ZINC-INDUCED METALLOTHIONEIN AND COPPER METABOLISM
IN INTESTINAL MUCOSA, LIVER, AND KIDNEY OF RATS

Philip G. Reeves², Ph.D., Kerry L. Rossow³, M.S., and Dennis J. Bobilya⁴, Ph.D.

United States Department of Agriculture
Agricultural Research Service
Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202-9034

ABSTRACT

Large doses of parenteral zinc (Zn) and/or the feeding of high Zn diets to animals or humans for long periods affects copper (Cu) metabolism. Previous work suggests that Zn-induced metallothionein (MT) in intestinal epithelial cells binds Cu and inhibits its absorption. This study was designed to determine the effects of treating rats with high dietary or parenteral Zn on Cu metabolism and its relationship to MT in the intestinal epithelium, liver and kidney. Six-week-old male rats were fed for one week a control diet containing 42 mg Zn and 6 mg Cu/kg. They were then divided into three groups. One group continued to receive the control diet while another received a similar diet containing 560 mg Zn/kg. A third group, fed the control diet, received a subcutaneous dose of 90 mg Zn/kg body weight every 2-3 days for the duration of the experiment. Rats from each group were killed on days 7 and 14. Low Cu status in Zn-treated rats was indicated by lower than normal serum Cu concentration, serum ceruloplasmin activity, low liver and kidney Cu concentrations and low cytochrome C oxidase activity. None of these changes, however, were related to an increase in Cu as a result of Zn-induced MT in the intestinal epithelial cell. Instead, as the MT concentrations rose, Cu concentration decreased. This study suggests that the effects of high Zn treatment on Cu status are not the result of the long-held theory that Zn-induced intestinal MT sequesters Cu and prevents its passage to the circulation. Instead, it may be caused by a direct effect of high luminal Zn concentrations on Cu transport into the epithelial cell.

KEY WORDS: Zinc, Copper, Metallothionein, Intestine, Kidney, Liver, Absorption, Rat

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²Correspondence to this author.

³Present address: Sanofi Diagnostics Pasteur, Inc., Chaska, MN 55318.

⁴Present address: Department of Animal and Nutritional Sciences, Kendall Hall, University of New Hampshire, Durham, NH 03824-3590, USA.
INTRODUCTION

Previous reports have shown that large doses of zinc (Zn) given to animals or humans, either by diet or parenterally, depress (Cu) status. It has been shown that the major site for this interaction between Zn and Cu occurs in the intestine where Zn reduces the absorption of Cu (1-3). Many hypotheses have been presented to explain why Zn reduces Cu absorption. One proposes that Zn competes with Cu for a Cu-binding protein in the mucosal cell that is thought to aid Cu absorption, and reduces the potential for Cu to be absorbed (4). Another suggests that Cu binds tightly to Zn-induced metallothionein (MT) in the intestinal epithelial cells (5-7). This bound Cu, then, is not available for transport across the basolateral membrane into the circulation. In time, the MT-bound Cu is lost through the intestinal tract as epithelial cells slough off. The latter hypothesis is widely accepted as the mechanism by which large doses of Zn impair Cu absorption (8-10). The binding of Cu to Zn-induced MT in liver is also thought to be one of the mechanisms by which Wilson's patients are detoxified of Cu when treated with Zn (11,12).

If Zn-induced intestinal MT effectively binds Cu in vivo, it seems reasonable to conclude that the concentration of Cu in intestinal cells would increase as the MT concentration increases. During recent studies we found that this was not the case. When rats were given high parenteral Zn, MT concentrations of intestinal epithelial cells increased 34-fold. However, concomitantly, the concentration of Cu increased less than 1.3-fold (13). Because these results did not conform to the hypothesis that Zn-induced MT effectively binds Cu in vivo, we did the following study to further examine these events.

METHODS

Sixty-three male Sprague-Dawley rats (Sasco, Lincoln, NE)5 weighing about 160 g were divided into three groups of 21 rats each. Rats in all three groups received a diet containing, by analysis, 42 mg Zn and 6 mg Cu/kg diet (Table 1) for one week. One group of rats continued to receive this diet for the duration of the experiment. Rats in a second group were fed a similar diet, but supplemented with additional Zn; it contained 560 mg of Zn/kg. A third group received the same diet as the first group and, in addition, each rat was given a subcutaneous injection of an emulsion of sunflower oil and Zn carbonate. The dose was given every 2-3 days in a portion equal to 1 mL of emulsion per kg body weight (BW) for the duration of the experiment. The dose was equivalent to 90 mg Zn/kg BW. This procedure was used to simulate continuous Zn absorption by allowing Zn to be slowly released into the circulation. As a result, rats fed the high-Zn diet consumed about 56 mg Zn • kg⁻¹ • d⁻¹ while those in the parenteral-Zn group received about 45 mg Zn • kg⁻¹ • d⁻¹.

After the rats had been on these regimens for one or two weeks, 7 and 14 rats, respectively, from each group were anesthetized with intraperitoneal injections of 50 mg sodium pentobarbital. Blood was drawn from the abdominal aorta into Monovette tubes (Sarstedt, Newton, NC) and allowed to clot at room temperature for one hour. Blood was centrifuged at 1,500g for 20 min and

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the serum was frozen until analyzed. A ten-centimeter section of the upper intestine, beginning at the pylorus, was excised and the lumen contents washed out with ice cold saline. The segment was slit open and the mucosal lining scraped off with the edge of a glass slide. Intestinal scrapings were frozen at $-20^\circ$ C until analyzed. Kidneys and part of the liver from each rat were excised and frozen.

### TABLE 1

Composition of the Basal Diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>305</td>
</tr>
<tr>
<td>Corn starch</td>
<td>300</td>
</tr>
<tr>
<td>Casein, high protein</td>
<td>150</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>50</td>
</tr>
<tr>
<td>Egg white solids</td>
<td>50</td>
</tr>
<tr>
<td>AIN-76 Mineral mix</td>
<td>35</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30</td>
</tr>
<tr>
<td>AIN-76 Vitamin mix</td>
<td>10</td>
</tr>
<tr>
<td>Choline premix</td>
<td>10</td>
</tr>
<tr>
<td>Biotin premix</td>
<td>10</td>
</tr>
</tbody>
</table>

1ICN Biochemicals, Cleveland, OH, Cat. #901521. 2Argo, CPC International, Englewood Cliffs, NJ. 3Teklad, Madison, WI, Cat. #160030. 4Mazola Oil, Best Foods, CPC International, Inc., Englewood Cliffs, NJ. 5Crisco Oil, Procter & Gamble, Cincinnati, OH. 6Teklad, Madison, WI, Cat. #160230. 7Teklad, Madison, WI, Cat. #170915. 8Teklad, Madison, WI, Cat. #160390. 9Teklad, Madison, WI, Cat. #40077. 10250 g of choline bitartrate per kg in powdered sugar. 1180 mg of d-biotin per kg in powdered sugar.

Proteins were precipitated from 0.5 mL of serum with 1.0 mL deionized water and 0.5 mL of 0.45 mol/L 5-sulfosalicylic acid in water. The mixture was allowed to sit at room temperature for one hr and then centrifuged at 2,000 g for 20 min. The resulting supernatant was analyzed for Zn and Cu by flame atomic absorption spectroscopy (AAS). Fresh serum was analyzed for ceruloplasmin activity (CPA) by the method of Schosinsky et al. (14).

Intestinal scrapings were divided into two portions. One portion was homogenized in five volumes of 0.23 mol/L 5-sulfosalicylic acid and allowed to stand at room temperature for one hr. After centrifugation, the supernatant was diluted appropriately and analyzed for Zn and Cu by AAS. The remaining portion was analyzed for MT by the $^{109}$Cd displacement method of Eaton and Toal (15).

The anterior half of each kidney and the right lobe of the liver from each rat were lyophilized to a constant weight and ashed in a muffle furnace. Residue from each sample was diluted to 5 mL in 0.1 mol/L HCl and analyzed for Zn and Cu by AAS. Another portion of each tissue was homogenized in appropriate buffers and analyzed for the activities of Cu-Zn superoxide
dismutase (SOD) (16) and cytochrome-C-oxidase (CCO) (17). Still another portion of each tissue was analyzed for MT concentration.

The data were analyzed by a two-way analysis of variance procedure (ANOVA; Crunch Statistical Software, Oakland, CA). Validity of the ANOVA is predicated upon the assumption that the variances are homogeneous. A test for homogeneity was done for each ANOVA by the method of Hartley (18). When the test was significant, i.e., variances were not homogeneous, the data were transformed to achieve or to approach homogeneity and analyzed. Data for each of the transformed parameters are presented in Table 2. Because the number of subjects per group varied greatly between weeks on experiment, Tukey's (19) methods were used to compare means. Although the analysis included both time and treatment, significant differences between means are shown only between treatments within each period.

**TABLE 2**

Ln Transformation of Data

<table>
<thead>
<tr>
<th></th>
<th>Week One of Experiment</th>
<th>Week Two of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hi Diet Zn</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>3.14±0.03</td>
<td>3.89±0.06</td>
</tr>
<tr>
<td>Intestinal Epithelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>5.55±0.06</td>
<td>6.90±0.10</td>
</tr>
<tr>
<td>MT</td>
<td>1.84±0.13</td>
<td>4.52±0.21</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>6.20±0.03</td>
<td>6.34±0.05</td>
</tr>
<tr>
<td>MT</td>
<td>1.78±0.07</td>
<td>2.07±0.22</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>6.01±0.02</td>
<td>6.16±0.04</td>
</tr>
<tr>
<td>MT</td>
<td>1.93±0.11</td>
<td>2.33±0.15</td>
</tr>
</tbody>
</table>

1When the test for homogeneity of variance between groups for each variable was significant by Hartley's \( F_{max} \) test, a natural log transformation of the data was performed. An ANOVA was then performed on this transformed data. 2High parenteral Zn.

**RESULTS**

Throughout the two-week experiment, there were no significant effects of treatment on weight gain of rats (Means ± SEM: control, 6.7 ± 0.3; high dietary Zn, 6.8 ± 0.2; high parenteral Zn, 6.9 ± 0.2 g/d).
Serum. Table 3 shows the effects of high Zn treatment on serum parameters. There were highly significant effects of treatment on serum Zn and Cu. During the first week, both high dietary Zn and high parenteral Zn caused an elevation in serum Zn to as much as 2.5 times that of the controls. However, after two weeks on the regimens, serum Zn in rats fed high dietary Zn was 40% lower the previous week. Parenteral Zn treatment, on the other hand, continued to elevate serum Zn during the second week (P<0.001). After one week, serum Cu concentrations were significantly (P<0.001) depressed in rats fed high dietary Zn but were significantly (P<0.001) elevated in those given parenteral Zn, as compared to control rats. After two weeks, however, serum Cu concentrations were severely depressed (P<0.001) in both groups treated with high Zn when compared to controls. Serum CPA followed the same course as serum Cu. By the end of the second week, CPA in the group treated with high parenteral Zn was barely detectable in 10 of 14 rats.

**TABLE 3**

Effect of High Dietary or High Parenteral Zn on the Concentrations of Zn and Cu and on the Activity of Ceruloplasmin (CPA) in Serum of Rats

<table>
<thead>
<tr>
<th></th>
<th>Week One of Experiment</th>
<th>Week Two of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hi Diet Zn</td>
</tr>
<tr>
<td>Zn, µmol/L</td>
<td>23.3±0.7$^a$</td>
<td>49.7±3.1$^b$</td>
</tr>
<tr>
<td>Cu, µmol/L</td>
<td>13.1±0.4$^a$</td>
<td>9.2±0.9$^b$</td>
</tr>
<tr>
<td>CPA, µkat/L</td>
<td>2.08±0.08$^a$</td>
<td>0.94±0.25$^b$</td>
</tr>
</tbody>
</table>

$^1$Mean ± SEM of 7 (1st wk) and 14 (2nd wk) replicates per mean. Means across groups with different superscript letters are significantly different from each other at P<0.05. $^2$Means±SEM of 14 replicates per mean. $^3$High parenteral Zn.

Intestinal Epithelial Cells. The effects of Zn treatment on Zn, MT and Cu in the intestinal epithelial cells are presented in Figure 1 and Table 2. After one week on the regimens, changes in Zn concentrations in the epithelial cells were similar to changes in MT concentrations (Figure 1, Panels Zn & MT). Throughout the study, MT and Zn concentrations were significantly (P<0.001) higher in the epithelial cells of rats treated with Zn than in controls. However, at the end of the second week, MT concentrations in the epithelial cells of rats fed high Zn diets were significantly lower (P<0.001) than at the end of the first week. When we subtracted the basal levels of Zn and MT from the stimulated values, the molar ratio of Zn:MT for both periods ranged from 6.6:1 to 7.8:1 for all groups except those given high-Zn in the diet for two weeks; this value was 15:1. When we took into account only those rats treated with Zn and determined the relationship between MT and Zn concentrations in individual rats, we found a highly significant positive correlation (Figure 2).

Treating rats with high Zn had a negative effect on the Cu concentration in the intestinal epithelial cells (Figure 1, Panel Cu). Both Zn treatments depressed Cu in the intestinal epithelial cells at both periods of sampling but were significant only after two weeks (P<0.01). Figure 2
shows that when the data were expressed on the basis of individual animals, no correlation between MT and Cu in the Zn-treated rats could be found.

Liver. Figure 3 shows the effects of high Zn treatment on liver parameters. After two weeks of treatment there was a two-fold elevation in liver Zn concentration in the group given high parenteral Zn, as compared to those fed high dietary Zn or control diets. Liver Zn appeared to be slightly elevated by high dietary Zn after one week, but the difference was not significant ($P > 0.05$).

Parenteral Zn but not high dietary Zn caused a large change in liver MT concentration (Figure 3, Panel MT). After one week of treatment, high parenteral Zn caused a six-fold increase in the concentration of MT over those receiving high dietary Zn. After two weeks, there was a twelve-fold difference between these two groups.

The effects of Zn treatment on liver Cu are shown in Figure 3, Panel Cu. The difference in liver Cu concentrations among groups were not significant after the first week of treatment. However, after the second week, both groups treated with Zn were similar to each other but both significantly ($P < 0.01$) lower than the controls.

To determine the degree of Cu status we measured the activities of two Cu-dependent enzymes in liver. Table 4 shows that both Zn treatments depressed CCO activity at both periods of measurement. However, the difference was significant ($P < 0.02$) only after two weeks on the regimens. There was a slight but significant ($P < 0.05$) effect on SOD activity during the second week in the group treated with high parenteral Zn compared with the control group.

Kidney. Figure 4 shows the effect of Zn treatment on kidney parameters. After one week on the regimens, the concentration of Zn was significantly ($P < 0.001$) increased in the kidneys of rats given high parenteral Zn as compared to the control rats. High dietary Zn also tended to elevate kidney Zn, but not significantly ($P < 0.06$). After two weeks, kidney Zn in rats given high parenteral Zn had risen to more than two-fold higher than that in either the control or the high dietary Zn groups.

MT concentration in kidneys followed the same pattern as Zn (Figure 4, Panel MT). There was no significant effect of high dietary Zn treatment on MT concentration in the kidneys compared to those fed the control diets. At the end of the first week, the MT concentration was

![Figure 1. Effect of normal dietary Zn (open), high dietary Zn (diag.), and high parenteral Zn (closed) on the concentration of Zn, MT, and Cu in intestinal mucosa. Bars represent the mean ± SEM of 7 (1st wk) and 14 (2nd wk) replicates. Bars within each panel with different superscripts are significantly different with $P \leq 0.05$.](image-url)
two-fold higher in the group treated with parenteral Zn than in the other two groups. By the end of the second week, MT concentration in the kidneys of the rats receiving parenteral treatment had increased to about nine times that of the other two groups.

The effect of Zn treatment on kidney Cu is shown in Figure 4, Panel Cu. One week on either high Zn treatments tended to depress the concentration of kidney Cu compared to controls, but the difference was not significant. After two weeks, however, both Zn treatments had depressed the concentration of kidney Cu and the difference was highly significant (P<0.001).

Treating rats with high dietary Zn did not affect Cu-dependent enzyme activities in kidney (Table 4). A statistical analysis using single degree of freedom comparisons, showed that parenteral Zn treatment significantly (P<0.02) depressed kidney CCO activity at both times of measurement when compared to the mean over the other two groups. Kidney SOD activity was not significantly affected by either treatment.

### TABLE 4

<table>
<thead>
<tr>
<th>Week One of Experiment¹</th>
<th>Week Two of Experiment²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>CCO</td>
<td>2.66±0.30</td>
</tr>
<tr>
<td>SOD</td>
<td>271±8</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>CCO</td>
<td>2.60±0.19</td>
</tr>
<tr>
<td>SOD</td>
<td>243±14</td>
</tr>
</tbody>
</table>

¹Mean±SEM of 7 replicates per mean. Means across groups with different superscript letters are significantly different from each other a P≤0.05. ²Mean±SEM of 14 replicates per mean. ³High parenteral Zn. ⁴CCO and SOD activities are expressed as nkat/mg protein.
DISCUSSION

It has been well documented that treatment with large doses of Zn compromises the Cu status of both animals or humans (1-4,10). It is believed that this effect is caused by the inhibition of Cu absorption when Cu binds to Zn-induced MT in the intestinal epithelial cells. This hypothesis is based on the demonstrations that MT has a greater affinity for Cu than for Zn and will preferentially bind Cu. It is reasonable to conclude, therefore, that Cu would displace Zn on Zn-induced MT in the intestinal epithelial cells and render it unavailable for transfer across the serosal membranes. It is also reasonable to conclude that the concentration of epithelial Cu would increase as MT concentration increased. Although this hypothesis has been widely accepted, data from past experiments, as well as the present one, do not support it.

Three studies have been used to support the hypothesis that Zn-induced MT binds Cu in the intestinal epithelial cell and renders it unavailable for absorption. Ogiso et al. (7) were the first to suggest this mechanism when they found that 10 g Zn/kg diets reduced Cu absorption in rats. There was a greater proportion of Cu bound to Sephadex-isolated MT from these rats than from those not fed high Zn. However, they also found that the high-Zn diet reduced the concentration of Cu in the intestinal tract by 50% or more.

Fischer et al. (5) determined the effects of feeding various concentrations of dietary Zn on Cu transfer through the mucosal and serosal membranes of isolated intestinal loops of rats. Although there was a significant decline in Cu transport of rats fed between 30 and 240 mg Zn/kg diet, there was not a significant change in the amount of Cu in the intestinal epithelial cells. They did not measure MT concentration, but based on data from other studies it could be estimated that much more MT would have been present in the intestinal epithelial cells of rats fed the higher amount of Zn than in those fed the lower amount. They did, however, isolate MT by gel filtration and determined the amount of Cu bound to the MT fraction. As dietary Zn increased eight-fold, Cu concentration in the MT fraction increased only 1.3-fold. They concluded that the mucosal Cu was bound almost exclusively to the protein fraction with a molecular weight similar to MT. They further concluded that Cu was bound to MT inside the mucosal cell and thus inhibited from passing into the circulation.

Work by a third group, Hall et al. (20), also suggested that intestinal MT was the causative factor in Zn-induced reduction of Cu absorption. This group fed rats four concentrations of Zn
ranging from 30 to 900 mg/kg diet and found that $^{64}\text{Cu}$ absorption was depressed only in those rats fed the highest level of Zn. Total Cu concentration in the intestinal epithelial cells was not measured, but they found a three-fold increase in $^{64}\text{Cu}$ in the intestinal mucosa, but only in the highest Zn group. MT was separated from the mucosal supernatant by gel filtration and the amount determined by measuring the concentration of Zn in each fraction; the assumption being that the amount of MT was proportional to the amount of Zn. Mucosal contents of MT in this fraction rose 27-fold in the high-Zn group but the concentration of Cu only increased four-fold. However, they surmised that this small change in MT-bound Cu might interfere with the transfer of Cu across the basolateral membrane.

The basic assumption in all these reports was that the elevated concentration of Cu found in MT isolated by gel filtration was representative of the form of Cu in the intestinal cell in vivo. This assumption may not be valid. Intact cells are highly compartmentalized, and under usual conditions, Cu and Zn are tightly bound to specific enzymes at active sites, or less tightly bound to other proteins and small, diffusible ligands. It is possible that Cu is compartmentalized separate from MT, and when the compartments are broken apart by homogenization, as in all these procedures, Cu would be redistributed from ligands of weak affinity to those of stronger affinity, such as MT. The metal composition of isolated MT may be very different from that inside the cell (21). It cannot be assumed, therefore, that Cu is bound to Zn-induced MT in the cell just because it appears to be bound to isolated MT.

The opposite state seems more typical. As a result of feeding high Zn, each of these studies either found a decrease in total cellular Cu or found no change in Cu concentration. Similar observations were made in the present study. That Cu status was greatly impaired by Zn treatment was clear from the observations that serum Cu concentration and ceruloplasmin activity were depressed. In addition, liver and kidney Cu concentration and liver cytochrome C oxidase activity were significantly lower than in controls. However, none of these changes appeared to relate to the sequestering of Cu by Zn-induced MT in the intestinal epithelial cells. In fact, the opposite was true. MT concentration in epithelial cells of Zn-treated rats increased by as much as 30-fold over controls while the Cu concentration decreased by 40%. The reduction in Cu concentration inside the intestinal epithelial cell suggests that the major effect of high lumenal Zn might be the inhibition of Cu transport into the cell. This in turn would result in reduced Cu transfer into the circulation.

FIG 4. Effect of normal dietary Zn (open), high dietary Zn (diag.), and high parenteral Zn (closed) on the concentration of Zn, MT, and Cu in kidney. Bars represent the mean \pm SEM of 7 (1st wk) and 14 (2nd wk) replicates. Bars within each panel with different superscripts are significantly different with $P<0.05$. 
Other work supports this suggestion. Oestreich and Cousins (22) used a vascular-intestinal perfusion technique to study the effect of high luminal Zn on Cu transport. In rats fed diets with Zn concentrations (30 mg/kg) too low to induce MT synthesis, they showed that elevated concentrations of luminal Zn decreased Cu concentrations in the mucosal cytosol as well as the amount of Cu transported to the vascular perfusate. Starcher (23), using chicks, showed that a single oral dose of 1 mg Zn per chick decreased the rate of $^{64}$Cu absorption by 67% and the amount of the isotope bound to epithelial proteins by 64%. Because these birds were not previously stimulated to produce abnormal MT concentrations in the intestinal cells, it suggests that Zn was inhibiting Cu absorption directly.

Other direct support for the suggestion that Zn directly inhibits Cu uptake by intestinal epithelial cells without the influence of MT was presented by Van Campen (24). He fed rats a normal Zn diet (45 mg/kg), a condition which should not elevate MT synthesis in the intestinal epithelial cells, and introduced, in situ, Zn and $^{64}$Cu solutions into the intestinal lumen so that the Zn:Cu ratio was approximately 150:1. After three hours, a 50% reduction in the body tissue uptake of $^{64}$Cu was observed in rats receiving high concentrations of Zn. In previous experiments, Van Campen and Scharfe (2) showed that Zn significantly reduced the amount of $^{64}$Cu leaving the intestinal lumen.

The present study suggests that during long-term treatment with high dietary Zn, the intestinal epithelial cells may have adopted a mechanism other than MT synthesis to prevent excessive Zn absorption. After one week of consuming high-Zn diets, serum Zn concentration was double that of the control group. Both mucosal Zn and MT concentrations were elevated and the calculated molar ratio of Zn to newly formed MT was 7.5:1. This is close to the maximal binding capacity of MT for Zn. However, after two weeks on this regimen, serum Zn was reduced by 40% compared to the first week, and although mucosal Zn remained unchanged, MT concentration was reduced by more than 60%. The Zn:MT molar ratio in this case was 15:1. This suggests that the mucosal cells had begun to adapt to high dietary Zn by lowering the synthesis of MT but retaining some other mechanism to prevent excessive Zn accumulation in the body.

Oral Zn therapy is very effective in preventing liver damage caused by excessive Cu deposition in Wilson's disease patients (12). Using a rat model, Lee et al. (11) loaded rats with Cu (750 mg Cu/kg diet) for eight weeks. Depot Zn treatment, much like that in the present experiment, was begun at three weeks and continued for eight more weeks. As a result of Zn treatment, SGPT activity was reduced, liver Cu was unchanged, liver MT increased five-fold, liver Zn increased about two-fold, and Cu in the liver cytosol (25,000 gr supernatant) decreased almost two-fold. They interpreted these results to suggest that Zn protected the liver from Cu toxicity by inducing MT that bound Cu. However, Zn-induced MT in the cytosol did not bind Cu. On the other hand, Zn treatment caused almost half the cellular Cu to remain with the 25,000g pellet. This suggests that if MT binds Cu, much of it became associated with the particulate fraction of the cell. The question remains, what artifacts are introduced by the redistribution of Cu during homogenization and centrifugation? It has been shown that liver Cu in Cu-loaded animals (25,26) and humans (27) is associated with the particulate fraction. Much of it can be extracted only with 2-mercaptoethanol while using freeze-thaw procedures.

The situation in the present study was much different from that described by Lee et al. (11); our diets contained normal concentrations of Cu. It could be argued that the reduced concentration of Cu in liver and kidney of our Zn-treated rats was because high-Zn prevented Cu
absorption from the diet. Therefore, not enough Cu was available to maintain normal tissue concentrations.

In summary, it was clear from many observations that Cu status was impaired in rats receiving the high Zn treatments, high dietary Zn or parenteral Zn. Serum Cu concentration and ceruloplasmin activity were depressed, liver and kidney Cu concentrations and liver cytochrome-C-oxidase activity were all significantly depressed. None of these changes, however, appeared to be related to the removal of Cu by Zn-induced MT in the intestinal epithelial cell. Instead, results showed that total intestinal mucosal Cu decreased when MT was induced by high Zn administration. This suggests that entry of Cu into the intestinal epithelial cells is inhibited by the presence of high Zn is the most likely mechanism by which high Zn impairs Cu status and not because Zn-induced MT sequesters Cu and prevents its transfer out of the cell.

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