Iron metabolism in chicks fed various levels of zinc and copper

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Four studies were conducted to determine the effect of high levels of zinc and copper on iron use of the chick. Chicks from the cross of New Hampshire × Single Comb White Leghorn were fed semi-purified diets with adequate (0.58–0.76 μmol Zn/g diet) and high (26.0–31.4 μmol Zn/g diet) levels of zinc and adequate (0.13–0.17 μmol Cu/g diet) and high (3.18–3.50 μmol Cu/g diet) levels of copper for 21 days. Fe-59 was fed in studies A–C and injected intraperitoneally in study D. Chicks fed high levels of zinc consistently showed decreased packed cell volumes and depressed concentrations of iron in livers and tibias. The anemia was not attributable to reduced feed and iron intakes. Chicks fed high rather than adequate amounts of zinc excreted 78% rather than 54% of an oral dose of Fe-59. The appearance of Fe-59 in plasma 1 hour after feeding the isotope decreased in chicks fed high rather than adequate levels of zinc. Ingestion of additional copper did not reverse the effects of ingesting high amounts of zinc on iron absorption. Ingestion of the high levels of zinc had minor effects on endogenous iron excretion. The ingestion of additional copper partially counteracted this effect.

Keywords: anemia; zinc; copper; iron; Fe-59; chick

Introduction

Ingestion of excess zinc (>22.9 μmol/g diet) by chicks has been shown to decrease body weight and feed intake,[1-4] to reduce hematocrit and hemoglobin with a microcytic and hypochromic anemia,[1-6] and to reduce tissue copper and iron concentrations.[3,4,7] The anemia caused by the ingestion of excess zinc could be the consequence of several different mechanisms.

Ingestion of excess zinc depresses feed, and accordingly iron, intake.[1-4] Ingestion of excess zinc has been reported by a number of investigators,[4,7-11] but not all investigators [11-13] to depress apparent absorption of iron. These discrepancies could reflect several factors. Storey and Greger[11] observed that although chronic ingestion of excess zinc depressed iron absorption, ingestion of one meal with excess zinc did not.

Other mechanisms for zinc-iron interactions may also exist. Ingestion of excess zinc by depressing copper use could ultimately alter iron transport and storage.[4,12,13] Accordingly, ingestion of excess zinc, by varying tissue concentrations of copper and copper-containing proteins, might even alter endogenous losses of iron; this has not been reported however.

The objectives of these experiments were the following: 1) to demonstrate that excess zinc intake results in anemia in chickens grown in a controlled environment and 2) to evaluate the impact of excess zinc intake on feed, and accordingly, iron intake, on iron absorption and on endogenous iron excretion.

Materials and methods

Experimental design

Four studies were conducted. In study A, chicks were assigned to a 2 × 3 factorial arrangement of treatments in which adequate (0.58 μmol Zn/g diet) and high (31.4 μmol Zn/g diet) levels of zinc; and adequate (0.17 μmol Cu/g diet), high (3.5 μmol Cu/g diet), and very high (8.34 μmol Cu/g diet) levels of copper were fed ad libitum. An additional group of chicks were fed the diet with adequate levels of zinc and copper to the level consumed on average by chicks fed excess zinc and very high levels of copper (limit fed, LF).

In studies B, C, and D, chicks were randomly assigned to a

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2 × 2 factorial arrangement of treatments in which adequate (0.73, 0.76, and 0.75 μmol Zn/g diet in studies B, C, and D, respectively) and high (26.3, 26.0, and 27.4 μmol Zn/g diet in studies B, C, and D, respectively) levels of zinc and adequate (0.14, 0.13, and 0.13 μmol Cu/g diet in studies B, C, and D, respectively) and high (3.43, 3.18, and 3.37 μmol Cu/g diet in studies B, C, and D, respectively) levels of copper were fed ad libitum. Diets were fed from hatching to 21 days of age to 7, 12, 8, and 8 chicks per treatment in studies A, B, C, and D, respectively. The diets in studies A–D contained 2.2, 1.7, 2.0, and 1.3 μmol Fe/g diet, respectively.

Animals and diets

In all four studies, chicks from the cross of New Hampshire × Single Comb White Leghorn were housed in individual stainless steel cages with wire floors in rooms maintained at 30° C with 24-hr lighting. The facilities met the requirements of the Institutional Animal Care and Use Committee.

Chicks in study A were fed the National Research Council[14] isolated soybean-starch based diet. In the remaining studies, the diet fed was a semi-purified, sucrose-casein based diet (*Table 1*).

Food consumption and body weights were recorded weekly in all studies. Deionized water was supplied ad libitum throughout the studies.

Radioisotope analyses

A gel capsule containing 74 kBq of Fe-59 (ferric chloride in 0.5 M HCl, 38.1 GBq/mmol Fe, New England Nuclear, Boston, MA, USA) in 0.1 g diet was placed in the crops of chicks on day 14 in study A and on day 20 in studies B and C. The chicks were also fed 1.0 g of additional diet. The chicks consumed the additional feed immediately because they were fasted overnight prior to receiving the capsule.

Loss of Fe-59 in excreta was measured for 1 week in study A. In study B, chicks were bled (via cardiac puncture using syringes rinsed with a heparin solution) 1, 2, 3, 4, and 5 hr after and were killed 6 hr after dosing with isotope. Chicks were divided so that each chick was bled only twice at 3-hr intervals. In study C, chicks were bled and killed 1 hr after dosing with isotope because the peak appearance of Fe-59 in plasma was found to occur after 1 hr in study C.

In study D, 7-day old chicks were injected intramuscularly with 183 kBq Fe-59 (ferric chloride in 0.5 M HCl, 85 GBq/mmol Fe, Dupont Company, Wilmington, DE, USA) to determine iron turnover. Chicks were bled at 5, 10, and 14 days after injection of the isotope. Red blood cell (RBC) pellets from heparinized blood were washed three times with phosphate buffer saline to remove any free isotope. Radioactivity in the pellets was determined. Biologic half-life of Fe-59 was obtained from the inverse slope resulting from plotting Bq/mL RBC or blood over time (days.)[15] Total blood volumes were calculated to be 7% of body weight. Fe-59 in excreta (for 2 weeks after injection of the isotope) and in tissues also was determined.

Radioactivity of excreta, sample capsules, and tissues in all studies was measured in an automatic gamma counter (Gamma Trac 1191 TM Analytic Inc., Elk Grove Village, IL, USA) set at the photopeaks of Fe-59. The radioactivity values were corrected for background and decay and then converted to Bq on the basis of the counting efficiency of Co-60 standard.

Other analyses

All chicks were killed via cervical dislocation. Organs (spleen, liver, and tibias) were cleansed of adhering material and weighed.

Table 1 Composition of semi-purified diets

Ingredient	Study A	Studies B–D
	g/kg	
Isolated soybean protein	250.0	—
Starch	598.0	—
Glucose	—	270.2
Sucrose	—	270.0
Casein	—	220.0
Egg albumin	—	80.0
Choline Cl (60%)	1.7	2.2
KH_2PO_4	10.0	13.8
KCl	1.0	—
NaCl	6.0	—
$NaHCO_3$	—	7.0
$CaCO_3$	14.8	18.3
$CaHPO_4$	16.4	12.0
$MgCO_3$	2.1	—
Mineral mix	10.0[a]	10.0[c]
Corn oil	40.0	30.0
Arginine Cl	—	6.0
Methionine	6.0	—
Glycine	4.0	—
Vitamin mix	10.0[b]	10.0[d]
Cellulose	30.0	50.0

[a] The mineral premix supplied the following in mg per kg of diet: MgO, 1000; $MnSO_4$-H_2O, 246; KI, 1.4; $CuSO_4$-$5H_2O$, 30; Na_2SeO_4, 0.2; $Fe(SO_4)_2$-H_2O, 500; $CoCl_2$, 1.7

[b] The vitamin premix supplied the following in mg per kg of diet: riboflavin, 17.5; thiamin-Cl, 15; Ca-pantothenic, 20; niacin, 50; pyridoxine-HCl, 6; folic acid, 6; biotin, 0.6; vitamin K-bisulfite, 1.5; vitamin B_{12}, 0.2; vitamin A (195,000 RE/g), 7; vitamin D_3 (10,000 μg/g), 11.2; vitamin E (364 mg D-L tocopherol/g), 125.

[c] The mineral premix supplied the following in mg per kg of diet: MgO, 1000; $MnSO_4$-H_2O, 200; KI, 1; Na_2SeO_4, 0.4; $Fe(SO_4)_2$-H_2O, 426.

[d] The vitamin premix supplied the following in mg per kg of diet: riboflavin, 40; thiamin-Cl, 5; Ca-pantothenic, 20; niacin, 50; pyridoxine-HCl, 7; folic acid, 2; biotin, 1.5; vitamin K-bisulfite, 2; vitamin B_{12}, 0.2; vitamin A (195,000 RE/g), 7; vitamin D_3 (10,000 μg/g), 3; vitamin E (227 mg D-L tocopherol/g), 200.

Diets and tissues were analyzed for zinc, iron, and copper by atomic absorption spectrophotometry (Perkin-Elmer Corp. Model 372, Norwalk, CT), USA.[16] Bovine liver standards (SRM #1577a) obtained from the National Bureau of Standards were analyzed with most of the sets of samples. The liver standards ($n > 17$) were found to contain 2.44 ± 0.02 μmol copper, 3.24 ± 0.07 μmol iron, and 1.90 ± 0.03 μmol zinc/g sample; the standard was certified to contain 2.49 μmol copper, 3.47 μmol iron, and 1.88 μmol zinc/g sample.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the general linear model that allowed the effects of zinc, copper, and their interactions to be determined in studies B, C, and D.[17] Mean treatment differences were determined by least square difference (LSD) with a level of statistical significance of 5%. Percentage data was transformed to arc sin angles prior to statistical analysis.

Results

Ingestion of excess zinc consistently decreased packed cell volumes. Although chicks fed high levels

Table 2 Hematological status of chicks fed various levels of zinc and/or copper

Diet zinc*	A	A	H	H	LF-A	A	H
Diet copper	A	H	A	H	LF-A	VH	VH
Packed cell volume (l)							
Study A*	0.26 ± 0.01†[a]	0.27 ± 0.01[a]	0.17 ± 0.01[c]	0.21 ± 0.01[b]	0.25 ± 0.01[a]	0.27 ± 0.01[a]	0.20 ± 0.01[b]
Study B‡	0.31 ± 0.02[a]	0.29 ± 0.04[a]	0.25 ± 0.07[b]	0.25 ± 0.01[b]	—	—	—
Study C‡	0.29 ± 0.04[a]	0.28 ± 0.08[ab]	0.25 ± 0.01[c]	0.26 ± 0.07[bc]	—	—	—
Study D‡	0.29 ± 0.06[a]	0.28 ± 0.08[a]	0.24 ± 0.08[b]	0.24 ± 0.04[b]	—	—	—

* A = adequate (0.58–0.76 μmol Zn/g diet and 0.13–0.17 μmol Cu/g diet); H = high (26.0–31.4 μmol Zn/g diet and 3.18–3.50 μmol Cu/g diet); VH = very high (8.34 μmol Cu/g diet); LF = limit fed.
† Values are mean ± SEM, n = 7 per treatment in study A, n = 12 in study B, n = 8 in studies C and D. Data analyzed by ANOVA and LSD tests applied to differentiate among means that were significantly ($P < 0.05$) different. In studies B–D, two-way ANOVA was applied to determine effect due to diet Zn, diet Cu, and their interaction. Means in a row without a common superscript letter differ significantly ($P < 0.05$).
‡ Effect of zinc was significant ($P < 0.01$) but effects of copper and their interaction were not statistically significant.

Table 3 Excretion and appearance of Fe-59 in tissues one week after ingestion of a meal labeled with Fe-59 among chicks in study A fed various levels of zinc and/or copper

Diet zinc*	A	A	H	H	LF-A	A	H
Diet copper	A	H	A	H	LF-A	VH	VH
Excreta (% of dose lost in week 1 after meal)							
	54 ± 4†[b]	58 ± 5[b]	78 ± 2[a]	78 ± 1[a]	62 ± 4[b]	58 ± 7[b]	77 ± 4[a]
Tissue appearance of Fe-59 (Bq/organ)							
Liver	211 ± 34	211 ± 33	106 ± 25	165 ± 84	148 ± 28	291 ± 86	100 ± 32
Tibia	25 ± 2[a]	24 ± 3[ab]	6 ± 1[bc]	5 ± 1[c]	15 ± 3[b]	26 ± 6[a]	5 ± 2[c]

* A = adequate (0.58 μmol Zn/g diet and 0.17 μmol Cu/g diet); H = high (31.4 μmol Zn/g diet and 3.50 μmol Cu/g diet); VH = very high (8.34 μmol Cu/g diet); LF = limit fed.
† Values are mean ± SEM, n = 7 per treatment in study A. Data analyzed by ANOVA and LSD tests applied to differentiate among means which were significantly ($P < 0.05$) different. Means in a row without a common superscript letter differ significantly ($P < 0.05$).

of zinc in soybean and starch-based diets in study A had significantly higher hematocrits when fed high rather than adequate levels of copper (*Table 2*), this small effect of copper intake was not observed in studies B, C, and D when casein and sucrose-based diets were fed. Limit-feeding did not affect the hematocrits of chicks in study A.

During the week after Fe-59 was orally administered in study A, excretion of Fe-59 was significantly greater when high levels of zinc were fed (*Table 3*). In study B, the amount of Fe-59 in plasma after the meal was greater at 1 hr than after 2, 3, 4, 5, or 6 hr (study 3, *Figure 1*). The appearance of Fe-59 into plasma after 1 hr was less in chicks fed the high rather than the adequate level of zinc. Copper intake had no effect on the appearance of Fe-59 in plasma.

One hr (study C) after the meal, less Fe-59 appeared in the liver of chicks fed high rather than adequate levels of zinc and/or copper (*Table 4*). The effects of zinc and copper appeared to be additive as the Zn × Cu interaction was statistically significant. Chicks fed either adequate levels of both zinc and copper or high levels of both elements accumulated more Fe-59 in tibias 1 hr after the meal than chicks fed adequate zinc with high copper. Six hours after the meal (study B), excess zinc depressed the appearance of Fe-59 in both livers and tibias. Intake of high rather than adequate levels of zinc and/or copper depressed Fe-59 appearance in spleen 6 hr after the meal.

One week after isotope was consumed (study A, *Table 3*), dietary treatments had no statistically significant effect on the amount of Fe-59 in livers, although there was a tendency for less Fe-59 to be present in livers of chicks fed high rather than adequate levels of zinc. The effect of dietary zinc on the amount of Fe-59 in tibias was statistically significant.

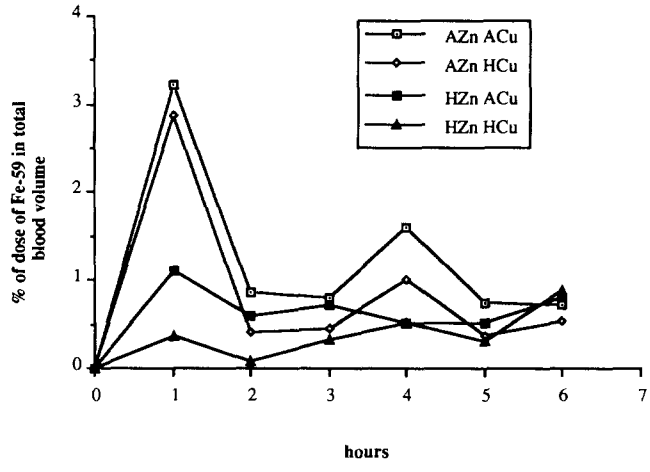

Figure 1 Incorporation of orally administered Fe-59 into blood of chicks fed various levels of zinc and/or copper in study B. Pooled SEM are 0.63, 0.18, 0.10, 0.16, 0.22, and 0.18 at 1, 2, 3, 4, 5, and 6 hours, respectively. A = adequate and H = high in codes for dietary treatments. Total blood volume was calculated as 7% of body weight.

Ingestion of high levels of zinc, but not copper, consistently depressed the concentration of iron in tibas and in livers in studies B–D (*Table 5*). In study A,

Table 4 Appearance of Fe-59 in tissues 1 and 6 hours after ingestion of a meal labeled with Fe-59 among chicks fed various levels of zinc and/or copper

Diet zinc*	A	A	H	H	Statistical significance of:‡		
Diet copper	A	H	A	H			
Tissue appearance of Fe-59 (Bq/organ)					Cu	Zn	Cu × Zn
Liver							
Study B (6hr)	45 ± 13†[a]	42 ± 16[a]	9 ± 2[b]	11 ± 3[b]	ns§	0.005	ns
Study C (1hr)	119 ± 20[a]	48 ± 13[b]	23 ± 5[bc]	10 ± 2[c]	0.001	0.0001	0.05
Tibia							
Study B (6hr)	41 ± 8[a]	38 ± 9[ab]	20 ± 6[b]	10 ± 4[c]	ns	0.005	ns
Study C (1hr)	13 ± 4[a]	5 ± 1[b]	8 ± 2[ab]	13 ± 1[a]	ns	ns	0.01
Spleen							
Study B (6hr)	6.0 ± 1[a]	1.4 ± 0.5[b]	1.5 ± 1.2[b]	1.1 ± 0.1[b]	0.0005	0.001	0.001

* A = adequate (0.73–0.76 μmol Zn/g diet and 0.13–0.14 μmol Cu/g diet); H = high (26.0–26.3 μmol Zn/g diet and 3.18–3.43 μmol Cu/g diet).
† Values are mean ± SEM, n = 12 per treatment in study B and n = 8 in study C.
‡ Data analyzed by 2 × 2 ANOVA and LSD tests applied to differentiate among means which were significantly ($P < 0.05$) different. Means in a row without a common superscript letter differ significantly ($P < 0.05$).
§ ns, not significant.

Table 5 Tissue iron concentrations of chicks fed various levels of zinc and/or copper

Diet zinc*	A	A	H	H	LF-A	A	H
Diet copper	A	H	A	H	LF-A	VH	VH
Liver Iron		(μmol/g wet tissue)					
Study A	0.86 ± 0.05†[bc]	0.86 ± 0.09[bc]	0.64 ± 0.02[c]	0.68 ± 0.05[c]	1.24 ± 0.11[a]	0.93 ± 0.11[b]	0.80 ± 0.05[bc]
Study B‡	0.95 ± 0.05[ab]	1.06 ± 0.07[a]	0.82 ± 0.05[b]	0.81 ± 0.07[b]	—	—	—
Study C‡	1.06 ± 0.05[a]	1.12 ± 0.09[a]	0.84 ± 0.05[b]	0.82 ± 0.02[b]	—	—	—
Study D‡	0.64 ± 0.02[a]	0.67 ± 0.02[a]	0.30 ± 0.01[b]	0.26 ± 0.01[b]	—	—	—
Tibia iron							
Study A	0.70 ± 0.04[a]	0.68 ± 0.05[a]	0.29 ± 0.04[b]	0.30 ± 0.02[b]	0.65 ± 0.05[a]	0.73 ± 0.05[a]	0.36 ± 0.01[b]
Study B‡	0.99 ± 0.07[a]	1.07 ± 0.05[a]	0.67 ± 0.09[b]	0.59 ± 0.07[b]	—	—	—
Study C‡	1.31 ± 0.09[a]	1.34 ± 0.05[a]	0.98 ± 0.05[b]	1.04 ± 0.05[b]	—	—	—
Study D‡	1.25 ± 0.09[a]	1.14 ± 0.06[a]	0.73 ± 0.05[b]	0.49 ± 0.02[b]	—	—	—
Spleen Iron							
Study B§	2.42 ± 0.21[bc]	2.90 ± 0.16[b]	2.20 ± 0.32[c]	3.60 ± 0.23[a]	—	—	—
Study D	1.12 ± 0.18	0.91 ± 0.13	1.18 ± 0.09	1.40 ± 0.13	—	—	—

* A = adequate (0.55–0.76 μmol Zn/g diet and 0.11–0.17 μmol Cu/g diet); H = high (26.0–33.9 μmol Zn/g diet and 3.18–3.98 μmol Cu/g diet); VH = very high (8.34 μmol Cu/g diet); LF = fed.
† Values are mean ± SEM, n = 7 per treatment in study A, n = 12 in study B, n = 8 in studies C and D. Data analyzed by ANOVA and LSD tests applied to differentiate among means that were significantly ($P < 0.05$) different. In studies B–D, two-way ANOVA was also applied to determine effect due to diet zinc, diet copper, and their interaction. Means in a row without a common superscript letter differ significantly ($P < 0.05$).
‡ Effect of zinc significant ($P < 0.01$) but effects of copper and their interaction were not statistically significant.
§ Effect of copper ($P < 0.01$) and of the interaction ($P < 0.01$) were significant; effect of zinc was not statistically significant.

the difference was clearer between chicks fed the high level of zinc and those pair-fed rather than ad libitum fed the adequate level of zinc.

We hypothesized that the tissue changes in Fe-59 and iron concentrations caused by ingestion of excess zinc could reflect interactions not only in absorptive processes but also in excretion. In study D, chicks fed adequate copper excreted less of an intraperitoneal dose of Fe-59 when fed high rather than adequate levels of zinc (*Table 6*). However, in general, differences in dietary intake of zinc and copper did not affect the $t_{1/2}$ or tissue distribution of injected Fe-59.

Discussion

There is no fully satisfactory method for monitoring iron absorption in chicks. The total collection of excreta as we did in study A is logical but extremely time consuming due to the consistency of chick excreta. The appearance of Fe-59 in blood after a meal could be misleading if animals in one treatment absorbed Fe-59 more slowly than those in the other treatments. However, the data in *Figure* 1 suggest that maximal appearance of Fe-59 in blood was the same for all treatments. Moreover, the losses of orally administered Fe-59 in excreta (*Table 3*) were the sum of unabsorbed iron, urinary losses, and endogenous gut losses. However, very little iron is lost in urine.[18] Thus, the greater loss of Fe-59 in excreta by chicks fed higher rather than adequate levels of zinc (*Table 3*) and the decreased appearance of Fe-59 in plasma (*Figure 1*) of chicks fed high rather than adequate zinc indicate that iron absorption was impaired by ingestion of high levels of zinc. Several investigators have

Table 6 Excretion, half-life, and appearance in tissue of injected Fe-59 in chicks fed various levels of zinc and/or copper in study D

Diet zinc*	A	A	H	H	Statistical significance of:		
Diet copper	A	H	A	H	Cu	Zn	Cu × Zn
Excretion of injected Fe-59 (% of dose excreted in 2 wk)							
	15.5 ± 2.6†[a]	9.5 ± 2.6[ab]	7.2 ± 0.7[b]	11.2 ± 1.6[ab]	ns§	ns	0.05
Half-life of Fe-59 (days)							
	26.9 ± 3.5	27.7 ± 4.9	28.2 ± 6.9	25.1 ± 4.9	ns	ns	ns
Tissue appearance of Fe-59 (Bq/organ)							
Tibia	100 ± 11	126 ± 16	96 ± 14	95 ± 17	ns	ns	ns
Liver	1087 ± 190	1423 ± 167	1092 ± 182	1045 ± 200	ns	ns	ns
Spleen	85 ± 10	119 ± 23	86 ± 25	53 ± 15	ns	ns	ns

* A = adequate (0.75 μmol Zn/g diet and 0.13 μmol Cu/g diet); H = high (27.4 μmol Zn/g diet and 3.37 μmol Cu/g diet). Means in a row without a common superscript letter differ significantly ($P < 0.05$).
† Values are mean ± SEM, n = 8.
‡ Data analyzed by 2 × 2 ANOVA and LSD tests applied to differentiate among means that were significantly ($P < 0.05$) different. A two-way ANOVA was also applied to determine effects due to a diet zinc, diet copper, and their interactions.
§ ns, not significant.

also suggested that high zinc intakes interfered with iron absorption in rats and humans.[8-10]

Excess zinc depressed the appearance of orally administered Fe-59 into livers after 1 and 6 hr (studies C and B, *Table 4*), but had less effect on the amount of Fe-59 in liver after 1 week (study A, *Table 3*). This could indicate that excess zinc slowed transport and storage processes. In theory this is possible if ingestion of excess zinc induced a relative copper deficiency. Rama and Planas[7] have shown that excess zinc depressed ceruloplasmin levels in chicks. Davis and Mertz[19] have noted that the release of iron from liver parenchymal cells and from the reticuloendothelial system appears to be reduced sometimes during copper deficiency. However, the very low levels of circulating ceruloplasmin in chicks suggest that other mechanisms are more likely.

Fisher et al.[20] have proposed that ingestion of excess zinc induced thionein that bound copper in the mucosal cells and ultimately induced a relative copper deficiency. However, in our studies, the addition of extra copper generally did not prevent the effects of excess zinc, including the effects of excess zinc on tissue distribution of Fe-59. We did observe, however, that when additional copper was added to the diet, excess zinc did not depress excretion of injected Fe-59. This might suggest that the additional copper facilitated iron transport so that excretion could occur.

We might have observed a greater effect of copper if more copper had been fed. However, ingestion of very high (8.34 μmol Cu/g diet) rather than high (3.5 μmol Cu/g diet) levels of copper in study A did not result in a greater effect of copper. Moreover, if we had fed much more copper, methionine metabolism would have been altered and growth would have been reduced further.[21]

The diets fed in study A had a different protein source (soy) than those fed in studies B–D (casein). This did not appear to alter the relative effects of the treatments but liver and tibia iron concentrations were lower in study A than in studies B and C. The lower spleen and liver iron concentrations of chicks in study D reflected the repeated blood collections.

Overall, these data indicate that ingestion of large amounts of zinc induced anemia primarily by depressing iron absorption in chicks. Ingestion of large amounts of zinc had minor effects on endogenous iron excretion. The ingestion of supplemental copper partially counteracted the effect of high levels of zinc on endogenous excretion of iron but did not alter zinc's effect on iron absorption.

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