[Chem. Pharm. Bull. 28(12)3595—3600(1980)]

Zinc Metabolism in the Liver of Rats orally administered Zinc Sulfate

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(Received June 26, 1980)

Zinc metabolism in rat liver was investigated after a single oral administration of zinc sulfate. After the administration of 20 mg Zn per 100 g body weight, the zinc accumulated in the liver and pancreas, but not significantly in the kidneys, heart, spleen or lungs. The amount of zinc excreted into the bile was much less than that accumulated in the liver. On the other hand, the elevation of zinc content in the serum caused a significant increase of zinc in the plasma membrane and the cytosol fractions of the liver cells. However, additional increase of zinc in the serum did not further enhance the elevation of zinc content in the plasma membrane and the cytosol fractions. Upon gel filtration on Sephadex G-75 of the serum and cytosol obtained after zinc administration, the accumulated zinc in the serum was mainly eluted at the void volume, while the metal in the cytosol was largely eluted at 1.8 times the void volume, suggesting that the protein-bound zinc in the serum is not directly transported into the liver cells. The present study suggests that the liver is a target organ of zinc in rats and that zinc taken up by the liver cells is stored by binding of the metal to cytosolic proteins.

Keywords——zinc; zinc metabolism in liver; bile excretion of zinc; plasma membrane; cytosol

Introduction

The essentiality of zinc in animal and human nutrition is well known.[2] Supplemental zinc in a practical diet is markedly accumulated in the liver, kidney, and pancreas of calves.[3,4] With rats, the same diet does not materially affect the zinc content of the liver and kidneys, suggesting that homeostatic control mechanisms for zinc are much more effective in this species than in calves.[5] However, many key aspects of zinc metabolism in the liver of rats are not fully understood. The present study was therefore undertaken to investigate zinc metabolism in the liver after a single oral administration of zinc sulfate to rats. We demonstrate that the liver is a target organ of zinc and that zinc taken up by the liver cells is stored by binding of the metal to cytosolic proteins.

Materials and Methods

Tissue Distribution of Zinc——Fifty male Wistar rats (Nippon Bio-Supp. Center, Tokyo, Japan), weighing approximately 100—130 g, were used in this experiment. The animals were fed on commercial lab. chow (Oriental Test Diet, Tokyo, Japan) and tap water freely, and any food uneaten after 2 hr was removed. Zinc sulfate was dissolved in demineralized water to final concentrations of 5, 10, 20 and 40 mg of Zn/ml. These solutions were given by single oral administration to rats fasted for 2 hr. At various times after zinc administration, all animals were bled by cardiac puncture under light anesthesia with ether. Blood samples collected by cardiac puncture were immediately centrifuged to obtain the serum. The liver was perfused with cold 0.25 M sucrose solution, removed and rinsed in cold 0.25 M sucrose solution. The stomach and duodenum

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2) E.J. Underwood, "Trace Elements in Human and Animal Nutrition," 3rd ed., Academic Press, New York, 1971, p. 208.
3) W.J. Miller, E.S. Wells, R.P. Gentry, and M.W. Neathery, *J. Nutr.*, **101**, 1673 (1971).
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were everted and rinsed in cold 0.25 M sucrose. The pancrease, kidneys, heart, spleen, and lungs were excised and rinsed in cold 0.25 M sucrose. All these tissues were obtained immediately after the bleeding. These tissues, about 0.2—0.3 g wet weight per test tube, were digested for 24 hr at 110° after adding 4 ml of nitric acid. The zinc concentration in tissues was determined by atomic absorption spectrophotometry after digestion.

Bile Excretion of Zinc——The abdomen was opened by a midline incision under light ether anesthesia. The common bile duct was then ligated, and the incision was closed with wound clips. Immediately after ligation of the bile duct, the animals were orally administered zinc (20 mg/100 g body weight). In the control rats the abdomen was opened by a midline incision without ligation of the bile duct. The zinc-administered animals were fed and watered for 3 hr, and then bled by cardiac puncture under light ether anesthesia. Blood samples were centrifuged immediately and the serum was separated. The liver was perfused and removed after the bleeding, and rinsed with cold 0.25 M sucrose solution. The zinc content in serum and liver was determined by atomic absorption spectrophotometry after nitric acid digestion for 24 hr at 110°.

Further, the bile duct was cannulated with PE-10 tubing under intraperitoneal 25% urethane anesthesia (0.6 ml/100 g). The tubing was secured in place, and then the incision was closed with wound clips. Zinc (10 and 20 mg/100 g) was orally administered immediately after the cannulation. The animals were put on a warm water bath ($38 \pm 1°$) to maintain the body temperature[6] and were fed and watered. The bile was collected for 3 hr, and its volume was measured using a pipet graduated in units of 0.01 ml. The zinc was determined by atomic absorption spectrophotometry. The amount of bile zinc was expressed in two ways: (i) content of bile zinc, defined as the excreted zinc (μg) per 100 g body weight of rats; and (ii) concentration of bile zinc defined as zinc (μg) per milliliter of bile.

Subcellular Distribution of Zinc——Zinc (20 mg/100 g) was orally administered to rats fasted for 2 hr before the experiments. The rats were bled by cardiac puncture 3 hr after zinc administration. The serum was separated from blood samples. The liver was perfused with cold 0.25 M sucrose solution, isolated, cut into small pieces, suspended 1: 4 in 0.25 M sucrose and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was spun at $150 \times g$ in a refrigerated centrifuge for 10 min to remove cell debris and the supernatant was spun at $400 \times g$ for 10 min to sediment plasma membrane fraction. The $400 \times g$ supernatant was further spun at $105000 \times g$ for 60 min and its supernatant (cytosol fraction) was collected. The amounts of zinc in the serum, plasma membrane and cytosol fractions were determined by atomic absorption spectrophotometry after digestion with nitric acid.

Gel Filtration of Zinc——The serum (120 mg protein/2 ml) and the cytosol fraction (150 mg protein/10 ml) were analyzed by gel filtration on columns (90×2.5 cm) of Sephadex G-75, with 1 mM Tris-ammonium, pH 8.6, as an eluent. The flow rate was about 15 ml/hr and 4.7 ml fractions were collected. Fractions were directly assayed for zinc content by atomic absorption spectrophotometry. The protein was determined by the method of Lowry *et al.*[7]

Statistical Method——The significance of differences between values was estimated by Student's t test. p values less than 0.05 were considered to indicate statistically significant differences.

Results

Tissue Distribution of Zinc

The amounts of zinc in soft tissues of rats 1, 3, 5, 12 and 24 hr after a single oral administration of zinc sulfate (20 mg Zn/100 g body weight) are shown in Fig. 1. Zinc in the stomach and duodenum markedly increased at 1 and 3 hr after the administration, and then decreased rapidly. Blood zinc increased linearly, reached a maximum at 3 hr and decreased to the control level at 5 hr. Zinc in the liver and pancreas was highest at 3 hr, and then decreased gradually. The amounts of zinc in the kidneys, heart, spleen and lungs were not significantly increased by zinc administration. Thus, the zinc absorbed in the body after a single oral administration accumulated largely in the liver and pancreas of rats.

The amounts of zinc in the liver and serum 3 hr after the administration of various doses of zinc sulfate are shown in Fig. 2. After the administration of 5.0 mg Zn/100 g, zinc in the liver significantly increased, but with higher doses (10, 20 and 40 mg Zn/100 g) lesser increments were observed. In contrast, the increase in blood zinc was not high, but was dose-dependent.

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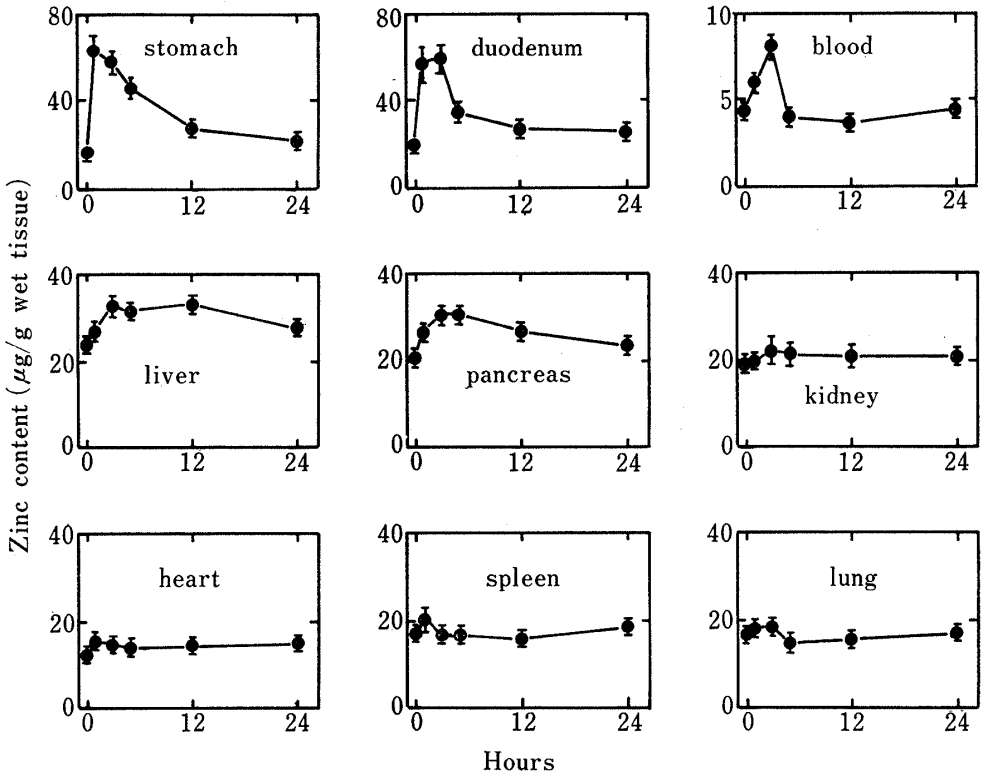

Fig. 1. Time Courses of Zinc Contents in the Tissues of Rats after a Single Oral Administration of Zinc Sulfate

Zinc (20 mg/100 g) was administered orally. Each point represents the mean of 5 animals. Vertical lines represent the SE.

TABLE I. Changes of Zinc Contents in the Blood and Liver of Rats after a Single Oral Administration of Zinc Sulfate

Treatment[a]		Zinc content (μg/g wet tissue)[b]	
		Blood	Liver
Intact rats	Control	4.3±0.13	23.7±1.20
	Zinc	11.2±1.77[c]	35.0±0.75[c]
Bile duct-ligated rats	Control	4.3±0.16	26.8±0.68
	Zinc	9.2±0.95[c]	32.1±1.80[c]

a) The rats were killed 3 hr after a single oral administration of zinc sulfate. Zinc (20 mg/100 g) was orally administered immediately after ligation of the bile duct.
b) Each value represents the mean ± SE.
c) $p<0.01$ as compared with the control.

TABLE II. Changes of Zinc Content in the Bile of Rats after a Single Oral Administration of Zinc Sulfate

Treatment[a]	Bile volume[b] (ml/100 g)	Bile zinc content[b]	
		(μg/100 g)	(μg/ml)
Control	1.48±0.04	0.41±0.01	0.29±0.01
Zinc(10 mg/100 g)	1.22±0.10[c]	1.01±0.01[d]	0.71±0.03[d]
Zinc(20 mg/100 g)	1.15±0.08[d]	3.12±0.33[d,e]	2.59±0.10[e]

a) The bile was collected for 3 hr after a single oral administration of zinc.
b) Each value represents the mean ± SE.
c) $p<0.05$ as compared with the control.
d) $p<0.01$ as compared with the control.
e) $p<0.01$ as compared with zinc (10 mg/100 g).

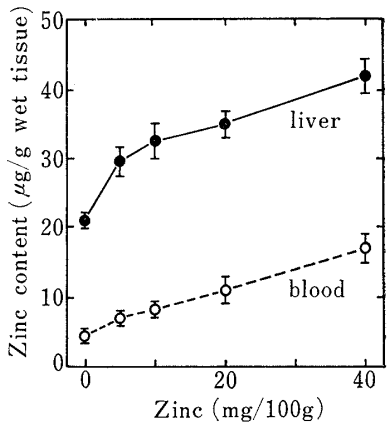

Fig. 2. Changes of Zinc Contents in the Liver and Blood of Rats after a Single Oral Administration of Zinc Sulfate

Zinc was administered orally, and the rats were bled 3 hr later. Each point represents the mean of 5 animals. Vertical lines represent the SE.

Bile Excretion of Zinc

When the zinc (20 mg Zn/100 g) was administered immediately after ligation of the bile duct, the increase of zinc content in the blood and liver at 3 hr after administration was not significantly altered from that of intact rats (Table I). Meanwhile, as shown in Table II, the zinc content was clearly increased in the bile collected for 3 hr after zinc administration, while the bile volume was significantly decreased. However, the amount of zinc excreted into the bile during 3 hr after zinc administration was much less than that accumulated in the liver at 3 hr after the administration. Thus, zinc accumulated in the liver was not rapidly excreted into the bile.

Subcellular Distribution of Zinc

The uptake of zinc from the serum by liver cells after a single oral administration of zinc was investigated. The time courses of increase in zinc content in the serum, the plasma membrane fraction and the cytosol fraction after a single oral administration of zinc are shown in Fig. 3. At 1 hr after the administration, zinc content in the serum was increased markedly, while those in the plasma membrane fraction and the cytosol fraction were not elevated significantly. At 3 hr after the administration, the cytosolic zinc level was markedly increased. This increase was maintained at 5 hr after zinc administration, although the serum zinc had returned to the control level at this time.

The zinc level in the serum was linearly elevated by increasing zinc doses (5, 10 and 20 mg Zn/100 g), while that in the plasma membrane fraction was elevated slightly (Fig. 4). The zinc in the cytosol was markedly raised by the dose of 5 mg Zn/100 g, but at higher doses (10 and 20 mg Zn/100 g) the cytosolic zinc level reached a plateau.

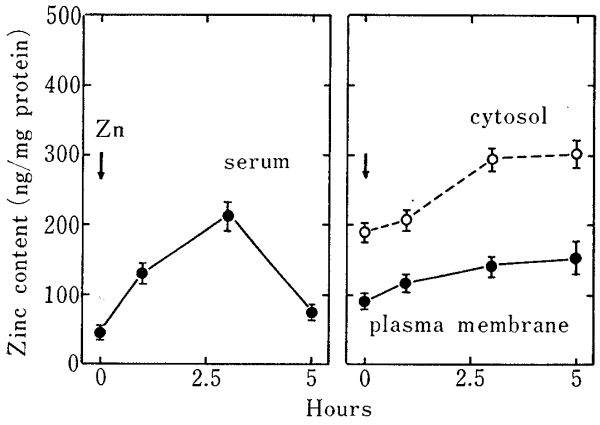

Fig. 3. Time Courses of Zinc Contents in the Serum, Plasma Membrane and Cytosol of Rat Liver after a Single Oral Administration of Zinc Sulfate

Zinc (20 mg/100 g) was administered orally. Each point represents the mean of 5 animals. Vertical lines represent the SE.

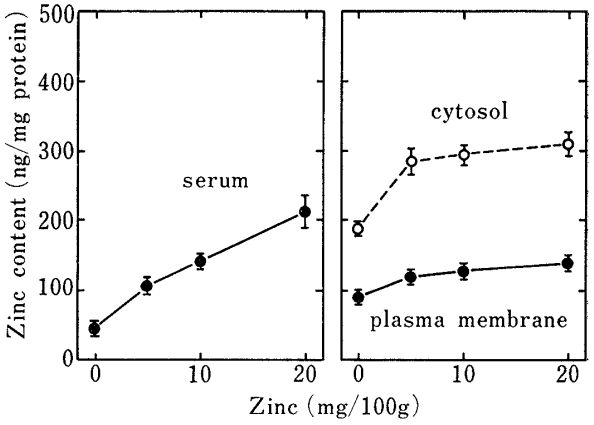

Fig. 4. Changes of Zinc Contents in the Serum, Plasma Membrane and Cytosol of Rat Liver after a Single Oral Administration of Zinc Sulfate

Zinc was administered orally, and rats were bled 3 hr later. Each point represents the mean of 5 animals. Vertical lines represent the SE.

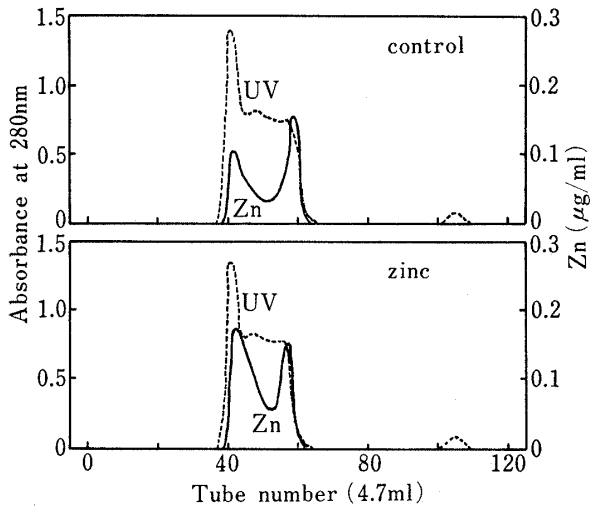

Fig. 5. Sephadex G-75 Chromatogram of the Serum of Rats after a Single Oral Administration of Zinc Sulfate

Zinc (20 mg/100 g) was administered orally, and rats were bled 3 hr later. Serum (120 mg protein/2 ml) was applied immediately after separation from blood.
----; absorbance at 280 nm, ——; content of zinc.

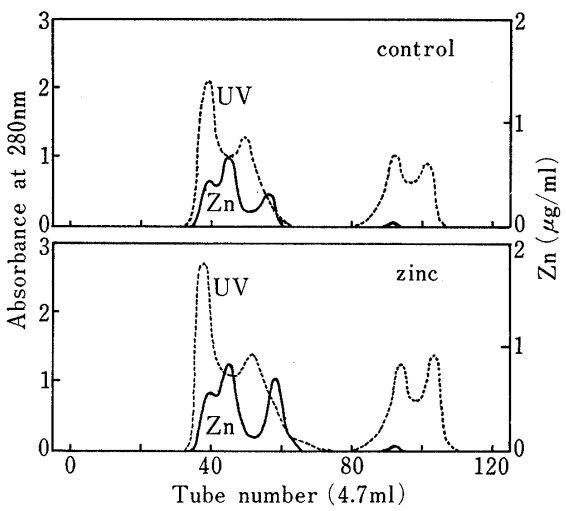

Fig. 6. Sephadex G-75 Chromatogram of the Cytosol of Rat Liver after a Single Oral Administration of Zinc Sulfate

Zinc (20 mg/100 g) was administered orally, and rats were bled 3 hr later. Cytosol (150 mg protein/10 ml) was applied immediately after centrifugation.
----; absorbance at 280 nm, ——; content of zinc.

Gel Filtration of Zinc in the Serum and Hepatic Cytosol

The gel filtration profiles on Sephadex G-75 of zinc in the serum and the cytosol 3 hr after administration of zinc (20 mg Zn/100 g) are shown in Fig. 5 and 6. The zinc-bound components in the serum of control rats were eluted as two peaks after elution of the bulk of soluble protein (Fig. 5). Upon zinc administration, the increment of zinc in the serum appeared as a higher first peak, which was eluted at void volume (Fig. 5). On the other hand, the cytosol of control rats was largely resolved into three components binding zinc (Fig. 6). Upon zinc administration, these three components all increased in zinc level. In particular, the amount of zinc in the eluate at 1.8 void volumes increased to approximately twice that of control rats. Thus, the administered zinc in the serum and the hepatic cytosol mainly bound to high molecular components which were different from each other.

Discussion

The accumulation of zinc in the liver of animals is well known, but zinc metabolism in this organ is not fully understood. In the present investigation, a single oral administration of zinc sulfate to rats produced a marked elevation of zinc content in the liver, and the amount of zinc excreted into the bile was much less than that accumulated in the liver. These results indicate that the liver may play a role in the storage of zinc given to rats.

The content of zinc in the serum was markedly elevated by zinc administration, and this increase was dose-dependent. However, the binding of zinc to the plasma membrane was not significantly potentiated by increasing levels of zinc in the serum. These results suggest that the plasma membrane in the liver cells may have a limited number of binding sites for zinc.

There was an increase in serum zinc as early as 1 hr after zinc administration. The serum zinc continued to increase, reaching a maximum at 3 hr, and returned to control levels by 5 hr after zinc administration. Meanwhile, the contents of zinc in the plasma membrane and cytosol of the liver cells were not significantly increased 1 hr after zinc administration, but at 3 and 5 hr, the plasma membrane and cytosolic zinc levels were clearly elevated. Thus,

an increase in serum zinc did not promptly cause significant increases of zinc in the plasma membrane and cytosol. From these results, it appears that the binding of serum zinc to the plasma membrane causes transport of the metal into the cytosol. Conversely, the cytosolic zinc did not decrease when the zinc level in the serum returned to control levels. Possibly zinc taken up by the liver cells was stored in the cytosol.

Furthermore, by gel filtration on Sephadex G-75, it was found that the increased zinc in the serum was mainly eluted at the void volume, while that in the cytosol was largely recovered at 1.8 void volumes. These results suggest that zinc in the serum and zinc in the cytosol bind to proteins which differ from each other in molecular weight. Presumably, zinc-binding protein in the serum does not itself enter the cytosol through the plasma membrane It is assumed that zinc bound to protein in the serum is rebound to the plasma membrane and transported into the cytosol.

Metallothionein can be induced in the cytosol by the administration of zinc to animals.[8] The restriction of food intake of zinc in rats causes a significant increase in liver zinc concentration, mainly as Zn-metallothionein.[9] *In vivo*[10] and *in vitro*,[11] Zn-metallothionein in liver cytosol is eluted at $V_e/V_o=1.8$ on a Sephadex G-75 column. Here, the increased zinc in the liver cytosol after oral administration of zinc to rats was mainly eluted at 1.8 void volumes upon gel filtration on Sephadex G-75. Thus, zinc held in the cytosol may be present as Zn-metallothionein.

The present investigation has shown that the liver is a target organ of zinc in rats, and that zinc taken up by the liver cells is stored by binding of the metal to proteins in the cytosol.

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