Values are expressed in μmol/kg of wet tissue or μmol/L of serum.
†Rats were fed diets containing either 50 or 350 mg of zinc/kg for 42 days. Each week throughout the period, 10 rats from each dietary group were killed, and tissue samples were taken for analysis. There were no significant differences between dietary groups or days except that the concentration of kidney copper increased with time. However, there was no significant effect of dietary zinc.

Table 2 Copper concentrations in the intestinal mucosa, serum, liver and kidney of rats slowly acclimated to weekly changes in dietary zinc from 50 to 350 mg zinc/kg diet*

			Zn Treatment†							
			Serum		Intestinal mucosa		Liver		Kidney	
	Diet Zn, mg/kg		Continual	Variable	Continual	Variable	Continual	Variable	Continual	Variable
Days	C	V	(C)	(V)	(C)	(V)	(C)	(V)	(C)	(V)
0	50	50	15.1 ± 1.1	15.1 ± 1.1	32.3 ± 2.0	32.3 ± 2.0	47.1 ± 2.7	47.1 ± 2.7	88.9 ± 9.2	88.9 ± 9.2
7	50	50	13.9 ± 0.6	14.1 ± 0.3	35.5 ± 1.3	35.6 ± 1.3	57.0 ± 2.4	51.6 ± 1.8	105.1 ± 12.2	92.9 ± 6.1
14	50	100	14.2 ± 0.5	13.8 ± 0.5	32.9 ± 2.0	32.8 ± 2.2	53.7 ± 1.6	54.7 ± 1.4	102.6 ± 9.2	108.4 ± 18.6
21	50	150	15.4 ± 0.7	14.9 ± 0.6	33.1 ± 1.6	33.2 ± 1.8	51.4 ± 0.6	58.4 ± 2.7	110.2 ± 7.1	108.4 ± 4.8
28	50	200	15.5 ± 0.9	17.6 ± 0.6	31.4 ± 1.0	30.4 ± 1.1	54.8 ± 1.7	56.8 ± 1.9	126.3 ± 8.5	123.8 ± 8.6
35	50	250	16.4 ± 0.9	15.9 ± 0.9	29.9 ± 1.7	32.7 ± 1.9	57.1 ± 1.6	57.5 ± 1.8	123.3 ± 6.5	121.8 ± 10.3
42	50	300	16.7 ± 0.6	17.6 ± 0.9	30.1 ± 2.0	31.2 ± 2.6	55.1 ± 1.6	57.8 ± 2.1	129.8 ± 9.6	129.4 ± 12.2
49	50	350	18.3 ± 0.9	15.6 ± 0.9	29.2 ± 1.8	35.9 ± 1.5	54.2 ± 1.1	56.5 ± 1.0	105.4 ± 6.2	108.6 ± 6.4

*Values are means ± SEM of 10 replicates. Values are expressed in μmol/kg of wet tissue or μmol/L of serum.
†Rats were fed two different regimens. One group received a diet containing 50 mg of zinc/kg continuously for 49 days, marked C. In the other group, the concentration of dietary zinc was increased by 50 mg/kg each week for the 49-day period, marked V. The values in the variable columns represent those obtained after the rats had consumed the respective dietary zinc concentration for 1 week. The only significant differences observed were increases in the amount of copper in the kidneys with time, except for day 49 when the concentration fell. There was no effect of increasing dietary zinc concentrations in kidney copper.

unknown, but two possibilities exist. One is that the intestinal mucosa adapts by reducing the rate of zinc absorption, and the other is that the adaptation response increases the rate of elimination of absorbed zinc from the body, or a combination of both. To my knowledge, no studies have been done to determine the effect of feeding high-zinc diets for prolonged periods on the absorption of zinc. It has been shown that zinc absorption from the intestine of rats fed normal zinc diets is biphasic; a carrier-mediated phase at low zinc concentrations and a passive phase (paracellular diffusion) at high concentrations.[8]

In preliminary studies (experiments not described) we showed that by using ligated intestinal loops[9] after 8 weeks on the experiment, the rate of absorption of ^{65}Zn from the intestinal lumen into the body of rats fed 350 mg of zinc/kg was only 60% ($P < 0.01$; $n = 3$) of that in rats fed 35 mg of zinc/kg. Similar differences occurred whether the lumenal incubation media contained 11.5 or 115 μmol zinc/L. This type of absorption experiment is difficult to interpret, however, because rats consuming high-zinc diets would have more zinc in the unstirred layer of the intestinal mucosa as well as a higher zinc concentration in the mucosal cell than those consuming diets with lower amounts of zinc. Consequently, the lower absorption rates might simply reflect the lower specific activity of ^{65}Zn at the sites of uptake and transport.[10] In addition, the concentrations of zinc used in the ligated loop experiment were in the range where zinc absorption could be regulated (carrier mediated). However, during normal dietary consumption, the lumenal zinc concentration in rats fed high-zinc could be high enough to be in the diffusional phase of absorption and not regulated. Thus, it seems doubtful that a 40% reduction in zinc transport rate, as measured by this procedure, could be a major contributor to near normal serum, liver, and kidney zinc concentrations in rats fed a 10-fold higher zinc concentration in their diet than controls.

Absorption is only one phase of the regulation of zinc homeostasis; excretion is the other.[11] One could argue that the adaptive response we observed was through the stimulation of a higher rate of excretion of zinc via the urine, through bile/pancreatic secretions, intestinal secretions, and sloughed mucosal cells. We did not measure these parameters, but based on the findings of others, loss through the bile/pancreatic secretions seems an unlikely mechanism. In two different experiments, Finley and Johnson[12] showed no significant difference in zinc secretions via the bile/pancreas in rats fed concentrations of dietary zinc ranging from 10 to 300 mg/kg for 7 weeks. They concluded that biliary/pancreatic secretions in the rat under these conditions were not major contributors to the maintenance of zinc homeostasis. Reinstein et al.[13] showed similar results, but over a shorter feeding period. It seems unlikely, therefore, that the reduction in concentrations of zinc in serum and tissues, as observed in the present study, could be accounted for by increased zinc excretions through this route.

What then is responsible for the apparent reduction of body zinc during high dietary zinc intakes? Finley and Johnson[12] suggested that an increase in the rate of zinc secretion by the intestine and/or an increased rate of turnover of intestinal mucosa cells could be contributing factors. Neither of these possibilities have been investigated.

Effects of feeding high-zinc diets on copper metabolism

Although it has been shown numerous times in the past that high dietary zinc affects copper status of rats through diminished copper absorption,[3,14–16] it was not apparent in the present study. Copper status as judged by concentrations of copper in serum, liver, and kidney was not affected by feeding high-zinc diets, or by the manner in which the diets were presented. However, the magnitude of the effect might depend on a number of factors including the amount of copper in the diet, the amount of zinc in the diet, composition of the diet, the age of the animal at the time the feeding trials begin, and/or the duration of the feeding trials. Fischer et al.[17,18] found that as little as 120 mg of zinc/kg of diet (6 mg of copper/kg) fed to weanling rats for 5 weeks significantly reduced the concentrations of copper in serum. Reeves et al.[3] showed a significant reduction in copper status of 6-week-old rats fed diets containing 560 mg of zinc/kg and 6 mg of copper/kg for 2 weeks. However, Hall et al.[19] could not find an effect on copper absorption at dietary concentrations of zinc below 900 mg/kg (3 mg of copper/kg) in 6-week-old rats. Likewise, Oestreicher and Cousins[20] did not find an effect of feeding diets containing 180 mg of zinc/kg (6 mg copper/kg) on copper absorption in 8-week-old rats. Thus, it was not too surprising to find in the present experiment that the copper status of 8-week-old rats fed 350 mg of zinc and 4 mg of copper/kg diet was not impaired. In a similar experiment (data not reported), we also did not observe an effect of feeding 350 mg/kg of zinc on copper status of rats fed 6 mg of copper/kg of diet. In the same study, we used the whole-body counting technique[21] to determine copper absorption and found no difference between rats fed 50 or 350 mg of zinc/kg of diet for 24 days.

Conclusions

This study showed some of the manifestations of adaptation to long-term intakes of high-zinc diets in rats. It demonstrated that the concentrations of MT and zinc in the intestinal mucosa, and zinc in serum, liver, and kidney, increased initially but then returned to near control values after about 4 weeks of feeding. These data suggest that during prolonged feeding of diets high in zinc, the rat adopts regulatory mechanisms to lower body zinc that are not involved with intestinal MT.

This study also points out the difficulties in interpreting physiological responses when the data are based on single-point observations with respect to time. Adaptation is a normal response of all animals when faced with changes in environmental conditions, including changes in diet.[1,2] In most animal studies where dietary nutrients may be deficient or in excess of required amounts, many of the physiological and biochemical changes observed are reflections of an adaptation response to these extremes. Depending on the point in time at which the observations are made, there may be no direct connection between the mechanism of action of a nutrient and the observed response. If multiple measurements over time are not made, we are placing limits on our interpretations of the data and may be missing vital information with regard to specific mechanisms involved in nutrient function.

Acknowledgments

The author wishes to thank Brenda Skinner for performing the various analyses, Jim Lindlauf for mixing and analyzing the diets, Denice Schafer and her staff for care of the animals, and Marie Swenson for management and preparation of the manuscript.

References

1 Schmidt-Nielsen, K. (1994). How are control systems controlled? *Am. Sci.* **82,** 38–44

2 Prosser, C.L. (1986). *Adaptational Biology: Molecules to Organisms,* John Wiley & Sons, New York, NY USA

3 Reeves, P.G., Rossow, K.L., and Bobilya, D.J. (1993). Zinc-induced metallothionein and copper metabolism in intestinal mucosa, liver, and kidney of rats. *Nutr. Res.* **13,** 1419–1431

4 National Research Council. (1985). *Guide for the Care and Use of Laboratory Animals,* National Institutes of Health, Bethesda, MD USA

5 Reeves, P.G., Nielsen, F.H., and Fahey, G.C., Jr. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition and ad hoc committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123,** 1939–1951

6 Eaton, D.L. and Cherian, M.G. (1991). Determination of metallothionein in tissues by cadmium-hemoglobin affinity assay. *Meth. Enzymol.* **205,** 83–88

7 Hoadley, J.E., Leinart, A.S., and Cousins, R.J. (1988). Relationship of ^{65}Zn absorption kinetics to intestinal metallothionein in rats: effects of zinc depletion and fasting. *J. Nutr.* **118,** 497–502

8 Davies, N.T. (1980). Studies on the absorption of zinc by rat intestine. *Br. J. Nutr.* **43,** 189–203

9 Hempe, J.M. and Cousins, R.J. (1989). Effect of EDTA and zinc methionine complex on zinc absorption by rat intestine. *J. Nutr.* **119,** 1179–1187

10 Evans, G.W., Johnson, E.C., and Johnson, P.E. (1979). Zinc ab-

sorption in the rat determined by radioisotope dilution. *J. Nutr.* **109,** 1258–1264.
11 Weigand, E. and Kirchgessner, M. (1980). Total true efficiency of zinc utilization: determination and homeostatic dependence upon the zinc supply status in young rats. *J. Nutr.* **110,** 469–480
12 Finley, J. and Johnson, P.E. (1992). Relative influence of amount of dietary zinc and infusion of protein into the duodenum on the amount of zinc in biliary/pancreatic secretions. *Nutr. Res.***12,** 1217–1228
13 Reinstein, N.H., Lönnerdal, B., Keen, C.L., and Schneeman, B.O. (1987). The effect of varying dietary zinc levels on the concentration and localization of zinc in rat bile-pancreatic fluid. *J. Nutr.* **117,** 1060–1066
14 Magee, A.C. and Matrone, G. (1960). Studies on growth, copper metabolism and iron metabolism of rats fed high levels of zinc. *J. Nutr.* **72,** 233–242
15 Van Campen, D.R. and Scaife, P.U. (1967). Zinc interference with copper absorption in rats. *J. Nutr.* **91,** 473–476
16 Ogiso, T., Mariyama, K., Sasaki, S., Ishimura, Y., and Minato, A. (1974). Inhibitory effect of high dietary zinc on copper absorption in rats. *Chem. Pharm. Bull.* **22,** 55–60
17 Fischer, P.W.F., Giroux, A., and L'Abbe, M.R. (1981). The effect of dietary zinc on intestinal copper absorption. *Am. J. Clin. Nutr.* **34,** 1670–1675
18 Fischer, P.W.F., Giroux, A., and L'Abbe, M.R. (1983). Effects of zinc on mucosal copper binding and on the kinetics of copper absorption. *J. Nutr.* **113,** 462–469
19 Hall, A.C., Young, B.W., and Bremner, I. (1979). Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. *J. Inorg. Biochem.* **11,** 57–66
20 Oestreicher, P. and Cousins, R.J. (1985). Copper and zinc absorption in the rat: mechanism of mutual antagonism. *J. Nutr.* **115,** 159–166
21 Heth, D.A. and Hoekstra, W.G. (1965). Zinc-65 absorption and turnover in rats. I. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. *J. Nutr.* **85,** 367–374