THE EFFECT OF ZINC LEVELS ON NITROGEN RETENTION IN PREADOLESCENT CHILDREN

by

Christine Renee Meiners

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Human Nutrition and Foods

APPROVED:

S. J. Ritchey, Chairman

Carl E. Polan

Raymond G. Cragle

Mary K. Korslund

Judy Driskell

Blacksburg, Virginia

December, 1975

ACKNOWLEDGEMENTS

The author is grateful to Dr. S. J. Ritchey, major advisor, for his guidance, patience, and encouragement throughout the study. Appreciation is also expressed to the children who participated in the study and the people who worked on the study, all of whom helped make this dissertation possible. She is also grateful to Dr. Mary Korslund, Dr. Raymond Cragle, Dr. Carl Polan, and Dr. Judy Driskell for their advice and service on the dissertation committee. The author would like to thank Dr. Kenneth Webb for his generous assistance in the amino acid analysis of the diet.

Thanks are due the author's husband for his unfailing encouragement throughout the study and the duration of her graduate program.

TABLE OF CONTENTS

Chapter

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 4

Zinc Deficiency 4
Metabolic Role of Zinc 7
Metabolic Interrelationships Involving Zinc 13
Evaluation of Zinc and Protein Nutrition 17

III. EXPERIMENTAL PROCEDURE 23

Experimental Design 23
Experimental Subjects, Management, Diet and Supplement Description 25
Urine and Fecal Collection 33
Nitrogen and Zinc Determination 34
Statistical Testing 36

IV. RESULTS AND DISCUSSION 37

Anthropometric and Biochemical Measurement on Subjects . . 37
Nitrogen Excretion 39
Nitrogen Balance 43
Zinc Excretion 48
Zinc Balance 52

V. SUMMARY AND CONCLUSIONS 55

LITERATURE CITED 58

APPENDIX I, Age, Height, Weight, Hemoglobin and Hematocrit of Subjects on Initial Day of Study 65

APPENDIX II, Typical Daily Schedule 67

APPENDIX III, Menus Served in Study 69

APPENDIX IV, Height and Weight of Subjects During Basal and Nitrogen Balance Period 74

APPENDIX V, Daily Urine Nitrogen for the Eight Days of Period I and Days 1-12 of Period II (g/day) 77

APPENDIX VI, Individual and Mean Daily Fecal Nitrogen for the Two Six-Day Periods in Period II (g/day) 80

APPENDIX VII, Individual Daily Total Nitrogen Excretion for Period II 82

APPENDIX VIII, Mean Daily Apparent Nitrogen Excretion by Treatment Group for Period II, g/day 84

APPENDIX IX, Individual Daily Urine Zinc Concentrations for the Three Compositing Periods of Period II 86

APPENDIX X, Individual Fecal Zinc for Period II 88

VITA 90

LIST OF TABLES

Table		Page
1.	Experimental Treatments During Days 9-20 of the Balance Study	24
2.	Comparison of Diet Protein and Whole Egg Protein for Determination of Amino Acid Supplement	31
3.	Summary of Heights and Weights of Subjects During Study	38
4.	Mean Daily Urine and Fecal Nitrogen and Daily Nitrogen Balance During Last 6 Days of Period II	40
5.	Nitrogen Excretion and Utilization	41
6.	Effect of Zinc, Essential Amino Acids or Zinc-Essential Amino Acids Interaction on Nitrogen Balance, g/day	46
7.	Mean Urine Zinc, Fecal Zinc and Daily Zinc Balance for the Last 6 Days of Period II	49
8.	Zinc Excretion and Utilization	50
9.	Effect of Zinc, Essential Amino Acids or Zinc-Essential Amino Acids Interaction on Zinc Balance, mg/day	53

CHAPTER I

INTRODUCTION

Mineral metabolism and utilization have received additional emphasis and taken on new importance in nutritional science during the past 20 years. Deficiency states for numerous trace elements have been defined in animals and man and recommended allowances have been generated for human populations. The nutritional role of zinc has received considerable focus as naturally occurring deficiencies have been reported in domestic animals (4) and in the human (11,13). In the 40 years since zinc was shown to be essential to life (1), comparatively little work has been done on its metabolic role and the nutritional interrelationships into which it may enter.

Naturally occurring zinc deficiencies are not completely due to dietary lack, because the metal is widely distributed in nature. Rather, zinc deficiency has been due to conditions that decreased the availability of dietary zinc. The difficulty in deleting zinc from the diet led early investigators to believe a zinc deficiency to be almost impossible (16). However, the effects of phytic acid in decreasing the availability of zinc is well known (62) and the effects of other dietary factors, such as calcium, have been documented to a lesser degree (34).

Zinc has been implicated in the utilization of dietary nitrogen in sheep (63). Hsu *et al.* (64), Lieberman *et al.* (20), and Terhune and

Sandstead (25) demonstrated that zinc was essential for protein and nucleic acid synthesis. However, the reports on experimental animals are clouded by the work of Mills et al. (17) who suggest that changes in appetites of animals receiving zinc deficient diets may be partially or completely responsible for reported observations. Newer methods of pair-feeding can eliminate extraneous appetite factors. It is possible now to do a complexity of studies involving zinc deficiency which were thought to be impossible a few years ago. But, what are the effects of marginal zinc intakes or binding of zinc by chelating molecules coexisting with zinc within the food item on protein metabolism in humans? There remain many unresolved questions concerning the effect on protein utilization at various dietary zinc levels.

Case histories have shown dramatic increases in growth and obvious protein anabolism due to introduction of zinc to zinc-deficient patients. However, very few controlled studies on animals or humans have been conducted to examine the interrelationship of zinc and protein. Increased protein utilization resulting from increased zinc has not been conclusively proven in test animals or man. This is particularly true when either protein or zinc are given in marginal amounts. It remains to be seen if zinc, in critical amounts, can affect protein utilization in man.

Nitrogen balance can be used to monitor the fate of dietary protein and protein utilization as it is affected by certain controlled experimental variables. Nitrogen balance is an appropriate technique (65) used to evaluate the effects of variation in protein quality and

dietary zinc quantities on protein utilization. No reports of controlled nitrogen balance studies were found in which the effects of different levels of dietary zinc and protein on nitrogen utilization were investigated in healthy human subjects. It remains to be seen if the observed cellular interrelationship of zinc and protein can be illustrated further by the nitrogen balance technique. It also is questionable if this can be seen in healthy, growing subjects who are not consuming zinc-deficient diets.

The objective of this research was to investigate the effects of variations in dietary zinc or protein quality on protein utilization. It was hypothesized that there is an effect on nitrogen balance due to variation in zinc quantity and protein quality in subjects consuming low-protein diets.

CHAPTER II

REVIEW OF LITERATURE

Zinc Deficiency

For over 100 years, zinc has been recognized as essential to many forms of plant and bacterial life. Only within the last 15 years, though, has the significance of zinc in practical animal nutrition been recognized. Due to its ubiquitious occurrence in nature, zinc was difficult to eliminate from human or experimental animals' diets. Therefore, the probability of zinc deficiency seemed remote. In 1934, Todd et al. (1) showed that zinc was essential to the growth of rats. Deficiency symptoms did not occur until only 2 ppm (μg/g) remained in the diet. In more recent studies by Macapinlac et al. (2) zinc-deficient rats exhibited anorexia, marked growth retardation, coarse and sparce hair growth, lymphocytopenia, and increased hematocrit values. Testes, but not ovaries, showed atrophy. Also seen were hyperkeratosis, acanthosis, and parakeratosis of the skin, esophagus, and forestomach. Hurley (3) demonstrated clearly the feasibility of producing zinc deficiency in rats consuming zero and 1 ppm of zinc. Diets of 40 ppm zinc supported nearly normal growth rates. Growth retardation in zinc deficient rats was accompanied by alopecia, dermal lesions, emaciation, abnormal posture, and often death. Zinc deficient females had abnormal estrous cycles and were unable to breed. Less

than half of marginally deficient female rats (fed 9 ppm zinc) delivered live young at term. Those pups were smaller and 98 percent showed gross congenital malformations. The rapid effect of the deficiency was seen to be precipitated by lack of mobilization of zinc from maternal stores, as was the case with other minerals such as calcium.

In 1955, Tucker and Salmon (4) reported that zinc cured and prevented parakeratosis in swine. Rations with 34 to 44 ppm zinc caused a zinc deficiency which was aggravated by added dietary calcium and phosphorus. It appeared that swine had a relatively high zinc requirement. O'Dell and coworkers (5,6) showed in 1958 that zinc was essential for growth and various other functions in birds. Zinc deficiency in female rabbits resulted in reduced feed intake, weight loss, low hematocrit, dermatitis, and reproductive failure (7). In fact, zinc has been shown to be required by calves, lambs, mice, dogs, quail, hens, and other experimental animals, including man (8).

Zinc was recognized as an essential nutrient for man in 1968 (9). The statement was made that persons eating a varied diet were not likely to develop zinc deficiency. The most recent revision of the Recommended Dietary Allowances (1974) provides recommended intakes of zinc ranging from 10 to 15 mg daily for children and adults (10).

Severe experimental zinc deficiency has never been produced in man, but the studies of Prasad (11,12,13) described Iranian and Egyptian male patients with natural dietary zinc deficiency. These men showed iron-deficiency anemia, hepatosplenomegaly, short stature, and marked hypogonadism. Zinc concentrations in hair, plasma, urine,

and sweat were low. Egyptian subjects showed various parasitic infestations. It was postulated that the zinc deficiency syndrome had its etiological origins in early childhood in both groups. Three major contributors to the condition were: (1) a diet high in cereals and legumes, (2) parasitic infestations whereby zinc-rich red blood cells are lost, and (3) geophagia, whereby natural chelating agents in soil bound zinc making it inaccessable for absorption. Excessive sweat loss by the subjects due to the warm climate was felt to contribute to zinc loss. Prasad (8) cited evidence for deficiency by showing an increased plasma ^{65}Zn turnover rate, decreased 24-hour exchangeable zinc pool, and decreased excretion of ^{65}Zn in stool and urine following intravenous injection of the isotope. Urinary excretion of stable zinc was also decreased. Oral administration of zinc resulted in accelerated growth rate, appearance of secondary sexual characteristics, and enlargement of external genitalia.

Until 1971-1972, all cases of overt zinc deficiency were reported in male patients. Because of this and because the testes, not the ovaries, contain large quantities of zinc, it was thought that the syndrome was limited to males. Ronaghy and Halsted (14) reported two females with zinc deficiency. They showed dwarfism, hypogonadism, iron-deficiency anemia, and geophagia. The anemia was quickly respondent to oral iron as was true of the male zinc-deficient dwarfs. Growth and sexual development occurred quickly when 120 mg of zinc sulfate was administered daily along with a well-balanced diet and an iron supplement. This diet, prior to the addition of zinc sulfate, resulted in gradual but much slower growth and sexual development. Thus, it was

confirmed that human growth and development was contingent upon adequate zinc intake.

Endocrine manifestations of zinc deficiency have been described by Sandstead et al. (15). In subjects discussed earlier (11,12,13), certain endocrine aberrations were seen. Hypopituitarism was manifested as hypogonadism, growth failure, and retarded osseous development. Decreased ACTH reserve and abnormal oral glucose tolerance were found in the patients. There appeared to be a functional relationship between zinc and pancreatic formation of insulin and glucagon. Zinc is concentrated in male genital tissues and fluids. The lack of normal sexual development seen in zinc-deficient males and females points to a zinc reproductive hormone relationship. It is possible that growth was retarded in general or specific areas because zinc was scarce and, due to some configurational criterion, protein synthesis was deterred and nongrowth or even catabolism predominated. Therefore, general growth retardation or specific areas of retardation are seen (15).

Metabolic Role of Zinc

In general, zinc functions by binding to molecules in biological systems to establish and maintain configurational relationships necessary for chemical and physical function. Examples are reported in the literature describing the role of zinc with respect to secondary, tertiary, and quaternary structure of proteins, the structure of nucleic acids, the transport and release of metabolites or large protein molecules such as enzymes and the protein hormones, and synthetic and catabolic reactions in the body (16).

Zinc is known to be an essential component of several metalloenzymes. These include: carbonic anhydrase, pancreatic carboxypeptidase, liver and yeast alcohol dehydrogenase, alkaline phosphatase, tryptophane desmolase, malic dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, and probably other pyridine nucleotide-dependent metallodehydrogenases. Also, zinc increased the activity of several other enzymes, apparently as a cofactor in some nonspecific manner (8). The primary enzymatic role of zinc may be in substrate binding or other catalytic functions. Zinc was known to act as a stereospecific binder for the coenzyme in horse liver alcohol dehydrogenase. Prasad (8) noted the likelihood that zinc-sensitive enzymes were capable of controlling cellular activity as a function of zinc availability to them. Mills et al. (17) found that pancreatic carboxypeptidase was the earliest to be affected by dietary lack of zinc. This occurred prior to any appetite or growth impairment in the animals. It is not known if this deactivation of the enzyme was due to deficiency of zinc for incorporation into the apoenzyme or to decreased production of the apoenzyme itself. Since no effect was seen in pancreatic trypsin and chymotrypsin as a result of zinc deficiency, the probability that apoenzyme formation was deterred seemed low. Prasad (8) speculates:

> . . . that in the growing cell there is a series of apoenzymes, each present at some low concentration, and with normal levels of zinc the apoenzymes combine with metal, thus forming functional zinc enzymes. But at limiting levels of zinc, the apoenzymes behave as a series of ligands, each competing for the available zinc ions according to their stability constants . . . . The apoenzymes or ligands with high stability constants will be satisfied first, while those with low stabilities will not form functional zinc complexes. The

> concentration of free zinc ions will decrease progressively as zinc is diluted by continuing growth, but among the apoenzymes or other macromolecular ligands, there will be first one then perhaps others whose decreasing activities will limit growth. Even when growth stops altogether, we can expect that some of the ligands with the tightest affinities for zinc might still be completely satisfied.

Prasad's concept can only be tested when the stability constants of at least two such ligands for zinc, including one limiting to growth, have been determined. Unfortunately, evidence to date shows that such apoenzymes do not accumulate in the zinc-deficient state.

The earliest biochemical lesion to be detected in zinc deficiency was a lowered RNA content of cells. This has been reported in experimental animals, plants, and fungus (7). Zinc and other metals were important normal constituents of several microorganisms (18). Scarcity of zinc may contribute to RNA destruction through a configurational defect, or zinc may be necessary to protect RNA from ribonuclease attack. Increasing ribonuclease activity has been reported in apple leaves with decreasing zinc concentration (19). Research on DNA and RNA synthesis in tissue culture (20,21), partially hepatectomized rats (22), and microorganisms (23,24) has suggested that zinc may be involved in the synthesis of these polynucleotides.

Mills *et al.* (17) have found small but consistent reductions in RNA concentration of the pancreas and livers of zinc-deficient rats as compared with pair-fed controls. However, the decrease of RNA was small compared to the dramatic cessation of growth that characterized zinc deficiency. They noted that, in the study of zinc deficiency, faulty conclusions can be made regarding DNA synthesis depending on the curation of zinc deficiency and its effect on appetite and the type of

pair-feeding employed. Other nutrients besides zinc may alter DNA formation due to lowered food intake. Furthermore, zinc deficiency radically reduced appetite in test animals. Pair-feeding experiments involving zinc-deficient animals and controls must be on a continuous basis whereby pair-fed control animals get the quantity of food eaten by the test animals over the same period of time. This accounts both for the quantity of diet the test animals consumed and the time interval of food consumption. This has been crucial in many biochemical tests.

Terhune and Sandstead (25) reported that zinc deprivation of rat dams from the 18th day of gestation through the 16th day of the neonatal period produced pups with greatly decreased activity of nuclear DNA-dependent RNA polymerase in their livers, particularly after day 10 of life. These pups were compared with pups from pair-fed (semi-starved) dams and pups whose mothers had free access to food. They mention that it is now known that RNA polymerase of E.coli is a zinc metalloenzyme (26), and that their results coincided with this fact. RNA polymerase contains about 2 gram-atoms of tightly bound zinc per mole of enzyme. Enzyme activity was seen to be directly correlated to zinc content. Similarly, Fujioka and Lieberman (21) reported suppression of DNA synthesis in rat liver cells due to chelation of available zinc by EDTA. They noted that zinc cations are both tightly and loosely bound by cells and that EDTA only binds the loosely bound zinc cations. Still, the remaining tightly bound zinc cations were unavailable for the process necessary to prepare the cell for DNA formation. Sandstead and Rinaldi (27) showed further that

dietary zinc deficiency in the rat impaired *in vivo* synthesis of DNA in the rat liver parenchymal cell. That the impairment was not due to inanation was shown by the rapid effect of a single injection of the metal into the severely deficient animals and the greater nuclear labeling of ^{3}H-thymidine into nuclei of pair-fed control animals.

Other invetigators have studied the role zinc may play in protein synthesis as a logical furtherance of its role in polynucleotide metabolism. Hove *et al.* (28) observed over 35 years ago that total plasma proteins decrease in zinc deficient rats. Since then, it has been shown many times that zinc plays an intrinsic role in protein synthesis (24) and more recently in wound healing (29). Stephen and Hsu (30) found that zinc deficiency significantly reduced the incorporation of thymidine-methyl-^{3}H into the skin and several organs of both intact and wounded animals as compared to pair-fed control animals. It was felt that protein metabolism is adversely affected in the zinc-deficient state through control of DNA.

Another approach was used by Prasad and Oberleas (31) who tested the activities of ribonuclease and deoxyribonuclease in several organs of zinc-deficient, continuously pair-fed and *ad libitum* fed control rats. Whereas DNase activities showed no difference between the zinc-deficient and pair-fed control rats, the activities of RNase were increased in the zinc-deficient tissues. Increased RNase activity may, in part, be responsible for decreased protein synthesis and growth retardation so commonly observed in many animal species, including man, suffering from zinc deficiency. Fernandez-Madrid *et al.* (32) further studied the biosynthesis of nucleic acids, collagen, and noncollagenous

proteins in developing connective tissue from zinc-deficient rats and their pair-fed, ad libitum controls. They found the RNA/DNA ratio was significantly lower in zinc-deficient connective tissue at 6 and 10 days as compared to pair-fed control rats. In 10-day samples of connective tissue, RNA and DNA were decreased. Also, a significant reduction in the proportion of large collagen-synthesizing polyribosomes and the content of labeled hydroxyproline on the polyribosomes from zinc-deficient animals was noted. Total collagen and also noncollagenous protein were found to be significantly decreased in zinc-deficient animals. These animals also showed greatly decreased incorporation of ^{14}C-thymidine into DNA. These results were shown to be due to zinc deficiency and not inanation. Their results, again, suggest a generalized impairment of protein synthesis with a secondary derangement of the biosynthesis of collagen.

Oberleas and Prasad (33) showed that quality and quantity of protein interrelate with quantity of zinc in maintaining rats. Weanling male rats were freely given diets of 4, 8, 12, 16 or 20 percent soybean protein with or without 55 mg zinc per kg of diet for 10 weeks. Of those given 4 or 8 percent protein without zinc, 75 percent died, compared with 8 percent in the groups given zinc. Growth rates of rats on 12, 16 and 20 percent protein diets did not differ when zinc was provided and soybean protein supplemented with zinc was shown to be comparable in quality to casein. Rats deprived of zinc showed a decrease in dry weights of testes, thymus, and kidneys and in the activities of alkaline phosphatase of bone and testes and alcohol dehydrogenase in bone.

Metabolic Interrelationships Involving Zinc

The effects of dietary components on zinc availability have been widely studied. Because of the many dietary components which affect zinc availability it is impossible to define a zinc requirement for all dietary regimens (34,35). The source of zinc must be taken into consideration. In general, zinc in animal products was more readily absorbed than that in plant products, particularly those arising from plant seeds. Smith et al. (36) used pigs to investigate the effects of added zinc to various protein and carbohydrate sources on the dietary zinc requirement. They found that with or without added zinc, pigs consuming milk proteins (containing 6-18 ppm zinc) had superior growth and no symptoms of zinc deficiency. All pigs receiving soybean protein rations (containing 16-22 ppm zinc) showed typical zinc-deficiency symptoms. The addition of 50 ppm zinc to this diet alleviated the deficiency symptoms; however, the addition of zinc to the milk protein rations did not improve performance. Zinc-deficient pigs fed 50 ppm zinc, 450 ppm EDTA, and autoclaved soybean protein showed marked alleviation of zinc deficiency. Similar findings were reported by Forbes and Yohe (37) after studies with rats. Rats fed casein or egg white as the source of protein required approximately 12 ppm zinc in the diet, while those fed soybean protein required 18 ppm. The apparent absorption of zinc by rats fed casein was 84 percent compared to 44 percent by those fed soybean protein. Studies with chickens (38,39,40) also showed that soybean protein caused a lower percentage absorption of zinc from the intestine. From these results, it is clear that the zinc in isolated soybean protein is bound so that it

is absorbed less efficiently than the zinc in animal proteins such as casein and egg white.

Detailing the effect of dietary protein sources on zinc absorption and excretion in chicks, Miller and Jensen (38) reiterated what Smith et al. (36) stated above. For five weeks after hatching, chickens were given a semi-purified diet based on glucose and isolated soybean protein, with 50 ppm zinc. Groups of 20 were then given the basal diet alone, basal with soybean protein autoclaved, basal with 0.3 percent EDTA, or with casein and gelatin 4:1 replacing the soybean protein for seven days. Each diet had 50 mCi each of ^{144}Ce and ^{65}Zn per kg. When the chickens were killed, it was found that casein and gelatin increased absorption of zinc anterior to the gizzard and in the small intestine, and they increased secretions of zinc into the duodenum.

One of the differences between vegetable and animal proteins which explains the differences in zinc absorption was the content of phytate, the hemophosphate ester of inositol (34). Phytate is inherent in certain plant sources of protein. Isolated soybean protein binds phytate tenaciously and, combined with phytate's ability to combine with complex metal ions, contributes to the lower availability of zinc from this and other plant seed origins. To test this relationship, O'Dell and Savage (41) added phytic acid to a casein-based diet and the growth response of chicks fed this diet was compared to those fed soybean protein. Phytic acid decreased the availability of zinc in the casein diet and produced deficiency symptoms similar to those seen in animals fed soybean protein containing a comparable level of phytate.

Similar results have been obtained by addition of phytic acid to diets based on free amino acids (42). Thus, phytic acid need not be bound to dietary protein to exert its zinc-binding effects.

In the late 1950s, Krotezer et al. (43) observed that autoclaving soybean protein increased the availability of the zinc contained within. Pigs absorbed and retained more zinc when the diet was autoclaved (36). The phosphate ester bonds are broken by autoclaving in an aqueous medium and the rate and extent of hydrolysis depends on the plant protein with which the phytate is associated.

With a high calcium level in the diet, increased levels of phosphate appeared to lower zinc absorption. At relatively high phosphorus concentrations, increasing calcium levels progressively hindered zinc absorption. Calcium and phytate interacted to affect zinc absorption in several animal studies (34,37,41). However, a human study testing this hypothesis did not show this, presumably due to the high content of animal protein in the diet (44). In the absence of phytate, excess calcium has had no detrimental effect on zinc absorption in pigs or chickens; but when the diet contained phytate, excess calcium increased its zinc-binding action. These results offer an explanation for the aggravating effect of calcium on parakeratosis observed when high levels of calcium were added to diets based on corn and soybean protein, but the lack of an effect of calcium when animal protein constituted the major source of protein (34). Chelating agents, both natural and man-made, can compete with phytate to increase zinc availability for absorption.

Oberleas and Prasad (33) found that on an equal protein basis, growth in rats given animal protein was comparable to that in rats given plant seed protein diets plus 55 mg/kg zinc. They suggested that for humans consuming diets of predominately cereal grains or seeds and legumes, supplementation of the diet with zinc may improve growth and well-being. This would be appropriate to vast segments of the world's population, including low-income residents of the southern United States.

The analysis of diets of low and high protein content and of single food items by Osis et al. (44) indicated that foods and diets high in protein were also high in zinc, whereas those foods and diets containing mostly carbohydrate were found to be much lower in zinc and nitrogen. They found variability in the zinc content of the meals and of average daily total dietary zinc intake by more than a factor of two. They interpreted this as an indication of the difficulty in assessing the zinc intake for a large segment of the population due to variation in protein and carbohydrate intake among people.

Recent studies by Van Campen and House (45) indicated that an important consequence of protein depletion may be a secondary zinc deficiency. They fed rats diets containing either 5 or 15 percent casein and either 9 or 33 ppm of zinc. Rats fed the 5 percent protein diets retained less of a single oral dose of ^{65}Zn than did those fed the 15 percent protein diets at either dietary zinc level. Rats fed diets containing 33 ppm of zinc retained less ^{65}Zn than did those fed 9 ppm zinc diets. Their results indicated that the low protein diet reduced absorption of orally administered ^{65}Zn and increased endogenous excretion of zinc. Rats fed the low protein diets also had decreased

zinc concentrations in plasma, liver, and small intestine. It would seem that when growth is limited by protein deficiency, certain tissues are unable to maintain normal zinc concentrations. This may reflect lack of either a carrier molecule or a binding site for zinc.

Evaluation of Zinc and Protein Nutrition

The nutritional parameters for zinc are difficult to define using balance studies, since the intestines are the major route of zinc excretion making it hard to evaluate true absorption and excretion (16). The zinc content of accessable tissues and fluids has been widely studied as an index to zinc nutritional status. The zinc content of hair and feathers has been shown to be lower in zinc-deficient animals (16,46). Erythrocytes and leukocytes contain appreciable quantities of zinc and are therefore often used as indices to zinc nutrition. Hambridge et al. (47) reported low levels of zinc in hair, impaired taste acuity, poor appetite, and suboptimal growth in children in the United States. Zinc supplementation relieved the deficiency symptoms.

The recent review of zinc nutrition in the United States by Sandstead (48) indicated the probable role of zinc in the utilization of dietary protein and several zinc-responsive conditions in the human. Growth failure in a number of instances (49,13,15) have shown marked improvement after supplemental zinc was given. These syndromes include dwarfism and poor development of the sexual organs, growth failure accompanied by a decrease in taste acuity in children in the United States (50), lowered sensitivity of taste and smell (51), and impaired

wound healing (29,52). Zinc interacts with protein metabolism on a molecular level, as discussed earlier, leading to growth failure and other manifestations described. Sandstead (48) evaluated the daily retention of zinc by individuals of various ages and physiological states. His findings suggested that some infants, pregnant women, teenage and college women, institutionalized individuals, and some living on low income diets have a marginal to deficient zinc intake and/or retention. This could be particularly harmful in the event of a physiologically stressful situation. Mills et al. (17) found that in dietary zinc deficiency, zinc stores were gradually depleted and correlated closely with the gradual growth failure seen clinically.

Zinc turnover studies have been performed in a limited number of normal individuals, dwarfs, and patients suffering from serious diseases. Prasad et al. (13) found a more rapid uptake of zinc by the tissues of dwarfs than by those of normal subjects. ^{65}Zn is a strong gamma-emitting radioisotope with a half-life of 245 days and therefore it has limited suitability with studies on human beings (16). Ronaghy et al. (53) conducted a supplementation study on a group of rural Iranian boys, ages 12-18, whose height was below the third percentile of the Iowa height standards. The boys were divided into three groups for supplementation with either a placebo, iron, or zinc in order to test whether sufficient zinc can promote accelerated growth and sexual development as a separate effect. The study lasted 17 months wherein supplementation continued for 5 months, was discontinued for 7 months, and reinstated for 5 more months. The most striking result proved to be the effect of zinc on accelerating the rate

of sexual maturation in the zinc test group. The boys given zinc showed a more rapid rate of weight gain, though the other subjects showed marked weight gain as well. Controlled studies of this nature, even with a relatively small number of subjects, are beneficial in delineating further the role of zinc metabolism and its necessity in the diet.

Engel et al. (54) reported a carefully controlled and extensive study of zinc requirement of preadolescent girls. Three balance studies were carried out on groups of 12 girls each, ranging between 7 and 10 years of age. Daily zinc intakes ranged from 4.6 to 9.3 mg/day and dietary protein from 0.6 to 3.0 g/kg body weight. In two of the studies, a linear relationship between zinc intake and retention was observed; however, the zinc retention in the third study was greater than the other two. The urinary excretion of zinc was relatively constant. Excluding the third study, zinc was equally well retained from diets in which protein was supplied by meats and milk as by cereals and legumes. Based on estimates of the difference in total body zinc content of young children and adults and on the zinc retention data, a dietary requirement of approximately 6 mg zinc per day appeared to be adequate for the preadolescent girl. The 1974 Recommended Allowances for children is based on these reports and the recognition that considerable zinc may be lost through the skin as suggested by Schraer (55). Unpublished data from this laboratory confirmed that

significant losses of zinc may occur through the skin of preadolescent girls.[1]

Similarly, Price and Bunce (56) studied the effect of nitrogen and calcium on the balance of zinc in preadolescent girls. Calcium was increased to an intake of 1.3 grams per day and nitrogen was elevated with either ammonium citrate or synthetic amino acids most limiting in the diets of the 7 to 9 year old female subjects. They reported that varying calcium intake from 300 to 1,300 mg/day had no effect on the zinc balance. Increasing dietary nitrogen appeared to improve zinc balance mainly through a reduction in fecal zinc. The intake of zinc was marginal in these diets, in an attempt to simulate a regimen typical of low income groups of the southeastern United States. They noted that despite the improved balance with the higher nitrogen diets, the average daily retention of 1.3 mg of zinc may not meet the needs of the growing child, since the sum of the calculated allowance for growth (0.25 mg) and possible dermal and sweat losses (1.75 mg) exceeded the observed retention by 0.7 mg. Therefore, the earlier figure from Engel et al. (54) may be a more realistic daily allowance given the variation in individual requirements for zinc inherent in the population of preadolescent girls.

Studies by Abernathy et al. (58) had shown that apparent nitrogen retention was linearly related to nitrogen intake over a range from 2.88 to 14.08 g nitrogen daily from mixed food sources. Testing of this with the typical low income southern diet (4 g nitrogen daily),

---

[1] Colleen H. Walls, "Cutaneous Mineral Loss in Preadolescent Girls" (unpublished Master's thesis, Virginia Polytechnic Institute and State University, 1974).

however, showed retention of considerably less nitrogen than predicted (59). Limiting essential amino acids were thought to cause the aberration. Abernathy et al. (57) conducted a study in 1970 designed to evaluate the effect of supplementation of diets which simulated those of low income southern families with amino acids most limiting with respect to egg protein. The diets contained 4 g of nitrogen and approximately 2,000 Kcal daily. They concluded that supplementation with lysine, threonine, and methionine was of little or no value in improving nitrogen utilization and that widespread fortification of foods with amino acids was not justified on the basis of their findings. Positive nitrogen balance was as contingent on total nitrogen supplementation as much as essential nitrogen supplementation.

Sandstead (60) questioned the results and conclusions reached by Abernathy et al. (57) citing this study as a classical example of subtle but important nutrient interrelationships. He suggested that zinc, being limiting in both the basal and amino acid enriched diets, could have been the cause of the lack of nitrogen balance improvement from addition of essential amino acids. There is little evidence in the literature to support the suggestion by Sandstead (61). Furthermore, if positive nitrogen balance is reliant upon zinc or other nutrient-nitrogen interrelationships, it seems unlikely that broad scale amino acid fortification will solve the problem anyway.

There is a paucity of data concerning the effect of variable zinc intake on the protein utilization of subjects of any age. Much work has been done with animals showing the metabolic ramifications of zinc deficiency and its effect on growth in animals. There is a need to know

if zinc can affect the protein utilization of subjects, particularly when the protein source is given in marginal amounts and of predominately plant origin. It has been well established that zinc affects protein metabolism on the cellular level and there is no question that zinc is required for normal growth and development in man and animals. Zinc deficiency has been shown to be reversible. Clearly, additional information is needed from controlled studies with human subjects to demonstrate the role of zinc in the utilization of other nutrients, particularly protein and amino acids, in the growing child. Also, additional data are needed to define the zinc requirement of the growing child, one of the most likely groups in the population to be severely affected by marginal intakes of zinc. This study was designed to provide additional data for the preadolescent child consuming marginal amounts of poor quality dietary protein, as in the basal diet.

CHAPTER III

EXPERIMENTAL PROCEDURE

Experimental Design

A 2×2 factorial study was designed to test the hypothesis that zinc may be a limiting nutrient in the utilization of dietary nitrogen. A basal protein diet was supplemented with isonitrogenous amounts of either essential amino acids or ammonium citrate and with two levels of zinc. The subjects were 23 preadolescent girls, because growth is an important factor in the metabolism of this age group. The effects on protein utilization of varying zinc and protein quality would be expected to be more pronounced. Also, these data are particularly important because previous studies in this area of research have not been reported in normal, growing children.

Subjects were recruited and randomly assigned to dietary treatments from outcome groups based on body weight (Table 1). The basal diet contained 23.8 g protein (N × 5.7) and 5.5 mg zinc. Supplements were added to the basal diet as follows:

I. Essential amino acids in amounts which, when added to those in the diet, provided a chemical score of 80 based on whole egg protein. Threonine, 0.234 g; valine, 0.244 g; methionine, 0.120 g; isoleucine, 0.332 g; lysine HCl, 0.334 g.

II. Threonine, 0.234 g; valine, 0.244 g; methionine, 0.120 g; isoleucine, 0.332 g; lysine HCl, 0.334 g. A zinc supplement of 0.0168 g zinc acetate.

III. Ammonium citrate, 1.123 g and zinc acetate, 0.0168 g.

Table 1

Experimental Treatments during Days 9-20 of the Balance Study

	Treatment[a]		No. of Subjects	Mean Weight on Day 1, kg
	g Nitrogen added	mg zinc		
Amino Acid	0.139[b]	10.5[c]	6	28.05
Ammonium Citrate	0.139[d]	5.5	6	29.05
Amino Acid	0.139[b]	5.5	6	29.78
Ammonium Citrate	0.139[d]	10.5[c]	5	30.92

[a]Basal diet provided 23.8 g protein (N × 5.7) and 5.5 mg zinc daily.

[b]Amino acids in the following amounts were added to the basal diet in order to attain a chemical score of 80 based on egg protein: Threonine, 0.234 g; valine, 0.244 g; methionine, 0.120 g; isoleucine, 0.332 g; lysine HCl, 0.334 g.

[c]0.168 g of zinc acetate added to provide 5.0 mg zinc.

[d]Ammonium citrate added to provide nitrogen equivalent to amount added from amino acids.

IV. Ammonium citrate, 1.123 g.

Treatments I, II, and IV had six subjects each, and treatment III had five subjects who completed the study.

The 21-day study was divided into two periods. Days 1-8 were an adjustment period in which all subjects consumed the basal diet only. The 8-day adjustment period (Period I) was used to stabilize nitrogen balance in the girls. It was assumed that the subjects were accustomed to eating more than 23.8 grams of protein per day. The experimental period (Period II) began on day 9 and continued through day 20. For each of these 12 days, all subjects received one of the four types of supplementation to their regular meals of the basal diet. All urine and feces were collected for each subject from day 1 through day 21. However, only those excretions corresponding to days 9-20, inclusive, were analyzed for nitrogen and zinc, and the resulting data were used in calculating individual nitrogen and zinc balances.

Experimental Subjects, Management, Diet and Supplement Description

Twenty-three female subjects, ranging in age from seven years and six months to nine years and nine months with a mean age of eight years and five months, were recruited from the Montgomery County area of southwest Virginia. Age, height, weight, and blood data are summarized in Appendix I. All subjects were assigned consecutive numbers beginning with 130. All samples were labeled with subjects' initials and number throughout the study.

Prior to the experimental period, each subject underwent a physical examination by a physician and was deemed healthy and free of

intestinal parasites. Parents supplied information regarding personal traits, food or other allergies, and previous state of health prior to admission into the study. They also signed a written consent form which detailed the purpose, design, and duration of the study.

A registered nurse examined each subject daily for minor injuries and illness likely to be contagious. The temperature of each subject was recorded each day. Subjects were weighed and measured every four days to monitor any weight change due to the diet. A physician was on call at all times to treat the children in case of emergencies. Only childrens' aspirin for headache and Donnagel for diarrhea were administered to the subjects by the nurse in times of need and record was made of each incident. One subject was being treated for allergies with a polaramine tablet daily and for one week one subject was treated for a cold with four penicillin tablets and two pseudophedrine tablets daily. These medications were thought not to interfere with nitrogen balance.

Subjects were housed in a dormitory near the Home Economics building containing the laboratory and metabolic kitchen. They were under the constant supervision of staff recreation workers or five adult female counselors who resided with the subjects. Counselors and recreation workers were trained in the logistics of fecal and urine collection, labeling, keeping daily notes, as well as maintaining high morale and rudimentary discipline. Counselors or recreation workers ate meals with the children. Subjects were informed about the importance of the study and the need for their conscientious participation.

The program was incorporated as part of a summer camp in which a daily schedule was established including active and quiet play, arts and crafts, tours and hikes, and other activities appropriate and desired by the subjects. A typical daily schedule of activities may be found in Appendix II. The children went swimming for one hour Monday through Friday of the three weeks of the study. It was not known how much pool water each child inadvertantly consumed while swimming and no controls on this could be attempted. The pool water was determined by analysis to contain .0105 μg/ml of zinc, an amount too low to affect the outcome of the data.

All food consumed by the subjects was prepared in the metabolic kitchen and served in an adjoining dining room. Dropped or spilled foods were weighed and replaced and all food and drinks were consumed completely. Foods and beverages were weighed in accordance with the prescribed diet. The menus cycled A, B, C, and D every four days for the duration of the study. The food items served were thought to be generally liked by this age group, gave color and textural interest, yet met the requirements of approximately 5.0 mg zinc and 24 g of protein per day (Appendix III).

The diet provided approximately 2,000 Kcal of energy daily for each subject. This was increased in 50 Kcal increments by addition of fondant candy (10 grams each) made of powdered sugar and margarine and/or extra Kool-Aid beverage providing 100 Kcalories per glass (25 g granulated sugar). These sources of additional calories were added to the daily regimen only if the subjects were losing weight.

Each morning all subjects received a multiple vitamin with iron tablet which ensured an adequate amount of vitamins and iron.[1]

All drinking water and water used in the preparation of beverages such as orange juice, lemonade, or Kool-Aid was first deionized by passing distilled water through a column of mixed-bed ion exchange resin. A double column of resin was set up in the dorm to supply ion-free drinking water to the subjects. This was the only drinking water allowed to the subjects. They could drink it ad libitum. When away from the dorm, the children took individual thermos bottles filled with ion-free water for drinking. The zinc content of the drinking water was monitored frequently in order to guarantee low to negligible levels of zinc. Zinc was always between .010 µg/ml and .025 µg/ml in dorm water and ion-free water produced in the laboratory for food preparation and drinking at meals.

Food composites were made in duplicate each day so that samples of the actual foods consumed could be analyzed for nitrogen and zinc. An extra serving of each food item was prepared and added to a Pyrex container throughout the day. Between meals this food was refrigerated. At each day's end the food to be composited, representing all foods and beverages consumed that day by any one subject, was transferred to a five-quart Waring blender using ion-free water. The food was homogenized, brought up to a standardized weight with ion-free water,

---

[1]One tablet contained the following: vitamin A, 5,000 IU; vitamin B, 15 IU; vitamin C, 60 mg; folic acid, 0.4 mg; thiamin, 1.5 mg; riboflavin, 1.7 mg; niacin, 200 mg; vitamin B6, 2.0 mg; vitamin B12, 6.0 mcg; vitamin D, 400 IU; iron, 18 mg.

and then rehomogenized for a constant amount of time. All margarine was left out of the food composites because it does not blend into solution. But foods, such as frosting, with margarine in them were included in compositing. The food homogenate, in the amount of 200 grams, was removed in duplicate, labeled as to menu, day, period, and duplicate, and then frozen. At the end of each four-day menu cycle period, the 200 g of daily food composites were thawed and 100 g removed from each. The 400 g of daily food composites were then thoroughly blended together and labeled as period composites for the appropriate four days. Nitrogen and zinc were determined on the four-day period composites.

The essential amino acid composition of the diet was measured prior to the study. Approximately 0.1 g of dried food composite for the period was put into 10 ml ampoules with 10 ml of 6N HCl added to each ampoule. Each ampoule was sealed and duplicates were hydrolyzed at 110°C for 12, 24, 36, 48, and 72 hours. After hydrolysis and cooling, the samples were filtered, the acid was removed by vacuum over NaOH flakes, the hydrolyzed amino acids were resuspended in buffer, and aliquots of hydrolysate-buffer mixtures were analyzed in an amino acid analyzer.[1] Concentrations of the essential amino acids in the diet were calculated from the chromatograms derived from the amino acid analysis on the hydrolysates. A regression was calculated for each amino acid concentration at each of the five time intervals. The resultant regression analysis on the data showed at what time in the

---

[1]Technicon TSH Automated Amino Acid Analyzer, Technicon Instruments Corporation, Tarrytown, NY 10591.

series the optimum concentration was seen. For all eight amino acids in the diets, no significant linear regression was established. Therefore, the amino acid concentration determined at any of the five time intervals of hydrolysis was reliable. A mean of the concentrations at each time interval was calculated as the concentration of each of the eight essential amino acids present in the diet. This figure was used in the calculation of chemical score and the amino acid supplementation in the study (see Table 2). The most limiting essential amino acids with respect to whole egg protein were threonine, valine, methionine, isoleucine, and lysine. The amino acids in the diet and 80 percent of each amino acid in 25 g whole egg protein are presented. The difference between the amount of each amino acid in the daily diet and the 80 percent figure for whole egg protein was the amount of each essential amino acid supplemented daily to those subjects receiving this treatment.

A stock solution of amino acids dissolved in ion-free water, 25 ml, was pipetted into the morning beverage of the subjects in groups I and III. After consumption of the beverage, each subject's glass was rinsed twice with deionized water and the rinse water was also consumed.

The amount of nitrogen supplemented to each subject daily in the form of amino acids was calculated to be 0.139 g/day. To insure that the treatment effect, if any, seen in the amino acid supplemented groups was due solely to essential amino acids and not to the nitrogen contained within, the basal and basal plus zinc groups also received a nitrogen supplement. This supplement was in the form of 1.123 g of

Table 2

Comparison of Diet Protein and Whole Egg Protein
for Determination of Amino Acid Supplement

Amino Acid	.80 × g AA/25 g Whole Egg Protein[a]	g AA/day Basal Diet	Difference[b]
Threonine	1.024	0.7898	0.234*
Valine	1.370	1.1264	0.244*
Methionine	0.672	0.4510	0.120*
Isoleucine	1.258	0.9262	0.332*
Leucine	1.764	1.7182	0.046
Phenylalanine	1.146	1.1308	0.015
Lysine	1.396	1.1260	0.156*

[a] Amino Acid Content of Foods and Biological Data on Proteins, Food Policy and Food Science Service, Nutrition Division, FAO, p. 122.

[b] The asterisk (*) denotes essential amino acid supplemented to basal diet.

ammonium citrate and provided 0.139 g nitrogen per day. An ammonium citrate stock solution, 5 ml, was pipetted daily into the appropriate subject's soup which was served at lunch. The "tang" of ammonium citrate was satisfactorily masked by the soups.

The amount of zinc in the daily diets was determined spectrophotometrically on wet-ashed samples from period food composites. Supplementation of zinc to 10.5 mg per day, slightly above the 1974 Recommended Dietary Allowance (10) for this age, was accomplished with zinc acetate. Zinc acetate is 29.79 percent zinc, and 0.0168 g of this compound supplied each child with 5.0 mg zinc each day. The zinc acetate was dissolved in deionized water to make a stock solution from which 10 ml was pipetted and added to the morning beverage of the proper subjects. After consumption of the beverage, each child's glass was rinsed twice with deionized water and the rinse water was also consumed. None of the adults eating with any of the groups knew the nature of the supplementation given the group with whom they ate.

On days 8, 14, and 20, nontoxic fecal markers were given to each subject prior to breakfast. Each marker contained 25 mg brilliant blue and 175 mg methyl cellulose as a filler. These two ingredients were mixed and stuffed into gelatin capsules. The marker was used so that feces belonging to treatment periods or subperiods could be distinguished. All feces for each subject containing the marker given on day 8 and those coming until, but not including, those marked on day 14 were analyzed with the day 14 marker and all those coming after, but not including, those marked on day 20 were likewise analyzed. Daily

nitrogen and zinc content of feces was calculated from analysis completed on the two 6-day collections.

Urine and Fecal Collection

All urine and feces for each subject were collected from day 1 through day 22, inclusive. A 24-hour urine collection began with the second voiding of a day and continued through the first voiding of the following day. Urine and fecal collection units were installed in the dorm within easy access from the subjects' rooms. Subjects enlisted the aid of a counselor when they used the units in order to avoid possible contamination, incomplete collection, or "mix-up" whereby one subject used another subject's bottle. Each subject had an individual space assigned on the urine collection unit. The urine passed through a stainless steel funnel into a clean, 2 l glass jar containing 10 ml of 4N HCl to retain ammonia. The children were not allowed to use the conventional bathrooms for anything but their evening showers. While they were away from the dormitory, urine was collected in clean, wide-mouthed 250 ml polyurethane bottles. The urine thus collected was transferred to each child's larger bottle in the dormitory collection unit. Any spillage of urine was recorded by a counselor.

All feces were collected into 1½-quart Pyrex casserole dishes placed in a specially designed collection unit. After voiding, a counselor labeled the casserole dish with the subject's number and initials, date, time, and noted the presence of markers. She then taped the lid on the casserole dish and placed it in a freezer for storage. Daily records were made of all bowel movements and anything

unusual such as urine contamination, presence of intestinal parasites, or unusual consistency. When the children were away from the dorm, they voided directly into the casserole dishes which were returned to the dorm and frozen.

Each morning, clean, acid-washed 1½-quart casseroles and 2 l urine collection bottles were delivered to the dorm. All urine and feces from the previous 24 hours were taken to the lab for analysis. The urine was transferred into a clean 2 l graduate cylinder and the volume recorded. The urine volume was then increased to 2,000 ml with deionized water and aliquots removed for daily nitrogen and creatinine determination, zinc analysis, and reserve. The remaining urine was discarded.

Feces were frozen in the lab until the end of a period. They were composited into two six-day groups representing the first and last six days of Period II, respectively. Feces were thawed, transferred to a weighed, five quart stainless steel Waring blender, and weighed. They were then blended to a uniform consistency with the addition of ion-free water, reweighed, and blended for a uniform amount of time at a constant rate of blender speed. Weights and final dilutions were recorded and aliquots were removed for zinc and nitrogen determination.

Creatinine was monitored daily so that there was a check on completeness of urine collection.

Nitrogen and Zinc Determination

Fecal and food nitrogen was determined using a modified Kjeldahl-Gunning-Arnold method (66). Urine nitrogen was analyzed

daily in an automated procedure utilizing a Technicon Auto Analyzer II.[1]

Zinc analysis was done on four-day composites of urine, beginning with day 1 of Period II, and daily zinc concentrations were calculated from this date. Urine, fecal, and food zinc was determined in wet-ashed samples (67) in a Perkin-Elmer atomic absorption spectrophotometer using a zinc lamp.[2] Food samples were 10 g, urine samples were 50 ml, and fecal samples were 3 g. Samples were weighed or measured into acid-washed 250 ml Pyrex beakers and 20 ml of concentrated nitric acid was added to initiate digestion. An acid-washed watch-glass was placed over the sample, followed by placing the beaker on a hot plate heated to 250°C to 300°C. Digestion to white crystals on the hot plate involved from two to several days, depending on the sample type. After digestion to crystals, 1 ml of concentrated HCl was added and the beaker was heated. The crystals were brought into solution using hot, deionized water and transferred to a 25 ml volumetric flask. The volume was taken to 25 ml and the sample, suspended therein, was read directly from this dilution with urine and food samples and from a further dilution of 10 ml of this dilution taken up to 25 ml with deionized water for the fecal samples. Zinc was expressed as mg/day in all samples.

---

[1]Technicon Auto-Analyzer II, Technicon Instruments Corporation, Tarrytown, NY 10591.

[2]Perkin-Elmer Spectrophotometer, Model 305, Perkin-Elmer Corporation, Norwalk, CN.

All glassware used in the wet-ashing process and all storage containers for diet supplements and excretory samples were acid-washed in 35 percent nitric acid solution and rinsed twice in ion-free water. This eliminated any zinc contamination from the environment.

Statistical Testing

Analysis of variance was completed on this 2×2 factorial experiment with unequal sample sizes using the Statistical Analysis System.[1] The experiment was a fixed-effects model utilizing two variables. Analysis of variance tested the effects on nitrogen and zinc balance resulting from the four treatments administered. This included the zinc effect, nitrogen effect, and zinc-nitrogen interaction effect.

---

[1]For a description of the Statistical Analysis System, see: A Users Guide to the Statistical Analysis System (Raleigh: North Carolina State University Student Supply Store, Jolayne Service, August, 1972).

CHAPTER IV

RESULTS AND DISCUSSION

Anthropometric and Biochemical Measurement on Subjects

The subjects' weight on the initial day of the study ranged from 22.3 kg to 40.2 kg with a mean of 29.5 kg, and heights ranged from 121.5 cm to 148.0 cm with a mean of 132.1 cm. On the initial day of the study, hemoglobin and hematocrit analysis was completed on each subject as a measure of general health to detect iron deficiency anemia. Hemoglobin values ranged from 13.99-15.56 g/100 ml in the group given amino acids plus zinc; 13.40-15.91 g/100 ml in those given ammonium citrate; 14.60-16.61 g/100 ml in those given amino acids; and 14.69-15.91 g/100 ml in those given ammonium citrate plus zinc. Hematocrit values ranged from 38.0-42.0 vol% in the group given amino acids plus zinc; 38.0-41.0 vol% in those given ammonium citrate; 37.0-42.0 vol% in those given amino acids, and 35.0-43.5 vol% in those given ammonium citrate plus zinc.

The height and weight data for the 23 subjects are summarized in Table 3. Additional details are shown in Appendix IV. Height of the children was stable during the study. The mean change in weight was .14±.56 kg. On day 12, it was observed that some of the subjects had lost small amounts of weight since their weighing on day 8. Therefore, on an individual basis the calorie level of the diet was increased

Table 3

Summary of Heights and Weights of Subjects during Study

	Height		Weight	
Subject No.	Day 1	Day 20	Day 1	Day 20
130	124.5	124.0	25.7	26.2
131	123.0	123.0	28.1	28.8
132	131.5	131.0	25.0	25.4
133	126.5	126.0	23.1	23.9
134	124.0	125.0	28.1	28.7
135	139.5	137.0	38.3	37.5
136	134.0	134.0	31.7	31.5
137	124.5	126.0	27.0	26.7
138	125.5	125.5	22.3	23.7
139	134.0	135.5	32.4	32.1
140	135.5	135.5	32.9	32.4
141	131.5	132.0	31.2	30.6
142	130.0	130.0	24.7	24.9
143	131.0	130.5	25.3	25.7
144	135.0	133.0	25.5	26.0
145	140.5	139.0	40.2	40.1
146	140.0	140.5	29.3	29.1
147	138.5	138.0	33.7	33.6
148	121.5	121.0	22.9	23.7
149	148.0	147.5	37.2	36.3
150	134.5	133.0	26.6	26.7
151	131.0	129.0	33.0	33.3
153	134.0	134.0	34.9	34.7
Mean	131.7	131.7	29.4	29.7
SD	6.6	6.4	5.2	4.7

to prevent weight loss by feeding fondant candy and sugar added to Kool-Aid beverage. The weight data of day 16 showed the weight change to be reversed in the subjects and a very slight weight gain continued throughout the study.

Nitrogen Excretion

Mean daily urine nitrogen was calculated for the individual subjects using the data from days 7-12 of Period II (see Table 4). The group given amino acids plus zinc excreted an average of 2.4±.26 g/day in urine; ammonium citrate, 2.52±.17 g/day; amino acids, 2.37±.10 g/day; and ammonium citrate plus zinc, 2.65±.10 g/day (see Table 5). The range of mean daily urine nitrogen both within and among groups was small. The overall range of mean daily urine nitrogen was from 1.99 g/day to 3.10 g/day, while the groups ranges were: amino acids plus zinc, 2.02-2.71 g/day; ammonium citrate, 2.29-2.73 g/day; amino acids, 1.99-2.90 g/day; and ammonium citrate plus zinc, 2.31-3.10 g/day.

Daily urine nitrogen was more irregular in Period I and generally more nitrogen was lost through urine during this time compared with Period II (Appendix V). Individual mean daily urine nitrogen ranged from 2.34 g/day to 4.93 g/day in Period I. During Period II, after adjustment to the low protein diet, urine nitrogen decreased and stabilized, ranging from 2.21 to 2.93 g/day (Appendix V). The overall stability of urine nitrogen excretion is evidenced by the small standard deviations of the individual and group daily urine nitrogen means. Fecal nitrogen was low relative to urine nitrogen.

Table 4

Mean Daily Urine and Fecal Nitrogen and Daily Nitrogen Balance during Last 6 Days of Period II

Treatment/ Subject	Mean Daily Urine Nitrogen g/day[a]	Daily Fecal Nitrogen g/day[b]	Daily Nitrogen Balance g/day[c,d]
Amino Acids & Zinc			
130 SRC	2.18	.966	1.17
131 CMW	2.02	.704	1.59
132 EL	2.56	.845	.91
133 JF	2.34	1.278	.70
134 KB	2.57	.715	1.03
135 NM	2.71	.972	.63
	$\bar{x}$=2.39±.26	$\bar{x}$=.91±.21	$\bar{x}$=1.00±.35
Ammonium Citrate			
136 KAP	2.73	1.060	.52
137 PCW	2.29	.834	1.19
138 ERV	2.52	.460	1.33
139 LB	2.62	1.029	.67
140 LL	2.61	1.017	.69
141 LAL	2.37	.957	.99
	$\bar{x}$=2.52±.16	$\bar{x}$=.89±.22	$\bar{x}$=.90±.32
Amino Acids			
142 MM	2.27	1.018	1.03
143 LS	1.99	1.128	1.20
144 DCP	2.90	.716	.70
145 TEM	2.55	.609	1.16
146 SP	2.18	1.095	1.04
147 DKL	2.37	1.023	.92
	$\bar{x}$=2.37±.31	$\bar{x}$=.93±.21	$\bar{x}$=1.01±.18
Ammonium Citrate and Zinc			
148 RM	2.31	1.084	.13
149 KL	3.10	.899	1.05
150 MB	2.37	.935	.72
151 SW	2.66	.548	1.11
153 CB	2.83	.766	.72
	$\bar{x}$=2.65±.32	$\bar{x}$=.85±.20	$\bar{x}$=.81±.39

[a]Mean of days 7-12, Period II. [b]Mean of days 7-12, period II.

[c]Gram N intake = 4.185 dietary + .139 supplemental N = 4.314 g/day total. [d]Nitrogen balance = intake - (urine + fecal).

Table 5

Nitrogen Excretion and Utilization

	Urine Nitrogen Mean±SD g/day	Fecal Nitrogen g/day	Digested N g/day[a]	Total Apparent Nitrogen Excretion Mean±SD, g/day	Nitrogen Retention Mean±SD g/day
Amino Acids	2.37±.10[b]	.93±.20[b]	3.26[b]	3.30±.18[b]	1.01±.18[b]
Amino Acids & Zinc	2.40±.26[b]	.91±.20[b]	3.28[b]	3.31±.35[b]	1.00±.35[b]
Ammonium Citrate & Zinc	2.65±.10[c]	.85±.20[c]	3.34[c]	3.50±.16[c]	.81±.39[c]
Ammonium Citrate	2.52±.17[b]	.89±.22[b]	3.30[b]	3.41±.10[b]	.90±.32[b]

[a]Nitrogen intake = 4.189 g/day.

[b]Mean of 6 individuals based on last 6 days of Period II.

[c]Mean of 5 individuals based on last 6 days of Period II.

Mean daily fecal nitrogen for all groups was slightly less than 1.0 gram per day (Table 4). There were no apparent differences in mean daily fecal nitrogen excretion between the four groups. The group supplemented with amino acids plus zinc averaged .91±.21 g/day; ammonium citrate, .89±.22 g/day; amino acids, .93±.20 g/day; and ammonium citrate plus zinc, .85±.20 g/day. Individual fecal nitrogen data for Periods I and II are also reported (see Appendix VI). Fecal nitrogen was stable within groups over the 12 days of Period II.

During the last six days of Period II, no significant differences in fecal nitrogen excretion were noted. Examination of the group means for the first and last six days of Period II showed no significant differences between any one group mean for days 1-6 and its corresponding mean for days 7-12. The means of the four groups for days 7-12 were more alike due to further adjustment by all subjects to the low protein diet. Generally, less nitrogen was lost via the feces during these last six days which indicated that the subjects were absorbing and retaining nitrogen with greater efficiency.

No significant differences in total apparent nitrogen excretion between the four groups were seen (Table 4). The amino acids group excreted an average of 3.30±.18 g/day; amino acids plus zinc, 3.31±.35 g/day; ammonium citrate plus zinc, 3.50±.16 g/day; and ammonium citrate, 3.41 g/day. There was very little difference between groups in digested nitrogen. Digested nitrogen ranged from 3.26-3.34 g/day between the four groups (Table 4). Individual daily total nitrogen excretion for Period II is presented in Appendix VII. Mean total apparent nitrogen excretion by treatment group is also summarized

(Appendix VIII). The coefficient of variation did not exceed 15 percent on any of the group means (Appendix VIII), indicating small variation in daily group means for the 12 days.

The ammonium citrate and ammonium citrate plus zinc groups virtually paralleled each other in total nitrogen excretion. This showed that zinc supplementation to the basal diet did not affect nitrogen excretion compared with the basal diet alone; therefore, it did not affect nitrogen utilization in the basal diet. Until day 6, total nitrogen excretion was more variable in the amino acid supplemented group. After day 6, all groups gradually decreased in total nitrogen excretion. The general conservation of nitrogen by the subjects and the further adjustment to the diets and supplements was indicated.

Nitrogen Balance

The nitrogen balance technique is the most widely used in the evaluation of amino acids and nitrogen requirements and utilization. Nitrogen equilibrium in the adult is attained when there are adequate supplies of essential amino acids and total nitrogen for tissue maintenance, and for replacement of nitrogen losses through the kidney, intestinal secretions, sweat, desquamation of epithelial cells, and synthesis of hair and nails (65). In the growing child, nitrogen equilibrium is also contingent upon the synthesis of new tissues; therefore, essential amino acids and total nitrogen must account for maintenance and growth. Nitrogen balance may be expressed as: $B = I - (U + F + S)$, where B is nitrogen balance, I is nitrogen intake,

U is urinary nitrogen, F is fecal nitrogen, and S is sweat nitrogen. Sweat nitrogen was not measured in this study. Sweat nitrogen has been measured previously on a similar group, consuming approximately the same amount of protein (68,72). About 0.2 g/day were lost through the sweat with normal activity (68). Nitrogen balance in the present study was calculated without inclusion of sweat nitrogen loss, assuming it to be constant.

It is possible for a subject to be in a state of nitrogen equilibrium while some tissues are in positive nitrogen balance and others are in negative balance simultaneously. Some cells have been shown to be incapable of synthesizing all the normally nonessential amino acids and, therefore, need them in exogenous sources (71). This clouds our assumptions concerning the essential amino acids. However, in the current nitrogen balance study, the total nitrogen status of the child was the focus of research.

Many important factors are known to greatly affect nitrogen balance and must be accounted for in research using this technique. Of these, there are physiological state, body protein reserves, calorie content of the diet, and the essential amino acids and nonessential nitrogen provided the subjects (65).

Individual mean daily nitrogen balances have been calculated on the basis of fecal and urinary nitrogen from the last six days of Period II (see Table 4). Nitrogen intake included 4.185 g/day from the basal diet plus .139 g/day in the supplements. Mean daily nitrogen balance ranged from +.13 g/day to +1.59 g/day in the subjects as a group. All subjects' daily nitrogen balances were positive. Analysis

of variance showed no significant differences ($p>.10$) in the individual mean daily balances for the subjects in the four treatment groups. There were essentially no differences in nitrogen utilization among the four groups. It can be seen, however, that the groups given amino acids had higher nitrogen retention compared with those given ammonium citrate. Zinc appeared to have no effect on the utilization of added essential amino acids to the basal diet. Supplementation with the five essential amino acids resulted in no effect on nitrogen utilization either. The absence of any treatment or interaction effects is illustrated further in Table 6.

The four group mean daily nitrogen balances have been summarized (see Table 5). The group given amino acids plus zinc averaged 1.00 g/day and there was no difference between this group and that given only amino acids which averaged 1.01 g/day. An earlier report by Abernathy et al. (57) showed a mean daily nitrogen retention of 0.40 g/day in the group given a basal diet identical to the present one with the addition of lysine, threonine, and methionine. Nitrogen balance was greater in the present study possibly because more limiting essential amino acids were added as supplements. Also, in the earlier study, a group given basal diet plus ammonium citrate (2.78 g/day) averaged 0.58 g/day nitrogen balance. This was lower than the comparable group in this study which averaged 0.90 g/day nitrogen retention.

When consideration is given to a growth increment of about 0.3 g/kg body weight (10) and 0.2 g/day for sweat (68), all individual daily nitrogen balances are only marginally positive. Also affecting

Table 6

Effect of Zinc, Essential Amino Acids or Zinc-Essential Amino Acids Interaction on Nitrogen Balance, g/day

	Amino Acids	Ammonium Citrate	$\bar{x}$ Effect
+Zinc	1.00	1.01	1.00[a]
-Zinc	0.90	0.81	0.86[a]
$\bar{x}$ Effect	0.95[b]	0.91[b]	

[a]Comparison of 1.00 and 0.86 showed no significant difference and therefore no zinc effect.

[b]Comparison of 0.95 and 0.91 showed no significant difference and therefore no nitrogen effect.

nitrogen balance are logistical problems in food consumption and excretion collection which generally cause nitrogen balance to be slightly more positive than actual.

Assuming that calorie content was not limiting, 23.8 g protein per day with or without added essential amino acids or the nitrogen equivalent barely sustained growth and maintenance. Positive nitrogen balances are characteristic to growing children consuming ample protein. Based on the current findings, it appears that zinc was not limiting in the nitrogen utilization of this diet by the subjects. Added zinc did not enhance nitrogen utilization in either the group fed the basal diet plus ammonium citrate or basal plus essential amino acids. The essential amino acids were no better utilized in the presence of supplemental zinc. The possibility that zinc was limiting in this diet and was the cause of the lack of better nitrogen utilization with an amino acid supplement does not seem to be valid, based on the current data (60). Perhaps a longer adjustment period was needed in which subjects consume about 5 mg zinc per day. This may deplete their zinc stores which, in a test diet period like the present one, may cause a zinc-amino acid treatment effect. In that case, the zinc status may better correlate to the Iranian and Egyptian subjects described by Prasad et al. (11,12,13) in which supplemental zinc drastically affected protein utilization.

In the study by Abernathy et al. (57), no advantage to nitrogen utilization was seen in supplementing the basal diet with the most limiting essential amino acids with regard to egg protein. Their results agree with those reported here. It must be concluded that zinc

supplementation did not enhance the nitrogen utilization of the subjects either with or without amino acid supplementation. Based on this and the previous study (57), large-scale fortification of foods with amino acids is not justified.

Zinc Excretion

Individual mean urinary zinc ranged from 0.28 mg/day to 0.96 mg/day (Table 7). Urine zinc appeared to be constant from group to group, regardless of intake. Urinary zinc was generally not greater in those groups given supplemental zinc. Mean daily urine zinc was highest in the group given ammonium citrate plus zinc. The group mean was 0.79±.11 mg/day for the ammonium citrate plus zinc group; amino acids plus zinc, 0.45±.14 mg/day; ammonium citrate, 0.60±.09 mg/day; and amino acids, 0.63±.12 mg/day (see Table 8). Urine for zinc analysis was analyzed in three four-day composites (Appendix IX). All subjects within each group excreted similar amounts, as seen by small standard deviations on the group means.

It appeared that the addition of zinc to the basal diet had no effect on urinary zinc. The values for the amino acids group and the ammonium citrate group are comparable, but slightly higher than similar groups reported by Price and Bunce (74) who studied zinc balance in preadolescent girls. The group given the basal 24 g protein diet plus 1.88 g/day ammonium citrate averaged .233 mg urinary zinc per day. The group given the three most limiting essentialy amino acids plus basal diet averaged .254 mg urinary zinc per day. McCance and Widdowson (75) completed ^{65}Zn studies on normal adults and reported

Table 7

Mean Urine Zinc, Fecal Zinc and Daily Zinc Balance for Last 6 Days of Period II

Treatment/ Subject	Mean Urine Zinc mg/day[a]	Fecal Zinc mg/day[a]	Daily Zinc Balance mg/day
Amino Acids & Zinc			
130 SRC	.47	8.09	1.94
131 CMW	.28	8.78	1.44
132 EL	.45	9.36	.69
133 JF	.43	6.66	3.41
134 KB	.38	8.57	1.55
135 NM	.68	8.18	1.64
	$\bar{x}$=.45±.13[b]	$\bar{x}$=8.27±.91	$\bar{x}$=1.77±.90
Ammonium Citrate			
136 KAP	.61	6.37	-1.48
137 PCW	.55	4.35	.60
138 ERV	.70	3.82	.93
139 LB	.65	4.98	-.13
140 LL	.65	4.61	.24
141 LAL	.45	4.13	.92
	$\bar{x}$=.60±.08	$\bar{x}$=4.71±.90	$\bar{x}$=.19±.91
Amino Acids			
142 MM	.53	4.89	.08
143 LS	.48	5.91	-.89
144 DCP	.68	6.39	-1.57
145 TEM	.83	3.81	.86
146 SP	.65	4.89	-.04
147 DKL	.60	4.78	.12
	$\bar{x}$=.63±.12	$\bar{x}$=5.11±.91	$\bar{x}$=-.24±.86
Ammonium Citrate and Zinc			
148 RM	.96	9.96	-.42
149 KL	.80	11.24	-1.54
150 MB	.65	9.15	.70
151 SW	.73	6.55	3.22
153 CB	.80	8.11	1.59
	$\bar{x}$=.79±.11	$\bar{x}$=9.00±1.78	$\bar{x}$=.71±1.83

[a]Data from last 6 days of Period II. [b]Mean±SD.

Table 8

Zinc Excretion and Utilization

	Urine Zinc Mean±SD mg/day	Fecal Zinc Mean±SD mg/day	Digested Zinc mg/day	Apparent Total Zinc Excretion, Mean±SD mg/day	Zinc Retention Mean±SD mg/day
Amino Acids	.63±.12[a]	5.11±.91[a]	.39[b]	5.11±.91[a]	-.24±.86[a]
Amino Acids & Zinc	.45±.14[a]	8.27±.91[a]	2.23[c]	8.27±.91[a]	1.78±.90[a]
Ammonium Citrate & Zinc	.79±.11[d]	9.00±1.73[d]	1.50[c]	9.00±1.78[d]	.71±1.8[d]
Ammonium Citrate	.60±.09[a]	4.71±.90[a]	.79	4.71±.90[a]	.19±.91[a]

[a]Mean of 6 individuals based on last 6 days of Period II. [b]Zinc intake = 5.5 mg/day.

[c]Zinc intake = 10.5 mg/day. [d]Mean of 5 individuals based on last 6 days of Period II.

that the subjects excreted about 0.3 mg zinc per day in urine. This did not vary with dietary intake of zinc.

Individual fecal zinc concentrations, based on the last six days of Period II, are presented in Table 7. Fecal zinc followed zinc intake closely for days 1-6 and days 7-12 of Period II (Appendix X). As McCance and Widdowson indicate (75), fecal zinc excretion normally parallels zinc intake within the limits of experimental error. Those groups given supplemental zinc excreted higher amounts, close to intake, compared with the groups given the 5.5 mg basal amount (Table 8). The group given amino acids plus zinc excreted an average of 8.27±.91 mg/day; ammonium citrate, 4.71±.90 mg/day; amino acids, 5.11±.91 mg/day; and ammonium citrate plus zinc, 9.00±1.78 mg/day. Within groups, there was slight variation between subjects (Table 7). The mean daily fecal zinc excretion for the two groups given supplemental zinc were very similar and it is clear that the form that supplemental nitrogen was in did not affect fecal zinc content.

The fecal zinc concentration for the nonzinc supplemented groups are higher than fecal zinc concentrations reported by Price and Bunce (74) in comparable groups. They report 3.39 mg/day for the ammonium citrate group compared to 4.71 mg/day in this study, and 3.25 mg/day in the amino acid supplemented group compared to 5.11 in this study. Their subjects ingested 1 mg less zinc than the present subjects, so the excretion values between the two studies can be considered agreeable.

Sweat zinc was not measured in this study, but sweat zinc figures have been reported for the same age and sex group consuming

similar diets and in moderate activity (69,76). Research has shown that about 0.143 mg zinc/day is lost in sweat by preadolescent children (69). Zinc balance was calculated on the basis of fecal and urine zinc in the present study, assuming sweat zinc to be a constant.

Total zinc excretion paralleled zinc intake (Table 8). Only the group supplemented with amino acids showed mean daily zinc excretion in excess of zinc intake. Mean daily zinc excretion was only 0.2 mg greater than intake in that group. Although some subjects had mean daily zinc excretion greater than zinc intake (Table 7), the mean daily total zinc excretion for three of the individual groups was not greater than the respective zinc intakes. Total zinc excretion averaged 8.72±.90 mg/day in the amino acids plus zinc group; ammonium citrate, 5.31±.92 mg/day; amino acids, 5.73±.85 mg/day; and ammonium citrate plus zinc, 9.79±1.83 mg/day.

Zinc Balance

Only the amino acid supplemented group showed a negative mean daily zinc balance (Table 9). The balance for that group was -0.24 mg/day; amino acids plus zinc, 1.78 mg/day; ammonium citrate, 0.19 mg/day; ammonium citrate plus zinc, 0.71 mg/day. Many subjects exhibited negative mean daily zinc balances in all groups (see Table 7). The groups given supplemental zinc had more positive mean daily zinc balances compared with those groups given the basal amount of zinc. There was a significant ($p<.01$) effect of added zinc whether added to the basal diet plus ammonium citrate or basal plus amino acids (Table 9).

Table 9

Effect of Zinc, Essential Amino Acids or Zinc-Essential Amino Acids Interaction on Zinc Balance, mg/day

	Amino Acids	Ammonium Citrate	$\bar{x}$ Effect
+Zinc	1.78	0.71	1.25[a]
-Zinc	-0.24	0.19	-0.03[a]
$\bar{x}$ Effect	0.77[b]	0.45[b]	

[a]Comparison of 1.25 and 0.03 showed a significant difference ($p<.01$) and therefore a zinc effect.

[b]Comparison of 0.77 and 0.45 showed no significant difference and therefore no nitrogen effect.

Zinc balance was shown to be greatly dependent upon dietary zinc concentration in the present study. This is further illustrated in Table 9.

Apparent absorption and retention of other minerals, such as calcium, have been shown to be enhanced by increasing dietary protein levels (77,78), but this is still a subject of experimental controversy as others have found just the opposite effect (79,80,81). Whether or not added protein quality enhances zinc balance is also questionable. The group given amino acids plus zinc conserved zinc in both urine and fecal excretion and therefore had higher zinc balance.

CHAPTER V

SUMMARY AND CONCLUSIONS

A 21-day metabolic balance study was conducted in the summer of 1975 in which the effects of added zinc and/or increased protein quality were measured on the nitrogen and zinc balance of 23 preadolescent girls. Subjects' average age was 8 years 5 months, average weight was 29.5 kg, and average height was 132.1 cm. The subjects were divided on the basis of weight into four treatment groups following the 2×2 factorial design of the study. The average weight of each group was approximately equal.

Groups were randomly assigned to treatments and after an eight day adjustment period with all children consuming daily 23.8 g protein, 2,000 Kcalories, and 5.5 mg zinc in the basal diet, the following supplements were administered to the subjects:

(a) The five essential amino acids most limiting with respect to egg protein. Supplementation was to 80 percent of whole egg protein. Threonine, .234 g; valine, .244 g; methionine, .120 g; isoleucine, .332 g; and lysine HCl, .334 g.

(b) An isonitrogenous amount of ammonium citrate matching the amino acid supplement: 1.123 g ammonium citrate supplying .139 g nitrogen daily.

(c) The amino acid supplement described in (a) above plus .0168 g zinc acetate supplying about 5.0 mg zinc.

(d) 1.123 g ammonium citrate plus .0168 g zinc acetate.

Supplementation was carried out for 12 days following the 8 day adjustment period.

All urinary and fecal excretions were collected for the entire 21 days of study. All food was prepared in the metabolic kitchen and served in an adjacent dining room. All foods were weighed and any food dropped or spilled by a child was weighed and replaced. The subjects drank only ion-free water and all beverages were prepared with ion-free water. Fecal and urine nitrogen were determined by a modified Kjeldahl-Gunning-Arnold method (66). Fecal and urine zinc data were obtained from wet-ashed samples using atomic absorption spectrophotometry. Daily nitrogen and zinc balances were calculated from the last six days of supplementation using the following equation: Balance (nitrogen or zinc) = Intake - (urine + fecal). Analysis of variance was performed on the 23 individual mean daily balances using the F-test.

Mean total excretion of nitrogen was greatest in the group given ammonium citrate plus zinc, .35 g/day, and least in the group given amino acids, 3.30 g/day. The other groups' mean total excretion were: amino acids plus zinc, 3.31 g/day; and ammonium citrate, 3.41 g/day. Nitrogen balance averaged 1.01 g/day in the group given amino acids, while the amino acids plus zinc group averaged 1.00 g/day; ammonium citrate, .90 g/day; and ammonium citrate plus zinc, .81 g/day. There was no effect of added zinc or added essential amino acids either separately or together on the nitrogen balance of these subjects. Zinc did not appear to affect nitrogen utilization in healthy subjects when protein was given in marginal amounts. The amino acids had no effect on nitrogen balance even when given in concert with 5.0 mg plus basal level of zinc. It must be concluded that in these healthy human subjects, there was no zinc effect on nitrogen utilization. Neither

supplementation with zinc nor amino acids was effective in improving nitrogen retention.

Mean daily zinc excretion was greatest in the ammonium citrate plus zinc group, 9.79 mg/day, and least in the ammonium citrate group, 5.31 mg/day. The amino acids plus zinc group excreted an average of 8.72 mg/day and the amino acids group excreted an average of 5.73 mg/day. Zinc excretion paralleled zinc intake and, as dietary zinc was increased, the increase in zinc excretion came predominately through fecal zinc. Mean daily zinc balance was highest in the amino acids plus zinc group, 1.78 mg/day. Mean daily zinc balance was lowest in the group given amino acids, -0.24 mg/day. The ammonium citrate group averaged 0.19 mg/day and the group given ammonium citrate plus zinc averaged 0.71 mg/day. Zinc balance followed zinc ingestion, being more positive with the 10.5 mg/day intake and low or negative with the 5.5 mg/day intake. There was a significant effect ($p<.01$) of added zinc on zinc balance. The present Recommended Dietary Allowance (10) of 10 mg zinc/day for this age group is necessary for positive zinc balance according to the present results. Further studies are needed to define clearly the zinc needs of this age group.

LITERATURE CITED

1. Todd, W. R., C. A. Elvenhjem, and E. B. Hart. 1934. Zinc in the nutrition of the rat. Am. J. Physiol. 107:146.

2. Macapinlac, M. P., W. N. Pearson, and W. J. Darby. In: Zinc Metabolism, edited by A. S. Prasad. Springfield, IL: Thomas, 1966, p. 142.

3. Hurley, L. S. 1969. Zinc deficiency in the developing rat. Amer. J. Clin. Nutr. 22:1332.

4. Tucker, H. E. and W. D. Salmon. 1955. Parakeratosis in zinc deficiency disease in pigs. Proc. Soc. Exptl. Biol. Med. 88:613.

5. O'Dell, B. L., P. M. Newberne, and J. E. Savage. 1958. Significance of dietary zinc for the growing chick. J. Nutr. 65:503.

6. _____, and J. E. Savage. 1957. Symptoms of zinc deficiency in the chick. Federation Proc. 16:394.

7. Shaw, N. A., H. C. Dickey, H. H. Brugman, D. L. Blamberg, and J. F. Witter. 1974. Zinc deficiency in female rabbits. Laboratory Animals 8:1.

8. Prasad, A. S. 1967. Nutritional metabolic role of zinc. Federation Proc. 26:172.

9. National Academy of Sciences. 1968. Recommended Dietary Allowances, Food and Nutrition Board, National Research Council, Washington, D. C.

10. National Academy of Sciences. 1974. Recommended Dietary Allowances, Food and Nutrition Board, National Research Council, Washington, D. C.

11. Prasad, A. S., J. A. Halsted, and M. Nadimi. 1961. Syndrome of iron deficiency anemia, hypogonadism, dwarfism, hepatosplenomegaly and geophagia. Amer. J. Med. 31:532.

12. _____, A. Miale, Jr., Z. Farid, H. H. Sandstead, A. S. Schulert and W. J. Darby. 1963. Biochemical studies on dwarfism, hypogonadism and anemia. Arch. Intern. Med. 11:407.

13. Prasad, A. S., A. Miale, Jr., Z. Farid, H. H. Sandstead, and A. R. Schulert. 1963. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. J. Lab. and Clin. Med. 61:537.

14. Ronaghy, H. A. and J. A. Halsted. 1975. Zinc deficiency occurring in females. Report of two cases. Amer. J. Clin. Nutr. 28:831.

15. Sandstead, H. H., A. S. Prasad, A. R. Schulert, Z. Farid, A. Miale, S. Bassilly, and W. J. Darby. 1967. Human zinc deficiency, endocrine manifestations and response to treatment. Amer. J. Clin. Nutr. 20:422.

16. Spivey Fox, M. R. 1970. The status of zinc in human nutrition. World Rev. of Nutr. and Diet. 12:208.

17. Mills, C. F., J. Quarterman, J. K. Chester, R. B. Williams, and A. C. Dalgarno. 1969. Metabolic role of zinc. Amer. J. Clin. Nutr. 22:1240.

18. Vallee, B. L. Zinc. In: Mineral Metabolism, edited by C. L. Comer and F. Brommer. New York: Academic Press, 1962, p. 443.

19. Kessler, B. In: Plant Analysis and Fertilizer Problems. Washington: Am. Inst. Biol. Sci., 1961, p. 314.

20. Lieberman, I., R. Abrams, N. Hunt, and P. Ove. 1963. Levels of enzyme activity and deoxyribonucleic acid synthesis in mammalian cells cultured from the animal. J. Biol. Chem. 238:3955.

21. Fujioka, M. and I. Lieberman. 1964. A zinc requirement for synthesis of DNA by rat liver. J. Biol. Chem. 239:1164.

22. Wacker, W. E. C. 1962. Nucleic acids and metals. III. Changes in nucleic acid, protein and metal content as a consequence of zinc deficiency in Euglena gracilis. Biochemistry 1:859.

23. Winder, F. G. and C. O. O'Hara. 1962. Effects of iron and zinc deficiency on the composition of Mycobacterium smegmatis. Biochem. J. 82:99.

24. _____, and J. M. Denneny. 1959. Effect of iron and zinc on nucleic acid and protein synthesis in Mycobacterium smegmatis. Nature 184:742.

25. Terhune, M. W. and H. H. Sandstead. 1972. Decreased RNA polymerase activity in mammalian zinc deficiency. Science 177:68.

26. Scrutton, M. C., C. W. Wu, and D. A. Goldwait. 1971. The presence and possible role of zinc in RNA polmerase obtained from Escherichia coli. Proc. Nat. Acad. Sci. USA 68:2497.

27. Sandstead, H. H. and R. A. Rinaldi. 1969. Impairment of deoxyribonucleic acid synthesis by dietary zinc deficiency in the rat. J. Cell Physiol. 73:81.

28. Hove, E., C. A. Elvehjem, and E. B. Hart. 1937. The physiology of zinc in the nutrition of the rat. Amer. J. Physiol. 119:768.

29. Pories, W. J. and W. H. Strain. In: Zinc Metabolism, edited by A. S. Prasad. Springfield, IL: Thomas, 1966, p. 378.

30. Stephen, J. K. and J. M. Hsu. 1973. Effect of zinc deficiency and wound healing on DNA synthesis in rat skin. J. Nutr. 103:548.

31. Prasad, A. S. and D. Oberleas. 1973. Ribonuclease and deoxyribonuclease activities in zinc-deficient tissues. J. Lab. and Clin. Med. 82:461.

32. Fernandez-Madrid, F., A. S. Prasad, and D. Oberleas. 1973. Effect of zinc deficiency on nucleic acids, collagen and noncollagenous protein of the connective tissue. J. Lab. and Clin. Med. 82:951.

33. Oberleas, D. and A. S. Prasad. 1969. Growth as affected by zinc and protein nutrition. Amer. J. Clin. Nutr. 22:1304.

34. O'Dell, B. L. 1969. Effect of dietary components upon zinc availability. A review with original data. Am. J. Clin. Nutr. 22:1315.

35. _____, L. E. Burpe, and J. E. Savage. 1972. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 102:653.

36. Smith, W. H., M. P. Plumlee, and W. M. Beeson. 1962. Effect of source of protein on zinc requirement of the growing pig. J. Animal Sci. 21:399.

37. Forbes, R. M. and M. Yohe. 1960. Zinc requirement and balance studies with the rat. J. Nutr. 70:53.

38. Miller, J. K. and L. S. Jensen. 1966. Effect of dietary protein source on zinc absorption and excretion along the alimentary tracts of chicks. Poultry Sci. 45:1051.

39. Savage, J. E., J. M. Yohe, E. E. Pickett, and B. L. O'Dell. 1964. Zinc metabolism in the growing chick. Tissue concentration and effect of phytate on absorption. Poultry Sci. 45:412.

40. Edwards, H. M., Jr. 1966. The effects of protein source in the diet on ^{65}Zn absorption and excretion by chickens. Poultry Sci. 45:412.

41. O'Dell, B. L. and J. E. Savage. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Expt'l. Biol. Med. 103:304.

42. Likuski, H. J. A., and R. M. Forbes. 1964. Effect of phytic acid on the availability of zinc in amino acid and casein diets fed to chicks. J. Nutr. 84:145.

43. Kratzer, F. H., J. B. Allred, P. N. Davis, B. J. Marshall, and P. Vohra. 1959. The effect of autoclaving soybean protein and the addition of ethylenediaminetetraacetic acid on the biological availability of dietary zinc for turkey poults. J. Nutr. 68:313.

44. Osis, D., L. Kramer, E. Wiatrowski, and H. Spencer. 1972. Dietary zinc intake in man. Amer. J. Clin. Nutr. 25:582.

45. Van Campen, D. and W. A. House. 1974. Effect of a low protein diet on retention of an oral dose of ^{65}Zn and on tissue concentration of zinc, iron and copper in rats. J. Nutr. 104:84.

46. Strain, W. H., L. T. Steadman, C. R. Landau, Jr., W. P. Berliner, and W. J. Pories. 1966. Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man. J. Lab. Clin. Med. 68:244.

47. Hambridge, K. M., C. Hambridge, M. Jacobs, and J. D. Baum. 1972. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. Pediat. Res. 6:868.

48. Sandstead, H. H. 1973. Zinc nutrition in the United States. Amer. J. Clin. Nutr. 26:1251.

49. Caggiano, V., R. Schnitzer, W. Strauss, R. K. Baker, A. C. Carter, A. S. Josephson, and S. Wallock. 1969. Zinc deficiency in a patient with retarded growth, hypogonadism, hypogammaglobulinemia and chronic infection. Am. J. Med. Sci. 257:305.

50. Henkin, R. I., P. J. Schecter, R. Hay, and C. F. T. Mattern. 1971. Idiopathic hypogeusia with dysheusia, hyposmia and dysamia: a new syndrome. J. Amer. Med. Assoc. 217:34.

51. Husain, S. L. 1969. Oral zinc sulfate in leg ulcers. Lancet 1:1069.

52. Ronaghy, H., M. R. Spivey Fos, S. M. Garn, H. Israel, A. Harp, P. G. Moe, and J. A. Halsted. 1969. Controlled zinc supplementation for malnourished school boys: A pilot experiment. Amer. J. Clin. Nutr. 22:1279.

53. Engel, R. W., R. F. Miller, and N. O. Price. 1966. Metabolic patterns in preadolescent children. XIII. Zinc balance. In: Zinc Metabolism, edited by A. S. Prasad. Springfield, IL: Thomas.

54. Schraer, K. K. 1972. Zinc balance in pregnant teenagers. Unpublished Master's thesis, University of California, Berkeley.

55. Price, N. O. and G. E. Bunce. 1972. Effect of nitrogen and calcium on balance of copper, manganese and zinc in preadolescent girls. Nutr. Reports Intl. 5:275.

56. Abernathy, R. P, S. J. Ritchey and J. C. Gorman. 1972. Lack of response to amino acid supplements by preadolescent girls. Amer. J. Clin. Nutr. 25:980.

57. _____, M. Speirs, R. W. Engel, and M. E. Moore. 1966. Effects of several levels of dietary protein and amino acids on nitrogen balance of preadolescent girls. Amer. J. Clin. Nutr. 19:407.

58. _____, S. J. Ritchey, M. K. Korslund, J. C. Gorman, and N. O. Price. 1970. Nitrogen balance studies with children fed foods representing diets of low income southern families. Amer. J. Clin. Nutr. 23:408.

59. Sandstead, H. H. 1973. Zinc as a unrecognized limiting nutrient: Letter to the editor. Amer. J. Clin. Nutr. 26:790.

60. Abernathy, R. P., S. J. Ritchey. 1973. Reply to Dr. Sandstead: Letter to the editor. Amer. J. Clin. Nutr. 26:791.

61. Oberleas, D., M. E. Muhrer, and B. L. O'Dell. 1966. The availability of zinc from foodstuffs. In: Zinc Metabolism, edited by A. S. Prasad. Springfield, IL: Thomas.

62. Somers, M., and B. J. Underwood. 1969. The influence of zinc deficiency in ram lambs upon the digestibility of dry matter and the utilization of nitrogen and sulfur in the diet. Australian J. Agr. Res. 20:889.

63. Hsu, J. M., W. L. Anthony, and P. J. Buchanan. 1969. Zinc deficiency and incorporation of ^{14}C-labeled methionine into tissue proteins in rats. J. Nutr. 93:425.

64. Pike, R. L. and M. L. Brown. 1969. Nutrition: An Integrated Approach. New York: John Wiley and Sons.

65. Association of Official Agricultural Chemists. Official Methods of Analysis. 7th ed. Washington, D. C., 1950.

66. Analytical Methods for Atomic Absorption Spectrophotometry. 1969. Revised edition. Perkin-Elmer Corporation, Norwalk, CN.

67. Howat, P. M., M. K. Korslund, R. P. Abernathy, and S. J. Ritchey. 1975. Sweat nitrogen losses by and nitrogen balance of preadolescent girls consuming three levels of dietary protein. Am. J. Clin. Nutr. 28:879.

68. Harrison, M. Sweat nitrogen losses in preadolescent boys consuming three levels of dietary protein. Unpublished Master's thesis, Virginia Polytechnic Institute and State University, 1975.

69. Bruning, J. L. and B. L. Kintz. 1968. Computational Handbook of Statistics. Chicago: Scott, Foresman and Co., p. 9.

70. Piez, K. A. and H. Eagle. 1958. The free amino acid pool of cultured human cells. J. Biol. Chem. 231:533.

71. Spence, N. P., R. P. Abernathy, ans S. J. Ritchey. 1972. Excretion of nitrogen in sweat by preadolescent girls consuming low protein diets. Amer. J. Clin. Nutr. 25:275.

72. Evans, G. W., C. I. Grace, C. Hahn. 1973. Homeostatic regulation of zinc absorption in the rat. Soc. Expt'l Biol. Med. 143:723.

73. Price, N. O. and G. E. Bunce. 1972. Effect of nitrogen and calcium on balance of copper, manganese and zinc in preadolescent girls. Nutr. Reports Int'l 5:275.

74. McCance, R. A. and E. M. Widdowson. 1942. The absorption and excretion of zinc. Biochem. J. 36:692.

75. Prasad, A. S., A. R. Schulert, H. H. Sandstead, A. Miale, Jr., and Z. Farid. 1963. Zinc, iron and nitrogen content of sweat in normal and deficient subjects. J. Lab. Clin. Med. 62:84.

76. McCance, R. A. and E. M. Widdowson. 1942. The effect of protein intake on the absorption of calcium and magnesium. Biochem. J. 36:684.

77. Hall, T. C. and H. Lehmann. 1944. Experiments on the practicality of increasing calcium absorption with protein derivatives. Biochem. J. 38:117.

78. Johnson, N. E., N. Alcantara, and H. Linkswiler. 1970. Effect of level of protein intake on urinary and fecal calcium and calcium retention in adult males. J. Nutr. 100:1425.

79. Margen, S. and D. H. Calloway. 1967. Effect of dietary protein on urinary calcium. Federation Proc. 26:629 (abstr).

80. _____, and D. H. Calloway. 1968. Effect of dietary protein on urinary calcium, magnesium and phosphorus. Federation Proc. 27:726 (abstr).

APPENDIX I

AGE, HEIGHT, WEIGHT, HEMOGLOBIN AND HEMATOCRIT OF SUBJECTS ON INITIAL DAY OF STUDY

Subject No.	Age: yrs	mos	Height cm	Weight kg	Hemoglobin g/100 ml	Hematocrit vol%
130	7	9	124.5	25.7	14.69	38.0
131	8	0	123.0	28.1	13.99	38.0
132	8	1	131.5	25.0	14.86	40.0
133	8	5	126.5	23.1	15.21	39.0
134	7	6	124.0	28.1	14.69	40.0
135	9	9	139.5	38.3	15.56	42.0
136	9	3	134.0	31.7	13.40	41.0
137	7	11	124.5	27.0	14.69	38.0
138	7	11	125.5	22.3	14.51	39.0
139	8	7	134.0	32.4	15.91	39.5
140	7	11	135.5	32.9	14.69	39.0
141	8	8	131.5	31.2	15.39	38.7
142	8	9	130.0	24.7	15.56	38.0
143	8	10	131.0	25.3	16.44	39.0
144	9	3	135.0	25.5	16.61	37.0
145	9	6	140.5	40.2	14.6	42.0
146	9	1	140.0	29.3	14.69	39.5
147	9	6	138.5	33.7	16.26	38.5
148	7	9	121.5	22.9	14.69	40.0
149	9	6	148.0	37.2	15.39	39.5
150	8	1	134.5	26.6	15.91	35.0
151	8	8	131.0	33.0	14.69	43.0
153	8	0	134.0	34.9	14.86	43.5
Mean	8	5	132.1	29.5	15.1	39.4
SD		8	6.5	5.0	.76	1.88

APPENDIX II

TYPICAL DAILY SCHEDULE

	Time	Activity
A.M.	7:00 - 7:45	Awaken and prepare for breakfast
	7:45 - 8:45	Eat breakfast
	8:45 - 10:30	Swimming
	10:45 - 11:15	Morning snack
	11:15 - 12:15	Indoor activities
	12:15 - 12:30	Prepare for lunch
P.M.	12:30 - 1:15	Eat lunch
	1:15 - 2:00	See registered nurse
	2:00 - 3:00	Field trip
	3:00 - 3:15	P.M. Snack
	3:15 - 5:15	Outdoor activities
	5:15 - 5:45	Prepare for dinner
	5:45 - 6:45	Eat dinner
	7:00 - 8:00	Yard recreation and/or indoor games, letter writing
	8:00 - 9:00	Shower and prepare for bed
	9:00	Bedtime

APPENDIX III

MENUS SERVED IN STUDY

Menu A

	Food Item	Amount, gm
Breakfast:		
	Pineapple juice	80
	Puffed rice	12
	Raisins	20
	Brown sugar	20
	Cream	20
	Bread, toasted	20
	Margarine	5
	Grape jelly	20
A.M. Snack:		
	Orange juice	100
	Cake, yellow	40
	Frosting, vanilla	30
Lunch:		
	Cream of celery soup, condensed	60
	Cream	30
	Lettuce	15
	Tomato, raw	40
	Salad dressing, French	10
	Bread	40
	Potato chips	20
	Prunes, uncooked	32
	Kool-Aid with sugar	25
P.M. Snack:		
	Kool-Aid with sugar	25
	Graham crackers	14
	w/ cream cheese	10
Dinner:		
	Baked beans	90
	w/ brown sugar	10
	Canned asparagus, drained	50
	Cabbage slaw	65
	w/ white sugar	5
	Canned peaches w/ syrup	100
	Bread	40
	w/ margarine	15
	Kool-Aid w/ sugar	25

Menu B

	Food Item	Amount, gm
Breakfast:		
	Orange juice	40
	Apple juice	80
	Canned apricots w/ syrup	50
	Bread, toasted	10
	Margarine	10
	Brown sugar	15
A.M. Snack:		
	Canned peaches, w/ syrup	50
	w/ cream, whipped	20
	w/ brown sugar	5
	Ginger bread	40
	Kool-Aid w/ sugar	25
Lunch:		
	Cream of chicken soup, condensed	30
	Cream	10
	Rice, cooked	70
	Margarine	5
	Lettuce	30
	Salad dressing, French	10
	Applesauce	100
	Krispie snack	30
	Bread	20
	Kool-Aid with sugar	25
P.M. Snack:		
	Banana	50
	Bread	20
	w/ margarine	5
	w/ brown sugar	10
	Kool-Aid with sugar	25
Dinner:		
	Ground beef, baked	15
	Baked onion	60
	w/ margarine	5
	Boiled potato	50
	w/ cream	10
	Corn pone	50
	w/ margarine	5
	w/ grape jelly	20
	Tomato, raw	40

Menu B, continued

Cake, yellow	50
Vanilla frosting	30
Kool-Aid with sugar	25

Menu C

	Food Item	Amount, gm
Breakfast:	Grape juice	120
	Rice Krispies	13
	w/ sugar	5
	w/ raisins	20
	w/ cream	15
	Bread, toasted	20
	w/ margarine	5
	w/ grape jelly	5
A.M. Snack:		
	Bread	40
	w/ margarine	10
	w/ grape jelly	15
	Kool-Aid with sugar	25
Lunch:	Tomato soup, condensed	60
	w/ cream	5
	Cheddar cheese	10
	Lettuce	15
	Mayonnaise	13
	Bread	20
	w/ margarine	5
	Cranberry freeze	150
	Kool-Aid with sugar	25
P.M. Snack:		
	Lemonade	60
	Potato chips	20
Dinner:		
	Noodles w/ tomato sauce	80
	Ground beef, cooked	20
	Cauliflower	50
	w/ margarine	10
	Pears with syrup	100

Menu C, continued

Applesauce cookies	30
Kool-Aid with sugar	25

Menu D

	Food Item	Amount, gm
Breakfast:		
	Orange juice	80
	Puffed wheat	12
	w/ brown sugar	15
	w/ cream	20
	Bread, toasted	20
	w/ margarine	5
	w/ grape jelly	10
A.M. Snack:		
	Bread	20
	w/ margarine	10
	w/ grape jelly	10
	Kool-Aid with sugar	25
Lunch:		
	Tomato soup, condensed	60
	w/ cream	10
	Bread	40
	Lettuce	15
	Tomato, raw	40
	Salad dressing, French	10
	Cake, yellow	50
	Frosting, vanilla	30
	Kool-Aid with sugar	25
P.M. Snack:		
	Applesauce cookies	30
	Kool-Aid with sugar	30
Dinner:		
	Beef-rice casserole	100
	Squash, yellow summer	50
	w/ margarine	10
	Corn pone	50
	w/ margarine	15
	w/ grape jelly	20
	Cherry-prune crisp	136
	Kool-Aid with sugar	25

APPENDIX IV

HEIGHT AND WEIGHT OF SUBJECTS DURING BASAL AND NITROGEN BALANCE PERIODS

Height Chart

Subject Number	Period I			Period II			Change cm
	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20	
130	124.5	124.5	124.0	124.0	124.5	124.0	0.00
131	123.0	123.5	123.0	123.0	124.0	123.0	0.00
132	131.5	131.5	130.5	131.0	131.0	131.0	-.50
133	126.5	126.5	126.5	126.0	126.0	126.0	-.50
134	124.0	124.0	123.5	124.5	123.5	125.0	1.00
135	139.5	139.0	138.0	139.0	137.0	137.0	1.50
136	134.0	134.0	134.0	134.5	134.0	134.0	0.00
137	124.5	125.0	125.0	124.5	124.5	126.0	1.50
138	125.5	125.5	124.5	123.5	125.0	125.5	0.00
139	134.0	133.0	134.0	134.5	134.5	135.5	1.50
140	135.5	136.5	136.0	135.5	134.5	135.5	0.00
141	131.5	131.5	131.5	131.5	132.0	132.0	.50
142	130.0	130.0	130.5	130.0	130.0	130.0	0.00
143	131.0	130.0	130.0	130.0	130.5	130.5	-.50
144	135.0	134.5	134.0	135.0	133.0	133.0	-2.00
145	140.5	142.0	140.5	140.5	-----	139.0	-1.50
146	140.0	140.0	140.0	140.0	140.5	140.5	.50
147	138.5	138.0	137.5	138.0	138.0	138.0	-.50
148	121.5	121.5	121.5	121.0	121.5	121.0	-.50
149	148.0	148.0	148.5	148.5	147.0	147.5	-.50
150	134.5	134.0	133.5	133.5	134.0	133.0	-1.50
151	131.0	130.5	130.5	130.0	130.0	129.0	-2.00
153	134.0	134.5	134.0	134.0	134.0	134.0	0.00
Mean	131.7	132.1	131.7	131.8	131.3	131.7	-.20
SD	6.6	6.7	6.7	6.8	6.3	6.4	1.23

Weight Chart

Subject Number	Period I			Period II			Change kg
	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20	
130	25.7	25.8	25.8	25.5	25.7	26.2	0.5
131	28.1	28.3	28.1	28.1	28.8	28.8	0.7
132	25.0	24.8	25.1	24.9	25.1	25.4	0.4
133	23.1	23.4	23.6	23.3	23.4	23.9	0.8
134	28.1	28.1	28.1	27.8	28.2	28.7	0.6
135	38.3	37.8	37.5	37.4	37.6	37.5	-0.8
136	31.7	31.5	31.8	30.9	31.6	31.5	-0.2
137	27.0	26.9	26.9	26.8	26.7	27.4	0.4
138	22.3	22.8	23.2	22.9	23.2	23.7	1.4
139	32.4	32.0	32.1	31.8	31.6	32.1	-.3
140	32.9	32.3	32.1	31.9	32.2	32.4	-.5
141	31.2	30.8	30.6	30.5	30.6	30.6	-.6
142	24.7	24.7	24.6	24.4	24.6	24.9	.2
143	25.3	25.3	25.3	25.0	25.2	25.7	.4
144	25.5	25.0	25.5	25.3	25.3	26.0	.5
145	40.2	40.1	40.3	39.5	----	40.1	-.1
146	29.3	29.3	28.8	28.9	29.1	29.1	-.2
147	33.7	33.3	33.5	33.1	33.1	33.6	-.1
148	22.9	23.2	23.0	23.1	23.3	23.7	.8
149	37.2	36.6	36.4	36.1	35.8	36.3	-.9
150	26.6	26.5	26.4	26.3	26.6	26.7	.1
151	33.0	32.8	32.9	32.8	32.8	33.3	.3
153	34.9	34.9	34.6	34.4	34.9	34.7	-.2
Mean	29.4	29.4	29.4	29.1	28.9	29.7	.14
SD	5.2	5.0	4.9	4.8	4.3	4.7	.56

APPENDIX V

DAILY URINE NITROGEN FOR THE EIGHT DAYS OF PERIOD I AND DAYS 1-12 OF PERIOD II (g/day)

Treatment / Subject	Day 1	2	3	4	5	6	7	8	Mean±SD daily urine nitrogen	Mean±SD of group
Amino Acids & Zinc										
130 SRC	4.46	4.60	1.34	1.74	2.70	2.78	4.34	2.62	3.07±1.26	
131 CMW	8.26	1.96	1.88	1.70	4.24	2.88	2.86	2.12	3.23±2.20	
132 EL	6.62	2.54	4.40	2.20	4.36	3.00	3.08	3.14	3.66±2.36	
133 JF	4.70	0.15	1.20	2.60	4.90	1.26	2.48	1.74	2.37±1.69	
134 KB	5.78	2.20	2.76	3.76	2.96	1.86	4.36	3.08	3.34±1.28	
135 NM	6.60	3.40	3.68	3.88	3.50	3.06	3.36	3.06	3.81±1.19	3.24±.56
Ammonium Citrate										
136 KAP	8.94	3.20	3.12	3.88	3.80	3.70	3.26	3.26	4.14±1.97	
137 PCW	5.94	3.10	3.30	3.76	2.20	3.30	3.74	3.84	3.64±1.09	
138 ERV	5.72	1.98	2.80	4.20	1.88	3.92	2.72	2.90	3.26±1.30	
139 LB	6.90	3.80	4.03	4.30	3.80	4.40	3.36	3.50	4.26±1.23	
140 LL	7.45	7.60	2.68	3.44	4.34	1.88	3.35	2.18	4.11±2.25	
141 LAL	5.16	3.66	2.90	1.96	0.15	4.00	1.66	4.60	3.01±1.68	3.73±.57
Amino Acids										
142 MM	3.72	2.16	2.48	2.47	2.66	2.32	2.70	2.10	2.57± .54	
143 LS	8.30	3.50	2.40	1.64	3.20	3.54	2.70	2.70	3.49±2.05	
144 DCP	7.48	3.90	1.30	3.10	4.72	2.14	4.46	3.46	3.82±1.87	
145 TEM	8.40	4.30	3.44	4.02	4.50	3.36	3.26	3.74	4.37±1.70	
146 SP	4.80	1.72	1.46	3.34	2.20	2.76	4.34	2.78	2.92±1.20	
147 DKL	7.60	2.06	1.60	2.96	2.90	3.00	1.28	2.10	2.93±2.00	3.35±.67
Zinc & Ammonium Citrate										
148 RM	4.82	2.60	2.64	1.74	3.90	1.68	2.70	2.64	2.84±1.05	
149 KL	8.80	4.16	5.00	5.14	4.34	4.10	3.94	4.00	4.93±1.64	
150 MB	8.34	----	1.72	1.58	6.80	3.60	3.26	3.50	4.11±2.80	
151 SW	5.36	4.94	3.74	3.08	2.28	4.34	2.80	3.68	3.77±1.09	
153 CB	----	1.44	3.80	2.90	5.80	3.16	2.20	4.30	3.32±1.37	3.79±.82

Treatment / Subject	Day 1	2	3	4	5	6	7	8	9	10	11	12	Mean±SD daily urine nitrogen	Mean±SD of group
Amino Acids & Zinc														
130 SRC	2.10	2.74	2.70	2.54	2.80	2.36	1.92	2.38	1.72	1.98	2.26	2.82	2.36± .37	
131 CMW	2.60	2.54	1.48	3.14	2.68	1.92	1.40	1.98	1.88	1.80	2.28	2.82	2.21± .55	
132 EL	2.12	3.08	2.94	1.70	3.74	2.18	2.70	2.48	2.40	2.32	2.64	2.84	2.60± .52	
133 JF	1.88	2.38	1.28	3.84	2.98	2.24	1.54	3.44	1.98	2.00	1.42	3.66	2.39± .87	
134 KB	1.94	3.08	1.60	3.88	2.88	2.74	2.34	2.66	2.08	2.86	2.48	3.02	2.63± .60	
135 NM	2.84	2.80	2.74	3.28	2.80	2.42	2.34	2.22	2.28	2.16	3.60	3.66	2.76± .52	2.49±.20
Ammonium Citrate														
136 KAP	1.94	2.80	3.00	3.06	3.40	2.70	1.82	3.68	2.50	2.50	2.66	3.22	2.77± .55	
137 PCW	3.02	1.50	4.12	2.80	2.28	4.26	1.30	1.40	3.28	2.42	2.62	2.76	2.65± .96	
138 ERV	1.65	3.08	3.44	2.72	3.04	2.40	2.28	3.20	2.26	2.28	2.48	2.64	2.63± .50	
139 LB	3.04	3.26	2.94	3.56	3.34	3.28	2.80	2.74	2.50	2.44	2.80	2.46	2.93± .37	
140 LL	2.29	2.12	3.60	3.56	2.46	2.80	2.52	2.74	2.26	2.30	2.48	3.38	2.71± .52	
141 LAL	2.58	2.78	1.70	2.28	3.48	2.68	2.52	2.68	1.88	1.90	2.28	3.00	2.48± .50	2.69±.15
Amino Acids														
142 MM	1.88	2.54	2.42	2.90	2.98	2.14	2.18	1.76	2.88	2.14	1.48	3.20	2.38± .53	
143 LS	1.57	2.30	2.70	3.10	2.66	2.48	2.28	2.48	1.98	2.54	2.34	2.82	2.44± .39	
144 DCP	1.78	4.32	2.34	1.96	3.92	2.86	3.84	1.76	3.80	2.70	3.14	2.20	2.89± .91	
145 TEM	1.75	4.30	3.00	3.48	3.68	3.02	1.76	3.08	2.40	3.18	2.54	2.38	2.88± .75	
146 SP	1.02	2.74	5.80	1.90	3.24	2.68	2.40	1.50	2.26	1.88	1.10	3.96	2.54±1.33	
147 DKL	1.01	4.12	1.52	1.96	4.08	2.68	2.72	2.74	2.20	2.08	2.14	2.38	2.47± .91	2.60±.22
Zinc & Ammonium Citrate														
148 RM	1.65	2.59	3.58	1.28	3.72	2.22	2.28	2.40	2.12	2.26	2.54	2.26	2.41± .68	
149 KL	3.85	3.68	2.24	4.28	3.66	3.48	3.40	2.58	2.76	3.18	3.22	3.46	3.32± .57	
150 MB	3.22	3.44	3.16	3.06	3.02	2.48	1.48	2.74	2.16	1.98	2.60	3.26	2.72±1.09	
151 SW	1.94	1.86	4.04	3.50	3.16	3.02	2.10	3.02	2.26	2.32	3.06	3.20	2.79± .68	
153 CB	2.60	3.08	2.92	3.40	3.40	2.68	2.64	2.48	2.26	3.60	2.20	3.82	2.92± .55	2.83±.33

APPENDIX VI

INDIVIDUAL AND MEAN DAILY FECAL NITROGEN FOR THE TWO SIX DAY PERIODS IN PERIOD II (g/day)

Treatment/ Subject	Days 1-6	Days 7-12
Amino Acids & Zinc		
130 SRC	.9746	.9661
131 CMW	1.1435	.7044
132 EL	1.0207	.8453
133 JF	.7373	1.2783
134 KB	.9136	.7154
135 NM	1.0637	.9723
	$\bar{x}$=.9755±.14	$\bar{x}$=.9136±.21
Ammonium Citrate		
136 KAP	1.2086	1.0596
137 PCW	.5137	.8341
138 ERV	.7570	.4604
139 LB	1.2893	1.0289
140 LL	1.0211	1.0168
141 LAL	.8227	.9569
	$\bar{x}$=.9354±.29	$\bar{x}$=.8927±.22
Amino Acids		
142 MM	1.1328	1.0178
143 LS	1.1112	1.1284
144 DCP	.5468	.7158
145 TEM	1.3708	.6094
146 SP	1.0155	1.0948
147 DKL	1.0194	1.0232
	$\bar{x}$=1.0328±.27	$\bar{x}$=.9315±.21
Zinc & Ammonium Citrate		
148 RM	.7296	1.0843
149 KL	.8217	.8995
150 MB	.5161	.9350
151 SW	.7869	.5475
153 CB	.9187	.7645
	$\bar{x}$=.7546±.15	$\bar{x}$=.8461±.20

APPENDIX VII

INDIVIDUAL DAILY TOTAL NITROGEN EXCRETION FOR PERIOD II

Treatment	Day												
Subject	1	2	3	4	5	6	7	8	9	10	11	12	Mean±SD
Amino Acids & Zinc													
130 SRC	3.075	3.715	3.675	3.515	3.775	3.235	2.887	3.347	2.687	2.947	3.227	3.787	3.231± .37
131 CMW	3.744	3.684	2.624	4.284	3.824	3.064	2.015	2.685	2.585	2.505	2.985	3.525	3.127± .48
132 EL	3.141	4.101	3.916	2.721	4.760	3.201	3.546	3.326	3.246	3.166	3.486	3.686	3.528± .54
133 JF	2.618	3.118	2.018	4.578	3.718	2.978	2.819	4.719	3.259	3.279	2.699	4.939	3.395± .91
134 KB	2.854	3.994	2.514	4.794	3.794	3.654	3.056	3.276	2.796	3.576	3.196	3.736	3.445± .62
135 NM	3.904	3.864	3.764	4.244	3.864	3.484	3.313	3.193	3.253	3.133	4.573	4.633	3.769± .51
Ammonium Citrate													
136 KAP	3.149	4.009	4.209	4.269	4.609	3.909	2.880	4.740	3.560	3.560	3.720	4.280	3.908± .55
137 PCW	3.534	2.014	4.634	3.214	2.794	4.774	2.135	2.235	4.115	3.255	3.455	3.595	3.321± .91
138 ERV	2.408	3.803	4.198	3.478	3.798	3.158	2.741	4.035	2.721	2.741	2.941	3.101	3.263± .59
139 LB	4.330	4.550	4.230	4.850	4.630	4.570	3.829	3.769	3.529	3.469	3.829	3.489	4.090± .49
140 LL	3.212	3.142	4.622	4.582	3.482	3.822	3.537	3.757	3.277	3.217	3.497	4.397	3.729± .52
141 LAL	3.403	3.603	2.523	3.103	4.303	3.503	3.477	3.637	2.837	2.857	3.237	3.957	3.370± .49
Amino Acids													
142 MM	3.013	3.673	3.553	4.033	4.113	3.273	3.198	2.778	3.898	3.157	2.498	4.218	3.451± .55
143 LS	2.683	3.413	3.813	4.213	3.773	3.593	3.409	3.609	3.109	3.669	3.469	3.949	3.559± .39
144 DCP	2.327	4.806	2.887	2.507	4.669	3.407	4.556	2.476	4.516	3.416	3.856	2.916	3.534± .93
145 TEM	3.121	6.671	4.371	4.851	5.051	4.391	2.370	3.690	3.010	3.790	3.150	2.990	3.955±1.19
146 SP	2.036	3.756	6.816	2.916	4.256	3.696	3.495	2.595	3.255	2.975	2.195	5.055	3.596±1.32
147 DKL	2.030	5.140	2.540	2.980	5.100	3.700	3.744	3.764	3.224	3.104	3.164	3.404	3.491± .91
Ammonium Citrate & Zinc													
148 RM	2.735	3.675	4.665	2.365	4.805	3.305	3.365	3.485	3.205	3.245	3.625	3.345	3.493± .68
149 KL	4.672	4.050	3.062	5.102	4.482	4.302	4.200	3.480	3.660	4.080	4.120	4.360	4.139± .54
150 MB	4.156	4.376	4.096	4.096	3.956	3.416	2.416	3.676	3.096	2.916	3.536	4.295	3.652± .59
151 SW	2.727	2.677	4.827	4.287	3.947	3.807	2.648	3.568	2.808	2.868	3.608	3.748	3.460± .71
153 CB	3.579	3.999	3.839	5.319	4.319	3.599	3.405	3.245	3.025	4.265	2.965	4.585	3.765± .55

Note: Total apparent nitrogen excretion = urine + fecal nitrogen.

APPENDIX VIII

MEAN DAILY APPARENT NITROGEN EXCRETION BY TREATMENT GROUP FOR PERIOD II, g/day

Treatment Group	1	2	3	4	5	6	7	8	9	10	11	12	Mean±SD
Amino Acids & Zinc	3.22	3.74	3.09	4.04	3.95	3.28	2.95	3.44	2.97	3.10	3.36	4.05	3.43±.41
Ammonium Citrate	3.35	3.52	4.07	3.93	3.93	3.95	3.10	3.63	3.34	3.20	3.44	3.80	3.32±.32
Amino Acids	2.53	4.42	3.99	3.58	4.46	3.67	3.47	3.15	3.51	3.35	3.05	3.75	3.58±.55
Ammonium Citrate & Zinc	3.41	3.68	3.94	3.85	4.14	3.53	3.22	3.49	3.15	3.51	3.57	4.04	3.35±.30

APPENDIX IX

INDIVIDUAL DAILY URINE ZINC CONCENTRATIONS FOR THE THREE COMPOSITING PERIODS OF PERIOD II

Treatment/ Subject	Days 1-4	Days 5-8	Days 9-12	Mean±SD
Amino Acids & Zinc				
130 SRC	1.000[a]	.500	.450	.650±.30
131 CMW	.200	.450	.200	.283±.14
132 EL	.400	.550	.400	.450±.08
133 JF	.650	.500	.400	.516±.12
134 KB	.350	.450	.350	.383±.06
135 NM	1.600	.950	.550	1.033±.53
Mean SD	.70±.52	.566±.19	.391±.11	
Ammonium Citrate				
136 KAP	.300	.550	.650	.500±.18
137 PCW	.850	.450	.600	.633±.20
138 ERV	.600	.700	.700	.666±.07
139 LB	1.050	.550	.700	.766±.26
140 LL	.450	.450	.750	.550±.17
141 LAL	1.400	.550	.400	.783±.13
Mean SD	.775±.40	.541±.09	.750±.12	
Amino Acids				
142 MM	.800	.600	.500	.633±.15
143 LS	1.600	.750	.350	.583±.64
144 DCP	.750	.650	.700	.700±.05
145 TEM	1.050	.800	.850	.900±.13
146 SP	.600	.950	.500	.683±.23
147 DKL	.550	1.100	.350	.666±.39
Mean SD	.891±.39	.808±.19	.541±.19	
Ammonium Citrate & Zinc				
148 RM	.700	.900	1.000	.866±.16
149 KL	.700	.800	.800	.866±.07
150 MB	.400	.550	.700	.666±.14
151 SW	.400	.500	.850	.550±.23
153 CB	.400	.600	.900	.633±.25
Mean SD	.520±.16	.670±.17	.850±.11	

[a] mg/day

APPENDIX X

INDIVIDUAL FECAL ZINC FOR PERIOD II

Treatment/ Subject	Days 1-6	Days 7-12
Amino Acids & Zinc		
130 SRC	8.085[a]	8.085
131 CMW	9.613	8.774
132 EL	9.796	9.364
133 JF	6.661	6.661
134 KB	8.680	8.569
135 NM	11.250	8.175
	$\bar{x}$=9.014±1.5	$\bar{x}$=8.271±.91
Ammonium Citrate		
136 KAP	6.443	6.371
137 PCW	3.807	4.347
138 ERV	4.975	3.815
139 LB	7.174	4.982
140 LL	4.860	4.607
141 LAL	9.696	4.134
	$\bar{x}$=6.159±2.11	$\bar{x}$=4.700±.91
Amino Acids		
142 MM	5.093	4.887
143 LS	4.962	5.910
144 DCP	4.607	6.385
145 TEM	5.372	3.805
146 SP	5.227	4.890
147 DKL	4.759	4.782
	$\bar{x}$=5.002±.31	$\bar{x}$=5.110±.91
Ammonium Citrate & Zinc		
148 RM	5.896	9.958
149 KL	9.107	11.244
150 MB	5.618	9.150
151 SW	8.195	6.554
153 CB	10.725	8.112
	$\bar{x}$=7.90±2.15	$\bar{x}$=9.00±1.78

[a] mg/day

The vita has been removed from the scanned document

THE EFFECT OF ZINC LEVELS ON NITROGEN RETENTION IN PREADOLESCENT CHILDREN

by

Christine R. Meiners

(ABSTRACT)

A 21-day metabolic balance study was designed to test the hypothesis that dietary zinc level affects protein utilization in growing children. Subjects' mean age was 8 years 5 months, mean weight was 29.5 kg, and mean height was 132.1 cm. The subjects were divided on the basis of weight into four treatment groups following a 2×2 factorial experimental design.

After an eight day adjustment period in which all subjects consumed a mixed diet known to contain 28.8 g protein, 5.5 mg zinc, and 2,000 Kcal, the four treatments were administered for 12 days. One group was supplemented with the essential amino acids found by analysis to be most limiting with respect to whole egg protein. Threonine, valine, methionine, isoleucine, and lysine were added in amounts to reach a chemical score of 80 based on whole egg protein. The sources and amounts of nitrogen were: food, 4.18 and supplements, 0.14 g/day. One group received these same amino acids plus a zinc supplement of 5.0 mg/day. One group received an isonitrogenous amount of ammonium citrate matching the nitrogen contained in the amino acids supplement. The last group received the isonitrogenous amount of ammonium citrate

plus 5.0 mg zinc. All supplements were given daily and were incorporated into meals.

All urinary and fecal excretions were collected and analyzed for nitrogen and zinc. Nitrogen analysis was accomplished using a modified Kjeldahl-Gunning-Arnold method and zinc was determined spectrophotometrically on wet-ashed samples.

Nitrogen retentions for the four groups were: amino acids plus zinc, 1.00; amino acids, 1.01; ammonium citrate, 0.90; and ammonium citrate plus zinc, 0.81 g/day. Analysis of variance on the balance data from the four groups showed no effect of zinc, added essential amino acids or the interaction of the two on the nitrogen balance of the subjects, although the trends of retention favored amino acids. There was a significant effect ($p<.01$) of added zinc on zinc balance, however. It was concluded that zinc did not affect nitrogen utilization in the growing child, but that zinc balance is affected by zinc quantity in the diet. The present Recommended Dietary Allowance of 10 mg zinc/day for this age group is the minimum necessary to maintain positive zinc balance.