CHAPTER II

LITERATURES REVIEW

The biological importance of zinc was first recognized when Raulin, in 1869, demonstrated that it was necessary for growth of Aspergillus niger (10). Subsequently, the ubiquitous distribution of zinc in nature was appreciated and the essentiality of zinc for higher plants, animals and men was also established.

The interest in zinc in human nutrition followed the exciting discoveries of Pories and Strain on the relation of zinc to wound healing (11), and of Prasad and co-workers on the relation of zinc deficiency to the occurrence of dwarfism and hypogonadism in malnourished adolescent boys in Iran and Egypt (11,12). A more profound deficiency state became apparent when Moynahan and Barnes, in 1973, showed that treatment with zinc induced a complete and rapid clinical remission in patient with acrodermatitis enteropathica (10, 13,14). There is now evidence that not only can zinc deficiency disease be found in malnourished populations, but also that disease and physical injury can alter zinc metabolism and excretion (12).

Zinc is essential for the activity of at least 90 enzymes participating in all the major metabolic pathways. Over 40 metalloenzymes exist in which zinc

is bound tightly to the apoenzyme in specific stoichiometric ratio and served one or more structural, regulatory, or catalytic functions (10). These include carbonic anhydrase, alkaline phosphatase, lactic dehydrogenase and carboxypeptidase. Zinc functions by maintaining spatial configurational relationships necessary for enzymatic action. In this role it helps to bind enzymes to substrates and may modify the molecular shape of enzymes by simutaneously combining with amino acids at different places on the protein, thus affecting secondary, tertiary and quaternary protein structure. A number of zinc metalloenzymes are involved in the regulation of cellular growth (12).

In addition to its function in enzyme systems, zinc participates in the metabolism of nucleic acids and the synthesis of proteins. Although its role is not completely understood, zinc appears to be an integral part of the RNA molecule of a number of species and is thought to help maintain stable molecular configuration. Zinc may also have an important role in cell division since zinc deficiency causes adverse effects on the incorporation of thymidine into the DNA of rats, Zinc is required for DNA synthesis, and the DNA-dependent RNA polymerase is a zinc dependent enzyme, as is thymidine kinase (12). There is evidence that zinc is important in fatty acid metabolism. Zinc-deficient animals have impaired collagen synthesis and poor wound healing.

Some evidence suggests that zinc deficiency alters the quantity and type of collagen cross links (10).

Zinc may also play an important role in taste and endocrine function. It has been suggested by Henkin that gustin and nerve growth factor which have been isolated from human and murine saliva respectively, are zinc metallo-proteins. They appear to combine with their respective taste buds and to be essential for their normal morphology and optimum function. Zinc influences insulin binding and degradation at the hepatocyte plasma membrane (10,15).

The role of zinc in reproduction has been suggested from animal studies in which a lack of zinc was associated with failure to maintain pregnancy and the development of fetal malformations such as missing digits or cleft palate, decreased fetal brain weight and behavioral abnormalities in the offspring (16,17).

Zinc absorption occurs mainly in the small intestine but most rapidly in the duodenum and proximal jejunum (10). The absorption of zinc is thought to be under homeostatic control, with increased total body zinc stores causing decreased absorption and vice versa. Zinc binding ligand (ZBL) which is present in human milk, is thought to facilitate the incorporation of zinc into the intestinal mucosa (10,18). Zinc-metallothionein (Zn-MTN), which has been identified in the

liver and small intestinal mucosa is also participated in the homeostatic regulation of zinc metabolism and absorption. It also provides a source of zinc at times of deprivation. Yet, the relationship between ZBL and intestinal Zn-MTN has not been clarified (10).

Zinc absorption is affected by level of zinc in the diet, chemical form in which zinc in ingested, and the presence of interfering substances (19,20,21). Zinc is equally well absorbed as the oxide, carbonate, sulfate or metal. Insoluble complex formed with phytic acid prevents zinc absorption. Both calcium and phosphorus depress zinc absorption. A mutual antagonism is apparent between zinc and copper during the absorptive process, taking place at the intestinal epithelium (20). Rats treated with vitamin D were observed to absorb more dietary zinc; however, this effect may be the result of increased need secondary to increased skeletal growth, rather than improvement in absorption (12).

Excretion of zinc in normal individuals is almost solely via the feces. Small amount of zinc is also found in urine and sweat. Urinary zinc excretion is less than 600 μg/day. The zinc concentration in neonatal urine may be 5 times that of the adult (10). Increased urinary excretion has been reported in patients with nephrosis, diabetes, alcoholism, hepatic cirrhosis and porphyria (12, 22).

Significant quantity of zinc can be lost in sweat, especially in hot climate where the volume of sweat may rise to 5 or more liters/day. The sweat of normal individuals was reported to contain an average of 1.15 ± 0.30 µg/ml Zn. The mean zinc level in the whole sweat of Zn-deficient patients was reduced to 0.6 ± 0.27 µg/ml. On this basis, a normal individual secreting 5 liters of sweat per day could lose 5 mg Zn/day and a Zn-deficient individual about 2 mg/day. In temperate climate the losses of zinc by this route would, of cause, be substantially smaller (20). There was considerable variation in the amount of zinc lost in sweat. The surface losses of zinc in preadolescent children and in the adult males were reported to be 0.14 mg/day and 0.5 mg/day respectively (23). Recently, Milne et al.(24) reported that the average whole body surface loss of zinc in men was 0.49 mg/day when average zinc intake was 8.3 mg/day and was 0.24 mg/day when average zinc intake was 3.6 mg/day.

A 70 kg man contains 1.4-2.3 g of zinc and a term neonate has about 60 mg (10). The liver, pancreas kidney, bone and voluntary muscles have the largest concentration. Other tissues with high concentrations include various parts of the eye, prostate gland, spermatozoa, skin, hair, fingernails and toenails. Plasma values average around 100 µg% (S.D.± 10 µg%) Women have lower values than men and concentrations in

children are lower than in adults. Red and white blood cells contain 6 to 8 times as much as the plasma (12). The zinc content of serum is 16% higher than that of plasma as the result of the release of zinc from platelets and unavoidable haemolysis. Plasma zinc level falls within hours of stress, such as surgery, trauma, inflammation and remains low with chronic stress (10).

Zinc deficiency may result from an inadequate dietary intake, malabsorption, increased body losses and intravenous feeding. Inadequate dietary intake results from protein-calorie malnutrition, vegeterianism, patients on protein-restricted diets, synthetic diets and weight reducing diets (10,25). Malabsorption may happen in patients with acrodermatitis enteropathica, celiac disease and other enteropathies, pancreatic insufficiency and immaturity of absorptive systems. Increased body losses may occur in a wide variety of conditions, such as starvation, burns, diabetes mellitus, diuretic treatment, proteinuria, hepatic disease, porphyria, chronic blood loss, parasitic infection, dialysis, and excessive sweating. Prolonged intravenous feeding carries a risk of zinc deficiency, that is, in part, secondary to the variable but low content of zinc in the administered solutions (10,26,27) and to the excessive urinary losses of zinc complexed with carbohydrates or amino acids, or both.

The clinical picture of zinc deficiency is most prominent in growing children. Symptoms include poor growth, loss of appetite, hypogonadism in males and loss of the senses of taste and smell (28). If the patient has had a surgical procedure, wound healing may be delayed (12). Cutaneous signs range from a rough skin to a severe eczematous dermatitis affecting the digits, perineum, mouth, and nasolabial folds (10).

The teratogenic effect of zinc deficiency in human has not been demonstrated; however, there are evidences demonstrating the effect in animals. Ingestion of a zinc deficient diet by rats during pregnancy results in a number of congenital anomalies, such as tail anomalies, clubfoot, syndactyly, hydrocephalus, scoliosis, and kyphosis (12,17). There is sufficient circumstantial evidence suggesting a relationship between maternal zinc status and congenital anomalies to urge further careful study of this problem.

Illnesses in which both serum and erythrocyte levels of zinc decreased include cirrhosis of the liver, hepatitis, nephrosis, malabsorption syndromes, chronic infectious diseases, myocardial infarction and hypothyroidism.

Deficiency of zinc has been associated with alterations in taste. Treatment with zinc, nickel or copper results in an improvement in taste perception.

Children with low hair zinc content and poor growth, who also had the loss of taste and smell showed reversal of the abnormalities following zinc supplementation (12).

Apparently, zinc toxicity is rare. It may result from inhalation of zinc, from excessive intakes when galvanized utensils are used in processing acid foods, or when metal containers are used for carbonated beverages. It may also result from home dialysis, sucking zinc alloy toys, prolonged oral zinc supplements and intravenous overdosage. Symptoms include anorexia, nausea, vomiting, lethargy, dizziness,diarrhea, and bleeding gastric erosions (10,12).

Dietary zinc requirements depend upon ages, functional activity, body stress, nature of the diet consumed and environment (29). An adult normally consumes 10 - 15 mg of dietary zinc a day (10). The following age-and sex-related daily dietary allowance have been recommended by the Food and Nutrition Board, National Academy of Science-National Research Council to be 3 mg for infants 0-6 months, 10 mg for children 1-10 years, 15 mg for adolescent children, adult men, and nonpregnant women, 20 mg for pregnant women, and 25 mg for lactating mothers (30).

Zinc should come from a balance diet that contains sufficient animal protein (12). Good dietary sources of zinc are meats, seafoods, whole cereals,

leguminous seeds and eggs (31). Among these, oysters the richest sources, may contain over 1000 µg/g Zn. White sugar, pome and citrus fruits are among the lowest in zinc content (31). Although cereals and vegetables have similar contents as compare to meats the bioavailability of zinc from plants sources is reduced by their high phytate (inositol hexaphosphate) content. Phytate may form complex with zinc and prevents its absorption. Complexation also occurs with fiber, hemicellulose, and clay. Both calcium and phosphate reduce the utilization of dietary zinc (10). The level and the bioavailability of zinc from food-stuffs may be altered during food preparations. Up to 80% of zinc in whole grain cereals can be lost during milling. For instance, white flours milled from North American hard wheat blends averaged 7.8 µg/g Zn, compared with 35 µg/g in the original wheats. Similar flour from Australian soft wheats averaged only 5 µg/g Zn, compared with 16 µg/g in the original wheats (31). Polished rice contains an average of 13.7 µg/g Zn, compared with an average of 16.4 µg/g Zn in unpolished rice (32).

The concentration of zinc in milk is relatively high. The reported values for cow's, ewe's and human milk lie between 3-5 µg/ml (33). Zinc concentration in milk is highest as in colostrum and decreasing at successive stages of lactation (34). Human milk contains a low molecular weight zinc binding ligand (ZBL) which,

probably, facilitates absorption. Unfortunately, such a ligand is not present in cow's milk, therefore, zinc available from this source, to the human infant, may be reduced (10).

Zinc content in daily diet varies a great deal, depending on the compositions of the diet. Diet rich in seafoods, for example, provides more zinc content than diet rich in vegetables. The American adult consuming a mixed diet, has an average intake of 10 to 15 mg Zn (12). The difference of zinc content not only depends on the compositions of diet, but also on the methods of preparation, environmental pollution and sources of food. The influence of fertilizers, soil conditions and levels of minerals in the soil on the concentration of zinc in plants has also been reported (31,35).

Copper was shown to be an essential nutrient for rats by Hart et al. in 1928. Since then, many metabolic functions, the adverse effects of copper deficiency and the interactions of copper with both essential and toxic elements have been characterized in experimental animals, farm animals and humans (36).

Copper is also a component of various enzyme systems. It is involved in such diverse functions as iron metabolism (ceruloplasmin), mitochondrial energy generation (cytochrome c), crosslinking of collagen

and elastin (lysyl oxidase), and normal central nervous system function (dopamine beta hydroxylase) (37).

Hypochromic microcytic anemia, identical to iron deficiency anemia, occurred from copper deficiency, is probably due to a disruption of iron metabolism. Normally, ceruloplasmin, the major form of copper in the blood, may aid the mobilization of iron from ferritin in the liver and other iron storage sites. The bone of copper deficient chicks contain higher than normal proportion of soluble collagen, and collagen crosslinking is impaired. Failure of the crosslink formation of collagen and elastin accounts for greater fragility and associated abnormalities. Cardiac failure, reported in animals raised on a copper deficient diet, may also result from failure of collagen and elastin crosslinking or from, as yet, an undefined muscular defect (4,38).

Copper deficiency affects the developing central nervous system. The disease described in subprimate species is manifested by locomotion incoordination associated with a lack of myelination of the spinal cord.

The animals with pigmented hair, wool or feathers, copper deficiency results in failure of melanin formation. Tyrosinase, a cuproenzyme, catalyzes the hydroxylation of tyrosine to DOPA (3,4,dihydroxyphenylalanine) and the oxidation of DOPA to a

quinone gives rise to melanin (4).

Copper is absorbed from the stomach and upper gut by at least two mechanisms. One mechanism, facilitated by amino acids, is an energy dependent process and may represent the absorption of copper complexes of amino acids. A smaller portion is absorbed by this mechanism. The bulk of the copper is absorbed by the second mechanism involving the binding to two protein fractions of the small intestine (4). Transition elements such as cadmium, zinc and molybdenum alter or interfere with copper metabolism as well as sulfate (39,40), ascorbic acid and phytate (41,42).

Absorbed copper appears in the blood stream within 15 minutes after ingestion (38). Copper concentrations are higher in serum than in plasma, and values depend on copper intakes. Average plasma values for women range from 87 to 153 µg% with a mean value of 120 µg%; for men the range is 89 to 137 µg% with a mean of 109 µg%. Approximately 90% of the copper in the plasma is bound to ceruloplasmin; the rest is bound to albumin (4).

Copper is excreted by two pathways. Fecal copper represents unabsorbed, dietary copper and copper released in the bile and that lost through the intestinal wall in the albumin-copper complex of the serum. Urinary copper accounts for a mere 4% of copper excreted (38). Small

amount of copper are also present in sweat and menstrual flow. Recently, Jacob et al. (23), reported that surface loss of copper was 0.34 mg/day. This accounted to the increase in copper requirement up to about 25-30% of normal daily requirement.

The distribution of the total body copper among the tissues varies with the species, ages, and copper status of the animals. An adult human body of 70 kg contains 80 to 120 mg of copper. Concentrations are highest in brain, liver, heart and kidney. Bone and muscle have lower concentrations but they contain one-half of the total because of their large mass.

The concentration of copper is greatest in the newborn and decreasing during the first year of life. Infants have an exceptional requirement of about 0.08 mg/kg of body weight. Older children need only half of this amount, and for adults, 0.03 mg/kg is sufficient (4).

Copper deficiency has not been reported in humans consuming a varied diet. Low serum copper values have been reported in patients receiving total parenteral nutrition (TPN) solutions without copper supplementation. Recommended copper supplementation in TPN solution is about 0.5 - 1.5 mg/day (26). Low serum copper and ceruloplasmin provide supportive evidence of copper deficiency. With the resumption of oral feeding, serum copper rises rapidly.

Three deficiency syndromes have been recognized in infants (4). One is manifested by moderate to severe anemia in infants whose diet is based on cow's milk. For complete recovery, therapy with both copper and iron is required since serum levels of both are low. The second syndrome is associated with chronic malnutrition and diarrhea. The use of modified cow's milk to alleviate the malnutrition contributed in some cases to the development of anemia. The third syndrome, Menke's kinky hair syndrome, is a sex-linked recessive defect of copper absorption. The infants have retarded growth, defective keratinization and pigmentation of the hair, hypothermia, degenerative changes in aortic elastin, abnormalities of the metaphyses of long bone and progressive mental deterioration. Brain tissue is practically devoid of cytochrome c oxidase, a cuproenzyme, and there is a marked accumulation of copper in the intestinal mucosa. Parenteral administration of copper result in transient improvement.

Decreased plasma copper is seen in patients with several malabsorption diseases such as celiac disease, tropical and non-tropical sprue, protein-losing enteropathies and nephrotic syndromes. Decreased serum copper, as well as decreased zinc, may result in alterations of taste perception.

Copper is toxic to man when it exists as the

unbound copper ion. In this way it acts as an inhibitor to many enzyme systems (43). There is no evidence of toxicity from environmental contamination other than from the industrial copper mining or processing (44). The ingestion of copper salts at levels ten times that found in normal diet leads to nausea and vomiting, possibly as a result of a disturbance of the balance between absorption and excretion (43).

Hypercupremia is developed in some patients with acute and chronic infections, patients with liver disease and patients with pellagra (4). In Wilson's disease, the excessive concentrations of copper in tissues, arises from metabolic defects involving absorbed copper, and not from the ingestion of excessive amount of copper (44).

While sufficient data are not available to set an RDA, copper intakes of 1.3 and 2 mg/day appear to maintain balance in adolescent girls and adults, respectively (4).

Copper is widely distributed and most diets provide about 2 mg/day (4). Foods high in copper are liver, kidney, oysters, chocolate, nuts, dried legumes, cereals, dried fruits, poultry, shellfish and animal tissues. Milk is, as poor in copper as it is in iron, containing 0.015 to 0.18 mg/liter (4,43). Daily amount of copper ingested from the diet is determined mainly

by the choices or selections of food making up the total diet. It is also influenced by the locality in which the foods are grown and by the extent of contamination with adventitious copper during processing, storage, and treatment with copper fungicides and anthelmintics. The refining of cereals results in a significant loss of copper, as with most other minerals. The mean copper content of the whole grain of North America hard wheats was reported to be 5.3 μg/g, whereas the copper content of the white flour made from these wheats averaged only 1.7 μg/g (45). Copper content of unpolished rice is about 4 μg/g while polished rice is about 3 μg/g (32).

The relationships of zinc as well as of copper or of both elements in cholesterol metabolism, have been presented by several reports. Burch et al. reported that the concentration of cholesterol in the serum of zinc-deficient pigs was lower than of normal pigs. Patel et al. found that the concentration of cholesterol in the serum of zinc-deficient rats was 44% of the control value (5). It was reported that oral ingestion of pharmacological doses of zinc lowered high-density lipoprotein-cholesterol (HDL-cholesterol) in man. Then, it was hypothesized that such a decrement in HDL-cholesterol could be associated with the atherogenic process. Freeland-Graves et al. (48) reported that pharmacological doses of zinc reduced HDL-cholesterol in woman, but the

reduction was transient and not dose-related. Koo et al. (49) demonstrated that an acute depletion of zinc in adult male rats produces a hypocholesterolemia and that the hypocholesterolemia is primarily due to a selective decline in high-density lipoprotein (HDL) fraction. They also demonstrated that cholesterol-fed rats have significant decreases in the serum level of HDL cholesterol and zinc. Hypercholesterolemia was produced in rats deficient in copper (5). Klevay (6,7) also suggested that a metabolic imbalance produced by either a relative or absolute deficiency of copper, characterized by a high ratio of zinc to copper, results in hypercholesterolemia. This hypercholesterolemia, in turns, leads to coronary heart disease. Fischer et al. (46), however, convinced that the dietary copper and zinc, at levels likely to occur in a normal mixed diet, are not significant factors in cholesterol metabolism. Recently, Koo et al. (47) indicated a significant correlation between the ratio of zinc to copper and total serum cholesterol, which was solely dependent upon serum zinc status rather than serum copper. Whether high ratio of zinc to copper, absolute or relative deficiency of copper or the level of zinc alone associated with serum cholesterol, is waiting for further research.