THE EFFECT OF VARIOUS DIETARY ZINC CONCENTRATIONS ON THE BIOLOGICAL INTERACTIONS OF ZINC-COPPER-IRON IN RATS

by

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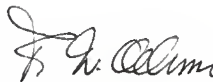
Approved by: 

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Abstract

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INTRODUCTION

Early studies concerning zinc, copper and iron have emerged the essentiality of these metals to the biological system (1,2). However the major impetus for research was associated with trace mineral deficiencies or toxicities. Most of our present knowledge, by the advent of purified diets, could highlighten the significance of trace mineral interactions. Such interactions may acknowledge the importance of so pervading nutritional deficiencies or toxicities in the biospheres especially in the developing countries. However, the mode of action of suspected or recognized trace element interactions remains, with few exceptions, to be elucidated.

The effect of dietary zinc supplementation on copper or iron biological availability, interactions, and metabolism is controversial (3,4,5,6). Likewise, the biochemical etiology of anorexia or growth retardation of zinc deprivation is a matter of conjecture (7,8,9,10).

From the foregoing argument, it is apparent that further study is required before a unifying hypothesis can be established for the effects of interactions or imbalances among zinc, copper and iron. Our study, therefore, will assist in understanding of situations in which risk to health exist from accumulation of potentially toxic elements, and will facilitate examining metabolic relationships between zinc, copper and iron to explain some

aspects of their mutually antagonistic effects. In more practical spheres a more detailed knowledge of how such interactions influence their availability will be of increasing value in estimating dietary recommendations, in establishment of new prophylactic measures and in assessment of situations in which a substantial risk to human and animal health in the developing countries may arise.

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THE RUNNING HEAD: DIETARY INTERACTIONS OF Zn,Cu and Fe.

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THE EFFECT OF VARIOUS DIETARY

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A B S T R A C T

Forty-two, male, weanling Sprague-Dawley rats were divided into three groups of 14 rats each and pair-fed a purified basal diet containing different zinc concentrations for 8 weeks. Group C, was fed a control purified basal diet containing 12 ppm zinc, 5 ppm copper and 35 ppm iron; group D was fed the basal diet containing <2 ppm zinc; group S was fed the basal diet supplemented with 1000 ppm zinc. The effects of the various dietary zinc concentrations on growth rate, feed efficiency, blood hemoglobin and hematocrit, relative organ weights, and protein concentrations of tissues, and the zinc, copper and iron content of blood, liver, kidney, spleen, heart and lung were studied. Blood samples were collected at 2,4,6 and 8 weeks and tissues were sampled at 4 and 8 weeks. Rats fed the zinc-deficient diet (D) had decreased weight gain and showed signs of zinc deficiency at d 5, but polydipsia and intermittent mild diarrhoea were also prominent and led to dehydration. The decreased weight gain in these zinc-deficient rats (D) suggest decreased protein synthesis may be of zinc deficiency mediated decreased insulin activity. A cyclical pattern of decreased and then normal food intake and weight gain occurred in the zinc-supplemented rats (S) from weeks 6 to 8. Copper concentrations increased in heart and lung by week 4, and decreased in liver and lung at week

8 in the rats of zinc-deficient diet (D). In rats fed the zinc-supplemented diet (S), copper concentrations decreased in liver and spleen and increased in kidney at week 4, and decreased in the liver and increased in the heart by week 8. Tissue iron concentrations in rats on the zinc-deficient diet (D) were consistently increased in all tissues, except liver, throughout the study. Rats receiving increased dietary zinc (S) generally had depressed iron concentrations in all tissues except heart. The fluctuations in tissues element concentrations with increased duration of the study is of variance with previous studies of shorter time frames. These data suggest an inconsistent antagonism between copper and zinc or other mechanisms may be involved in copper homeostasis. If tissue concentrations reflect intestinal uptake, an apparent competition and/or inhibition occurs between iron and zinc in the intestine with zinc deficiency but not in zinc supplementation. The relative dietary proportions of zinc, copper and iron and time effect influence zinc, copper and iron metabolism at the intestinal and cellular transport level.

Keywords:

Zinc, copper, iron, dietary element and interaction, rats, zinc deficiency, zinc supplementation, weight gain, feed efficiency, total protein, hemoglobin, hematocrit, organ weight, tissue concentrations.

INTRODUCTION

Early studies have been conducted to elucidate the nutritional role of zinc, copper, and iron in man and animals [1,2,3]. The role of zinc has been of particular interest [4,5,6,7]. While these studies have examined bioavailability, metabolism, deficiency and toxicity of individual compounds,interactions between these elements have been less studied.

The dietary essentiality of zinc for rats was established more than 50 years ago [3], and the importance of zinc in human and animal health [8,9] has been thoroughly reviewed [10]. Dietary zinc could change metabolism of other divalent cations [11]. However,the effect of high dietary zinc on the utilization of copper is controversial. Although zinc and copper exhibited mutual antagonism at intestinal absorptive level [12,13,14], high dietary zinc increased tissue concentrations of copper by increasing metallothionein (MT) synthesis [6,15]. In contrast, other investigators [5,7,16,17] demonstrated that excess dietary zinc decreased the tissue concentrations of copper.

Although decreased food intake and growth inhibition are the only overt signs of zinc deficiency [18,19,20], the mechanism of how the lack of zinc is responsible is not known. Reduced food intake has been corrected with zinc supplementation or by decreasing dietary

proteins in zinc-deficient rats [18]. The anorexia of zinc deficiency may be induced by abnormalities in endogenous opiate regulation [21]. The growth inhibition seen in zinc deficiency has been attributed to decreased food intake [20,21,22], reduced feed conversion efficiency [23,24,25], an inhibited secretion of growth hormone [26,27],or poor growth of skeletal muscles [19].

Anemia and reduced tissue iron concentrations have been reported in mice, rats and chicks due to high dietary zinc [7,28,29,30,31], but these have not been seen in humans [32]. This inverse relationship between zinc and iron has been rationalized by the hypothesis that zinc and iron share a common absorptive pathway [29,33],but other studies have failed to demonstrate this [34]. Administration of inorganic iron has depressed the absorption of inorganic zinc, but not when zinc was in organic form [35,36] or given with a meal [37]. Since the copper-dependent enzyme, ceruloplasmin, has been shown to govern the rate at which Fe (II) is released from liver ferritin, and is converted to plasma transferrin-Fe (III) [38], zinc has been postulated to alter iron metabolism by interfering with copper metabolism [30,39,40,41,42].

Data on the interactions of zinc, copper and iron vary with differences in diet formulations, relative dietary ratios [5,7], the duration of study [7], and differing test methodology. Accordingly, we designed an 8-week feeding

study in which standardized test diets were used [43]. The object of this study was to illuminate the metabolic lesions of zinc deficiency and supplementation with special reference to zinc, copper, and iron concentrations and interactions organs and tissues of rats.

MATERIALS AND METHODS

ANIMALS AND DIET:

Forty-two weanling , male, Sprague-Dawley rats (Sasco Inc,Omaha,NE) were individually housed in stainless steel, wire-bottomed cages and fed a standard rat diet (Purina rat chow, St. Louis, MO) and allowed free choice water during the acclimation period. The facilities fulfilled the standards of the American Association for Accreditation of Laboratory Animal Care. After 7 days of acclimation the rats were randomly assigned to one of three experimental dietary groups of 14 rats each and fed purified basal diet (Purina Inc.,Richmond,Indiana) for 8 weeks that vary only in the zinc concentrations (Table 1). The basal control diet (C) was formulated to contain 12 ug zinc/g, 35 ug iron/g and 5 ug copper/g. The zinc-deficient basal diet (D) contained less than 2 ug zinc/g, while the zinc-supplemented basal diet (S) contained 1000 ug zinc/g.

To ensure pair feeding, the rats were fed 15 g/rat/day for the 8 weeks till the termination of the experiment. Free access toe deionized water was allowed throughout the study. Food consumption was established by measuring the unconsumed feed daily and body weights were determined weekly. Our own analysis [44] verified that zinc concentrations were 13-13.2, <2 ug zinc/g and 1000-1090, in the control, zinc-deficient and zinc-supplemented diets respectively. Copper and iron concentrations were constant

at 5-7 and 35 ug/g respectively in each of the three diets.

SAMPLING AND ANALYSIS:

Heparinized whole blood samples were collected bi-weekly for hemoglobin and hematocrit evaluation under mild ether anaesthesia by cardiac puncture . At the end of the fourth week, 5 animals/experimental group , selected at random, were sacrificed by an overdose of ether anaesthesia. Liver, kidney, spleen, heart and lung were excised ,cleaned of connective tissues,twice rinsed in deionized water, and stored at $-20^{0}C$ for determination of zinc, copper, iron and protein concentrations. The internal organ weights and mean wet organ weight : body weight ratio were also determined. This was repeated for the remaining rats at the end of the 8 -week feeding period.

A portion of the whole blood was analyzed for hemoglobin and hematocrit by direct aspiration using an automatic Coulter Counter (Model S-plus IV, Counter Electronics Inc, Hialeah, FL). The remaining whole blood as well as the liver, kidney, spleen, heart and lung were analyzed for zinc, copper and iron concentrations by atomic absorption spectrophotometry [44].

The total protein content in liver, spleen, heart, kidney, and lung was determined by the Bio-Rad protein mircoassay method (BIO RAD,Richmond,Ca). The standard reference solution employed was bovine gamma globulin (1.29 mg/ml). For the protein assay, 0.8 ml of standard,

appropriately diluted samples and blanks were mixed with 0.2 ml of concentrated dye reagent and placed in clean ,dry test tubes. Samples were vortexed and measured at 595 nm against blanks using a Hitachi Perkin-Elmer (Tokyo, Japan) spectrophotometer. Concentrations were then read directly from a standard curve.

Tissues were prepared for elemental analysis by a wet ash procedure. Using pre-washed forceps and scissors, 5 g of minced tissues with an equivalent amount of deionized water homogenized in acid-washed tubes until a uniform slurry was formed. One gram of slurry was then placed into a tared 100 ml acid-washed beaker. Whole blood was prepared by pipetting 1 ml of blood directly into a tared beaker. At least two blanks were prepared using deionized water. Nine milliliters of concentrated nitric acid (70-71%) was added to each sample and allowed to digest overnight in an acid hood. Perchloric acid (69-72%) was added the next morning to establish a nitric:perchloric ratio of 9:2 or 9:1 for tissues or blood respectively were gently heated. Small increments of nitric acid were added as appropriate to avoid tarring. Beakers were removed from heat prior to complete dryness when about 0.1-0.2 ml of residual acid remained. Appropriate amounts of 2% nitric acid in relation to tissue weights were added and swirled to dissolve the digestion residue. Beakers and ashed samples were weighed and the samples transferred to an acid-washed polypropylene sample

containers (25-30 ml size). The sample zinc, copper and iron concentrations were measured by atomic absorption spectrophotometry with a Perkin-Elmer,Model 306 atomic absorption spectrophotometer equipped with a one-slot burner head, an acetylene flame, appropriate hollow-cathode lamp, and recorder. Wavelengths used were 214 nm for zinc, 248 nm for iron and 325 nm for copper. Standard reference solutions for each element were purchased from Fisher Scientific Company, Fairlawn.NJ.

Calculations for the element concentrations were carried out using the following formulae:

Net weight of sample = (beaker + sample)-(tare weight of beaker).

Net weight of ashed sample = (beaker + ashed sample)-(tare weight of beaker).

Wet ash dilution = (net weight ashed sample)/(net weight of sample).

Total dilution = (wet ash dilution)x(analysis dilution).

Sample mineral (ppm) = (total dilution)x(ppm diluted sample).

All data were statistically analyzed by analysis of variance using SAS. Comparison between treatment means was performed on the basis of the Fisher's LSD only when a significant overall treatment effect was observed (F-test ,$p<0.05$) [45].

RESULTS

Body Weight and Feed Efficiency:

Table 2 summarizes the body weight and feed efficiency ratios of the rats fed the zinc-deficient (D) and zinc-supplemented diets (s) for 8 weeks. Rats on the zinc-deficient diet (D) were anorexic at the first week and, although did not lose weight, thereafter they grew more slowly than rats fed the control diet (C) or the zinc-supplemented diets (s). The mean body weight of rats fed a zinc-deficient diet (d) was 79.7% and 77.8% of controls by the end of week 4 and 8, respectively. That of zinc supplemented group (S) was 101.9% and 97.8% of the controls (Table 2). Feed efficiency (g of body weight gain/g of feed consumed) was reduced for the zinc-deficient rats (D) throughout the study, but was not significantly different in the zinc-supplemented rats (S) when compared to the controls. The signs manifested by the zinc-deficient group (D) were anorexia, poor growth, rough coat, hyposthesia, intermittent mild diarrhoea and polydipsia was also prominent. Rats fed the zinc supplemented diet (S) showed no signs of toxicity, but they lost weight during week 7. This was compensated for during week 8 (Fig 1).

Organ Weight:

At week 4 and 8, the internal organ weights (expressed as gram wet weight) of the zinc-deficient rats (D) were significantly reduced in all organs except spleen

when compared to the paired-fed controls(C) (Table 3). In contrast, no significant variation occurred in the zinc-supplemented rats (S), although liver and kidney weights were slightly greater than in the controls at week 4 (Table 3). At week 8 heart, spleen and lung weights of the zinc-supplemented rats (S) were significantly less ($p<0.05$) and than the paired-fed controls (C) (Table 3). The mean relative organ wet weight:body weight (g wet tissue/g body weight percent) of the zinc-deficient (D) and the zinc-supplemented rats (S) was similar or greater than that of control rats (C) (Table 4).

Hemoglobin and Hematocrit:

The rats on the zinc-deficient (D), had increased hemoglobin concentration . Zinc supplementation (S) had no effect on hemoglobin concentrations when compared to controls (C) (Fig 3) throughout the study.

The hematocrit was significantly increased in the zinc-deficient (D) group (Fig 4) but no effect was observed on hematocrit in the zinc supplemented group (S) during the 8-week course of the study.

Organ Protein Concentrations:

The protein concentrations (mg/g tissues) of the organs at week 4 and week 8 decreased significantly ($p<0.05$) in the zinc-deficient rats (D) but remained unchanged in the lung at week 8 (Fig 2). Similarly, protein concentrations generally decreased significantly in the organs of the zinc-

supplemented rats (S), although they increased in the kidneys at week 4 and showed no alteration in the heart and lung at week 8 when compared to control organs (Fig 2).

Blood Element Concentrations:

The blood zinc, copper and iron concentrations at weeks 2,4,6 and 8 are given in Table 8. Blood zinc concentrations in the zinc-deficient group (D) were decreased in weeks 2 and 8 , but not at weeks 6 and 8. In the zinc-supplemented group (S) blood zinc concentrations were increased at week 2, were not significantly varied at weeks 4 and 6 and were decreased significantly at week 8 when compared to the controls (C) (Table 8).

Blood copper concentrations were generally unaffected by feeding the zinc-deficient (D) or zinc-supplemented (S) diets, although reduced blood copper levels occurred at weeks 6 and 8 in the zinc-deficient rats (8) (Table 8).

Blood iron concentrations increased considerably with a peak at week 6 in the rats fed the zinc-deficient diet (D), whereas in the rats given zinc supplementation (S) the iron level was increased only at week 2 and remained at control concentrations at the other weeks (Table 8).

Organ Zinc Concentrations:

Zinc concentrations in the heart and lung of rats fed the deficient diet (D) were significantly lower than the controls (C) and were similar of controls (C) in the

liver, spleen and kidney at week 4. However, a significant decrease in zinc concentration was observed in all organs except the heart at week 8 (Table 5). Zinc concentrations were higher than those of controls ($p<0.05$) in all organs of rats fed the zinc-supplemented diet (S), except for lung and heart which were unaffected at the end of week 4 and 8, respectively (Table 5).

<u>Organ Copper Concentrations:</u>

At the end of the fourth week, copper concentrations were significantly increased in heart and lung and were not altered in liver , kidney and spleen of the zinc-deficient rats (D). At week 8 the copper concentrations had decreased in the liver and lung and were not different in the kidney, heart and spleen of the same zinc-deficient (D) group (Table 6). In the rats fed the zinc-supplemented diet (S) copper concentrations were decreased in liver and spleen, were increased in kidney and were not altered in other of organs at week 4. At week 8 copper concentrations in group S were decreased in liver, increased in heart and were not changed in lung, kidney and spleen (Table 6).

<u>Organ Iron Concentrations:</u>

In general ,iron concentrations were significantly increased in all the organs of the zinc-deficient rats (D), however at week 4 liver had a lower concentration than the controls and no deviation was observed in the kidney, and at

week 8 liver and kidney were not different from controls (Table 7). Conversely, the iron concentrations in the major organs of the zinc-supplemented rats (S) were generally decreased, although no variation was detected from the controls in kidney at week 4 and in the heart and spleen at week 8 (Table 7). Only the heart that had increased iron concentrations at week 4 in the zinc-supplemented diet (S) group.

DISCUSSION

Body weight and feed efficiency in zinc-supplemented rats (S) did not show significant difference from controls (C) (Table 2). Contrary to our observations, other investigators reported increased [5,6] or decreased [7,46] body weights concomitant with zinc supplementation. Our data suggest that these differences may be explicable to their short-term study or that our 1000 ppm zinc may not be sufficiently toxic for such reactions to be emphasized. However, the cyclical pattern in food intake and weight gain expressed in the zinc-supplemented (S) rats in weeks 6-8 remained to be explained (Fig 1). Rats fed the zinc-deficient diet (D) exhibited signs of zinc deficiency as reported in other studies [3,18,20,47], but inaddition polydipsia and intermittent diarrhea were also prominent in our study.

The organ protein contents displayed no significant differences at week 4 and decreased afterwards in the zinc-supplemented rats (S) than in pair-fed controls (Fig 2). This view is further strengthened by the observation of similar responses in the internal organ weights (Table 3) and body weights (Table 2) in the zinc-supplemented (S) group. These data make it is difficult to agree with other finding [6] that protein synthesis increases with zinc supplementation. This may be due to the use by the others [4,6] of experimental designs of shorter

duration which were not sufficient to produce effects we have observed. The body weights (Table 2), internal organ weights (Table 3) and protein contents (Fig 2) of the rats fed the zinc-deficient diets (D) were 20%, 19-38% and 13-22% lower, respectively after the 8-week study than in the pair-fed controls (C). Whereas zinc deficiency has been repeatedly described to have profound effect on growth, speculations of mechanisms involved are associated with marked change in appetite and food intake [18,20,22], anorexia of abnormal opiate regulation [21], lower feed conversion efficiency [23,24,25] or through inhibited secretion of growth hormone (GH) at marginal (27] or severe [26] zinc deficiency. However, the actual mechanism is not well understood.

The results with proteins highlighted important implications that zinc deficiency <u>per se</u> has a restrictive effect on growth independent of reduction in food intake in accord with previous studies (18,19). Further, zinc deficiency has been shown to adversely influence the growth of skeletal muscles as anabolic and catabolic phases of food intake increases the overall energy requirements [19]. Since our data (Table 4) emphasized that the mean organ weight: body weight ratio was proportionally greater in the zinc-deficient (D) than in the pair-fed controls (C), the reduction in the body mass was greater as well. It has been shown that zinc deficiency decreases insulin secretion

indicated by high total insulinlike activity (TILA) [26]. Insulin is an important regulator of fuel metabolism by promoting uptake of branched chain amino acids by muscles, accelerates entry of glucose and fatty acids in muscles and adipose cells and inhibits intracellular degradation of proteins [48]. Abnormality of Insulin metabolism may, therefore, contribute to decreased protein synthesis and subsequently body weight loss. Thus growth inhibition showed in the zinc-deficient rats (D) may has been mediated , inaddition to anorexia, via impairment of muscle protein synthesis and asymmetry of total energy as result of abnormality of insulin metabolism. Further investigation will be required to clarify the biochemical etiology of the poor growth and anorexia of zinc deficiency.

Fluid intakes decrease in zinc deficiency [8]. Diarrhoea of zinc deficiency may be directly associated with zinc deficiency [49], increased fecal sodium losses [50] or may be secondary to waned immunocompetence [51]. Polydipsia and mild intermittent diarrhea were evident in rats fed our zinc-deficient diet (D), and resulted in increased hematocrit values (Fig 4) suggesting hemoconcentration and dehydration . These data find support in other reports [9,47]. As proposed in other studies [52,53], osmotic fragility of erythrocytes was not observed in the zinc-deficient rats (D) since egg white was used as a source of protein in the test diets and discoloration of plasma or

urine was not observed.

Although our dietary zinc level was strictly deficient (< 2 ppm), yet zinc retention was well restored in organs at week 4 (Table 5) suggesting increased intestinal absorption [54,55] or zinc resorption from tissues other than internal organs. Chen et al [56] have speculated that increased liver zinc concentration is attributable to increased metallothionein (MT) in the liver soluble fraction when rats fed 1000 or 2000 ppm zinc in the diet. Although tissue zinc concentrations increased significantly (0-36%) at week 4 ,it then deceased significantly and consistently in the liver of the zinc-supplemented rats (S) (Table 5) at week 8 compared to the controls (C). This suggests that liver MT may not be the only factor for zinc accumulation in the liver or that their effect may be exhibited on short-term basis. This indicate that other (unknown) mechanism may be involved in decreasing the uptake by the hepatocytes. Furthermore, zinc concentrations were significantly decreased in blood of the zinc-supplemented (S) rats at week 8. An explanation for this reduction may be due to decreased zinc flux consequent to increased intestinal thionein binding. This has been reported elsewhere [57]. An alternative interpretation could be increased zinc efflux as part of homeostatic regulatory process of zinc excretion [54,58].

Excess dietary zinc reduces copper absorption

[7,14,16] via induction of MT synthesis in the mucosal cells [17] or mutual antagonism at the absorptive site [13,59]. Other investigators [15], in contrast, reported induction of MT synthesis with increased tissue copper concentrations. Copper concentrations in our study were inconsistent in organs (Table 6) or not altered in blood (Table 8) of rats fed the zinc-supplemented (S) diets at weeks 4 and 8. Likewise, copper concentrations significantly altered and were inconsistent in various tissues of rats fed the zinc-deficient diet (D) as determined at weeks 4 or 8. It is evident from our study that tissue copper concentrations influenced with time and zinc dietary status. Furthermore, irrespective of their luminal concentrations, the inverse relationship between copper and zinc at the absorptive level shown in other studies [13] is not always consistent with time. Copper status therefore not only a reflect of mutual antagonism between copper and zinc at the intestinal level [13], but likely other mechanisms interfere with copper metabolism at intestinal and/or cellular level.

The ingestion of excess zinc has caused anemia and depressed hematocrit and reduced iron concentrations in tissues of several animal species [18,29,30,39]. Iron and zinc interactions are thought to be mediated via changes in copper metabolism [30,38,39,40,41]. In our study, iron retention was consistently depressed in all tissues except heart (Table 7), and no change was observed on hemoglobin

(Fig 3), hematocrit (Fig 4) or blood iron concentration (Table 8) in the zinc-supplemented rats (S). Conversely, tissue iron concentrations (except for liver), blood iron concentration, hematocrit and hemoglobin were significantly increased in the rats on zinc-deficient diets (D). Although reported that zinc and iron are absorbed by different metabolic pathways [34], other studies have reported inter-element absorption competition [33,60]; zinc or iron absorption is increased when the respective competitor is deficient [39] in the diet . This suggests that these metals may share part of the same intestinal absorptive mechanism. Our findings support that competition between iron and zinc at an absorptive level occurs, as documented by tissue concentrations reflecting intestinal uptake [29,33,60,61]. The hematocrit, hemoglobin, and blood iron concentrations resulting from dietary zinc supplementation suggest that other mechanisms interfering with iron biological utilization. In view that ceruloplasmin, a copper-containing enzymes has been demonstrated to be involved in release and conversion and incorporation of Fe(II) to Fe(III) in plasma transferrin [38], zinc could influence iron metabolism by interfering with copper metabolism.

Variations and conflict in conclusions from studies involving interactions between zinc, copper and iron would reflect not only differences in the relative ratios of these elements in the test diets used but also the study

duration during which such interactions are allowed to become evident through the ways employed. Expert committee should realize that the widespread occurrence of marginal or impaired trace element nutrition in population at large may lead to emergence of overt signs of deficiency in patients who develop situations predisposing to that deficiency. Future element interactions dietary experiments should extent the time during which various dietary ratios are fed to more closely approximate the long term dietary condition that exists in various areas of this world's developing countries.

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TABLE 1: INGREDIENTS OF THE BASAL DIET USED IN THE CONTROL OF THIS STUDY.

INGREDIENTS	AMOUNT (%)
Egg Whites	13.50
Sucrose	31.1995
Solka Floc	3.00
RP Vitamin Mix	2.00
Special Mineral Mix #2	5.00
DL-Methionine	0.10
Choline chloride	0.20
Biotin	0.0005
Corn Oil	2.5
Lard	2.5
Dextrin	40.00

Specifications:

Protein 12.15%

Fat 5.0%

Fiber 3%

Carbohydrates 73.2%

Digestible energy 3.86 Kcal/g

Iron 35 ppm

Copper 5 ppm

Zinc <2ppm for the deficient diet, 12ppm for the control diet and 1000 ppm added as zinc carbonate (0.1895%) for the zinc-supplemented diet.

TABLE 2: BODY WEIGHT AND FEED EFFICIENCY IN GROUP OF 14 RATS EACH FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Values are Mean ± SE,g)

GROUP	BODY WEIGHT (g)			% CONTROL[1]		F.EFFICIENCY[2]	
	WK0	WK4	WK8	WK4	WK8	WK4	WK8
C	85.8±2.5[3]	209.7±2.9[a]	261.5±4.1[a]	0	0	0.31	0.21
D	88.7±2.5	166.2±2.7[b]	199.7±3.7[b]	79.7	77.8	0.23	0.17
S	90.4±2.5	212.5±2.6[a]	256.2±3.5[a]	101.9	97.8	0.31	0.20

[1] Percent of body weight compared to controls.

[2] Gram of body weight gain/g of food consumed.

[3] Body weight within weeks that don't share a common superscribt are significantly ($p<0.05$) different by Fisher's LSD.

TABLE 3: ORGAN WEIGHTS (g) OF RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Values are Mean Organ Wet Weight ± SE in g)

ORGAN/DIET	WEEK 4	WEEK 8
LIVER		
C	7.5 ± 0.40[b]	7.9 ± 0.40[b]
D	6.2 ± 0.40[a]	6.2 ± 0.40[a]
S	8.4 ± 0.40[b]	8.0 ± 0.30[b]
KIDNEY		
C	0.7 ± 0.03[b]	0.9 ± 0.03[b]
D	0.6 ± 0.03[a]	0.9 ± 0.03[a]
S	0.8 ± 0.03[b]	0.8 ± 0.03[b]
SPLEEN		
C	0.5 ± 0.04[a]	0.6 ± 0.04[b]
D	0.4 ± 0.04[a]	0.4 ± 0.03[a]
S	0.4 ± 0.03[a]	0.5 ± 0.03[c]
HEART		
C	0.8 ± 0.04[b]	0.9 ± 0.04[b]
D	0.6 ± 0.04[a]	0.7 ± 0.03[a]
S	0.8 ± 0.04[b]	0.7 ± 0.03[a]
LUNG		
C	1.3 ± 0.10[b]	1.3 ± 0.10[b]
D	0.9 ± 0.10[a]	1.0 ± 0.10[a]
S	1.1 ± 0.10[b]	1.1 ± 0.10[a]

Means within a column for each organ with the same superscript are not significantly different at the 0.05 level by Fisher's LSD.

TABLE 4: MEAN ORGAN WEIGHT:BODY WEIGHT RATIO IN RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Values are Mean ± SE in g/g percent)

ORGAN	WEEK 4	WEEK 8
LIVER		
C	3.6 ± 0.30	3.0 ± 0.30
D	3.9 ± 0.50	3.0 ± 0.30
S	4.0 ± 0.20	3.1 ± 0.30
KIDNEY		
C	0.4 ± 0.04	0.3 ± 0.02
D	0.4 ± 0.03	0.3 ± 0.01
S	0.4 ± 0.02	0.3 ± 0.02
SPLEEN		
C	0.2 ± 0.02	0.2 ± 0.02
D	0.3 ± 0.10	0.3 ± 0.02
S	0.2 ± 0.03	0.2 ± 0.02
HEART		
C	0.4 ± 0.02	0.3 ± 0.01
D	0.4 ± 0.03	0.3 ± 0.03
S	0.3 ± 0.04	0.3 ± 0.02
LUNG		
C	0.6 ± 0.03	0.5 ± 0.10
D	0.5 ± 0.04	0.5 ± 0.10
S	0.5 ± 0.10	0.4 ± 0.01

TABLE 5: CONCENTRATIONS OF ZINC ($\mu g/g$ WET TISSUE) IN TISSUES OF RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Expressed as Mean ± SE in $\mu g/g$ wet tissue)

ORGAN/DIET	WEEK 4	WEEK 8
LIVER		
C	22.1 ± 0.9^{a}	26.9 ± 0.8^{b}
D	19.7 ± 0.9^{a}	22.7 ± 0.7^{a}
S	24.9 ± 1.0^{b}	24.7 ± 0.7^{c}
KIDNEY		
C	20.1 ± 0.6^{a}	19.2 ± 0.5^{b}
D	18.7 ± 0.6^{a}	15.3 ± 0.5^{a}
S	27.3 ± 0.7^{b}	22.8 ± 0.5^{c}
SPLEEN		
C	17.4 ± 0.6^{b}	18.7 ± 0.5^{b}
D	19.4 ± 0.6^{a}	17.3 ± 0.5^{a}
S	19.7 ± 0.6^{a}	20.1 ± 0.4^{c}
HEART		
C	15.6 ± 0.9^{b}	15.2 ± 0.8^{ab}
D	18.5 ± 0.9^{a}	13.6 ± 0.8^{a}
S	20.7 ± 1.0^{a}	17.5 ± 0.7^{b}
LUNG		
C	19.3 ± 0.5^{b}	19.1 ± 0.5^{b}
D	16.0 ± 0.5^{a}	16.9 ± 0.4^{a}
S	19.0 ± 0.5^{b}	21.0 ± 0.4^{c}

Means within a column for each organ with the same superscript are not significantly different at the 0.05 level by Fisher's LSD.

TABLE 6: CONCENTRATIONS OF COPPER (μg/g WET WEIGHT) IN TISSUES OF RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Expressed as Mean ± SE in μg/g wet tissue)

ORGAN/DIET	WEEK 4	WEEK 8
LIVER		
C	3.3 ± 0.1[a]	4.3 ± 0.1[b]
D	3.6 ± 0.1[a]	3.6 ± 0.1[a]
S	2.8 ± 0.1[b]	3.4 ± 0.1[a]
KIDNEY		
C	4.9 ± 0.5[a]	5.7 ± 0.4[a]
D	6.1 ± 0.5[a]	5.4 ± 0.4[a]
S	6.4 ± 0.5[b]	5.8 ± 0.4[a]
SPLEEN		
C	2.2 ± 0.1[a]	1.4 ± 0.1[a]
D	2.0 ± 0.1[a]	1.5 ± 0.1[a]
S	1.9 ± 0.1[b]	1.5 ± 0.1[a]
HEART		
C	6.7 ± 0.3[b]	4.6 ± 0.3[a]
D	7.7 ± 0.3[a]	4.8 ± 0.2[a]
S	6.8 ± 0.3[b]	5.4 ± 0.2[b]
LUNG		
C	2.1 ± 0.1[b]	1.9 ± 0.1[b]
D	2.4 ± 0.1[a]	1.5 ± 0.1[a]
S	2.4 ± 0.1[b]	1.9 ± 0.1[b]

Means within a column for each organ with the same superscript are not significantly different at the 0.05 level by Fisher's LSD.

TABLE 7: CONCENTRATIONS OF IRON ($\mu g/g$ WET TISSUE) IN TISSUES OF RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Expressed as Mean ± SE in $\mu g/g$ wet tissue)

ORGAN/DIET	WEEK 4	WEEK 8
LIVER		
C	210.8 ± 5.3^{b}	193.3 ± 4.9^{a}
D	178.4 ± 5.3^{a}	185.0 ± 4.5^{a}
S	165.8 ± 5.9^{a}	137.3 ± 4.2^{b}
KIDNEY		
C	145.0 ± 4.1^{a}	157.9 ± 3.7^{b}
D	141.4 ± 4.1^{a}	176.8 ± 3.4^{a}
S	113.8 ± 4.5^{b}	136.0 ± 3.2^{c}
SPLEEN		
C	204.7 ± 10.1^{b}	195.2 ± 9.2^{a}
D	291.3 ± 10.1^{a}	204.0 ± 8.5^{a}
S	138.5 ± 11.2^{c}	191.7 ± 8.0^{a}
HEART		
C	87.7 ± 3.3^{b}	68.6 ± 3.0^{b}
D	107.3 ± 3.3^{a}	77.5 ± 2.7^{a}
S	109.0 ± 3.6^{a}	65.0 ± 2.6^{b}
LUNG		
C	93.6 ± 2.7^{b}	81.0 ± 2.7^{b}
D	130.6 ± 2.7^{a}	95.3 ± 2.3^{a}
S	76.6 ± 2.7^{c}	72.0 ± 2.2^{c}

Means within a column of each organ with the same superscript are not significantly different at the 0.05 level by Fisher's LSD.

TABLE 8: WHOLE BLOOD ZINC, COPPER AND IRON CONCENTRATIONS (ug/g WHOLE BLOOD) IN RATS FED CONTROL (C), ZINC-DEFICIENT (D) OR ZINC-SUPPLEMENTED (S) DIETS FOR 8 WEEKS.

(Expressed as Meam±SE in ug/g whole blood)

ELEMENT	WEEK 2	WEEK 4	WEEK 6	WEEK 8
ZINC				
C	5.4 ± 0.3^{b}	4.9 ± 0.3^{ab}	5.6 ± 0.3^{a}	5.9 ± 0.3^{b}
D	4.2 ± 0.3^{a}	4.5 ± 0.3^{a}	5.3 ± 0.3^{a}	4.8 ± 0.3^{a}
S	6.4 ± 0.3^{c}	5.5 ± 0.3^{b}	5.5 ± 0.3^{a}	5.0 ± 0.2^{a}
COPPER				
C	0.9 ± 0.03^{a}	0.8 ± 0.04^{a}	1.0 ± 0.04^{b}	0.8 ± 0.04^{b}
D	0.8 ± 0.03^{a}	0.8 ± 0.04^{a}	0.8 ± 0.04^{a}	0.7 ± 0.03^{a}
S	0.8 ± 0.03^{a}	0.8 ± 0.04^{a}	0.9 ± 0.04^{b}	0.8 ± 0.03^{b}
IRON				
C	516.9 ± 15.2^{b}	698.5 ± 18.0^{a}	765.4 ± 18.0^{b}	556.1 ± 16.4^{a}
D	607.1 ± 15.2^{a}	670.4 ± 18.0^{a}	829.1 ± 18.0^{a}	577.1 ± 15.2^{a}
S	562.2 ± 15.2^{c}	676.8 ± 18.0^{a}	755.4 ± 18.0^{b}	543.5 ± 15.2^{a}

Means within a column for each element with the same superscript are not significantly different at the 0.05 level by Fisher's LSD.

(1) Figure 1: Average weight gain (g)/week in rats fed Control (c), Zinc-Deficient (D) or Zinc-Supplemented (S) diets for 8 weeks.

(2) Figure 2: Organ protein concentrations (mg/g) in rats fed Control (C), Zinc-Deficient (D) or Zinc-Supplemented (S) diets for 8 weeks.

(3) Figure 3: Hemoglobin concentrations (g%) in rats fed Control (C), Zinc-Deficient (D) or Zinc-Supplemented (S) diets for 8 weeks.

(4) Figure 4: Hematocrit values (%) in rats fed Control (C), Zinc-deficient (D) or Zinc-Supplemented (S) diets for 8 weeks.

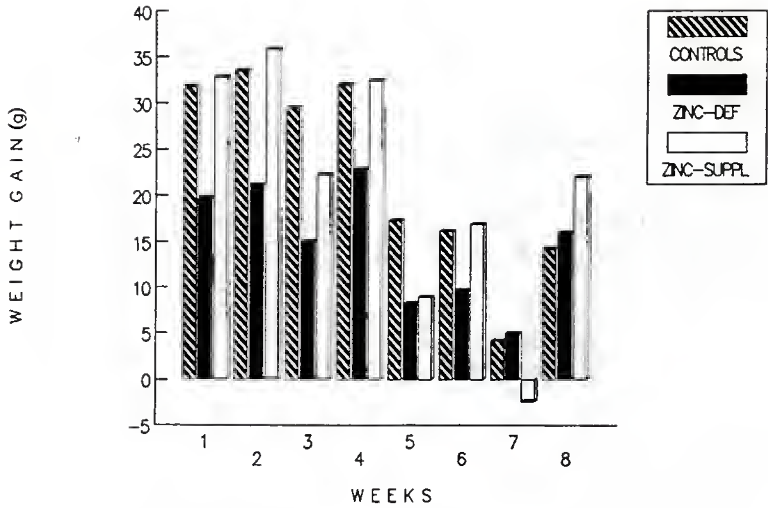

FIGURE 1

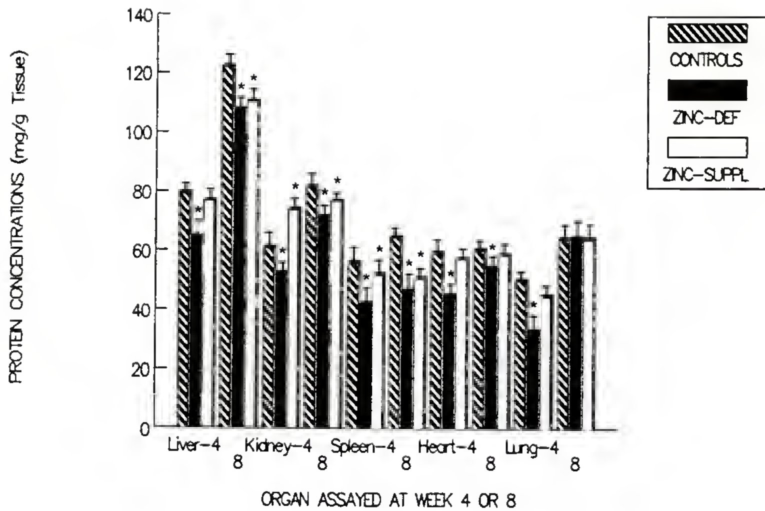

FIGURE 2
* Values for an organ are significantly different from controls by Fisher's LSD ($p < 0.05$).

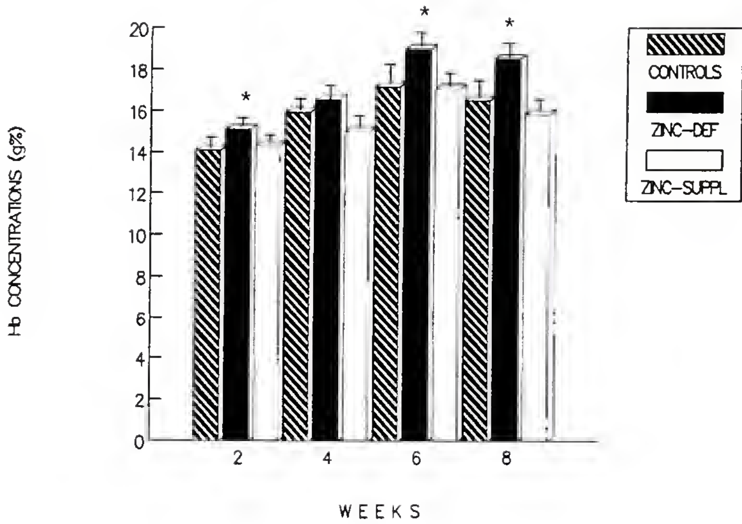

FIGURE 3
* Values for a week are significantly different from controls by Fisher's LSD ($p < 0.05$).

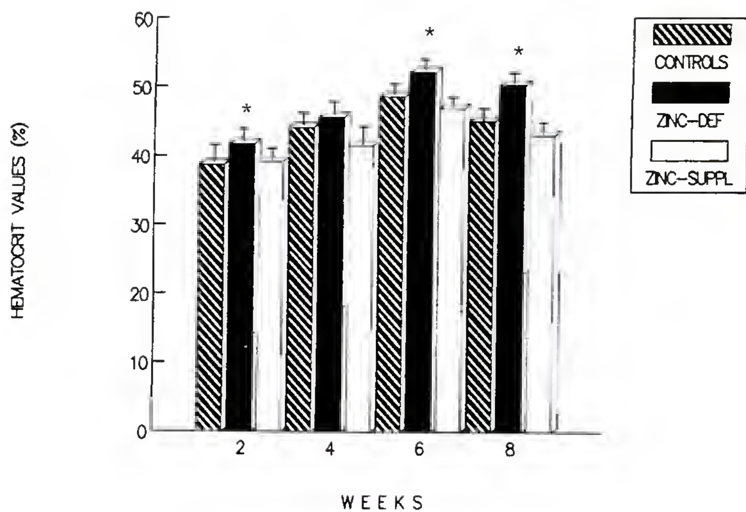

FIGURE 4

* Values for a week are significantly different from controls by Fisher's LSD ($p < 0.05$).

APPENDIX I

A REVIEW ON ZINC, COPPER AND IRON: BIOCHEMICAL ROLES, METABOLISM, DEFICIENCY, TOXICITY AND INTERACTIONS

A REVIEW ON ZINC, COPPER AND IRON: BIOCHEMICAL ROLES, METABOLISM, DEFICEINCY, TOXICITY AND INTERACTIONS

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INTRODUCTION

The concern of trace elements in the biological system began over a century ago. Early investigation regarding iron, cell respiration and oxidative processes (1) and nutritional role of zinc (2) founded most of the present research understandings.

The advent of purified diets permitted a new approach to study a wide range of naturally occurring nutritional ailments in man and animals. The essentiality of zinc (3), copper and iron (4) for growth in rats has been established 50 years ago. In 1950's, it was evident that either deficiencies or toxicities of certain minerals were often precipitated by concurrent occurrence or absence of other elements or nutrients in the environment. Such nutritional interrelationships was shown by Dick (5) upon investigating interactions of copper, molybdenum and inorganic sulphate. These interactions may acknowledge the importance of so pervading nutritional deficiencies or toxicities in the environment. However, the extent of possible dietary interactions and dietary forms of many trace elements remain, with few exceptions, to be elucidated.

Z I N C

O R I G I N :

Zinc has been utilized for industrial and ornamental purposes for almost 2000 years, but its history was obscured until the middle age era (2). Early, zinc has been shown to be a constituent (23%) of semitic bronze (6) and brass (2) and in formulation of a compound to the Babylonians of 3000 B.C. (7). The first revealed zinc piece was in a form of idol (8). Zinc has been thought to be the component of several ores (8). In Asia, zinc has been introduced for utilitarian purposes since before (9) or after the beginning of Christian era (10). The laboratory preparation of zinc metal was speculated to be practiced by the Chinese (11) and Indians (12) in the twelfth century. It has been thought that Meditteranians were the first to use zinc ointment as curative remedy of skin lesions. However, the first documented oral zinc therapy was the use of zinc sulphate in treatment of gleet and leucorrhea (13).

ESSENTIALITY AND REQUIREMENTS:

Zinc nutrition of man and animals has become an area of intensive investigation. Early studies showed that zinc is nutritionally essential for higher plants (14) and animals (15). Todd and associates (1934), using semi purified diets, had brought the first evidence that zinc is essential dietary factor for rat growth (3). In 1955, Tucker and Salmon (16) reported that zinc is a prophylactic and curative remedy for parakeratosis in pigs. O'Dell and co-workers (17) showed that zinc is a required nutrient for growth, feathering and skeletal development in poultry. The first evidence of zinc deficiency in man has been aroused by Prasad and collaborates (18). Subsequent studies identified the severe acquired zinc deficiency syndrome (19). Acrodermatitis enteropathica has been encountered in man due to genetic zinc deficiency (20).

Quantitative assessment of dietary zinc requirements have been determined in a number of animal species. Zinc requirement for rat was estimated at 11-18 ppm depending on protein source (21). The minimum requirement for growing rats has been estimated at 12 ppm on casein or egg white diets (21). Soybean diets significantly depressed growth (22) and render zinc unavailable for rats due to presence of high phytate content (21,23). Swenerton and Hurley (24) reported that 60 ppm was inadequate and 100 pp was adequate in preventing long-term testicular changes on

diets containing soybean. Maximum growth in guinea pigs necessitates presence of 12 ug dietary zinc added a casein-based diet (25).

In female baby pigs, 45 ug/g zinc has been found satisfactory for growth based on purified soybean protein diets (26), but 46 ug/g zinc improved growth rate in weanling pigs (27). Increments of dietary calcium to twice normal or higher level required increase of zinc level well above 45 ug/g to prevent signs of parakeratosis in pigs (16,28). On a corn-based meal , 35 ug/g zinc was shown to be satisfactory for reproductive performance of breeding sows (27).

Lambs need 18 to 30 ug zinc/g egg white diets for optimal growth (29), and that 76 ug/ Kg body weight per day was estimated as net requirement for maintenance (30). Underwood and Somers (31) suggested 17 ug dietary zinc for convenient growth in rams, but it was not adequate for testicular growth and spermatogenesis.

Dietary zinc of 8 to 9 ppm was found adequate for growth in calves (32), although 10 to 14 ppm is necessary to maintain normal plasma zinc level (27). Intake of 2.65 mg zinc per day was needed to maintain a 50 Kg calf (33). On the basis of minimal urinary, fecal and body surface losses, Weigand and Kirchgessner (34) assigned 53 ug/Kg body weight a daily net zinc requirement for maintenance of lactating cows.

Chicks grew well on 35 to 40 ug zinc/g soybean protein-type diets (17), and 25 to 30 ug/g on animal protein source (35). Monkeys require 15 ug dietary zinc for optimal growth (36).

Estimates for human needs have been derived from metabolic balance studies (37) and regression analysis of apparent zinc absorption and retention (38). The bioavailability of zinc from various sources was determined at 20% in terms of fractional absorption (39). In one study, adult human diets has been shown to supply from 5 to 22 mg zinc per day (40), but most mixed diets provide 12 to 15 mg/day (41). A balance study in healthy adults receiving 11-15 mg zinc/day had shown adequacy or slight positive response (42). An experimental study of zinc deficiency had suggested minimal endogenous losses of zinc to be 146 mg via feces and urine and 0.81 via integument (43).

Turnland and associates (44) suggested that 15 mg dietary zinc is adequate for elderly males, but it was also estimated at 8.8 mg zinc/day (45). A remarkable retention of zinc was observed in preadolescent children receiving 6 to 18 mg zinc/day (46). In preadolescent girls (47) 6 mg zinc per day was shown to be adequate, but Richie et al (48) suggested 7 to 8 mg/day. Based on zinc balance studies (49) and assuming 30 ug zinc/g of fat free tissues, zinc is required at approximately 150 ug/Kg body weight or a total of 725 ug/day for normal growth in infants between 1 and 3

months; 67 ug/Kg body weight for 3 to 6 months; 40 ug/Kg body weight for 6 to 9 months and 25 ug/Kg body weight for 9 to 12 months (27).

Zinc balance during the third trimester of pregnancy was determined by Swanson and collaborates (51). That zinc is daily retained primarily in the placenta during the last 20 weeks of gestation has been hypothesized by Sandstead (52,53). Zinc requirements and retention was difficult to achieve in preterm infants (50).

Zinc content of some forages and animal foods has been comprehensively reported (45,55). White sugar, pome and citrus provide negligible proportions of zinc in the diet (56). Wheat germ bran and oysters are among the richest sources (56). Canned vegetables has been reported to contain 40 to 80% less zinc than fresh vegetables (57). Zinc contents of cow's milk vary from 3 to 8 ug/g (58,59). The bioavailability of zinc varies considerably with percentage absorption (60,61) and type of meal consumed (61).

The recommended daily allowance is 3 mg for infants less than 6 months of age, 5 mg for older infants, 10 mg for preadolescent children, 15 mg for adolescents and adults, 20 mg during pregnancy and 25 mg during lactation (62).

C H E M I S T R Y

Zinc is a bluish-white metal with a density of 7.133 g/cc. Its atomic number is 30, atomic weight is 65.37 and it is placed in group IIb of the periodic table. Zinc is a divalent element (2^+) with electron configuration of 2-8-18-2.Zinc is derived from zinc blende (sphalerite) and marmatite,which are zinc sulphide ores. The former constitute about 90% of our today's metallic zinc. Zinc exists in hydrated form in neutral and acidic aqueous solutions,but the hydroxide is precipitated in alkaline solution. Zinc compounds possess chemical properties that make them useful as oxides, carbonates, sulphides, phosphates, chlorides and organic complexes. Because of its known electrochemical activity, zinc is utilized in galvanizing iron and steel to protect against their structural degradation.zinc readily forms an alloy with other metals. Zinc oxide is commonly used in paints and in vulcanization of rubber. Other useful zinc inorganic compounds include; zinc sulphate, zinc chloride, zinc sulphide, zinc carbonate, zinc borate, zinc cyanide, zinc nitrate, zinc phosphate, zinc permangenate and zinc peroxide (56).

BIOCHEMISTRY

Since the essentiality of zinc as a growth factor has been determined (15,63), it is obvious that the element is involved in various metabolic processes leading to normal development.Zinc has been found to be incorporated in over 70 metalloenzymes (64), which have significant metabolic roles.Moreover, zinc is apparently required to maintain the structure of apoenymes (64).

The first biological function of zinc was determined by Keilin and Mann (65) on isolation of carbonic anhydrase (EC 2.2.1.1) from bovine erythrocytes. Three forms of carbonic anhydrase have been identified (66), in which zinc is tightly bound to the active site (67) to potentiate its catalytic activity (68). This enzyme plays an important role in rapid dehydration of bicarbonate in lung and hydration of carbon dioxide in other tissues (69). The metal has been shown as a constituent of alcohol dehydrogenase (EC 1.1.1.1.) of microorganisms, peanuts and mammalian liver (70,71). Using NAD as a co-factor, dehydrogenase canalizes the oxidation of ethanol, vitamin A and certain steroids, and that it reduces aldehydes and ketones in the presence of NADH (71,72). Carboxypeptidases are zinc metalloenzymes catalyzing the hydrolysis of c-terminal peptide bonds of peptides and proteins (73). Carboxypeptidase A (EC 3.4.2.1.) isolated from beef pancreatic glands (74) is known to contain one gram of zinc per molecular weight of 34.600

(75). Carboypeptidase B (EC 3.4.2.2.) of the pancreatic secretion (76,77) contains one gram of zinc per molecular weight of 34.300 (12). Alkaline phosphates (EC 3.1.3.1.), a zinc metalloenzyme (78,79) of E.Coli (80) has been shown to contain 4 atoms of zinc which are necessary for activity and structural integrity of the enzyme (81). Alkaline phosphate catalyzes the hydrolysis of a variety of phosphate esters including; B-glycerophosphate (82), paranitrophenylphosphate (53), and phenol-phthalein monophosphate (84).

That zinc is required for DNA synthesis was demonstrated by Buchanan and Hus (85). Further, zinc deficiency has been reported to influence adversely the cell cycle phases (G^1,S,G^2 and mitosis) of Euglena gracilis (64) indicating the vital role of zinc in DNA synthesis (86). The inhibitory effect of EDTA on activity of DNA polymerase (87) and DNA transferase (88) provided further proof suggesting that a protein bound metal atom is required for enzyme activity. Some investigators (86) suggested that the decreased activity of deoxythimidine may be responsible for the initial depression of DNA synthesis. Three nucleotide polymerases have been identified as zinc-dependent enzymes; DNA polymerase (EC 2.7.7.7.) containing 2 to 4 gram atom of zinc per mole (89); DNA dependent RNA polymerase (EC 2.7.7.6.), containing 2 gram of zinc per mole (90); and DNA dependent DNA polymerase (91). Other zinc-dependent enzymes include; dehydropeptidase, aminopeptidase, enolase,

arginase, tripeptidase, histidine deaminase and L-Mannosidase (92). Sanstead et al (93) provide evidence that zinc play a role in the maintenance of poly nucleotide conformation.

On a fat-free basis, total body zinc concentrations has been found to be 20 to 30 ug/g in rat, cat and pig (94), and cow (95) and sheep (96). Large proportions (50-60%) of zinc are confined to highly oxidative (97) muscles of humans (2) and lactating cows (98). In bones and integument, zinc concentrations have been estimated at 100-250 ug/g (99), and 20 ug/g (96) on dry weight basis. Only less than 0.5% of total body zinc is found in blood of adult human (2) or sheep (96) of which 75-80% in the red cells and 12-22% in plasma (27).

M E T A B O L I S M

ABSORPTION:

The mechanism and control of zinc absorption are still difficult to comprehend, however it appears that zinc absorption is influenced by a number of environmental factors (100). The percentage absorption of zinc has been demonstrated to be 5 to 10% in rats (101), 20 to 30% in growing chicks (102) and 50.8% in humans (103). In rat, a number of studies have determined the major sites of zinc absorption to be the duodenum, jejunum and ileum (104,105,106). Davies and associates (22) indicated that zinc absorption is 60% from duodenum, 30% from ileum and 10% from jejunum in fasted rats, but Antoson et al (107) reported 60% from the ileum and 20% from both jejunum and duodenum in none fasting rats. It has been shown that zinc uptake by rat duodenum is saturable at lumen zinc level of 0.8 mM (104), but another study (108) suggested a lower level of 100 uM with maximum absorption capacity at 7.4 nmol/min. One third of oral zinc dose is absorbed in the abomasum of cattle (109). Zinc absorption was greatest in the distal and proximal ends of the intestines of calves (110). In chicks, proventriculus and small intestine constitute the major routes for zinc absorption (111).

Cousins (112) has proposed 4 phases for zinc absorption; uptake by the intestinal cells, transfer through mucosal cells, entry to the portal circulation and re-

secretion of zinc intestinal cells. Citrate, picolinate, histidine, glutamate and ethylenediaminetetraacetic acid (EDTA) were experimentally suspected to facilitate zinc absorption as binding ligands (113,114,115), yet the exact physiological role remains to be elucidated. Suso and Edwards (102) suggested that presence of chelating agents in the ingested food enhance zinc absorption from the gut. Hahn and associates (116) demonstrated that N-N-N-trimethyl-1,2-ethandiamine is zinc-binding ligand in swine duodenum. Subsequent studies (117) reported that prostaglandin E_2 plays an important role in zinc binding and transport across the intestinal mucosa. Zinc absorption decreased with dietary phytate (23), high phosphate level (118) or excess dietary calcium (119), and increases in the presence of certain amino acids, peptides or EDTA (120). It has been shown that coffee, dairy products and brown bread have tendency to reduce zinc absorption in humans (121).

There is inverse relationship between metallothionein synthesis and zinc absorption (122). About 20 to 30% of cytosol zinc is confined to metallothionein fraction (123), and that it contributes to zinc homeostasis between lumen and portal circulation by retarding zinc transfer from the cell (112). Transfer of zinc from cell to portal circulation has been found sluggish when compared to the uptake process in rat (104,124).

Zinc supply status contributes greatly to overall

zinc homeostasis (125,126). However, Solomons and Cousins (100) suggested that high circulatory zinc, by inducing intestinal metallothionein synthesis, enhances mucosal zinc to accumulate both dietary and endogenous zinc contributing to zinc homeostasis. Physiological factors such as age (127), pregnancy and lactation (27) were reported to have influential effects on the level of zinc absorption.

INTERMEDIARY METABOLISM:

The absorbed zinc will be bound to albumin (128), of which 30 to 40% will be liberated to the circulation (129) with subsequent distribution to other tissues at various concentrations and turnover rates (129,130). It has been shown that alpha-macroglobulin, transferrin, ceruloplasmin, hepatoglobin and gamma globulin also bind significant amounts of zinc (93). A small amount of zinc exist as an ultrafiltrable fraction mostly bound to amino acids histidine, glutamine, threonine, cystine and lysine (93). Circulating zinc will also be pooled into red blood corpuscles and soft tissues rather than to be excreted (61). Intraperitoneal injection of zinc had affected both zinc turnover and distribution (27), but other studies (131) reported that zinc retention is enhanced and turnover is decreased in soft tissues and organs except bones and with restricted dietary diets.

The uptake of zinc by cells involve rapid

saturable uptake process which may be carrier mediated followed by slower phase that is apparently passive (132,133). Furthermore, increased uptake may be stimulated via glucocorticoides with excess quantities being bound to newly synthesized metallothionein (134). About 60 to 80% of intracellular zinc occur in cytosol, 10% in the nuclear portion and only small amounts in the mitochondria and ribosomes (135,136). However,there is evidence that most of cytosol zinc is bound to protein (123,137), while few or surplus amounts are bound to metallothionein under normal (138) or increased (137,139) zinc dietary states, respectively. This suggest that metallothionein has a complementary role particularly in hepatic zinc metabolism (140,141). Liver cytosol has been reported as a major compartment for zinc metabolism in rats (123), chicks (137), cattle (139) and humans (132), and that it provides necessary zinc binding components in amounts and proportions coinciding with zinc status and age of the animal (142).

Zinc appears to play an important role in inducing synthesis of metallothionein mRNA (143,144). In its bound form, zinc is an essential factor in maintaining the configuration of RNA (147), DNA (148), ribosomes (149) and biomembranes (150). Glucocorticoides (145) and catecholamines (146) indirectly stimulate metallothionein synthesis probably by potentiating zinc entry into hepatic cells.

EXCRETION:

Under normal dietary circumstances feces constitute the major route for zinc excretion (45,151,152,153), however 2 to 10% may be recovered in urine. Fecal excretion included both unabsorbed dietary zinc and endogenous fecal excretion of zinc,a primary mechanism for zinc homeostasis (129,154). Endogenous fecal excretion of zinc has been demonstrated to increase with increased dietary zinc (155), and to decrease on low zinc diets (27). Biliary, pancreatic and mucosal cell routes have been demonstrated to be the major sources for endogenous fecal excretion of zinc (122,156,157). Urinary excretion of zinc was estimated at 300 to 500 ug/day in adults (158,159), 380 ug/day in preadolescent children (48) and approximately 187 ug/day in young children (160). Urinary excretion of zinc was less than 1 mg/day in sheep and calves and approximately 0.25 ug/ml in dairy cows (27). Urinary zinc is a function of filtration of plasma zinc (161), rate of urine production and amounts of creatinine excreted (162,163). Dermal and sweat zinc losses (43,164), and menstrual losses (165) were reported as minor excretory routes of zinc.

ZINC DEFICIENCY

Only a few cases of frank zinc deficiency have been reported in cattle (2) and sheep (166). Using experimental poor zinc diets, primary zinc deficiency has been demonstrated in man (167,168). Conditioned zinc deficiency is characterized by hypogonadism and nutritional dwarfism in man (169), which could be ameliorated on zinc supplementation (170,171). Conditioned zinc deficiency may a sequel to excessive renal (172) and intestinal (173) excretion and to increased requirements in neoplastic diseases (174,175), burns (176) and pregnancy (175).

Food consumption is significantly reduced in man and animals with zinc deficiency (177). Food consumption is variable (178,179), and that it less affected when rats fed a 5% rather than 20% protein diet. Dietary intakes is increased following zinc repletion in zinc-deficient rats (180).

Growth retardation is an overt feature in all species showing signs of zinc deficiency including rats (179,181), lambs and calves (32) and fetuses (182). Growth inhibition may be associated with decreased thymidine kinase activity with subsequent depression in DNA synthesis and cell division (178,183). Other possible causes for growth retardation may include abnormalities of endogenous opiates regulation (183), increased protein catabolism (184), hypogeusia (185) and decreased levels of growth hormone

(186). Moreover, anorexia nervosa of humans has been attributed to zinc deficiency (187). Skeletal deformities due to zinc deficiency have been demonstrated in chicks, poults and quail (17,188). Thickening and shortening of bones (27), stiffness of joints in calves (189) and sheep (166), and skeletal malformations in rats (182) were observed on feeding zinc depleted diets.

There was evidence of relationship between subnormal hair and plasma zinc levels and the incidence of atherosclerosis and myocardial infarctions (190,191), however the mode of action awaits explanation. Although zinc therapy has been shown to be beneficial in curing chronic leg ulcerations (192) and bed sores (193), the effect of zinc on wound healing is a matter of conjecture (194). Erythrocyte counts and hematocrits of rats (195) and Japanese quail (188) increased and leukocytes of baby pigs (27) decreased with dietary zinc deficiency. Increased osmotic fragility is a feature encountered in erythrocytes of zinc-deficient rats (196). Some studies (197) reported a drop in plasma protein levels, but others (188) did not. Deficient plasma zinc levels have been reported in acute and chronic infections (198), in uncured pernicious anemia (199) and in cirrhosis and liver diseases (200).

Impaired DNA synthesis in the liver of zinc-deficient rat has been demonstrated in many studies (201,202). The testes contents of zinc, total proteins, DNA,

and RNA were shown to be reduced in zinc deficiency (203). Zinc is necessary for regulation of cell mitosis (204), and that protein synthesis is impaired in zinc deficiency (205) possibly as a consequence of abnormally produced or inhibited ribonucleic acid synthesis.

Thymidine kinase activity, an enzyme required for DNA synthesis and mitotic division of cell (86) and pancreatic carboxypeptidase activity (195) have been found to be depressed in tissues of zinc-deficient rats. Subnormal levels of alkaline phosphatase were encountered in tissues of zinc-deficient calves (206), pigs (207), rats (208) and man (209). Similar alterations were obtained with carbonic anhydrase levels in the blood of zinc-deficient calves (210), rats (211) and dwarfs (40). Zinc-dependent enzymes including lactic dehydrogenase (LDH), malic dehydrogenase (MDH), and NADH levels were reduced in testes, bones, oesophagus and kidneys of zinc-deficient rats (212) and baby pigs (213).

Dark adaptation (214), protein metabolism and uptake (215,216), and insulin metabolism (217,218) were found to be adversely affected in zinc-deficient rats. An appreciable alterations in carbohydrate metabolism and adipose tissues were revealed in zinc deficiency (219), but Quarterman and Florence (220) found that these changes are modified with feeding patterns. The composition of essential fatty acids is adversely affected by zinc deficiency (221).

Hyperkeratinization and dermatitis (27), parakeratosis (31) and poor feathering (17) are clinical manifestations of zinc deficiency in animals and birds. Diarrhoea has been shown in zinc deficiency (20) with subsequent losses in sodium and potassium (222). Zinc deficiency could induce immunosuppression in monkeys (223) guinea pigs (224), mice (225) and man (226). Spermatogenesis and development of secondary sex organs were shown to be adversely affected by zinc deficiency (31,227). Acrodermatitis enteropathica (20) and pancreatic insufficiency (228) have been implicated in the development of malabsorption syndrome in man. Hypogeusia (185), impaired healing (229,230), ischemic leg ulcerations (231) and sickle cell disease (232) are yet other consequences of chronic zinc deficiency.

Brain function and maturation are adversely affected during embryonal life (203), early childhood (233) and early adolescence in monkeys (223). Deleterious effects encountered in offspring's learning abilities when born to dams deficient in zinc (234). In uncured acromegaly, zinc levels were considerably lower compared to controls (235). Behavioral abnormalities are associated with zinc deficiency (236) and could be alleviated with zinc supplementation (237).

ZINC TOXICITY

Zinc is generally a none toxic element to mammals and birds, however toxicities have been demonstrated in a number of species due to oral, parenteral or environmental exposures (238,239). Natural or accidental zinc poisoning has been reported in sheep and calves (238) as well as dogs (240). In young animals, an intake of 5000 ug/g zinc oxide has resulted in growth retardation concomitant with high mortality rates, while less severe effects were encountered with a lesser dose (241).

Ruminants are more susceptible to zinc toxicity as compared to other species probably due to ill-effects on microorganisms (2), volatile acid concentrations (241) or cellulose digestion in the rumen (242). Although zinc supplementation reduced plasma copper concentrations to levels indicative of deficiency (243), it has been revealed beneficial in improving hemoglobin , RBC, cell volume and total protein level of cattle (244). High dietary zinc (1700 ug/g) induced signs of deprived appetite, depressed weight gain and feed efficiency in steers and heifers (245), but increased tissue concentration of zinc and iron and decreased liver copper concentrations (238).

Iron and zinc concentrations in liver and kidney of chronic zinc-poisoned sheep and calves (500-1000 ug/g) were significantly elevated with no significant alterations in copper contents (238). Similar alterations were observed

in weanling lambs receiving 1500 ug/g diet (241). However, Davies and co-workers (246) observed,in addition, extensive renal damage in suckling lambs on feeding 134 ug/g diet. Moreover, Bires (247) showed that blood levels of zinc and copper remained high following parenteral administration of copper oxide in sheep. Pregnant sheep showed high incidence of abortion and stillbirths when fed 750 ug zinc/g diet (245). The clinical manifestation of copper intoxication in sheep was described as inappetence,loss of condition, diarrhoea, profound weakness, icterus and anemia (238). Kidney is the most affected organ in experimental zinc toxicity of sheep (248) with high zinc concentrations in liver and pancreas (248,249).

Decreased hematocrit (250), anorexia (251), microcytic hypochromic anemia (252,253), reduced copper concentrations (253), growth retardation (254,255) have been reported in rats receiving excess zinc intakes. However, increased or unchanged copper concentrations (254,255) and increased weight gain (254) were also reported. Other studies claimed that hemoglobin and growth rate (256) and copper levels (255) remained unchanged in rats fed high dietary zinc. A decrease in iron concentrations was observed in some studies (250,253) especially in liver (255,256) and kidneys (255) in rats fed zinc supplemented diets. Zinc concentrations were high in all tissues except liver, heart and lung of rats (254). Conversely , zinc concentrations

were high in heart ,liver ,kidney and spleen of zinc-supplemented rats (187). An appreciable drop in cytochrome oxidase activity (251) and increased prostaglandin activity (257) were also reported in rats fed high levels of zinc.

Richard and co-workers (258) described a combination of zinc toxicity,copper deficiency and anemia in swill-fed pigs. Pigs were tolerant to 500 ug zinc/g diet (259,260), but levels of 5000 ug/g have resulted in increased zinc and decreased copper concentrations in plasma and liver (261). In another study (262), a level 1000 ug zinc/g diet revealed no ill-effects in weanling pigs, but Brink and associates (263) have reported reductions in weight gain, feed intake and feed efficiency, arthritis and gastritis in weanling pigs when fed 2000, 4000 or 8000 ug zinc/g diet.

No significant effect observed in hemoglobin, hematocrit, glucose, BUN, total proteins and enzymes of monkeys fed high dietary zinc (264). However, hypocupremia and hyperchlestrolemia were observed in female monkeys fed a zinc-supplemented diet (264). In humans, oral zinc did not produce ill-effects ,however acute and chronic toxicities were reported with gastrointestinal disorders as main features (2). Moreover, hypocupremia has been shown in patients with acrodermatitis enteropathica (265) or sickle cell anemia (93) receiving zinc therapy.

Excess dietary zinc has been proposed to prevent

copper intoxication since it alleviated copper accumulation in the liver when added to high copper diets (266,267). Other investigators suggested that a mutual antagonism between zinc and copper is expressed when luminal concentration of the respective congener is increased (268,269). It has been suggested that high dietary zinc exerts its antagonistic effect by potentiating the synthesis of copper-binding ligand,probably metallothionein, thus reducing its absorption and bioavailability to tissues (270). Cox and Harris (253) reported that excess dietary zinc produces an early and marked loss of hepatic iron and copper and showed that zinc does not interfere with iron absorption. Conversely, Settlemire and Martone (271) indicated such interference at the absorptive level. Elevation of dietary calcium level from 0.7 to 1.1% was proved to effective in protecting against high dietary zinc levels (272).

C O P P E R

ORIGIN:

Copper has long been recognized to be essential factor for growth and development of living matter. Over 2000 years ago, Hindu, Egyptian and Assyrian alchemists have prescribed copper and its compounds as a remedial therapy for many human maladies (92). Early, copper was found to be incorporated at 7% in turacin (273), a red porphyrin of certain bird feathers and in hemocyanin, a blood component of certain snails (274). That copper is essential element in rats was first reported by McHargue (275) and Hart (4). In 1937, Bennetts (276) described nutritional disorders of copper in lambs,enzootic neonatal ataxia, when borned to copper-deficient ewes in Australia. In early 1950's (5), the existence of the three-way interaction between copper, molybdenum and inorganic sulphate has been emphasized.

ESSENTIALITY AND REQUIREMENTS:

The essentiality of copper has been reported as early as 1817 (92), which then paved the way for the study in different animal species. The growth-stimulating effect of copper is not fully understood ,but it is partially described in some studies (277). Discrepancies regarding responses to supplementary copper is attributable to mutual antagonism between iron and copper, copper and zinc (270) or calcium, zinc and copper (278).

A supplement of 0.005 ug Cu/day improved growth and hemoglobin concentrations in rats fed milk diets (279). The minimum requirement for a 70-gram rat fed a 10 gram diet daily was proposed at 1 ppm for hemoglobin production, 3ppm for growth and 10ppm for melanin production in hair (56). Copper deficiency developed in rats when fed $\leq$1 ug Cu/g diet (270). An amount of 5 ppm has been recommended as adequate nutrient requirement for growth, gestation or lactation in rat (280). However, the minimum requirement for maintenance remains to be elucidated (280). Kirchgessner and Spoerl (281), however, showed that 8 ug Cu/g starch-casein based diet is required for pregnancy and lactation in rat. Anemia, hair depigmentation and stunted growth were expressed in guinea pigs fed diets containing 0.5-0.7 ppm copper (282).

Ullery and co-workers (283) observed no significant differences in treatments when baby pigs were fed diets providing 6,16 or 106 ppm copper. Copper levels of

4 ug/g copper was proposed adequate for growth in pigs up to 90 Kg live weight (270). Addition of 250 ug/g copper to normal pig rations improved weight gain of growing pigs (284) and that daily weight gain and feed efficiency increased by 8% and 5.5%, respectively (285). Another study reported average increase of 9.7% in weight gain and 7.9% increase in feed efficiency (286). Adverse effects manifested by reduced live weight gain and feed efficiency (266) mortality and skin lesions (305) were also observed in animals fed added 250 ug/gm copper sulphate.

Few data available describing the minimum copper requirement for growth and production in poultry. However, 4-5 ug/g copper is found adequate since other antagonistic elements are meager (287). A positive input was observed at levels of 75 and 225 ug/g copper, but levels of 300 ug/g inhibited growth in poultry (270). A level of 100 ug/g copper improved weight gain of 9-week old chick and duckling 8-63 days of age (288). A supplementary copper of 250 ug/g improved chick growth on fish meal,wheat and added tallow-based diet, whereas no response or depressed growth observed when copper was added to a maize-soybean diet (270).

Cross-bred sheep were found to be well maintained at intakes of 1 mg Cu/day (289). Nonetheless, copper levels of % mg/day was found to be insufficient to merino sheep, and that 10 mg/day or 10 ug/g was shown to be satisfactory (290). Dick (5) described that molybdenum intakes of 0.5

mg/day can induce antagonistic effect in copper retention in sheep. However, Suttle (291) indicated that such effect is insignificant. Parenteral administration of 30-40 mg copper glycenate or CuCaedtate has been found satisfactory in sheep (292), if given at intervals of 3-6 months. Another study showed that a single dose of 40-60 mg copper glycinate, methionate or CuCaedetate is adequate in pregnant ewes (293). however, death was reported in ewes injected parenterally with copper compounds (294).

Cattle grazing in pastures providing 7-14 ug/g copper and 3-20 ug/g molybdenum developed copper deficiency (295). Salt licks containing 0.5 -1.0% copper sulphate or 5-7 Kg/hectare of copper have been found satisfactory to meet requirements of grazing livestock (270). Furthermore, parenteral administration of copper glycenate or copper calcium-ethylenediaminetetraacetic acid (CuCaedetate) in doses of 120-140 in cattle were shown to be effective preventive measures against copper deficiency if given at intervals of 3-6 months (292). However, another study (290) reported that a single dose of 100 mg CuCaedetate may be adequate in cattle.

In humans, a daily copper intake of 80 ug/Kg has been recommended for infants (30,62). Based on balance studies, safe and adequate copper intakes were determined at 2-3 mg/day (30,62) or 1.3 mg/day (292,297) in adults and children. However, 1.3 mg copper has been estimated as daily

requirement in preadolescent girls (298).

Food sources as oysters, organ meals, green vegetables and many varieties of fish are considered copper rich meals (100 ug/100 Kg), while cheese, bread,mutton,beef and milk are poor sources (49,53). Soft water could provide copper in amounts varying from 0.4 mg/day (299) to 1.4 mg/day (270) and may cause chronic copper poisoning in infants (300).

The bioavailability of copper has been shown to be little affected by phytate or fiber (52,53). Conversely, another study (297) indicted that bioavailability of copper is affected by fiber phytate,starch and copper deficiency. Other nutrient components such as ascorbic acid (236), zinc (301,302), silver, mercury and cadmium (303) and iron (304) could induce antagonistic influence at the absorptive and metabolic levels.

CHEMISTRY

Copper has an atomic number of 29 and molecular weight 63.54. It occupies a position between nickel and zinc in the subgroup IB of the periodic table. Copper comprises about 0.007% of the earth's crust. Copper exists as sulphide ores exemplified by chalcocite (Cu_2S), covellite (CuS), chalcopyrite ($CuFeS_2$) and chalcanthite ($CuSo_4.5H_2o$).

Copper valencies vary between 1+ and 2+. Despite the fact that copper does not replace hydrogen from solutions, it is readily soluble in oxidizing acids such as nitric acid, sulfuric acid and ferric sulfate. Copper is soluble in ammonia and ammonium salts in presence of air and in sodium and potassium cyanide. Copper is incorporated in alloys such as cupronickels, aluminium bronze, silicone bronze and manganese bronze. In manufacture, copper is utilized in copper sheet and strip, generators, light bulbs, telephones and light and power lines. (56).

B I O C H E M I S T R Y

Like many other trace elements, copper pursue a number of biological functions represented by proteins and enzymes. Copper proteins have been encountered in molds (galactose oxidase), bacteria (azurin), plants (laccase) and animals (ceruloplasmin) with molecular weight ranging from 10,790 to 7 million (92). Copper proteins are conviently classified into two main divisions depending on their color; blue and non-blue. Many plant oxidases belong to blue class, whereas those of animal origin belong to non-blue class (92). Copper is able to reduce oxygen to water or carbon peroxide (306).

Unlike other enzymes, cuproenzymes possess high potentiality for oxygen depending on the number of incorporated copper atoms (307). All cuproenzymes containing 4 atoms, except cytochrome oxidase, are capable of reducing oxygen to wate, while those containing 1 to 2 atoms reduce oxygen to hydrogen peroxide (307). Hydroxylase, a cuproenzyme, is known to incorporate one molecular oxygen into its substrate with subsequent hydroxylation (308,309). Cytochrome oxidase is involved in energy supply for muscle contraction (309,310) in aerobic organisms mainly in the heart (92), flight muscles of birds and insects (310), liver mitochondria (311) and gray matter of brain (312). Ceruloplasmin (ferroxidase) occurs in mammalian serum (313) with molecular weight of 150,000 and eight copper atoms/

mole protein (314,315). The number of copper atoms bound to each molecule is a matter of conjecture (314,316). As an ion transport enzyme, ceruloplasmin activity has been studied in vitro (317,318) and vivo (319). It has been shown that copper ceruloplasmin plays a vital role in copper (320,321) and iron transport (768) and in regulation of biogenic amines (322,323). Superoxide dimutase is known to protect cells from oxygen damage (92). Only relatively few amine oxidases which are involved in oxidative deamination (324).

Two types of copper-binding proteins has been reviewed; metallothionein and (Cu-,Zn)-thionein. Metalothionein of horse liver and kidney contains 0.14 copper atoms/ mole (325). Bremner and Young (326) speculated that some zinc atoms of (Cu-,Zn)-thionein of rat liver could be substituted by copper atoms. Subsequent studies isolated another copper-binding protein having the same amino acid composition, except cysteine, from bovine (327) and human (328) fetal liver. Copper-chalatin (329), is a copper-binding protein which was isolated from yeast and liver cytosol of rat (330), rabbit and chicken (329). A copper-binding protein with similar amino acid composition, but lower cysteine content, and molecular weight of 7,000, 9,000 or 12,000 has been isolated from liver of human (331), rat (332) and pig (333) and rat intestine (92). Thus, copper loading will induce synthesis of copper-substituted metallothionein,(Cu-,Zn)-thionein (326), or specific copper-

binding proteins with lower cystein content (334). With exception of albocuprein I (330), copper has been shown to exist in cuprous form in proteins (330,331). Copper-binding proteins have been shown to be involved in detoxification (333) and bioenergetics (329), but their precise role remains to be elucidated.

METABOLISM

ABSORPTION:

Copper bioavailability is substantially influenced by the nutritional status of the individual, its chemical form and interaction with other dietary factors (270). Although absorption occurs via the entire alimentary tract, larger proportions take place in human stomach (335) and large intestine of sheep (270). There is sufficient evidence that copper absorption is increased in copper-deficient subjects than with copper adequate state (336,337). However, copper absorption increases in saturated state (338). The potential role of metallothionein as a transport and interaction site for copper and zinc has been reviewed by Cousins (339). Due to the hypothesis that metallothionein has strong affinity to copper than zinc, copper absorption is greatly influenced by metallothionein (270). Other unknown factors in bile, pancreatic juice, intestinal and gastric secretions were known to be limiting factors for copper absorption (231,340). Copper absorption increases in malgenancies and decreases with estradiol administration, despite increased ceruloplasmin activity (341). Moreover, copper absorption has been shown to vary considerably depending on moisture content (342,343) and presence of organic ligands (334) in the feed. Sulphate and sulphite also reduce copper absorption by forming insoluble complexes (270). Phytate can reduce assimilation of copper

(345). Dietary protein are known to enhance copper biological availability (346,347).

Underwood (348) illuminated the essentiality of exogenous factors, especially other mineral, in influencing of copper status of an individual. The mutual antagonistic effects of copper and zinc have been demonstrated in many studies (268,349,350). Metal ions such as zinc, cadmium, silver, mercury, induce antagonistic effects to copper absorption (351,352), possibly by competing at the copper-binding protein in intestinal wall (353). Pharmacological doses of zinc was shown to induce copper deficiency in humans , which was ameliorated on copper supplementation (354). Excessive dietary zinc could induce metallotheinin synthesis which preferentially render copper unavailable to biological systems (269). An evidence of copper-selenium interaction represented by reduction of peroxidase activity has been evident, however the mechanism is not known (355). Excessive dietary calcium was reported to potentiate copper toxicity in swine (278) and to reduce copper absorption in mice (278). Interaction of copper with molybdenum or ascorbic acid plays a vital role in copper bioavailability (337,356). The negative effect of ascorbic acid on copper absorption was demonstrated in chicks, rats, and humans (337,357,358), and that the mechanism may be mediated by reduction of cuprous ions or formation of stable complex.

INTERMEDIARY METABOLISM:

Despite the majority of absorbed copper is bound to ceruloplasmin, copper may also be transported loosely bound to albumin or certain amino acids (270). The latter mean of transportation renders copper readily available for liver and other tissues (56). Dietary copper is a powerful determinant of cholesterolemia (359). Liver represents the main site of copper metabolism (360), and that uptake by hepatocytes is saturable and temperature dependent (361,362). Cyanide and n-ethylmaleimide (362) induced significant inhibition of copper uptake by hepatocytes. However, Saltman and collaborates (361) did not demonstrate significant change by other metal ions. Amino acids, on the other hand, have been shown to enhance copper by hepatocytes probably through complexation with copper (363).

Inside liver parenchymal cells, copper accumulates in mitochondria, microsomes, nuclei and soluble fraction in proportions varying with age (364,365), strain (136,366) and copper status of animal (364,367). It has been demonstrated that more than half of incorporated copper is confined to supernatant fractions, possibly metallothionein, SOD and other copper enzymes (368,369). Phenobarbital administration has been found to increase microsomal copper content of rats receiving normal diet (56).

Copper enzymes has been widely reviewed (370).

Ceruloplasmin and erythrocuprein are the vehicles that transport copper from liver to target organs (56). Ceruloplasmin enzyme is thought to be involved in defense mechanism against neoplasm, infection, inflammation and stress (339,371). Furthermore, ceruloplasmin displays a variety of functions including oxidation of biogenic amines, iron transportation and destruction of super oxide radicals (372). Iron mobilization involves conversion of Fe^{2+} to Fe^{3+} with subsequent reduction of ceruloplasmin-bound Cu^{2+} to Cu^{1+} (373). Serum iron concentrations, ceruloplasmin levels and hemoglobin are directly correlated in the biological system (219,374). Anemia could be alleviated with copper (4). Iron metabolism is impaired with severe depression of ceruloplasmin activity, although depressed ceruloplasmin levels does not always reflect anemia (227,372). Lysyl oxidase, a copper metalloenzyme necessary for cross-linking elastin and collagen fibers which is necessary for tissue maturation and bone formation (375,376). Recently, lysyl oxidase has been hypothesized to cause sudden infant death syndrome (SIDS) by affecting lung tissues (377). Some copper metalloenzymes may be involved in immunocompetence and inflammatory responses (378). The physiological roles of some copper-dependent enzymes including; albucuprien I and II, neurocuprein, pink copper proteins and diamine oxidase are not fully understood (379,380,381,382).

EXCRETION:

Most of unabsorbed copper is excreted in feces, while most of the absorbed portion is actively excreted through the biliary system in all species (56,270). Biliary excretion of copper represent the major excretory route in humans (383,384,385), dogs and pigs (386,387) and mice and poultry (388). However, biliary excretion of copper revealed no significant increase in Wilson's disease despite considerable accumulation of element in the liver (335). Literature had shown disparity regarding copper concentration in urine (152,385). However, copper in concentrations of 3.8-6.6 ug/L (389,390) or a daily copper excretion of 5-50 ug in adult urine was considered normal (270). Increased serum an biliary levels and decreased urinary excretion of copper have been observed in adrenalectomized and hypophysectomized cats (270). Increased urinary losses of copper is induced by increased molybdenum intakes (391). Moreover, urinary excretion of copper increased in patients with liver cirrhosis and biliary obstruction (270). Minor excretory routes of copper were sweat (392) and normal menstrual flow (393). Larger quantities (0.4 mg/day) of copper, imposing no nutritional hazard, could be excreted during highest human lactation (56).

COPPER DEFICIENCY

Frank copper deficiency, associated with soil and crop copper, has been reported in cattle (394). Blood disorders including anemia (395), leucopenia (396) and neutropenia (397) have been shown in copper deficiency. Since ceruloplasmin is essential in oxidation and incorporation of iron into transferrin (398), its deficiency will impede the release of iron from liver and reticuloendothelial system (399) with subsequent development of anemia. Such evidences were not noticed by other investigators (400,401,402), but increased breakdown of heme was postulated as a result of increased accumulation of iron in the liver (403). Moreover, it has been reported that the life-span of red cells is shortened in copper-deficient pigs (404).

Copper deficiency is associated with skeletal abnormalities in rabbits (405), chicks (406), pigs (397), sheep (407,408) and children (409). Skeletal abnormalities was not observed in lambs fed copper-deficient diets, but rather encountered in lambs borne to copper-deficient ewes (410). Such skeletal abnormalities and reduced strengths of collagen and elastin were attributed to reduced amine oxidase or lysyl oxidase activities (376,411).

Neonatal ataxia of lambs is a nervous disorder, demyelinating encephalopathy, associated with copper status of animal, and has also been termed "swayback" (412). The

conditions is caused by copper deficiency in lambs, ewes or pastures (413) ,and could be ameliorated on copper supplementation (413). Other studies, however, did not demonstrate such relationship (412,414). Swayback has been developed experimentally in molybdenum- (415) and lead-supplemented (412) lambs. Neonatal ataxia has also been reported in goats (416), guinea pigs (282), young pigs (417) and rats (418).

Intermittent scouring has been reported in copper deficiency in cattle (419,420). "Peat scours" was ascribed to high dietary molybdenum in New Zealand cattle (373). "Falling disease", a cardiac abnormality described in cattle grazed on copper-deficient pasture (416), but was not encountered in horses and sheep grazed in the same area (421). Acute heart failure and hypertrophy of heart and brain were shown in pigs (422) and rats (423) as a result of copper deficiency. Mortalities related to abnormality of elastic tissues of aorta were reported in copper-deficient guinea pigs (414,422), chicks (424) and pigs (425). Such aortic lesions may be attributed to defective activity in lysyl oxidase (426,427) or amino oxidase (428), copper-dependent enzymes. Experimental copper deficiency has led to dissecting aneurysm in chicks (418). Decreased in ATP levels (429), systemic blood pressure (430,431,432) and norepinephrine levels (433) were reported in copper deficiency.

There is apparent discrepancies regarding the effect of copper deficiency on the ratio of saturated and unsaturated fatty acids (336,434). In rats, copper deficiency has led to accumulation of n-3 and n-6 longer-chain polyunsaturated fatty acids in triglycerides of various tissues (435). Increased lipid peroxidation due to inhibition of SOD, catalase and glutathione peroxidase in the liver (436) and erythrocyte membrane (437) has been encountered in copper deficiency (355,436). It has been hypothesized that hypercholesterolemia of copper-deficient rats is caused by suppression of cholesterol degradation process (438).

Fetal death and infertility in sheep, guinea pig and rat (439,440), reduced hatchability in hens (441) and delayed or suppressed estrus in animals (420) are yet other consequences of copper deficiency. Recently, congenital copper deficiency has been hypothesized to cause sudden infant death syndrome (377).

Several studies have demonstrated a relationship between copper deficiency and impairment of glucose metabolism (442,443,444). There is hypothetical evidence that copper plays essential role against inflammation (445,446). Immunosuppression has been described in rats as a result of depressed SLC proliferation (447). Decreased pigmentation of hair, achromotrichia, has been noticed in several animal species, but not in pigs (270). Impaired

keratinization is another sequelae of copper deficiency (408,426).

COPPER TOXICITY

Studies on copper toxicity has begun over a century ago. In 1874, Harnack (448) emphasized subcutaneous lethal doses of copper in dogs, rabbits and frogs. Gallipe (449), on the other hand, noted copper poisoning in farm animals. Early, the accumulation of copper in liver and other tissues of copper-poisoned animals has been reviewed (270). Chronic copper toxicity syndrome in man, Wilson's disease, has been shown to develop following accumulation of copper in renal tubules, brain and cornea as a result of increased copper-binding proteins (450,451).

Of all ruminants, sheep is relatively the most susceptible species to copper toxicosis (452). Copper toxicity in sheep has been associated with copper-supplemented diets (453,454), dietary mineral supplements (455), swine feed or waste (456,457), overdosage in swayback disease (458) or molluscicide-sprayed pastures (459). Diets deficient in molybdenum (460), high in heliotrope or clover (452) may predispose for copper toxicity. Chronic copper poisoning is characterized by accumulation of copper principally in cytosol (461) of liver (462,463) of sheep, and clinically manifested by hemolytic crisis, drop in hematocrit level and hemoglobinemia (464). Recently, it has been suggested that copper toxicity in sheep could be alleviated via complexation by ciliate protozoa (465).

Enzymes such as serum glutamic oxaloacetic

transaminase (466,467), liver-specific arginase (468), glutamate dehydrogenase and sorbitol dehydrogenase (466) were found to be significantly elevated in copper poisoning. Copper was shown to be sequestered in hepatic lysosomes (469) whose density and volume increased significantly 48 hours before onset of hemolytic crises (470). Following hemolytic crisis, plasma methemoglobin (471) and blood urea levels (466) may be elevated. Liver necrosis of copper toxicity is thought be associated with release of lysosomal enzymes (470). Renal tubular degeneration and necrosis (472) developed when renal copper accumulation reached 240 ug/g wet weight (466).

Goats are more tolerant to copper toxicity than sheep (473). However, acute copper toxicity has been encountered in goats with lesions of diarrhea, jaundice, hepatic necrosis and hemosiderosis (474). Copper concentrations rose from 19 to over 2000 ppm in liver, 7 to over 300 ppm in kidney, 3 to over 140 ppm in spleen and 9 to over 30 ppm in heart (475).

In cattle, moderate dietary copper concentrations was not injurious (476), and increased weight gain of calves (477). The toxic dose in cattle has been estimated 3 to 12 times the dose required to correct copper deficiency (478). Acute poisoning is characterized by an abrupt increase of hepatic copper to over 2000 ug/g dry weight with subsequent centrilobular necrosis (479). In chronic copper poisoning

the onset was insidious and characterized by hemoglobinuria and hemoglobinemia and icterus (480,481).

Pigs showed increased growth rate at 250 ug/g added dietary copper only when suitable amounts of zinc and iron (482) or proteins (252) were added. Two fold elevation of copper was encountered in liver, kidney, bile and gastrointestinal tract of 250 pp copper-supplemented boars (483). High levels of hepatic copper is the only overt sign of copper toxicity in swine (484). Females are more susceptible than males (278), and that dietary copper level of 700 ppm was considered fatal to pigs (485).

Rats appear to be tolerant to copper toxicity. A dose of 250 ug/g developed no sign of toxicity (486), but feed conversion increased in males and decreased in females (487). Acute toxicity with larger doses had resulted in increased alkaline phosphatase (488) and copper in liver particulates, but not cytosol (489). In chronic toxicity, copper accumulated in liver in proportions directly correlated to copper dosage (490) particularly in subcellular particles (491,492), despite increased lysosomal levels (493). Organs affected other than liver were kidney (34) and heart (494). Biphasic serum copper elevation was observed in rats fed excess copper for 15 weeks (495). Copper toxicity in rats could be potentiated in rats by feeding heliotrope alkaloids (496), sodium azide (497), carbon tetrachloride or alpha-naphthylisothiocyanate (498).

Oysters (499), penicillamine (500) and ciliate protozoa (465) have been found to be effective in copper-laden animals.

I R O N

ORIGIN:

Iron is necessary to biological mechanisms and therefore exists in all living matter. It occurs in ferrous form in igneous and metamorphic rocks and in ferric state in sedimentary rocks (501). Iron salts are generally confined to poorly drained acid soils (502). Sedimentary iron has been extensively reviewed (503,504), predominantly as ferric oxyhydroxides. Carbonacious deep sea sediments (505) and oceanic oxide-rich sediment (506) estimated to contain up to 11% and 15% iron, respectively. The most important iron ores are sedimentary in nature and most iron stones are distributed around the Atlantic and Indian oceans (507).

ESSENTIALITY AND REQUIREMENTS:

Over a century ago iron was discovered to be a constituent of blood (348). Early studies regarding the essentiality and involvement of iron in oxidative mechanisms has been reviewed (1). Iron-containing flavoprotein were subsequently discovered (508,509), and it became evident that lack of dietary iron inhibit iron-dependent enzymatic processes (510,511).

Swine are devoid to iron balance due to their rapid growth and low iron stores at birth (287). Piglets must receive 21 mg/Kg (512) to attain adequate iron , while 125 mg is necessary for full growth (513). The recommended dietary allowance,however, for growing pig has been estimated at 80 mg iron/Kg dry matter (514).

Iron requirements of chicks has been suggested at 50 ug/g iron (501) and as high as 75-80 u/g diet in another study (511). However, it has been recommended that 80 ug/g is necessary for the first 8 weeks of age and 40 ug/g for the end of 18 weeks in poultry (515). Another study (511) showed that chicks require 75-80 mg iron/Kg when fed a soybean-protein or a casein-gelatin based diet.

Matrone et al (516) suggested 30-60 mg iron/day for normal hemoglobin and growth in calves fed milk diets from birth to 40 weeks of age, but Blaxtar et al (517) proposed 100 mg iron/day. Estimate amounts to 40 ug iron/g diet was reported adequate for calves 17 days to 11 weeks of

age when fed a fat-supplemented skim milk diet (518). Milking cows require 50-60 mg/day, while pregnant cows need 60-80 mg iron/day (519).

The minimal requirements of growing lambs was estimated at 25 ppm but not more than 40 ppm (520). Iron requirements for mature sheep are limited, but 10-15 mg/day was estimated when animal feed averages 10-15 ppm iron (519).

In humans, iron requirements for growth through infancy, childhood and adolescence amount to 30 mg iron/Kg increase in body weight (501). The recommended daily allowances have been estimated at 10-15 in infants and children ages 5-10, 10-11 mg in males and females ages 11 to 15 and 18 mg for lactating or pregnant women (62).

The richest sources of iron are meat meals and fish meals (400-600 ug/g), blood (3000 ug/g), egg white. cocoa, cane molasses, parsley and shell fish (515). Foods that are poor in iron are milk, milk products, white four, white sugar, bread and most fruits (515). Intermediate sources are muscle meat, poultry, nuts and green vegetables (515).

There are some studies concerning animal feed analysis (521,522,523). Animal feeds were estimated to contain 100-250 mg /Kg grasses, 200-300 mg/Kg legumes, 30-60 mg/Kg cereal grains and 100-200 mg/Kg oil seed meals (523). Iron content of some inorganic materials fed to livestock

amounts to more than 500 ug/Kg; these include ground limestone, rock phosphate, ground oyster shells and dicalcium phosphate (523).

CHEMISTRY

Iron has an atomic number of 26 and atomic weight of 55.847. It is classified in Group VIIIB of the periodic table. Iron is tetramorphous and comprises approximately 5% of the earth's crust. Iron occurs in delta, gamma, beta and alpha forms at temperatures above 1400^0C, below 1400^0C, 895^0C and 766^0C respectively. Iron ores are hematite (Fe_2O_3), brown ore, magnetite (Fe_2O_4) and siderite. Iron valency could be 2+ or 3+ in oxidation states, 4+ in perfferite (FeO_3^{2-}) and 6+ in ferrate (FeO_4^{2-}). Nonoxidizing acids convert iron to ferrous ion with subsequent reduction of hydrogen ion to hydrogen gas. Oxidizing agents induce formation of ferrous ion in presence of excess metal and ferric ion in presence of excess oxidizing agent. Rusted iron is principally ferric oxide (56).

BIOCHEMISTRY

Total body iron content is influenced by age, nutritional status and species of the animal (515). Human body contains about 60-70 ug/g (524), while that of rat contains about 50 ug/g of the whole body (525). Iron is predominantly confined to hemoglobin and it was estimated at 57% in dogs (515) and 60-70% in humans (526). Iron content has been shown to be affected by sex in rats (527), mice and birds (528) but not in humans (524).

Blood iron is mainly represented by hemoglobin and transferrin in a ratio of 1000:1 (515). and by ferritin in human blood (529), serum (530) and leukocytes (529). Serum transferrin or siderophilin is an iron-binding protein (531) in all vertebrate species (532). Transferrin is a glycoprotein with two iron-binding sites (533) and molecular weight of 76.000 in humans (515). Serum transferrin has been shown to play a role in iron transportation (534) and in body defense mechanism against infection (535). Serum ferritin levels has been correlated to total body iron stores (536,537), but not when concentrations are low (538). It was reported that ferritin is a useful diagnostic tool of storage iron (534). Walters and associates (537) suggested that 1 ng/ml of serum corresponds to about 8 mg of storage iron. Serum ferritin has been found to increase in liver diseases (515) and in leukemia (539).

Hemopexin (540) and hepatoglobin (541,542) are glycoproteins secreted by liver. Following intravascular hemolysis, hemopexin binds heme, while hepatoglobin binds hemoglobin to liver for catalysis and iron conservation, a process that is receptor-mediated (543,544,545).

Iron is stored in two main forms; ferritin and hemosiderin, occurring in all tissues with highest concentrations in liver, spleen and bone marrow (546). In its crystalline form, ferritin contains up to 20% iron, while hemosiderin contains up to 35% iron in a form of ferric hydroxide (515). It has been proposed that hemosiderin provides a useful diagnostic measure for body iron stores (547) and iron deficiency anemia (548). The amounts and proportions of ferritin and hemosiderin are affected by a number of factors (549,550). Ferritin-hemosiderin ratio was little affected in with chronic or acute hemolytic anemia (515), iron-deficient chicks (551), but not with iron supplementation (552). Iron is predominantly deposited as hemosiderin in patient with hemochromatosis and transfusional siderosis (549), nutritional siderosis in cattle (553), copper and cobalt deficiency in sheep (554) or when iron levels in liver and spleen are beyond 2000 ug/g (552).

Iron occurs as ferritin of lactoferrin in milk (555) as well as saliva and sweat (556). Lactoferrin and ferritin are similar in their iron-binding sites, however

they differ in their immunological specificity (557), amino acid and peptide composition (556) and electrophoretic motility (555).

METABOLISM

ABSORPTION:

Intestinal mucosa tend to regulate iron absorption (558) with most absorption encountered at duodenum (501). Due to restricted pathways of iron excretion (559), about 5-10% of iron is absorbed depending of the food source and 10-20% in deficient subjects (56). In general, iron in ferrous form is highly absorbed compared to ferric form (560). Iron from heme sources is directly absorbed into mucosal cells, while iron of nonheme and iron protein compounds needs to be reduced to ferrous form for effective absorption (561). Normal gastric juice is capable to accomplish these transformation (562,563). The bioavailability of iron from soybean is greater than from corn or wheat in humans (564,565).

Regulation of iron absorption is not mediated via a hormonal factor in plasma (566), but Bhargavra and Gabbe (567) suggested that iron absorption is influenced by erythropoietic activity of the erythron. Mucosal transferrin has been suggested to be involved in transmucosal transport of iron (428,568), and that its concentration was found to be directly proportional to iron uptake (428). Simpson and collaborates (569) reported ferric iron uptake by brush border membrane viscles. Another study (570) demonstrated that apomucosal transferrin may be involved in transportation of iron between cells and brush border.

Unlike inorganic iron, heme iron is absorbed directly by intestinal epithelial cells and subsequently released in nonheme form (561,571). Gastroferrin, a glycoprotein of gastric juice, has been postulated to regulate iron absorption (562), and its deficiency has been correlated to the development hemochromatosis (515) and iron deficiency anemia (572).

Dietary interactions of iron with cobalt, zinc, copper, cadmium and manganese contribute considerably to interference with iron absorption possibly by competing at the absorption site (515). A supplement of 10 or 100 fold of cobalt could reduce or inhibit iron absorption (573) indication shared absorptive pathway. This has been confirmed when iron and cobalt absorption increased in iron-deficient rats (574) and iron deficiency in humans (575). High dietary phosphorus decreased iron absorption (576) and increased fecal excretion of iron in presence of low or high intakes of calcium (577).

Histidine, lysine and cysteine could influence iron absorption (578,579). Several studies showed that ascorbic acid increases iron absorption (580,581,582), but no effect was revealed on hemoglobin, iron absorption (561) or iron utilization from wheat sources in rats (583). Phytate significantly depressed iron absorption when added to human diets (584,585). However, sodium phytate did not affect iron utilization when added to rat diets (586,587),

nor did it affect iron utilization from wheat bran in rats (583,588). Iron absorption is enhanced in the presence of organic acids such as citric, lactic, malic, pyruvic, succinic and tartaric (589,590). Meat content "meat factor", fish, poultry or MFP enhanced iron absorption (590,591) compared to vegetable foods (565), possibly by the formation of luminal iron transport carrier (592) through amino acid cysteine (593,594). Carbohydrates such as starch, lactose, glucose and sucrose (595), ferric fructose (596), fructose (597) and sorbitol (598) enhanced iron absorption and retention. Bicarbonate or phosphate therapy produced no significant impact on iron absorption, whereas desferroxamine, by contrast, reduced iron absorption significantly (599,600), and it is therefore necessary to counteract iron overdosage (524,601). Of beverages, tea (602) and coffee (603) showed inhibitory effect upon iron absorption.

The potential uptake of iron is considerably modified by disease state (515). Iron absorption increased in hemolytic anemia, plastic anemia, pernicious anemia ,pyridoxin deficiency and hemochromatosis (604) and in increased erythropoiesis (605), but decreased in transfuginal polythcythemia (606).

INTERMEDIARY METABOLISM:

Following absorption, iron is normally bound to

serum transferrin or siderophilin (531) almost in all vertebrate species (532). Diurnal fluctuations in serum iron has been observed in man (607), pigs (608) and dogs (609). Species variation in serum iron and total iron binding capacity (TIBC) is small, however average values encountered were high in sheep, pigs and cattle (608,610,611) than humans.

Incorporation of plasma iron into liver, spleen and bone marrow ferritin is mediated via ATP and ascorbic acid (612,613). It has been shown that molybdenum and xanthine oxidase facilitate release of iron from hepatic ferritin to plasma (398,613), but Osaki and Sirirech (614) did not detect such release. Ceruloplasmin (ferroxidase I) has been shown to participate considerably in mobilization of iron from its stores (219,615).

Among metabolic cycles of iron hemoglobin cycle is the largest,and that 70% of plasma iron turnover is confined to bone marrow (91). The process of hemoglobin synthesis involves transfer of iron via transferrin (616). Ferric iron transported to bone marrow is reduced and released into ferrous form before being incorporated into protoporphyrins during biosynthesis of heme (617,618). Transferrin-iron complex is taken up by immature reticulocytes with subsequent release of transferrin to plasma (619,620). About 80% of iron is transformed to hemoglobin within mitochondria of erythroid cell (621). The adequacy of iron supplement

versus erythroid need may be monitored by increased cell protoporphyrin (390). Iron of senescent or defective red cells in reticuloendothelial cell is catabolized and either taken up by transferrin (609), ceruloplasmin (622) or stored as ferritin (623).

Iron storage forms are ferritin, hemosiderin with highest concentration in liver, spleen and bone marrow (515). Ferritin is a water soluble compound containing 20% iron, whereas hemosiderin is water insoluble and contains 35% iron (624,625). The hemosiderin:ferritin ratio was found to be increased in favor of ferritin when iron concentrations were high (1000-4000 ug/g) in liver and spleen of rats and rabbits (626) and humans (549). The reverse was true in poultry (551) and at low iron storage concentrations (549). Iron is stored as nonheme iron at 100 ug/g in bone marrow of normal humans (627) and in relatively small amounts in the muscles (627,628). In healthy subjects, there was evidence of direct correlation between serum ferritin and iron stores in the body (536,537), but this relationship was not longer maintained in disease states (629) or at low concentrations (538). Hepatic uptake and storage of iron is potentiated when erythron uptake decreases (630).

EXCRETION:

Early studies have suggested limited excretion of

iron from the body (559) even in the occurrence of iron overload (631). Using chemical balance studies (632) and radioiron techniques (633,634), the amount of excreted iron was estimated at 0.2 and 0.3-0.5 mg/day in normal human adult respectively. Iron was shown to be excreted in bile at 1 mg/day mostly from hemoglobin breakdown, and that it may be absorbed by 40% before reaching feces (635). Green and co-workers (634) estimated iron losses in adult male. It has been demonstrated that iron losses are directly correlated with iron stores (636) or inversely correlated with absorption (637). Urinary iron excretion increased in presence of chelating agents (deferroxamine) (638) and in nephrotic syndrome (639).

Iron losses through integument amount to 0.5 mg/day in healthy adults (640) and as high as 6.5 mg/day in some instances (641), which was claimed to contribute to the incidence of iron deficiency in the tropics (641). A net loss of 0.8-1.0 mg iron/day was estimated in urine, feces, and sweat in women and men of average size (642). Iron losses amount to 0.4 mg/day during lactation, but menstrual losses were considerably variable among women (643,644). Menstrual losses have been estimated at 4-26 mg/period in young women (645), 2-59 mg/day in adolescent girls (646) and 27-37 mg was regarded as upper limit for normal loss (644).

IRON DEFICIENCY

Iron deficiency syndrome is expressed by three stages; depletion of iron stores, latent iron deficiency and overt iron deficiency anemia (647,501). The clinical manifestations of iron deficiency are associated with listlessness, fatigue, sore tongue, angular stomatitis and dysphagia in human adults and by anorexia, depressed growth and immunosuppression in children and young growing animals (648,515). Gastric achlorhydria, gastritis, atrophy (649) and reduced plasma proteins (650) were observed in iron-deficient infants and children. Some studies indicated that loss in weight gain is a late consequence of iron deficiency in rats (651,652) but not in children (653,654). One study demonstrated that anemia is associated with 78% of anemic pregnant women and that there is a correlation between maternal and newborn hematopoiesis (655).

Iron deficiency anemia was encountered in pigs fed copper as a growth factor (656,657) and in baby pigs exclusively confined to sow's milk (515). Iron deficiency anemia has been reported to occur in calves and lambs only under heavy helminth infestation (658) or when reared under milk-based diets (546,659). A diversity of methodology has been utilized to monitor iron deficiency with certainty (660,661,662). However, values of transferrin iron saturations of less than 15% are used as criterion of iron deficiency (647).

Dietary iron deficiency has been had resulted in lipid abnormalities and increased triglycerides in rats (663,664) and chicks (665). Hyperlipidemia was reversibly correlated to iron supplementation in rat (666). One study (667) suggested mild impairment in essential fatty acid metabolism in moderately (12 mg/Kg) iron-deficient rats.

A significant drop in myoglobin content has been reported in iron-deficient piglets and puppies (668), chicks (551) and rats (551,651). Furthermore , young animals (668) and skeletal muscles (551,669) are most vulnerable to myoglobin loss in iron deficiency. However, chronic iron deficiency anemia of moderate or severe degree did not induce significant alterations in enzyme activity, capillary density or myoglobin content of human skeletal muscles (342).

Biochemical measurements showed that cytochrome oxidase is reduced in intestinal mucosa in iron-deficient subjects (670,671), and in intestinal mucosa and skeletal muscles of iron-deficient rats (672). Decreased cytochrome C activity (651) and catalase activity (673) were encountered in iron-deficient rats, piglets and calves. Aconitase activity depressed in iron-deficient patients (51), and normal or decreased in tissues of iron deficient rats (510).

Although the relationship between human iron status and susceptibility to infection is controversial (674), impaired cell-mediated immune response and

bactericidal activity in children (675) and impairment of antibody formation in rats (676) were ascribed to iron deficiency. Enhanced susceptibility to E.Coli infection was observed in iron-deficient piglets (677)

One study (678) speculated that iron deficiency induces behavioral abnormality in children and adults, however the mechanism and extent in which iron deficiency is responsible remains to be established. In rats, spontaneous basal activity diminished in presence of iron deficiency, but it was independent of severity of anemia and corrected upon iron therapy (679). Reduction in scholastic achievement reported in association with iron deficiency (680), but not in another study (681). Pica or alotteriophagia was observed in iron-deficient patients (682,683), but direct relationship was difficult to achieve. However, deficiency of monoamine oxidase in brain of iron-deficient rats (684) and humans (685,686) raised the likelihood of that this enzyme may be associated with behavioral disorders of iron deficiency.

The potentiality of manganese absorption increased in iron deficiency (574,464) and decreased in individuals with adequate iron stores (464). The absorption of cobalt (574,575) and lead (687.688) have been shown to be increased in iron-deficient rats. Cadmium absorption increased by 4 to 6-fold in iron deficient rats (689). There was no significant change regarding absorption of cesium,

magnesium, calcium and copper in iron-deficient rats (574).

Iron deficiency has been shown to depress cell proliferation of cheek pouch epithelium of hamster (690). Several studies have demonstrated injury to mitochondria in various tissues of iron-deficient animals and humans (691,692). A significant drop in serum folic acid has been observed in iron-deficient subjects (693), which may be a sequelae to decreased red cells life-span (694,695), ineffective erythropoiesis (696) or lactation (697) superimposed in iron deficiency.

IRON TOXICITY

Although iron intake levels of 50 mg/day (698) or 25-75 mg/day (515) is regarded safe for humans, accidental toxicity have been reported in children (699,700) and may be fatal (701,702). Fatalities in adults were associated with suicidal intent (703,704). The adult lethal dose was estimated at 200-250 mg iron/Kg body weight (702). Iron toxicity in animals requires much higher levels of iron in presence of high in takes of cobalt, zinc, manganese, or copper than when such intakes are low or normal (515).

The clinical stages of acute iron poisoning have been described (705,706) and may show marked leucocytosis, metabolic acidosis, hyperbilirubinemia and deranged blood coagulation (682,707). The onset or shock is an indication of serious prognosis (706) and the case mortality rate was 11-17% (706,708). Foals in the immediate postnatal life are highly susceptible to acute iron toxicity from low levels (16 mg/Kg body weight) probably potentiated by vitamin E and selenium deficiency (709).

Chronic iron toxicity has been reported in individuals with idiopathic hemochromatosis (710) or refractory anemia (711,712,713). Fibrosis (714), carcinomas (715) and occasionally cholangiomas (716) were the synopsis of idiopathic hemochromatosis. Idiopathic hemochromatosis is characterized by iron deposits in parenchymal tissues of many organs (713,714) especially in liver (714). In

refractory anemia, repeated blood transfusion could cause iron deposition predominantly in reticuloendothelial cells of liver, spleen and bone marrow (710). Thalassemia major is a genetic syndrome depicted by increased erythroid activity with subsequent excessive iron absorption from intestine (717,718). Chronic parenchymal iron overload exhibited specific clinical manifestation including liver dysfunction, diabetes, cardiac failure, endocrine abnormalities, arthritis, and abnormal skin pigmentation (501).

Chelating agents such as deferroxamine (601) and ethylenediaminetetraacetic acid (EDTA) (696,719) have been utilized to cure iron overload. Bicarbonate and phosphate have been also described to treat iron overload, but Dean et al (719) showed that this was ineffective in reducing iron absorption in pigs. Other attempts of reducing iron toxicity was accomplished by using sodium dihydrogen phosphate that renders unabsorbed iron into insoluble complex (699,69).

INTERACTIONS OF ZINC, COPPER AND IRON

The potentiality of absorption, utilization and excretion of trace elements including zinc, copper and iron and subsequently their physiological, pharmacological or toxicological roles in the body may be appreciably influenced by the presence of other dietary elements, their concentrations or chemical composition.

INTERACTION BETWEEN ZINC AND COPPER:

Despite the hypothesis that zinc and copper are apparently antagonistic in the biological systems, their doses and relative ratios required to produce such interactions is controversial (720,721,722,723).

It has been reported 40 years ago that zinc high dietary zinc (1.0%) induced anemia in rats which could be alleviated on copper supplementation (724). Van Reen (1953) demonstrated decreased activity of cytochrome oxidase (copper-dependent enzyme) in the liver of rats fed 500-700 ug zinc/100 g diet, which was reversed on copper supplementation (224). Ritchie et al (1963) showed that copper toxicity in pigs was alleviated by dietary zinc supplementation (266). Detrimental responses were also observed by hill and associates when 100 ppm zinc was added to the diet of zinc-deficient chicks (268). Subsequent isotope experiments in rats suggested that zinc interfere with copper metabolism by decreasing utilization and

increasing excretion but had little effect on absorption (725).

High dietary and oral zinc therapy had induced hypocupremia and increased fecal losses of copper (352,370,726,727). Some studies have described that excess oral zinc had resulted in anemia (302,728) and leukopenia (729,730), that responded to copper supplementation (729), suggesting zinc-induced hypocupremia. Increased copper retention in mucosal cells with concomitant decrease in copper absorption was observed in rats fed high dietary zinc compared to those with a lower zinc in their diet (389,731,732). Conversely, radioisotope in vitro studies revealed significant depression of zinc absorption by high dietary copper (733). These results were subsequently confirmed (269), and that a competition and/or inhibition of copper or zinc absorption occurred when the concentration of the other is elevated (269). This approach was thus exploited for treatment of Wilson's disease (734) and sickle cell anemia (728).

Investigations regarding serosal transport mechanisms (735), the potential role of metallothionein in zinc (122,126) and copper absorption (339) and its preferential binding of copper (122,389,736) propose that this protein may be the site of mutual antagonism. There is hypothetical evidence that zinc induces metallothionein synthesis in liver of humans (737) and rats (738) and kidney

of rats (739) which may render excessive copper nontoxic.

Rats fed diets containing 0.4% or more zinc revealed significant drop in tissues copper and iron (253), cytochrome oxidase and catalase (740,741). Both zinc and copper supplements were associated with decreased deposition of respective element in liver of rat (721). Depot parenteral injection of zinc increased urinary copper excretion and plasma copper concentration in rats (187). However, Kang et al (255) demonstrated unchanged copper concentrations in all tissues of rats fed based diets containing deficient or supplemented levels of zinc. Song and collaborates (254), in contrast, reported increased copper concentrations in liver, kidney and spleen in zinc-supplemented rats. Copper concentrations also increased in tissues of rats fed zinc-deficient diets (742,743).

While it was evident that increased dietary zinc acts as a metabolic antagonist in ewe lambs (744), dietary zinc supplementation enhanced copper deficiency and zinc toxicity in pregnant ewes (745). It has been shown that a genetic factor may be involved in mutual competition between zinc and copper in sheep (267). Supplementary zinc could alleviate high plasma aspartate transaminase (ASPT) levels and liver storage of copper resulted from chronic copper toxicosis in sheep (746).

Although copper supplementation increased weight gain and feed efficiency in growing pigs (747), another

study (748) reported skin lesions similar to those of zinc deficiency which was alleviated on zinc supplementation (56).

In young horses high dietary zinc had resulted in skeletal abnormalities due to hypocuprosis (749). Mean daily intake of 12-15 mg zinc/Kg live weight depressed plasma copper concentrations to levels considered to indicate copper deficiency in cows and calves (750). The adverse effect of zinc supplementation on copper status was moderated by the presence of phytate in the chow of monkeys (264).

INTERACTION BETWEEN ZINC AND IRON:

Interaction between zinc and iron is controversial. It was reported that zinc absorption decreased following intakes of large quantities of nonheme iron and a single dose of inorganic zinc (751,752,753), but was not when zinc was administered in an inorganic form (63,753) or when zinc and iron were administered with meal (754). Other investigators (158,755) observed reduced circulatory zinc levels in pregnant women and infants, although it was not reported in other studies (756,757,758). Adverse effects of high iron intakes on zinc absorption was not observed in infants receiving iron fortified formulas (759). In contrast, high dietary zinc induced anemia and decreased tissue iron concentrations in rats and chicks

(756,760), but such effect was not revealed in humans (38,63).

Early isotope studies on iron metabolism in rats fed high dietary zinc suggested that zinc does not interfere with iron absorption but interfere with iron utilization (725). However, the antagonistic effect of high dietary zinc on iron was thought to be at the absorptive level (761,762) or metabolic level involving alteration in copper metabolism (271,760). It has been speculated that zinc affects iron metabolism by interfering with iron incorporation into or its release from ferritin or by shortening life-span of RBC (763). It was reported that empty stomach enhances the inhibitory effects of excessive iron on zinc, although it was not observed when supplements were provided with a zinc ligand or meal (754). Despite the proposal that zinc and iron are absorbed by different pathways (764), other studies (93,751) had shown inter-element competition at the absorptive site and that zinc and iron absorption increased when the concentration of respective element is deficient (765).

In a radioisotope study (250), an intake of excessive ^{59}Fe in one test meal or several weeks did not alter tissue contents of zinc or apparent absorption of ^{65}Zn. Conversely, chronic high dietary ^{65}Zn depressed hematocrit, reduced of ^{59}Fe absorption and retained less ^{59}Fe in tibias and liver of rats, although no effect was

noticed with intake of high zinc in one test meal (250). Zinc and iron concentrations in liver, kidney and pancreas of female domestic fowl were significantly elevated when fed diets containing 6 or 20g added zinc/Kg (766). Other studies showed that high dietary zinc induced a marked loss of hepatic iron (158,253) and decreased amounts and percentage of ferritin-bound iron in liver of rat (271). However, it has been suggested that feeding normal, subnormal or toxic amounts of zinc may also cause a marked decrease of iron in kidney and liver of rat (255).

Feeding zinc-deficient diets had resulted, although not different from controls, in increased iron concentrations of plasma fraction in dams and fetuses (767) and only increased testes iron contents in another study (212). In zinc-deficient rats intestinal contents of ^{59}Fe was found to increase appreciably (761). It was hypothesized that iron and zinc are absorbed by different metabolic pathways and that there is increased turnover of zinc in iron-deficient rats possibly due to shorted life span or increased zinc concentration of erythrocytes (764). Dietary iron deficiency enhanced absorption of iron and zinc whereas iron deficiency due to bleeding enhanced the absorption of iron only (311).

INTERACTION BETWEEN COPPER AND IRON:

The first indication of iron-copper interaction

was demonstrated 60 years ago when copper supplementation alleviated anemia in iron-supplemented rats (4). Frieden (768) speculated that copper-dependent anemia is attributed to subnormal activity of ferroxidase (ceruloplasmin) resulting in hypoferremia. However, this argument has not been confirmed by other studies due to the finding that anemia was absent in rats (400,769) and mice (227,770) when ceruloplasmin levels were low. A reduction in cytochrome activity rather than ceruloplasmin was another alternative explanation for copper-dependent anemia in animals (770). However, Gipp and co-workers (656) demonstrated that the microcytic hypochromic anemia of high dietary copper was attributed to impairment of iron absorption and that this impairment was reversed by ascorbic acid. Weisenberg and collaborates (402), in contrast, showed that iron supplementation alleviated anemia in copper-deficient rats without elevating ceruloplasmin or cytochrome oxidase activities.

It has been shown that iron is a potent dietary antagonist of copper metabolism in cattle, and that 250 mg iron/Kg was adequate to reduce copper to levels indicative of copper deficiency (771,772). The reverse was obtained in experimental rats (773) and that no inhibitory effect was observed on intestinal copper absorption. Moreover, obvious hemoglobin response was obtained in rats fed milk-based diets (774,775) or in copper-deficient suckling mice (40)

when given iron preparations. It has been demonstrated that iron-fortified infant formulas were associated with reduced absorption of copper by 13% and retention by 12% of intakes (759). It was revealed that high dietary iron depressed copper absorption only in copper-deficient rats (776). However, high iron with ascorbic acid caused severe anemia in copper-deficient rats and decreased plasma ceruloplasmin by 44% in copper-adequate rats (776).

On the other hand, dietary copper levels up to 60 ug/g had no effect on liver iron concentrations, but 120 ug/g copper resulted in 50% decrease in liver iron (777). Iron levels high or less than those recommended by National research council (30) have been found beneficial to counteract the adverse effect of high dietary copper on iron utilization (656,657,778). However, increased dietary copper was not required upon provision of supplementary iron (656). High dietary copper could induce a decrease in liver iron, an increase in liver copper and anemia in pigs (657,779) indicating interference with iron utilization.

Iron and copper concentrations in liver of rat tend to decrease with increased dietary supplementation of respective element (721). Copper-deficient female rats had higher serum iron concentrations than copper-deficient male rats indicating that copper and iron relationships may be influenced by sex of animal (447).

SUGGESTED FUTURE RESEARCH

Within the past several decades many studies have been conducted to extrapolate trace elements nutritional risks to human and animal health. Although the advent of purified diets and the selection of the most relevant animal model have permitted a new approach to study zinc, copper and iron, the understanding and detection of nutrient metabolic and antagonistic effects is far from complete.

Further research is needed to assess zinc status and adequate dietary intakes in both humans and animals. More studies should be conducted on factors affecting bioavailability of zinc. A more detailed study on metallothionein in relation to zinc, copper and iron may be of increase value to explain their mutual antagonistic effects. The biochemical etiology of anorexia and growth inhibition of zinc deprivation needs to be elucidated. More biochemical research may be needed to study the influence of zinc status on impaired brain biochemistry or function as well as behavior by using appropriate animal models. The efficacy of therapeutic use of zinc to alleviate toxic effects of copper, lead, cadmium or calcium is questionable. The role of zinc deficiency on immune function, pregnancy and birth defects is in need of determination.

More sensitive devices are needed to detect

marginal or impaired copper nutrition in human and animal populations. Further research should be encouraged on the influence of dietary and endocrine factors on copper absorption. The growth-stimulating effect of copper is not fully understood especially for pigs. The precise role of copper-dependent proteins in detoxification and bioenergetics require further investigation. There is evidence of copper-zinc, copper-iron, copper-selenium and copper-ascorbic acid interactions, but mode of action is obscured. Studies should be conducted to highlight the role of lysyl oxidase in sudden infant death syndrome (SIDS). The physiological role of copper-dependent proteins including albocupreins, neurocupreins and diamine oxidases need to be established. More information is required on the relationship dietary copper and impairment of carbohydrates metabolism or inflammations.

Additional research on enhancing or inhibiting factors of iron are required to understand the intricacies of availability. The interaction between iron and its physiological binding proteins as well as other dietary factors particularly copper, zinc and lead needs to be clarified. Research on molecular and cellular aspects of iron metabolism should be promoted to clarify the mechanisms of absorptions, transport and metabolism. The relationship between human iron status, behavior and defense mechanism should be defined. More information is required on the

cytotoxicity and carcinogenicity of excess iron. More efficient therapeutic and prophylactic agents should be sought to improve the treatment of iron-laden patients.

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APPENDIX II

Individual Animal Data on the Effect of Various Dietary Zinc Concentartions on the Biological Interactions of Zinc-Copper-Iron in Rats

T	AN	WK.	LIVER			KIDNEY			HEART			SPLEEN			LUNG		
R	#		Zn (ppm)	Cu (ppm)	Fe (ppm)	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe
C	1	4	21.40	2.98	215.82	19.72	4.13	127.20	18.39	7.36	69.48	19.89	2.15	200.56	19.17	1.85	96.90
C	4	4	22.56	3.25	202.12	19.25	4.17	143.90	17.10	7.65	88.53	15.37	2.05	194.67	20.62	2.16	90.32
C	11	4	25.35	3.31	231.80	21.37	6.03	158.42	12.48	5.82	87.39	16.20	2.03	207.62	18.99	2.00	95.96
C	13	4	21.13	3.38	210.42	19.95	5.83	144.38	11.60	6.02	90.19	19.50	2.46	210.22	18.25	2.43	100.6
C	14	4	20.12	3.64	193.88	20.09	4.08	150.98	18.50	6.58	102.8	15.77	2.10	210.22	19.51	2.26	84.24
C	3	8	28.39	4.03	189.92	21.26	6.27	161.60	16.39	4.79	67.89	19.25	1.39	196.79	20.96	1.90	81.87
C	5	8	26.49	4.26	199.44	22.10	5.79	155.74	17.85	4.44	66.65	17.44	1.23	192.83	18.74	1.92	81.04
C	6	8	24.88	4.35	190.78	17.85	7.04	159.60	14.82	4.40	68.50	20.12	1.67	191.95	20.09	2.00	80.35
C	7	8	28.40	4.34	194.16	18.22	4.50	158.58	14.64	4.65	68.67	17.42	1.33	196.82	17.70	1.72	80.94
C	9	8	26.83	4.44	198.14	17.39	4.43	155.94	14.13	4.50	71.18	18.96	1.58	198.00	17.83	1.83	81.00
C	12	8	26.52	4.06	187.20	18.42	6.00	155.74	13.64	4.65	68.68	19.07	1.38	194.89	ND	ND	ND

Organ Concentrations of Zinc, Copper and Iron of Controls (C)

TRT	AN #	WK	LIVER Zn (ppm)	LIVER Cu (ppm)	LIVER Fe (ppm)	KIDNEY Zn	KIDNEY Cu	KIDNEY Fe	HEART Zn	HEART Cu	HEART Fe	SPLEEN Zn	SPLEEN Cu	SPLEEN Fe	LUNG Zn	LUNG Cu	LUNG Fe
D	1	4	19.27	3.38	191.94	17.91	5.53	131.06	18.65	8.29	120.19	18.67	2.28	311.19	14.84	2.37	140.48
D	3	4	19.17	3.26	157.18	17.80	6.74	122.06	18.31	8.14	117.97	20.50	2.25	292.07	17.49	2.47	123.43
D	8	4	21.41	3.89	179.06	18.15	6.81	142.62	21.21	8.48	100.87	20.07	1.90	295.82	18.28	2.23	125.93
D	11	4	20.49	3.62	163.92	20.73	4.81	155.22	20.04	6.41	102.19	19.20	1.62	281.79	14.33	2.25	116.70
D	14	4	18.37	3.77	199.76	18.86	6.67	156.00	14.49	7.03	95.22	18.69	2.07	275.38	15.24	2.74	146.30
D	5	8	26.56	3.58	179.16	16.21	3.98	171.60	13.24	4.68	76.37	17.34	1.47	210.00	16.18	1.42	94.70
D	6	8	22.88	3.54	183.08	15.89	4.70	171.42	13.41	4.75	78.42	17.55	1.43	232.59	16.97	1.40	95.81
D	7	8	21.85	3.54	191.44	14.44	6.01	177.06	13.69	4.94	78.23	18.72	1.65	215.34	18.76	1.62	95.31
D	9	8	22.77	3.52	178.02	14.72	3.79	176.68	12.78	4.80	79.72	16.69	1.40	109.52	15.52	1.50	95.14
D	10	8	22.55	3.48	180.36	14.71	7.47	185.08	14.86	4.66	75.28	16.86	1.37	216.40	17.08	1.51	94.30
D	12	8	21.49	3.68	190.88	14.99	5.89	179.76	14.43	4.84	79.37	18.28	1.46	214.71	17.19	1.52	95.02
D	13	8	20.98	3.78	192.08	15.95	5.88	176.30	12.91	4.96	75.37	15.65	1.50	229.48	16.28	1.42	96.69

Organ Concentrations of Zinc, Copper and Iron in Zinc–Deficient Rats (D)

T	AN	WK	LIVER			KIDNEY			HEART			SPLEEN			LUNG		
R T	#		Zn (ppm)	Cu (ppm)	Fe (ppm)	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe
S	1	4	24.88	2.63	157.72	25.98	5.83	134.32	16.28	6.11	124.16	19.71	1.76	134.86	19.06	2.36	65.58
S	2	4	20.27	2.88	165.76	23.82	6.86	166.88	20.42	6.53	95.95	21.14	2.21	161.22	18.19	2.13	70.90
S	5	4	26.42	3.02	188.74	28.01	6.94	112.02	20.54	6.16	108.86	20.31	1.62	142.17	18.37	2.14	77.56
S	9	4	21.35	2.85	156.06	28.26	6.66	100.46	21.68	8.67	94.91	18.31	1.93	142.42	20.26	2.74	86.89
S	14	4	26.75	2.67	160.48	27.08	6.30	108.34	24.44	6.11	107.95	20.57	2.31	134.55	19.06	2.41	82.27
S	3	8	24.81	3.31	153.00	21.11	5.66	144.42	18.31	5.66	65.84	20.67	1.44	209.81	20.38	2.04	73.35
S	4	8	23.85	3.21	153.44	25.32	6.12	135.04	18.15	5.57	64.30	19.22	1.42	209.91	20.63	2.01	73.47
S	6	8	19.72	3.11	128.72	23.31	4.45	136.76	17.20	5.35	65.20	20.29	1.62	164.61	20.66	2.02	70.54
S	7	8	26.41	3.11	140.86	22.84	6.33	137.06	17.66	5.51	64.41	20.91	1.46	203.78	23.13	2.11	71.40
S	8	8	25.40	3.42	128.54	21.92	7.51	137.76	17.77	5.37	65.09	18.31	1.36	180.76	20.36	1.92	72.40
S	10	8	23.94	3.54	124.92	23.10	5.67	134.42	17.22	5.17	65.83	21.24	1.63	175.92	20.63	1.71	70.43
S	11	8	26.47	3.53	128.74	23.85	5.83	135.70	16.66	5.26	63.36	19.52	1.48	173.09	21.82	1.74	72.67
S	13	8	27.33	3.91	140.28	20.55	5.06	126.46	17.04	5.16	66.11	21.01	1.40	215.48	20.75	1.92	71.87

Organ Concentrations of Zinc, Copper and Iron in Zinc–Supplemented Rats (S)

TRT	N #	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C	1	89.6	125.5	161.1	197.3	228.9	ND	ND	ND	ND
C	2	75.1	100.5	120.8	ND	ND	ND	ND	ND	ND
C	3	76.2	105.5	144.1	178.5	212.2	231.2	252.3	259.1	268.3
C	4	87.4	122.0	156.2	181.4	215.5	ND	ND	ND	ND
C	5	92.1	123.6	160.1	185.8	220.9	227.4	243.2	245.0	265.4
C	6	87.2	120.8	154.5	180.5	216.1	230.3	240.8	246.8	265.8
C	7	76.8	114.4	143.3	168.3	203.4	214.2	232.0	237.7	246.1
C	8	77.5	112.0	144.7	ND	ND	ND	ND	ND	ND
C	9	100.6	134.3	170.6	194.9	230.3	345.2	263.8	264.2	280.5
C	10	75.7	110.5	150.5	ND	ND	ND	ND	ND	ND
C	11	108.1	133.7	169.4	195.8	222.5	ND	ND	ND	ND
C	12	83.9	122.9	160.5	179.6	214.3	231.0	244.0	248.4	260.8
C	13	79.0	99.2	120.0	142.8	161.2	ND	ND	ND	ND
C	14	92.5	122.9	160.5	182.5	213.9	ND	ND	ND	ND

Body Weight/Week of Controls (C)

TRT	N #	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
D	1	94.3	116.6	144.1	155.3	182.2	ND	ND	ND	ND
D	2	83.5	101.8	123.8	132.4	173.2	183.9	190.8	ND	ND
D	3	87.9	112.0	112.2	135.6	155.7	ND	ND	ND	ND
D	4	94.7	119.6	ND	ND	ND	ND	ND	ND	ND
D	5	103.9	115.9	125.9	124.8	128.6	125.7	135.0	144.2	158.2
D	6	104.4	123.8	159.3	168.0	193.0	195.8	202.4	201.1	215.7
D	7	81.7	102.9	136.7	158.7	188.6	197.8	199.4	204.5	221.8
D	8	85.5	101.8	111.0	123.2	137.2	ND	ND	ND	ND
D	9	80.6	103.0	124.0	134.3	162.4	164.7	171.8	174.3	194.4
D	10	98.7	118.7	151.7	173.0	200.6	208.5	218.9	228.5	245.5
D	11	76.4	94.4	121.4	125.2	ND	ND	ND	ND	ND
D	12	88.1	115.7	150.3	169.0	195.3	188.5	211.9	218.6	247.7
D	13	103.8	121.3	119.5	132.6	136.0	138.4	150.4	158.9	158.2
D	14	77.1	95.1	113.7	145.5	168.0	ND	ND	ND	ND

Body Weight/Week (g) of The Zinc-Deficient Rats (D)

TRT	N #	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
S	1	84.6	122.6	158.6	183.1	217.1	ND	ND	ND	ND
S	2	82.2	118.0	152.7	177.1	207.8	ND	ND	ND	ND
S	3	96.0	124.1	166.1	191.1	220.6	229.5	247.9	245.8	ND
S	4	94.9	133.6	170.2	194.0	231.7	233.1	254.5	253.2	275.5
S	5	90.0	125.1	162.4	184.2	217.8	ND	ND	ND	ND
S	6	91.4	125.7	164.2	181.5	209.0	220.7	229.6	209.0	227.2
S	7	95.4	126.5	162.7	178.4	213.9	221.1	241.8	241.1	264.1
S	8	104.4	124.8	160.6	178.3	210.0	214.3	234.5	235.4	258.2
S	9	91.2	126.6	158.0	177.6	210.7	ND	ND	ND	ND
S	10	85.9	126.8	158.9	181.5	203.4	209.4	226.7	230.6	252.7
S	11	83.2	117.1	153.0	171.9	208.1	220.0	230.8	228.1	244.3
S	12	82.8	103.3	142.9	ND	ND	ND	ND	ND	ND
S	13	96.3	130.0	160.2	184.2	221.3	232.2	249.4	252.6	276.4
S	14	86.2	120.0	155.4	172.5	205.3	ND	ND	ND	ND

Body Weight (g)/ Week of the Zinc–Supplemented Rats (s)

TRT	NIMA #	DATE WEEKS	Zn (ppm)	BLOOD Cu (ppm)	Fe (ppm)
C	1	4	5.16	0.78	703.58
C	2	2	7.17	0.91	429.92
C	3	2	4.59	0.76	479.17
C	3	6	5.06	1.09	736.08
C	3	8	5.84	0.71	538.50
C	4	4	4.99	0.86	665.94
C	5	6	5.51	1.07	792.77
C	6	2	5.65	0.86	551.45
C	6	6	5.82	1.04	772.13
C	6	8	6.36	0.73	572.58
C	7	8	5.87	0.82	554.71
C	8	2	4.97	0.83	497.10
C	9	6	5.86	0.90	735.21
C	9	8	4.05	0.84	544.32
C	10	2	4.82	0.83	518.86
C	11	4	4.27	0.67	647.82
C	12	2	4.92	0.85	623.01
C	12	6	5.72	0.93	791.04
C	12	8	7.38	0.80	558.19
C	13	4	4.76	0.74	719.71
C	14	2	5.33	0.93	519.03
C	14	4	5.09	0.83	756.08

Blood Zinc, Copper and Iron Concentrations of the Controls (C)

TRT	ANIMAL #	DATE (WEEK)	Zn (ppm)	BLOOD Cu (ppm)	Fe (ppm)
D	1	4	3.98	0.67	674.08
D	2	6	5.27	0.90	870.14
D	3	2	3.94	0.95	590.40
D	3	4	4.83	1.07	678.83
D	4	2	4.13	0.79	594.72
D	5	6	5.68	0.86	868.92
D	5	8	4.79	0.64	580.76
D	6	6	4.71	0.82	804.35
D	6	8	4.45	0.65	578.14
D	7	2	5.19	0.80	616.22
D	8	2	4.63	0.63	601.69
D	8	4	3.99	0.83	611.62
D	9	6	5.06	0.80	809.69
D	9	8	4.44	0.68	557.62
D	10	8	4.55	0.68	578.68
D	11	2	4.34	0.77	611.04
D	11	4	4.69	1.03	641.94
D	12	2	3.96	0.86	594.36
D	12	6	5.75	0.96	792.36
D	12	8	4.05	0.75	566.89
D	13	2	3.74	0.85	631.28
D	13	8	7.38	0.62	576.19
D	14	4	4.68	0.74	783.01

Blood Concentrations of Zinc, Copper and Iron of the Zinc-Deficient Rats (D)

TRT	ANIMAL #	DATE (WEEK)	Zn (ppm)	BLOOD Cu (ppm)	Fe (ppm)
S	1	4	5.25	0.77	684.98
S	2	2	5.34	0.93	627.36
S	2	4	5.10	0.93	568.93
S	3	2	5.22	0.81	552.35
S	3	6	5.79	1.04	743.71
S	3	8	5.12	0.73	535.09
S	4	6	5.60	1.02	742.86
S	4	8	5.26	0.69	539.07
S	5	4	5.71	0.69	680.45
S	6	2	6.16	0.77	532.80
S	6	6	5.55	0.92	777.67
S	6	8	4.93	0.82	526.24
S	7	8	4.91	0.94	596.48
S	8	2	7.61	1.00	567.44
S	8	8	4.93	0.75	702.48
S	9	4	5.76	0.75	702.48
S	10	8	5.01	0.76	526.18
S	11	6	5.34	0.94	735.89
S	11	8	5.05	0.90	573.76
S	12	2	5.99	0.70	573.28
S	13	2	7.78	0.88	555.98
S	13	6	5.17	0.97	777.23
S	13	8	5.02	0.80	ND
S	14	2	6.75	0.80	527.26
S	14	4	5.54	0.76	747.01

Blood Concentrations of Zinc, Copper and Iron of the Zinc–Supplemented Rats (S)

TRT	ANIMAL #	DATE (WEEK)	PROTEIN CONTENT [mg/g] LIVER	KIDNEY	SPLEEN	HEART	LUNG
C	1	4	79.0	63.2	56.2	60.0	48.3
C	3	8	120.2	81.2	62.5	62.1	66.0
C	4	4	78.0	62.5	55.7	60.2	48.7
C	5	8	119.6	82.1	62.3	60.4	60.8
C	6	8	126.3	80.0	65.4	58.7	64.8
C	7	8	121.9	83.2	66.1	59.7	63.1
C	9	8	122.4	80.7	66.0	61.2	66.1
C	11	4	78.5	62.4	54.1	58.6	49.7
C	13	4	80.0	64.0	58.1	58.3	51.2
C	14	4	80.5	60.7	55.1	59.1	52.0

Organ Protein Concentrations of the Controls (C)

TRT	ANIMAL #	DATE (WEEK)	PROTEIN CONTENT [mg/g] LIVER	KIDNEY	SPLEEN	HEART	LUNG
D	1	4	66.1	52.2	40.7	40.8	30.8
D	3	4	64.3	53.4	44.3	46.1	36.2
D	5	8	114.1	70.1	44.6	60.1	66.0
D	6	8	104.7	72.8	48.2	54.5	60.8
D	7	8	108.2	72.3	49.3	48.7	66.2
D	8	4	63.7	50.1	44.1	48.2	33.1
D	10	8	106.2	71.3	45.2	55.7	64.7
D	11	4	63.3	51.7	40.7	44.3	30.1
D	12	8	104.9	70.8	45.5	52.8	64.6
D	14	4	65.2	54.1	41.8	45.3	34.2

Organ Protein Concentrations of the Zinc-Deficient (D)

TRT	ANIMAL #	DATE (WEEK)	LIVER	PROTEIN CONTENT [mg/g] KIDNEY	SPLEEN	HEART	LUNG
S	1	4	77.1	74.1	51.3	56.6	45.8
S	2	4	75.8	72.3	49.8	57.1	44.7
S	3	8	106.2	74.6	51.7	64.0	60.8
S	4	8	114.1	78.1	50.6	58.8	66.3
S	5	4	76.3	74.8	50.9	58.1	43.7
S	7	8	112.2	78.0	52.1	54.9	66.2
S	9	4	76.7	73.8	53.1	57.9	44.1
S	10	8	109.3	75.6	48.3	58.2	64.4
S	11	8	110.1	76.3	49.8	57.7	63.7
S	14	4	77.3	73.7	53.4	56.5	44.8

Organ Protein Concentrations of the Zinc-Supplemented Rats (S)

TRT	ANIMAL #	DATE (WEEK)	HEMOGLOBIN (g%)	HEMATOCRIT (%)
C	1	4	15.3	44.2
C	2	2	14.1	38.2
C	3	2	12.8	35.9
C	3	6	15.9	44.9
C	3	8	15.9	42.1
C	4	4	15.1	41.6
C	5	6	16.9	46.1
C	5	8	17.7	47.9
C	6	2	ND	ND
C	6	6	18.3	52.5
C	6	8	ND	ND
C	7	8	17.2	46.6
C	8	2	14.1	38.6
C	9	6	16.8	48.2
C	9	8	12.9	37.4
C	10	2	13.6	37.9
C	11	4	16.1	45.5
C	12	2	15.0	42.3
C	12	6	17.9	50.8
C	12	8	18.8	51.3
C	13	4	15.7	42.3
C	14	2	14.9	39.5
C	14	4	17.5	47.4

Hemoglobin Concentrations and Hematocrit Values of the Controls (C)

TRT	ANIMAL #	DATE (WEEK)	HEMOGLOBIN (g%)	HEMATOCRIT (%)
D	1	4	16.0	45.5
D	2	6	18.9	52.8
D	3	2	15.9	44.3
D	3	4	16.5	45.5
D	4	2	14.9	40.9
D	5	6	19.1	50.7
D	5	8	19.0	50.4
D	6	6	17.9	49.9
D	6	8	18.5	49.0
D	7	2	15.3	42.1
D	7	8	19.0	52.4
D	8	2	14.2	38.4
D	8	4	15.5	41.0
D	9	6	19.5	53.4
D	9	8	17.6	48.6
D	10	8	19.4	52.9
D	11	2	15.4	43.7
D	11	4	17.7	49.1
D	12	2	14.6	40.8
D	12	6	19.4	53.9
D	12	8	18.5	50.4
D	13	2	15.4	41.7
D	13	8	17.4	48.1
D	14	4	17.1	47.0

Hemoglobin Concentrations and Hematocrit Values
of the Zinc-Deficient Rats (D)

TRT	ANIMAL #	DATE (WEEK)	HEMOGLOBIN (g%)	HEMATOCRIT (%)
S	1	4	15.1	41.2
S	2	2	14.5	39.3
S	2	4	13.4	36.3
S	3	2	14.6	40.0
S	3	6	17.7	49.5
S	3	8	16.5	44.0
S	4	6	16.6	45.8
S	4	8	ND	ND
S	5	4	15.2	42.0
S	6	2	14.6	40.4
S	6	6	16.7	45.9
S	6	8	16.3	44.8
S	7	8	15.6	43.0
S	8	2	14.4	38.8
S	8	8	16.1	44.2
S	9	4	15.1	41.3
S	10	8	15.7	42.6
S	11	6	17.6	47.8
S	11	8	15.4	41.8
S	12	2	14.0	38.5
S	13	2	14.2	39.1
S	13	6	16.7	45.5
S	13	8	15.1	41.0
S	14	2	13.9	38.2
S	14	4	16.4	45.9

Hemoglobin Concentrations and Hematocrit Values
of the Zinc–Supplemented Rats (S)

TRT	ANIMAL #	DATE (WEEK)	ORGAN WET WEIGHT [g] LIVER	KIDNEY	SPLEEN	HEART	LUNG
C	1	4	8.5	0.80	0.5	0.9	1.3
C	3	8	8.4	0.74	0.7	0.9	1.3
C	4	4	7.7	0.80	0.4	0.8	1.3
C	5	8	6.9	0.86	0.6	0.8	1.2
C	6	8	9.2	0.88	0.5	0.8	1.0
C	7	8	7.2	0.84	0.6	0.8	1.4
C	9	8	8.6	0.94	0.7	0.9	1.8
C	11	4	7.4	0.65	0.5	0.8	1.3
C	12	8	7.5	0.90	0.7	0.9	1.3
C	13	4	6.7	0.65	0.4	0.6	1.0
C	14	4	7.2	0.75	0.5	0.7	1.4

Organ Wet Weight (g) of the Controls (C)

TRT	ANIMAL	DATE	ORGAN WET WEIGHT [g]				
	#	(WEEK)	LIVER	KIDNEY	SPLEEN	HEART	LUNG
D	1	4	7.0	0.65	0.6	0.6	0.9
D	3	4	7.2	0.60	0.5	0.6	0.9
D	5	8	4.3	0.56	0.3	0.5	1.0
D	6	8	5.9	0.66	0.3	0.6	0.9
D	7	8	7.3	0.71	0.4	0.7	0.8
D	8	4	5.4	0.50	0.4	0.7	0.8
D	9	8	6.7	0.66	0.3	0.6	0.9
D	10	8	7.8	0.79	0.5	0.9	1.1
D	11	4	4.7	0.55	0.3	0.5	0.8
D	12	8	7.2	0.80	0.4	0.8	1.1
D	13	8	4.5	0.61	0.3	0.6	0.8
D	14	4	6.6	0.50	0.4	0.7	0.9

Organ Wet Weight (g) of the Zinc-Deficient Rats (D)

TRT	ANIMAL #	DATE (WEEK)	ORGAN WET WEIGHT [g] LIVER	KIDNEY	SPLEEN	HEART	LUNG
S	1	4	9.4	0.75	0.5	0.7	1.0
S	2	4	8.0	0.80	0.5	0.7	1.3
S	3	8	8.7	0.89	0.7	0.7	1.2
S	4	8	8.2	0.87	0.6	0.8	1.2
S	5	4	8.5	0.75	0.4	0.8	1.2
S	6	8	7.3	0.77	0.4	0.6	1.0
S	7	8	9.5	0.70	0.6	0.7	1.1
S	8	8	8.1	0.83	0.5	0.7	1.1
S	9	4	8.4	0.75	0.4	0.8	1.1
S	10	8	6.9	0.72	0.5	0.7	1.1
S	11	8	8.1	0.94	0.5	0.8	1.0
S	13	8	7.5	0.90	0.4	0.7	1.1
S	14	4	7.7	0.70	0.4	0.8	1.1

Organ Wet Weight (g) of the Zinc–Supplemented Rats (S)

THE EFFECT OF VARIOUS DIETARY ZINC CONCENTRATIONS ON THE BIOLOGICAL INTERACTIONS OF ZINC-COPPER-IRON IN RATS

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A B S T R A C T

Forty-two, male, weanling Sprague-Dawley rats were divided into three groups of 14 rats each and pair-fed a purified basal diet containing different zinc concentrations for 8 weeks. Group C, was fed a control purified basal diet containing 12 ppm zinc, 5 ppm copper and 35 ppm iron; group D was fed the basal diet containing <2 ppm zinc; group S was fed the basal diet supplemented with 1000 ppm zinc. The effects of the various dietary zinc concentrations on growth rate, feed efficiency, blood hemoglobin and hematocrit, relative organ weights, and protein concentrations of tissues, and the zinc, copper and iron content of blood, liver, kidney, spleen, heart and lung were studied. Blood samples were collected at 2,4,6 and 8 weeks and tissues were sampled at 4 and 8 weeks. Rats fed the zinc-deficient diet (D) had decreased weight gain and showed signs of zinc deficiency at d 5, but polydipsia and intermittent mild diarrhoea were also prominent and led to dehydration. The decreased weight gain in these zinc-deficient rats (D) suggest decreased protein synthesis may be of zinc deficiency mediated decreased insulin activity. A cyclical pattern of decreased and then normal food intake and weight gain occurred in the zinc-supplemented rats (S) from weeks 6 to 8. Copper concentrations increased in heart and lung by week 4, and decreased in liver and lung at week 8 in the rats of zinc-deficient diet (D). In rats fed the

zinc-supplemented diet (S), copper concentrations decreased in liver and spleen and increased in kidney at week 4, and decreased in the liver and increased in the heart by week 8. Tissue iron concentrations in rats on the zinc-deficient diet (D) were consistently increased in all tissues, except liver, throughout the study. Rats receiving increased dietary zinc (S) generally had depressed iron concentrations in all tissues except heart. The fluctuations in tissues element concentrations with increased duration of the study is of variance with previous studies of shorter time frames. These data suggest an inconsistent antagonism between copper and zinc or other mechanisms may be involved in copper homeostasis. If tissue concentrations reflect intestinal uptake, an apparent competition and/or inhibition occurs between iron and zinc in the intestine with zinc deficiency but not in zinc supplementation. The relative dietary proportions of zinc, copper and iron and time effect influence zinc, copper and iron metabolism at the intestinal and cellular transport level.

Keywords:

Zinc, copper, iron, dietary element and interaction, rats, zinc deficiency, zinc supplementation, weight gain, feed effeciency, total protein, hemgolobin, hematocrit,oragn weight, tissue concentrations.