THE ABILITY OF A BASAL RATION TO ALLEVIATE
BIOLOGICAL CHANGES IN RATS RESULTING FROM
PREVIOUS CONSUMPTION OF HIGH ZINC DIETS

by

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7079

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science in Home Economics

Greensboro
May, 1965

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SCOTT, ALICE SMITH. The Ability of A Basal Ration to Alleviate Biological Changes in Rats Resulting from Previous Consumption of High Zinc Diets. (1965) Directed by: Dr. Aden C. Magee. pp. 92.

The ability of a basal ration to alleviate the adverse effects of a high level of dietary zinc on growth, hemoglobin, liver mineral constituents, and bone mineralization in young male rats was studied.

Zinc toxicity resulted in a marked decrease in growth and hemoglobin levels. Significant decreases in liver copper and iron deposition associated with zinc toxicity did not occur until the second week. A marked increase in liver zinc was produced by the 0.75% level of zinc. Bone calcium was significantly decreased from the beginning while the decrease in bone phosphorus did not occur until the fourth week. A highly significant increase in bone magnesium and bone zinc occurred.

The basal ration improved the subnormal growth and the depressed liver iron deposition of the young male rats, and completely alleviated the adverse effect of zinc on hemoglobin, liver copper, and liver zinc. The basal ration produced a further decrease in bone calcium and caused the bone magnesium level to drop below the normal level of the control animals. The decrease in phosphorus and the increase in bone zinc were improved by the basal ration but were not returned to the normal level of the control animals.

ACKNOWLEDGEMENTS

The author wishes to express her sincere gratitude to her advisor, Dr. Aden C. Magee, for his guidance and advising during this study. Appreciation is also extended to the members of the advisory committee, Miss Louise Lowe, Mrs. Mary Dickey, and Dr. Bruce Eberhart, for their constructive criticisms, and to Miss Nena Philbrick for her technical assistance.

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CHAPTER I

INTRODUCTION

For many years nutritionists have been interested in the effects of mineral toxicities on the animal organism, and numerous research studies have been conducted to increase knowledge concerning the mechanisms involved. One of the most recent minerals to be investigated has been that of zinc. Although the adverse effects of zinc on the animal have been described, the method of the interference of zinc with various biological systems in the body has not been determined.

Previous studies have revealed that rats fed high levels of dietary zinc (0.5 to 1.0%) displayed a depressed growth rate; hypochromic, microcytic anemia; a decrease in liver iron and liver copper; an increase in liver zinc; an interference with the normal deposition of calcium and phosphorus in the bones; and an increase in the deposition of zinc in the bones.

In experiments designed to determine how zinc interferes with various metabolic pathways, many researchers have utilized the technique of adding various supplements to the diets of zinc-fed rats in an effort to alleviate and/or prevent the abnormal changes produced by excess dietary zinc. The primary emphasis has been on preventing the adverse effects of zinc in the beginning, and no studies have been conducted to determine how existing conditions in the animal body resulting from zinc toxicity can be corrected. Some of the supplements previously used may complicate metabolic conditions in the animal rather than aid in the alleviation of the adverse effects of zinc. The addition of various supplements may further upset the delicate nutrient balance which exists in the animal body.

The abnormal conditions in the animal resulting from zinc toxicity have been well described, but the permanency or non-permanency of these conditions has not been determined. Although excessive zinc may result in permanent biological changes in the body, certain abnormal conditions may not be permanent and can be corrected. This study was directed toward the solution of some of these problems because it might give additional information pertaining to the alleviation and/or correction of existing conditions caused by nutrient toxicities.

CHAPTER II

REVIEW OF LITERATURE

Although Sutton and Nelson (1) first reported that high levels of dietary zinc were associated with decreased growth and lowered hemoglobin levels of rats in 1937, approximately twenty years passed before any attempts were made to determine how excessive zinc was interfering with the growth and hemoglobin formation mechanisms of the animal.

In 1946 Smith and Larson (2) found that a liver extract would alleviate the adverse effect of zinc on growth. Supplements of copper or a mixture of copper, iron, and cobalt prevented the lowering of hemoglobin levels of the zinc-fed rats, but these minerals had no effect on the subnormal growth of the zinc-fed rats. Smith and Larson concluded that the effects of zinc on growth and hemoglobin formation were separate and distinct. Although Duncan _et al._ (3) and Grant-Frost and Underwood (4) reported that copper supplements improved the growth of zinc-fed rats, the results of several studies (5, 6, 7) confirmed the earlier findings of Smith and Larson (2) that copper supplements could not alleviate subnormal growth associated with zinc toxicity.

In addition to subnormal growth and lowered hemoglobin levels, zinc toxicity has been shown to be associated with decreases in catalase, cytochrome oxidase, xanthine oxidase and δ-aminolevulinate dehydratase activities (3, 5, 8, 9, 10), decreases in tissue copper and iron levels (4, 6, 7), marked increases in liver and bone zinc deposition (7, 11, 12, 13), and marked decreases in bone calcium and phosphorus levels (13, 14, 15, 16).

In attempting to determine how zinc interferes with various metabolic pathways in the animal body, various researchers have added supplements to high zinc diets to see if the adverse effects of zinc on various biological systems could be prevented or alleviated. Results of several studies indicated that dietary supplements of liver, distiller's dried solubles, and calcium and phosphorus could alleviate the adverse effect of zinc toxicity on weight gain (2, 7, 12, 13, 17). McCall _et al_. (18) reported that the severity of zinc toxicity on weight gain was dependent upon the source and the level of dietary protein, but Magee and Spahr (12) observed that high levels of dietary protein accentuated the severity of zinc toxicity. Copper supplementation has been shown to be effective in preventing the adverse effect of zinc toxicity on various enzyme systems (3, 5, 7, 10) and liver copper deposition (4, 6, 7). Recently, Chang (17) has shown that calcium and phosphorus supplements will partially alleviate the adverse effect of zinc on hemoglobin levels and on liver copper deposition.

While the results of the studies previously mentioned show how the adverse effect of zinc toxicity on the animal body can be prevented, the mechanisms involved still remain unsolved. No studies have been conducted to determine if excessive zinc results in permanent damage to the metabolic systems involved. There is also no information available to indicate how an established zinc toxicity condition can be corrected. An indication of a possible method of correcting a zinc toxicity condition has been observed by Magee.[1] He found that the adverse effect of zinc toxicity on hemoglobin levels of rats can be alleviated by removing the excess zinc from the diet and placing the animals on a normal basal ration. There is the possibility that other adverse conditions in the rats associated with zinc toxicity could be similarly alleviated by this method. Such a study might also give an indication of whether some of the metabolic pathways subject to interference by zinc are permanently damaged.

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[1] A. C. Magee, 1964. Unpublished data.

CHAPTER III

EXPERIMENTAL PROCEDURES

The primary objectives of this study were (a) to investigate the ability of a basal ration to correct certain biological changes in young rats which resulted from previous consumption of a high zinc diet and (b) to determine the length of time that a high level of zinc can be consumed by rats that would result in abnormal conditions which could not be alleviated by a basal ration. Criteria used to evaluate the effectiveness of the basal ration were weight gain; hemoglobin level; copper, iron, and zinc levels of the liver; and the deposition of calcium, phosphorus, magnesium, and zinc in the bone.

Sixty weanling male albino rats[1] of the Sprague-Dawley strain were used in the study. The animals were divided into six groups. All groups, except Group I and Group VI, consisted of ten animals. Group I consisted of fourteen animals, and there were six animals in Group VI. The animals were housed in individual wire-bottom cages in an air conditioned room maintained at 76° F. The animals were randomized into replications according to initial body weight. Group numbers and animals within a replication

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[1]Purchased from Holtzman Company, Madison, Wisconsin

were assigned at random to individual cages. All animals received food and water *ad libitum*. Each animal was weighed weekly during the experimental period.

All animals in Group I received a non-fortified basal ration the entire experimental period. Animals in Group II-V received the basal ration plus 0.75% of zinc and the basal ration. Rats in Group VI received the basal ration plus 0.75% of zinc the entire experimental period.

A feeding procedure was followed so that the animals in Groups II, III, IV, and V received the high zinc ration for one, two, three, and four weeks, respectively. At the end of the predetermined time on the high zinc diet, four animals from the particular group in question were sacrificed. The remainder of the animals in these groups (II-V) were placed on the basal ration for four weeks. For comparative purposes, randomly selected control animals from Group I were sacrificed. Animals in Group I which were not killed at various intervals throughout the experiment received the basal ration for a total of eight weeks. Animals in Group VI received the high zinc diet for eight weeks.

The composition of the basal ration is shown in Table 1. Zinc in the carbonate form was added to the basal ration at the expense of cornstarch for the high zinc diet.

Prior to sacrificing, blood samples were taken from the tails of the animals. Hemoglobin determinations were

TABLE 1

COMPOSITION OF THE BASAL DIET

Constituents	Per Cent
Casein[a]	20
Corn starch[b]	61
Vegetable fat[c]	10
Mineral mix[d]	4
Vitamin mix[e]	2
Cellulose[f]	2
Cod liver oil[g]	1

[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; $FePO_4.2H_2O$, 1.470; $MnSO_4$, 0.020; $MgSO_4$, 9.000; $KAl\ (SO_4)_2.12H_2O$, 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. 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 the Nutritional Biochemicals Corporation, Cleveland, Ohio.

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

[g]E. R. Squibb and Sons, New York. This cod liver oil is listed to contain 1700 U. S. P. units of Vitamin A and 170 U. S. P. units of Vitamin D per 1 gram.

made on these blood samples by the method of Shenk et al. (19).

Livers of all animals were removed at the time of sacrifice and weighed. A small amount (0.5-1.0 gram) of each liver was removed and dried in an oven at 35°C to provide dry weight data. The remainder of each liver was prepared for mineral analyses by ashing with nitric and perchloric acids on a hot plate. Three ml. of 0.6N HCl were used to dissolve the ash of each sample, and redistilled water was used to bring the volume to 25 ml. Copper and iron contents of the liver samples were determined by the methods of Parks et al. (20) and Kitzes et al. (21), respectively, as modified by Matrone et al. (22). Zinc contents of the livers were determined by means of an atomic absorption spectrophotometer.[2]

As each animal was sacrificed, one femur was removed and frozen until mineral analyses were made on the bones. The bones were thawed, cleaned of muscle and fat, dried at 35°C in an oven, and weighed on an analytical balance. The bones were prepared for subsequent mineral analyses by the wet-ashing technique previously mentioned. The ash of each bone was dissolved in three ml. of 0.6N HCl and brought to a volume of 100 ml. with redistilled water. One ml. of this solution was rediluted to 25ml. Appropriate aliquots of

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[2]Model 303 Atomic Absorption Spectrophotometer, Perkin-Elmer Corporation, Norwalk, Connecticut.

the second dilution were taken for the calcium and phosphorus determinations. Magnesium determinations were made by using appropriate aliquots of the first dilution of each bone sample. One ml. of the original dilution of each sample was rediluted to 10 ml., and appropriate aliquots were taken for the zinc determinations.

Calcium, phosphorus, and magnesium contents of the bones were determined by the methods of Weybrew _et_ _al_. (23), Simonsen _et_ _al_. (24), and Simonsen _et_ _al_. (25). Zinc contents of the bones were determined with the atomic absorption spectrophotometer.

A slight modification was necessary in the calcium and magnesium methods because the bone samples had a high degree of alkalinity. In order to prevent co-precipitation of calcium and magnesium, 0.2 ml. of 6N HCl was added to the test solution directly following the addition of the indicator, brom cresol green.

A randomized block design was used in the study, and all data were subjected to an analysis of variance. Statements of significance are based on odds of at least 19 to 1 ($p \leq 0.05$). Least significant difference (L.S.D.) values for each criterion, except weight gain, studied were calculated to give an indication of the difference between two group means that is required to show significance.

CHAPTER IV

RESULTS AND DISCUSSION

Growth and Hemoglobin Data

Detailed data obtained during this study are presented in Appendix A. The mean values of weight gain and hemoglobin levels are presented in Tables 2 and 3, respectively.

Growth

A high level of dietary zinc resulted in a highly significant decrease ($p \leq 0.01$) in weight gain in young rats (Table 2). The amount of growth retardation associated with zinc toxicity increased as the length of time a 0.75% level of zinc was consumed by the animals increased. When the zinc-fed rats were placed on the basal ration for a period of four weeks, the average growth rates of all the zinc-fed groups of animals were equal to that of the control animals. There was no significant difference in the weight gains between the control animals which received the basal diet only and the animals which were given the 0.75% zinc diet for one week and the basal for four weeks.

Hemoglobin

Mean hemoglobin values obtained from the various groups of animals are given in Table 3. Analysis of the

TABLE 2

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON WEIGHT GAIN OF YOUNG RATS PRODUCED BY 0.75% ZINC

Weeks on Diets	Dietary Regimen: Basal	Dietary Regimen: 0.75% Zn	Weeks on Diets	Dietary Regimen: Basal	Dietary Regimen: 0.75% Zn[a] Followed by Basal
	Weight gain in gm.			Weight gain in gm.	
1	40 (14)[b]	28 (10)	5	244 (6)	233 (6)
2	94 (14)	64 (10)	6	284 (6)	250 (6)
3	150 (10)	93 (10)	7	317 (6)	250 (6)
4	202 (6)	110 (10)	8	352 (6)	289 (6)
8	352 (6)	133 (5)			

[a]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[b]Each figure in ( ) represents the number of animals composing the mean for each group.

TABLE 3

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON HEMOGLOBIN LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b] 0.75% Zn	Basal
	gm. Hb/ 100 ml.		gm. Hb/ 100 ml.	
1	10.69[c]	1	8.71	12.79
2	11.69[c]	2	7.64	13.16
3	11.50	3	6.93	12.98
4	12.60	4	4.84	13.01
8	13.85	8	2.65[d]	
	L.S.D.[e] 0.05	.394		
	L.S.D. 0.01	.523		

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

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 7 animals.

[d]Figure represents the mean of 5 animals.

[e]Least significant difference at specified probability levels.

results (Appendix C, Table 3) indicated that a level of 0.75% zinc resulted in a highly significant decrease ($p \leq 0.01$) in the hemoglobin levels of young rats. The adverse effect of zinc on hemoglobin level was more pronounced the longer the animals received the high zinc diet. When the zinc-fed rats were placed on the basal for four weeks, the hemoglobin concentrations returned to levels which were higher than those observed in the control animals.

Liver Mineral Constitutents

Detailed information on the effects of 0.75% of zinc on liver copper, iron, and zinc is shown in Appendix A. Mean values of these criteria are presented in Table 4, 5, and 6, respectively.

Copper

As the length of time the animals received the high zinc diet increased, a progressive decrease in liver copper deposition was observed (Table 4). However, a significant decrease in liver copper did not occur until animals had received zinc for two weeks. When the excess zinc was removed from the diet and the animals were placed on the basal ration, the depressed liver copper levels returned to levels which were not significantly different from those found in the control animals.

TABLE 4

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES OF LIVER COPPER LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Diet	Dietary Regimen: 0.75% Zn	Dietary Regimen: Basal[b]
	mcg. Cu/gm. dry wt.		mcg. Cu/gm. dry wt.	
1	23.27	1	19.85	18.29
2	11.16	2	8.50	14.94
3	13.13[c]	3	5.85	13.76
4	13.75	4	1.79	14.48
8	15.06	8	1.34	
	$L.S.D._{0.05}$[d]	7.11		
	$L.S.D._{0.01}$	9.52		

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figure represents the mean of 3 animals.

[d]Least significant difference at specified probability levels.

Iron

A 0.75% level of dietary zinc resulted in highly significant decreases ($p \leq 0.01$) in liver iron concentrations of young rats. With increasing weeks on zinc, a progressive decrease in liver iron resulted (Table 5). The difference in the liver iron levels of the rats as compared to the control animals was more pronounced after two weeks on zinc. When the zinc-fed experimental rats were placed on a basal ration for four weeks, an increase in liver iron resulted in all groups. The decrease in liver iron was alleviated by a basal ration in rats consuming the high zinc diet as long as three weeks, but did not alleviate the decrease in liver iron in the animals which had consumed the high zinc diet for a period of four weeks.

Zinc

Analysis of the data (Appendix C, Table 3) indicated that a consumption of 0.75% of zinc was associated with a highly significant increase ($p \leq 0.01$) in liver zinc (Table 6). As the number of weeks on the zinc diet increased, the level of zinc in the liver greatly increased. Placing the experimental animals on a basal diet resulted in a marked decrease in liver zinc accumulation. The liver zinc levels in the experimental animals were reduced to the liver zinc levels of the control animals with the basal ration.

TABLE 5

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON LIVER IRON LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen 0.75% Zn	Dietary Regimen Basal[b]
	mcg. Fe/gm. dry wt.		mcg. Fe/gm. dry wt.	mcg. Fe/gm. dry wt.
1	192.25	1	131.34	289.95
2	246.87	2	113.90	243.28
3	297.64	3	139.83	249.16
4	398.64	4	167.96	278.37
8	386.09	8	157.15	

L.S.D.[c] 0.05 78.21

L.S.D. 0.01 104.64

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Least significant difference at specified probability levels.

TABLE 6

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON LIVER ZINC LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b] 0.75% Zn	Basal
	mcg. Zn/gm. dry wt.		mcg. Zn/gm. dry wt.	
1	80.53[c]	1	398.57	84.47
2	70.89	2	799.02	84.23
3	65.90[c]	3	758.63	80.85
4	79.77	4	863.76	86.21
8	70.55	8	525.03	
	L.S.D.[d] $_{0.05}$		211.74	
	L.S.D. $_{0.01}$		287.77	

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 3 animals.

[d]Least significant difference at specified probability levels.

Bone Mineral Deposition

Detailed data obtained during this study are presented in Appendix B. The mean values of bone calcium, phosphorus, magnesium, and zinc levels are presented in Tables 7, 8, 9, and 10, respectively.

Calcium

Analysis of the data (Appendix C, Table 4) indicated that the addition of 0.75% of zinc to the diets of weanling rats produced a highly significant difference ($p \leq 0.01$). In general, as the rats remained on zinc for an increasing number of weeks, the calcium levels of the bones decreased. The basal ration failed to alleviate the decrease in bone calcium in the zinc-fed animals (Table 7). Instead, a further decrease in the calcium levels was observed after the experimental animals had consumed the basal ration for four weeks.

Phosphorus

Analysis of the data (Appendix C, Table 4) indicated that a significant decrease ($p \leq 0.05$) in bone phosphorus levels did not occur until the animals had consumed the high zinc diet for four weeks (Table 8). When the zinc-fed rats were placed on a basal ration for four weeks, a general increase in the phosphorus levels occurred. At the end of the four weeks feeding period of the basal ration, no significant differences in the phosphorus levels existed

TABLE 7

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON BONE CALCIUM LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b] 0.75% Zn	Basal
	mg. Ca/gm. dry wt.		mg. Ca/gm. dry wt.	
1	567.93[c]	1	528.80	407.28
2	648.56	2	504.30	337.18
3	403.34[c]	3	565.36	292.60
4	357.73	4	326.08	281.94
8	434.69	8	279.58	
	L.S.D.[d] 0.05	166.85		
	L.S.D. 0.01	226.76		

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 3 animals.

[d]Least significant difference at specified probability levels.

TABLE 8

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON BONE PHOSPHORUS LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b] 0.75% Zn	Dietary Regimen[b] Basal
	mg. P/gm. dry wt.		mg. P/gm. dry wt.	
1	69.29[c]	1	64.69	87.93
2	68.17	2	66.14	86.79
3	94.57[c]	3	82.57	85.63
4	89.83	4	56.29	64.76
8	90.63	8	52.07	
	L.S.D.[d] 0.05	28.23		
	L.S.D. 0.01	38.36		

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 3 animals.

[d]Least significant difference at specified probability levels.

between the control animals and the experimental animals.

Magnesium

The addition of 0.75% dietary zinc to the diet of young rats, in general, resulted in highly significant increases ($p \leq 0.01$) in bone magnesium deposition (Table 9). However, when the excess zinc was removed from the diet, bone magnesium dropped to levels which were significantly lower ($p \leq 0.01$) than those found in the control animals.

Zinc

Analysis of the data (Appendix C, Table 4) indicated that the intake of 0.75% zinc was associated with a highly significant increase ($p \leq 0.01$) in deposition of zinc in the bones (Table 10). Young rats fed a high level of zinc showed a marked increase in bone zinc as compared to the control animals. When the previously fed zinc animals were placed on a basal ration, a highly significant decrease ($p \leq 0.01$) in bone zinc accumulation occurred. These levels of bone zinc, however, were still greater than those observed in the control animals.

TABLE 9

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON BONE MAGNESIUM LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b]: 0.75% Zn	Dietary Regimen[b]: Basal
	mg. Mg/gm. dry wt.		mg. Mg/gm. dry wt.	
1	6.53[c]	1	7.09	4.38
2	4.49	2	5.81	4.05
3	5.23[c]	3	5.28	4.20
4	4.69	4	5.63	4.26
8	4.38	8	4.18	
	L.S.D.[d] $_{0.05}$	.328		
	L.S.D. $_{0.01}$	.445		

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 3 animals.

[d]Least significant difference at specified probability levels.

TABLE 10

THE ABILITY OF A BASAL RATION TO ALLEVIATE THE ADVERSE CHANGES ON BONE ZINC LEVELS OF YOUNG RATS[a] PRODUCED BY 0.75% ZINC

Rats Fed Basal Diet Only		Rats Fed High Zinc and Basal Diets		
Weeks on Diet		Weeks on Zinc	Dietary Regimen[b] 0.75% Zn	Basal
	mg. Zn/gm. dry wt.		mg. Zn/gm. dry wt.	
1	1.78[c]	1	11.96	4.30
2	1.60	2	32.90	8.26
3	1.88[c]	3	36.43	10.19
4	1.37	4	39.18	11.31
8	1.78	8	39.33	
	L.S.D.[d] 0.05	5.67		
	L.S.D. 0.01	7.71		

[a]Each figure represents the mean of 4 animals unless otherwise indicated.

[b]Randomly selected animals from the various groups receiving the 0.75% Zn diet were placed on a basal diet for a period of 4 weeks.

[c]Figures represent the mean of 3 animals.

[d]Least significant difference at specified probability levels.

CHAPTER V

GENERAL DISCUSSION

Many of the biological changes associated with zinc toxicosis reported by previous researchers were observed throughout this study. Some of these biological changes were subnormal growth rates, decreases in blood hemoglobin levels, decreases in liver copper and iron levels, increases in liver zinc levels, decreases in bone calcium and phosphorus levels, and increases in bone magnesium and zinc levels.

The results of this study suggest that, although zinc retards the growth of young rats, the mechanism which controls growth does not appear to be damaged by the excess zinc. When the excess zinc was removed from the diet, the animals exhibited growth rates which were equal to those found in the animals receiving only the basal ration. If the growth mechanism had been damaged, such growth rates would not have been expected.

Although the presence of a high level of zinc in the diets of young rats prevents the normal formation of hemoglobin, the results of this study indicate that zinc does not permanently damage the mechanism involved with hemoglobin synthesis. Normal hemoglobin levels were observed in the zinc-fed rats when the extra zinc was removed from the diet and these rats were placed on the basal ration. Perhaps, zinc interferes with hemoglobin formation through its adverse effect on one or more of the necessary hemopoietic factors, such as copper and iron. The excess zinc may facilitate the removal of such factors from the site of hemoglobin formation. Once the excess zinc has been removed from the diet, the interference is eliminated. The hemopoietic factors move back to the site of hemoglobin formation, and normal hemoglobin synthesis is resumed.

Although high levels of zinc resulted in marked decreases in liver copper levels, this abnormal condition does not appear to be permanent. When excess zinc was removed from the diet, normal deposition of copper in the liver occurred. The fact that the basal ration resulted in a reversal of the depressed liver copper condition would indicate that additional supplements to the diet, such as extra copper, are not needed to combat the adverse effect of zinc on liver copper deposition.

Normal levels of liver iron were restored in rats fed the high zinc diet for two weeks when these animals were placed on the basal ration for four weeks. However, when the animals were kept on the high zinc diets for three and four weeks, the level of iron found in the livers of these animals after maintenance on the basal were lower than those found in the livers of comparable control animals. Although the reason for the failure of the basal ration to completely alleviate the adverse effect of zinc on iron metabolism as it did on copper metabolism is not apparent, there is the possibility that the basal ration does not contain a sufficient amount of iron to supply the amount of iron that is required to make-up for the losses in hemoglobin and tissue iron. There is the possibility that if the zinc-fed rats had been allowed to consume the basal ration for a period of time that was longer than four weeks, the liver iron levels would have returned to normal.

The marked increase in liver zinc deposition in zinc-fed rats is in agreement with the findings of previous investigators (7, 11, 12, 13). However, this marked increase in zinc deposition in the liver of young rats is not permanent. When the excess zinc was removed from the diet, excessive zinc in the liver apparently moved out, and normal liver zinc levels were reached. The data also indicate that a maximum level of abnormal zinc concentration was reached. The data revealed that the same amount of zinc accumulated in the livers of the animals in Groups V and VI. However, the liver weights of the animals in Group VI were heavier than those of the animals in Group V, and this probably accounted for the decrease in liver zinc accumulation observed in the animals receiving the high zinc diet for eight weeks.

High levels of dietary zinc resulted in lowered bone calcium levels which could not be alleviated by removing the excess zinc from the diet and placing the animals on a basal ration. The fact that a further decrease in bone calcium levels occurred when the zinc-fed rats were placed on the basal diet indicates that the mechanism controlling the movement of calcium into the bones is permanently damaged. The movement of zinc from the bones of the zinc-fed rats placed on the basal ration could possibly have facilitated additional movement of calcium from the bones thereby resulting in a further loss of this mineral from this tissue.

Although phosphorus is closely associated with calcium in the bones, the pattern for the movement of bone phosphorus observed in this study was not the same as the pattern of the movement of bone calcium. The levels of bone phosphorus in animals fed the high zinc diet for two weeks followed by the basal diet were higher than the bone phosphorus levels found in the control animals. When rats received the high zinc diet for four weeks, the marked decrease in bone phosphorus could not be completely alleviated with the basal ration.

Previous investigators (12, 13, 17) have reported that the addition of calcium and phosphorus supplements to the diets of zinc-fed rats alleviated the adverse effect of zinc on calcium and phosphorus deposition in the bones. Jowsey and Gershon-Cohen (26) reported that a high calcium diet reversed conditions of experimental osteoporosis in cats produced by a low-calcium diet. There is the possibility that a fortified diet containing supplements of calcium and phosphorus could completely alleviate the adverse effect of zinc on calcium and phosphorus deposition in the bone.

In this study the feeding of a high zinc diet was associated with increases in the deposition of magnesium in the bones of young rats. This finding is not in agreement with the observations of others (13,17). Since bone magnesium levels of the zinc-fed rats decreased when these animals were placed on the basal ration, there is the possibility that the movement of zinc from the bones facilitated a movement of magnesium from the bones that was similar to that observed in the calcium data.

The marked increase in bone zinc deposition in rats consuming high levels of dietary zinc was similar to findings of other researchers (13, 17). When the zinc-fed rats were placed on the basal ration, a highly significant decrease in bone zinc deposition occurred. However, the levels of bone zinc in these animals were still significantly higher than those found in the control animals. These results would suggest that the presence of zinc in the diet elevates the level of zinc in the bones to a concentration that can not be completely alleviated by a basal ration. There is a possibility, however, that if the zinc-fed animals had been kept on the basal ration for a longer period of time, bone zinc concentrations in these animals would have more closely approached those levels found in the control animals.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

The present study was conducted (a) to determine the length of time that a high level of zinc can be consumed by rats that would result in abnormal conditions which could not be alleviated by a basal ration and (b) to investigate the ability of a basal ration to correct certain biological changes in young rats which resulted from previous consumption of a high zinc diet.

A level of 0.75% dietary zinc was fed to the experimental animals for predetermined lengths of time before placing the animals on the basal ration for four weeks. A basal ration was fed to the control animals for the entire experiment.

Criteria used to evaluate the effectiveness of the basal ration were weight gain; hemoglobin levels; copper, iron, and zinc levels of the liver; and the deposition of calcium, phosphorus, magnesium, and zinc in the bones. The data collected from all phases of the experiment were subjected to statistical analyses.

Experimental results showed that a high level of dietary zinc produced a highly significant decrease in weight gain in young rats. However, once the excess zinc was removed from the diet, the animals exhibited a growth rate which was equal to that found in the animals receiving only the basal ration. These results suggest that the growth mechanism had not been damaged, and that, in time, the experimental animals would possibly equal the control animals in weight. Thus, the adverse effect of zinc on growth could possibly be alleviated completely with a basal ration. Zinc toxicosis was also related to a marked decrease in the hemoglobin levels of young rats. When the zinc-fed rats were placed on the basal ration for four weeks, the hemoglobin concentrations returned to levels which were higher than those observed in the control animals. These results suggest that zinc apparently did not damage the mechanism involved with hemoglobin formation, and that a basal ration was effective in alleviating the adverse effects of zinc on hemoglobin. A level of 0.75% dietary zinc produced a similar effect on copper. The high dietary zinc produced a significant decrease in liver copper in the animals which received the zinc diet for two or more weeks. Once the excess zinc was removed from the diet, the animals exhibited copper levels which were equal to those found in the animals receiving only the basal ration. Additional supplements were not needed to alleviate the adverse effect of zinc on liver copper. A highly significant decrease in liver iron was associated with zinc toxicity. The adverse effect on liver iron was alleviated in the animals which had consumed zinc for three weeks, but not in the animals which consumed zinc for four weeks. Zinc apparently affected the mechanism involved with iron deposition to a greater extent than the mechanism involved with copper. A marked increase in liver zinc which resulted from the high zinc diet was alleviated by a basal ration. Apparently, the liver zinc deposition mechanism was not damaged. There was a progressive decrease in weight gain, hemoglobin levels, liver iron and copper deposition and an increase in liver zinc deposition as the length of time the animals consumed the 0.75% level of zinc increased.

Zinc toxicity adversely affected the mineral content of the bones in young rats. Excess zinc produced a marked decrease in calcium levels. A further decrease in the calcium levels was observed after the experimental animals had consumed the basal ration for four weeks. Apparently the mechanism involved in the deposition of calcium in the bones was damaged, and the basal ration was ineffective in its repair. Bone phosphorus deposition was not affected until the animals had consumed zinc for four weeks. The basal ration was partially effective in alleviating the decrease in bone phosphorus. The addition of 0.75% dietary zinc to the diet of young rats, in general, resulted in highly significant increases in bone magnesium deposition.

The removal of excess zinc from the diet resulted in a drop in bone magnesium levels which were significantly lower than those found in the control animals. Apparently, the mechanism was damaged to some degree and the basal ration was unable to alleviate the adverse effect of zinc. As in the liver, excess zinc accumulated in the bones of the young rats. The basal ration produced a significant decrease in bone zinc accumulation, yet, the condition was not completely alleviated in the four weeks period on the basal ration.

Recommendations for Further Investigations

The results of this study substantiate the proposed hypothesis that a basal ration will alleviate some of the adverse effects associated with zinc toxicity. However, the basal ration failed to alleviate completely the adverse effects of zinc on growth; liver iron deposition; bone calcium, magnesium, and zinc deposition. Apparently, some damage has been done by the excess zinc to the various mineral regulating mechanisms. Further information is needed before the nature of the zinc interferences with the above criteria can be clarified.

The adverse effects of zinc on growth, liver iron, and bone zinc can possibly be alleviated by extending the time the zinc-fed rats consumed the basal ration. The basal ration produced a marked increase in growth and liver iron and a significant decrease in bone zinc in the four weeks period. Possibly, greater improvement would occur with an extended period on the basal.

Of considerable interest was the further decrease in bone calcium deposition in the zinc-fed animals when placed on a basal ration. A balance study may be used to determine where the calcium goes after leaving the bones. Radioisotope studies may be used to determine the source and amount of endogenous calcium excreted in the urine of zinc-fed rats when placed on a basal ration.

Contrary to previous findings, zinc appeared to have a stimulatory effect on magnesium causing a significant increase in bone magnesium levels. However, when the zinc-fed animals were placed on the basal ration for four weeks, the bone magnesium levels dropped significantly below the control animals. The intriguing question is, what happened to the magnesium which moved out of the bones? A balance study may be used to determine where this magnesium went after leaving the bones. Also, a radioisotope study may be used to determine the source and amount of endogenous magnesium excreted in the urine of zinc-fed rats when placed on a basal ration.

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APPENDIX A

DETAILED DATA SHOWING THE EFFECTS OF TREATMENT ON GROWTH, HEMOGLOBIN, LIVER COPPER, LIVER IRON, AND LIVER ZINC

TABLE 1

WEIGHT DATA FOR GROUP I

Replications	Initial Weight	Weight at End of Week 1	2	3	4	5	6	7	8
		grams							
1	60	95	150	208	257	297	336	365	403
2	58	95	149	204	257	299	342	386	420
3	57	102	155	213	265	312	348	381	428
4	58	99	152						
5	59	98	153	213	265				
6	61	105	169	236	297				
7	60	102	156						
8	59	94	140	193	237				
9	59	103	163						
10	57	90	141	198	249	295	337	368	398
11	59	99	150	204	256	300	346	382	412
12	55	91	140	200	251				
13	55	97	156						
14	56	101	156	212	265	308	340	368	398
Mean	58	98	152	208	260	302	342	375	410

TABLE 2

WEIGHT DATA FOR GROUP II

Repli- cations	Initial Weight	Weight at End of Week							
		1	2	3	4	5	6	7	8
					grams				
1	59	90	133	184	236	269			
2	59	91	152	207	270	315	358	400	435
3	57	86	127	198	254	299			
4	58	91							
5	60	76							
6	62	91							
7	57	75	133	188	240	285	322	360	388
8	59	93	145	201	252	299			
9	55	84							
10	57	90	142	191	239	276			
Mean	58	87	139	195	249	291	340	380	412

TABLE 3

WEIGHT DATA FOR GROUP III

Repli-cations	Initial Weight	Weight at End of Week							
		1	2	3	4	5	6	7	8
					grams				
1	60	83	108	161	198	238	289		
2	58	91	130	184	250	294	339	368	396
3	56	94	134	187	230	277	318		
4	58	84	108						
5	61	93	124						
6	61	93	136						
7	57	94	132	176	231	275	318	340	365
8	59	82	108	157	213	243	277		
9	56	92	120						
10	58	89	128	186	236	275	309		
Mean	58	90	123	175	226	267	308	354	381

TABLE 4

WEIGHT DATA FOR GROUP IV

Repli- cations	Initial Weight	Weight at End of Week							
		1	2	3	4	5	6	7	8
					grams				
1	62	94	122	137	165	217	257	294	
2	59	87	129	146	164	196	233	253	273
3	57	86	121	162	208	259	309	341	
4	57	90	123	157					
5	61	84	110	119					
6	60	82	114	141					
7	57	85	120	139	184	249	316	357	389
8	58	88	130	166	218	269	298	319	
9	55	55	63	90					
10	57	83	120	160	199	246	277	298	
Mean	58	83	115	142	190	239	282	310	331

TABLE 5

WEIGHT DATA FOR GROUP V

Replications	Initial Weight	Weight at End of Week							
		1	2	3	4	5	6	7	8
		grams							
1	61	95	138	174	196	224	294	345	376
2	58	87	123	144	159	203	280	317	348
3	57	92	136	167	182	206	264	305	314
4	58	90	129	164	189				
5	60	90	123	157	167				
6	63	92	126	141	135				
7	62	89	135	172	205	262	324	357	382
8	59	92	126	148	149	171	218	283	334
9	55	83	115	112	124				
10	57	82	126	161	179	212	271	298	331
Mean	59	89	128	154	169	213	275	318	348

TABLE 6

WEIGHT DATA FOR GROUP VI

Replications	Initial Weight	Weight at End of Week							
		1	2	3	4	5	6	7	8
					grams				
1	61	91	125	156	186	155	148	165	205
2	59	87	123	158	181	194	203	210	205
3	56	88	135	154	184	190	183	176	[a]
4	57	90	142	177	187	191	185	175	162
5	59	82	117	148	173	192	199	204	192
6	58	84	122	158	186	201	200	199	196
Mean	58	87	127	159	183	187	186	188	192

[a]The animal died prior to termination date.

TABLE 7

HEMOGLOBIN DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level
				mg./100 ml. blood				
1	I	9.97	1	II	9.17	1	II	12.86
		12.43			9.37			13.20
		11.06			8.29			14.43
		8.60			10.20			11.92
		12.26			8.00			11.14
		9.28			7.20			13.20
		11.20						
Mean		10.69			8.71			12.79

TABLE 7--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level
			mg./100 ml. blood					
2	I	12.00	2	III	7.66	2	III	12.77
		10.77			8.23			13.40
		12.60			7.89			13.49
		10.06			8.00			12.86
		12.52			6.77			12.09
		11.52			7.26			14.34
		12.34						
Mean		11.69			7.64			13.16

TABLE 7--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level
			mg./100 ml. blood					
3	I	12.77	3	IV	10.69	3	IV	11.37
		11.46			6.17			13.57
		11.77			5.49			13.66
		11.69			5.46			12.69
		10.92			8.49			13.03
		10.40			5.26			13.57
Mean		11.50			6.93			12.98

TABLE 7--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level
			mg./100 ml. blood					
4	I	12.60	4	V	5.49	4	V	12.86
		12.86			4.80			13.40
		12.52			4.20			14.63
		12.77			4.57			12.77
		12.26			3.47			11.52
		12.69			6.23			12.86
Mean		12.60			4.84			13.01

TABLE 7--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Hemoglobin Level	Weeks on Diet	Group	Hemoglobin Level
		mg./100 ml. blood			
8	I	13.03	8	VI	4.43
		14.23			2.27
		14.03			2.02
		14.43			2.31
		13.86			2.20
		13.49			
Mean		13.85			2.65

TABLE 8

LIVER COPPER DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level
			mcg./gm. dry weight					
1	I	30.04	1	II	12.70	1	II	17.48
		15.52			31.87			14.21
		26.99			2.23			11.74
		20.52			12.74			11.44
Total		93.07			59.54			54.87
Mean		23.27			19.85			18.29

TABLE 8--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level
			mcg./gm. dry weight					
2	I	10.94	2	III	16.87	2	III	16.23
		9.89			6.50			17.00
		12.12			2.71			12.35
		11.69			7.92			14.16
Total		44.64			34.00			59.74
Mean		11.16			8.50			14.94

TABLE 8--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level
			mcg./gm. dry weight					
3	I	12.64	3	IV	6.11	3	IV	12.95
		12.90			.78			14.20
		13.86			1.72			13.17
					14.77			14.70
Total		39.40			23.38			55.02
Mean		13.13			5.85			13.76

TABLE 8--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level
			mcg./gm. dry weight					
4	V	12.96	4	V	1.40	4	V	13.18
		12.12			1.61			14.34
		15.53			1.56			16.58
		14.52			2.60			13.80
Total		55.03			7.16			57.90
Mean		13.75			1.79			14.48

TABLE 8--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Copper Level	Weeks on Diet	Group	Copper Level
		mcg./gm. dry weight			
8	I	15.37	8	VI	1.21
		14.16			1.63
		14.94			1.29
		15.75			1.23
Total		60.22			5.36
Mean		15.06			1.34

TABLE 9

LIVER IRON DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level
			mcg./gm. dry weight					
1	I	146.82	1	II	206.11	1	II	376.31
		180.56			88.87			247.67
		176.10			126.02			262.68
		265.50			104.36			273.13
Total		768.98			525.36			1159.79
Mean		192.25			131.34			289.95

TABLE 9--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level
			mcg./gm. dry weight					
2	I	223.48	2	III	113.68	2	III	211.64
		258.97			93.06			240.77
		242.39			104.96			185.75
		262.65			143.89			334.97
Total		987.49			455.59			973.13
Mean		246.87			113.90			243.28

TABLE 9--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level
				mcg./gm. dry weight				
3	I	323.45	3	IV	143.88	3	IV	253.63
		277.10			156.44			283.86
		285.13			116.07			218.88
		304.89			142.94			240.27
Total		1190.57			559.33			996.64
Mean		297.64			139.83			249.16

TABLE 9--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level
					mcg./gm. dry weight			
4	I	291.32	4	V	142.32	4	V	160.60
		482.30			129.59			246.28
		491.61			207.95			418.86
		329.31			191.97			287.73
Total		1594.54			671.83			1113.47
Mean		398.64			167.96			278.37

TABLE 9--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Iron Level	Weeks on Diet	Group	Iron Level
		mcg./gm. dry weight			
8	I	384.81	8	VI	140.51
		415.98			125.19
		357.97			178.43
		385.63			184.45
Total		1544.39			628.58
Mean		386.09			157.15

TABLE 10

LIVER ZINC DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
				mcg./gm. dry weight				
1	I	78.42	1	II	24.09	1	II	108.56
		77.19			698.92			77.72
		85.99			18.01			72.90
					853.27			78.68
Total		241.60			1594.29			337.86
Mean		80.53			398.57			84.47

TABLE 10--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
					mcg./gm. dry weight			
2	I	69.98	2	III	777.05	2	III	85.36
		60.86			686.11			82.54
		74.63			660.71			83.60
		78.10			1072.19			85.42
Total		283.57			3196.06			336.92
Mean		70.89			799.02			84.23

TABLE 10--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mcg./gm. dry weight					
3	I	64.53	3	IV	598.48	3	IV	80.99
		73.43			853.71			80.49
		59.75			699.77			78.82
					882.54			83.11
Total		197.71			3034.50			323.41
Mean		65.90			758.63			80.85

TABLE 10--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mcg./gm. dry weight					
4	I	80.73	4	V	669.98	4	V	73.68
		75.64			637.73			99.13
		85.46			975.44			87.18
		77.25			1171.90			84.86
Total		319.08			3455.05			344.85
Mean		79.77			863.76			86.21

TABLE 10--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
		mcg./gm. dry weight			
8	I	72.75	8	VI	503.80
		65.16			504.24
		71.37			567.65
		72.92			524.43
Total		282.20			2100.12
Mean		70.55			525.03

APPENDIX B

DETAILED DATA SHOWING THE EFFECTS OF TREATMENT ON BONE CALCIUM, BONE PHOSPHORUS, BONE MAGNESIUM, AND BONE ZINC

TABLE 1

BONE CALCIUM DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level
				mg./gm. dry weight				
1	I	452.80	1	II	616.96	1	II	374.42
		436.19			631.35			371.61
		814.81			362.03			347.75
					504.87			535.33
Total		1703.80			2115.21			1629.11
Mean		567.93			528.80			407.28

TABLE 1--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level
			mg./gm. dry weight					
2	I	808.28	2	III	366.91	2	III	365.54
		933.23			560.20			344.39
		375.91			653.52			313.85
		476.81			436.55			324.93
Total		2594.23			2017.18			1348.71
Mean		648.56			504.30			337.18

TABLE 1--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level
			mg./gm. dry weight					
3	I	346.77	3	IV	523.06	3	IV	302.93
		451.27			614.17			251.16
		411.98			517.13			280.17
					607.06			336.14
Total		1210.02			2261.42			1170.40
Mean		403.34			565.36			292.60

TABLE 1--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level
			mg./gm. dry weight					
4	I	372.94	4	V	291.61	4	V	257.48
		476.62			304.77			232.49
		325.44			306.66			318.57
		255.91			401.29			319.20
Total		1430.91			1304.33			1127.74
Mean		357.73			326.08			281.94

TABLE 1--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Calcium Level	Weeks on Diet	Group	Calcium Level
		mg./gm. dry weight			
8	I	528.36	8	VI	341.29
		420.57			231.25
		443.03			304.30
		346.78			241.48
Total		1738.34			1118.32
Mean		434.69			279.58

TABLE 2

BONE PHOSPHORUS DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level
			mg./gm. dry weight					
1	I	56.84	1	II	70.76	1	II	100.41
		59.03			69.64			86.62
		92.00			60.16			58.45
					58.21			106.24
Total		207.87			258.77			351.72
Mean		69.29			64.69			87.93

TABLE 2--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level
			mg./gm. dry weight					
2	I	105.89	2	III	60.80	2	III	73.26
		90.83			64.06			97.75
		36.02			60.44			101.45
		39.94			79.27			74.69
Total		272.68			264.57			347.15
Mean		68.17			66.14			86.79

TABLE 2--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level
			mg./gm. dry weight					
3	I	103.52	3	IV	88.13	3	IV	101.22
		78.14			65.13			41.10
		102.04			85.87			100.54
					91.15			99.67
Total		283.70			330.28			342.53
Mean		94.57			82.57			85.63

TABLE 2--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level
			mg./gm. dry weight					
4	I	101.55	4	V	47.02	4	V	54.07
		97.69			55.74			54.35
		91.45			67.50			59.26
		68.63			54.89			91.34
Total		359.32			225.15			259.02
Mean		89.83			56.29			64.76

TABLE 2--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Phosphorus Level	Weeks on Diet	Group	Phosphorus Level
		mg./gm. dry weight			
8	I	107.69	8	VI	38.58
		96.81			67.67
		59.71			33.41
		98.29			68.63
Total		362.50			208.29
Mean		90.63			52.07

TABLE 3

BONE MAGNESIUM DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level
					mg./gm. dry weight			
1	I	7.16	1	II	5.33	1	II	4.58
		5.59			6.34			4.38
		6.84			10.23			4.44
					6.45			4.10
Total		19.59			28.35			17.50
Mean		6.53			7.09			4.38

TABLE 3--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level
			mg./gm. dry weight					
2	I	5.26	2	III	5.87	2	III	4.28
		3.58			5.21			4.25
		4.41			5.64			4.11
		4.72			6.52			3.57
Total		17.97			23.24			16.21
Mean		4.49			5.81			4.05

TABLE 3--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level
			mg./gm. dry weight					
3	I	5.30	3	IV	5.07	3	IV	4.70
		4.64			5.41			3.45
		5.74			4.66			3.98
					6.00			4.68
Total		15.68			21.14			16.81
Mean		5.23			5.28			4.20

TABLE 3--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level
					mg./gm. dry weight			
4	I	4.91	4	V	5.33	4	V	4.57
		4.36			5.60			4.09
		4.66			6.08			4.02
		4.81			5.53			4.37
Total		18.74			22.54			17.05
Mean		4.69			5.63			4.26

TABLE 3--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Magnesium Level	Weeks on Diet	Group	Magnesium Level
		mg./gm. dry weight			
8	I	4.28	8	VI	4.38
		4.51			3.56
		4.24			4.50
		4.50			4.28
Total		17.53			16.72
Mean		4.38			4.18

TABLE 4

BONE ZINC DATA

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mg./gm. dry weight					
1	I	1.14	1	II	16.28	1	II	4.21
		2.69			4.53			3.47
		1.51			12.54			4.70
					14.49			4.83
Total		5.34			47.48			17.21
Mean		1.78			11.69			4.30

TABLE 4--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mg./gm. dry weight					
2	I	1.49	2	III	30.22	2	III	7.81
		1.64			32.59			9.05
		1.39			33.46			8.57
		1.86			31.31			7.61
Total		6.38			127.58			33.04
Mean		1.60			31.90			8.26

TABLE 4--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mg./gm. dry weight					
3	I	1.72	3	IV	40.67	3	IV	10.74
		2.17			44.05			10.88
		1.76			40.59			9.45
					20.40			9.70
Total		5.65			145.71			40.77
Mean		1.88			36.43			10.19

TABLE 4--Continued

Basal-Fed Rats			Zinc-Fed Rats Before Basal Feed			Zinc-Fed Rats After Basal Feed		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
			mg./gm. dry weight					
4	I	1.51	4	V	43.10	4	V	11.39
		1.53			39.80			10.12
		1.09			40.65			11.33
		1.35			33.18			12.40
Total		5.48			156.73			45.24
Mean		1.37			39.18			11.31

TABLE 4--Continued

Basal-Fed Rats			Zinc-Fed Rats		
Weeks on Diet	Group	Zinc Level	Weeks on Diet	Group	Zinc Level
		mg./gm. dry weight			
8	I	1.99	8	VI	31.90
		1.71			40.25
		1.74			46.80
		1.69			38.38
Total		7.13			157.33
Mean		1.78			39.33

APPENDIX C

ANALYSES OF VARIANCE DATA

TABLE 1

ANALYSES OF VARIANCE OF WEIGHT GAIN DATA
BEFORE CHANGE OVER TO BASAL RATION

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Group I vs. Group II			
Total	19	1141	
Between Categories	1	616	616**
Within Categories	18	525	29
Group I vs. Group III			
Total	19	6205	
Between Categories	1	4381	4381**
Within Categories	18	1824	101
Group I vs. Group IV			
Total	19	30468	
Between Categories	1	16018	16018**
Within Categories	18	14450	803
Group I vs. Group V			
Total	19	50761	
Between Categories	1	42687	42687**
Within Categories	18	8074	449
Group I vs. Group VI			
Total	9	420329	
Between Categories	1	418541	418541**
Within Categories	8	1788	224

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

TABLE 2

ANALYSES OF VARIANCE OF WEIGHT GAIN DATA AFTER CHANGE OVER TO BASAL RATION

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Group I vs. Group II			
Total	11	2092	
Between Categories	1	397	397
Within Categories	10	1695	170
Group I vs. Group III			
Total	11	6162	
Between Categories	1	3334	3334**
Within Categories	10	2828	283
Group I vs. Group IV			
Total	11	20445	
Between Categories	1	12740	12740**
Within Categories	10	7705	771
Group I vs. Group V			
Total	11	15942	
Between Categories	1	12097	12097**
Within Categories	10	3845	385

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

TABLE 3

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

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Hemoglobin	
Total	82	1036.03	
Category	13	955.07	73.47**
Replications	5	6.42	
Error	64	74.54	1.16
		Liver Copper	
Total	54	2726.08	
Category	13	1747.02	134.38**
Replications	3	41.00	
Error	38	938.06	24.68
		Liver Iron	
Total	55	544383.51	
Category	13	422770.82	32520.83**
Replications	3	4741.59	
Error	39	116871.10	2996.69
		Liver Zinc	
Total	53	6059792.33	
Category	13	5116975.52	393613.50**
Replications	3	150892.97	
Error	37	791923.84	21403.34

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

TABLE 4

ANALYSES OF VARIANCE OF BONE CALCIUM, BONE PHOSPHORUS, BONE MAGNESIUM, AND BONE ZINC DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Bone Calcium	
Total	53	1260000.88	
Category	13	756795.63	58215.04**
Replications	3	11598.01	
Error	37	491606.74	13286.66
		Bone Phosphorus	
Total	53	24865.48	
Category	13	10247.68	788.28
Replications	3	543.78	
Error	37	14075.02	380.40
		Bone Magnesium	
Total	53	69.16	
Category	13	47.01	3.61**
Replications	3	2.89	
Error	37	19.26	.52
		Bone Zinc	
Total	53	12647.44	
Category	13	12035.32	925.79**
Replications	3	45.72	
Error	37	566.40	15.30

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