THE EFFECT OF ZINC TOXICITY ON THE CALCIUM, PHOSPHORUS, AND MAGNESIUM CONTENT OF BONE AND BLOOD IN YOUNG RATS

by

Alta Kennedy Stewart

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Approved by

Allen C. Magee
Director

APPROVAL SHEET

This thesis has been approved by the following committee of the Faculty of the Graduate School at the Woman's College of the University of North Carolina, Greensboro, North Carolina.

Aden C. Magee
Thesis Director

Oral Examination
Committee Members

J. Sandra Sparks
Naomi G. Wanner
Marguerite N. Felton
F. V. Sperry

June 22, 1962
Date of Examination

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CHAPTER I

INTRODUCTION

Aristotle believed the food of plants and animals to be composed of four "essential elements"--earth, air, fire and water. While our present day concepts of nutrition differ radically from such early beliefs, information in this branch of science is by no means complete. One area where information is lacking is that of the trace elements. Over the last hundred years, and especially in the last thirty years, a steadily increasing number of minerals, occurring in minute amounts in the animal body, have had definite physiological roles assigned to them. Two different kinds of investigations have advanced out understanding of the role of these minerals: (1) the investigation of various naturally occurring diseases of man and animal shown to be due to a dietary deficiency, or excess, of a particular trace element and (2) the investigation of the effects on animals of specially constituted diets, deliberately designed to have an abnormally low, or high, content of the trace mineral under study.

Of the twenty-two trace elements known at the present time, six have been shown to be essential for the maintenance of the higher forms of animal life. Zinc is one of these six. It was early discovered in plant and animal tissue. Its universal occurrence in nature, frequently

in concentrations approaching that of iron, has established its fundamental importance to all living cells and has stimulated research into the specific physiological functions with which it is concerned. Its influence on growth, its presence in various enzyme systems and the discovery of biochemical lesions resulting from its deficiency or excess, have all provided basic data which will demand verification and expansion.

The extent of the changes which occur in zinc-fed animals is only now being recognized. Although gross symptoms have been described, the sites and the modes of interference of zinc in processes such as bone development and mineralization have not been clarified; nor has information been provided on possible ways and means of alleviating these harmful effects.

This study was undertaken with the purpose of extending present information in this area of investigation and of providing additional knowledge on the physiological reaction of the animal body to stress. It will attempt to clarify, if possible, the mechanism(s) of the interference of zinc with calcium and phosphorus in bone mineralization and to determine the effect of zinc toxicity on the metabolism of magnesium in young rats.

CHAPTER II

REVIEW OF LITERATURE

The work of Todd, Elvehjem and Hart, in 1934, gave the first indication that zinc was essential for the normal growth of animals.[1] In 1937, Sutton and Nelson reported that the limits of tolerence of young rats to zinc was between 0.5% and 1.0% of the diet.[2] They observed that levels of dietary zinc below 0.5% resulted in no adverse effects on the animals, while animals receiving 0.5% had reduced growth rates, poor reproduction and lowered hemoglobin levels. When rats were fed 1.0% zinc, death occurred in ten to twelve weeks. More recently, Van Reen has reported growth depression in young rats on an intake of 0.3% of zinc.[3]

High levels of dietary zinc have been shown to interfere with copper metabolism, to inhibit certain enzyme systems, and to interfere with iron metabolism in young rats. Smith and Larson observed that the depression in hemoglobin associated with zinc toxicity could be alleviated by supplements of dietary copper.[4] Van Reen noted a beneficial

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[1] W.R. Todd, C.A. Elvehjem and E.B. Hart, Am. J. Physiol., 107 146 (1934).

[2] W.R. Sutton and V.E. Nelson, Proc. Soc. Exptl. Biol. Med., 36, 211 (1937).

[3] R. Van Reen, Arch. Biochem. Biophys., 46, 337 (1953).

[4] S.E. Smith and E.J. Larson, J. Biol. Chem., 163,29 (1946).

effect of copper supplimentation on the depressed activity of liver cytochrome oxidase and catalase.[5] Duncan et al. reported that copper overcame the decreased heart cytochrome oxidase activity associated with zinc toxicity.[6] Grant-Frost and Underwood observed that lowered copper levels of the kidney and the liver of zinc-fed rats could be overcome by additional dietary copper.[7] An early and marked loss of liver iron has responded favorably to iron supplementation.[8] Magee and Matrone, studying effects of zinc toxicity on hemoglobin formation, liver copper and iron content, and heart cytochrome oxidase activity, concluded that zinc interfered with copper metabolism by decreasing the utilization and increasing the excretion of copper. Interference with iron utilization appeared to be a direct effect rather than one mediated through the copper metabolism.[9]

In 1951, Sadasivan reported that high levels of dietary zinc were associated with decreases in the calcium, phosphorus and total ash content of the femur of rats.[10] He also noted a significant increase in

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[5] Van Reen, op. cit.

[6] G.D. Duncan, L.F. Gray and L.J. Daniel, Proc. Soc. Exptl. Biol. Med., 83, 625 (1953).

[7] D.R. Grant-Frost and E.J. Underwood, Australian J. Exptl. Biol. Med. Sci., 36, 339 (1958).

[8] D.H. Cox and D.L. Harris, J. Nutrition, 70, 514 (1960).

[9] A.C. Magee and G. Matrone, J. Nutrition, 72, 233 (1960).

[10] V. Sadasivan, Biochem. J., 48, 527 (1951).

phosphorus excretion and a decrease in intestinal phosphatase activity of rats fed 0.5% and 1.0% zinc.[11, 12]

Similar toxic effects have been observed in other animals. Gross bone changes and an arthritic condition characterized by swollen joints have been reported in the weanling pig.[13, 14] The results of Thompson and co-workers indicated a lowered net retention of both calcium and phosphorus in lambs fed supplements of zinc sulfate.[15]

Although these latter studies indicate that high levels of dietary zinc prevent the normal development and the mineralization of the bone in various species, the exact mechanism(s) of this zinc interference remains an enigma. The apparant antagonistic relationship between zinc and calcium is controversial. As Forbes has pointed out, this relationship may not exist under all conditions.[16] Although an interrelationship between zinc and phosphorus is indicated, information concerning this aspect of the problem is lacking. There is also the possibility that interrelationships exist between zinc and other minerals necessary for bone development. Since magnesium has been shown

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[11] V. Sadasivan, Biochem. J., 49, 186 (1951).

[12] V. Sadasivan, Biochem. J., 52, 452 (1952).

[13] R.E.R. Grimmett, I.G. McIntosh, E.M. Wall and C.S.M. Hopkirk, New Zealand J. Agr., 54, 216 (1937)..

[14] M.F. Brink, D.E. Decker, S.W. Terrill and A.H. Jensen, J. Animal Sci., 18, 836 (1959).

[15] A.H. Thompson, S.L. Hansard and M.C. Bell, J. Animal Sci., 18, 187 (1959).

[16] R.M. Forbes, Federation Proceedings, 19, 643 (1960).

to be an essential constituent in bone formation and is closely associated with calcium and phosphorus metabolism, knowledge of the effect of excess zinc on magnesium would be desirable. It is evident that much more information must be made available before a final, definite statement can be made on the interference of zinc in bone mineralization.

CHAPTER III

EXPERIMENTAL PROCEDURES

The primary objectives of this study were to investigate (a) the nature of the interference of zinc in bone mineralization, (b) the possibility of changes in blood calcium and phosphorus associated with zinc toxicity, and (c) the possibility of zinc interference with magnesium metabolism.

The series of four experiments, which constitute Part I of the study, were designed to show the effect of zinc toxicity on levels of calcium, phosphorus and magnesium in bone and blood in the presence and the absence of other dietary supplements, and to determine the relative time when changes in bone calcium, phosphorus and magnesium begin to occur. Changes in bone zinc were also studied.

In Part II of the study, metabolism units were utilized to determine the effect of zinc toxicity on the net retention of calcium, phosphorus and magnesium.

I. GROWTH, BONE FORMATION AND BLOOD COMPOSITION OF RATS FED HIGH LEVELS OF ZINC

Since this phase of the study consisted of four experiments, procedures pertaining to a specific experiment will be discussed separately. Several procedures were common to all of these experiments, and these procedures are discussed in the following paragraphs.

Weanling male albino rats, descendants of the Sprague-Dawley strain, were used in all phases of the study.[1] Animals of a particular experiment were housed in individual wire-bottom cages in a controlled enivronment of 74° - 78° and 40% to 50% humidity. Food and water were offered ad libitum. Animals used in each experiment were randomized into replications according to initial body weights. Animals and test treatments within a replication were assigned at random to individual cages. The length of each experiment was four weeks. A weekly weight record was kept for all animals.

The basal diet, shown in Table I, also served as the control treatment. This diet, by analysis, contained 0.52% calcium, 0.55% phosphorus, 0.04% magnesium and 10 ppm. of zinc. In all experiments, supplements were added to the basal diet at the expense of starch. Zinc was fed as the carbonate; supplements of calcium, phosphorus and magnesium were in the carbonate, phosphate and sulfate forms, respectively.

At the end of each experiment, blood samples were obtained from the animals by means of a heart puncture. All blood samples from rats receiving the same treatment in a particular experiment were pooled. Serum was obtained from each pooled sample of blood by centrifuging. A serum filtrate was prepared using specified amounts of blood serum, distilled water and 20% trichloracetic acid. Measured amounts of the filtrate were taken for mineral determinations.

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[1]Purchased from Holtzman Company, Madison, Wisconsin.

TABLE I

COMPOSITION OF THE BASAL DIET

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

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

[b]Globe Easy-flow Conrstarch 3366, Greensboro, North Carolina.

[c]"Crisco," Proctor and Gamble Company, Cincinnati, Ohio.

[d]Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[e]Each 100 gm. of vitamin mix contained the following: 1 mg. biotin, 5 mg. folic acid, 0.1 mg. of 0.1% vitamin B_{12} (with mannitol), 25 mg. thiamine, 25 mg. pyridoxine, 50 mg. 2-methyl napthoquinone, 50 mg. riboflavin, 50 mg. nicotinic acid, 150 mg. Ca-panthothenate, 500 mg. p-amino benzoic acid, 5 gm. inositol, 7.5 gm. choline, 30 gm. DL-methionine, and 56.6 gm. corn starch.

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

[g]Each 1000 gm. of diet contained 24 drops of oleum percomorphum, Mead Johnson and Company, Evansville, Indiana.

Rats from randomly selected replications were sacrificed, and the femurs of each animal were removed. The bones were cleaned of muscle and fat by rubbing with a rough cloth, dried at 35° C, and weighed on an analytical balance. The bones were prepared for mineral analysis by wet ashing with nitric and perchloric acids on a hot plate. The ash of each pair of bones was dissolved in 0.6 N HCl and brought to a volume of 100 ml. with distilled water. Five ml. of this solution were rediluted to 100 ml., and appropriate aliquots of the second dilution were taken for the calcium and the phosphorus determinations. Appropriate aliquots of the first dilution of each bone sample were taken for the magnesium and the zinc determinations.

Calcium was determined by the method of Weybrew et al.,[2] phosphorus by the method of Simonson et al.,[3] and magnesium by a method outlined by Simonson et al.[4] The method of McCall, Davis and Stearns was used to determine zinc.[5]

Due to the high alkalinity of the bone samples, a slight modification in the calcium procedure was necessary. In order to maintain the required pH of 5 and to prevent co-precipitation of magnesium with calcium, three drops of 6N HCl were added to the test sample immediately after the addition of the brom cresol green indicator.

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[2] J.A. Weybrew, G. Matrone and H.M. Baxley, Anal. Chem., 20, 759 (1948).

[3] D.G. Simonson, M. Wertman, L.M. Westover and J.W. Mehl, J. Biol. Chem., 166, 747 (1946).

[4] D.G. Simonson, L.M. Westover and M. Wertman, J. Biol. Chem., 169, 39 (1947).

[5] J.T. McCall, G.K. Davis and T.W. Stearns, Anal. Chem., 30, 1345 (1958).

II. NET RETENTION OF CALCIUM, PHOSPHORUS AND MAGNESIUM

In the metabolism experiment, four animals received the basal diet and four animals a diet containing 0.75% of zinc. To insure uniform adaptation, all animals were maintained on the basal diet for a preliminary period of five days.

Each animal used in this experiment was placed in an individual metabolism cage. The cages were fixed as shown in Figure I, so that the urine and feces could be collected separately.

During the experimental period of four weeks duration, fresh amounts of feed were given daily, and the food consumption of each animal was measured. Water was available at all times. To reduce food spillage, the feed intake was limited to fifteen grams during the first and second weeks and to twenty grams during the third and fourth weeks. The experimental period was divided into four consecutive seven-day collection periods. All urine and feces samples from an individual animal were pooled for each seven-day period. Measured amounts of the pooled urine and feces were ashed, dissolved in 6N HCl, and made up to volume with distilled water. Calcium, phosphorus, magnesium and zinc determinations were made on appropriate aliquots of these samples by the methods previously described.

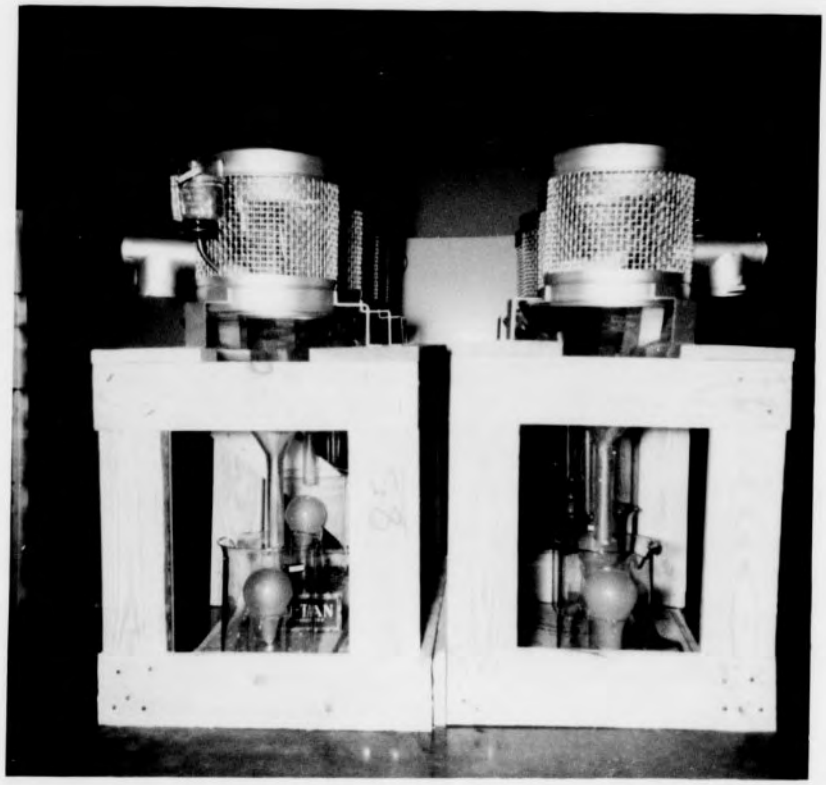

FIGURE 1

ARRANGEMENT OF APPARATUS USED IN METABOLISM EXPERIMENT

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I

The experiment was designed to test the effects of three levels of dietary zinc on (1) growth rate, (2) calcium, phosphorus, magnesium and zinc content of bone, and (3) calcium, phosphorus and magnesium levels in the blood. Although a minimum level of 0.5% of dietary zinc is usually necessary to cause a significant reduction in the growth of rats, there is the possibility that a lower level of zinc will significantly change the mineral composition of the bone and the blood. Thus, 0.25%, 0.50% and 0.75% levels of dietary zinc were used as the test treatments in this experiment.

Detailed data from the experiment are given in Appendix A, Tables 1-5 while the means of growth rate, bone calcium, phosphorus, magnesium and zinc, and serum calcium, phosphorus and magnesium are shown in Table II.

Analyses of the data (Appendix C, Table 1) revealed that the addition of 0.75% of zinc to the diet resulted in a highly significant decrease ($p \leq 0.01$) in weight gain and a significant decrease ($p \leq 0.05$) in bone calcium and bone phosphorus. The data also indicate that a level of 0.25% of dietary zinc is associated with a highly significant increase ($p \leq 0.01$) in the deposition of zinc in the bone, although no significant changes occurred in the content of bone calcium and

TABLE II

EFFECT OF ZINC ON GROWTH, SERUM COMPOSITION, AND BONE MINERALIZATION (EXPERIMENT I)

Level of dietary zinc	Weight gain[a] at 4 weeks	Bone constituents[a]				Serum constituents[c]		
		Ca	P	Mg	Zn	Ca	P	Mg
%	gms	mg./gm. dry weight				mg./100 ml.		
None	188	146.29	66.04	3.45	0.09	13.73	11.92	3.27
0.25	197	143.82	54.01	3.37	0.58	13.74	12.72	3.07
0.50	161[b]	131.37	40.78	3.44	1.34	10.31	13.40	3.39
0.75	113[b]	107.41	39.57	3.44	2.34	12.15	11.92	2.87

[a]Each figure is the mean of 6 animals unless otherwise indicated.

[b]Mean of 7 animals

[c]Each figure represents the determination for a pooled sample of serum from 6 animals.

phosphorus at this level of zinc intake. The dividing line for the toxic effect of zinc on these two minerals would appear to fall between the 0.25% and the 0.50% levels. Zinc had no significant effect on the magnesium content of the bones.

The effect of increased dietary zinc on serum calcium, phosphorus and magnesium concentrations was inconsistent. Although serum calcium values were depressed at the two highest levels of zinc intake, serum phosphorus levels were either increased or remained at control levels. The changes which occurred in serum magnesium showed no distinctive pattern of increase or decrease. Failure to note any consistent effects, in calcium at least, may be due to the fact that serum levels of this mineral are primarily controlled by the parathyroid hormone and are not readily influenced by dietary intake.[1]

Experiment II

Although Cox and Harris have reported that the effect of zinc toxicity on certain body constituents was evident within the first week, no experiments have been conducted to determine how soon significant changes begin to occur in the metabolism of calcium, phosphorus and magnesium in animals maintained on high zinc diets.[2] In order to obtain this information, sixty-four animals were divided equally into four groups. One group was maintained as controls; the other three groups were fed diets containing 0.25%, 0.50% and 0.75% of zinc,

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[1] E.W. Crampton and L.E. Lloyd, "Fundamentals of Nutrition," W.H. Freeman and Company, San Francisco, 1959, p. 221.

[2] Cox and Harris, loc. cit.

respectively. At weekly intervals, four animals from each group were sacrificed and levels of bone calcium, phosphorus, magnesium and zinc and serum calcium, phosphorus and magnesium were determined.

The data (Table III and Appendix A, Tables 6-9) showed a significantly reduced weight gain ($p \leq 0.01$. in animals receiving zinc supplemented diets. This harmful effect upon weight gain, noticed in the second week, resulted in a final mean gain of 172 grams for the control animals but only 155 grams, 125 grams and 112 grams for animals receiving diets containing 0.25%, 0.50% and 0.75% of zinc, respectively.

Of the minerals studied, the clearest and most definite patterns were found in the calcium and zinc values in the bone. As the zinc content of the diet increased, progressively larger amounts of zinc were deposited in the bone accompanied by a progressive lowering of calcium levels. Since the changes in the concentrations of these two minerals were judged highly significant ($p \leq 0.01$) during the first week of the experimental period, it could be assumed that the toxic effect of zinc on calcium was an immediate one. As before, 0.75% zinc produced the greatest lowering in the calcium content of the bone. At this intake, calcium values were only slightly higher during the fourth week of the experiment than during the first week. This would indicate one of two possibilities: either a progressive slowing in the rate at which calcium was deposited in the bone, or an increased rate of withdrawal of calcium from the bone.

Phosphorus presented a more variable pattern and one difficult to interpret. A puzzling drop in control values occurred during the latter part of the experiment. From the results obtained in

TABLE III

PROGRESSIVE STAGES IN THE EFFECT OF ZINC ON GROWTH, SERUM COMPOSITION, AND BONE MINERALIZATION (EXPERIMENT II)

Treatment	Weight[a] gain	Bone constituents[b]				Serum constituents[c]		
		Ca	P	Mg	Zn	Ca	P	Mg
	gm.	mg./gm/ dry weight				mg/100 ml.		
Control								
First week	39	140.85	26.19	2.57	.13	9.58	12.95	2.40
Second week	87	150.57	34.41	2.59	.08	12.79	12.75	2.66
Third week	134	156.01	36.56	2.81	.13	11.05	12.76	2.76
Fourth week	172	167.72	17.75	2.56	.15	11.39	12.15	1.57
0.25% Zinc								
First week	33	131.79	21.31	2.72	.40	10.02	13.16	2.56
Second week	76	143.39	42.32	2.54	.54	12.70	12.42	2.67
Third week	121	144.58	20.10	2.75	.77	13.00	13.93	3.05
Fourth week	155	154.23	19.90	2.60	.78	11.17	11.60	2.50
0.50% Zinc								
First week	31	113.19	36.56	2.56	.61	9.63	11.91	2.27
Second week	67	122.50	23.94	3.21	.90	10.35	12.96	2.87
Third week	107	142.32	25.38	2.71	1.77	11.10	12.99	3.14
Fourth week	125	143.62	39.86	2.96	1.68	10.01	11.95	1.73
0.75% Zinc								
First week	27	116.06	16.88	2.50	1.01	9.75	11.66	2.39
Second week	61	114.19	43.18	2.54	1.46	11.83	11.58	2.60
Third week	88	125.56	29.87	2.83	2.39	11.01	11.99	2.47
Fourth week	112	121.66	19.81	2.55	2.39	10.93	11.18	3.03

[a]Each figure represents the mean of 16, 12, 8 and 4 animals for weeks 1,2,3 and 4, respectively.

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

[c]Each figure represents the determination for a pooled sample of serum from 4 animals.

Experiment I, a continued rise in these values would have been expected. Although the pattern of change at each individual level of zinc was not a distinct one, the overall picture was a final bone content of phosphorus equal to, or only slightly higher than, the levels of phosphorus observed during the first week. At the lowest and highest levels of zinc intake, the effect of zinc was noticeable during the third week; at the intermediate level, during the second week. However, the changes which did occur were not significantly different from each other in any one week of the experiment. The considerable variation observed in animals on the same treatment could have been a major reason for the failure to detect significant changes in bone phosphorus. Bone magnesium values showed variation from week to week and from diet to diet, but the observed differences were found to be not statistically significant. Zinc, regardless of the concentration used, appeared to have no effect on the levels of magnesium in the bone.

No distinct pattern could be defined for the serum constituents under investigation. Only during the fourth week was there any apparent trend. At this time, the serum calcium and phosphorus levels of rats fed the high zinc diets were slightly and consistantly below those of the control group. With magnesium, the trend was one of increased serum levels associated with the feeding of zinc.

Experiment III

The results of Experiment I and Experiment II showed a definite effect on bone mineralization at the higher levels of zinc intake. The major change noted was a decreased calcium and phosphorus content of the bone. Since Toepher and Sherman obtained improved levels of bone

calcium and phosphorus using calcium supplements under conditions of normal nutrition, there was the possibility that increasing the dietary level of calcium and phosphorus would counteract the toxic zinc effects observed in bone.[3] In order to test this possibility, Experiment III was designed to show the effects of adding calcium and phosphorus supplements to high zinc diets. In this experiment, supplemental levels of 0.4% each of calcium and phosphorus were added to diets containing 0.75% zinc. Supplements of 0.08% of magnesium were also utilized.

The results of Experiment III are presented in Table IV and Appendix A, Tables 10-14. Supporting the previous findings, statistical analysis of the data revealed that increased levels of zinc are associated with a highly significant decrease ($p \leq 0.01$) in growth rate. The growth data indicated that the control animals gained approximately twice as much as animals receiving 0.75% zinc in the diet. When supplements of 0.4% calcium, 0.4% phosphorus and 0.08% magnesium were added to the high zinc diet, alone or in combination, an improved weight gain occurred. Greatest improvement was noted in those animals receiving a diet supplemented with both calcium and phosphorus followed by the combination supplement of calcium, phosphorus and magnesium. The addition of either calcium, calcium plus magnesium, or phosphorus plus magnesium resulted in intermediate increases in weight gain of approximately the same magnitude. No significant improvement in weight gain was observed when supplements of either phosphorus or magnesium were added to the

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[3] E. Toepher and E. Sherman, J. Biol. Chem., 115, 684 (1936).

TABLE IV

EFFECT OF VARIOUS SUPPLEMENTS ON RATS FED HIGH LEVELS OF ZINC (EXPERIMENT III)

Treatment	Weight gain[a] at 4 weeks	Bone constituents[b]				Serum constituents[c]		
		Ca	P	Mg	Zn	Ca	P	Mg
	gm.	mg./gm. dry weight				mg./100 ml.		
Control	182	162.86	43.38	2.98	0.11	11.86[d]	11.33[d]	2.93[d]
0.75% Zinc	97	110.94	25.39	2.99	2.69	11.05	10.85	3.17
0.75% Zn + 0.4% Ca	126	133.94	26.99	2.92	1.72	12.16	13.52	3.61
0.75% Zn + 0.4% P	106	109.44	32.03	2.77	1.95	11.52	13.20	3.32
0.75% Zn + .08% Mg	113	100.18	35.11	3.20	2.29	10.05	12.07	3.54
0.75% Zn + 0.4% Ca + 0.4% P	154	158.21	37.71	3.00	1.06	10.95	12.97	2.49
0.75% Zn + 0.4% Ca + .08% Mg	129	145.33	37.67	3.48	1.43	11.64	12.51	3.07
0.75% Zn + 0.4% P + .08% Mg	128	117.24	40.48	3.11	1.54	11.53	11.54	2.81
0.75% Zn + 0.4% P 0.4% Ca + .08% Mg	139	125.94	33.77	3.18	0.84	10.15	12.05	2.67

[a] Each figure represents the mean of 6 animals unless otherwise indicated.

[b] Each figure represents the mean of 4 animals.

[c] Each figure represents the determination for a pooled sample of serum from 6 animals unless otherwise indicated.

[d] Mean of 5 animals.

high zinc diets. This improved growth rate, obtained by adding calcium and phosphorus to a toxic zinc diet, is in disagreement with the findings of Brink and co-workers.[4] They failed to observe any significant interaction between zinc and calcium in weanling pigs receiving diets containing 0.05% to 0.4% zinc and a supplement of 1.0% calcium. These investigators, however, did note a trend towards the alleviation of the poor daily weight gain at the highest zinc-calcium supplemented intake.

Changes in bone calcium, phosphorus, magnesium and zinc followed a pattern similar to that observed in Experiment I. The addition of 0.75% of zinc to the basal diet resulted in a decrease in calcium and phosphorus content of the bone; a sharply defined increase in the amount of zinc entering the bone, and no discernible change in magnesium levels. In general, supplements of calcium, alone or in combination with the other minerals, resulted in increased amounts of calcium in the bone. The most effective supplement used was calcium plus phosphorus which increased the calcium bone values to near those of rats fed the control diet. This was an increase of approximately 40% over the levels observed for rats fed the non-supplemented 0.75% zinc diet. With a calcium and magnesium combination, a marked percentage increase in calcium values also occurred. The addition of either phosphorus or magnesium, supplied singly, had no apparent effect on the accumulation of calcium in the bone.

The addition of either phosphorus or magnesium, but not calcium, to the high zinc diet was associated with increases in the accumulation

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[4]Brink, Decker, Terrill and Jensen, loc. cit.

of phosphorus in the bone. Additional increases in bone phosphorus were observed when supplemental combinations of calcium and phosphorus, calcium and magnesium, and phosphorus and magnesium were used.

The highest values for bone magnesium were obtained when magnesium alone or in combination with calcium and phosphorus was added to the high zinc diet. Such values exceeded those of the control group. The significant difference ($p \leq 0.05$) between treatments appeared to occur between the group of diets not supplemented with magnesium and the group of diets to which magnesium had been added.

The degree to which zinc entered the bone was closely related to the type of supplementation. The addition of either calcium or phosphorus to the high zinc diet was associated with a marked reduction in the amount of zinc deposited in the bone, while the addition of magnesium had no apparent effect on bone zinc. A combination of all three supplements tested in this experiment resulted in a mean bone zinc level that was only seven times greater than the mean value for the controls. The mean bone zinc level of the rats fed the non-supplemented high zinc diet was some twenty times higher than that of the controls.

The control values for calcium, phosphorus and magnesium in the serum were within normal limits, With dietary supplementation a small, but in some cases noticeable, variation from the control values occurred. The highest value in each group was produced by the calcium suplemented diet and, in each instance, this value exceeded the control value for that particular group. Thus, calcium was instrumental in increasing the levels of serum calcium, phosphorus and magnesium. The effectiveness of a phosphorus supplement in increasing the concentration

of phosphorus in the serum and of supplemental magnesium in raising serum magnesium was secondary to the effectiveness of calcium but was greater than any combination of the three elements.

In general, serum levels of phosphorus and magnesium showed greater increases than did serum calcium levels which are controlled by the parathyroid hormone. Such increases would appear to be a direct reflection of the higher dietary intake rather than a more extensive withdrawal of phosphorus and magnesium from the bone.

Experiment IV

The levels of calcium and phosphorus supplements used in Experiment III partially alleviated the adverse effect of zinc on bone calcium and phosphorus. It could be reasoned that increased levels of these minerals would completely alleviate this effect. Experiment IV was designed to compare the effects of three levels of calcium and phosphorus supplements on the calcium and phosphorus contents of the bones of zinc-fed rats. The levels of supplements tested are given in Table V. All supplements, alone and in combination, were added to diets containing 0.75% zinc.

Increasing the calcium supplement from 0.4% to 0.8% resulted in a significant increase in the bone calcium of zinc-fed rats. A supplement of 1.2% calcium gave the same response as did the 0.8% supplemental level. It is also interesting to note that the mean bone calcium values of zinc-fed rats supplemented with either 0.4% calcium and phosphorus or 0.8% calcium and phosphorus were no different from the mean bone calcium values of the rats supplemented with 0.8% calcium and 1.2%

TABLE V

EFFECT OF CALCIUM AND PHOSPHORUS SUPPLEMENTS ON RATS FED HIGH LEVELS OF ZINC (EXPERIMENT IV)

Treatment	Weight gain[a] at 4 weeks	Bone constituents[a]			
		Ca	P	Mg	Zn
	gms.	mg./gm. dry weight			
Control	148	163.36	40.74	2.41	0.10
0.75% Zn	104	116.41	32.13	2.32	1.29
0.75% Zn + 0.4% Ca	124	132.29	35.21	2.18	1.03
0.75% Zn + 0.4% P	113	118.29	32.06	2.28	0.99
0.75% Zn + 0.8% Ca	131	149.12	30.48	2.18	0.99
0.75% Zn + 0.8% P	116	124.57	28.89	2.27	0.68
0.75% Zn + 1.2% Ca	120	146.70	33.45	2.27	1.13
0.75% Zn + 1.2% P	119	126.01	31.51	1.91	0.40
0.75% Zn + 0.4% Ca + 0.4% P	131	145.98	28.31	2.42	0.76
0.75% Zn + 0.8% Ca + 0.8% P	132	146.56	37.95	1.79	0.28[b]
0.75% Zn + 1.2% Ca + 1.2% P	115	156.66	32.02	1.41[b]	0.26[b]

[a]Each figure represents the mean of 6 animals unless otherwise indicated.

[b]Mean of 5 animals.

calcium. A supplement of 1.2% calcium and phosphorus completely alleviated the marked decrease in the calcium level of the bones of the zinc-fed animals, since the mean calcium value of these rats was not significantly different from that of the control animals. Phosphorus supplementation alone had no apparent effect on bone calcium. It is possible that the beneficial effect of phosphorus in combination with calcium is primarily one of maintaining the proper calcium-phosphorus ratio essential for normal bone development.

In this experiment the addition of zinc did not significantly decrease the bone phosphorus levels as was observed in previous experiments, and none of the supplements tested had any effect on bone phosphorus. No particular pattern was apparent for the minor changes which did occur. It would appear that the effect of zinc on the phosphorus content of the bone is of a more variable nature and is secondary to the effect of zinc on bone calcium.

No significant reduction in the magnesium content of the bone occurred with an intake of 0.75% zinc, and no significant increase in the levels of bone magnesium resulted from supplementation. The lowest values of bone magnesium observed were in animals receiving 1.2% calcium and phosphorus. This finding is in agreement with the conclusions reached by O'Dell in a recent review of the relationship of the magnesium requirement of animals to other dietary constituents.[5] The evidence presented in this review indicated that excess phosphorus will

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[5] B.L. O'Dell, Federation Proceedings, 19, 648 (1960).

decrease the absorption of magnesium in the animal body. Although a calcium-magnesium antagonism has also been shown to exist, this condition generally occurs on a high calcium and a deficient magnesium intake.

As observed in Experiment III, supplements of calcium and phosphorus effectively prevented the entry of excess zinc into the bone. In this experiment, however, phosphorus supplementation prevented the increase in bone zinc to a greater degree than calcium supplementation. In general, a combination of the two supplements was more effective than either supplement used alone. The zinc-fed animals supplemented with either 0.8% calcium and phosphorus or 1.2% calcium and phosphorus had mean bone zinc values which approached the mean value of the control animals.

Analysis of the weight gain data (Appendix C, Table 4) indicated a significant increase in weight gain when calcium and phosphorus were given in combination in the diet at either the 0.4% or the 0.8% level. There was essentially no difference in the ability of one level over the other in promoting an increase in weight gain. Since a 1.2% calcium and phosphorus supplement was beneficial in promoting proper bone mineralization, but not growth, there is the indication that a different mechanism of zinc interference is involved in each of the processes. However, at such high levels of calcium and phosphorus intake, the possibility of toxic effects from these two minerals, as well as from zinc, should be considered. The adverse effect of high levels of

calcium and phosphorus on several animal species has been shown by various researchers.[6,7,8]

Experiment V

The reduced calcium and phosphorus content of bone noted in the previous experiments would indicate the possibility of increased excretion of calcium and phosphorus. A metabolic study was carried out to determine the effect of a toxic zinc intake on absorption, utilization and net retention of these two minerals and magnesium. Two groups of four animals each were maintained in metabolism cages for four weeks. One group received the basal diet; the other group was fed a diet containing 0.75% zinc. Each of the four consecutive collection periods covered seven days.

Data presented in Table VI and Appendix B, Tables 1-3, showed a marked decrease in the net retention of calcium and phosphorus in animals receiving supplementary zinc, as compared with control animals. This data is presented graphically in Figure 2. The observed decrease in net retention occurred as early as the first week. The maximum change in retention for both calcium and phosphorus came during the second week and this appeared to be the period in which the toxic zinc effect was greatest. During this second week calcium levels fell to approximately one-fifth the control values and phosphorus levels fell

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[6] B.L. O'Dell, E.R. Morris and W.O. Regan, J. Nutrition, 70, 103 (1960).

[7] R.M. Forbes and M. Yohe, J. Nutrition, 70, 53 (1960).

[8] E.R. Morris and B.L. O'Dell, J. Nutrition, 75, 77 (1961).

TABLE VI

CALCIUM, PHOSPHORUS, AND MAGNESIUM BALANCE SUMMARY (EXPERIMENT V)

Item	Collection periods[a]							
	1		2		3		4	
	Basal	0.75% Zn	Basal	0.75% Zn	Basal	0.75% Zn	Basal	0.75% Zn
	total mg./collection period[b]							
Calcium								
Intake	339.23	319.83	393.71	358.80	409.43	379.44	471.78	405.23
Urine excretion	0.60	1 1.97	0.49	1.60	1.13	2.13	1.84	1.99
Feces excretion	115.65	217.80	114.51	314.96	192.00	282.15	238.92	339.46
Net retention	222.98	100.05	245.71	42.24	216.30	95.16	231.02	63.78
Phosphorus								
Intake	344.74	405.95	516.16	455.42	536.78	481.61	618.51	514.34
Urine excretion	103.63	52.38	142.81	103.77	164.95	98.72	189.97	100.78
Feces excretion	56.11	147.35	60.21	200.50	82.01	151.25	104.34	184.34
Net retention	285.01	206.22	313.14	151.15	289.82	231.64	327.20	229.22
Magnesium								
Intake	23.43	18.92	27.19	21.22	28.28	22.44	32.58	23.97
Urine excretion	0.74	0.83	0.14	1.77	1.09	0.94	0.66	1.09
Feces excretion	6.82	8.10	8.07	10.12	8.68	10.84	10.99	10.27
Net retention	15.88	9.94	18.97	9.32	18.50	10.66	20.93	12.62

[a]Each collection period consisted of seven consecutive days. Feces samples of all animals on a particular diet were combined for a collection period, as were all urine samples.

[b]Each figure represents the mean of four animals.

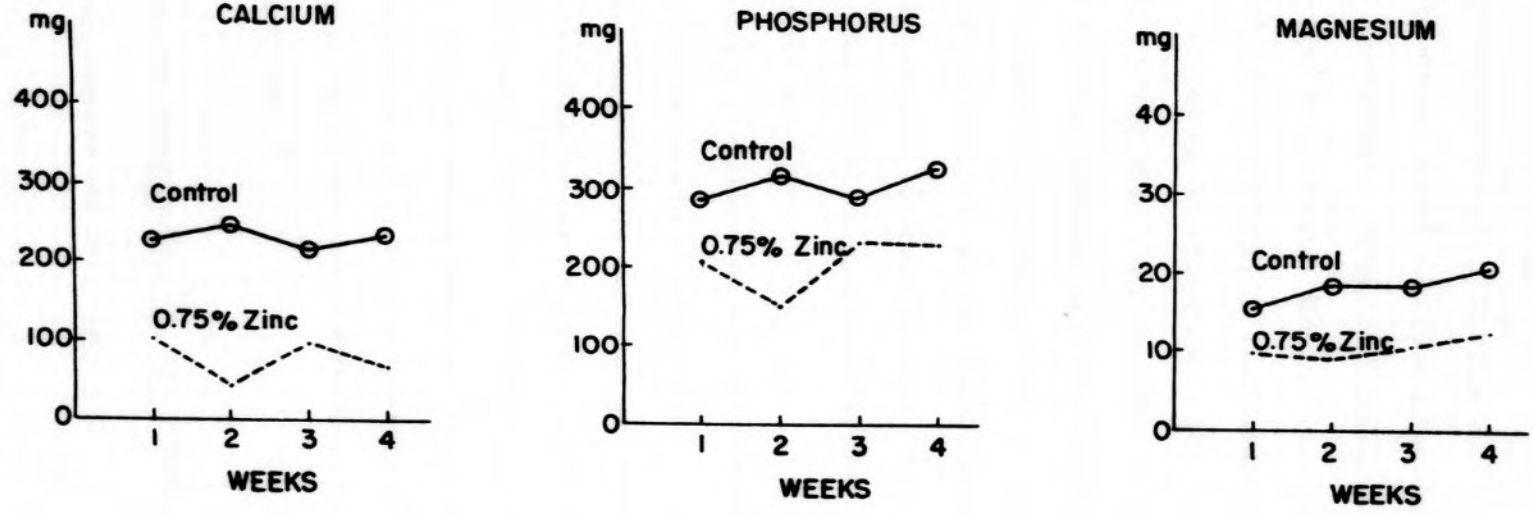

FIGURE 2

EFFECT OF ZINC ON NET RETENTION OF CALCIUM, PHOSPHORUS AND MAGNESIUM

to one-half the control values. All other collection periods (first, third and fourth weeks) showed a calcium retention for animals on the high zinc diet averaging two-fifths the control diet and a phosphorus retention averaging three-fourths the control diet.

The intake of a high level of zinc produced a dramatic change in the pathways of excretion for phosphorus. It repressed the excretion of this mineral via the urine and stimulated its excretion in the feces. This result is in close agreement with the findings of Sadasivan.[9] Because urinary excretion of phosphorus was reduced in zinc-fed rats, he concluded that the minerals of the bones were not directly mobilized. The accompanying increase observed in fecal excretion suggested that zinc prevented assimilation of phosphorus by interfering with its absorption from the intestine.

The pathway of calcium excretion was not changed by the addition of zinc to the diet but a 40% to 50% increase in excretion through the feces did occur. Excretion via the urine was also intensified but gradually stabilized during the last week of the study to comparable amounts for both control and zinc-fed animals. Thompson and co-workers have presented evidence that in lambs, at least, the high fecal excretion could be accounted for by both an increase in fecal endogenous calcium and a lowering in the actual absorption of calcium from the gastro-intestinal tract.[10]

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[9] V. Sadasivan, Biochem. J., 49, 186 (1951).

[10] Thompson, Hansard and Bell, loc. cit.

A reduction in net retention of magnesium was observed during the first collection period and continued throughout the experiment. In view of previous data, this probably reflects the influence of zinc on magnesium at sites in the body other than the bone.

CHAPTER V

GENERAL DISCUSSION

Many of the gross symptoms of zinc toxicosis reported by other researchers were observed throughout the various phases of this study. Some of these symptoms were lack of appetite, retarded growth, small skeletal size, yellow and coarsened fur and increased irritability.

I. EFFECT OF ZINC ON GROWTH

The relationship between reduced food consumption and the retarded growth associated with the feeding of high levels of zinc has not been clearly defined. Earlier researchers felt that the reduction in food consumption by itself was insufficient to account for the entire effect of zinc on growth. This viewpoint has been strengthened by a more recent observation that the lowered food consumption is associated with a rise in metabolic rate and a subsequent decrease in the efficiency with which food is used by the body.[1] The depressing effect of zinc on weight gain could be, therefore, a result of an interference with some growth mechanism, while the decrease in food intake results from the decreased growth.

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[1] E.J. Underwood, "Trace Elements in Human and Animal Nutrition," Academic Press, New York, 1956, p. 222

Previously, only liver and protein from a soybean source have been effective in alleviating the decrease in weight gain resulting from the feeding of excess zinc.[2,3,4] Results obtained in this investigation provide the first indication that a specific mineral(s) is also capable of improving the subnormal growth. Supplementing a high zinc diet with calcium and phosphorus resulted in a marked increase in weight gain. A smaller, but still noticeable increase in weight gain, was evident with calcium alone. Although phosphorus alone was largely ineffective in promoting weight gain, it appears to be essential for establishing and maintaining the proper calcium-phosphorus ratio conducive to normal growth. Highest weight gains were obtained on those diets which had calcium-phosphorus ratios of approximately 1:1 to 2:1, except when the zinc-fed animals received a supplement of 1.2% calcium and phosphorus. Since Forbes and Yohe have shown this level of calcium to be toxic to growth, it is possible that a condition of calcium toxicity, as well as zinc toxicity, existed when a supplement of 1.2% calcium was used. When the calcium-phosphorus ratio fell to as low as 0.3:1 or rose as high as 3:1, a poorer weight gain resulted.

In this study, zinc toxicity did not significantly decrease the weight gain of young rats until the second week. Significant changes in bone development occurred in zinc-fed rate during the first week.

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[2] Smith and Larson, loc. cit.

[3] Magee and Matrone, loc. cit.

[4] J.T. McCall, V. Mason and G.K. Davis, J. Nutrition, 74,51 (1961).

[5] Forbes and Yohe, loc. cit.

The results of this study also indicate that a level of 0.5% zinc is associated with significant decreases in bone calcium and phosphorus, but not weight gain, at the end of four weeks. This would suggest the possibility that the interference of zinc with bone mineralization precedes the interference with growth. The amount of growth that will occur in animals fed high levels of zinc might depend, therefore, upon the amount of skeletal development that takes place.

The manner in which calcium alleviates the toxic effect of zinc on growth is not clear. The problem is further complicated because the exact mechanism of zinc interference with growth has not been determined. It may be that the addition of calcium to a high zinc diet causes the removal of excess zinc from the body by some means so that near normal conditions with respect to zinc absorption and utilization exist. Under certain conditions, calcium is capable of forming insoluble complexes with other minerals. A definite calcium-zinc relationship has already been established. In general, the zinc-fed animals with improved weight gains on supplemented diets also had less accumulation of zinc in the bones. The formation of an insoluble calcium-zinc salt would be one effective means of making zinc less available. It should be pointed out that phosphorus in combination with calcium has a marked influence on the alleviation of the subnormal growth. This could indicate that a more efficient removal of excess zinc results when a proper dietary ratio of calcium and phosphorus is maintained.

Another possibility is that the improvement in growth noted with added calcium and phosphorus is a result of the improvement in skeletal

development. It seems logical that an increase in skeletal size would result also in an increase in weight.

II.. INFLUENCE OF ZINC ON BONE MINERALIZATION

Calcification involves a constant, dynamic interchange of ions between the bone structure and the body fluids bathing this structure. Bone is no longer considered an inert mass of mineral salts. It is both enzymatic and non-enzymatic in character and consists of the precipitation and deposition of calcium, phosphorus and lesser minerals into a collagenous organic matrix. The present study indicates an apparent interference of zinc with some phase(s) of calcification. Two possible mechanisms may be suggested. They are: (1) interference of zinc with calcium and phosphorus absorption and (2) interference of zinc with a specific enzyme system related to bone calcification.

The first possibility is suggested by an examination of the data from Experiment V. These data have shown an increased excretion of calcium and phosphorus in rats injecting 0.75% zinc. Other experimants in the study have indicated a lowered calcium and phosphorus content of the bone at this same level of zinc. Observed changes in the pathways of excretion of calcium would appear to indicate a primary failure in absorption as shown by an increased excretion via the feces. The increase in excretion via the urine would indicate, to a smaller extent, failure in the normal utilization of calcium or possibily mobilization of calcium from the bones. The decreased excretion of phosphorus in the urine and the concomitant increase in the feces suggests that zinc interferes with the absoprtion of phosphorus. It is

well known, however, that calcium and phosphorus may be partly re-excreted into the large intestine as endogenous calcium and phosphorus. It is, therefore, difficult to say whether the increased amounts of these two minerals found in the feces represent only a failure in absorption. Thompson and co-workers have shown both an increased endogenous excretion and a decreased absorption of calcium from the gut of lambs receiving 0.5% and 1.0% zinc.[6] Using radiotracer techniques, they obtained data showing an endogenous excretion of calcium which rose from 28 mg./day on the basal diet to 39.9 mg./day on either level of zinc. Calcium absorption fell from 41.9% in animals on the basal diet to 21.2% for animals on high levels of zinc. Phosphorus absorption was also decreased from 88.3% to 81.6%.

The second mechanism suggested for the interference of zinc in bone calcification involves the relationship of zinc to a specific group of enzymes, the phosphatases. Robinson, in 1932, produced important evidence that a phosphatase, occurring in active bone and cartilage, hydrolyzed organic phosphates in the blood.[7] The increased concentration of inorganic phosphate resulting from this action was thought to initiate precipitation. A more recent theory suggests that phosphatase participates in the process of calcification but not in the direct manner described by Robinson. In this theory, phosphatase is

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[6] Thompson, Hansard and Bell, loc. cit.

[7] R. Robinson, "The Significance of Phosphoric Acid Esters in Metabolism", New York University Press, New York, 1932, p. 124.

thought to be concerned with preparation of the organic matrix in which the phosphate ions are precipitated.[8] Interference of zinc in the activity of liver phosphatase has been demonstrated. Sadasivan has shown that the poor assimilation of phosphorus from the intestine is associated with a significant decrease in intestinal phosphatase.[9] If a similar interference occurred with bone phosphatase activity, it is highly possible that zinc could affect calcium and phosphorus metabolism at both the cellular level and at the site of absorption.

The over-all results of this study indicate an antagonistic effect of zinc on the normal deposition of both calcium and phosphorus in the bone of growing rats which can be alleviated with calcium and phosphorus supplementation. If the primary effect of zinc on calcium and phosphorus is to promote excess excretion of these minerals from the body, it is logical to assume that this loss is replaced with the addition of calcium and phosphorus supplements. The possibility of added calcium and phosphorus facilitating the removal of excess zinc from the body has already been discussed in connection with growth. The ideas presented in that discussion could also apply to bone mineralization. In view of the relative ease with which the abnormal increase in bone zinc and the decrease in normal deposition of calcium and phosphorus in the bone can be prevented, it would appear that the

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[8] F. C. McLean and M. R. Urist, "Bone", The University of Chicago Press, Chicago, 1955, p. 58.

[9] V. Sadasivan, Biochem. J., 52, 452 (1952).

effect of zinc on bone mineralization is not permanent and irreversible.

III. EFFECT OF ZINC ON MAGNESIUM METABOLISM AND UTILIZATION

The results of the metabolism experiment indicate a definite effect of zinc on the metabolism of magnesium, even though bone and serum magnesium levels did not appear to be changed with a high intake of dietary zinc. The data would suggest that the normal deposition of magnesium in the bone is not affected by zinc, although the net retention of magnesium is less. Since magnesium is a co-factor for many important enzyme systems involved in glycolysis, phosphorylation, purine and pyrimidine synthesis, and nitrogen metabolism, it is possible that the primary effect of zinc on metabolism would be in connection with one or more enzymatic reactions in the animal body.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

I. SUMMARY

This study was conducted to determine the effects of high levels of dietary zinc on the growth and the bone mineralization of young rats, in the presence and in the absence of various supplements.

Specific experiments were designed to (1) investigate the changes which occurred in the levels of bone and serum mineral constituents of animals receiving excess zinc, (2) observe the time at which these changes begin to occur, and (3) determine the possible beneficial effects of the use of calcium, phosphorus, and magnesium supplements.

Criteria used as measures of the response of animals to individual treatments were weight gain, bone levels of calcium, phosphorus, magnesium and zinc; and the concentration of calcium, phosphorus and magnesium in the serum. All data were subjected to statistical analyses.

A metabolism experiment was conducted to determine the effects of zinc on the absorption, the utilization and the net retention of calcium, phosphorus and magnesium.

Experimental results showed that the addition of high levels of zinc to the diets of young rats led to marked decreases in weight gain. Although a level of 0.75% dietary zinc was required to produce significant decreases in weight gain at four weeks, the results

indicated that a level of 0.5% zinc would probably significantly decrease weight gain within six weeks. The adverse effect of zinc on growth could be alleviated with supplements of calcium and phosphorus. Zinc toxicity resulted in a consistent and marked decrease in the calcium content of the bone and a less consistent, but still noticeable, decrease in the level of bone phosphorus. Zinc apparently had no effect on bone magnesium levels. A level of 0.5% dietary zinc was sufficient to cause a reduced bone calcium and phosphorus content. This marked decrease in bone calcium was observed in animals which had been on the high zinc diet for only one week. The adverse effect of zinc on bone calcium and phosphorus could be alleviated by supplementing the high zinc diets with calcium and phosphorus. The importance of maintaining a proper calcium-phosphorus ratio was emphasized by the results of some of the experiments.

There was a marked increase in the accumulation of zinc in the bones of rats on the high zinc diets. A level of 0.25% zinc was sufficient to significantly increase the amount of zinc in the bones. Also, the results indicated that this marked increase in bone zinc occurred during the first week the animals were on experiment.

The results of the metabolism experiment indicate that high levels of zinc cause decreases in the net retention of calcium, phosphorus and magnesium. The effect of zinc on the net retention of these elements was evident during the first week of experimental conditions. A significant increase in the fecal and the urinary excretion of calcium occurred in the rats receiving the high zinc diet, indicating that zinc is interfering with the absorption and the utilization of calcium. The

increase in fecal excretion and the decrease in urinary excretion of phosphorus in these same animals would suggest that zinc primarily interferes with the absorption of phosphorus. There is also evidence that zinc increases both the urinary and the fecal excretion of magnesium. This would suggest that zinc affects both the absorption and the utilization of this mineral.

No marked changes in serum calcium, phosphorus and magnesium were observed in any of the experiments. Of the minimal changes which did occur, none appeared significant.

II. RECOMMENDATIONS FOR ADDITIONAL INVESTIGATION

The results of this study have shown that an increase in the excretion of calcium and phosphorus occurs when rats are fed excess zinc. Both a failure in absorption and changes after absorption, possibily enzymatic in nature, are implicated. More information, however, is necessary before the nature of zinc interference with calcium and phosphorus metabolism is clarified. Some areas of future study which could furnish this required information are:

(a) Radioisotope studies to determine the amounts and the sources of endogenous calcium and phosphorus excreted in the urine of rats fed high levels of zinc.

(b) Metabolism studies to determine the effects of calcium and phosphorus supplements on the absorption, utilization and net retention of zinc.

(c) Enzymatic studies to determine the effects of zinc on bone phosphatase activity.

Liver extract and protein from a soybean source have been helpful in alleviating the subnormal growth pattern in zinc-fed rats. Similar results were obtained in this study with the use of calcium and phosphorus supplements. It would be of interest to examine the possibility of interrelationships between the mechanisms by which each of these results is achieved.

Although no definite conclusions could be made from the blood data gathered in this study, there is an indication that more detailed experiments could yield important information concerning the metabolism of these minerals during conditions of zinc toxicity.

While zinc apparently has no effect on the level of magnesium in the bone, results of the metabolism experiment show a definite effect of zinc on magnesium metabolism. Since many of the enzyme systems in the animal body require magnesium as a co-factor, it is possible that zinc interferes with some of these enzymes.

Manganese is required for proper bone development in species such as rabbits, chickens, rats and mice. A deficiency of this mineral leads to gross bone malformation. In the present investigation, the color of the bone ash indicated qualitatively a lack of manganese in the bones of rats receiving excess zinc. A study of the effect of zinc toxicity on bone manganese is therefore suggested. Since a manganese deficiency has been shown to be related to a reduction in bone phosphatase activity, this relationship should not be overlooked.

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APPENDIX

APPENDIX A

DETAILED DATA SHOWING THE EFFECTS OF TREATMENTS TESTED IN THE VARIOUS EXPERIMENTS

TABLE 1. EFFECT OF TREATMENTS TESTED IN EXPERIMENT I ON THE GROWTH OF RATS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Treatment	Replications 1	2	3	4	5	6	7	Total	Mean
	4 weeks weight gain (gm.)								
1	175	215	203	193	164	180		1130	188
2	205	207	191	205	195	179		1182	197
3	179	149	135	168	156	171	169	1127	161
4	111	155	129	88	113	84	114	794	113
Total	670	726	658	654	628	614	283	4233	

TABLE 2. EFFECT OF TREATMENTS TESTED IN EXPERIMENT I ON BONE CALCIUM LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Treatment	Replication 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	176.94	127.68	132.22	148.54	130.30	162.05	877.73	146.29
2	121.43	147.58	155.54	159.64	149.71	141.02	874.92	143.82
3	82.68	146.37	133.50	150.65	140.51	134.48	788.19	131.37
4	82.76	138.49	111.36	99.14	124.28	87.66	643.69	107.41
Total	463.81	560.12	532.62	557.97	544.80	525.21	3184.53	

TABLE 3. EFFECT OF TREATMENTS TESTED IN EXPERIMENT I ON BONE PHOSPHORUS LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	71.00	61.37	62.58	72.67	52.00	76.61	396.23	66.04
2	46.33	83.34	44.79	24.20	77.10	48.28	324.04	54.01
3	26.11	62.90	43.31	36.87	33.42	44.06	246.67	40.78
4	31.93	32.05	42.74	39.84	61.50	29.36	237.42	39.57
Total	175.37	239.66	193.42	173.58	224.02	198.31	1204.36	

TABLE 4. EFFECT OF TREATMENTS TESTED IN EXPERIMENT I ON BONE MAGNESIUM LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	mg/gm. dry weight							
1	3.63	3.29	3.38	3.44	3.44	3.54	20.72	3.45
2	3.07	3.46	3.50	3.17	3.67	3.34	20.20	3.37
3	3.07	3.48	3.58	3.47	3.61	3.43	20.65	3.44
4	3.30	3.44	3.53	3.56	3.36	3.45	20.64	3.44
Total	13.07	13.68	13.99	13.64	14.08	13.77	82.21	

TABLE 5. EFFECT OF TREATMENTS IN EXPERIMENT I ON BONE ZINC LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	0.10	0.14	0.06	0.08	0.09	0.08	0.55	0.09
2	0.58	0.73	0.65	0.50	0.56	0.48	3.49	0.58
3	1.45	1.65	0.96	1.50	1.62	0.88	8.06	1.34
4	2.98	2.47	2.07	2.18	2.37	1.95	14.02	2.34
Total	5.12	4.97	3.74	4.26	4.65	3.38	26.12	

TABLE 6. EFFECT OF TREATMENTS TESTED IN EXPERIMENT II ON BONE CALCIUM LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

Replications

	Treatment	1	2	3	4	Total	Mean
		mg./gm. dry weight					
Week 1	1	135.08	130.49	146.48	148.13	560.18	140.05
	2	127.19	135.38	128.27	136.32	527.16	131.79
	3	107.76	117.01	106.70	121.27	452.74	113.19
	4	114.26	126.02	119.47	104.48	464.23	116.06
	Total	484.29	508.90	500.92	510.20	2004.31	
Week 2	1	149.19	147.51	151.87	153.69	602.26	150.57
	2	152.89	128.65	146.86	145.16	573.56	143.39
	3	97.94	126.94	133.22	131.88	489.98	122.50
	4	114.70	106.55	112.32	123.20	456.77	114.19
	Total	514.72	509.65	544.27	553.93	2122.57	
Week 3	1	148.46	162.64	144.61	168.32	624.03	156.01
	2	133.07	156.05	153.20	136.00	578.32	144.58
	3	142.28	121.93	155.47	149.58	569.26	142.32
	4	112.65	139.59	135.42	114.49	502.15	125.56
	Total	536.46	580.21	588.70	568.39	2273.76	
Week 4	1	160.87	179.75	179.42	150.83	670.87	167.72
	2	159.99	150.58	151.21	155.12	616.90	154.23
	3	156.05	137.37	136.65	144.42	574.49	143.62
	4	126.39	114.98	117.22	128.04	486.63	121.66
	Total	603.30	582.68	584.50	578.41	2348.89	

TABLE 7. EFFECT OF TREATMENTS TESTED IN EXPERIMENT II ON BONE PHOSPHORUS LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

	Treatment	Replications 1	2	3	4	Total	Mean
		mg./gm. dry weight					
Week 1	1	24.36	26.99	32.95	20.44	104.74	26.19
	2	13.17	15.02	16.02	41.01	85.22	21.31
	3	25.14	28.38	35.36	42.47	131.35	32.84
	4	11.78	18.06	17.22	20.44	67.50	16.88
	Total	74.45	88.45	101.55	124.36	388.81	
Week 2	1	17.94	41.68	18.06	56.96	134.64	34.41
	2	79.73	46.46	17.40	25.68	169.27	42.32
	3	14.43	19.23	15.06	47.04	95.76	23.94
	4	50.09	34.05	59.20	29.37	172.71	43.18
	Total	162.19	141.42	109.72	159.05	572.38	
Week 3	1	11.40	35.86	25.93	73.05	146.24	36.56
	2	15.59	21.29	18.00	25.53	80.41	20.10
	3	37.62	21.88	15.04	26.99	101.53	25.38
	4	34.94	39.78	15.21	29.54	119.47	29.87
	Total	99.55	118.81	74.18	155.11	447.65	
Week 4	1	22.99	18.35	14.73	14.94	71.01	17.75
	2	17.79	15.34	25.22	21.26	79.61	19.90
	3	43.50	37.09	45.90	32.94	159.43	39.86
	4	16.03	25.10	20.58	17.54	79.25	19.81
	Total	100.31	95.88	106.43	86.68	389.30	

TABLE 8. EFFECT OF TREATMENTS TESTED IN EXPERIMENT II ON BONE MAGNESIUM LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

		Replications					
	Treatment	1	2	3	4	Total	Mean
		mg./gm. dry weight					
Week 1	1	2.61	2.21	2.81	2.67	10.29	2.57
	2	2.54	2.78	2.82	2.74	10.89	2.72
	3	2.28	2.82	2.44	2.70	10.25	2.56
	4	2.30	2.43	2.67	2.61	10.01	2.50
	Total	9.73	10.23	10.73	10.72	41.41	
Week 2	1	2.11	2.69	2.67	2.87	10.35	2.59
	2	2.13	2.42	2.67	2.94	10.16	2.54
	3	3.90	3.73	2.76	2.46	12.86	3.21
	4	2.43	2.55	2.42	2.76	10.16	2.54
	Total	9.57	11.39	10.53	11.03	43.52	
Week 3	1	2.39	2.88	2.65	3.33	11.25	2.81
	2	2.88	2.64	2.71	2.78	11.01	2.75
	3	2.91	2.75	2.56	2.64	10.86	2.71
	4	2.78	3.06	2.79	2.70	11.33	2.83
	Total	10.96	11.33	10.71	11.45	44.45	
Week 4	1	2.44	2.45	2.38	2.97	10.24	2.56
	2	2.60	2.19	2.81	2.80	10.40	2.60
	3	2.93	3.10	2.86	2.93	11.82	2.96
	4	2.54	2.86	2.56	2.25	10.21	2.55
	Total	10.51	10.60	10.61	10.95	42.67	

TABLE 9. EFFECT OF TREATMENTS TESTED IN EXPERIMENT II ON BONE ZINC LEVELS

Treatments

1 - Control
2 - 0.25% Zinc
3 - 0.5% Zinc
4 - 0.75% Zinc

		Replications					
	Treatment	1	2	3	4	Total	Mean
		mg./gm. dry weight					
Week 1	1	0.13	0.18	0.08	0.13	0.52	0.13
	2	0.41	0.46	0.29	0.44	1.59	0.40
	3	0.43	0.86	0.55	0.60	2.44	0.61
	4	1.44	1.03	0.73	0.85	4.04	1.01
	Total	2.40	2.53	1.64	2.01	8.59	
Week 2	1	0.08	0.09	0.08	0.07	0.32	0.08
	2	0.44	0.60	0.64	0.47	2.15	0.54
	3	0.98	0.64	1.09	0.87	3.59	0.90
	4	1.65	1.16	1.67	1.38	5.85	1.46
	Total	3.15	2.49	3.48	2.79	11.91	
Week 3	1	0.13	0.15	0.13	0.11	0.52	0.13
	2	0.99	0.56	0.77	0.77	3.09	0.77
	3	1.84	1.81	1.84	1.60	7.09	1.77
	4	2.72	2.19	2.41	2.23	9.55	2.39
	Total	5.68	4.71	5.15	4.71	20.25	
Week 4	1	0.15	0.12	0.16	0.16	0.59	0.15
	2	1.10	0.55	0.78	0.69	3.12	0.78
	3	1.54	1.76	1.82	1.62	6.74	1.68
	4	2.52	2.21	2.51	2.31	9.55	2.39
	Total	5.31	4.64	5.27	4.78	20.00	

TABLE 10. EFFECT OF TREATMENTS TESTED IN EXPERIMENT III ON THE GROWTH OF RATS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.08% Mg
6 - 0.75% Zn + 0.4% Ca + 0.4% P
7 - 0.75% Zn + 0.4% Ca + 0.08% Mg
8 - 0.75% Zn + 0.4% P + 0.08% Mg
9 - 0.75% Zn + 0.4% Ca + 0.4% P + 0.08% Mg

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	4 week weight gain (gm.)							
1	198	204	(184)[a]	160	174	174	1094	182
2	86	76	105	101	85	132	585	97
3	103	121	127	165	134	106	756	126
4	104	104	103	71	136	118	636	106
5	117	105	142	120	108	87	679	113
6	157	163	133	142	166	164	925	154
7	126	125	107	136	157	128	779	129
8	150	114	151	98	91	164	768	128
9	134	129	170	97	139	170	839	139
Total	1175	1141	1222	1090	1190	1243	7061	

( )[a] indicates calculated missing plot value.

TABLE 11. EFFECT OF TREATMENTS TESTED IN EXPERIMENT III ON BONE CALCIUM LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.08% Mg
6 - 0.75% Zn + 0.4% Ca + 0.4% P
7 - 0.75% Zn + 0.4% Ca + 0.08% Mg
8 - 0.75% Zn + 0.4% P + 0.08% Mg
9 - 0.75% Zn + 0.4% Ca + 0.4% P + 0.08% Mg

Treatment	Replications 1	2	3	4	Total	Mean
	mg./gm. dry weight					
1	174.07	168.93	171.47	136.96	651.43	162.86
2	114.03	124.21	102.29	103.24	443.77	110.94
3	114.09	131.85	154.93	134.88	535.75	133.94
4	104.82	132.80	107.43	92.69	437.74	109.44
5	117.04	99.40	88.17	95.70	400.31	100.18
6	151.23	153.40	182.33	145.89	632.85	185.21
7	124.41	145.05	187.32	124.55	581.33	145.33
8	139.28	103.76	107.20	118.72	468.96	117.24
9	132.16	114.83	121.04	135.72	503.75	125.94
Total	1171.13	1174.23	1222.18	1088.35	4655.89	

TABLE 12. EFFECT OF TREATMENTS TESTED IN EXPERIMENT III ON BONE PHOSPHORUS LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.08% Mg
6 - 0.75% Zn + 0.4% Ca + 0.4% P
7 - 0.75% Zn + 0.4% Ca + 0.08% Mg
8 - 0.75% Zn + 0.4% P + 0.08% Mg
9 - 0.75% Zn + 0.4% Ca + 0.4% P + 0.08% Mg

Treatment	Replications 1	2	3	4	Total	Mean
	mg./gm. dry weight					
1	49.54	44.94	38.40	40.64	173.52	43.38
2	31.52	20.44	31.81	17.79	101.56	25.39
3	45.58	20.64	24.42	17.32	107.96	26.99
4	36.05	37.26	25.02	29.77	128.10	32.03
5	52.52	29.41	38.29	20.22	140.44	35.11
6	50.46	38.62	29.03	32.74	150.85	37.71
7	41.39	26.54	34.95	47.80	150.68	37.67
8	40.59	45.42	51.29	24.62	161.92	40.48
9	35.04	30.29	35.78	33.95	135.06	33.77
Total	382.69	293.56	308.99	264.85	1250.09	

TABLE 13. EFFECT OF TREATMENTS TESTED IN EXPERIMENT III ON BONE MAGNESIUM LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.08% Mg
6 - 0.75% Zn + 0.4% Ca + 0.4% P
7 - 0.75% Zn + 0.4% Ca + 0.08% Mg
8 - 0.75% Zn + 0.4% P + 0.08% Mg
9 - 0.75% Zn + 0.4% Ca + 0.4% P + 0.08% Mg

Treatment	Replications 1	2	3	4	Total	Mean
	mg./gm. dry weight					
1	3.21	2.70	3.20	2.80	11.91	2.98
2	2.93	3.15	3.14	2.74	11.96	2.99
3	3.08	2.71	2.77	3.10	11.67	2.92
4	2.93	2.79	2.73	2.63	11.07	2.77
5	3.70	2.86	3.24	3.00	12.80	3.20
6	3.17	2.70	3.33	2.81	12.01	3.00
7	3.44	3.43	3.62	3.44	13.93	3.48
8	3.25	2.69	3.69	2.84	12.46	3.11
9	3.54	2.81	3.00	3.37	12.73	3.18
Total	29.25	25.85	28.73	26.72	110.55	

TABLE 14. EFFECT OF TREATMENTS TESTED IN EXPERIMENT III ON BONE ZINC LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.08% Mg
6 - 0.75% Zn + 0.4% Ca + 0.4% P
7 - 0.75% Zn + 0.4% Ca + 0.08% Mg
8 - 0.75% Zn + 0.4% P + 0.08% Mg
9 - 0.75% Zn + 0.4% Ca + 0.4% P + 0.08% Mg

Treatment	Replications 1	2	3	4	Total	Mean
	mg./gm. dry weight					
1	0.08	0.14	0.09	0.11	0.42	0.11
2	3.38	2.70	2.49	2.17	10.75	2.69
3	0.87	2.16	2.04	1.81	6.87	1.72
4	1.62	2.32	2.39	1.47	7.79	1.95
5	2.68	1.82	1.85	2.83	9.17	2.29
6	1.17	0.99	1.03	1.04	4.24	1.06
7	1.68	1.30	1.55	1.18	5.71	1.43
8	1.44	1.87	1.73	1.14	6.18	1.54
9	0.66	0.99	1.02	0.68	3.35	0.84
Total	13.57	14.30	14.20	12.41	54.49	

TABLE 15. EFFECT OF TREATMENTS TESTED IN EXPERIMENT IV ON THE GROWTH OF RATS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.8% Ca
6 - 0.75% Zn + 0.8% P
7 - 0.75% Zn + 1.2% Ca
8 - 0.75% Zn + 1.2% P
9 - 0.75% Zn + 0.4% Ca + 0.4% P
10 - 0.75% Zn + 0.8% Ca + 0.8% P
11 - 0.75% Zn + 1.2% Ca + 1.2% P

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	4 weeks weight gain (gm.)							
1	144	139	132	160	140	165	889	148
2	62	127	117	100	106	112	624	104
3	113	126	125	114	138	129	745	124
4	119	106	118	106	121	108	678	113
5	139	132	126	128	125	136	786	131
6	117	128	110	128	118	94	695	116
7	121	132	130	112	109	115	719	120
8	126	123	116	126	109	112	713	119
9	134	131	133	119	143	128	788	131
10	125	123	115	154	131	145	793	132
11	111	101	117	128	123	110	690	115
Total	1311	1368	1339	1375	1363	1354	8110	

TABLE 16. EFFECT OF TREATMENTS TESTED IN EXPERIMENT IV ON BONE CALCIUM LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.8% Ca
6 - 0.75% Zn + 0.8% P
7 - 0.75% Zn + 1.2% Ca
8 - 0.75% Zn + 1.2% P
9 - 0.75% Zn + 0.4% Ca + 0.4% P
10 - 0.75% Zn + 0.8% Ca + 0.8% P
11 - 0.75% Zn + 1.2% Ca + 1.2% P

Replications

Treatment	1	2	3	4	5	6	Total	Mean
				mg./gm. dry weight				
1	176.70	174.12	151.60	150.61	156.78	170.36	980.17	163.36
2	108.21	136.20	107.47	98.27	118.28	130.02	698.45	116.41
3	126.85	140.91	119.30	116.27	147.28	143.10	793.71	132.29
4	112.11	103.99	118.09	113.80	132.58	129.14	709.71	118.29
5	147.43	138.28	160.64	138.05	152.65	157.65	894.70	149.12
6	128.60	130.88	129.63	125.33	123.72	109.28	747.44	124.57
7	147.69	146.97	152.94	147.81	132.53	152.26	880.20	146.70
8	127.33	144.53	124.67	115.36	129.41	114.76	756.06	126.01
9	139.18	158.75	163.18	118.10	146.16	150.50	875.87	145.98
10	156.02	145.53	115.26	153.31	148.38	160.88	879.38	146.56
11	151.07	149.44	170.09	159.37	146.16	163.84	939.97	156.66
Total	1521.19	1569.60	1512.87	1436.28	1533.93	1581.79	9155.66	

TABLE 17. EFFECT OF TREATMENTS TESTED IN EXPERIMENT IV ON BONE PHOSPHORUS LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.8% Ca
6 - 0.75% Zn + 0.8% P
7 - 0.75% Zn + 1.2% Ca
8 - 0.75% Zn + 1.2% P
9 - 0.75% Zn + 0.4% Ca + 0.4% P
10 - 0.75% Zn + 0.8% Ca + 0.8% P
11 - 0.75% Zn + 1.2% Ca + 1.2% P

Treatments	Replications 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	42.64	43.07	41.78	52.45	35.74	28.74	244.42	40.74
2	30.83	27.02	39.73	38.82	25.60	30.76	192.76	32.13
3	44.46	38.86	27.72	27.72	42.70	29.82	211.28	35.21
4	44.35	40.40	29.82	24.94	25.13	27.75	192.39	32.06
5	35.34	40.52	23.89	18.73	37.52	26.86	182.86	30.48
6	23.90	34.10	28.55	39.53	31.42	15.82	173.32	28.89
7	36.80	39.15	41.08	25.90	25.17	32.59	200.69	33.45
8	46.02	31.86	22.86	19.59	37.75	30.96	189.04	31.51
9	31.67	26.64	30.77	32.16	26.06	22.57	169.87	28.31
10	40.66	46.32	23.23	29.58	51.66	36.24	227.72	37.95
11	32.88	43.80	38.57	21.68	24.57	30.61	192.11	32.02
Total	409.55	411.77	348.00	331.10	363.32	312.72	2176.46	

TABLE 18. EFFECT OF TREATMENTS TESTED IN EXPERIMENT IV ON BONE MAGNESIUM LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.8% Ca
6 - 0.75% Zn + 0.8% P
7 - 0.75% Zn + 1.2% Ca
8 - 0.75% Zn + 1.2% P
9 - 0.75% Zn + 0.4% Ca + 0.4% P
10 - 0.75% Zn + 0.8% Ca + 0.8% P
11 - 0.75% Zn + 1.2% Ca + 1.2% P

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	1.35	2.18	2.25	2.99	2.89	2.80	14.46	2.41
2	1.40	2.40	2.29	2.31	2.69	2.82	13.91	2.32
3	2.80	2.12	1.86	1.75	2.82	1.75	13.10	2.18
4	1.96	2.48	2.49	2.05	2.35	2.38	13.71	2.28
5	1.70	2.45	1.63	2.25	2.82	2.22	13.07	2.18
6	2.52	2.19	1.84	2.29	2.54	2.21	13.59	2.27
7	1.39	2.32	2.12	2.67	2.49	2.61	13.60	2.27
8	1.56	2.31	1.67	2.12	1.95	1.85	11.46	1.91
9	3.04	1.83	2.04	2.50	2.65	2.43	14.49	2.42
10	1.41	1.47	1.86	1.61	2.68	1.68	10.71	1.79
11	1.01	0.84	1.39	1.54	2.23	(1.42)[a]	8.43	1.41
Total	20.14	22.59	21.44	24.08	28.11	24.17	140.53	

[a] ( ) indicates calculated missing plot value.

TABLE 19. EFFECT OF TREATMENTS TESTED IN EXPERIMENT IV ON BONE ZINC LEVELS

Treatments

1 - Control
2 - 0.75% Zn
3 - 0.75% Zn + 0.4% Ca
4 - 0.75% Zn + 0.4% P
5 - 0.75% Zn + 0.8% Ca
6 - 0.75% Zn + 0.8% P
7 - 0.75% Zn + 1.2% Ca
8 - 0.75% Zn + 1.2% P
9 - 0.75% Zn + 0.4% Ca + 0.4% P
10 - 0.75% Zn + 0.8% Ca + 0.8% P
11 - 0.75% Zn + 1.2% Ca + 1.2% P

Treatment	Replications 1	2	3	4	5	6	Total	Mean
	mg./gm. dry weight							
1	0.11	0.09	0.06	0.09	0.14	0.11	0.60	0.10
2	0.65	1.22	1.18	1.76	1.66	1.17	7.63	1.29
3	1.45	0.88	1.41	0.84	1.41	0.20	6.19	1.03
4	0.73	1.03	0.48	1.03	1.11	1.56	5.95	0.99
5	1.10	1.08	0.76	0.69	1.08	1.26	5.96	0.99
6	0.63	0.30	0.57	0.83	1.03	0.74	4.09	0.68
7	0.65	0.78	0.91	1.26	1.78	1.45	6.81	1.13
8	0.24	0.34	0.41	0.49	0.61	0.31	2.39	0.40
9	0.59	0.59	0.62	1.42	0.89	0.44	4.55	0.76
10	0.23	0.41	0.16	(0.49)[a]	0.36	0.49	2.16	0.28
11	0.15	0.08	0.27	0.30	0.48	0.30	1.57	0.26
Total	6.52	6.80	6.83	9.21	10.54	8.00	47.89	

( )[a] indicates calculated missing plot value.

APPENDIX B

METABOLISM DATA

TABLE 1. EFFECT OF ZINC ON THE NET RETENTION OF CALCIUM

(EXPERIMENT V)

Collection period 1

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	363.94	332.49	345.97	314.52	320.97	311.80	325.56	320.97
Mg. excreted in the urine	0.49	0.52	1.17	0.21	1.93	1.46	2.27	2.23
Mg. excreted in the feces	119.05	108.99	145.65	88.91	173.34	215.64	248.62	233.61
Net retention	244.40	222.98	199.15	225.40	145.70	94.70	74.67	85.13

Collection period 2

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	399.89	404.38	386.41	384.16	348.48	371.41	353.07	362.24
Mg. excreted in the urine	0.58	0.54	0.38	0.47	1.44	1.32	3.41	1.24
Mg. excreted in the feces	196.38	131.81	110.61	151.22	295.39	311.66	342.65	310.15
Net retention	202.93	272.03	275.42	232.47	51.65	58.43	8.01	50.85

TABLE 1. (continued)

Collection period 3

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	395.39	433.58	408.87	399.89	378.29	378.29	389.75	371.41
Mg. excreted in the urine	1.29	0.71	1.31	1.23	3.14	2.87	1.38	1.11
Mg. excreted in the feces	233.90	184.23	187.69	162.18	260.69	245.79	327.68	294.45
Net retention	160.20	248.64	219.87	236.48	114.46	129.63	60.69	75.85

Collection period 4

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	491.99	467.28	456.05	471.78	398.92	426.43	434.14	371.41
Mg. excreted in the urine	2.50	2.29	1.58	0.97	1.00	4.07	1.53	1.36
Mg. excreted in the feces	282.61	236.49	215.88	220.70	324.67	308.04	397.35	345.78
Net retention	206.88	228.50	238.59	250.11	73.25	114.32	43.26	24.27

TABLE 2. EFFECT OF ZINC ON THE NET RETENTION OF PHOSPHORUS

(EXPERIMENT V)

Collection period 1

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	477.14	435.90	453.58	412.34	407.40	395.76	413.22	407.40
Mg. excreted in the urine	106.50	96.75	113.00	98.25	52.00	51.50	57.75	48.25
Mg. excreted in the feces	58.78	53.52	61.04	51.09	(150.02)[a]	138.54	148.80	152.03
Net retention	311.86	285.63	279.54	263.00	205.38	205.72	206.67	207.12

Collection period 2

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	524.26	530.15	506.59	503.65	442.32	471.42	448.14	459.78
Mg. excreted in the urine	151.75	149.75	133.00	136.75	77.58	87.50	139.50	110.50
Mg. excreted in the feces	(53.12)[a]	56.73	62.36	68.62	193.40	237.00	182.31	189.28
Net retention	319.39	323.67	311.23	298.28	171.13	146.92	126.33	159.80

TABLE 2. (continued)

Collection period 3

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	518.37	568.44	536.04	524.26	480.15	480.15	494.70	471.42
Mg. excreted in the urine	191.00	170.28	164.86	133.64	103.46	67.44	115.54	108.43
Mg. excreted in the feces	97.74	80.91	75.48	73.92	216.81	109.35	158.63	120.21
Net retention	229.63	317.25	295.70	316.70	159.88	303.36	220.53	242.78

Collection period 4

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	645.02	612.62	597.90	618.51	506.34	541.26	538.35	471.42
Mg. excreted in the urine	205.40	193.62	179.49	169.37	92.01	75.07	119.36	116.66
Mg. excreted in the feces	151.73	95.32	75.70	94.59	192.93	133.47	222.55	188.41
Net retention	287.89	323.68	342.71	354.55	221.40	332.72	196.44	166.35

( )[a] indicates calculated missing plot value.

TABLE 3. EFFECT OF ZINC ON THE NET RETENTION OF MAGNESIUM

(EXPERIMENT V)

Collection period 1

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	25.13	22.96	23.89	21.72	18.98	18.44	19.26	18.98
Mg. excreted in the urine	0.12	1.56	0.40	0.89	0.98	0.79	0.64	0.94
Mg. excreted in the feces	6.14	6.54	7.17	7.43	9.35	8.42	8.37	6.40
Net retention	18.98	14.86	16.32	13.40	8.65	9.23	10.25	11.64

Collection period 2

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	27.62	27.93	26.69	26.53	20.61	21.97	20.88	21.42
Mg. excreted in the urine	0.19	0.11	(0.15)[a]	0.14	1.98	1.97	1.38	(1.77)[a]
Mg. excreted in the feces	8.36	7.11	8.75	8.07	9.38	8.64	12.15	10.32
Net retention	19.07	20.71	18.79	18.32	9.25	11.36	7.35	9.33

TABLE 3. (continued)

Collection period 3

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	27.31	29.94	28.24	27.62	22.37	22.37	23.05	21.97
Mg. excreted in the urine	1.44	0.89	1.12	0.92	1.64	1.52	0.34	0.29
Mg. excreted in the feces	10.79	8.78	6.68	8.48	8.45	12.18	11.37	11.37
Net retention	15.08	20.27	20.44	18.22	12.28	8.67	11.34	10.33

Collection period 4

	Control Replications				0.75% Zinc Replications			
	1	2	3	4	1	2	3	4
Mg. per total feed intake	33.98	32.27	31.50	32.58	23.59	25.22	25.09	21.97
Mg. excreted in the urine	1.26	0.60	0.28	0.49	1.28	1.01	0.97	1.11
Mg. excreted in the feces	13.59	12.73	7.11	10.56	6.87	13.49	11.11	9.64
Net retention	19.11	18.94	24.11	21.53	15.44	10.72	13.01	11.22

( )[a] indicates calculated missing plot value.

APPENDIX C

ANALYSES OF VARIANCE DATA

TABLE 1. ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT I

Source of Variation	Degrees of freedom	Sum of squares	Mean square
		Growth	
Total	23	32,769.8334	
Replications	5	1,904.8334	380.9666
Treatments	3	25,593.8334	8,531.2777**
1,2 vs. 3,4	1	18,928.1666	18,928.1666**
1 vs. 2	1	225.3333	225.3333
3 vs. 4	1	6,440.3333	6,440.3333**
Error	15	5,271.1666	351.4111
		Bone calcium	
Total	23	14,339.6837	
Replications	5	1,579.0199	315.8039
Treatments	3	6,027.9035	2,009.3011*
1,2 vs. 3,4	1	4,287.2247	4,287.2247**
1 vs. 2	1	0.6580	0.6580
3 vs. 4	1	1,740.0208	1,740.0208
Error	15	6,732.7603	448.8506
		Bone phosphorus	
Total	23	7,136.3346	
Replications	5	874.3784	174.8756
Treatments	3	2,795.6213	921.8737*
1,2 vs. 3,4	1	2,324.2080	2,324.2080**
1 vs. 2	1	434.2830	434.2830
3 vs. 4	1	7.1302	7.1302
Error	15	3,466.3349	234.8889
		Bone magnesium	
Total	23	0.5834	
Replications	5	0.1586	0.0317
Treatments	3	0.0280	0.0093
Error	15	0.3968	0.0264

TABLE 1. (continued)

Source of Variation	Degrees of Freedom	Sum of squares	Mean square
		Bone zinc	
Total	23	18.5343	
Replications	5	0.5957	0.1191
Treatments	3	17.2600	5.7533**
1,2 vs. 3,4	1	13.5751	13.5751**
1 vs. 2	1	0.7252	0.7252**
3 vs. 4	1	2.9601	2.9601**
Error	15	0.6786	0.0452

* Significant ($p \leq 0.05$)

** Highly significant (≤ 0.01)

TABLE 2. ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT II

Source of variation	Degrees of freedom	Sum of squares	Mean square
	Growth		
Week 2			
Total	47	7,530	
Replications	11	887	80.64
Treatments	3	4,735	1,578.00**
Error	33	1.908	57.82
	Bone calcium		
Week 1			
Total	15	2,656.2485	
Replications	3	106.5641	35.5200
Treatments	3	251,078.6610	655.6378**
Error	9	582.7708	64.7500
	Bone phosphorus		
Week 4			
Total	15	3,328.1154	
Replications	3	872.5431	290.8477
Treatments	3	583.9242	194.6414
Error	9	1,871.6481	207.9609
	Bone magnesium		
Week 4			
Total	15	1.1448	
Replications	3	0.0281	0.0093
Treatments	3	0.4480	0.1493
Error	9	0.6687	0.6743
	Bone zinc		
Week 1			
Total	15	2.0704	
Replications	3	0.1215	0.0405
Treatments	3	1.6602	0.5534**
Error	9	0.2887	0.6320

*Significant ($p \leq 0.05$)

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

TABLE 3. ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT III

Source of variation	Degrees of freedom	Sum of squares	Mean square
	Growth		
Total	53	53,625.8149	
Replications	5	1,765.7037	353.1407
Treatments	8	32,619.1482	4,077.3936**
2,4,5 vs. 3,7,8	1	4,511.3611	4,511.3611**
6,9 vs. 7,8	1	1,962.0416	1,962.0416*
6 vs. 9	1	616.3333	616.3333
Error	40	19,240.9630	481.0241
	Bone calcium		
Total	35	23,789.7741	
Replications	3	1,029.2579	343.0859
Treatments	8	15,931.5928	1,991.4491**
2,4,5 vs. 3,6,7	1	9,130.2905	9,130.2905**
1 vs. 3	1	1,115.1552	1,115.1552
2 vs. 9	1	299.8000	299.8000
Error	24	6,828.9234	284.5384
	Bone phosphorus		
Total	35	3,430.5605	
Replications	3	43,409.0280	303.2289
Treatments	8	1,123.8380	104.4797*
2,3 vs. 5,9	1	272.0850	272.0850*
2,3 vs. 6,7	1	529.1150	529.1150**
1 vs. 6,7	1	86.2983	86.2983
Error	24	1,397.0358	58.2098
	Bone magnesium		
Total	35	3.5115	
Replications	3	0.9478	0.3159
Treatments	8	1.2894	0.1611*
1,2,3,4 vs. 5,6,8,9	1	0.3591	0.3591*
Error	24	1.2743	0.0530

TABLE 3. (continued)

Source of variation	Degrees of freedom	Sum of squares	Mean square
		Bone zinc	
Total	35	23.4409	
Replications	3	0.2523	0.0841
Treatments	8	19.4690	2.4336**
2,5 vs. 3,4	1	1.7292	1.7292**
3,4,8 vs. 6,7,9	1	2.5026	2.5026**
6 vs. 7	1	0.2738	0.2738
6 vs. 9	1	0.0968	0.0968
Error	24	3.7196	0.1549

*Significant ($p \leq 0.05$)

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

TABLE 4. ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT IV

Source of variation	Degrees of freedom	Sum of squares	Mean square
	Growth		
Total	65	16,326.9849	
Replications	5	275.5303	55.1060
Treatments	10	8,744.1515	874.4151**
5,9,10 vs. 4,6,11	1	2,567.1111	2,567.1111**
1 vs. 5,9,10	1	1,250.0000	1,250.0000**
2 vs. 4,6,8,11	1	648.6750	648.6750*
Error	50	7,307.3031	146.1460
	Bone calcium		
Total	65	23,108.1012	
Replications	5	1,211.0511	242.2102
Treatments	10	15,201.4119	1,520.1411**
2 vs. 3,5,7	1	3,110.7642	3,110.7642**
1 vs. 11	1	134.6700	134.6700
11 vs. 7,9,10	1	472.5762	472.5762**
Error	50	6,695.6832	133.9127
	Bone phosphorus		
Total	65	4,312.6585	
Replications	5	755.9830	151.1966
Treatments	10	837.4993	83.7499
Error	50	2,719.1762	54.3835
	Bone magnesium		
Total	64	535.6410	
Replications	5	3.4874	0.6974
Treatments	10	5.7255	0.5725
Error	49	526.4281	10.5285
	Bone zinc		
Total	64	14.4518	
Replications	5	1.1751	0.2350
Treatments	10	9.3345	0.9334**
1 vs. 10,11		0.1785	0.1785*
6,9 vs. 8		0.4158	0.4158**
2 vs. 3		0.1728	0.1728*
2,7 vs. 4,5		0.2677	0.2677**
4,5 vs. 3,7		0.0499	0.0499
Error	49	3.9422	0.0712

*Significant ($p \leq 0.05$)

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