A STUDY OF THE EFFECTS OF CALCIUM AND PHOSPHORUS SUPPLEMENTS ON THE MINERAL METABOLISM OF YOUNG RATS FED HIGH LEVELS OF ZINC

by

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Young male rats were used to investigate the effect of zinc toxicity on the metabolism of calcium, phosphorus, magnesium, zinc, copper, and iron in the presence and the absence of calcium and phosphorus supplements.

Results of the study indicated that calcium and phosphorus supplementation prevents the marked decreases in the retention of calcium and phosphorus in young rats associated with zinc toxicity. The data suggest that calcium and phosphorus supplements furnish sufficient amounts of these minerals to replace the amounts lost by the adverse action of zinc and facilitate the removal of some of the excess zinc from the animal body.

The addition of calcium, phosphorus, or zinc supplements to diets of young rats resulted in decreases in the percentage of the total magnesium intake that was retained.

Zinc toxicity was associated with a marked increase in the apparent absorption and retention of zinc. Calcium and phosphorus supplements added to the high zinc diet resulted in additional increases in zinc retention. Animals receiving diets without added zinc were in negative zinc balances.

Zinc toxicity resulted in a marked increase in the apparent absorption and retention of copper. In general, the addition of calcium or phosphorus to the high zinc diet

resulted in additional improvements in copper retention.

Calcium and phosphorus supplements partially alleviated the adverse effect of zinc toxicity on weight gain, hemoglobin level, and liver copper accumulation. Supplements of these two minerals also prevented the marked increase in liver zinc deposition associated with zinc toxicity.

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CHAPTER I

INTRODUCTION

In the normal animal delicate balances often exist between various essential nutrients which can be upset if one or more of the nutrients involved in a specific interrelationship is present in the diet in abnormal concentrations. The physiological behavior of animals consuming ingredients not generally considered dietary essentials has stimulated research in many areas of nutrition, particularly in the area of trace mineral excesses. Of great interest has been the biological response of animals to diets containing high levels of zinc.

Zinc toxicosis is associated with several adverse conditions in the animal, such as depression in weight gain, interference with bone mineralization, and interference with the metabolic pathways of calcium, copper, iron, magnesium, and phosphorus. Although many of the symptoms of the zinc toxicity syndrome have been characterized, the sites and the modes of interference of zinc with the various metabolic processes involved remain enigmas.

A recent report has shown that calcium and phosphorus supplements will alleviate the adverse effects of zinc on the weight gains and on the deposition of calcium

and phosphorus in the bones of young rats. The mechanism(s) involved, however, have not been determined. Furthermore, there is the possibility that the interrelationships between zinc and other elements, other than calcium and phosphorus, which exist in the animal body may be influenced by calcium and phosphorus supplementation. The present investigation was directed towards the elucidation of some of these problems because it might serve as the means of obtaining additional information pertaining to the roles of certain mineral nutrients in the animal body.

CHAPTER II

REVIEW OF LITERATURE

Sutton and Nelson (1, 2) reported that high levels of dietary zinc had adverse effects on the growth, hemoglobin levels, and reproductive capabilities of rats in 1937, but almost a decade passed before additional research was conducted to characterize the zinc toxicity syndrome. In 1946 Smith and Larson (3) reported that the etiologies of the subnormal growth and the anemia associated with zinc toxicosis were separate and distinct. Subsequent experimentation revealed that high levels of dietary zinc resulted in lowered catalase, cytochrome oxidase, and xanthine oxidase activities (4, 5, 6, 7), decreases in tissue copper and iron levels (8, 9, 10), increases in liver zinc levels (10, 11), and interfered with the normal metabolism of calcium and phosphorus (12, 13, 14).

Although the exact mechanism(s) involving the interference of zinc with growth has not been determined, results of several studies indicated that dietary supplements of liver and distillers dried solubles can alleviate the effect of zinc toxicity on weight gain (3, 10, 15). Magee and Matrone (10) reported that the factor(s) of liver extract that alleviated subnormal growth appeared to reside in the

organic portion of the extract. McCall et al. (16) reported that the severity of zinc toxicity on growth depended upon the source and the level of dietary protein. Magee and Spahr (15), however, found that high levels of dietary protein accentuated, rather than reduced, the severity of zinc toxicity, and their data indicated that dietary protein per se was not the primary factor involved in the growth depression. Recently, Stewart and Magee (17) have reported that dietary supplements of calcium and phosphorus will partially alleviate the depression in weight gains of young rats fed high levels of zinc.

Van Reen (4) observed beneficial effects of copper supplements on the depressed activities of liver catalase and cytochrome oxidase. Depression in heart cytochrome oxidase activity could also be prevented with supplements of copper (5, 10). Copper supplementation prevented the marked decreases in tissue copper levels, but iron supplementation did not prevent the decreases in tissue iron levels associated with zinc toxicity (10). Magee and Spahr (15) reported that a 20% level of distillers dried solubles added to a high zinc diet significantly reduced liver zinc accumulation.

Sadasivan (12) observed that high levels of zinc were associated with marked decreases in bone calcium and phosphorus levels in rats. Other reports indicate that zinc toxicosis results in decreases in the retentions of calcium, phosphorus, and magnesium in rats (13, 14, 17) and decreases

in the retentions of calcium and phosphorus in lambs (18).

Stewart and Magee (17) found that calcium and phosphorus supplements alleviated the antagonistic effect of zinc on the normal deposition of calcium and phosphorus in the bones of young rats. Although the results of their study indicated that the calcium and phosphorus supplements facilitate the removal of excess zinc from the animal body so that near normal conditions exist with respect to zinc absorption and utilization, the exact mechanism(s) involved remains an enigma. There is the possibility that a study of the effects of calcium and phosphorus on the retentions of these minerals by zinc-fed rats may yield additional information concerning the calcium-phosphorus-zinc interrelationship. If the primary action of calcium and phosphorus supplements is to facilitate the removal of zinc, there is the possibility that these supplements could alleviate the adverse effect of zinc on copper and iron metabolism.

CHAPTER III

EXPERIMENTAL PROCEDURES

The primary objectives of this study were to investigate (a) the nature of the interference of zinc with calcium, phosphorus, and magnesium metabolism and (b) the effects of calcium and phosphorus supplements on the metabolism of copper, iron, and zinc in zinc-fed rats.

The study was divided into two parts. Part I pertains to a metabolism experiment designed to show the effect of zinc toxicity on the retentions of calcium, phosphorus, magnesium, copper, and zinc in young rats in the presence and absence of calcium and phosphorus supplements. In part II the effects of calcium and phosphorus supplements on weight gains, hemoglobin levels, and the deposition of copper, iron, and zinc in the livers of rats fed high levels of zinc were studied.

Young male albino rats[1] were used for all phases of the study. The animals were housed in individual wire-bottom metabolism cages which were fixed so that feces and urine could be collected separately. Fresh feed was

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[1]Sprague-Dawley rats purchased from Holtzman Company Madison, Wisconsin.

provided daily, and food consumption records for each animal were kept. The animals had free access to distilled water at all times. The animals used in the study were randomized into replications according to initial body weights. Experimental diets and animals within a replication were assigned at random to individual cages.

A 2^3 factorial design was utilized and included two levels of zinc, calcium, and phosphorus. The dietary supplements were fed alone and in combination to furnish, in addition to the control, the following experimental diets: 0.8% calcium, 0.8% phosphorus, 0.8% calcium + 0.8% phosphorus, 0.75% zinc, 0.75% zinc + 0.8% calcium, 0.75% zinc + 0.8% phosphorus, and 0.75% zinc + 0.8% calcium + 0.8% phosphorus.

The composition of the basal diet used in the study is given in Table 1. Chemical analyses of representative samples of this basal showed that it contained an average of 0.51% of calcium, 0.55% of phosphorus, 0.05% of magnesium, 5 ppm. of copper, and 3 ppm. of zinc. Supplements were incorporated into the basal at the expense of equal amounts of starch. Zinc was fed as the carbonate; supplements of calcium and phosphorus were in the carbonate and potassium dihydrogen phosphate forms, respectively.

Mineral Balance Study

Thirty-two animals, averaging 53 grams in weight initially, were divided equally into eight groups. Each

TABLE 1

COMPOSITION OF THE BASAL DIET

Constituents	Per cent
Casein[a]	19
Corn starch[b]	63
Vegetable fat[c]	10
Mineral mix[d]	4
Vitamin mix[e]	2
Cellulose[f]	2
Oleum percomorphum[g]	-

[a]Vitamin Test Casein, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b]Globe Easy-flow Corn Starch 3367, Corn Products Sales Company, Greensboro, North Carolina.

[c]Crisco, Procter and Gamble Company, Cincinnati, Ohio.

[d]Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio. The composition of this salt mixture is listed as: (in per cent) $CaCO_3$, 21.000; $CuSO_4.5H_2O$, 0.039; $FePO_4 \cdot 2H_2O$, 1.470; $MnSO_4$, 0.020; $MgSO_4$, 9.000; $KAl(SO_4)_2 \cdot 12H_2O$, 0.009; KCl, 12.000; KH_2PO_4 31.000; KI, 0.005; NaCl, 10.500; NaF, 0.057; and $Ca_3(PO_4)_2$, 14.900.

[e]Each 100 gm. of vitamin mix contained: (in milligrams) 0.1% vitamin B_{12} (with mannitol), 0.1; biotin, 1; folic acid, 5; thiamine·HCl, 25; pyridoxine·HCl, 25; 2-methyl-naphthoquinone, 50; riboflavin, 50; nicotinic acid, 50; Ca pantothenate, 150; p-aminobenzoic acid, 500; (in grams) inositol 5; choline chloride, 7.5; DL-methionine, 30; and corn starch, 56.6. All vitamins and methionine were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[f]Alphacel, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[g]Each kilogram of diet contained 24 drops of oleum percomorphum, Mead Johnson and Company, Evansville, Indiana.

group received one of the experimental diets previously mentioned.

The experimental period covered a total of four weeks, and during this time one collection period was utilized. The collection period was conducted during the last week the animals were on the dietary regimens. Respective excreta from an individual animal were collected daily, pooled, and kept in sealed containers in a refrigerator until they could be analyzed. Samples of the pooled fecal and urine collections from each animal for the repsective collection period were prepared for mineral analyses by ashing with nitric and perchloric acids on a hot plate. The ash of each sample was dissolved in 3 ml. of 0.6N HCl and brought to a volume of 100 ml. with redistilled water. Representative samples of the eight experimental diets were also prepared for mineral analyses by the same procedures. Calcium, phosphorus, magnesium, copper, and zinc determinations were made on appropriate aliquots of the feed, fecal, and urine samples by the methods of Weybrew et al. (19), Simonsen et al. (20), Simonsen et al. (21), Parks et al. (22) as modified by Matrone et al. (23), and McCall et al. (24), respectively.

Growth, Hemoglobin, and Liver Mineral Study

Sixty-four animals were utilized for this phase of the investigation and were divided equally into eight groups. Each group received one of the experimental diets previously mentioned. The animals were kept on their respective

dietary regimens for four weeks. At the end of the experimental period, blood samples were taken from the tails of each animal. Hemoglobin determinations, by the method of Shenk et al. (25), were made on the blood samples. All animals were killed, and the liver of each was removed and weighed. A small portion of each liver was dried separately at 35°C in order to provide dry weight data. The remainder of each liver was prepared for mineral analyses by the wet-ashing procedure previously mentioned. The ash of each liver sample was dissolved in 0.6N HCl and brought to a volume of 25 ml. with redistilled water. Copper and zinc determinations were made on appropriate aliquots of the liver samples by the methods previously mentioned. Iron determinations were made on the samples using the method of Kitzes et al. (26), as modified by Matrone et al. (23).

CHAPTER IV

RESULTS AND DISCUSSION

Mineral Balance Study

Detailed data gathered during this phase of the investigation are presented in Appendix A, Tables 1-6.

Calcium

Zinc toxicity resulted in a highly significant decrease ($p \leq 0.01$) in the retention of calcium by young rats which could be prevented by supplementing the diet with calcium (Table 2). In the absence of zinc, phosphorus supplementation resulted in an increase in calcium retention, but when phosphorus was added to a high zinc diet, a negative calcium balance resulted in the animals. Supplements of calcium, in the absence of zinc, resulted in marked improvements in calcium retention. The greatest amount of calcium retention occurred in animals fed the basal diet supplemented with calcium and phosphorus.

The data indicated that zinc results in a significant increase in the fecal excretion of calcium and a corresponding decrease in the apparent absorption of calcium. The addition of calcium to the high zinc diet furnished sufficient amounts of this mineral to replace that lost by the

TABLE 2

CALCIUM BALANCE SUMMARY[a]

Diet	Ca Intake (mg./day)	Ca Excreted in (mg./day) Feces	Urine	Ca Absorbed (mg./day)	(%)	Ca Retention (mg./day)	(%)
Basal	66.93	38.99	0.51	27.94	41.7	27.43	40.9
Basal + 0.8% Ca	288.11	141.49	1.15	146.62	50.8	145.70	50.5
Basal + 0.8% P	82.05	35.40	1.61	46.65	56.8	45.04	54.9
Basal + 0.8% Ca + 0.8% P	292.14	89.61	2.04	202.53	69.3	200.49	68.6
Basal + 0.75% Zn	61.38	55.25	0.78	6.13	10.0	5.35	8.7
Basal + 0.75% Zn + 0.8% Ca	208.13	123.19	0.43	84.94	40.8	84.51	40.6
Basal + 0.75% Zn + 0.8% P	79.38	88.59	2.05	- 9.21[b]	-	-11.26[b]	-
Basal + 0.75% Zn + 0.8% Ca + 0.8% P	232.59	131.21	1.16	101.38	43.5	100.22	43.1

[a]Each figure is the mean of 4 animals.

[b]A negative percentage value would result from the negative number. Such a value is meaningless; therefore, it has not been calculated.

adverse effect of zinc on calcium metabolism. In the absence of zinc, phosphorus had no marked influence on fecal calcium, but when phosphorus was added to the high zinc diet, a marked increase in fecal calcium excretion resulted. Calcium supplements, in the absence of zinc, resulted in a marked improvement in the apparent absorption of calcium.

Zinc caused a slight increase in the urinary excretion of calcium, but the presence of phosphorus supplements was associated with marked increases in urinary calcium. Rats receiving only calcium supplements had increased urinary calcium levels. Supplements of calcium, however, prevented the adverse effect of zinc on urinary calcium excretion.

Phosphorus

The presence of calcium, phosphorus, and zinc supplements in the diets of young rats was associated with marked decreases in the percentage of the phosphorus intake retained by the animals (Table 3). The data indicated that 0.75% zinc had the greatest adverse effect on phosphorus retention. Phosphorus supplementation was associated with marked improvements in the amounts of phosphorus retained even though the percentage phosphorus retention was lower than that observed in the control animals. The data also indicate that calcium supplements resulted in slightly better percentage phosphorus retention than did phosphorus supplements.

In this study the addition of calcium, phosphorus, and zinc to the diet was associated with increases in fecal

TABLE 3

PHOSPHORUS BALANCE SUMMARY[a]

Diet	P Intake (mg./day)	P Excreted in Feces (mg./day)	P Excreted in Urine (mg./day)	P Absorbed (mg./day)	P Absorbed (%)	P Retention (mg./day)	P Retention (%)
Basal	71.37	14.02	21.45	57.35	80.3	35.90	50.3
Basal + 0.8% Ca	75.15	43.09	2.77	32.06	42.6	29.29	38.9
Basal + 0.8% P	176.42	22.06	96.52	154.36	87.4	57.84	32.7
Basal + 0.8% Ca + 0.8% P	190.77	51.38	73.64	139.39	73.0	65.75	34.4
Basal + 0.75% Zn	61.18	32.80	16.61	28.38	46.3	11.77	19.2
Basal + 0.75% Zn + 0.8% Ca	74.59	44.95	9.91	29.64	39.7	19.73	26.4
Basal + 0.75% Zn + 0.8% P	164.36	47.48	85.49	116.88	71.1	31.39	19.1
Basal + 0.75% Zn + 0.8% Ca + 0.8% P	187.79	72.85	61.23	114.94	61.2	53.71	28.6

[a]Each figure is the mean of 4 animals.

phosphorus levels. Calcium and zinc supplements, alone and in combination, were associated with decreases in the amounts of phosphorus excreted in the urine. Supplements of phosphorus were associated with highly significant increases ($p \leq 0.01$) in urinary phosphorus. Although there was a marked increase in the amount of phosphorus intake in rats fed diets containing the phosphorus supplement, the marked increase in urinary phosphorus in rats receiving these diets was sufficient to result in a lower percentage of phosphorus retention.

Magnesium

The main effects of phosphorus and zinc were to decrease the amount of magnesium retained, while the main effect of calcium was to increase the amount of magnesium retained (Table 4). However, the percentage of the total magnesium intake retained was less in the animals supplemented with calcium, phosphorus, and zinc than in the animals receiving only the basal. Although calcium and zinc supplements added separately to the basal resulted in decreases in magnesium retention, the combination of both supplements was associated with an increase in the amount of magnesium retained by the animal. The presence of added dietary phosphorus was associated with decreases in the amounts of magnesium retained in all cases.

The addition of high levels of calcium, phosphorus, or zinc resulted in increases in the amount of magnesium

TABLE 4

MAGNESIUM BALANCE SUMMARY[a]

Diet	Mg Intake (mg./day)	Mg Excreted in (mg./day)		Mg Absorbed		Mg Retention	
		Feces	Urine	(mg./day)	(%)	(mg./day)	(%)
Basal	6.87	1.96	0.20	4.91	71.5	4.71	68.6
Basal + 0.8% Ca	7.37	4.08	0.14	3.29	44.6	3.15	42.7
Basal + 0.8% P	6.94	3.16	0.21	3.78	54.5	3.57	51.4
Basal + 0.8% Ca + 0.8% P	8.03	3.66	0.13	4.37	54.4	4.24	52.8
Basal + 0.75% Zn	6.20	2.73	0.19	3.47	56.0	3.28	52.9
Basal + 0.75% Zn + 0.8% Ca	8.29	3.41	0.37	4.88	58.9	4.51	54.4
Basal + 0.75% Zn + 0.8% P	6.53	3.79	0.18	2.74	42.0	2.56	39.2
Basal + 0.75% Zn + 0.8% Ca + 0.8% P	8.04	4.32	0.11	3.72	46.3	3.61	44.9

[a]Each figure is the mean of 4 animals.

excreted in the feces and corresponding decreases in the apparent absorption of magnesium. The main effect of calcium was to decrease urinary magnesium, but the main effects of phosphorus and zinc were to increase the excretion of magnesium in the urine.

Zinc

All animals which did not receive a high level of zinc were in a negative zinc balance since they were excreting more zinc than they were receiving from their respective diets (Table 5). When zinc was added to the diet, a highly significant increase ($p \leq 0.01$) in the apparent absorption and retention of zinc resulted. In the absence of zinc, calcium and phosphorus supplements were associated with slight improvements in the apparent absorption and retention of zinc. When calcium and phosphorus supplements were added to the high zinc diet there was an increase in the amount of zinc that was absorbed and retained.

Calcium and phosphorus supplements, in the presence of zinc, were associated with substantial increases in the fecal excretion of zinc. Although increases in the amount of zinc in the urine occurred in animals receiving each dietary supplement, the results indicated that zinc and calcium had a greater effect on urinary zinc than did phosphorus.

Copper

Although all animals in this study were in negative

TABLE 5

ZINC BALANCE SUMMARY[a]

Diet	Zn Intake (mg./day)	Zn Excreted in (mg./day)		Zn Absorbed		Zn Retention	
		Feces	Urine	(mg./day)	(%)	(mg./day)	(%)
Basal	0.039	0.710	0.029	- 0.671	-[b]	- 0.700	-
Basal + 0.8% Ca	0.113	0.067	0.100	0.460	40.7	- 0.054	-
Basal + 0.8% P	0.052	0.266	0.051	- 0.214	-	- 0.265	-
Basal + 0.8% Ca + 0.8% P	0.058	0.317	0.037	- 0.282	-	- 0.296	-
Basal + 0.75% Zn	45.423	21.625	0.123	23.798	52.4	23.675	52.1
Basal + 0.75% Zn + 0.8% Ca	81.707	37.542	0.188	44.165	54.0	43.977	53.8
Basal + 0.75% Zn + 0.8% P	62.480	14.100	0.094	48.380	77.4	48.286	77.3
Basal + 0.75% Zn + 0.8% Ca + 0.8% P	55.045	18.597	0.066	36.448	66.2	36.382	66.1

[a]Each figure is the mean of 4 animals.

[b]Negative percentage values are meaningless and have not been calculated.

copper balance (Table 6), the results indicate that zinc toxicity was associated with a marked increase in copper retention. In the absence of zinc, calcium added to the basal had no effect on copper retention, while phosphorus supplementation was associated with a decrease in copper retention. In combination, however, these supplements were associated with a marked improvement in copper retention. In the presence of zinc, calcium and phosphorus added separately to the diet were associated with an additional improvement in copper retention. However, when both supplements were added in combination to the high zinc diet, the improvement in copper retention was not as good as that observed when either supplement was added separately to the high zinc diet. These results indicate a significant calcium x phosphorus interaction with respect to copper metabolism.

Animals receiving the high zinc diets excreted less copper in the feces than did those not receiving supplemental levels of zinc, and the data indicated that zinc significantly improved the apparent absorption of copper. The main effect of calcium and phosphorus supplements was to improve the apparent absorption of copper by decreasing the fecal excretion of copper. Calcium and phosphorus supplements appeared to have the greatest effect on the urinary excretion of copper.

TABLE 6

COPPER BALANCE SUMMARY[a]

Diet	Cu Intake (mcg./day)	Cu Excreted in (mcg./day)		Cu Absorbed (mcg./day)	Cu Retention (mcg./day)
		Feces	Urine		
Basal	76.21	459.23	1.32	-383.02	-384.34
Basal + 0.8% Ca	70.89	436.59	5.29	-365.70	-370.99
Basal + 0.8% P	61.21	481.26	6.39	-420.05	-426.44
Basal + 0.8% Ca + 0.8% P	71.72	283.27	3.31	-211.55	-214.86
Basal + 0.75% Zn	45.96	88.71	1.54	- 42.75	- 44.29
Basal + 0.75% Zn + 0.8% Ca	57.20	79.44	2.06	- 22.24	- 24.30
Basal + 0.75% Zn + 0.8% P	57.43	60.36	0.97	- 2.93	- 3.90
Basal + 0.75% Zn + 0.8% Ca + 0.8% P	62.74	89.92	3.01	- 27.18	- 30.19

[a]Each figure is the mean of 4 animals.

Growth, Hemoglobin, and Liver Mineral Study

Detailed data showing the effects of calcium and phosphorus supplements on the weight gains, hemoglobin levels, and liver mineral deposition in the presence and absence of zinc are given in Appendix B, Tables 1-5. Mean values of these criteria are presented in Table 7.

Growth

A high level of dietary zinc resulted in a highly significant decrease ($p \leq 0.01$) in weight gain which could be partially alleviated with supplements of 0.8% calcium or 0.8% calcium plus 0.8% phosphorus. Supplements of 0.8% phosphorus did not prevent the subnormal growth associated with zinc toxicity in this study.

Hemoglobin

Analysis of the results indicated that a 0.75% level of dietary zinc was associated with a highly significant decrease ($p \leq 0.01$) in hemoglobin level. Supplements of calcium and phosphorus, in the absence of zinc, had no apparent effect on hemoglobin level. In the presence of zinc, only a combination of calcium and phosphorus supplement partially alleviated the adverse effect of zinc on hemoglobin levels.

Liver copper

The addition of 0.75% zinc to the diets of young rats resulted in a highly significant decrease ($p \leq 0.01$)

TABLE 7

EFFECTS OF CALCIUM, PHOSPHORUS, AND ZINC SUPPLEMENTS ON WEIGHT GAINS, HEMOGLOBIN LEVELS, AND LIVER COPPER, IRON, AND ZINC VALUES[a]

Level of Zinc (%)	Levels of Calcium and Phosphorus (%)			
Ca	0	0.8	0	0.8
P	0	0	0.8	0.8
	Weight gain at 4 weeks (gm.)			
0	169	167	164	162
0.75	126	138	125	149
	Hemoglobin (gm./100 ml blood)			
0	13.4	12.9	13.4	13.3
0.75	7.0	7.6	7.8	10.8
	Liver copper (mcg./gm. dry weight)			
0	10.2	12.3	11.4	12.5
0.75	3.6	3.6	2.8	6.3
	Liver iron (mcg./gm. dry weight)			
0	276.1	254.2	270.7	207.5
0.75	113.5	119.6	112.1	150.8
	Liver zinc (mcg./gm. dry weight)			
0	35.3	32.1	42.0	50.7
0.75	349.3	241.8	189.3	89.2

[a]Each figure is the mean of 8 animals.

in liver copper accumulation. In the absence of zinc, calcium and phosphorus supplements were associated with increases in liver copper deposition. These increases, however, were not statistically significant. The data also indicated that a supplement of 0.8% calcium plus 0.8% phosphorus partially prevented the adverse effect of zinc on liver copper.

Liver iron

The addition of 0.75% zinc to the diets of young rats resulted in a highly significant reduction ($p \leq 0.01$) in liver iron level. Although calcium and phosphorus supplements, either singly or in combination, did not significantly prevent the marked decrease in liver iron associated with zinc toxicity, there was some improvement in liver iron when both of these minerals were added to the high zinc diet. In the absence of zinc, calcium appeared to have a depressing effect on liver iron deposition when it was added to the diet. Phosphorus had no apparent effect on liver iron in the presence or absence of zinc when it was the only supplement added to the basal.

Liver zinc

A high level of dietary zinc resulted in an increase in liver zinc level which was significant at the 1% level of probability. When supplements of calcium were added to the high zinc diet, there was a significant reduction ($p \leq 0.05$) in liver zinc accumulation. Supplements of

phosphorus resulted in zinc levels which were significantly different from those of the zinc-fed rats at the 1% level of probability. A combination of calcium and phosphorus completely prevented the increase in liver zinc associated with zinc toxicosis because the mean zinc level of the zinc-fed animals receiving these minerals was not significantly different from the mean zinc level of the animals receiving only the basal diet.

CHAPTER V

GENERAL DISCUSSION

Results of this investigation indicate that the antagonistic effect of zinc on the normal excretion and retention of calcium and phosphorus in young rats can be alleviated with calcium and phosphorus supplements. Evaluation of the data suggests that the beneficial action of these supplements is two-fold. There is evidence that calcium and phosphorus supplements added to high zinc diets furnish sufficient amounts of these minerals to replace the amounts lost by the adverse action of zinc. The results of the zinc balance portion of the study showing a marked increase in fecal zinc levels in zinc-fed rats supplemented with calcium support the proposal of Stewart and Magee (17) that this supplement partially alleviated the adverse effect of zinc on the animal body by facilitating the removal of excess zinc from the system. The improvement in hemoglobin levels, the increase in liver copper and iron deposition, and the decrease in liver zinc accumulation in zinc-fed rats supplemented with calcium and phosphorus also support the thesis that part of the beneficial effect of these supplements is to remove excess zinc from the body. If the only action of the calcium and phosphorus supplements was to

replace those amounts lost by the adverse effect of zinc, one could reason that the excess zinc would still be present in the animal body and would continue to adversely affect copper and iron levels in the liver and hemoglobin concentrations. The data, however, suggest that both mechanisms are probably involved.

The antagonistic effect of zinc on calcium absorption and retention appears to involve a fairly simple mechanism in that a level of 0.8% calcium supplement completely alleviated the adverse effect of zinc toxicity on calcium metabolism. The adverse effect of zinc toxicity on phosphorus metabolism, however, seems to be more complex since a level of 0.8% of phosphorus did not result in an increase in phosphorus retention in zinc-fed rats. Although the apparent absorption of phosphorus was significantly increased in rats fed the high zinc diet supplemented with phosphorus, there was a corresponding increase in the urinary excretion of phosphorus which offset the increased apparent absorption of this mineral. This condition actually resulted in a decrease in the percentage of phosphorus retained by the animal.

In this study the addition of 0.8% of phosphorus to the basal resulted in an improvement in calcium retention. In the presence of zinc, however, phosphorus supplementation resulted in a negative calcium balance in the animals. These results indicate that the levels of dietary calcium

and phosphorus used in this study do affect the calcium balance of rats under certain conditions. Whiting and Bezeau (27) have reported that the apparent absorption and retention of calcium was not influenced by the addition of calcium to the diets of pigs. Miller et al. (28) have reported that dietary levels of phosphorus above 0.5% did not affect the calcium balance of pigs.

The effects of calcium and phosphorus supplementation, in the absence of zinc toxicity, on the apparent absorption and retention of phosphorus in rats under the conditions of this study were similar to those observed in the pig by Whiting and Bezeau (27) and Miller et al. (28). The presence of calcium prevented the marked decrease in the percentage of phosphorus retained associated with zinc toxicity better than did phosphorus supplementation. Even in the absence of zinc, the addition of phosphorus supplements resulted in greater decreases in the percentage of phosphorus retained than did the addition of calcium supplements.

Although both calcium and phosphorus supplementation improved the apparent absorption and retention of zinc in zinc-fed rats, the data indicate that phosphorus has a greater influence on zinc metabolism when rats are fed toxic levels of zinc. In the absence of extra zinc, calcium appeared to have a greater influence on zinc metabolism than did phosphorus.

The overall results of this investigation indicate that the levels of calcium, phosphorus, and zinc in the diets of young rats have marked influences on the excretion, apparent absorption, and retention of zinc. Forbes and Yohe (29), however, reported that the apparent absorption and urinary excretion of zinc in rats were not influenced by additional dietary calcium.

The results of this study furnish additional support for a definite calcium and zinc interrelationship in the rat and indicate the existence of a phosphorus and zinc interrelationship. However, the overall results of this study suggest a combined interrelationship involving calcium, phosphorus, and zinc instead of two separate and distinct interrelationships. The data indicate that the dietary levels of all three of these minerals have a significant influence on the requirement of any one element, as far as the rat is concerned, and that all three minerals should be considered when evaluating the dietary adequacy of an experimental ration with respect to calcium, phosphorus, and zinc.

The effect of zinc toxicity on the apparent absorption and retention of magnesium was similar to that reported by Stewart and Magee (17). The results also indicate that calcium supplements can alleviate the adverse effect of zinc toxicity on the retention of magnesium by young rats. The adverse effects of calcium or phosphorus

supplements, in the absence of zinc, on the apparent absorption of magnesium by the rat were in agreement with those observed by Toothill (30). Presumably, the beneficial effect of supplemental calcium on the metabolism of magnesium in zinc-fed rats is to remove excess zinc from the body so that it is not present to interfere with the normal metabolism of magnesium.

The results of the copper balance was surprising because the adverse effect of zinc toxicity on copper metabolism has been well documented by numerous reports. The results of this study, however, indicate that zinc toxicity actually improves the apparent absorption and retention of copper. The retention of copper was increased even more when extra phosphorus was added to the high zinc diet.

The effects of the calcium and phosphorus supplements on the weight gains of zinc-fed rats were similar to results reported by Stewart and Magee (17). Addition of an 0.8% level of calcium or phosphorus supplement to the diets of animals receiving no extra zinc did not result in any apparent abnormalities in growth since the mean weight gains of the animals receiving the basal supplemented with calcium and/or phosphorus were essentially the same as the mean weight gain of the control animals.

The decrease in liver iron level associated with the addition of 0.8% calcium to the basal supports the idea that a calcium and iron interrelationship exists in the

animal body. Moore et al. (31), however, have emphasized that the interrelationship between calcium and iron is probably non-specific and is presumably typical of many similar interrelationships in mineral metabolism. Results of this study also indicate that phosphorus may have an influence on this calcium and iron interrelationship.

The results of this investigation showing that calcium and phosphorus supplements could prevent the marked accumulation of zinc in the liver paralleled very closely the results observed by Stewart and Magee (17) in connection with bone zinc levels. The data of this study also indicate that phosphorus has a greater influence on liver zinc deposition than does calcium.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

An investigation was conducted to determine the effect of high levels of dietary zinc in the presence and absence of calcium and phosphorus supplements on the growth and the metabolism of calcium, phosphorus, magnesium, copper, iron, and zinc in young rats. Criteria used as measurements of the responses of animals maintained on the various dietary regimens were the levels of calcium, phosphorus, magnesium, copper, and zinc retained by the animals; weight gain; hemoglobin level; and liver copper, iron, and zinc levels.

This study was conducted in two parts. In the first part of the study, a metabolism experiment was conducted to determine the effects of high levels of zinc on the apparent absorption, the utilization, and the retention of calcium, phosphorus, magnesium, copper, and zinc in the presence and absence of supplemental levels of calcium and phosphorus. The effects of calcium and phosphorus supplements on the weight gains; hemoglobin levels; and the deposition of copper, iron, and zinc in the livers of zinc-fed rats were studied in the second part of the investigation.

Results of the study revealed that zinc toxicity resulted in highly significant decreases in the retention of calcium which could be prevented with calcium supplements. Calcium supplementation resulted in marked improvements in calcium retention. Phosphorus supplementation caused an increase in calcium retention in the absence of zinc, but zinc-fed rats supplemented with added phosphorus were found to be in a negative calcium balance.

Calcium and zinc supplementation resulted in decreases in the amount of phosphorus retained, but phosphorus supplementation was associated with an increase in the total phosphorus retained. All three supplements, however, were associated with decreases in the percentage of phosphorus intake that was actually retained. Calcium supplementation resulted in a better percentage of phosphorus retention than did supplements of phosphorus.

The percentage of total magnesium intake retained was lower in animals supplemented with calcium, phosphorus, and zinc than in animals receiving only the basal diet. The decrease in magnesium retention was associated primarily with increases in the amounts of magnesium excreted in the feces.

Zinc toxicity resulted in a highly significant increase in the retention and the apparent absorption of zinc. The addition of calcium and phosphorus supplements to the high zinc diet resulted in additional increases in

zinc retention and the percentage of total zinc intake that was retained. In the absence of zinc, a supplement of calcium resulted in a significant increase in the apparent absorption of zinc, but the amount absorbed was not sufficient to overcome the loss of zinc in the urine associated with calcium supplementation.

Although all animals maintained on the various dietary regimens used in this study were in negative copper balances, the presence of high levels of dietary zinc significantly improved copper retention. In general, calcium supplementation improved copper retention, while supplements of phosphorus, in the absence of calcium, showed a tendency to decrease copper retention. The improvement in copper retention in zinc-fed rats was primarily associated with a significant decrease in the fecal excretion of copper.

Results of this study showed that a high level of dietary zinc decreased weight gains, hemoglobin levels, liver copper and iron levels and increased liver zinc levels. A combination of calcium plus phosphorus supplement partially alleviated the adverse effect of zinc on weight gain, hemoglobin level, and liver copper accumulation. Supplements of these two minerals also prevented the marked increase in liver zinc deposition associated with zinc toxicity.

The results of this study indicate that an interrelationship between calcium, phosphorus, and zinc exists

in the animal body. Some of the data also indicate that all three of these minerals should be considered when evaluating the dietary adequacy of an experimental ration with respect to calcium, phosphorus, and zinc.

Recommendations for Additional Investigations

Results of this study substantiate the proposed thesis that an interrelationship exists between phosphorus and zinc in the animal body. The mechanism(s) involving the interference of phosphorus metabolism by zinc, however, may be more complexed than the one involving the interference with calcium metabolism. Although calcium supplementation prevented the decrease in the total percentage of calcium retained associated with zinc toxicity, phosphorus supplementation did not prevent the adverse effect of high levels of zinc on the percentage of phosphorus retained by the animal. Additional information is needed to clarify the means whereby zinc interferes with phosphorus metabolism.

The effect of zinc on phosphorus metabolism may be mediated through the inactivation or inhibition of some of the important energy transferring or transporting enzyme systems of the animal body because these systems require phosphorus. Zinc has been shown to interfere with various phosphatase enzymes, and there is the possibility that this mineral could interfere or inhibit other phosphorus-dependent enzymes such as kinases, phosphorylases, or pyrophosphorylases.

Although the results of this study indicate that

zinc interferes primarily with the absorption of magnesium, there is the possibility that zinc also interferes with enzymes which require magnesium as a cofactor. Many of the enzymes requiring magnesium are involved with energy transfer and with various stages in the pathways connected with the metabolism of carbohydrates, lipids, and proteins. If zinc toxicity results in a magnesium deficiency condition, then it is possible that certain enzymes which require magnesium for activation would be adversely affected by high levels of dietary zinc.

A decrease in liver zinc accumulation and an increase in the apparent absorption and retention of zinc resulted when calcium and phosphorus supplements were added to the high zinc diet. These findings prompt the intriguing question of where is the excess zinc accumulating in the animal body under the experimental conditions of this study. There is the possibility that zinc is accumulating in other organs of the body. Additional studies are needed to determine the exact fate of the excess zinc which is present under the conditions utilized in this study.

Previous results have indicated that zinc interferes with copper metabolism. Results of this study, however, indicate that although zinc may interfere with certain phases of copper metabolism it may also improve the apparent absorption and retention of copper. Additional studies are needed to clarify these supposedly contradictory findings.

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APPENDIX A

MINERAL BALANCE DATA

TABLE 1

MINERAL ANALYSES OF DIETS USED IN THE STUDY

Diets

1 - Control
2 - 0.8% Ca
3 - 0.8% P
4 - 0.8% Ca + 0.8% P
5 - 0.75% Zn
6 - 0.75% Zn + 0.8% Ca
7 - 0.75% Zn + 0.8% P
8 - 0.75% Zn + 0.8% Ca + 0.8% P

Diets	Zn[a]	Ca[a]	P[a]	Mg[b]	Cu[b]
1	0.003	5.12	5.46	0.52	5.83
2	0.008	20.32	5.30	0.52	5.00
3	0.004	6.26	13.46	0.53	4.67
4	0.004	20.00	13.06	0.55	4.91
5	4.447	6.04	5.99	0.61	4.50
6	6.200	17.09	5.66	0.63	4.34
7	5.554	6.48	14.61	0.58	5.11
8	3.834	16.20	13.08	0.56	4.37

[a]Expressed as mg./gm. of diet on a fresh weight basis.

[b]Expressed as mcg./gm. of diet on a fresh weight basis.

TABLE 2

CALCIUM BALANCE DATA

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
		Total amount per collection period (mg.)							
Ca intake	1	373.76	2072.64	619.74	2040.00	462.77	1623.55	492.48	1458.00
	2	506.88	2113.28	494.54	2040.00	516.86	1760.27	615.60	1668.60
	3	501.76	2052.32	594.70	2040.00	354.59	1230.48	473.04	1684.80
	4	491.52	1828.80	1588.44	2060.00	384.64	1213.39	641.52	1701.00
Total		1873.82	8067.04	2297.42	8180.00	1718.86	5827.69	2222.64	6512.40
Mean		468.46	2016.76	574.36	2045.00	429.72	1456.92	555.66	1628.10
Fecal Ca	1	239.11	892.17	269.66	435.19	426.25	986.83	435.19	1000.15
	2	250.50	925.66	196.48	797.60	396.99	1181.37	528.66	636.56
	3	293.48	762.63	258.29	617.46	316.24	791.82	311.70	1170.51
	4	308.63	1381.32	266.76	658.96	407.48	489.43	1204.84	866.64
Total		1091.72	3961.78	991.19	2509.21	1546.96	3449.45	2480.39	3673.86
Mean		272.93	990.45	247.80	627.30	386.74	862.36	620.10	918.47

TABLE 2--Continued

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
Urinary Ca	1	1.27	3.26	11.58	15.51	2.61	0.95	6.40	4.04
	2	4.53	15.28	6.67	4.06	3.74	2.03	11.08	7.24
	3	2.78	4.43	7.14	13.45	1.20	1.49	8.02	4.51
	4	5.62	9.31	19.59	23.97	14.38	7.50	32.03	16.75
Total		14.20	32.28	44.98	56.99	21.93	11.97	57.53	32.54
Mean		3.55	8.07	11.25	14.25	5.48	2.99	14.38	8.14
Ca retention									
	1	133.38	1177.21	338.50	1589.30	33.91	635.77	50.89	453.81
	2	251.85	1172.34	291.39	1238.34	116.13	576.87	75.86	1024.80
	3	205.50	1285.26	329.27	1409.09	37.15	437.17	153.32	509.78
	4	177.27	438.17	302.09	1377.07	-37.22	716.46	-396.35	817.61
Total		768.00	4072.98	1261.25	5613.80	149.97	2366.27	-316.28	2806.00
Mean		192.00	1018.25	315.31	1403.15	37.49	591.57	- 79.09	701.50

TABLE 3

PHOSPHORUS BALANCE DATA

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
		Total amount per collection period (mg.)							
P intake	1	398.58	540.60	1332.54	1332.12	461.23	537.70	1110.36	1177.20
	2	540.54	551.20	1063.34	1332.21	515.40	582.98	1387.95	1347.24
	3	535.08	535.30	1278.70	1332.12	353.41	407.52	1066.53	1360.32
	4	524.16	447.10	1265.24	1345.18	383.36	560.34	1037.31	1373.40
Total		1998.36	2074.10	4939.82	5341.54	1713.40	2088.54	4602.15	5258.16
Mean		499.59	518.53	1234.96	1335.38	428.35	522.14	1150.14	1314.54
Fecal P	1	97.95	320.00	160.78	291.56	255.15	333.31	344.00	331.84
	2	77.56	267.98	106.87	380.13	288.84	419.95	339.29	578.31
	3	94.42	328.66	131.31	272.80	182.75	231.00	201.50	526.60
	4	122.70	298.77	218.61	494.22	251.54	274.44	444.77	602.95
Total		392.53	1206.41	617.57	1438.71	918.28	1258.70	1329.56	2039.70
Mean		98.16	301.60	154.39	359.68	229.57	314.68	332.39	509.93

TABLE 3--Continued

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
Urinary P	1	11.76	14.08	487.45	662.53	62.77	18.05	584.26	331.97
	2	182.17	45.89	634.27	355.16	83.71	140.20	984.36	501.56
	3	228.01	11.38	719.25	464.89	68.19	20.25	444.57	482.14
	4	178.60	6.14	861.47	579.25	250.50	99.14	380.45	398.90
Total		600.54	77.49	2702.44	2061.83	465.17	277.64	2393.64	1714.57
Mean		150.14	19.37	675.61	515.47	116.29	69.41	598.41	428.64
P retention									
	1	288.87	206.52	684.31	378.03	143.31	186.34	182.10	513.39
	2	280.81	237.33	322.20	596.83	202.85	22.83	64.30	267.37
	3	212.65	195.26	428.14	594.43	102.47	156.27	420.46	351.58
	4	222.80	181.09	185.16	271.71	-118.68	186.76	212.09	371.55
Total		1005.13	820.20	1619.81	1841.00	329.95	552.20	878.95	1503.89
Mean		251.28	205.05	404.95	460.25	82.49	138.05	219.74	375.97

TABLE 4

MAGNESIUM BALANCE DATA

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
		Total amount per collection period (mcg.)							
Mg intake	1	40.04	53.04	52.47	56.10	46.97	59.85	44.08	50.40
	2	51.48	54.08	41.87	56.10	52.46	65.92	55.10	57.68
	3	50.96	52.52	50.35	56.10	35.99	46.08	42.43	58.24
	4	49.92	46.80	49.82	56.65	39.04	60.36	41.18	58.80
Total		192.40	206.44	194.51	224.95	174.46	232.21	182.70	225.12
Mean		48.10	51.61	48.63	56.24	43.62	58.05	45.68	56.28
Fecal Mg	1	11.81	26.43	23.02	22.53	20.83	30.60	22.83	26.76
	2	10.38	23.98	19.81	28.39	21.64	30.87	35.19	38.13
	3	14.80	30.62	21.61	16.29	12.81	16.82	15.38	34.66
	4	17.84	33.14	24.17	35.19	21.29	17.10	32.61	21.33
Total		54.83	114.17	88.61	102.40	76.57	95.39	106.01	120.88
Mean		13.71	28.54	22.15	25.60	19.14	23.85	26.50	30.22

TABLE 4--Continued

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0.75	0.75	0.75	0.75	0.75
Urinary Mg	1	1.45	0.59	0.64	0.59	0.67	0.18	0.99	0.72
	2	0.75	1.71	0.90	0.53	0.65	0.18	1.68	0.97
	3	0.73	0.74	0.72	1.73	1.09	0.14	1.41	0.57
	4	2.53	0.88	3.67	0.31	2.92	9.78	1.03	0.88
Total		5.46	3.92	5.93	3.16	5.33	10.28	5.11	3.14
Mean		1.36	0.98	1.48	0.79	1.33	2.57	1.28	0.79
Mg retention									
	1	26.78	26.02	28.81	32.98	25.47	29.07	20.26	22.92
	2	40.35	28.39	21.16	27.18	30.17	34.87	18.23	18.58
	3	35.43	21.16	28.02	38.08	32.09	29.12	25.55	23.01
	4	29.55	12.78	21.98	21.15	14.83	33.39	7.54	36.59
Total		132.11	88.35	99.97	119.39	102.56	126.45	71.58	101.10
Mean		33.03	22.09	24.99	29.85	25.64	31.61	17.90	25.28

TABLE 5

ZINC BALANCE DATA

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
		Total amount per collection period (mg.)							
Zn intake	1	0.219	0.816	0.396	0.408	342.419	589.000	422.104	345.060
	2	0.297	0.832	0.316	0.408	382.442	638.600	527.630	394.902
	3	0.294	0.808	0.380	0.408	262.373	446.400	405.422	398.736
	4	0.288	0.720	0.376	0.412	284.608	613.800	394.334	402.570
Total		1.098	3.176	1.468	1.636	1271.842	2287.800	1749.510	1541.268
Mean		0.275	0.794	0.367	0.409	317.961	571.950	437.378	385.317
Fecal Zn	1	0.306	0.612	0.329	0.555	227.753	289.995	131.149	147.329
	2	0.277	0.318	0.258	0.312	174.044	356.042	35.898	151.278
	3	0.582	0.487	0.352	0.720	46.900	54.488	113.265	136.019
	4	18.723	0.472	6.517	7.311	156.815	350.673	114.351	86.093
Total		19.888	1.889	7.456	8.898	605.512	1051.198	394.663	520.719
Mean		4.972	0.472	1.864	2.225	151.378	268.800	98.666	130.180

TABLE 5--Continued

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
Urinary Zn	1	0.034	0.024	0.090	0.008	0.340	0.908	0.217	0.148
	2	0.227	0.321	0.129	0.084	0.606	0.325	0.594	0.211
	3	0.045	0.182	0.156	0.237	0.452	0.230	0.192	0.788
	4	0.498	2.228	1.050	0.709	2.068	3.787	1.623	0.702
Total		0.804	2.807	1.425	1.038	3.446	5.250	2.626	1.844
Mean		0.201	0.702	0.356	0.259	0.862	1.312	0.657	0.462
Zn	1	- 0.121	0.180	-0.023	-0.155	114.326	298.097	290.738	197.583
	2	- 0.157	-0.193	-0.071	0.012	207.792	282.233	491.138	243.413
	3	- 0.333	0.139	-0.128	-0.545	215.021	391.682	291.985	261.929
	4	-18.933	-2.032	-7.191	-7.608	125.745	259.340	278.360	315.776
Total		-19.544	-1.520	-7.413	-8.296	662.884	1231.352	1352.221	1018.701
Mean		- 4.886	-0.380	-1.853	-2.074	165.721	307.838	338.055	254.675

TABLE 6

COPPER BALANCE DATA

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
		Total amount per collection period (mcg.)							
Cu intake	1	425.59	510.00	462.33	500.82	346.50	412.30	388.36	393.30
	2	577.17	520.00	368.93	500.82	387.00	447.02	485.45	450.11
	3	571.34	505.00	443.65	500.82	265.50	312.48	373.03	454.48
	4	559.68	450.00	438.98	505.73	288.00	429.66	362.81	458.85
Total		2133.78	1985.00	1713.89	2008.19	1287.00	1601.46	1609.65	1756.74
Mean		533.45	496.25	428.47	502.05	321.75	400.37	402.41	439.19
Fecal Cu	1	2551.99	5226.61	3466.04	3074.81	1398.10	622.16	513.10	749.76
	2	3149.96	3422.11	2775.10	2857.93	363.63	586.99	478.45	499.89
	3	3186.87	485.04	3751.10	186.26	353.42	363.14	356.01	725.13
	4	3969.48	3090.79	3483.06	1812.62	368.78	652.07	344.67	543.07
Total		12858.30	12224.55	13475.30	7931.62	2483.93	2224.36	1692.23	2517.85
Mean		3214.58	3056.14	3368.83	1982.91	620.98	556.09	423.06	629.46

TABLE 6--Continued

Item	Rep.	Supplement (per cent added to diet)							
		Ca 0	0.8	0	0.8	0	0.8	0	0.8
		P 0	0	0.8	0.8	0	0	0.8	0.8
		Zn 0	0	0	0	0.75	0.75	0.75	0.75
Urinary Cu	1	7.11	25.19	45.92	43.59	3.04	11.95	2.10	29.00
	2	8.29	83.79	103.43	12.00	2.53	-	4.10	4.35
	3	8.57	11.10	14.69	11.32	16.78	9.76	10.68	26.04
	4	12.92	28.12	11.99	25.65	20.83	21.62	10.35	24.75
Total		36.89	148.20	179.03	92.56	43.18	43.33	27.23	84.14
Mean		9.22	37.05	44.76	23.14	10.79	14.44	6.81	21.04
Cu retention	1	-2133.51	-4741.80	-3049.63	-2617.58	-1054.64	-221.81	-126.83	-385.46
	2	-2581.08	-2985.90	-2509.60	-2369.11	20.84	-139.97	2.90	- 54.13
	3	-2624.10	8.86	-3322.14	- 303.24	- 104.70	- 60.42	6.34	-296.69
	4	-3422.72	-2668.91	-3059.07	-1332.54	- 101.61	-244.03	7.79	-108.97
Total		-10761.41	-10387.75	-11940.44	-6015.99	-1240.11	-666.23	-109.80	-845.25
Mean		-2690.35	-2596.94	-2974.11	-1504.00	- 310.63	-166.56	- 27.45	-211.31

APPENDIX B

GROWTH, HEMOGLOBIN, AND LIVER MINERAL DATA

TABLE 1

GROWTH DATA

Treatments

1 - Control
2 - 0.8% Ca
3 - 0.8% P
4 - 0.8% Ca + 0.8% P
5 - 0.75% Zn
6 - 0.75% Zn + 0.8% Ca
7 - 0.75% Zn + 0.8% P
8 - 0.75% Zn + 0.8% Ca + 0.8% P

Rep.	Treatments 1	2	3	4	5	6	7	8
	4 weeks weight gain (gm.)							
1	115	115	197	197	157	165	156	146
2	207	205	151	178	173	201	162	171
3	191	204	200	186	109	131	112	177
4	174	124	159	184	124	170	115	178
5	163	174	139	154	126	117	98	115
6	162	168	161	147	120	116	96	146
7	163	160	143	156	118	120	92	122
8	173	183	161	98	80	80	70	138
Total	1348	1333	1311	1300	1007	1100	900	1193
Mean	169	167	164	162	126	138	112	149

TABLE 2

HEMOGLOBIN DATA

Treatments

1 - Control
2 - 0.8% Ca
3 - 0.8% P
4 - 0.8% Ca + 0.8% P
5 - 0.75% Zn
6 - 0.75% Zn + 0.8% Ca
7 - 0.75% Zn + 0.8% P
8 - 0.75% Zn + 0.8% Ca + 0.8% P

Rep.	Treatments 1	2	3	4	5	6	7	8
	mg./100 ml. blood							
1	12.86	13.66	14.43	14.55	7.77	7.37	9.83	11.14
2	13.66	14.43	11.37	13.95	6.80	7.37	9.29	14.23
3	14.35	13.66	14.86	13.29	8.54	6.23	8.00	12.09
4	14.86	13.03	15.57	14.55	8.43	8.54	7.14	11.92
5	13.29	13.12	13.40	12.69	5.74	7.48	8.18	9.29
6	12.34	12.00	12.60	12.34	5.06	9.72	7.42	8.60
7	12.77	12.09	12.69	13.03	6.08	6.92	7.09	8.66
8	12.77	11.46	12.52	12.26	7.65	7.20	5.54	10.26
Total	106.90	103.45	107.44	106.66	56.07	60.83	62.49	86.19
Mean	13.40	12.93	13.43	13.33	7.01	7.60	7.81	10.77

TABLE 3

LIVER COPPER DATA

Treatments

1 - Control
2 - 0.8% Ca
3 - 0.8% P
4 - 0.8% Ca + 0.8% P
5 - 0.75% Zn
6 - 0.75% Zn + 0.8% Ca
7 - 0.75% Zn + 0.8% Ca + 0.8% P
8 - 0.75% Zn + 0.8% Ca + 0.8% P

Rep.	Treatments 1	2	3	4	5	6	7	8
	mcg./gm. dry weight							
1	9.51	13.57	17.09	13.90	5.18	6.34	6.57	4.70
2	14.73	2.82	18.44	14.40	3.89	3.82	3.20	11.26
3	5.85	11.86	3.20	12.00	5.03	6.10	4.38	10.43
4	6.09	13.68	1.42	13.93	6.54	3.27	3.59	8.88
5	12.68	-	15.87	10.46	2.14	1.25	1.05	2.59
6	8.96	20.59	12.04	11.13	2.02	4.14	2.43	2.91
7	11.93	11.54	10.73	8.74	1.75	1.12	0.48	2.30
8	11.66	11.66	12.24	15.27	2.20	2.44	0.65	7.29
Total	84.41	85.82	91.03	99.83	28.75	28.48	22.35	50.36
Mean	10.18	12.26	11.38	12.48	3.59	3.56	2.79	6.30

TABLE 4

LIVER IRON DATA

Treatments

1 - Control	5 - 0.75% Zn
2 - 0.8% Ca	6 - 0.75% Zn + 0.8% Ca
3 - 0.8% P	7 - 0.75% Zn + 0.8% P
4 - 0.8% Ca + 0.8% P	8 - 0.75% Zn + 0.8% Ca + 0.8% P

	Treatments							
Rep.	1	2	3	4	5	6	7	8
	mcg./gm. dry weight							
1	216.20	209.34	234.83	218.31	85.67	126.99	111.96	150.62
2	314.85	331.45	280.98	175.43	141.31	110.06	100.18	173.67
3	283.45	346.35	268.34	256.06	146.30	165.40	123.57	203.52
4	269.47	287.10	219.98	167.68	117.37	98.10	147.46	179.90
5	289.41	178.74	362.63	235.54	152.93	112.41	109.04	102.49
6	216.96	208.26	310.29	244.07	90.70	103.25	96.71	146.93
7	323.97	324.38	276.35	198.24	106.61	139.22	97.34	131.52
8	294.57	148.20	211.81	165.03	120.96	108.61	110.28	117.60
Total	2208.88	2033.82	2165.21	1660.36	961.85	964.03	896.54	1206.25
Mean	176.91	254.23	270.65	207.55	120.23	120.50	112.07	150.78

TABLE 5

LIVER ZINC DATA

Treatments

1 - Control	5 - 0.75% Zn
2 - 0.8% Ca	6 - 0.75% Zn + 0.8% Ca
3 - 0.8% P	7 - 0.75% Zn + 0.8% P
4 - 0.8% Ca + 0.8% P	8 - 0.75% Zn + 0.8% Ca + 0.8% P

	Treatments							
Rep.	1	2	3	4	5	6	7	8
	mcg./gm. dry weight							
1	21.4	24.9	26.9	36.5	265.3	227.1	137.3	131.2
2	19.7	25.3	21.1	31.5	300.0	91.5	92.6	69.9
3	23.0	53.1	28.5	43.9	-	127.9	81.1	82.4
4	-	18.2	39.8	22.8	172.0	84.2	98.5	75.4
5	47.5	34.4	53.1	77.5	409.0	350.0	217.1	99.5
6	53.2	49.6	83.4	83.4	436.2	373.1	330.6	41.3
7	44.8	25.5	51.7	56.7	433.1	263.6	258.1	102.9
8	37.6	24.6	31.1	53.6	429.6	416.4	291.0	111.3
Total	247.2	256.4	355.7	405.9	2445.2	1934.2	1506.4	713.9
Mean	35.3	32.1	42.0	50.7	349.3	241.8	189.3	89.2

APPENDIX C

ANALYSES OF VARIANCE

TABLE 1

ANALYSES OF VARIANCE OF RETENTION DATA
FOR EACH MINERAL STUDIED

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Calcium	
Total	31	8,390,759.67	
Replications	3	172,644.14	57,548.05
Treatments	7	7,231,592.14	1,033,084.59**
Zn	1	1,407,032.48	1,407,032.48**
Ca	1	5,278,089.85	5,278,089.85**
P	1	125,945.53	125,945.53**
ZnCa	1	168,047.78	168,047.78**
ZnP	1	132,688.47	132,688.47**
CaP	1	119,261.18	119,261.18**
ZnCaP	1	626.49	626.49
Error	21	172,644.14	8,221.15
		Phosphorus	
Total	31	894,558.19	
Replications	3	89,283.49	29,761.16
Treatments	7	500,970.66	71,567.24**
Zn	1	127,657.73	127,657.73**
Ca	1	24,390.12	24,390.12
P	1	307,361.32	307,361.32**
ZnCa	1	20,550.23	20,550.23
ZnP	1	567.76	567.76
CaP	1	20,442.92	20,442.92
ZnCaP	1	0.37	0.37
Error	21	304,304.04	14,490.67

TABLE 1--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Magnesium	
Total	31	1,713.10	
Replications	3	157.26	52.42
Treatments	7	744.91	106.42*
Zn	1	74.24	74.24
Ca	1	47.82	47.82
P	1	70.15	70.15
ZnCa	1	240.35	240.35*
ZnP	1	64.07	64.07
CaP	1	107.90	107.90
ZnCaP	1	142.38	142.38
Error	21	810.93	38.61
		Zinc	
Total	31	703,249.34	
Replications	3	9,497.09	3,165.70
Treatments	7	646,874.84	92,410.69**
Zn	1	578,331.57	578,331.57**
Ca	1	1,985.90	1,985.90
P	1	7,261.36	7,261.36
ZnCa	1	1,482.50	1,482.50
ZnP	1	6,942.28	6,942.28
CaP	1	26,501.49	26,501.49**
ZnCaP	1	24,369.75	24,369.75**
Error	21	48,877.41	2,232.26

TABLE 1--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Copper	
Total	31	65.38	
Replications	3	4.27	1.42
Treatments	7	46.89	6.73**
Zn	1	41.10	41.10**
Ca	1	1.17	1.17
P	1	0.54	0.54
ZnCa	1	1.30	1.30
ZnP	1	0.16	0.16
CaP	1	0.56	0.56
ZnCaP	1	1.48	1.48
Error	21	14.22	0.68

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).

TABLE 2

ANALYSES OF VARIANCE OF FECAL EXCRETION DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Calcium	
Total	31	3,586,241.37	
Replications	3	81,825.04	27,275.01
Treatments	7	2,389,851.89	369,617.30**
Zn	1	210,723.83	210,723.83
Ca	1	1,750,339.21	1,750,339.21**
P	1	4,882.20	4,882.20
ZnCa	1	52,174.19	52,174.19
ZnP	1	229,662.37	229,662.37*
CaP	1	132,749.01	132,749.01
ZnCaP	1	12,921.09	12,921.09
Error	21	1,114,564.44	53,074.50
		Phosphorus	
Total	31	597,686.48	
Replications	3	37,956.43	12,655.14
Treatments	7	458,234.10	65,462.01**
Zn	1	111,738.01	111,738.01**
Ca	1	225,370.52	225,370.52**
P	1	85,029.66	85,029.66**
ZnCa	1	10,671.51	10,671.51
ZnP	1	16,929.38	16,929.38
CaP	1	4,443.18	4,443.18
ZnCaP	1	4,103.50	4,103.50
Error	21	101,487.01	4,832.71

TABLE 2--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Magnesium	
Total	31	1,575.25	
Replications	3	156.86	52.29
Treatments	7	801.83	114.55*
Zn	1	47.14	47.14
Ca	1	356.58	356.58**
P	1	184.99	184.99*
ZnCa	1	48.61	48.61
ZnP	1	33.87	33.87
CaP	1	76.57	76.57
ZnCaP	1	54.08	54.08
Error	21	758.58	36.12
		Zinc	
Total	31	348,189.31	
Replications	3	15,395.17	5,131.72
Treatments	7	261,395.17	37,408.75**
Zn	1	200,654.99	200,654.99**
Ca	1	9,632.20	9,632.20
P	1	17,425.74	17,425.74*
ZnCa	1	10,815.49	10,815.49
ZnP	1	16,923.63	16,923.63*
CaP	1	2,816.04	2,816.04
ZnCaP	1	3,592.90	3,592.90
Error	21	70,932.89	3,377.76

TABLE 2--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Copper	
Total	31	66.92	
Replications	3	3.73	1.24
Treatments	7	49.37	7.05**
Zn	1	45.42	45.42**
Ca	1	0.83	0.83
P	1	0.43	0.43
ZnCa	1	1.20	1.20*
ZnP	1	0.22	0.22
CaP	1	0.36	0.36
ZnCaP	1	0.93	0.93*
Error	21	3.73	0.18

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).

TABLE 3

ANALYSES OF VARIANCE OF WEIGHT GAIN, HEMOGLOBIN, LIVER COPPER, LIVER IRON, AND LIVER ZINC DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Weight gain	
Total	63	95,542.75	
Replications	7	19,835.75	2,833.65
Treatments	7	24,719.25	3,531.32**
Zn	1	18,632.25	18,632.25**
Ca	1	2,025.25	2,025.25
P	1	110.25	110.25
ZnCa	1	2,652.25	2,652.25
ZnP	1	49.00	49.00
CaP	1	650.00	650.00
ZnCaP	1	600.25	600.25
Error	49	30,987.75	632.40
		Hemoglobin	
Total	63	555.17	
Replications	7	34.30	4.90
Treatments	7	463.67	66.24**
Zn	1	394.37	394.37**
Ca	1	9.17	9.17**
P	1	19.72	19.72**
ZnCa	1	16.70	16.70**
ZnP	1	12.28	12.28**
CaP	1	7.30	7.30
ZnCaP	1	4.14	4.14
Error	49	57.19	1.17

TABLE 3--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
		Liver copper	
Total	62	1,647.90	
Replications	7	71.01	10.14
Treatments	7	969.01	138.43**
Zn	1	891.10	891.10**
Ca	1	41.59	41.59
P	1	3.11	3.11
ZnCa	1	12.66	12.66
ZnP	1	0.25	0.25
CaP	1	1.69	1.69
ZnCaP	1	18.61	18.61
Error	48	607.88	12.66
		Liver iron	
Total	63	382,652.69	
Replications	7	2,312.36	330.34
Treatments	7	285,230.25	40,747.18**
Zn	1	254,974.50	254,974.50**
Ca	1	2,116.23	2,116.23
P	1	901.65	901.65
ZnCa	1	15,369.80	15,369.80**
ZnP	1	5,513.81	5,513.81
CaP	1	7.74	7.74
ZnCaP	1	6,346.51	6,346.51
Error	49	95,110.08	1,941.02

TABLE 3--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Liver zinc	
Total	63	1,017,473.37	
Replications	7	143,876.49	20,553.78
Treatments	7	666,073.04	95,153.29**
Zn	1	454,141.21	454,141.21**
Ca	1	23,893.43	23,893.43**
P	1	60,417.64	60,417.64**
ZnCa	1	32,492.25	32,492.25**
ZnP	1	95,552.85	95,552.85**
CaP	1	304.50	304.50
ZnCaP	1	1,821.15	1,821.15
Error	49	207,523.84	4,235.18

*Significant ($p \leq 0.05$)

**Highly significant ($p \leq 0.01$)