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Zinc-Binding Protein: Relationship to Short Term Changes in Zinc Metabolism[1,2] (39479)

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A variety of functions has been suggested for metallothionein (MTN), a low molecular weight cytoplasmic metalloprotein. These include roles in detoxification of Cd, Hg or Zn (1, 2), hepatic storage of Zn and Cu (3), Cu absorption (4), and Zn metabolism (5, 6). Recently we proposed a dual function for a Zn-binding protein (ZnBP), which is similar to MTN, in the homeostatic regulation of Zn metabolism. Synthesis of both mRNA and ZnBP appeared to be required for plasma Zn uptake into liver cells where ZnBP appeared to serve as a storage protein (7). Furthermore, we found that intestinal ZnBP bound newly absorbed dietary Zn and suggested the protein may serve to antagonize absorption by competing for Zn with an intracellular chelator in mucosal cells. In order for ZnBP to serve these functions, its synthesis and degradation must be readily responsive to a metabolic signal. Previously we demonstrated that parenterally administered Zn resulted in changes in serum Zn concentrations prior to changes in liver or intestinal ZnBP synthesis (8). It therefore appeared plausible that serum Zn may serve as a short-term metabolic modulator of Zn metabolism via its influence on ZnBP synthesis. The experiments described here demonstrate that the short-term response of liver and intestinal ZnBP formation is related to fluctuations in serum Zn which can be brought about by changes in dietary Zn level as well as parenterally administered Zn.

Materials and methods. Weanling male rats (Sprague-Dawley, Madison, Wisconsin) were housed individually in stainless steel cages and fed *ad lib.* a standard laboratory chow that contained 50 ppm Zn until a weight of 150 g was attained, at which time the rats were used for experiments. For the studies involving the parenteral Zn dose, 25 μmole of Zn (as $ZnSO_4$ in 0.9% saline) was injected (ip) and 0, 8, 24, or 48 hr later the rats were killed by decapitation and the blood was collected. The livers and intestinal mucosa from each rat within each treatment group were pooled and homogenized in 0.25 *M* sucrose-10 m*M* Tris-HCl (pH 8.6). The 105,000 g supernatant was applied to a column (2.6 × 50 cm) packed with Sephadex G-75 and eluted as described previously to isolate ZnBP (7). Chromatographic fractions and serum were measured for Zn content by atomic absorption spectrophotometry.

In studies involving dietary manipulations, the rats were housed as above except they were given resin demineralized H_2O and fed either a Zn deficient diet or the same diet supplemented (150 ppm Zn) with $ZnSO_4$. The Zn deficient diet (<1 ppm Zn) was that described previously, except that spray-dried egg white was used as the protein source and Zn was omitted from the mineral mix (9). The rats were depleted of Zn by feeding the Zn deficient diet for a 24 hr period. Subsequently they were fed the 150 ppm Zn-supplemented diet for 24 hr and were then fed the Zn deficient diet for an additional 24 hr period. One-third of the rats were killed following each of the three feeding periods. The serum, liver, and intestinal mucosa were collected, prepared and analyzed as described above.

In a third study, rats were fed the Zn deficient diet for 7 days after which time the animals were fed the Zn deficient diet supplemented with 0, 25, 75, or 150 ppm Zn (as $ZnSO_4$) for 24 hr. The serum, liver, and intestinal mucosa were collected, prepared, and analyzed as described above.

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Results and discussion. The Sephadex G-75 elution profiles of both liver and intestinal muscosal cytosol and the corresponding serum Zn concentration from rats fed a standard stock diet (50 ppm Zn) are shown in Fig. 1. Under these conditions serum Zn is relatively constant (1.2 μg/ml). The liver cytosol fractionated into 3 Zn-containing peaks (left, top profile). Peak I represented high mol wt Zn-containing proteins. Peak II has been shown to possess both superoxide dismutase and carbonic anhydrase activity (10). The third peak, which corresponded to a molecular weight of 6,000–12,000 daltons, was designated as ZnBP. Intestinal mucosal cytosol (right, top profile) also fractionated into 3 Zn-containing peaks. In this case Peak I, eluted near the void volume of the column, again comprised the high mol wt Zn-containing proteins, however the second Zn peak in the mucosal profile corresponded to ZnBP. Both liver and intestinal mucosal ZnBP were found to have identical chromatographic properties on a standardized G-75 column. The third Zn peak in the mucosal cytosol profile corresponded to a very low mol wt (<1,000 daltons) Zn-chelating complex that has been implicated to function in intestinal Zn absorption (7, 11).

The remaining profiles in Fig. 1 show changes in Zn distribution among the chromatographic fractions from liver and mucosal cytosol and the serum Zn concentration at 8, 24, or 48 hr following parenteral Zn administration. At 8 hr postinjection serum Zn was significantly elevated above control levels ($P < 0.01$) to 11.0 μg/ml and both liver and intestinal ZnBP showed a modest accumulation of Zn. However, by 24 hr serum Zn had declined significantly ($P < 0.01$), while Zn bound to cytoplasmic ZnBP had increased in both tissues. These data support our suggestion that ZnBP synthesis may be required for the uptake of Zn from the blood into these tissues (7, 8). By 48 hr following the Zn injection ZnBP-

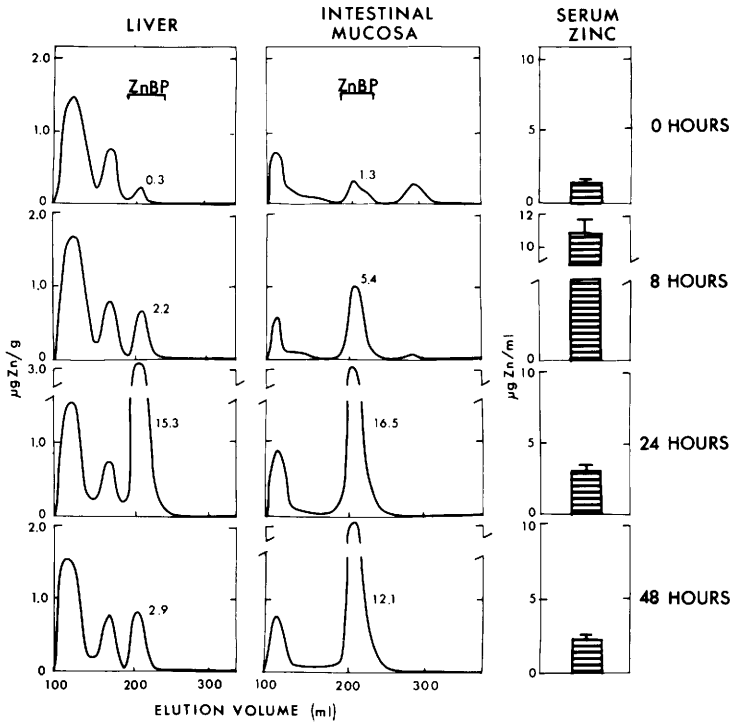

FIG. 1. Sephadex G-75 elution profiles of cytosol-bound zinc from liver and intestinal mucosa and the serum zinc concentration of zinc adequate rats. The rats were killed 0, 8, 24, and 48 h after an injection (ip) of 25 μmole of Zn^{2+} as $ZnSO_4$. ZnBP is the zinc-binding protein fraction and the number next to each ZnBP peak is the total ZnBP-bound zinc (μg Zn/g fresh tissue). Fractions eluted between 100–140 ml comprise Peak I of cytosol from both tissues and fractions eluted between 150–180 ml comprise Peak II of liver cytosol. Each serum Zn value represents the mean ± SEM of three rats.

bound Zn had declined in the liver, but not in mucosa, from the value obtained at 24 hr and the serum Zn content decreased to within normal levels.

We investigated the nature of the ZnBP response in animals fed diets of varied Zn content for short periods of time (24 hr). The changes in chromatographic behavior of liver and mucosal cytoplasmic Zn from rats subjected to a Zn depletion-repletion-depletion regime achieved by feeding either the Zn deficient diet (<1 ppm Zn) or the same diet supplemented with 150 ppm Zn is shown in Fig. 2. Initially (Day 0) a small quantity of Zn was bound to intestinal and liver ZnBP and serum Zn levels were within normal limits. This agreed with previous data that demonstrated when steady state levels of dietary Zn are maintained large amounts of ZnBP are not found (5-8, 10, 12). After the Zn deficient diet was fed for 24 hr (Day 1) the major change in soluble Zn was that bound to ZnBP. Both liver and mucosal ZnBP-bound Zn declined to trace amounts and serum Zn decreased to 0.4 μg/ml. If these Zn depleted animals were subsequently fed the Zn supplemented diet (150 ppm Zn) for 24 hr (Day 2), there was a substantial increase in the Zn content of both liver and intestinal mucosal cytosol. This increase occurred almost entirely in ZnBP-bound Zn and was approximately equal in both tissues. The serum Zn concentration was significantly ($P < 0.01$) elevated, to 5.2 μg/ml, which was five times that found in control rats. If the rats were subsequently fed the Zn deficient diet for an additional 24 hr (Day 3), the major decline in soluble Zn was accounted for by ZnBP-bound Zn. Serum Zn levels declined to near normal limits.

Comparison of the data in Figs. 1 and 2 indicated that the magnitude of the response of Zn accumulation by ZnBP was reasona-

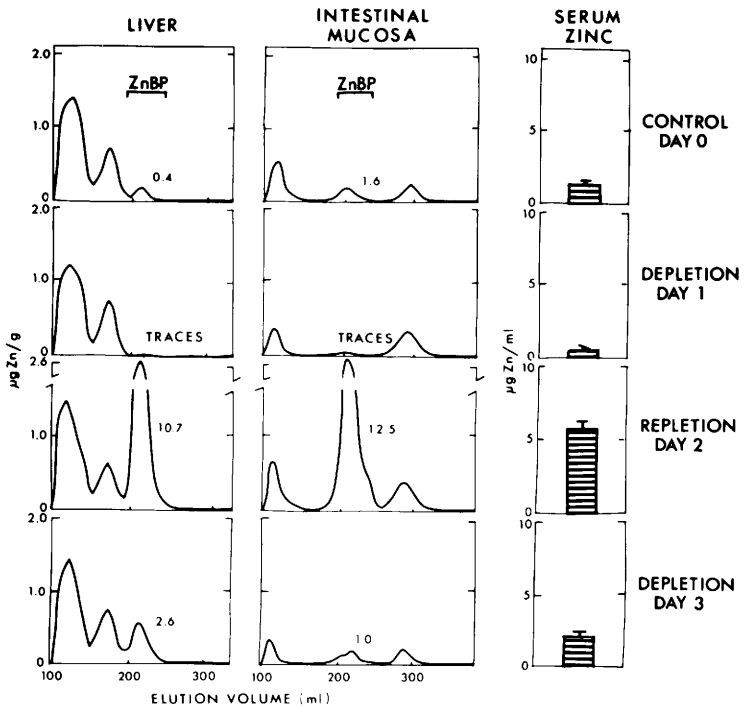

FIG. 2. Sephadex G-75 elution profiles of cytosol-bound zinc from liver and intestinal mucosa and the serum zinc concentration of rats. The rats were fed a Zn deficient diet for 24 hr (Day 1, Depletion), then were fed a Zn sufficient diet for 24 hr (Day 2, Repletion), and finally were fed the Zn deficient diet for 24 hr (Day 3, Depletion). ZnBP is the zinc-binding protein fraction and the number next to each ZnBP peak is the total ZnBP-bound Zn (μg Zn/g fresh tissue). Fractions eluted between 100-140 ml comprise Peak I of cytosol from both tissues and fractions eluted between 150-180 ml comprise Peak II of liver cytosol. Each serum Zn value represents the mean ± SEM of three rats.

bly comparable whether Zn was administered parenterally or was fed. Zn absorption is known to increase after feeding a Zn deficient diet (12). The large accumulation of Zn in both liver and intestinal ZnBP following Zn depletion and repletion as well as the elevated serum Zn levels are indicative of above normal Zn absorption from the dietary supply. The relative Zn content of the higher molecular weight proteins were not affected by the changes in dietary Zn. Therefore ZnBP is the cytoplasmic protein component that is dynamically correlated to changes in serum Zn concentrations.

A final series of experiments were conducted to investigate the effects of a 7 day Zn depletion followed by a 24 hr repletion period where varying levels of supplemental Zn were fed. Accumulation of Zn by liver and intestinal ZnBP appeared to follow different courses for the varying Zn levels (Fig. 3). The increase in liver ZnBP-bound Zn was found to be exponential. In the intestine there was a moderate increase in ZnBP-bound Zn up to 75 ppm of Zn supplementation. When 150 ppm was fed, there was a substantial accumulation of Zn in mucosal ZnBP and a concomitantly smaller increase in serum Zn compared to when 75 ppm was fed. This latter observation would correspond to increased hepatic uptake and decreased absorption (7).

Previous experiments have shown that changes in serum Zn concentration, in response to parenterally administered Zn, preceded changes in tissue ZnBP-bound Zn levels. This indicated that perhaps serum Zn served as a modulator of Zn metabolism by initiating the appearance of ZnBP (7). High and near-toxic levels of dietary Zn have been shown to produce elevated levels of liver MTN (6). Thus MTN was previously believed to serve a detoxification role. The present data demonstrate that changes in

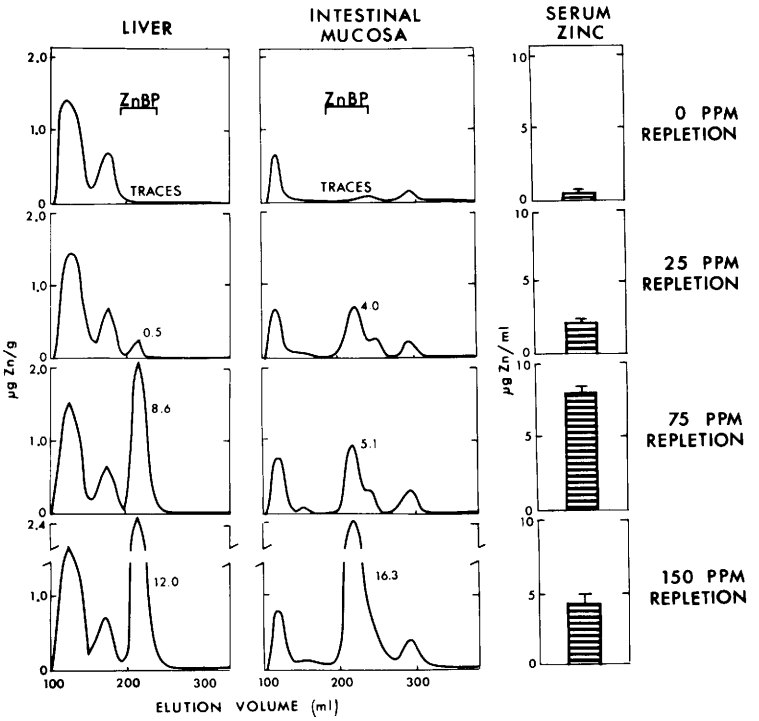

FIG. 3. Sephadex G-75 elution profiles of cytosol-bound zinc from liver and intestinal mucosa and the serum zinc concentration of rats. The rats were fed a Zn deficient diet for 7 days and then were fed the same diet but supplemented with 0, 25, 75, or 150 ppm Zn as $ZnSO_4$ for 24 hr. ZnBP is the zinc binding protein fraction and the number next to each ZnBP peak is the total ZnBP-bound zinc (μg Zn/g fresh tissue). Fractions eluted between 100–140 ml comprise Peak I of cytosol from both tissues and fractions eluted between 150–180 ml comprise Peak II of liver cytosol. Each serum Zn value represents the mean ± SEM of three rats.

serum Zn concentrations and liver and intestinal ZnBP-bound Zn are rapid and significant in response to subtle changes in dietary Zn level. These results suggest that ZnBP is part of the mechanism that regulates the daily metabolism of zinc in animals. Preliminary evidence from our laboratory as well as others (10) suggests that ZnBP has characteristics identical to those of MTN. The remarkable similarity in cysteine content, metal to protein ratio, dimorphism when subjected to DEAE-ion exchange chromatography and a lack of absorbance at 280 nm are all consistent with the known characteristics of MTN. An amino acid analysis of a zinc binding protein obtained from rats given a parenteral zinc load has shown conclusively that metallothionein is the protein which was isolated (10). It therefore appears that MTN is the protein which responds to the treatments described above.

Summary. The short-term changes in hepatic and intestinal mucosal zinc binding protein (ZnBP) bound Zn and in serum Zn concentration were investigated following either parenterally administered Zn or changes in dietary Zn. Both treatments resulted in similar changes in ZnBP-bound Zn. Repletion of Zn depleted rats resulted in increased hepatic and intestinal ZnBP-bound Zn and subsequent depletion decreased ZnBP-bound Zn in both tissues. These results demonstrate that ZnBP formation and degradation responds readily to fluctuations in dietary Zn level. Serum Zn was correlated to the appearance of ZnBP in both tissues and could be the metabolic signal that influenced the amount of Zn associated with ZnBP. The data suggest that ZnBP serves a regulatory function in zinc metabolism. Finally preliminary evidence suggests that ZnBP may be identical to metallothionein.

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