TISSUE DISTRIBUTION OF ZINC IN THE RAT AS RELATED TO DIETARY ZINC REQUIREMENT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
BRUNA E. RUKSAN
1968

ABSTRACT

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By Bruna E. Ruksan

Two experiments, involving a total of 89 weanling rats, were conducted to determine the requirement of zinc for normal growth. The relationship between the level of zinc in the diet with appetite, growth rate, food efficiency and zinc concentration of the tissues was studied. A study on the interrelation between copper and zinc for low concentration in the diet was also included.

In the first experiment anorexia and growth retardation was observed in the group on the basal diet, and two animals of this group died after a few days with diarrhea. The relation between zinc content of the diet and appetite, growth rate, food efficiency and bone zinc was highly significant ($P < 0.01$). No differences were observed in the zinc content in kidneys and liver with the zinc in the diet. However, copper concentration of liver decreased with the increment of zinc in the diet.

In the second experiment, part A the results obtained for appetite, growth rate and food efficiency confirmed the observation of the first experiment and indicated that the dietary requirement of the rat for zinc approximates 10 ppm.

From the previous observations on the significant increase in zinc content of bones with the zinc level of diet, the group of rats on basal diet was interchanged with the groups on 10 ppm and 15 ppm zinc diets, in order to study the effect of previous diets on growth rate, appetite, food efficiency, zinc content of bones, liver

and kidneys and copper concentration of kidneys and liver.

On the repletion with zinc in the deficient group, a high increment in appetite, growth rate and zinc content of bones was observed. While on depletion with basal diet in the 10 ppm and 15 ppm zinc diet groups, the appetite was decreased, the weight was arrested and the zinc content of bones decreased significantly, but neither liver nor kidney concentration of zinc was affected.

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biochemistry

1968

ACKNOWLEDGMENT

The author wishes to express her sincere appreciation to Dr. R. W. Luecke for his guidance and interest throughout the course of this investigation.

The author also wishes to thank the members of Department of Biochemistry for their helpful suggestions and assistance.

Acknowledgment is also due to the Food and Agriculture Organization of the United Nations (FAO) and Instituto Nacional de Tecnologia Agropecuaria (INTA)-Argentina for the fellowhsip, which made possible this project.

The author is especially grateful to her parents, relatives, friends and members of FAO-INTA Project for the encouragement, understanding and love throughout the course of these studies.

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I. INTRODUCTION

The zinc requirement in the diet by the animals for the normal growth and development is variable and depends upon the other nutrients present and their concentration in the diet.

The value existing for the zinc requirement for rats, for normal growth fed a diet with egg white as a protein source is 12.6 ppm of zinc (Forbes and Yohe, 1960). Luecke et al. (1968) showed that high levels of supplementary biotin were required to prevent symptoms of biotin deficiency from appearing and that resulted in an improvement of growth of rats on the zinc supplemented diet but not in the animals on deficient diet.

There are conflicting reports in the literature on the antagonism existing between zinc and copper at low concentrations. Davis (1958) reported that increasing the level of zinc in the diet causes a depression of the copper values in the liver, but only when the copper levels in the diet approach those of low normal levels of around 5 to 10 ppm. This was not observed by Bunn and Matrone (1966) who found that the liver copper concentration only decreased by the action of zinc and cadmium together, but no effect was observed by either one individually. Reinhold et al. (1967) did not find any effect of low zinc intake upon the concentration of copper in the tissues.

Macapinlac et al. (1965) observed zinc concentrations in bone to decrease by two-thirds in rats when fed diets low in zinc. Also it was suggested by many investigators that bone might play an important

role in stored zinc, releasing it for use by other critical organs.

From the studies by Forbes, (1961); Likuski and Forbes, (1965); Fox and Harrison, (1964) and Hurley et al., (1964) the zinc concentration in bone ash appeared to be a sensitive reflection of zinc absorption, especially at suboptimal intake in the young growing animals.

The present series of investigations were initiated in order to 1--determine the effects of supplemental zinc fed at various levels on appetite, growth rate and food efficiency to weanling rats fed egg-white protein diet supplemented with biotin. 2--Evaluate the zinc concentration in tissue in relation to the zinc content of the diet as a possible use as an aid in the diagnosis of zinc deficiency. 3--The study of interrelation of zinc-copper in tissues was also included in these experiments in order to obtain more information on the antagonism of these elements at low concentration in the diet. In order to attain these objectives analysis for copper and zinc in tissues were made, in addition to collecting growth and food consumption data.

II. REVIEW OF LITERATURE

Zinc an Essential Micronutrient

Zinc was first demonstrated to be an essential nutrient for living organisms when Raulin (1869) showed that it is necessary for the growth of Aspergillus niger. This finding was confirmed forty years later by Bertrand and Javillier (1911). Previous attempts by Bertrand and Benzon (1922) and others to demonstrate the essentiality of zinc in animals were unsuccessful because the purified diets which they employed in their experiments were deficient in other essential nutrients. Convincing evidence concerning the need for zinc for the normal growth and development of mice was reported by Bertrand and Bhattacherjee (1934, 1935) and Todd et al. (1934) and in rats by Stern et al. (1935).

Follis et al. (1941) in their histological studies of zinc deficient rats found alterations in the skin consisted of a hyperkeratinization, thickening of the epidermis, intra and intercellular edema and loss of hair follicles with preservation of the subaceous glands.

The early work with the rat and mouse indicated that the zinc requirement is less than 5 ppm of diet. Therefore, it was generally assumed that a deficiency of this element would not occur in other species such as swine, cattle and poultry when fed diets made up of natural feed ingredients such as corn, soybean oil meal (Luecke, 1965).

In 1953 Kernkamp and Ferrin, reported the incidence in pigs of a dermatitis termed parakeratosis which had been experienced for

eleven years. The indidence of the disease was as high as 60 percent. Thomas and Eden (1954) also reported the occurrence of a skin disease in pigs in Great Britain which they termed nutritional dermatitis. A relationship of zinc to parakeratosis was suggested by data reported by Curtin (1954). There were no cases of dermatitis in pigs receiving a diet containing cottonseed meal when supplemented with 2 mg of zinc per lb of diet (Brinegar and Hunter, 1955).

Tucker and Salmon (1955) elucidated the essential role of zinc in the prevention and cure of this disease. They suggested that the levels of calcium or phosphorous or both are contributing factors in the incidence of the disease. The influence of Ca and P as well as other nutrients on zinc metabolism were studied later by Luecke et al. (1956-1957), Lewis et al. (1957), Bellis and Philp (1957), and others.

Following the discovery of the essentiality of zinc in the nutrition of pigs investigations were extended to other animals. O'Dell and Savage (1957) have briefly reported that added zinc stimulated the growth and the bone length of chicks fed a semipurified soybean protein diet containing approximately 50 ppm of zinc. They concluded that the zinc in soybean protein was not available in comparison with the zinc of a similar diet containing casein. This study was confirmed by Morrison and Sarett (1958), O'Dell et al. (1958) and Young et al. (1958).

Supplee et al. (1958) observed that zinc and potassium were needed for growth, prevention of perosis and normal feathering in poults fed a soybean protein ration. Kratzer et al. (1958) confirmed the importance of zinc in rations for poults.

The abnormal bone condition appears to arise from a failure of

cartilage development in the epiphyseal plate region of the long bones and decreased osteoblastic activity in the thin bony collar (Keinholz et al. 1961).

Few studies have been made on the nature and occurrence of this deficiency in ruminants. The first indication that zinc deficiency could arise in grazing cattle came from Guiana where Legg and Sears (1960) found that it was responsible for outbreaks of parakeratosis in cows and yearlings and poor growth in young stock, since these manifestations responded to oral or parenteral administration of zinc.

Haaranen and Hyppola (1961) described work done in Finland where a syndrome characterized by skin lesions, poor reproduction and low milk production in housed dairy cattle responded to zinc therapy.

In the Netherlands, Grashius (1963-1964) noted a syndrome, where the affected animals had a poor reproductive performance, low milk yield, eczematous scabs and loss of hair around the muzzle, neck and tail. The zinc contents of herbage grazed by affected stock was 19 to 83 ppm. Underwood (1962) has suggested that the minimum zinc requirement for grazing cattle is about 30 ppm while Haaranen (1963) indicated a requirement of about 45 ppm. Hartmaus (1965) who reinvestigated the problem on these Finish farms, vigorously contested Grashius's findings claiming that the condition was probably attributable to "poor management" and not to a deficiency of zinc (Mills et al., 1967).

In 1960 Halsted and Prasad described a sindrome occurring in human males characterized by severe iron deficiency anemia, hypogonadism, dwarfism, hepatosplenomegaly and geophagia, which they observed in villagers in Iran suffering from malnutrition. Despite hepatosplenomegaly the liver function tests were normal except for serum alkaline phosphatase

which was consistently elevated. They pointed out the possibility of zinc deficiency as an explanation of hypogonadism, dwarfism and changes in alkaline phosphatase.

One year later "hypogonadal dwarfism" was described in male subjects residing in the Egyptian Nile Valley with severe iron deficiency anemia, schistosomiasis and ancyclostomiasis (Prasad, 1961). The zinc level of plasma, red cells and hair was reduced in these subjects and ^{65}zinc turnover studies appeared to confirm the presence of zinc deficiency. Minor differences between the patients of Egypt and Iran include the following:

A. Geophagia was common in Iran but was not found in Egyptian cases.

B. Hookworm and schistosomal infection were not present in Iranian cases but were in Egypt (Prasad et al., 1963).

Coble et al. (1966 b) concluded that multiple factors are present that may affect the growth and development of rural male Egyptians. Inadequate information on development potential and effect of environment on these subjects and the observation that they ultimately obtain normal maturation and stature without therapy or change in zinc levels further complicate definition of the role of zinc in their delayed maturation.

The possibility remains, however, that the slower growth curves and smaller ultimate statures of rural male Egyptians in comparison to their upper socioeconomic Cairo counterparts are in part a result of inadequate zinc nutrition. A recent finding of inadequate growth hormone rise following insulin induced hypoglycemia in both retarded and control subjects with low plasma zinc levels indicates that a defect in

pituitary reserve is present (Coble et al., 1966 a).

Biochemical Functions of Zinc

Zinc-Enzyme Interrelations:

Keilin and Mann (1939-1940) showed zinc as an integral part of the enzyme carbonic anhydrase which catalyzes the reversible reaction between carbon dioxide and water. It was demonstrated that erythrocyte carbonic anhydrase also catalyzes the hydration of acetaldehyde and pyridine aldehydes. The complete inhibition by acetozolamide also suggest the necessity of the zinc ion for these processes (Pocker and Meany, 1967).

Carbonic anhydrase has been isolated from various sources, mainly mammalian red blood cells. Bovine erythrocyte carbonic anhydrase, homogeneous by ultracentrifugal and electrophoretic analysis was prepared by Lindskog (1960). In 1962 Lindskog and Malmstrom, indicated that only one zinc ion in each molecule of erythrocyte bonine carbonic anhydrase, is an obligatory component for the enzymatic hydration of CO_2.

Since the first evidence in 1939 of the fundamental role for zinc in metabolism, about fifteen to twenty zinc-containing metalloenzymes have been isolated and purified from a variety of organisms and tissues from diverse species, in the last fifteen years, thereby indicating the general metabolic importance of the element.

Vallee and Neurath (1954-1955) have demonstrated that zinc is a part of carboxypeptidase of bovine pancreatic juice. This enzyme degrades polypeptides in a sequential fashion, beginning at the C terminus. Two carboxypeptidases A and B, are involved in protein digestion

in the duodenum. Carboxypeptidase A, exhibits maximal catalytic activity with C terminal aromatic residues, while carboxypeptidase B is specific for C terminal basic residues. Carboxypeptidase A is a metalloenzyme of molecular weight 34,300 containing one atom of zinc per molecule of protein (Mahler, 1966). It occurs in the pancreatic juice in the form of a zymogen (Coleman et al., 1960). This finding explains previous observations in which it was demonstrated that about 6.5 percent of the dose of ^{65}zinc administered to dogs was eliminated in their pancreatic juice within five days (Vallee, 1957). A variety of chelating agents such as phenanthroline (Quiocho and Richard, 1966); acetylation and iodination of tyrosine residues (Coleman et al., 1966); substitution of other metal ions for the native zinc atom (Coleman and Vallee, 1960) and irradiation with ultraviolet light (Piras and Vallee, 1966, 1967 a, b) alter the catalytic specificity of carboxypeptidase A.

Because of the similarities in composition and mechanism of action of carboxypeptidase A and B, it was of interest to compare the structure of the active center peptides of both carboxypeptidases. The lack of homology between the cysteinyl peptides of carboxypeptidases A isolated by Sampath et al. (1963) and carboxypeptidase B studied by Wintersberger (1965) may suggest that these two enzymes have evolved independently. Alternatively, it might be suggested that the difference in amino acid sequence in the region of the zinc-binding thiol, merely serves as a support for the crucial metal atom but, itself, is not a part of the active site.

Alcohol dehydrogenase of yeast (Vallee and Hoch, 1955 a, b) and of the equine liver (Theorell et al., 1955; Vallee and Hoch, 1956) was found to contain four and two moles of zinc per mole of protein respec-

tively and cannot be removed without causing irreversible changes in protein structure. Removal of the zinc of yeast alcohol dehydrogenase leads to dissociation of the molecule into subunits (Kagi and Vallee, 1960), while in horse liver alcohol dehydrogenase (LADH) protein denaturation and aggregation results (Druyan and Vallee, 1962). Apparently the metal-protein interaction is responsible in part of the tertiary and quaternary structures of these enzymes.

The LADH enzyme has a molecular weight of 84,000, binds two moles of coenzyme per mole of protein and catalyzes the reversible oxidation of alcohols to aldehydes. The SH groups of two cysteinyl residues per mole of protein have been found essential for the activity of this enzyme, one per active enzymatic site. Carboxymethylation of these groups with iodoacetate inactivates the enzymes, and the prior binding prevents the reaction (Li and Vallee, 1965).

Alcohol dehydrogenase has been purified from human liver also and identified as a metalloenzyme by Von Wartburg et al., (1964). It is quite similar to the horse liver enzyme enzymatically and structurally and has broad substrate specificity, including methanol and ethyleneglycol.

Glutamic dehydrogenase of beef liver, which catalyzes the reversible oxidative deamination of glutamate, was reported by Adelstein (1957) to be a zinc metalloenzyme. The average zinc content was 3.4± 1.0 atoms per molecule. Like alcohol dehydrogenase it appears to bind one NAD molecule per atom of zinc. Glutamic dehydrogeanse, having a molecular weight of 1,000,000 is a much larger molecule than other pyridine nucleotide dependent enzymes that have been studied.

Lactic dehydrogenase of mammalian tissues has also been found

to be a zinc metalloenzyme (Vallee and Wacker, 1956).

The detection of zinc in these enzymes may explain the high concentration of this element in the liver and the retina, since it has been shown that LADH oxidizes vitamin A and reduces retinene probably being identical with retinene reductase. Thus far, no other metals have been found to occur in similar dehydrogenases (Vallee, 1959).

In addition to the dehydrogenases presented, more recent investigations have shown that D-glyceraldehyde-3-phosphate dehydrogenase, isolated from bovine, crayfish and bakers yeast is a zinc metalloenzyme, with two moles of zinc per mole of enzyme, apparently bound by means of cysteine and histidine (Keleti, 1964).

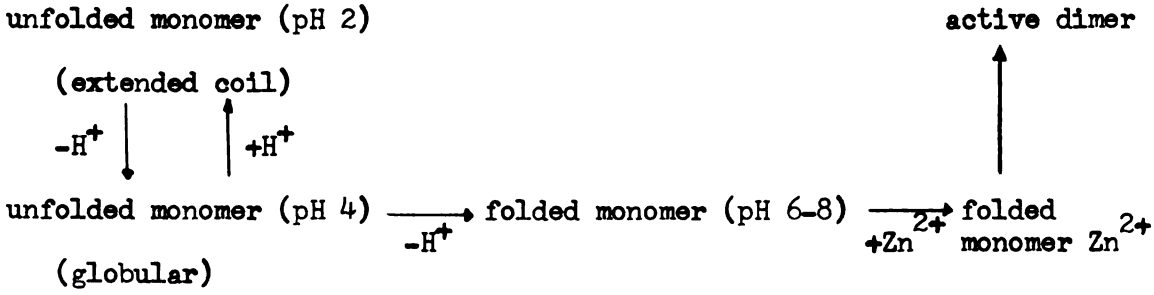
17-β-hydroxysteriod dehydrogenase activity was stimulated by zinc ions for the placental (Langer and Engel, 1958), and adrenal (Dahm and Breuner, 1964) enzymes. No significant stimulation was observed in the case of testicular 20-α-hydroxysteroid dehydrogenase (Shikita et al., 1967).

The isolation of a zinc-dependent hexokinase from Neurospora crassa led to the suggestion that this is a zinc metalloenzyme. Three to fourfold increases were found in zinc-sufficient, as compared to zinc-deficient organism. The hexokinase was not isolated in homogeneous form and, indeed, showed significant phosphoglucose isomerase activity. The partially purified enzyme was inhibited by EDTA (Medina and Nicholas, 1957).

The role of zinc in regulating the activity of alkaline phosphatase has been of continued interest since some preparations of this enzyme have been reported to constitute zinc metalloenzymes and also to exhibit properties of metal-enzyme complexes.

Mathies (1958) has established that alkaline phosphatase from swine kidneys is a zinc enzyme. The enzyme contains significnat amounts of zinc, magnesium and copper. He demonstrated a direct proportionality between increases in specific activity and zinc content of the enzyme. The best preparation contained 0.15 percent of firmly bound zinc and removal of any part of the remaining zinc by dialysis resulted in directly proportional losses in enzymic activity.

Reynolds and Schlesinger (1967) in their study of the refolding and reassociation of subunits of alkaline phosphatase from Escherichia coli found that the active dimer is a zinc metalloprotein and contains three zinc atoms per cimer. The metal is not required to form the refolded monomer, but is essential for dimerization process.



Snaith and Levvy (1968) have found that α-mannosidase is an active zinc-protein complex readily dissociable at pH 5, the pH optimum enzyme activity and the zinc ion can be displaced by cadmium, cobalt and other bivalent ions with almost complete loss of enzyme activity. These observations apply equally to the enzyme from rat epididymis, jack bean meal and the limpet, Patilla vulgata. α-Mannosidase seems to be present in all mammalian tissues. Its activity is high in the male genital tract where it responds directly to androgens.

In addition to the metalloenzymes, there have been numerous

reports on a variety of enzymes whose activity is increased by addition of zinc ions (Vallee, 1959). Most of them lack specificity as evidenced by the fact that they are activated by other ions also. Some of these zinc enzyme complexes are: arginase, carnosinase, histidine deaminase, lecithinase, aminopeptidase, enolase, yeast aldolase, oxalacetic decarboxylase, several peptidases and serum intestinal phosphatase.

Harvey et al. (1967) purified a phosphodiesterase from carrot that possesses exonucleolytic activity which degrades both oligodeoxyribonucleotides and oligoribonucleotides starting from a free hydroxyl on the 3' end and producing 5' mononucleotides. Divalent ions Mg^{2+}, Mn^{2+}, Ca^{2+} and Zn^{2+} increase activity while EDTA inhibits.

Requirement for zinc for DNA synthesis has been demonstrated by Fujioka and Lieberman (1964). Wacker (1962) found that RNA of Euglena gracilis is decreased; that of amino acids is increased and the DNA content is doubled.

The formation of either polysomes or non-specific aggregation of ribosomes under the influence of zinc ions was obtained from the seed of Pesim arvense by Barker and Rieber (1967).

Zinc-Hormone Interrelations:

The significant amount of zinc in the pancreas and the role of zinc in the crystallization of insulin led to speculation that there might be some functional relationship between zinc and the secretion of insulin by the pancreas. In 1938 Scott and Fisher reported that the pancreas of the diabetic contained some 50 percent less zinc than did that of nondiabetics. Later it was showed that when the results

were expressed in a fat-free basis, there was no significant difference between zinc content of diabetic and normal pancreas.

The relation of zinc to the pancreas and insulin has been studied histochemically, using dephenylthiocarbazone. Variable amounts of ainz were found in both the alpha and beta cells of the islets of Langerhans. The amount of zinc in the beta cells varied directly with insulin secretion in response to physiological stimuli. However the mechanisms for insulin production were not inactivated by irradiation of the islets with ^{65}zinc, or by injecting 2 to 3 mc of ^{65}zinc to the rat. Nor does ^{65}zinc administration produce any changes in the blood sugar level in rats. Consequently the possible biochemical interrelationship between insulin and zinc is still an unsolved problem.

Some findings suggest a relationship between zinc content of the alpha cells and glucagon secretion. However, further research will be required to establish a zinc-glucagon relationship.

Reports have appeared in the literature suggesting a relationship between zinc and other hormones, especially the adrenocorticotrophic hormone (ACTH). Treatment of ACTH preparation with zinc ($Zn(OH)_2$) increases and prolongs its physiological action as in insulin. Although pure preparations of growth hormone from pituitaries contain practically no trace of zinc. The problem remains unsolved according to Orten (1965).

Since the first suggestion by Bertrand and Vladesco (1920-1921) that high concentration of zinc of the genital tissue might be involved in male reproductive function. The high concentrations of zinc in male sex organs and fluids of various species was confirmed by many investigators. But the biochemical forms and functions of zinc in these glands

and their secretions have been only partially elucidated and only a few functions are known. The carbonic anhydrase content of these tissues is high, but only 3.5 percent of the total zinc of the dorsolateral rat prostate can be accounted for by this enzyme. Recently, a highly active α-mannosidase was isolated from the male genital tract and it responds directly to androgens (Snaith and Levvy, 1968).

Millar et al. (1957) found that zinc deficiency in rats results in degeneration of the testes, hypoplasia of the coagulating glands, the seminal vesicles and prostate, and relative or complete decrease in the numbers of sperm in the epididymes. All changes produced by zinc deficiency, except the testicular atrophy, were reversed when supplemental zinc was added to the diet. If testicular atrophy had occurred, neither testis nor epidymis regained normal size, function or zinc concentration. It appears that the impaired development of the male accessory sex organs in rats on zinc deficient diet may be a result of severe inanition which in turn causes reduced gonatropin secretion from the pituitary and a consequent fall in androgen production.

Savage (1968) observed that the zinc requirement of hens for egg production is appreciably lower than the amount required for hatchability or for optimum growth and feathering in chicks and poults. Since as the hens become depleted of body stores of zinc, embryos show skeletal anomalies which are characterized by faulty limb and trunk development.

Interrelation With Other Nutrients

There are several factors which affect the ability of the animal to utilize zinc as it is present in foods (Luecke, 1965). Since the suggestion by Tucker and Salmon (1955) that the level of calcium or phosphorous or both are contributing factors in the incidence of parakeratosis. A number of investigators became interested in the relation between calcium, zinc, iron, phosphorus, phytate, vitamin D, copper, cadmium and other nutrients.

The ability of animals to absorb zinc varies with the chemical form in which it is ingested. Zinc in the form of the oxide, carbonate, sulfate or metal appears to be equally available to the chick, whereas the zinc in the ores sphalerite composed mostly of zinc sulfide and franklinite of oxides of zinc, iron and manganese are largely unabsorbed. Since zinc as zinc oxide is quite available, therefore, it would appear that the crystal structure that results from the mixture of these metallic oxides prevent the zinc in franklinite from being available for chicken (Edwards, 1959).

Different protein sources also vary greatly in their capacity to supply zinc for the needs of rats, chicks and pigs. Soybean meal was the principal source of protein in the rations associated with a zinc deficiency. In 1960 Lease et al. found that the feeding of sesame meal rations produced gross sings of zinc deficiency although the rations contained about 52 ppm of zinc.

Some peculiarity of plant proteins was suspected as a factor in the development of a zinc deficiency (Anonymous 1957; 1961; 1967). This was shown by the observation that purified rations containing

casein produced good gorwth and normal skin in swine even when the level of zinc was no greater than 10 ppm. The chicks fed a similar diet, with the exception that the protein source was casein and gelatin, instead of soybean, showed no improvement in growth when zinc was added.

Phytate was early suspected as the factor in soybean and sesame meals that might be affecting the zinc availability. The confirmation for this was obtained by the addition of phytic acid to casein rations fed chicks that increased their requirement for zinc (Anonymous, 1964).

In another study to evaluate the interrelation between zinc, calcium and phytate, young pigs were fed a casein diet to which 1.4 percent phytic acid was added. The ration contained 14 ppm of zinc and 1.5 percent of calcium. The weekly weight gain decreased from 1.68 to 0.25 pounds when phytic acid was incorporated in the diet. The growth depression could be alleviated by the supplementation of the diet with 100 ppm of zinc (Oberleas et al., 1962).

Byrd and Matrone (1965) studied the influence of calcium on the incorporation of zinc into the phytate complex. They found that when the molar ratios of calcium and zinc were 1 to 1 or 2 to 1, the calcium decreased the absorption of zinc, by the phytate, but when the ratio of calcium to zinc was 100 to 1, the presence of calcium in the solution increased the incorporation of zinc into the complex to the point where 99 percent of it was present as the phytate. In the absence of calcium, none of the zinc was incorporated into phytate. Since rations that are associated with the development of parakeratosis generally contained 30 to 100 ppm of zinc and 1 to 2 percent of calcium, it was postulated that calcium initiated a coprecipitation with zinc to form insoluble

phytates. The adverse effect of the phytic acid can only be overcome by adding enough zinc to the ration.

When the calcium and zinc were both present in the same solution during precipitation a greater amount of phytate precipitate was formed (Oberleas et al., 1966). This suggestion stems from the work of Byrd and Matrone (1965) who found a greater zinc loss from solution when the molar ration for these two elements was 100 to 1. All of the previous evidence has emphasized the inhibitory effect of calcium on zinc absorption in the presence of phytates. It would appear that the deleterious influence of plant proteins in zinc absorption was related to their phytate content. Plumlee et al. (1960) and Smith et al. (1962) have found that the utilization of zinc in various proteins is correlated with the level of phytic acid in the protein. They also reported an improvement in zinc utilization when EDTA (ethylenediaminetetracetic acid) was added to a soybean protein ration.

Kratzer and Starcher (1963) suggested that EDTA improves the effectiveness of added zinc as well as increases the utilization of zinc already present in the basal diet. Other similar chelating agents were studied and their effectiveness were determined by Vohra and Kratzer (1964).

It is interesting to speculate concerning the presence in common foods of naturally occurring chelating agents capable of reducing or enhancing the biological availability of zinc and other trace elements (Pond, 1965). Scott and Zeigler (1963) presented results indicating that certain natural feedstuffs, such as casein, liver extract, contain a factor or factors capable of improving both absorption and metabolic utilization of zinc in similar way to ethylendiaminetetracetic acid.

It appears that the active principles in the natural feedstuffs may be natural chelates which normally function to improve transport and utilization of required mineral nutrient.

Smith et al. (1960) found that pigs fed the autoclaved ration exhibited improved performance, a lower incidence of parakeratosis, a greater zinc retention and a lowered zinc excretion. Autoclaving reduced the chick's or poult's need for added zinc on isolated soybean protein or sesame diets (Kratzer et al., 1959; Lease et al., 1960) and it has been suggested that this reduction is due to destruction of phytic acid (O'Dell et al., 1964). They found that autoclaving for four hours reduced the phytic acid of isolated soybean protein to 13 percent of the original amount. Lease in 1966 studied the effect of autoclaving sesame meal on its phytic acid content and on the availability of its zinc to the chick. The author found that after autoclaving for two hours phytic acid was not reduced. Autoclaving for four hours reduced the phytate from 1.00 to 0.78 percent which was not statistically significantly different from the unautoclaved meal. The difference may be due to a difference in zinc-binding factors between the two materials or to destruction of phytic acid in the isolated soybean protein concomitant with, but not related to the release of zinc.

Oberleas et al. (1962) concluded that the action of phytate on zinc availability takes place in the gastrointestinal tract rather than at the cell level. Phytic acid also decreased the availability of zinc in the absence of protein, with amino acids serving as the nitrogen source to as great an extent as when casein was present in the diet (Likuski and Forbes, 1964). That the problem of zinc availability

may not be simple matter of binding to phytate is suggested by a recent work from the university of Wisconsin (Anonymous, 1967). Dahmer et al. (1966) found that the addition of histidine to low zinc, soybean meal ration alleviated the leg abnormality but did not affect other zinc deficiency symptoms such as poor growth or low zinc content of bone.

Nielsen et al. (1967) studied the effect of histidine and its various metabolites on chicks fed low-zinc diets. They reported that histidine at 1.0 percent and 2 percent of the diet or histamine at 0.2 percent of the diet prevented the "arthritic-like" or "perosis-like" syndrome in zinc deficient chicks fed soy protein diets, while having little or no effect on other symptoms of zinc deficiency. Nielsen et al. (1968 a, b) continued their studies with histamine, as well with other anti-arthritic agents and found that they prevented leg abnormalities, but had little or no effect on other symptoms of zinc deficiency. The authors concluded that the leg defect was "arthritis like" in certain respects as it responded to various anti-arthritic agents.

Lewis et al. (1957 a) demonstrated that zinc was readily removed from an in vitro solution during the precipitation of calcium phosphates brought about by increasing from 3 to 6 the pH of solutions of calcium, phosphate and zinc. These conditions were said to simulate somewhat the change in pH of the ingesta during its passage from the pig's stomach to small intestine. Increasing the Ca:P ratio of these solutions markedly increased the amount of zinc removed from solution. This type of phenomenon may explain the detrimental effect of a high-calcium diet on parakeratosis, but do not explain it completely as was suggested by the authors.

Phytate occurs in nature as the calcium magnesium salt of phytic acid, and thus as a component of such a complex and as a companion Group II element magnesium might be expected to act as does calcium in increasing zinc uptake in the presence of phytate. But the effect of added magnesium on the chicks dietary zinc requirements apparently was not influenced by variation in the magnesium, zinc, calcium and phytate content of the protein sources. Safflower meal was the highest in these factors and casein ration the lowest, in neither case did added magnesium increase the chick's dietary requirement (Lease and Williams, 1966).

Another interesting possibility concerning the absorption of zinc is that suggested by Worker and Magicowsky (1961) who have proposed that the absorption of many divalent ions may have their absorption influenced by the level of vitamin D in the diet. The pronounced effect of calcium on zinc nutrition and the role of vitamin D in calcium absorption and metabolism suggest a possible effect of dietary vitam D on zinc utilization. Becker and Hoekstra (1966) found that the effect of dietary vitamin D on uptake of oral ^{65}zinc was most pronounced when the vitamin was suddenly added to the diet of previously vitamin D-deficient rats. At 60 hours the skeletal, but not soft tissue, uptake of oral or injected ^{65}zinc was significantly increased by the supplementation of vitamin D to vitamin D deficient rats. However, skeletal specific activity (% ^{65}zinc per g) was increased by increased by vitamin D only with orally administered ^{65}zinc. The authors concluded that the increased absorption of dietary zinc attributed to vitamin D probably results not from a direct effect of the vitamin, but from a homeostatic response to the increased need for

zinc which accompanies inhanced growth and calcification.

A number of observations concerning copper-zinc interactions have been made in connection with studies that have dealt with the effects of excessive dietary zinc. Zinc toxicity in the rat is manifested by a hypochromic, microcytic anemia and poor growth (Sutton and Nelson, 1937; Smith and Larson, 1946) indicating that the interaction is not a simple one and that the zinc toxicity syndrome has an effect not only on copper metabolism but also on iron metabolism as well.

Davis (1958) reported that copper has a marked and inverse relationship to zinc, at least within the liver of animals. It was noted that when copper levels in the liver rose the values above 3,000 ppm, there was a reduction of zinc from a normal value of 300 ppm to levels approaching 1 ppm. Conversely, increasing the level of zinc in the diet caused a depression in the copper concentration of the liver, but only when copper levels in the diet approached those of borderline or normal of around 5 to 10 ppm.

Cox and Harris (1960) demonstrated that excess of dietary zinc results in an accumulation of zinc in the liver with an early and marked loss of liver iron. The data suggest that the reduction of iron is responsible for the production of the anemic condition and presumably the depression of the activity of some iron containing enzymes. A lowered liver copper may also occur and the data indicate that it may be the result of the reduced liver iron rather than an effect of the zinc.

Van Campen and Scaife (1967) studied the site or sites at which zinc interferes with copper. They found that high levels of zinc depressed the absorption of ^{64}copper when both zinc and the ^{64}copper

were placed directly into the isolated duodenum segment. If the ^{64}copper was put into intestinal segment and the zinc was given intraperitoneally, no depression in ^{64}copper absorption resulted. The results indicate that zinc does not depress copper absorption by first building up to critical levels in some non-intestinal tissue, and subsequently, interfering with copper absorption; since the tissue zinc concentrations were comparable in both groups of rats given either intraperitoneally and intraduodenally zinc. The evidence, rather indicates that the depression of copper absorption by high levels of zinc is mediated either in or on the intestine.

Hoefer et al. (1960) and Ritchie et al. (1963) have published that rather high levels of copper (125-250 ppm) will alleviate the parakeratosis syndrome in swine. In some cases a copper supplement was nearly as effective as a zinc supplement. Wallace et al. (1960) presented fragmentary evidence of such and effect of copper. While O'Hara et al. (1960) on the contrary found that zinc deficiency appeared in pigs fed a high level of copper but not in those receiving a similar diet without copper. Work at Wisconsin has repeatedly shown no beneficial effect of copper in curing or preventing zinc deficiency in swine. This confusing picture is difficult to rationalize. The mechanism is not presently understood, but the importance of dietary agents of various proteins may also play an important role (Hoekstra, 1964).

The cadmium-zinc antagonism has been most widely studied, but Hill et al. (1963) have demonstrated in the chick that copper and iron are also antagonized by cadmium. The cadmium toxicity resulted in a reduced growth rate, mortality, microcytic hypochromic anemia and

atony and elongation of the gizzard. The growth depression and gizzard abnormality were corrected by increased dietary zinc. The mortality was reversed by added copper and increased dietary iron partially corrected both the mortality and the growth depression, indicating a previously unsuspected iron component of cadmium toxicity.

Cadmium appears to be very firmly bound in the body, as evidenced by studies showing a virtual absence of cadmium turnover in mice (Cotzias et al. 1961) and by the gradual accumulation of cadmium in the tissues of animals and man throughout life (Schroeder et al., 1963).

The suggestion that cadmium competes with zinc in important cellular sites is supported by the permanent sterility produced in male animals by injecting a single dose of a cadmium salt and its prevention or at least its delay, by simultaneous administration of large amounts of zinc (Hoekstra, 1964).

Zinc in Tissue

Lechartier and Bellany (1877) noted the presence of zinc in the biological matter. In the same year Raoult and Breton confirmed the occurrence of zinc in human liver. For fifty years the interest in the biological role of this element was only sporadic. Difficulties in methodology limited most investigations to qualitative determinations, but it was realized that the element was a common constituent of plants and animals.

Lutz (1926) critically examined the available literature on the distribution of zinc in biological matter and concluded, "in few if any, natural biologic materials which have been analyzed has there been reported failure to detect zinc. We may, therefore, consider as

established the fact that zinc is a universal and normal mineral constituent of all biological material." He found it present in all organs of the rat, cat and man, and that bone, skin and hair contain high zinc levels compared to most soft tissues of the body. The methods employed by subsequent workers were also diverse; the precision, accuracy and the base lines comparison were quite variable.

The quantitative and qualitative aspects of the zinc content of animal tissues have been treated in detail by Vallee (1959) and by Underwood (1962). The concentration in most of the soft tissues of the body approximates 20-30 ppm, which is some 10-15 times that of copper and less than half that of total iron. In contrast to the copper, the mammalian newborn does not consistently have higher concentrations of total body zinc than do mature animals of the same species. There is no evidence of appreciable fetal storage of zinc as normally occurs with iron and copper. During suckling whole body zinc concentration rises substantially from newborn levels in the rat and the pig but not in the cat or the guinea pig. The liver and spleen of the rat, rabbit and pig contain higher levels of zinc at the end of the suckling period than at the beginning, whereas in the kitten which is born with higher levels in these organs than the other species, there is a fall during suckling (Widdowson, 1950; Spray and Widdowson, 1951). The importance of the colostrum milk which is 4 to 5 times richer in zinc than later milk, was demonstrated by fostering newborn mice on the mothers in later lactation. A pronounced reduction in whole body zinc was achieved (Nishimura, 1953). This indicates that the young mammal can readily obtain its zinc requirement from maternal milk.

Zinc occurs in all living cells in varying concentrations. The zinc concentration in most of the organs approximates 25 ppm on the fresh-weight basis and considerably higher concentration of zinc is found in bone, hair and wool and portions of the prostate and the eye. Except in the liver and kidneys zinc concentration is not appreciably changed by alteration of zinc intake, but the capacity of the body to store zinc in any of its organs other than in bone is limited (Keinholz et al., 1964; Moses and Parker, 1964; Hoekstra et al., 1956; and Turk, 1965).

In the young growing animal the zinc concentration in the bone ash is a sensitive reflection of zinc absorption, especially at sub-optimal intake levels (Forbes, 1961; Likuski and Forbes, 1965; Fox and Harrison, 1964 and Hurley et al., 1964).

The major portion of zinc in whole blood is in the erythrocytes (75-85 percent) where it occurs mainly as a constituent of carbonic anhydrase. Determination of zinc content and carbonic anhydrase activity have implied that they are mutally dependent variables (Vallee et al., 1949). Erythrocytes are rich sources of pyridine nucleotide dependent dehydrogenases, it is possible that the fraction of zinc may be associated with some of these enzymes (Vallee et al., 1956).

Leucocytes contain 3 percent of whole blood zinc, but each leucocyte contains 25 times as much zinc as each erythrocyte. Human leucocytes contain a metalloprotein, with a zinc to protein ratio of 3 mg of zinc per g of protein, however, no enzymatic activity in this complex nor any correlation between the zinc content of leucocytes and the activity of several zinc containing enzymes has been demonstrated (Underwood, 1962).

Halsted et al. (1968) found that the blood platelets contain a significant amount of zinc. This, in part, accounts for the fact that serum zinc is 16 percent higher than is plasma zinc.

In blood serum, zinc exist in at least two fractions, a firmly bound form which is said to amount to 34 percent, and a loosely bound zinc amounting to 66 percent of the total zinc content. The firmly bound zinc protein is a globulin that satisfies the criteria of metal-protein complex. The loosely-bound complex appears to be concerned primarily with zinc transport. Neither substance has been shown to exhibit enzymatic properties (Vikbladh, 1951).

Several studies have shown that large oral doses of zinc significantly increase whole blood and plasma zinc in rats and rabbits (Underwood, 1962). Dreosti et al. (1968) recently reported that in rats receiving a zinc deficient diet for only one day, showed a plasma zinc decrease of 38 percent (from 95.9 to 60.1 µg percent) in pregnant animals and 55 percent (from 110.3 to 50.4 µg percent) in weanling males. Food restriction elicited a similar but less marked response in the pregnant females, but not in the young males. Miller and Miller (1962) reported that zinc-deficient calves exhibit a reduced blood zinc content.

The tissue of the eye, especially the choroid of carnivora and particularly the tapetum lucidum, contains higher concentrations of zinc than any other animal organ; this amounts in some species to as much as 13 percent of the dry tissue. The function of zinc in eye tissues remains unexplained. Some zinc in the retina may be incorporated in part in the retinene reductase which catalyzes the interconversion of vitamin A alcohol and aldehyde; and may point to a vitamin A

zinc interrelationship, since no other metals have been found to occur in similar dehydrogenases (Forbes, 1967; Vallee, 1959).

Since the reports by Bertrand and Vladesco (1921), several groups of workers have confirmed the finding of high concentration of zinc in the male sex organs and fluids of various species. The prostate gland in particular concentrates zinc, although there are wide variations between species in the distribution of this element in the gland. Zinc deficiency in rats results in degeneration of testes, hypoplasia of the coagulating glands, the seminal vesicles and prospate, and relative or complete decrease in the numbers of sperm in the epididymis. All changes produced by zinc deficiency except the testicular atrophy, were reversed when supplemental zinc was added to the diet (Millar et al., 1958, 1960). There is a correlation between zinc content and carbonic anhydrase activity of prostatic tissue; the role of zinc in normal sperm function remains obscure since zinc deficiency produces testicular degeneration and aspermia, as well as markedly reducing the zinc concentration in the total male reproductive tract. The carbonic anhydrase accounts for only 3 percent of the total zinc present. There is no correlation between zinc content and the activity of either acid or alkaline phosphatase. It is interesting that the necrotizing effect of dithizone on the prostate of the rat and the dog is correlated with formation of a fine intracellular deposit of zinc dithizone. This indicates that a large portion of the zinc present is not firmly bound to protein that it is unavailable for chelation (Forbes, 1967).

In normal liver and mammary tissue cells zinc is present in the nuclear mitochondrial and "supernatant" fraction, with the highest

concentrations occurring in the supernatant fluid and microsomes (Thiers and Vallee, 1957; Bartholomelew et al., 1959). The data reported on the intracellular distribution of zinc in rat liver in terms of micrograms of zinc per milligram of nitrogen was found: nuclei, 0.77; mitrochondria, 0.42; microsomes, 0.65; supernatant, 2.0; and reconstituted whole liver, 1.05 (Edwards et al., 1961; Cotzias and Papavasiliou, 1964). These findings are consistent with the view that the majority of the known zinc-containing enzymes are in the supernatant fraction of the liver cell (Thiers and Vallee, 1957).

It is apparent that zinc accumulates in cartilage of the bones at sites of calcification and, once deposited in the calcified tissue, is firmly bound (Haumont, 1961; Vincent, 1963; 1965; Gilbert and Taylor, 1956). Haumont and McLean (1965) in a later histochemical and autoradiographic study of zinc and the physiology of bone established that the stain produced by dithizone in their sections, shown to be associated with the locus critical for calcification, results from a reaction with zinc. From this they concluded that zinc is in some way bound to the initiation of mineralization of preosseous tissue, whether in the formation of osteous, or in endochondral or subperiosteal ossification. While their autoradiographic studies showed that zinc is present in calcified tissue; and it has proved that zinc is progressively incorporated within the preosseous tissue as mineralization occurs and that it is still present in the fully calcified tissue.

Alexander and Nasbaum (1962) found that the shaft of the rat femur had a lower zinc concentration 330 ppm of ash than the head of the femur, 430 ppm, but they did not find an elevation of zinc in the

bones with age, in order to confirm the previous observation reported by Taylor (1961).

These observations do not tell whether zinc in bone is associated with the bone mineral or with the organic matrix. While the evidence is not conclusive, all indications point to a specific effect, on an organic constituent of bone, rather than to any association with the mineral itself. Since it appears to play a part in the sequence of events leading to calcification, and in view of its known association with hormones and enzyme systems, it may be fair to assume that it helps to catalyze the calcification process, but such a mechanism, if it exists, has not been demonstrated (Haumont and McLean, 1965).

III. EXPERIMENTAL PROCEDURE

General

Weanling male rats of the Sprague-Dawley strain were obtained from a local breeding farm around 21 days of age and immediately placed on experiment. The general procedure for lotting, management and record keeping was essentially the same in the two experiments. All lots were distributed as evenly as possible for weight and the animals were kept in individual stainless steel cages with free access to deionized distilled water. Food was provided *ad libitum* in aluminum feeders with stainless steel covers. Food consumption and growth data were collected every 48-72 hours.

Spray dried egg white solid was used as the source of protein since it has a very low zinc content. The vitamin-glucose mixture of the basal diet was modified by increasing the level of biotin so that the complete diet contained 4 mg/kg of this vitamin according to the previous observations of Luecke *et al.* (1968). The authors observed that the increase was necessary since preliminary studies with the unmodified diet containing 0.2 mg of biotin per kg resulted not only in growth failure but the appearance of scaly seborrheic type of dermatosis, often during the second week of the experiment. They also observed other symptoms included progressive alopecia, particularly in the areas around the mouth and eyes in some instances a spastic gait and kangaroo-like posture.

In general, all the overt symptoms suggested a clinical manifes-

tation of biotin deficiency, but it was never noted in the zinc-supplemented group. They found that the use of the modified vitamin-glucose mixture containing high levels of biotin did not alter the poor growth shown by the zinc deficient group, but resulted in marked improvement in growth of the supplemented group.

All diets were adequately fortified with minerals to provide the required levels except for zinc. The zinc was supplemented at different level to the diets as zinc sulfate. Analysis for zinc and copper were performed on all rations throughout the study. The methods for determining zinc and copper of the diets were essentially the same as those used for liver and kidney assays.

The statistical results have been computed by the CDC 3600 available for research work at the Computer Center of Michigan State University.

Trace Element Assay of Tissue

The rats were placed under light anesthesia with ether and killed by removing as much blood as possible by heart puncture. Liver, kidneys and bones were removed within a few minutes after exsanguination and washed several times with deionized water, placed in metal-free Saran and frozen with dry ice. The tissues were stored in the frozen state until analysis were performed. Adhering tissue was removed from bones after boiling in water for one minute. They were then fat extracted first with alcohol and later with ether and ashed at 600°C. The ash was taken up in concentrated hydrochloric acid, suitable dilutions made and the analysis carried out with a Perkin-Elmer Model 303 atomic absorption spectrophotometer using a single

zinc cathode tube with absorption measured at 213.7 Å.

Dry matter was determined on the liver and kidney tissue in an oven at 90°C. The nitric-perchloric acid method was utilized for wet washing. The procedure followed was similar to that outlined by Johnson et al. (1959), except that a smaller amount of tissue was used (0.3-1.2 g for kidneys and 0.8-9.1 g for liver on the fresh basis). All analysis were made in duplicate.

After digestion was complete and the residue had been evaporated to dryness, it was dissolved in deionized water with addition of hydrochloric acid and transferred to a volumetric flask. For zinc additional dilutions were made. The determination of copper was done on the original dilution by atomic absorption spectrophotometry using a single copper cathode tube with absorption measured at 324.1 Å. Control blanks were determined simultaneously on the purity of cleaning and digesting reagents.

Experiment I

The Effect of Varying Levels of Dietary Zinc:

The purpose of this experiment was to determine the zinc requirement of growing rats fed the egg white protein diet, and its effect on growth, appetite and tissue concentrations of zinc and copper.

The analysis of tissues for copper and zinc were made to study the relationship of zinc content of the diet with tissue concentration and the copper-zinc interrelationship. The interest in the determination of relationship of zinc content of the diet to the zinc content of tissues arose in part, from the need of a sensitive method for the

diagnosis of zinc deficiency which might prove superior to blood serum levels, since the low serum yield in small animals and frequent hemolysis of the cellular material proved troublesome.

The determination of relationship between copper and zinc in the tissues at low levels of dietary zinc was the other point of this study since there is little existing information on this point.

The composition of the basal zinc-low diet is shown in Tables 1, 2 and 3. The diet was found by analysis to contain 0.78 ppm of zinc. All animals were fed to appetite, and deionized water was available at all times.

Forty weanling male rats were equally divided into 5 groups of 8 animals each, and fed basal diet supplemented with zinc sulfate, at levels of 0, 5, 10, 15 and 20 ppm.

Experiment II

Part A

The Effect of Varying Levels of Dietary Zinc:

This experiment was designed by similarly to Experiment I in order to confirm the values obtained and to provide additional data on weight gain, appetite, food efficiency and zinc content of bones. Smaller weanling rats which are presumably more sensitive to zinc deficiency were used.

A total of 49 rats was used in this experiment in which zinc sulfate was added to basal diet to provide 0, 5, 7.5, 10, 12.5 and 15 ppm of zinc. Eight animals were placed on each treatment, except for the zero group which had nine. Details of feeding and caging the

TABLE 1

Composition of Basal Diets

Ingredient	Percentage of Diet
Glucose monohydrate[a]	57.7
Egg white solids (spray-dried)[b]	20.0
Corn oil	10.0
Cellulose[c]	3.0
Salt mix[d] (Table 2)	3.7
Vitamin-glucose mix[e] (Table 3)	5.0
Vitamin A, D, E and K oil[f]	0.5
Ethoxyquin[g]	0.1

[a]Cerelose, Corn Products Company, Argo, Illinois.

[b]General Biochemicals, Inc., Chagrin Falls, Ohio.

[c]Solka Floc. Brown Company, Berlin, New Hampshire.

[d]Similar to that used by Phillips, P. H. and E. B. Hart. J. Biol. Chem., 109:657. 1935. Reagent grade salts were used and $ZnCl_2$ was omitted.

[e]Composition similar to that used by Forbes, R. M. and M. Yohe. J. Nutr., 70:53. 1960. Except that the level of biotin was increased from 0.004 to 0.080 g/kg of mixture to prevent biotin deficiency.

[f]Vitamin A and D concentrate: 2000 IU vitamin A and 250 IU vitamin D with the addition of 10 mg menadione and 600 mg α-tocopherol per 100 g of A and D oil.

[g]Santoquin (1-2 Dihydro-6-ethoxy-2,2,4 trimethylquinoline) Monsanto Chemical Co.

TABLE 2

Composition of Salt Mix

Ingredient	Percentage of Salt Mixture
Calcium carbonate	30.000
Calcium phosphate, monobasic	7.500
Cobalt chloride	0.005
Copper sulfate	0.030
Ferric Citrate	2.750
Magnesium sulfate	0.510
Potassium iodide	0.080
Potassium phosphate, dibasic	32.200
Sodium chloride	16.700
Zinc chloride	0.025

The mineral mix was provided by G.B.I. under the name of Phillips and Hart No. 4 Salt Mix. It was made with reagent grade salts from which zinc chloride was omitted. The original Phillips and Hart diet was modified by addition of cobalt chloride and manganese sulfate was omitted.

TABLE 3

Composition of Vitamin-Glucose Mix

Ingredient	Amount
Vitamin B_{12} in mannitol	0.04 mg.
Biotin	8.00 mg.
Folic acid	1.00 mg.
Pyridoxine HCl	8.00 mg.
Riboflavin	12.00 mg.
Thiamine mononitrate	20.00 mg.
Calcium pantothenate	32.00 mg.
Nicotinic acid	50.00 mg.
Choline chloride	3,000.00 mg.
Cerelose	to complete 100 g.

Composition similar to that used by Forbes, R. M. and M. Yohe. J. Nutr. 70:53, 1960, except that the level of biotin was increased from 0.004 to 0.080 g/kg of mixture to prevent biotin deficiency. The chlorotetracycline was omitted.

animals were similar to that utilized in the preceding experiment and the basal diet had the same composition.

Part B

The Effect of Interchanging Different Zinc Level Diets:

It was decided that the results obtained merited further investigation. The animals of groups fed 0, 10 and 15 ppm of zinc in the diet were utilized for further study.

The rats of zero ppm of supplemental zinc were fed 10 and 15 ppm of zinc while the rats of these latter two groups were placed on 0 ppm zinc diet. Two animals of each group were killed as controls, four rats of control group were placed on 10 ppm zinc diet and the other three on 15 ppm zinc diet. Three animals of 10 ppm of zinc diet and four of 15 ppm zinc diet were placed on the basal diet, while the other two of each group were kept on the original diet as positive controls. No control was carried with zero ppm zinc group, because of insufficient number. On the other hand the animals were very weak, and might not have survived the additional three weeks of the experiment. The animals were killed at first and third week of the experiment for a study of the concentration of the zinc in the tissues.

In this part consequently the effect of the interchange of diets on appetite and growth of rats was studied. Second it was important to determine to what degree the animal's previous food intake history played on growth and appetite. Other investigations have suggested that the bone may serve to store excess zinc, which to some degree, can be released for the use by other critical tissues (Savage, et al., 1964). Accordingly the zinc concentration in the bones and liver was studied after one and three weeks of switchover.

IV. RESULTS AND DISCUSSION

Experiment I

Differences resulting from the supplementation of different levels of zinc to the basal diet were highly significant ($P < 0.01$) for food intake, growth and food efficiency and are shown in Table 4.

The poor growth of the rats on the basal diet was accompanied by a unkempt aspect, the hair was greasy, sparce and coarse, and a red brownish color appeared on the head of some of the animals of this group. Also two rats developed diarrhea at twenty-first day and one died on the twenty-fourth and the other on the twenty-seventh day. But neither the diarrhea or other symptoms were observed in any of the rats of zinc supplemented groups, even with the group on 5 ppm zinc that did not grow at a normal rate.

Anorexia was observed early in the rats on basal diet. After only 96 hours the animals consumed 50 percent of the amount consuming during the first 48 hours, and approximately 40 percent of the amount consumed by the animals fed 10 ppm or more of zinc in the diet. The same low food intake was maintained for the overall four weeks of the experiment by this group. The relative intake declined to one-fourth of the 10 ppm of zinc diet by the end of third week. Similar observations were reported by Macapinlac *et al.* (1965).

Prasad *et al.* (1967) suggested that the difference in weight gain can be accounted for partly on the basis of anorexia; however

TABLE 4

Response of Rats Fed Different Levels of Zinc in Experiment I

Concentration of Zinc in the Diet (ppm)	0	5	10	15	20
No. rats[a]	8	8	8	8	8
Days fed diet[b]	21	21	21	21	21
Avg. initial wt. (g)	59.5	59.5	59.3	59.4	59.4
Avg. final wt. (g)	75.6	134.5	199.5	195.5	189.4
Total wt. gain per animal (g)	16.1±2.1	75.0±6.1	140.4±5.1	135.9±6.3	130.0±4.8
Avg. daily gain (g)	0.8	3.6	6.7	6.5	6.2
Total diet consumed per animal (g)	108.3±2.9	201.3±8.0	302.6±8.4	291.1±9.8	282.9±10.2
g of food/g weight gain	7.5±0.9	2.8±0.2	2.2±0.1	2.2±0.1	2.2±0.0

[a]Two rats of 0 ppm zinc diet died at 24 and 27 days of the experiment.

[b]The experiment was continued for 28 days. The results obtained for the additional week do not present any difference. But these values are used together with those of the experiment II later in order to obtain a more complete evaluation of zinc level in the diets with the variables studied.

approximately three times as much food was required by 0 ppm zinc diet group for each gram of body weight gained as was required by 10 ppm or higher zinc level diet, suggesting a metabolic derangement of some type.

Analysis of the residual food of the basal diet group was made to determine how much contamination from the environment was present by the method of feeding utilized. The zinc content in the residues were 1.52 ppm and 1.04 ppm of zinc in the diet after first and second week respectively, while the original determination gave 0.78 ppm of zinc for the same diet. Even though the analysis for the other diet residues were not performed it is possible to assume that after the first week the concentration of zinc in the diets should be very similar to its original concentration at all times.

The growth curves of the experimental rats on different diets are shown in Figure 1. From these results and those presented in Table 4 it would appear that under the conditions of the experiment the requirement of the rat for zinc approximates 10 ppm of diet.

Vallee (1959) reported that the zinc content of soft tissues is normal, but that of bone is lowered in zinc deficiency. Similar observations were made by other investigators. Savage et al. (1964) found that the bones of zinc depleted chicks contained about one-third as much zinc as controls. Macapinlac (1965) found that the zinc concentration of the deficient animals were markedly reduced in the testes and the femur, but not in the other tissues examined. Taylor (1961) indicated that the zinc content of the femur, pelvis and humerus expressed as µg of zinc/g wet bone do not differ significantly from each other at any age. They also observed that the zinc concentration

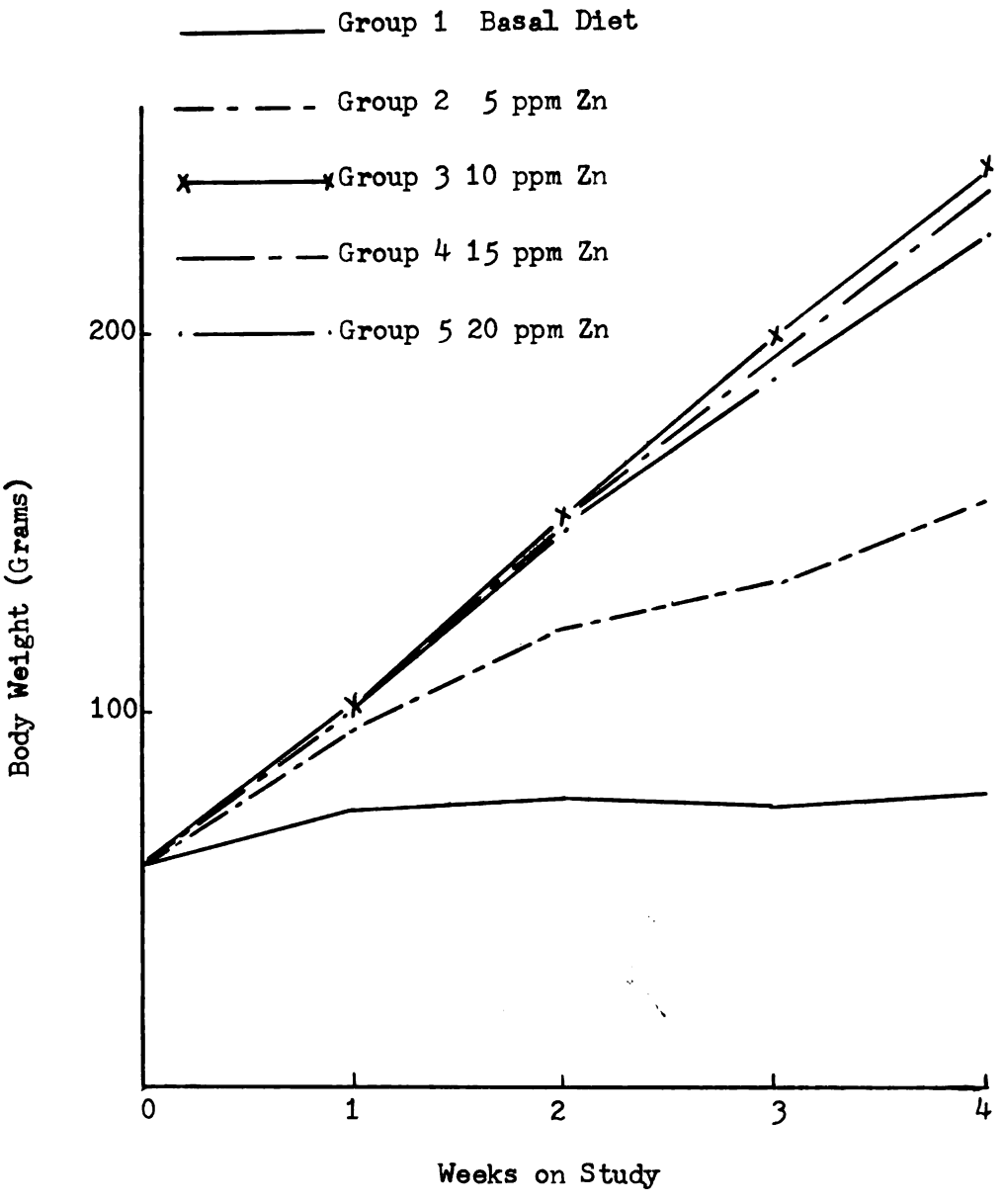


Figure 1. Experiment I--Growth Curves of Rats Fed Different Levels of Zinc.

in bones increased with the age, but this was not confirmed by Alexander and Nusbaum (1962) who found 30 percent more zinc in the ends than in the shaft and no variation with age in their experiment conducted over 414 days.

Femur was utilized for the zinc and percentage of ash determinations. The results are shown in Table 5. The zinc concentration of the bones of the different groups were highly correlated with the zinc concentrations in the diet ($P < 0.01$). Also a significant decrease ($P < 0.01$) was observed in the percentage of ash with the zero ppm zinc diet group. Hurley et al. (1964) also observed a decrease of ash content of femurs in deficient females.

TABLE 5

Effects of Variation on Zinc Content of Diets on Concentration of Zinc in Bones

Item	0 ppm	5 ppm	10 ppm	15 ppm	20 ppm
No. of rats	8	8	8	8	8
Days fed diet	28[a]	28	28	28	28
Percentage of ash[b]	57.1±0.5	60.3±0.6	60.1±0.5	60.3±0.3	60.7±0.2
Zn concentration[c]	70.8±3.5	87.5±2.8	186.5±4.1	238.2±4.4	256.4±3.8

[a]Except for the two rats that died on days 24 and 27.

[b]Percent on the fat-free, dry basis.

[c]Expressed in ppm of zinc on the dry, fat-free basis.

The zinc content of the liver and kidneys showed no significant differences (Table 6), except for the content of zinc in liver of the two animals that died before the experiment was finished (Appendix

Table 2). The lack of significant differences in the zinc content of the liver and kidneys was observed previously by Savage et al. (1964) with chicks, who suggested that these critical organs might hold zinc tenaciously. Keinholz et al. (1964) found that 10 ppm dietary zinc maintained zinc concentration of many body components of hens equal to those produced by feeding 70 ppm zinc diet and, if the animal grows and develops at all, its tissues maintain the normal zinc concentration. There was no tendency for the rats fed excess zinc to store the element in liver or kidney as was the case with some other nutrients.

TABLE 6

Effect of Different Levels of Zinc in the Diet
on Copper and Zinc Concentration in Livers and Kidneys

Item	o ppm	5 ppm	10 ppm	15 ppm	20 ppm
Liver copper[a]	15.2±1.7	9.7±0.5	5.6±0.9	6.8±0.6	5.4±0.5
Liver zinc[b]	93.6±8.4	77.9±2.6	77.6±2.7	83.5±2.0	84.9±2.5
Kidney copper[c]	23.0±2.3	19.1±1.6	17.0±0.2	16.1±0.5	17.1±1.3
Kidney zinc[d]	87.9±0.6	79.6±2.5	87.2±1.4	92.5±1.7	86.1±2.7

[a]The value is the average of the analysis for 8 livers and is expressed in ppm of copper on dry weight basis.

[b]Idem as for copper.

[c]The value is the average of the three pairs of kidneys and is expressed in ppm of copper on dry weight basis.

[d]Idem as for copper in kidneys.

A number of observations concerning copper-zinc antagonism have been reported in connection with the effects of excessive dietary concentration of one or the other of these elements in the diet, but only

a few studies have been made of their interactions with low zinc diets. Davis (1958) observed that increasing the level of zinc in the diet causes a decrease of copper values in the liver, but only when the copper levels in the diet approach those of a low normal value of about 5 to 10 ppm copper.

Reinhold et al. (1967) found no evidence of low zinc intake on the copper concentration in liver and kidneys, but their diets contained higher concentration of copper based on the mineral mix composition bases, since there was no copper analysis reported for the diets fed.

The depression of the copper values in the liver observed was highly significant ($P < 0.01$). The copper content in liver for the groups fed 10 ppm, 15 ppm and 20 ppm of zinc in diets were 5.6 ppm, 6.8 ppm and 5.4 ppm of zinc which are below the 10-50 ppm of copper concentration in liver reported for normal adults rats or humans (Underwood, 1962). A decrease in the copper content of the liver of this group may have been due to dilutional phenomena, but Van Campen and Scaife (1967) suggested from their studies, that a depression of copper absorption by high levels of zinc is mediated either in or on the intestine; the same antagonistic effect might be present for the low concentrations. A marked reduction in liver catalase and cytochrome oxidase was observed by Van Reen (1953) for high diets, but there is no evidence on this point, and it might be related to the copper reduction observed in this experiment.

Experiment II

Part A

The results of Experiment II, part A for food intake, growth, food efficiency and zinc content in bones are reported in Table 7. Bones were analyzed from only three of the six groups at the end of third week, since the other groups were retained for further study. One rat of the group fed 10 ppm zinc diet died on fifteenth day of experiment but the reasons were not determined.

The values obtained for appetite, growth, food efficiency and zinc content in bones, in general confirm the values in the Experiment I. Consequently the values of both experiments were submitted to a statistic study to obtain a more complete picture of the relationship between the zinc concentration of the diets and the other variables. The analysis of bones of the two experiments were determined in the first experiment after four weeks and in the second after three weeks of experiment. However on the basis of the studies of Alexander and Nusbaum (1962), who did not find any variation of zinc concentration with time, the values were grouped together.

In the Figure 2 is shown the relationship of weight gain to the concentration of zinc in the diet for all diets studied after three weeks on the experiments.

Similar correlations were obtained for food consumption and for food efficiency, but they are not shown. Thus the results of both experiments indicate that the zinc requirement of the rat approximates 10 ppm of the diet.

In Figure 3 it is important to note that the linear increase of

TABLE 7

Response of Rats Fed Different Levels of Zinc in Experiment II

Concentration of Zinc in the Diet (ppm)	0	5	7.5	10	12.5	15
No. of rats	9	8	8	7[a]	8	8
Days fed diet	21	21	21	21	21	21
Avg. initial wt. (g)	47.4	47.3	47.1	47.1	47.3	47.4
Avg. final wt. (g)	66.4	125.0	170.3	170.7	167.5	178.0
Total wt. gain per animal (g)	19.0±1.3	77.6±3.8	123.1±2.9	123.6±3.2	120.3±3.7	130.6±2.5
Avg. daily gain (g)	0.9	3.7	5.9	5.9	5.7	6.2
Total diet consumed (g)	100.2±3.1	183.8±7.0	250.6±3.8	238.6±6.0	232.8±5.4	259.0±3.4
g food/g weight gain	5.4±0.28	2.37±0.05	2.04±0.04	1.93±0.02	1.94±0.02	1.98±0.02
Bone ash %	---	57.10±0.5	58.0±0.3	---	56.8±0.4	---
Zn in bones	---	81.6±3.2	125.5±5.2	---	236.0±4.6	---

[a]One of the rats died on fifteenth day of the experiment.

[b]No values for 0 ppm, 10 ppm and 15 ppm zinc diets were obtained, since the rats were utilized for the part B of the Experiment II.

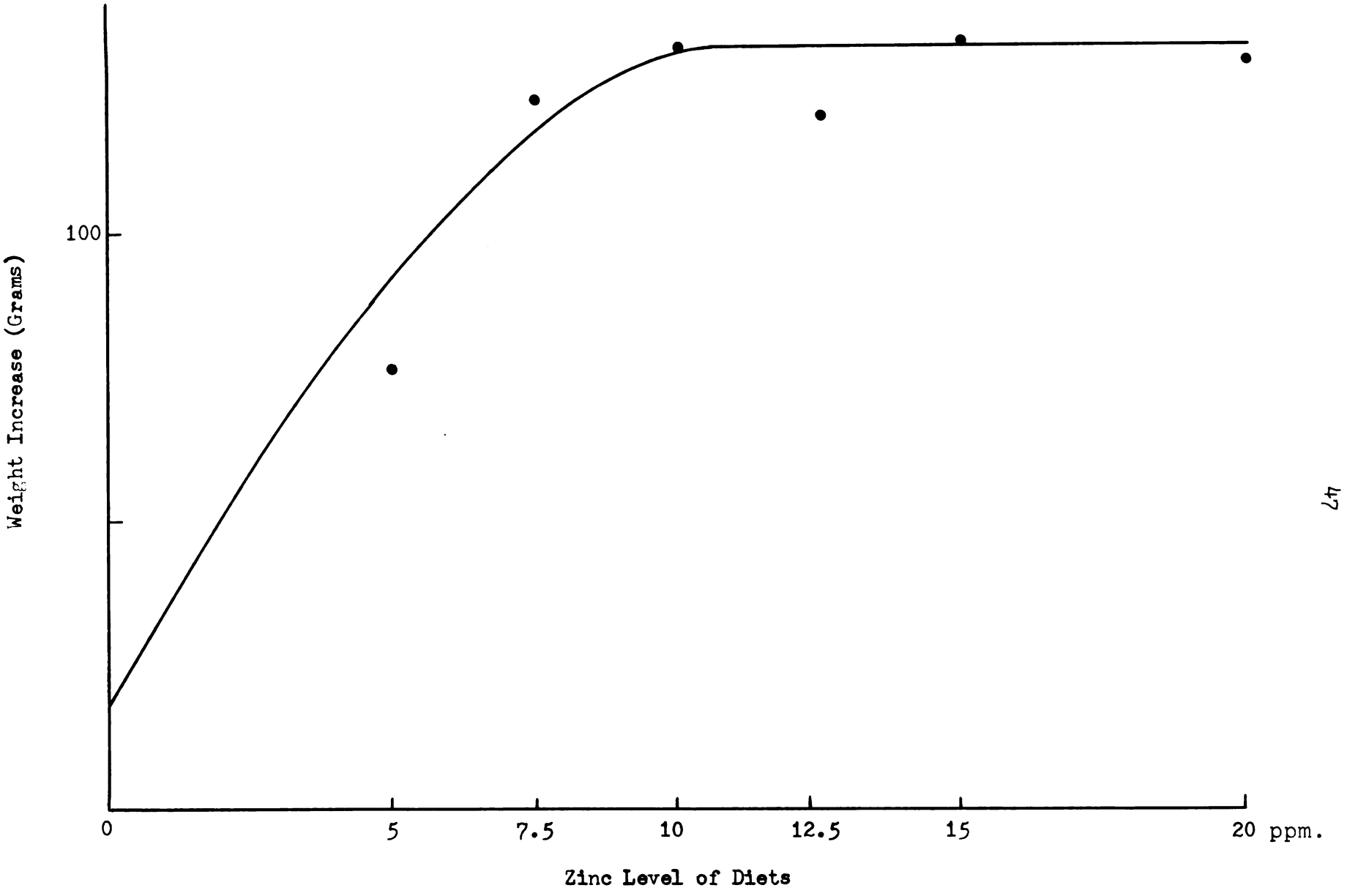


Figure 2. Relation of Weight Increase to Zinc Diet Levels

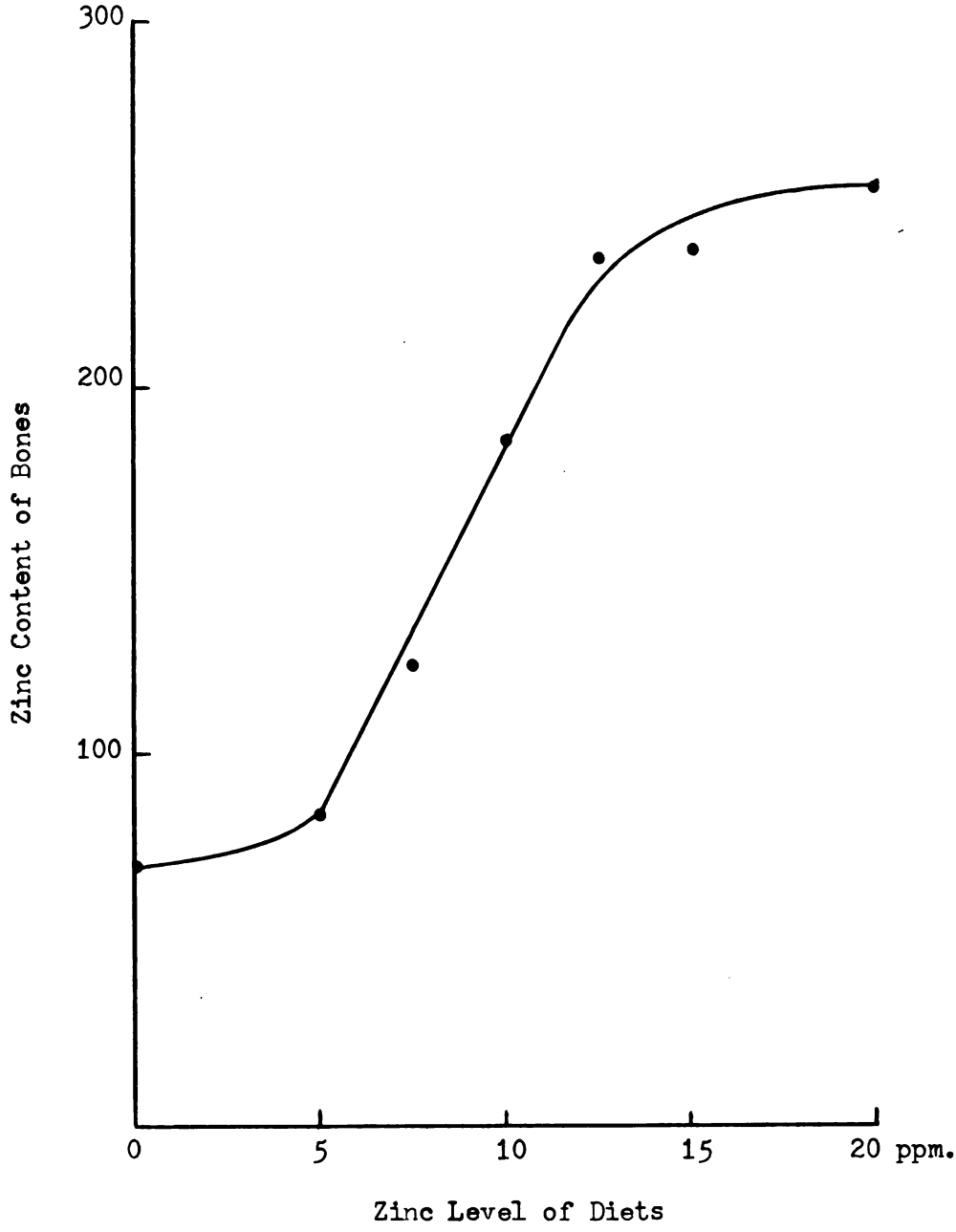


Figure 3. Relation Between Bone Zinc and Zinc Diet Levels

zinc concentration of the bones with respect to zinc content of the diets is between 5 and 12.5 ppm. It is also observed that only a small difference in the concentration of bone zinc occurs between basal and 5 ppm zinc diet. This indicates that the animal utilizes at maximum the zinc available for growth and that the amount of zinc is not sufficient for the requirements of normal development. The growth rate was 50 percent of the 10 ppm zinc diet group, and consequently there is not an excess of zinc for storage in the bones.

For the higher concentrations of zinc in the diets the increment in bone zinc content is smaller. This may be due to some regulating mechanism or to a decrease in efficiency of the absorption mechanisms, since the immediate requirement of the animals for normal growth are satisfied.

Although the essentiality of zinc for growth and metabolism of the bone has not been established its presence in the deficient animals at similar concentration (70-80 ppm) in the kidneys and liver on a dry basis suggests that its presence is more than accidental.

Part B

The results of Experiment II, part B, are reported in the Table 7. The initial values of food intake are the average of the last 48 hours on the original diet. The final food consumption is also the average of 48 hours, even if it was checked every 24 hours.

The appetite of the repleted rats increased 50 percent in the first 24 hours, about 100 percent at 48 hours and up to 300 percent at 96 hours. The consumption later increased slowly up to 450 percent during the subsequent two weeks. Quarterman (cited by Mills, 1967) noted

an increase in appetite after four hours of feeding a repleted diet. In this case the increment of food consumption was 50 percent after 24 hours; nevertheless, it was noted that after the animal tried the new diet, the animal immediately became more interested in it. The response of the first hours was not determined and might not be detectable, but a change in the interest of the animals was evident in a few minutes. The mechanism related to this quick response is being studied by Quarterman. The current evidence suggests that enzymes other than the presently known zinc enzymes are more sensitive to zinc deficiency, it is possible that the same enzymes also play an important role in repletion. The activities of amylase and of liver and kidney catalase, which are not zinc dependent enzymes have been shown to decrease in zinc deficiency (Robertson and Burns, 1963). It is of interest that if amylase is involved in the depletion it may be that the same enzyme plays some role or other similar in the fast response due to repletion, since α-amylase (1-4 glucan 4 glucanohydrolase) is present in saliva and pancreatic juice.

The homeostatic mechanisms which acts at the sites of absorption and on intestinal excretion are a reflection of mechanisms resulting in homostatic conservation of the zinc. It was observed that a very high absorption exceeding 80 percent occurred for the low zinc content diets, but its importance in the fast response due to repletion is unknown.

Similar responses to appetite, growth, food efficiency, was observed for both groups of animals on 10 and 15 ppm zinc diet, even appearing a little higher for the 15 ppm zinc diet. One rat on the basal diet had diarrhea in the latter part of Experiment II, part A and

placed on 10 ppm zinc diet to determine whether it could be corrected by administration of only a zinc supplemented diet. In the first experiment two animals from the group on the basal diet died in a few days from diarrhea. The rat responded to the supplemented diet similarly to those of the other animals and the diarrhea was stopped.

The inverse picture was observed with the rats of groups from 10 and 15 ppm zinc diets when placed on the depleted diet (basal diet). Again both groups responded quite similarly but the effect was quicker on rats previously fed 10 ppm zinc diet. The food intake decreased to 66 percent at 72 hours for rats previously fed 10 ppm zinc diet, while 80 percent consumption was observed for the rats on the 15 ppm zinc diet at 96 hours. More depression of food consumption was observed a little later and subsequently a marked increase in food intake was maintained for 24 hours in rats on the 10 ppm zinc diet and 72 hours in rats on the 15 ppm zinc diet. After that a second depression of food consumption occurred and the intake was maintained around 50 percent of the controls for the last two weeks of the experiment.

The difference observed in the animals to repletion and to depletion of zinc may be influenced by the presence of a zinc supplemented diet in the digestive tract, which delays the response of the animal to the new diet.

The zinc content of the bones of deficient rats on repletion increased from 65.3 ppm to 78.2 ppm of zinc for the 10 ppm zinc diet and to 135.3 for the 15 ppm zinc diet, respectively, after one week. The values were 100.4 ppm and 172.5 ppm zinc for the 10 ppm and 15 ppm zinc diet, respectively, after three weeks. All of the values are expressed on dry, fat-free bone basis. Here the difference between

TABLE 8

Response of Rats Fed Different Levels of Zinc to Switchover the Diets - Experiment II, Part B

ONE WEEK

Diet ppm Zinc	Original Diet ppm Zinc	No. Rats	Avg. Daily Food Consumption g		Body Weight		Zinc in Bones		Zinc in Liver		Copper in Liver	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
10	0	4	4.2	12.0	66.7	116.2	65.3	78.2	72.2	76.9	16.0	12.7
15	0	3	4.2	13.0	66.7	122.7	65.3	135.3[a]	72.2	77.5	16.0	10.9
0	10	4	13.7	7.0	173.7	173.0	131.2[a]	104.4	--	78.3	--	9.3
10 (control)	10	2	13.7	17.0	170.0	205.5	131.2	--	--	83.5	--	8.3
0	15	4	16.0	12.7	177.7	187.5	216.5	143.5	78.6	75.3	6.0	8.0
15	15	2	16.0	17.0	177.5	216.0	216.5	--	78.6	--	6.0	--

THREE WEEKS

10	0	2	4.2	18.8	66.5	239.0	65.3	100.4	72.2	74.7	16.0	9.4
15	0	2	4.2	17.3	67.5	220.0	65.3	172.5	72.2	77.5	16.0	11.2
0	10	2	13.7	8.3	175.0	174.0	131.2	93.1	--	74.1	--	9.1
10	10	2	13.7	17.3	170.0	268.5	131.2	151.2	--	83.5	--	8.3
0	15	2	16.0	8.7	180.5	183.5	216.5	121.5	78.6	86.4	5.9	7.4
15	15	2	16.0	17.8	177.5	270.5	216.5	217.7	78.6	86.6	6.0	6.5

[a]Only one value.

the two groups of animals placed on 10 ppm and 15 ppm zinc diet was even higher than that observed for food consumption and growth rate.

The zinc content of the bones of rats on a basal diet that were previously on 10 ppm and 15 ppm zinc diet, decreased from 131.2 ppm to 104 ppm and from 216.5 to 143.5 ppm in one week and to 93.1 ppm and 121.5 ppm of zinc at the end of the third week, respectively.

The zinc content of the bones decreased 30 percent during the first week. Alexander and Nusbaum (1962) found that the ends of the bones contained 30 percent more zinc than did the shaft. The zinc from the ends may be released for maintaining the homostasis since at the time of osteogenesis zinc is still free; while the zinc of calcified bone is literally sequestered, neither Ca-EDTA nor dithizone can react with it and the zinc apparently is not available for ionic exchange (Haumont and McLean, 1965).

V. SUMMARY

Experiment I

Forty weanling rats were fed a diet containing egg white solids as a source of protein, supplemented with zinc as zinc sulfate at levels of 0 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm for four weeks.

The manifestations of zinc deficiency syndrome in the rat, which include anorexia and growth retardation, was presented by the group on the basal diet. The appetite, growth rate and food efficiency were significantly related to the zinc content in the diet. Diarrhea occurred in two animals on basal diet, which died, a few days later. But it was not observed in any of the animals of other groups.

The zinc content of the bones increased linearly with the concentration of the zinc in the diet.

No differences were observed in the zinc concentration in kidneys and liver with the zinc diet. Copper concentration of the liver decreased significantly with the increase of zinc in the diet.

Experiment II

Part A

Forty-nine weanling rats were placed on this experiment and fed for three weeks. The composition of the basal diet was the same as used for the Experiment I and it was supplemented with zinc as zinc sulfate at levels 0 ppm, 5 ppm, 7.5 ppm, 10 ppm, 12.5 ppm and 15 ppm of

zinc. The results obtained for appetite, growth rate and food efficiency confirmed the observations of the Experiment I.

Only the analysis of bones for the groups 5 ppm, 7.5 ppm and 12.5 ppm zinc in the diet were done at the end of third week, since the other three groups were retained for further study. The concentration of zinc in the bones was significantly related to the zinc contain of the diet.

Part B

The rate of the basal group were interchanged with the rats on 10 ppm and 15 ppm zinc diets to test the hypothesis that bone plays an important role in stored and released zinc for use by critical organs during the depletion and to see if the previous history of the animal exerts some influence on the appetite and growth rate, as well as the concentration of zinc in the tissues.

The response of both groups of rats placed on basal diet showed a similar picture in the rats previously the 15 ppm zinc diet, the depression of appetite was observed later and to a lower degree.

The rats from basal diet on repletion fed 10 ppm and 15 ppm zinc diets showed a very similar response and a very rapid increase in food intake. The concentration of zinc in bones increased faster in the animals on 15 ppm zinc diet than on 10 ppm. Also a decrease in copper concentration was observed with the increase in zinc levels of diet, but neither liver nor kidney concentration of zinc was affected.

VI. CONCLUSIONS

Supplementation of egg white protein diet with zinc at levels of 10 ppm supplies the minimum requirement by the weanling rats for normal growth. Inanition accompanying the deficiency plays a significant role in the growth rate and food efficiency.

Bone zinc concentration is linearly increased with dietary levels of this element between the limits studied.

Zinc concentration of the liver and kidney showed no significant alteration in relation to the zinc content of the diet at the levels used.

Copper content of the liver was decreased significantly with the first increment of zinc in the diet (5 ppm).

On the repletion with zinc in the deficient group, the zinc content of bones increased significantly. Also observed was an high increment in growth rate and appetite.

On the depletion the zinc content of bones was reduced significantly in one week, it was not sufficient to maintain the normal growth rate and the appetite.

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APPENDIX TABLE 1

Zinc Content of Diets by Analysis

Experiment I

Level of Zinc in the Diet (ppm)	0	5	10	15	20
By analysis	0.80	5.08	10.83	15.69	18.91

Experiment II

Level of Zinc in the Diet (ppm)	0	5	7.5	10	12.5	15
By analysis	0.78	4.50	6.80	9.29	12.30	14.30

APPENDIX TABLE 2

Exp. I--Gain Weight, Food Intake, Bone Ash, Zinc in Bones, Livers and Kidney, and Copper in Livers and Kidneys

Rat No.	Weight gain, g.	Food intake	Bone ash %	Zn in bones ppm	Zn in liver ppm	Cu in liver ppm	Zn in kidney ppm	Cu in kidney ppm
Lot I - Basal diet - 0 ppm Zn								
1	11	103	58.7	87.2	78.1	12.8	86.7	24.1
2	10	102	56.3	68.6	90.5	13.4	88.7	26.3
3	24	108	58.5	75.3	79.2	12.6	--	--
4	19	101	57.5	59.3	79.1	10.4	--	--
5	13	113	55.3	75.1	143.7	23.7	88.2	18.7
6	9	100	57.1	66.2	89.0	13.1	--	--
7	23	117	58.3	56.9	75.5	14.3	--	--
8	20	122	54.9	77.4	113.3	21.4	--	--
Mean	(16.1)	(108.3)	(57.1)	(70.8)	(93.6)	(15.2)	(87.9)	(23.0)
SE	2.1	2.9	0.5	3.5	8.4	1.7	0.6	2.3
Lot II - 5 ppm Zn								
9	84	195	60.3	84.0	88.0	8.6	80.6	16.0
10	65	182	57.3	82.0	86.0	11.8	83.4	20.8
11	68	203	60.7	84.3	76.7	8.5	74.9	20.6
12	95	215	60.0	87.0	66.8	11.6	--	--
13	57	215	59.7	81.9	71.4	9.4	--	--
14	65	194	61.9	90.6	79.2	8.1	--	--
15	62	166	62.6	106.1	81.5	9.9	--	--
16	104	240	59.6	84.1	73.9	9.7	--	--
Mean	(75)	(201.2)	(60.3)	(87.5)	(77.9)	(9.7)	(79.6)	(19.1)
SE	6.1	8.0	0.6	2.8	2.6	0.5	2.5	1.6

APPENDIX TABLE 2--CONTINUED

Rat No.	Weight gain, g.	Food intake	Bone ash %	Zn in bones ppm	Zn in liver ppm	Cu in liver ppm	Zn in kidney ppm	Cu in kidney ppm
				Lot III - 10 ppm Zn				
17	134	295	62.5	208.4	71.4	4.3	86.8	17.4
18	161	338	60.6	174.0	69.5	6.9	85.0	16.9
19	141	284	61.3	172.9	69.6	9.2	89.8	16.7
20	123	307	57.9	185.8	79.7	3.9	--	--
21	144	314	59.9	185.2	84.4	6.8	--	--
22	135	282	59.8	180.8	83.4	2.5	--	--
23	160	330	59.2	191.8	89.0	2.6	--	--
24	124	271	59.4	193.3	73.9	8.5	--	--
Mean	(140.3)	(302.6)	(60.1)	(186.5)	(77.6)	(5.6)	(87.2)	(17.0)
SE	5.1	8.4	0.5	4.1	2.7	0.9	1.4	0.2
				Lot IV - 15 ppm Zn				
25	116	244	59.6	232.4	87.9	7.0	92.3	15.5
26	125	290	59.2	224.7	80.8	5.3	95.6	17.1
27	159	313	59.6	225.5	87.1	10.9	89.6	15.6
28	144	301	60.7	243.4	91.8	7.1	--	--
29	116	273	60.6	256.8	86.7	7.1	--	--
30	147	324	59.9	250.3	76.2	5.5	--	--
31	124	268	61.7	234.6	77.0	5.4	--	--
32	158	316	60.9	258.7	80.7	6.5	--	--
Mean	(135.9)	(291.1)	(60.3)	(238.2)	(83.5)	(6.8)	(92.5)	(16.1)
SE	6.3	9.8	0.3	4.4	2.0	0.6	1.7	0.5
				Lot V - 20 ppm Zn				
33	140	289	60.1	254.2	76.3	5.5	80.8	16.7
34	114	261	60.1	249.9	92.2	4.4	89.3	19.4
35	119	271	60.5	279.0	87.5	4.7	88.1	15.1
36	135	284	60.4	253.6	83.1	4.5	--	--
37	118	251	60.7	242.5	76.3	4.3	--	--
38	155	347	61.3	257.3	90.6	8.1	--	--
39	131	279	61.3	261.5	93.2	6.6	--	--
40	128	281	61.2	252.8	79.9	5.1	--	--
Mean	(130.0)	(282.9)	(60.7)	(256.4)	(84.9)	(5.4)	(86.1)	(17.1)
SE	4.8	10.2	0.2	3.8	2.5	0.5	2.7	1.3

APPENDIX TABLE 3

Exp. II--Gain Weight, Food Intake, Bone Ash, Zinc in Bones

Rat No.	Weight gain, g	Food Intake, g	Bone Ash %	Zn in Bones ppm
		Lot I - Basal diet - 0 ppm Zn		
1	14	91		
2	19	97		
3	24	121		
4	22	103		
5	17	90		
6	14	98		
7	17	95		
8	20	103		
9	24	104		
Mean	(19.0)	(100.2)		
SE	1.3	3.1		
		Lot II - 5 ppm Zn		
10	73	185	54.6	88.0
11	79	175	57.7	88.1
12	69	166	55.9	86.7
13	84	212	58.5	87.4
14	63	155	57.7	67.3
15	96	208	57.9	69.9
16	86	193	58.6	75.8
17	71	176	55.9	89.5
Mean	(77.6)	(183.8)	(57.1)	(81.6)
SE	3.8	7.0	0.5	3.2

APPENDIX TABLE 3--CONTINUED

Rat No.	Weight gain, g	Food Intake, g	Bone Ash %	Zn in Bones ppm
		Lot III - 7.5 ppm Zn		
18	127	269	58.3	126.7
19	116	253	58.7	140.9
20	125	246	58.6	114.6
21	127	249	58.4	116.2
22	114	240	58.1	134.9
23	136	255	58.5	110.1
24	128	258	56.9	110.5
25	112	235	56.4	148.9
Mean	(123.1)	(250.6)	(58.0)	(125.5)
SE	2.9	3.8	0.3	5.2
		Lot IV - 10 ppm Zn		
26	135	264		
27	128	247		
28	129	251		
29	120	228		
30	127	231		
31	112	219		
32	--	--		
33	114	230		
Mean	(123.6)	(238.6)		
SE	3.2	6.0		
		Lot V - 12.5 ppm Zn		
34	123	247	56.8	232.5
35	136	254	57.8	250.9
36	116	231	54.5	227.1
37	105	211	57.9	251.8
38	121	235	57.9	248.2
39	122	233	56.9	237.0
40	108	212	56.6	221.8
41	131	239	56.3	219.0
Mean	(120.3)	(232.8)	(57.1)	(236.0)
SE	3.7	5.4	0.4	4.6

APPENDIX TABLE 3--CONTINUED

Rat No.	Weight gain, g	Food Intake, g	Bone Ash %	Zn in Bones ppm
		Lot VI - 15 ppm Zn		
42	132	260		
43	118	238		
44	137	263		
45	129	257		
46	137	267		
47	139	269		
48	125	257		
49	128	261		
Mean	(130.6)	(259.0)		
SE	2.5	3.4		