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Accumulation and Depletion of Zinc in Chick Tissue Metallothioneins[1]

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ABSTRACT Two experiments were conducted with Hubbard broiler chicks on the metabolism of high levels of dietary zinc. In the first experiment, chicks were fed a basal diet or the basal diet plus 500, 1,000, 2,000 or 4,000 ppm zinc, and in the second experiment chicks were fed the basal diet or the basal diet plus 1,000, 2,000, 4,000, 8,000 or 16,000 ppm zinc, using zinc acetate. Zinc was found to be associated with 4 peaks when the cytosols from tissues (liver, kidney, pancreas and intestinal mucosa) were eluted through columns of G-75 Sephadex. Although the zinc content of all four peaks increased with increased zinc content of the diet, the greatest elevation occurred in metallothionein (MT). This protein was purified from all four tissues (liver, kidney, pancreas and intestinal mucosa) and the amino acid analysis revealed the presence of about 30% cysteine. Zinc accumulated to the greatest extent (μg in MT/g tissue) in MT of the pancreas. The zinc in MT disappeared very rapidly when chicks which had been fed a diet containing high levels of zinc were fed a low zinc diet, indicating the extreme lability of this metal in this protein. The results suggest the involvement of MT in zinc homeostasis. J. Nutr. *109:* 1720–1729, 1979.

INDEXING KEY WORDS metallothionein · zinc · chickens · liver · pancreas · kidney · intestinal mucosa

Metallothionein (MT) is a unique protein first identified in equine renal cortex (1), but is now known to be a ubiquitous protein in tissues of many species of animals. Its unique properties include low molecular weight, high sulfhydryl and metal content, and the absence of aromatic amino acids (2). The initial work on MT originated from interest in the physiological function of cadmium (1); however the protein has repeatedly been shown to contain large amounts of zinc as well (2–5). Further evidence for a relationship of MT to zinc metabolism is work showing that the inhibition of MT synthesis also affects zinc metabolism (6), and that tissue MT levels are very sensitive to the zinc status of the animals (7, 8).

In a preliminary study we found excess zinc to be stored entirely with MT in the cytosols in rat liver (9). This was confirmed in a more extensive subsequent study, but the zinc which had accumulated with MT was found to be extremely labile (10). Much of the zinc in liver and pancreas of calves fed 600 ppm zinc in the diet was associated with a low molecular weight protein (MW), presumably MT

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9275

11), suggesting that young bovine species respond similarly to rats as far as zinc accumulation in tissue proteins is concerned. Other studies have been conducted on the appearance of a zinc-binding protein in pancreas of rats (12). Therefore, it was of interest to determine whether the chick would be similar to these animals as far as zinc accumulation with MT. The accumulation and depletion of zinc was studied in liver, kidney, intestinal mucosa and pancreas.

MATERIALS AND METHODS

Two experiments were conducted with Hubbard broiler chicks. In the first study, chicks were fed a basal diet or this diet plus 500, 1,000, 2,000, or 4,000 ppm zinc for 4 weeks. Reagent grade zinc acetate was used as the zinc source. Each dietary treatment was replicated three times with five males and five females per replicate. The day-old chicks were brooded and reared in a chick battery.[4] The composition of the basal diet is shown in table 1. Trace mineral mix and selenium and molybdenum supplement without zinc were supplied as outlined by Briggs (13). The vitamin levels in the premix were based on Gordon (14). Four to 6 chicks from each replicate were killed by dislocating the head from the neck and the liver and kidneys were excised and frozen (−20°) until analyzed for zinc or prepared for the column chromatography work. Some of the chicks which were fed the diet containing 4,000 ppm zinc were fed the basal diet up to 7 days in order to follow the depletion of zinc from MT.

In the second experiment, the same basal dietary composition, breed of chicks and brooding-rearing methods were followed as in experiment 1. Chicks (30 birds/diet) were fed either the basal diet (table 1) or this diet plus 1,000, 2,000, 4,000, 8,000 or 16,000 ppm zinc; all were fed these diets for 5 weeks. The birds were killed as previously described, and liver, kidney, pancreas and small intestine excised. The liver, kidney and pancreas were frozen (−20°) until zinc analysis and the column chromatography were done. After excising, the small intestines were rinsed with distilled water and cut longitudinally. The mucosal cells were removed by scraping the serosa with a spatula, and frozen (−20°) until the analyses were done. Some of the birds which had been fed the diet containing 4,000 ppm zinc for 5 weeks were fed the basal diet for up to 22 days, so that the rate of depletion of zinc from MT in the tissues could again be studied.

After thawing, tissues from four birds of each treatment group were pooled and homogenized (4 g/6 ml) in cold isotonic NaCl solution with a Potter-Elvehjem homogenizer. The homogenate and successive supernatant fractions were then centrifuged at 37,000 × g for 30 minutes and at 113,000 × g for 150 minutes to obtain respectively crude nuclei and mitochondrial (CN), microsomal (MC) and soluble (cytosol) fractions (S). A total of 8 ml of the cytosol were chromatographed on G-75 Sephadex gel filtration columns to separate the soluble zinc proteins. In the second experiment, the MT fractions from the gel filtration step were subsequently chromatographed on DEAE-cellulose [5] columns with

TABLE 1

Composition of basal diet

Component	Percent
Glucose monohydrate[1]	39.24
Soybean meal (44% protein)	49.00
Soybean oil	4.00
Salt N (Briggs w/o Zn)[2]	6.00
Salt N (Se + Mo supplement)[2]	0.10
Vitamin mix[3]	0.03
Vitamin and amino acid premix[4]	1.63

[1] Cerelose 2001, obtained from Schuss Wholesale Grocery, Portland, Oregon. [2] Obtained from Nutritional Biochemicals (Ref. 13). [3] Obtained from Nutritional Biochemicals. Supplied per kilogram of premix: 30,197.0 mg thiamin; 33,853.0 mg riboflavin; 93,231.2 mg pantothenic acid; 27,861.0 mg pyridoxine; 33,853.0 mg vitamin K (menadione); 1,354.1 mg biotin; 406,236.3 mg niacin; 16,926.5 mg folic acid; 338.5 mg vitamin B_{12}. [4] Supplied per kilogram of premix: 1,227.2 mg ascorbic acid; 306.9 mg p-aminobenzoic acid; 1,866.8 mg vitamin E (D α tocopheryl acetate); 61,363.2 mg Inositol; 183,598.8 mg choline; 613,020.5 IU retinyl Palmitate; 122,401.2 IU cholecalciferol; 7,670.4 mg BHT; 306,916.2 mg DL-methionine (98%); 122,726.5 mg glycine.

[4] Jamesway battery, Jamesway Manufacturing Company, Fort Atkinson, Wisconsin.

[5] Whatman DE52 cellulose, obtained from Whatman Inc., 9 Bridewell Place, Clifton, New Jersey 07014.

TABLE 2

Growth rate, mortality, feed conversion and feed consumption for birds

Dietary treatments	Ave. body wt	Mortality	Feed	
			Conversion	Consumption
	g	*dead/total*	*g food/g body wt*	*g/bird*
Basal	745	3/30	1.59	1199
Basal + 500 ppm Zn	784	2/30	1.64	1279
Basal + 1,000 ppm Zn	818	0/30	1.63	1333
Basal + 2,000 ppm Zn	792	0/30	1.62	1272
Basal + 4,000 ppm Zn	720	1/32	1.61	1151

a salt gradient to determine the number of MT species in each chick tissue. The main Zn-binding fraction from DEAE cellulose column chromatography was further purified by hydroxylapatite [6] column chromatography using phosphate buffer as previously described.[7] The subcellular fractions were digested with nitric and perchloric acids and analyzed for zinc by atomic absorption spectrophotometry.[8] The eluted fractions from the columns were analyzed for the metal by direct aspiration into the atomic absorption spectrophotometer. The total zinc content in each tissue fraction and the amount in each protein peak from the gel filtration step was calculated and expressed as μg zinc per fraction, or per peak per g fresh tissue. Amino acid analyses were performed on the MT preparations with an amino acid analyzer [9] (15), after the samples had been subjected to performic acid oxidation (16). The Student's *t*-test and analysis of variance were used to statistically evaluate the data (17).

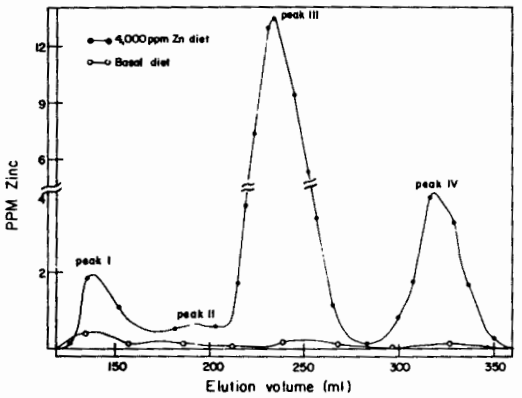

Fig. 1 Sephadex G-75 chromatography of cytosol from pancreas of chicks fed a basal diet or this diet plus 4,000 ppm zinc. Eight milliliters of cytosol from pancreas of four chicks were chromatographed on a sephadex G-75 column (2.0 × 94 cm) and eluted with 0.5 M Tris-Cl buffer, pH 8.4, at 4°. Fractions of 4 to 5 ml were collected at a flow rate of 24 to 30 ml/hour and analyzed for zinc. The amount of zinc in each fraction was calculated from its volume and zinc concentration, and the amount in each peak by summing the amount in each fraction in the peak (tissues are from chicks of experiment 2).

RESULTS

The 4 week growth rate, mortality, feed conversion and feed consumption for the birds in experiment 1 are shown in table 2. There were no significant differences between the chicks fed the basal diet and the various zinc supplemented treatments in growth rate, mortality and feed conversion. However, the chicks fed the diet supplemented with 4,000 ppm zinc appeared to consume slightly less total feed per chick (during the 4 week period) than the other dietary treatments.

Shown as an example, figure 1 illustrates the elution pattern of the zinc proteins in the cytosol from pancreas of birds in experiment 2 fed either the basal diet or this diet plus 4,000 ppm zinc. Four zinc peaks were eluted from this column. The first (peak I) elutes at the void volume, the

[6] Bio-gel HT, obtained from Bio-Rad Lab., Richmond, California.

[7] Chen, R. W. & Whanger, P. D. (1979) Purification, amino acid composition and immunity of rat liver and kidney metallothioneins. Biochem. J. (submitted).

[8] Jarrell-Ash, Model No. 82-516, 590 Lincoln Street, Waltham, Massachusetts. Zinc was determined at the 2,139 A° line, with a gas mixture of air and acetylene.

[9] Beckman amino acid analyzer, Model 120 B, Palo Alto, California.

second (peak II) elutes at about 30,000 MW, the third (peak III) at MW of 10,000, and the fourth (peak IV) at MW of about 800. Although zinc accumulated in all of these peaks, the greatest accumulation by far was in peak III, which is MT, as was demonstrated by amino acid analysis (table 5).

In experiment 1, zinc gradually accumulated in MT up to 2,000 ppm zinc in the diet (fig. 2). The addition of 4,000 ppm zinc to the diet markedly increased the zinc content of this fraction. The zinc content of the other peaks in the cytosol increased only slightly with increased zinc content in the diet (data not shown) but was similar to the patterns shown in figure 4.

Zinc rapidly depleted from MT in both liver and kidney when chicks which had been fed the diet containing 4,000 ppm zinc for 4 weeks were fed the basal diet (fig. 3). The zinc in MT reached the basal level within 5 days with no further depletion after 7 days of feeding the basal diet. This indicates that the zinc is very labile in liver and kidney MT.

The data for 5 week growth rate, mortality, feed conversion and feed consumption for experiment 2 are shown in table 3. After 3 weeks, only four chicks which were fed the basal diet with 16,000 ppm zinc were alive; therefore, these birds were killed at this time for tissue zinc and metallothionein analyses. For some unknown reason,

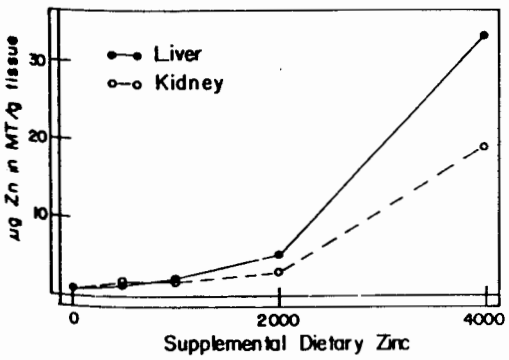

Fig. 2 Accumulation of zinc in liver and kidney MT of chicks fed a basal diet or this diet plus either 500, 1,000, 2,000 or 4,000 ppm zinc. The amount of zinc in MT/g tissue was calculated as indicated in figure 1. Each point represents the results obtained when tissues from four chicks were pooled for analysis (experiment 1).

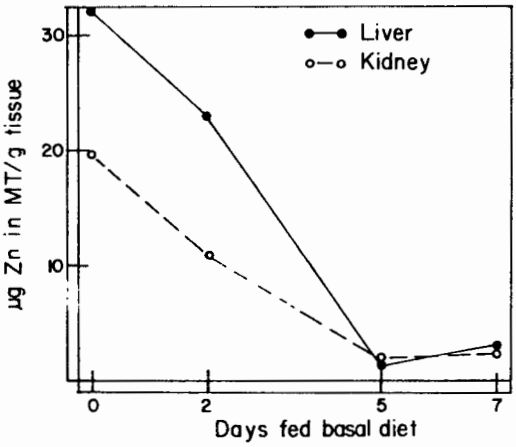

Fig. 3 Depletion of zinc from liver and kidney MT of chicks which had been fed the diet containing 4,000 ppm zinc and changed to the basal diet. The amount of zinc in MT per g tissue was calculated as indicated in figure 1. Each point represents the results obtained when tissues from four chicks were pooled for analysis (experiment 1).

eight out of 10 chicks died in one replicated pen of the 1,000 ppm zinc level. For the chicks fed the basal diet with 8,000 ppm zinc, the average body weight, feed consumed per chick and feed conversion were significantly lower than those of chicks fed the basal diet or basal diet plus either 1,000, 2,000 or 4,000 ppm zinc. It appears from these data that the initial toxic effect of zinc in this type of diet is somewhere between 4,000 and 8,000 ppm.

The accumulation of zinc on peaks I through IV of the cystosol from liver, kidney, pancreas and intestinal mucosa of birds fed the various diets in experiment 2 is shown in figure 4. Very little change in the zinc content of peaks I, II and IV occurred in kidney and liver, but the zinc content increased slightly in these peaks with increasing dietary zinc in intestinal mucosa and pancreas. In both of these tissues, zinc in peak IV accumulated to the greatest extent when 8,000 ppm zinc was included in the diet. In all four tissues, however, the majority of the excess zinc accumulated in peak III, which is MT. With 4,000 ppm dietary zinc, this metal accumulated to the greatest concentration (μg Zn/g tissue) in MT in the pancreas, followed by the intestinal mucosa, liver

TABLE 3

Growth rate, mortality, feed conversion and feed consumed for birds fed the basal diet or this diet plus either 1,000, 2,000, 4,000, 8,000 or 16,000 ppm zinc to four weeks of age (experiment 2)

Dietary treatments	Ave. body wt	Mortality	Feed	
			Conversion	Consumption
	g	*dead/total*	*g food/g body wt*	*g/bird*
Basal	778[1,b]	3/30	1.97[a]	1,458[b]
Basal + 1,000 ppm Zn	723[b]	12/30[2]	2.09[a]	1,283[b]
Basal + 2,000 ppm Zn	706[b]	2/30	1.87[a]	1,316[b]
Basal + 4,000 ppm Zn	714[b]	4/30	1.77[a]	1,258[b]
Basal + 8,000 ppm Zn	162[a]	24/30	5.00[b]	786[a]
Basal + 16,000 ppm Zn	—	30/30	—	—

[1] Values with different superscript letters are significantly different at $P < 0.05$. [2] For some unknown reason, eight of 10 chicks died from one replicated pen.

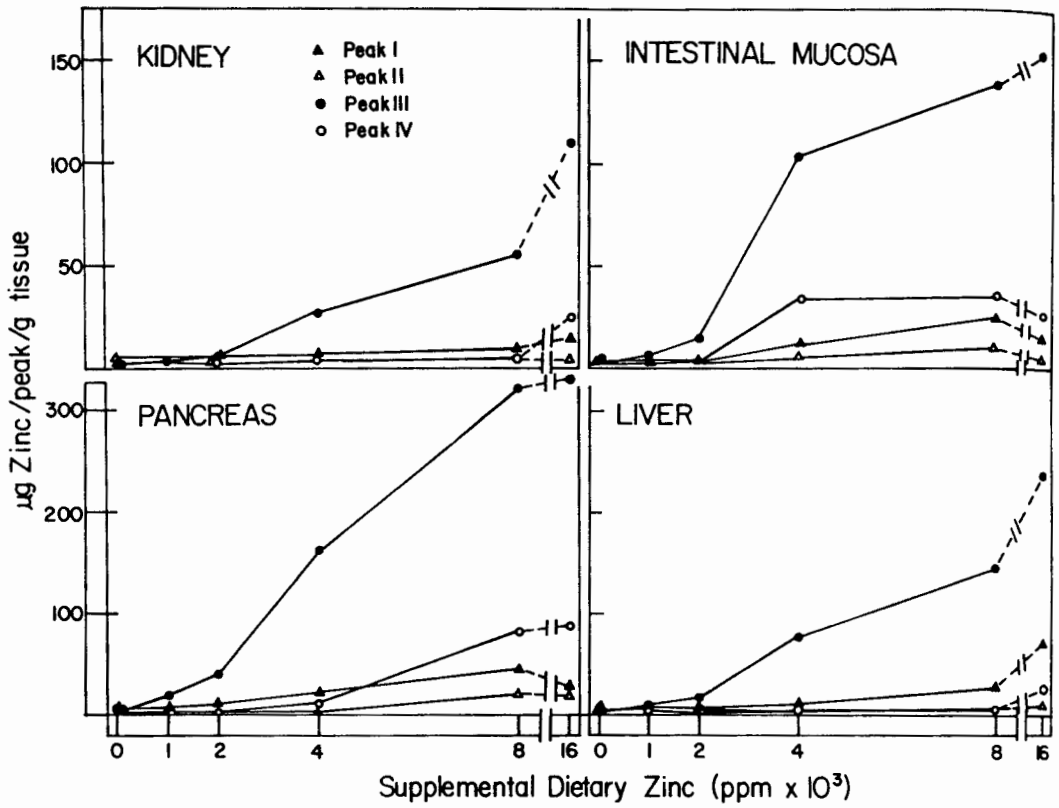

Fig. 4 Accumulation of zinc in cytosolic proteins in pancreas, liver, kidney and intestinal mucosa of chicks fed basal or basal plus either 1,000, 2,000, 4,000, 8,000 or 16,000 ppm zinc in the diet. The gel filtration and calculation of amount of zinc in peaks I through IV per g tissue were conducted as indicated for figure 1. Since the chicks which were fed the diet with 16,000 ppm zinc were killed 2 weeks earlier than the others, the extension of the lines to these data points is represented by dashed lines. Each point represents the results obtained when tissues from four chicks were pooled for analysis (experiment 2).

TABLE 4

Subcellular zinc distribution in various tissues of chicks fed the basal diet supplemented with various levels of zinc[1] (experiment 2)

Zn supplementation or depletion	Tissues and fractions[2] Liver			Kidney			Intestinal mucosa			Pancreas		
	CN	MC	S	CN	MC	S	CN	MC	S	CN	MC	S
Basal	3.2	1.4	14.9	1.7	1.2	11.6	1.2	1.3	6.5	1.7	3.0	12.5
+1,000 ppm	13.0	1.9	22.6	1.8	3.1	14.4	15.0	1.3	16.1	2.4	2.0	29.7
+2,000 ppm	15.2	9.0	31.9	11.9	3.2	17.3	23.1	2.0	25.6	19.9	18.5	54.9
+4,000 ppm	13.9	5.1	96.5	7.5	3.0	42.2	78.3	10.1	157.0	22.3	52.5	202.3
+8,000 ppm	22.0	20.5	189.2	8.3	6.2	75.8	45.0	20.9	212.4	1,000.0	45.0	470.0
+16,000 ppm	610.0	38.3	344.6	97.0	10.1	169.2	236.1	19.7	190.1	1,135.0	49.2	457.1
Depletion (+4,000 ppm group)												
Day 1	20.0	9.4	91.4	22.5	5.0	40.3	41.1	13.1	75.8	120.0	30.5	194.2
Day 2	24.6	2.1	36.1	12.3	2.8	17.0	16.0	12.6	43.0	19.5	6.8	116.8
Day 4	10.3	8.9	44.3	7.7	2.0	17.9	12.3	2.1	30.4	108.0	10.8	140.7
Day 7	18.3	5.3	23.3	10.5	2.7	12.4	8.3	1.1	8.5	42.0	19.6	41.9
Day 11	5.5	5.2	18.7	9.6	1.2	13.2	9.3	1.4	10.3	14.4	6.8	25.2
Day 22	3.1	2.4	11.1	2.0	1.4	9.6	1.4	2.2	7.8	4.5	2.0	8.8

[1] Values are μg zinc in each fraction per g pooled tissues from 10 chicks. [2] CN = crude nuclear + mitochondrial fraction MC = microsomal fraction; S = soluble fraction.

and kidneys in decreasing order. Even though the chicks fed the diet with 16,000 ppm zinc were 2 weeks younger, the amount of zinc in the different peaks was either as high or higher than in the various peaks from tissue cytosols of chicks fed the diet with 8,000 ppm zinc.

Due to the freezing and thawing of tissues, the mitochondrial and crude nuclear fractions could not be easily separated by differential centrifugation. Therefore, the combined fraction of crude nuclei and mitochondria was prepared by one step centrifugation. As the amount of zinc was increased in the diet, there was a corresponding increase of zinc in the crude nuclear plus mitochondrial and soluble fractions of liver, kidney, pancreas and intestinal mucosa (table 4). Zinc, however, accumulated to the greatest extent in the soluble fraction of these four tissues and small changes occurred in the zinc content of the microsomes with the various diets.

Similar to the first experiment, zinc disappeared very rapidly from tissue MT when birds which had been fed the diet with 4,000 ppm zinc were changed to the basal diet (fig. 5). Zinc disappeared from pancreas MT at the slowest rate, followed by the liver and intestinal mucosa, and fastest from the kidney. The half-life of zinc in MT was calculated to be respectively 2.3, 1.5, 1.2 and 0.9 days for pancreas, liver, intestinal mucosa and kidney. Since the pancreas contained the greatest concentration of zinc (μg metal/g tissue), the amount of zinc loss per day was greatest from this tissue. Thus, zinc in MT appears to be very labile in all tissues studied.

There was predominantly one species of MT (MTP_{II}) in all four tissues of the chick (fig. 6). There appears to be slightly more of the first MT (MTP_{I}) species in pancreas

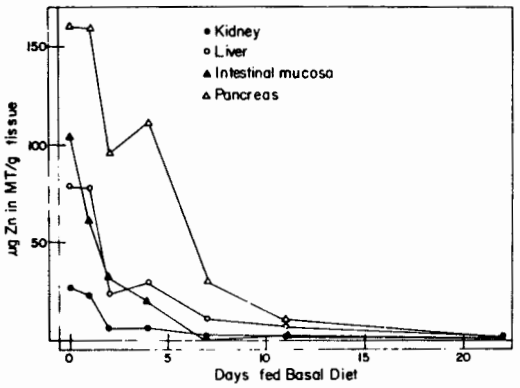

Fig. 5 Depletion of zinc from pancreas, liver, kidney and intestinal mucosa MT of birds which had been fed the diet with 4,000 ppm zinc and changed to the basal diet. The column conditions and calculations of the amount of zinc in MT/g tissue were the same as indicated for figure 1. Each point represents the results obtained when tissues from four chicks were pooled for analysis (experiment 2).

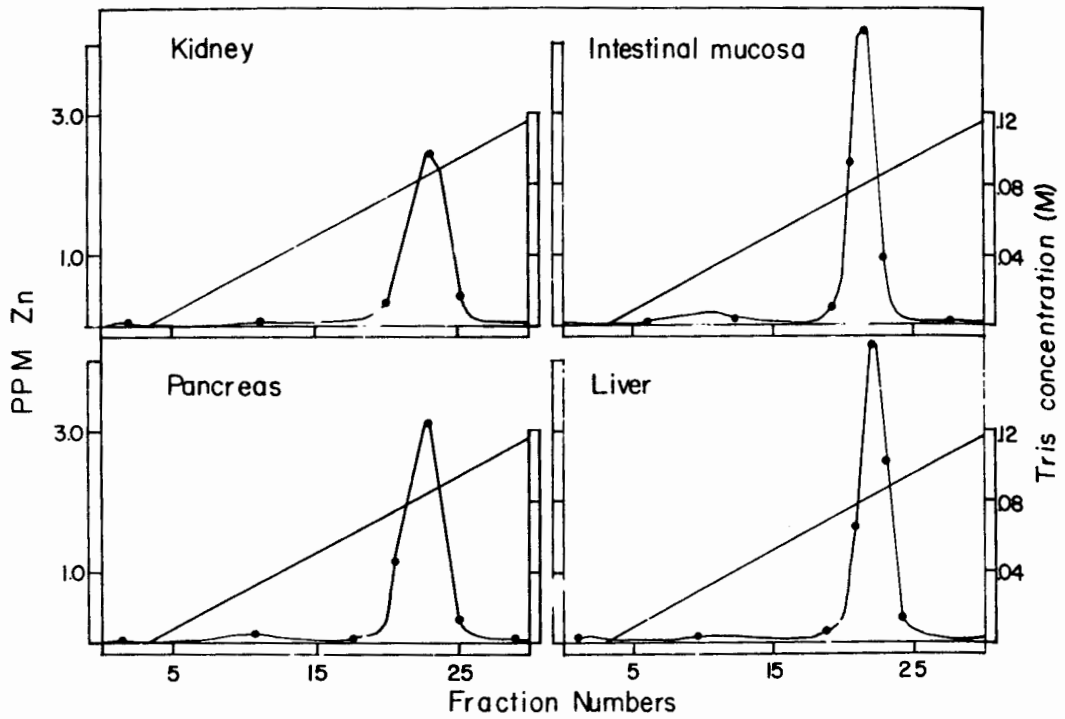

Fig. 6 Chromatography of the MT fractions from the gel filtration steps on DEAE cellulose. The samples are from birds fed the diet with 4,000 ppm zinc. The samples from the gel filtration step were eluted through small DEAE (DE52) cellulose columns (2.5 × 6 cm) with a gradient of 0.004 to 0.32 M Tris buffer, pH 8.4, at a flow rate of 24 ml/hour, collecting 4 ml per fraction. Each sample was analyzed for zinc. Since there were no peaks eluted after the major MT species, the graphs show only the patterns through 0.12 M Tris buffer (experiment 2).

and intestinal mucosa than in the other two tissues. The amino acid analysis of the purified zinc peak III from all four tissues of chicks fed 4,000 ppm zinc in the diet revealed a very high content of cysteine (27.6 to 32%), and essentially the absence of aromatic amino acids (table 5). Isoelectric focusing gels revealed predominantly one protein in all of these preparations, suggesting high purity. Thus, these characteristics of zinc peaks III indicate that they are indeed MT.

DISCUSSION

Similar to the rat (10) and the young calf (11), excess zinc accumulates in MT of the tissues of chicks. As the zinc was increased in the diet, a corresponding increase of zinc occurred almost predominantly in MT of the cytosol. In contrast to the rats, however, there was greater accumulation of zinc in peaks I, II and IV of the cytosol in chick tissues. The zinc content of these peaks in rat tissues was essentially unaffected by the zinc level in the diet (10).

The nature of zinc in peaks I and II has not been investigated in our laboratory, but we have done some work with peak IV. We have subsequently purified this peak from gel filtration step with Biogel P_2 gel chromatography and high pressure liquid chromatography. Our evidence indicates that it is not a prostaglandin, as has been suggested by others (18). However, prostaglandin was found to increase the absorption of zinc by 70% in rats, but an inhibitor of the release of prostaglandin, indomethacin, was found to decrease the absorption by 60% (18). Furthermore, O'Dell et al. (19) have obtained some evidence for a relationship of zinc to prosta-

TABLE 5

Amino acid composition of metallothionein (zinc peak III) isolated from liver, kidney, pancreas and intestinal mucosa of chicks[1]

Amino acid	Liver	Kidney	Pancreas	Intestinal mucosa
		mole %		
Cysteine[2]	31.8	27.6	31.0	32.0
Aspartic acid	9.8	9.7	9.8	9.6
Methionine[2]	1.9	2.6	2.0	1.8
Threonine	1.7	2.3	1.9	1.7
Serine	12.6	10.8	11.7	12.0
Glutamic acid	3.8	4.2	3.9	3.6
Proline	5.0	5.4	5.7	5.3
Glycine	6.5	7.3	6.7	6.7
Alanine	9.7	8.9	9.3	9.7
Valine	1.8	2.4	1.7	1.6
Isoleucine	0	1.0	0.2	0
Leucine	0	1.0	0.2	0.5
Histidine	0.9	1.8	1.3	0.9
Lysine	9.3	8.8	9.4	9.0
Arginine	5.0	4.8	4.9	4.9

[1] Tyrosine and phenylalanine were detected in all preparations at less than 0.5%. [2] Determined respectively as cysteic acid and methionine sulfone.

glandin metabolism. When toxic doses of aspirin, an inhibitor of prostaglandin synthesis, were given to pregnant rats receiving a diet adequate in zinc, pathologic signs analogous to those of zinc deficiency were produced. Thus, there appears to be a relationship between prostaglandin synthesis and zinc metabolism, but our data do not indicate that peak IV is this compound. A low MW zinc binding ligand (similar to peak IV) has been reported to be absent in intestinal mucosa of young rats (newborn to 14 days of age), but is present in rats after 16 days of age (20). Thus, the available evidence indicates that the compound(s) in peak IV may be involved with zinc metabolism.

Dietary cadmium will result in an increased zinc content of peak IV[10]; however, this element is not deposited in this peak. Thus, in addition to zinc, cadmium is another dietary variable which will influence the zinc levels in peak IV.

Similar to the rat (10), the accumulated zinc in tissue MT of chicks is also very labile (figs. 3 and 5). We had originally proposed that MT is a storage protein for zinc analogous to ferritin for iron (9). However, the rapid disappearance of zinc from MT when rats (10) or chicks (present work) were fed a low zinc diet raises questions concerning this role. It is still probable that MT could be serving as a temporary zinc storage protein. A small amount of zinc appeared to be transferred from the MT fraction to peaks I and II during zinc depletion in the rat (10), suggesting some zinc in MT is available to other systems. Injections of ^{65}Zn-thionein in rats have also provided evidence that zinc from MT can be transferred to other tissue proteins and reutilized similar to inorganic salts of this metal (21). Thus, zinc in MT appears to be metabolically active.

The tissue levels of MT are extremely sensitive to zinc (7, 8). MT in both liver and intestinal mucosa responds rapidly to altered dietary zinc (7). This is consistent with our data showing an 8-fold increase of MT synthesis after only 1 day of feeding a diet high in zinc (8). An additional 2–3 fold increase over the first day of MT synthesis occurred after 2 days of feeding this diet. In addition to zinc and other metals such as cadmium, copper or mercury, we have also shown that the age of the animal,[10] the sulfur status,[10] and various stresses (22) will affect MT levels in tissues. Also, infection (23) and starvation

[10] Oh, S. H., Deagen, J. T. & Whanger, P. D. (1978) Unpublished data.

(3) have been shown to influence tissue levels of this protein. Thus, these are examples where environmental conditions must be rigorously controlled in order to obtain adequate results with this protein. These factors no doubt contribute to the widely different values for the half-life of MT reported in the literature (8, 24, 25).

Weight gains of broiler chicks were not reduced by up to 2,400 ppm added zinc to the diet (26). In the present studies, 4,000 ppm zinc did not significantly depress the growth of chicks, but 8,000 and 16,000 ppm zinc decreased the growth rate. In fact, at these levels zinc was toxic. Another report has indicated that 1,500 ppm for chicks resulted in only decreased growth with no deaths (27). Thus, the zinc in the diet used in the present study was not as toxic as in their diet.

The metabolism of zinc is dependent upon the age and species of the animal. We have found the half-life of MT to be almost twice as long in 6-month old rats as in 1-month old ones.[10] Feeding 600 ppm zinc to young calves resulted in an accumulation of this metal with MT in liver and pancreas, but feeding this level of zinc to adult cows did not affect the tissue zinc levels (11). Unlike the calf and weanling pig, the metabolism of zinc in the young chick does not appear to change appreciably with increasing maturity (26). Thus, age and species are factors which must be taken into consideration in metabolic studies with zinc.

There was predominantly one species of MT in tissues of the chick (fig. 6), which is in contrast to the rat and rabbit. There are two species of MT of about equal concentration in rat liver and kidney (28, 29) and the rabbit liver (5). Predominantly one species of MT is present in equine liver and kidney (2) and in bovine and porcine liver.[10] The significance of this variation between species of animals is not known. The amino acid analyses of zinc peak III from all four tissues revealed that they were MT (table 5). This amino acid composition is very similar to that reported by Weser et al. (4) for chick liver MT. Thus, this indicates that the major protein for binding excess zinc in tissues is MT.

In conclusion, it appears that MT is involved in zinc homeostasis in the chick as has been shown for the rat (10) and calf (11). The exact function of MT, however, in zinc metabolism is not known. The rapid disappearance of zinc from MT when chicks are fed a diet low in zinc raises questions concerning its role as a zinc storage protein. However, it could play a role as a temporary storage protein for this metal, in which its involvement would be in counteracting zinc toxicity when this metal is present in diets at high concentrations.

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