Effect of zinc injection on Zn binding in cytosols of several tissues of kids

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1. An experiment was conducted with goat kids to determine the effect of zinc injection on Zn binding in cytosols of several tissues of kids.

2. Following the Zn load, plasma Zn increased for 8 h then decreased. Zn injection significantly increased the Zn contents of the liver and kidney.

3. Injection of Zn into kids stimulated the production of Zn in a fraction with an apparent molecular weight of approximately 10000 in the cytosols of the liver, kidney and small intestinal mucosa. It is suggested that these fractions probably correspond to metallothioneins. Rumen papilla did not synthesize Zn-containing protein in response to an acute administration of Zn.

4. Zn injection significantly decreased the Cu content of the liver and affected the distribution of Cu in hepatic cytosol fraction, suggesting an interaction between these two elements.

5. The volume of bile collected in the gall bladder and its Zn content were markedly increased by Zn injection, suggesting that bile is one of the Zn excretion routes in kids.

Zinc metabolism is regulated homoeostatically in ruminants with normal or low dietary Zn intakes (Miller *et al.* 1967; Miller *et al.*, 1968; Miller, 1969; Neathery *et al.* 1973). But homoeostatic control mechanism function is less effective in calves on high Zn intake (Kincaid, Miller, Gentry *et al.* 1976). With high Zn intake, Zn contents increased in some tissues including pancreas, kidney and liver of calves (Kincaid, Miller, Gentry *et al.* 1976). Zn injection as well as Zn ingestion led to an accumulation of the metal in both liver and intestinal mucosa of rats (Richards & Cousins, 1976). Several recent reports have indicated that excess Zn is bound to a low molecular protein (metallothionein) in some tissues (Richards & Cousins, 1975; Chen *et al* 1977). Recent investigation has shown that increasing dietary Zn of calves from 42 to 642 mg/kg elevated the Zn content of liver and pancreas by 600 and 1400 % respectively (Kincaid, Miller, Fowler *et al.* 1976). The elevated Zn in liver and pancreas involved a substantial increase in all intracellular fractions, with by far the largest amount associated with a low molecular weight protein (Kincaid, Miller, Fowler *et al.* 1976).

Information is limited on the effect of an acute administration of Zn on Zn metabolism in ruminants. This research was designed to determine whether Zn injection into goat kids could induce the synthesis of Zn-binding components in several tissues.

METHODS

Eight Japanese native male kids 13–14 weeks of age and weighing 6 kg were used. They were weaned at 7 weeks of age, after which they were offered concentrate (commercial calf starter) and hay (Italian ryegrass) *ad lib.* Concentrate and hay contained 86 and 33 mg Zn/kg and 9·7 and 6·5 mg Cu/kg respectively. Kids were injected, intraperitoneally, with Zn (as $ZnSO_4.7H_2O$ in a solution containing 9 g sodium chloride/l) at a dose rate of 8 mg Zn/kg body-weight, or an equal volume of NaCl solution at 09.00 hours. They were fasted for 24 h after Zn or control injections. Blood samples were taken from the jugular vein just before injection, 4, 8 and 24 h after injection.

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The animals were killed 24 h after injection. The liver, kidney, small intestine and rumen were removed and bile in the gall bladder was collected. The liver and kidney were stored at −20° until required. The small intestine and rumen were rinsed thoroughly with ice-cold NaCl solution (9 g/l). Intestinal mucosa cell and rumen papilla were harvested by scraping with a microscope slide. The liver, kidney, intestinal mucosa and rumen papilla were homogenized in 2–3 vol (w/v) 0·01 M-Tris-acetate buffer (pH 8·6) containing 0·25 M-sucrose at 4°. The homogenate was centrifuged at 100000 *g* for 60 min at 4°. The supernatant fraction (cytosol fraction) was frozen (−20°) until the analyses were done. A portion of the liver and kidney was ashed by the method of Reitz *et al.* (1960) and the Zn content was measured by atomic absorption spectrophotometry (AAS). Plasma and bile were diluted with demineralized water and the Zn content measured directly by AAS.

A portion (2·5 ml) of cytosol fraction (100000 *g* supernatant fraction) was separated on a column of Sephadex G-75 (900 × 16 mm) using 0·01 M-Tris-acetate buffer (pH 8·6) containing 0·1 g NaN_3/l, as eluant. The flow-rate of buffer was approximately 33 ml/h and 4·9 ml fractions were measured for Zn content by AAS and for absorbance at 280 nm. The concentrations of Cu in the liver and in the fractions eluted from Sephadex G-75 columns were determined by AAS, since an interaction on the effect of Zn on hepatic Cu have been reported in ruminants (Bremner & Marshall, 1974; Mjor-Grimsrud *et al.* 1979).

RESULTS AND DISCUSSION

Plasma Zn

Plasma Zn concentration in the Zn-injected kids increased rapidly in the first 4 h after injection from 0·89 to 5·51 μg/ml, then more slowly to a maximum of 5·99 μg/ml at 8 h (Fig. 1). This was similar to the observation that intraperitoneal injection of Zn into rats increased serum Zn markedly for 8 h (Richards & Cousins, 1975). By 24 h post-injection, the excess Zn had been cleared from the blood of the Zn-injected kids. This relatively rapid decrease in plasma Zn was partly due to the accumulation of Zn in liver and kidney as described later. The concentration of plasma Zn in the control kids remained fairly constant throughout the experiment.

Zn and Cu contents of the liver, kidney and bile

Zn injection significantly increased the Zn contents of the liver and kidney (Table 1). Of the increased Zn in the liver of the Zn-injected kids 74 % was found in the cytosol fraction. Of the increased Zn in the kidney of the Zn-injected kids 72 % was found in the cytosol fraction.

Zn injection significantly decreased the Cu content of the liver. These results suggest that excess Zn may have the effect of eliminating hepatic Cu. Bremner *et al.* (1976) reported that liver Cu concentration was reduced by Zn supplementation of a diet. Over half (69 %) of the Cu in the liver of the control kids was in the soluble fraction. The proportion of hepatic Cu in the soluble fraction was unaffected by Zn injection (70 %). This value was similar to those obtained with the livers in rats (Evans *et al.* 1970), but was considerably higher than those obtained with the livers in calves (Bremner & Marshall, 1974) and sheep (Saylor & Leach, Jr. 1980). The Cu content of the kidney was decreased by Zn injection, but the difference between the control and Zn-injected groups was not significant.

The volume of bile in the gall bladder of the Zn-injected kids was much higher than that of the control kids. The concentration of Zn in the bile of the Zn-injected kids was much higher than that of the control kids. These results suggest that bile is one of the Zn excretion routes in kids.

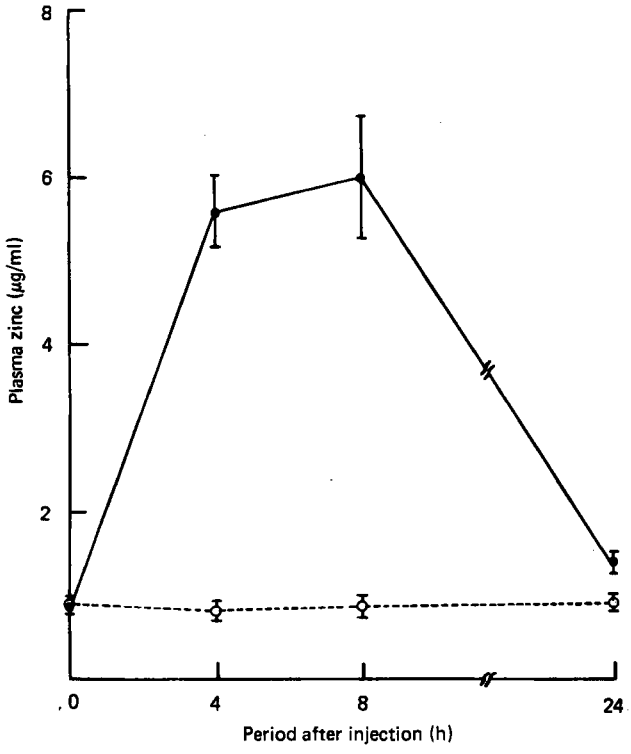

Fig. 1. Changes in plasma zinc (μg/ml) of kids with period (h) after intraperitoneal injection of Zn (●) or saline (9 g sodium chloride/l) control (○).

Table 1. *Effect of zinc injection into kids on the concentrations of Zn and Cu in the liver (μg/g fresh liver), kidney (μg/g fresh kidney) and bile (μg/ml)*

(Mean values with their standard errors for four kids/treatment; the volume (ml) of bile collected in the gall bladder is given in parentheses)

	Liver				Kidney					
	Total		Cytosol		Total		Cytosol		Bile	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Concentration of Zn										
Control	52	3	29	2	30	2	10	1	0·3 (4±1)	0·1
Zn-injected	136	4**	91	4**	59	4**	31	3**	5·0 (44±5)**	0·8**
Concentration of Cu										
Control	59	3	41	5	1·7	0·3	†		†	
Zn-injected	20	5**	14	5**	2·3	0·2	†		†	

** Values significantly different from the control group ($P < 0.01$).
† Not determined.

Gel filtration on Sephadex G-75

Fig. 2 gives the elution pattern on Sephadex G-75 of liver cytosol from the Zn-injected kids or the control kids. The chromatography of the hepatic cytosol from the control kids demonstrated that Zn was bound to two peaks (peaks 1 and 2). The Sephadex G-75 column

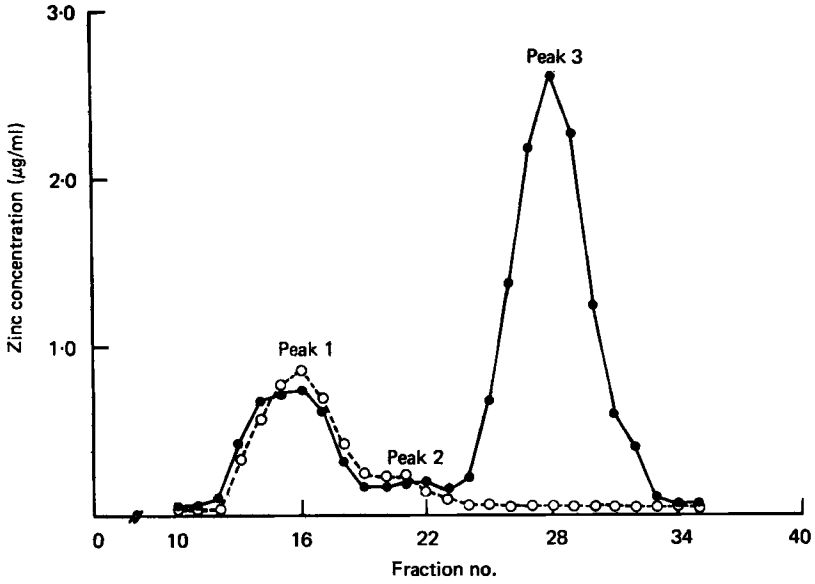

Fig. 2. Fractionation on Sephadex G-75 of liver cytosols of zinc-injected kids (●) and saline (9 g sodium chloride/l) control kids (○).

was calibrated with the following proteins, bovine serum albumin, chymotrypsinogen and cytochrome C by the method of Andrews (1965). From their elution volumes, peaks 1 and 2 have been found to correspond to molecular weight of approximately 70000 and 30000 respectively. When the hepatic cytosol from the Zn-injected kids was chromatographed, a third peak (peak 3), corresponding to a molecular weight of approximately 10000, was eluted in addition to the previous two peaks. Of the increase in liver Zn content of the Zn-injected kids 74 % is accounted for by an increase in peak 3. The distribution of Zn in the soluble fraction from kidneys was similar to that observed for the liver (Table 2). Much of the Zn in renal cytosol of the Zn-injected kids was associated with a molecular weight of approximately 10000 (peak 3). Of the increase in kidney Zn content of the Zn-injected kids 68 % is accounted for by an increase in peak 3.

The chromatography of the intestinal mucosa cell cytosol from the control kids showed that the cytosol separated into two Zn-containing peaks (peaks 1 and 3) (Table 2). A second peak (peak 2) was not separated clearly from peak 1. Zn injection did not increase the Zn content of peak 1, but there was a remarkable increase in the content of peak 3. There was quite low absorbance at 280 nm observed with peak 3 fractions in the cytosols of the liver, kidney and intestinal mucosa of the Zn-injected kids.

Recent reports have indicated that Zn injection induces the formation of low-molecular-weight soluble proteins in rats and that excess Zn accumulates almost exclusively in this fraction (Richards & Cousins, 1975; Chen *et al.* 1977). This protein has been identified as metallothionein and has been found in a number of species, including chicken (Weser *et al.* 1973), rat (Bremner & Davies, 1975), rabbit (Nordberg *et al.* 1972) and man (Pulido *et al.* 1966). Zn proteins in goat liver have been reported by Mjor-Grimsrud *et al.* (1979). The present study demonstrates that injection of Zn into kids stimulated the production of Zn in a fraction (peak 3) with a molecular weight of approximately 10000 in the cytosols

Table 2. *Effect of zinc injection into kids on the distribution of Zn and Cu among fractions isolated by gel filtration on Sephadex G-75 of supernatant fractions of homogenates of the liver, kidney and small intestinal mucosa*

(Mean values with their standard errors for four kids/treatment)

	Concentration of metal in supernatant fraction (μg/g fresh tissue)		Concentration of metal in fraction (μg/g fresh tissue)					
			Peak 1		Peak 2		Peak 3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver								
Concentration of Zn								
Control	29	2	21·9	1·2	4·3	0·4	2·8	0·2
Zn-injected	91	4	21·1	1·6	5·3	0·8	64·6	1·5
Concentration of Cu								
Control	41	5	19·4	1·4	3·5	0·4	18·4	1·6
Zn-injected	14	5	0·6	0·6	0·9	0·9	12·0	3·2
Kidney								
Concentration of Zn								
Control	10	1	6·7	1·2	1·3	0·2	2·0	0·2
Zn-injected	31	3	7·1	2·9	2·1	0·3	21·8	3·7
Small intestinal mucosa								
Concentration of Zn								
Control	10	2	6·9	0·8	1·6	0·2	1·9	0·2
Zn-injected	19	4	7·0	1·4	1·1	0·5	11·0	2·5

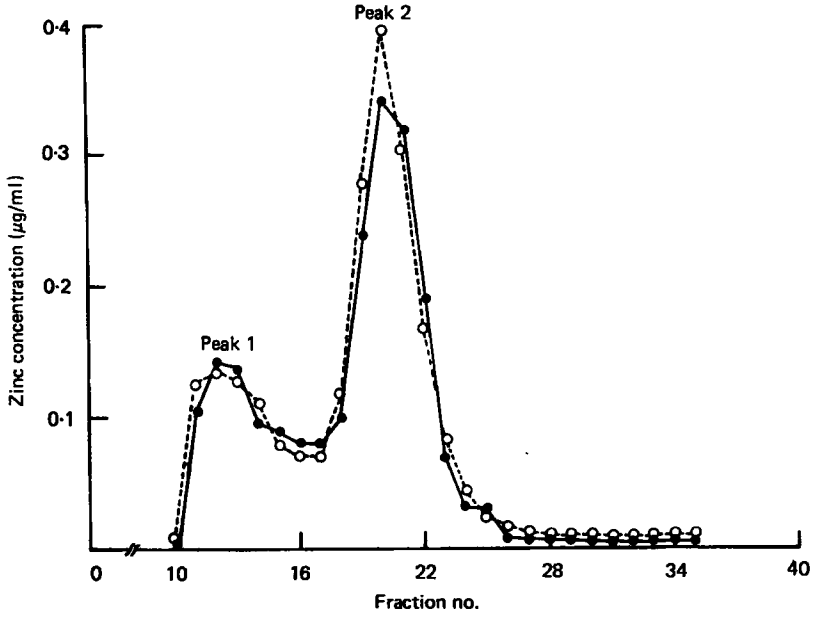

Fig. 3. Fractionation on Sephadex G-75 of rumen papilla cytosols of zinc-injected kids (●) and saline (9 g sodium chloride/l) control kids (○).

of liver, kidney and intestinal mucosa. From an apparent molecular weight of 10000 of this fraction and its quite low absorption at 280 nm, it is suggested that peak 3 fractions obtained in this study probably correspond to metallothioneins. A dual role for metallothionein in Zn metabolism has been proposed, wherein metallothionein serves as uptake and storage of excess Zn in liver cells to prevent its toxic effect and a sequestration function to control Zn absorption in intestinal cells (Richards & Cousins, 1975). The results presented here suggest that Zn-containing components (peak 3) could serve both functions in Zn metabolism in kids.

Three Cu fractions were obtained, two major Cu fractions (peaks 1 and 3) and one minor fraction (peak 2) in the hepatic cytosols of the control kids (Table 2). Cu was apparently associated with all Zn-containing fractions in the hepatic cytosol. Zn injection caused a marked reduction in the amount of Cu associated with peak 1 and there was one major Cu-containing fraction (peak 3) obtained. The Cu content in peak 3 with the Zn-injected kids was lower than that with the control kids. These results suggest that excess Zn supplied by Zn injection may affect the distribution of Cu in hepatic cytosol fraction. Evidence for such an interaction on the effect of Zn on hepatic Cu has been presented by Saylor *et al.* (1980) who found that the proportion of Cu in metallothionein fraction increased from 52·8 % in the unsupplemented sheep to 62·5 % in those fed supplemental Zn primarily due to a decrease in the Cu content of a high-molecular-weight fraction.

The chromatography of the rumen papilla cytosol from the control kids demonstrated that two main Zn-containing peaks were found, with approximate molecular weight from their elution volumes of $\geqslant$ 70000 and 30000 respectively (Fig. 3). The distribution of Zn in the soluble fraction from the Zn-injected kids was similar to that observed with the control kids. In both liver and kidney cytosols, peak 2 fractions were minor Zn-containing components, but there was an appreciable amount of Zn in peak 2 for rumen papilla cytosol. There is some evidence that Zn is absorbed from the rumen wall (Arora *et al.* 1969). Unlike the liver, kidney and small intestinal mucosa cytosols, Zn injection did not induce the synthesis of peak 3 for rumen papilla cytosol.

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