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CLINICAL, BIOCHEMICAL, AND PHARMACOLOGICAL ROLE OF ZINC

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INTRODUCTION

Although zinc had been known to be essential to the growth of microorganisms for over one hundred years, it was not until 1934 that zinc was shown to be necessary for the growth and well-being of the rat (1, 2). Clinical manifestations in zinc-deficient animals include growth retardation, testicular atrophy, skin changes, and poor appetite.

In 1961, it was first suspected that zinc deficiency may occur in man (3); this was confirmed in 1963 (4–6). In studies from Iran (7, 8), it was clearly demonstrated that zinc is a principal limiting factor in the nutrition of children and adolescents and that this probably accounted for growth retardation so commonly seen there.

Recent reports indicate that marginal deficiency of zinc in man is probably widespread and common throughout the world including the USA (9, 10). It is also evident that not only nutritional deficiency but also conditioned deficiency of zinc may complicate many diseased states.

In recent years, many biochemical and physiological roles of zinc have been reported. The purpose of this paper is to elucidate the clinical, biochemical, and pharmacological role of zinc in human subjects.

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ETIOLOGY OF ZINC DEFICIENCY

Nutritional

In the fall of 1958, a 21-year-old patient at Saadi Hospital, Shiraz, Iran, who looked like a 10-year-old boy, was brought to my attention. In addition to dwarfism, he had severe anemia, hypogonadism, hepatosplenomegaly, rough and dry skin, mental lethargy, and geophagia (3). The nutritional history was interesting in that he ate only bread made of flour. The intake of animal protein was negligible. He also consumed nearly one pound of clay daily. The habit of geophagia (clay-eating) is not uncommon in the villages around Shiraz. During a short period of time, we found ten additional similar cases.

Although there was no evidence for blood loss, the anemia was due to iron deficiency. We concluded that this may have been due to a lack of availability of iron from the bread, greater iron loss due to excessive sweating in the hot climate, and the adverse effect of geophagia on iron absorption. In every case the anemia was completely corrected by oral administration of iron.

It was difficult to explain all of the clinical features solely on the basis of tissue iron deficiency, inasmuch as growth retardation and testicular atrophy are not seen in iron-deficient experimental animals. The possibility of a zinc deficiency was considered. Zinc deficiency was known to produce growth retardation, testicular atrophy, and skin changes in animals. Inasmuch as heavy metals may form insoluble complexes with phosphates, we speculated that some factors responsible for decreased availability of iron in these patients may also adversely affect the availability of zinc.

Subsequently, similar patients were encountered in Egypt (4–6). Their dietary history was also similar, except that geophagia was not documented. These subjects were documented to have zinc deficiency. This conclusion was based on the following evidence: 1. The zinc concentrations in plasma, red cells, and hair were decreased. 2. Radioactive zinc-65 studies revealed that the plasma zinc turnover rate was greater, the 24-hour exchangeable pool was smaller, and the excretion of zinc-65 in stool and urine was less in the patients than in the control subjects.

Further studies in Egypt showed that the rate of growth was greater in patients who received supplemental zinc as compared to those who received iron instead or those receiving only animal protein diet consisting of bread, beans, lamb, chicken, eggs, and vegetables (6, 11). Pubic hair appeared in all cases within 7 to 12 weeks after zinc supplementation was initiated. Genitalia size became normal, and secondary sexual characteristics developed within 12 to 24 weeks in all subjects receiving zinc. On the other hand, no such changes were observed in a comparable length of time in the

iron-supplemented group or in the group on an animal protein diet alone. Thus, the growth retardation and gonadal hypofunction in these subjects was related to zinc deficiency.

In the Middle East, excessive intake of phytate, which is known to decrease the availability to zinc for absorption, was considered to be a principal factor responsible for zinc deficiency. Excessive loss of zinc by sweating and blood loss due to parasitic infestations in Egypt were considered to be additional factors accounting for zinc deficiency in human subjects.

In 1966, Coble et al (12) reported a follow-up study of patients with dwarfism and hypogonadism from Kharga Oasis, originally studied by Prasad et al (13) in 1963. Three years later, a majority of the dwarfs studied showed an increase in growth and gonadal development without any specific treatment. The plasma zinc concentrations in these patients in 1965 were not altered compared to their levels in 1962. The authors concluded that these cases merely represented examples of delayed maturation. In another paper, Coble et al (14) demonstrated low plasma zinc levels in normal rural male Egyptians. These results were interpreted to show a lack of relationship between growth and status of zinc in the human body, and the essentiality of zinc in human nutrition was questioned. It is clear from the reports of Coble et al (12, 14) that those dwarfs from Kharga oasis who showed increased growth and hypozincemia demonstrated delayed maturation, and up to the ages of 18–19 failed to attain heights that one would expect to see in Egyptians of an upper socioeconomic level and normal Americans who showed normal plasma zinc levels. The same was true for "normal" rural Egyptian males who showed delayed maturation and smaller ultimate statures compared to their upper socioeconomic counterparts in Cairo. Racially and culturally speaking, the normal subjects from Cairo, belonging to an upper socioeconomic group, are the same as rural "normals." Thus, the so-called rural normals had abnormal growth patterns. Therefore, these observations could be interpreted to show that a low plasma zinc level indeed correlated with slower growth rate and delayed maturation as reported by Coble, Schulert & Farid (12) and Coble et al (14).

Further studies in Iran clearly demonstrated that zinc is a principal limiting factor in the nutrition of children (7). It was also evident from several studies conducted in the Middle East that the requirement of zinc under different dietary conditions varied widely. For instance, in the studies reported by Prasad et al (4–6) and Sandstead et al (11), 18 mg of supplemental zinc with a diet containing adequate animal protein and calories was sufficient to produce a definite response with respect to growth and gonads but in studies where the subjects continued to eat the village diet, up to 40 mg of zinc supplement were required to show some growth effect (7).

Halsted et al (8) published results of their study in a group of 15 men who were rejected at the Iranian Army Induction Center because of "malnutrition." A unique feature was that all were 19 or 20 years of age. Their clinical features were similar to those of zinc-deficient dwarfs reported earlier by Prasad et al (3–6). They were studied for 6 to 12 months. One group was given a well-balanced nutritious diet containing ample animal protein plus a placebo capsule. A second group was given a capsule of zinc sulfate containing 27 mg of zinc. A third group was given the same diet plus the diet alone without additional medication for six months, followed by the diet plus zinc for another six-month period. The development in subjects receiving the diet alone was slow and the effect on height increment and onset of sexual function was strikingly enhanced in those receiving zinc. The zinc-supplemented boys gained considerably more height than those receiving ample protein diet alone. The zinc-supplemented subjects showed evidence of early onset of sexual function, as defined by nocturnal emission in males and menarchy in females. The two women described in this report were from the hospital clinic and represented the first cases of dwarfism in females due to zinc deficiency (8).

A brief mention should be made regarding the prevalence of nutritional zinc deficiency in human populations throughout the world. Clinical pictures similar to those reported by us in zinc-deficiency dwarfs have been observed in many countries such as Turkey, Portugal, and Morocco. Also, zinc deficiency should be prevalent in other countries where primarily cereal proteins are consumed by the population.

Research on the nutritional status of zinc in infants and young children has been very limited. However, the importance of adequate zinc nutrition in the young is apparent from data on other mammals. Dietary zinc requirements are relatively high in the young as compared with those of mature animals of the same species, and the effects of dietary insufficiency are particularly severe. Furthermore, several of the major features of zinc deficiency, such as growth retardation and impaired learning ability, are peculiar to the young animal.

A clinical syndrome similar to that of "adolescent nutritional dwarfism" has been identified in younger children in Iran, though failure of sexual maturation is not evident prior to adolescence. This syndrome is most common in small rural communities in which there is also a high incidence of adolescent nutritional dwarfism.

Nutritional Zinc Deficiency in Children in the United States

In 1972, symptomatic zinc deficiency was reported in a number of Denver children (10). These children were identified as a result of a survey of trace element concentrations in the hair of apparently normal children from middle and upper income families.

There was no apparent cause of the relatively poor growth in the majority of children with low hair zinc. Growth retardation is one of the earliest manifestations of zinc deficiency in the young animal, and the correlation between the low hair zinc levels and low growth percentiles in these children suggested a causal relationship.

Anorexia is another prominent and early feature of zinc deficiency in animals, and most of the children with low hair zinc levels in the original Denver study also had a history of poor appetite. In particular, the consumption of meats was very limited despite access to larger quantities. As animal products are the best source of available zinc, it is quite possible that the dietary zinc intake was inadequate.

It has been calculated recently that substantial sections of the population of the United States are at risk from suboptimal zinc nutrition. Those at particular risk from a deficiency of this metal include subjects whose zinc requirements are relatively high, for example, at times of rapid growth, and people subsisting on low income diets. Although a severe or moderately severe deficiency of zinc on nutritional basis is unlikely to be seen in the developed countries, a marginal deficiency may occur frequently.

Zinc Nutrition and Deficiency in Infants

Hair and plasma zinc levels are exceptionally low in infants in the United States compared to other age groups including the neonate, older children, and adults. It appears unlikely that these low levels can be regarded as entirely normal for this age, because levels are not equally low in other countries where comparable data have been obtained (15). Similar considerations apply equally to the low plasma zinc levels reported for infants in this country. Plasma concentrations in infants in Sweden and Germany have been reported to be no lower than those for adults in these countries.

Several factors, including difficulty in achieving positive zinc balance in early postnatal life and a "dilutional" effect of rapid growth, may contribute to zinc depletion in infants. A unique factor in the United States that may contribute to zinc deficiency is the low concentration of this metal in certain popular infant milk formulas. Zinc supplementation of all of these formulas has not been routine, though it is likely to become a universal practice in the near future, following the recent recommendations of the Food and Nutrition Board of the National Academy of Sciences with respect to zinc intake in infants.

The majority of infants with low levels of zinc in plasma and hair have not had any detectable signs of zinc deficiency. However, it appears that those at the lower end of a spectrum of zinc depletion, as manifested by low levels of hair and plasma zinc, and perhaps those who remain moderately depleted for a prolonged period of time, do develop symptomatic zinc deficiency (15). In the original Denver study, 8 of 93 infants and children

aged less than 4 years had hair zinc levels less than 30 ppm, and 6 of these manifested declining growth percentiles and poor appetite. It was noted also that the high percentiles of the older children with hair zinc levels less than 70 ppm first declined during infancy; thus, if their poor growth resulted from an insufficiency of this nutrient, the latter must have commenced during infancy. It is conceivable that once a deficiency state has been established, the resulting anorexia may tend to perpetuate the deficiency state. Experience with a number of infants who had low levels of zinc in plasma and hair, and who have responded favorably to zinc supplementation, indicates that some cases of failure to thrive in infancy may be caused by zinc deficiency. Anorexia has been a prominent feature in these infants, and one case manifested a bizarre form of pica which improved dramatically following zinc supplementation (15). Currently, however, there is a lack of controlled studies of dietary zinc supplementation in these infants.

OTHER CAUSES OF ZINC DEFICIENCY

Alcohol

Alcohol induces hyperzincuria (16). The mechanism is unknown. A direct effect of alcohol on the renal tubular epithelium may be responsible for hyperzincuria. Acute ingestion of alcohol did not induce zincuria in some experiments (17); however, Gudbjarnason & Prasad (16) reported increased urinary zinc excretion following alcohol intake. This effect was evident when complete urine collection was analyzed for zinc during first 3-hr and second 3-hr periods, following ingestion of 6 oz of chilled vodka.

The serum zinc level of the alcoholic subjects tends to be lower in comparison to the controls. An absolute increase in renal clearance of zinc in the alcoholics demonstrable at both normal level and low serum zinc concentration has been observed (18). Thus the measurement of renal clearance of zinc may be clinically utilized for etiological classification of chronic liver disease due to alcohol in different cases. Excessive ingestion of alcohol may lead to severe deficiency of zinc (19).

Liver Disease

Vallee et al (20) initially described the abnormal zinc metabolism that occurs in patients with cirrhosis of the liver. These investigators demonstrated that patients with cirrhosis had low serum zinc, diminished hepatic zinc, and paradoxically, hyperzincuria. These observations led them to suggest that zinc deficiency in the alcoholic cirrhotic patient may be a conditioned deficiency that was somehow related to alcohol ingestion. These observations have been confirmed by other investigators.

Saldeen & Brunk (21) have shown that parenteral zinc salts protect rat liver from damage by carbon tetrachloride. These studies suggest that zinc exerts a protective effect on the liver.

Gastrointestinal Disorders

Zinc deficiency has been reported in patients with steatorrhea (9, 22). In an alkaline environment, zinc would be expected to form insoluble complexes with fat and phosphate analogous to those formed by calcium and magnesium. Thus, fat malabsorption due to any cause should result in an increased loss of zinc in the stool.

Exudation of large amounts of zinc protein complexes into the intestinal lumen may also contribute to the decrease in plasma zinc concentrations that occur in patients with inflammatory disease of the bowel. It seems likely that protein-losing enteropathy due to other causes may also impair zinc homeostasis. Another potential cause of negative zinc balance is a massive loss of intestinal secretions.

Renal Diseases

The potential causes of conditioned deficiency of zinc in patients with renal diseases include proteinuria and failure of tubular reabsorption (9). In the former instance, the loss of zinc-protein complexes across the glomerulus is the mechanism. In the latter an impairment in the metabolic machinery of tubular reabsorption due to a genetic abnormality or toxic substances would result in zinc loss. While low plasma zinc concentrations have been described in patients with massive proteinuria, no reports of low plasma levels of zinc in patients with tubular reabsorption defects have appeared in the literature.

In patients with renal failure, the occurrence of conditioned zinc deficiency may be the result of a mixture of factors which at present are ill defined. If 1,25-dihydroxycholecalciferol plays a role in the intestinal absorption of zinc, an impairment in its formation by the diseased kidney would be expected to result in malabsorption of zinc. It seems likely that plasma and soft tissue concentrations of zinc may be "protected" in some individuals with renal failure by the dissolution of bone that occurs as a result of increased parathyroid activity in response to low serum calcium. In experimental animals, calcium deficiency has been shown to cause release of zinc from bone. In some patients who are successfully treated for hyperphosphatemia and hypocalcemia, the plasma zinc concentration may be expected to decline because of the deposition of zinc along with calcium in bone. Thus, in the latter group in particular, a diet low in protein and high in refined cereal products and fat would be expected to contribute to a conditioned deficiency of zinc. Such diet would be low in zinc. The patients

reported by Mansouri et al (23) who were treated with a diet containing 20–30 g of protein daily and who had low plasma concentrations of zinc, appear to represent such a clinical instance. Presumably the patients of Halsted & Smith (24) were similarly restricted in dietary protein. In other patients with renal failure whose dietary protein was not restricted, plasma zinc concentrations were not decreased. Patients on dialysis had even higher levels, particularly following dialysis. Apparently, zinc deficiency may not be a problem in patients on dialysis if their dietary consumption of protein is not restricted.

Neoplastic Diseases

The occurrence of conditioned deficiency of zinc in patients with neoplastic diseases will obviously depend upon the nature of the neoplasm. Anorexia and starvation, plus avoidance of foods rich in available zinc, are probably important conditioning factors. An increased excretion of zinc subsequent to its mobilization by leukocyte endogenous mediator (LEM) in response to tissue necrosis may be another factor.

Burns and Skin Disorders

The causes of zinc deficiency in patients with burns include losses in exudates. Starvation of patients with burns is a well-recognized cause of morbidity and mortality. The contribution of conditioned zinc deficiency to the morbidity of burned patients is not defined. Limited studies indicate that epithelialization of burns may be improved by treatment with zinc. Such a finding is consistent with the beneficial effect of zinc on the treatment of leg ulcers and the well-defined requirement of zinc for collagen synthesis (25–28).

In psoriasis, the loss of large number of skin cells may possibly result in zinc depletion. The skin contains approximately 20% of the body zinc. Thus, if the loss of epithelial cells is great enough, it is conceivable that the massive formation of new cells by the skin could lead to conditioned deficiency. Low levels of plasma zinc have been reported in some patients with extensive psoriasis (29, 30); however, others have not been able to confirm these findings (31, 32).

Parasitic Infestations

Blood loss due to parasitic diseases may contribute to conditioned deficiency of zinc. Such appears to have been the case in the zinc-responsive "dwarfs" reported from Egypt (4–6). As red blood cells contain 12–14 μg of zinc/ml, infections with hookworm and/or schistosomiasis that are severe enough to cause iron deficiency will probably contribute to the occurrence of zinc deficiency.

Iatrogenic Causes

Possible iatrogenic causes of conditioned deficiency of zinc include use of antimetabolites and antianabolic drugs. Treatment with some of these drugs make patients feel ill. They become anorectic and may starve. With catabolism of body mass, urinary excretion of zinc is increased. Commonly used intravenous fluids are relatively zinc free. Thus, under usual circumstances, a negative zinc balance could occur in patients who are given antimetabolites, antianabolic agents, or prolonged intravenous therapy.

Failure to include zinc in fluids for total parenteral nutrition (TPN) is another example of iatrogenically induced conditioned deficiency of zinc. A decline of plasma zinc has been observed in several patients given TPN fluids containing less than 1.25 mg of zinc daily (9, 33). In some cases urinary zinc loss is excessive (33). Some patients on TPN have developed a clinical picture resembling acrodermatitis enteropathica. Typical severe zinc deficiency occurred in one patient following penicillamine therapy for Wilson's disease (34).

Diabetes

Some patients with diabetes mellitus have been found to have increased urinary losses of zinc (35). The mechanism is unknown. Presumably, some of them may become zinc deficient, though in general, the plasma zinc is not affected.

Collagen Diseases

In patients with inflammation such as rheumatoid arthritis, lupus erythematosus, infection, or injury, two factors may lead to zinc deficiency. The loss of zinc from catabolized tissue (36) and mobilization of zinc by leucocytic endogenous mediator (LEM) (37) to the liver and its subsequent excretion in the urine may account for conditioned zinc deficiency in such cases. Recently, a beneficial effect of zinc therapy in patients with rheumatoid arthritis has been reported (38).

Pregnancy

Plasma concentration of zinc decreases in human pregnancy (39). Presumably, the decrease reflects in part the uptake of zinc by the fetus and other products of conception. It has been estimated that the pregnant woman must retain approximately 750 μg of zinc per day for growth of the products of conception during the last two thirds of pregnancy. Thus, when zinc deficiency occurs in pregnancy, a conditioning factor is the demand of the fetus for zinc. Studies in the rat suggest that the placenta actively provides zinc to the fetus (40). If the diet of the pregnant woman does not include liberal amounts of animal protein, the likelihood of conditioned deficiency

of zinc is increased, as zinc is probably less available from food derived from grains and other plants. The possible importance of zinc deficiency in human pregnancy is implied by the observations of Hurley et al (41), Caldwell et al (42), and Halas et al (43). Zinc deficiency in pregnant rats was shown to cause fetal abnormalities, behavioral impairment in the offspring, and difficulty in parturition in the mother (41).

Caldwell et al (42) were the first to show that in both prenatal and postnatal nutrition even a mild zinc deficiency in rats had profound influence on behavior potential despite an apparently adequate protein level in the diet. Recently, it has been observed that zinc deficiency in fetal and suckling rats results in adverse biochemical effects in the brain (43). The adverse effects on the brain of the suckling rats are apparently not readily reversible, as behavioral testing of nutritionally rehabilitated 60–80 day-old male rats has shown that they performed poorly on a Tolman-Honzik maze when compared to pair-fed and ad libitum–fed control rats (44). These findings suggested that zinc deficiency during the critical developmental period of the rat brain induces poorly reversible abnormalities which are manifested by impaired behavioral development.

Hurley and co-workers (41) have shown that short-term depletion of zinc in maternal rats results in a wide variety of congenital anomalies in the offspring. In view of the important role of zinc in nucleic acid metabolism, Hurley & Shrader (45) have proposed that impaired deoxyribonucleic acid (DNA) synthesis in zinc-deprived embryos prolongs the mitotic cycle and reduces the number of normal neural cells, leading to malformations of the central nervous system. It is tempting to speculate that the exceptionally high rates of congenital malformations of the central nervous system as reported from the Middle East (46) might be caused by maternal zinc deficiency.

Plasma zinc is known to decrease following use of oral contraceptive agents (47, 48). Our recent data indicate that whereas the plasma zinc may decline, the zinc content of the red blood cells increases as a result of administration of oral contraceptive agents. This phenomenon may merely mean a redistribution of zinc from the plasma pool to the red cells. Alternatively, oral contraceptive agents may enhance carbonic anhydrase (a zinc metalloenzyme) synthesis, thus increasing the red cell zinc content.

Genetic Disorders

SICKLE CELL DISEASE Recently, deficiency of zinc in sickle cell disease has been recognized (49). Inasmuch as zinc is an important constituent of erythrocytes, it is possible that long-continued hemolysis in patients with sickle cell disease might lead to a zinc-deficient state.

In a study reported by Prasad et al (49, 50), zinc in plasma, erythrocytes, and hair was decreased and urinary zinc excretion was increased in sickle cell anemia patients as compared to controls. Erythrocyte zinc and daily urinary zinc excretion were inversely correlated in the anemia patients ($r = -0.71$, $p < 0.05$), suggesting that hyperzincuria may have caused zinc deficiency in these patients. Carbonic anhydrase, a zinc metalloenzyme, correlated significantly with erythrocyte zinc ($r = + 0.94$ $p < 0.001$). Plasma ribonuclease (RNase) activity was significantly greater in anemia subjects than in controls, consistent with the hypothesis that sickle cell anemia patients were zinc deficient.

In spite of the tissue zinc depletion in sickle cell anemia patients, the mean urinary excretion of zinc was higher than in the controls. This may have been a direct result of increased filtration of zinc by the glomeruli owing to continued hemolysis, or there may have been a defect in tubular reabsorption of zinc somehow related to sickle cell anemia, a possibility that cannot be excluded at present. Continued hyperzincuria may have been responsible for tissue depletion of zinc as suggested by a significant negative correlation between values for 24-hr urinary zinc excretion and erythrocyte zinc. At this stage, however, one cannot rule out additional factors such as predominant dietary use of cereal proteins and other nutritional factors that affect zinc availability adversely, thus accounting for zinc deficiency. Further work is warranted for proper elucidation of the pathogenesis of zinc deficiency in sickle cell anemia.

ACRODERMATITIS ENTEROPATHICA Acrodermatitis enteropathica was described in 1943 by Danbolt & Close (51), and the clinical and pathological features have been delineated by numerous investigators (52). Prior to the serendipitous discovery of diiodohydroxyquinolone therapy in 1953 by Dillaha and co-workers (53), patients with acrodermatitis enteropathica invariably died from cachexia, usually with terminal respiratory infection. Although diiodohydroxyquinolone has been used successfully for the therapy of this condition for 20 years, the mechanism of drug action has never been elucidated. It now seems possible that the efficacy of diiodohydroxyquinolone might be related to the formation of an absorbable zinc-chelate, inasmuch as diiodohydroxyquinolone is a derivative of 8-hydroxyquinolone, a chelating agent (54).

In 1973, Barnes & Moynahan (55, 56) studied a two-year-old girl with severe acrodermatitis enteropathica who was being treated with diiodohydroxyquinolone and a lactose-deficient synthetic diet. The clinical response to this therapy was not satisfactory, and the physicians sought to identify contributory factors. They found that the concentration of zinc in the patient's serum was profoundly reduced, and, therefore, they administered

oral zinc. The skin lesions and gastrointestinal symptoms cleared completely, and the patient was discharged from the hospital. When zinc was inadvertently omitted from the child's regimen, she suffered a relapse which promptly responded when oral zinc was reinstituted. In their initial reports, Barnes & Moynahan (55, 56) attributed zinc deficiency in this patient to the synthetic diet.

It was later appreciated that zinc might be fundamental to the pathogenesis of this rare inherited disorder and that the clinical improvement reflected improvement in zinc status. Support for zinc deficiency hypothesis came from the observation that a close resemblance between the symptoms of zinc deficiency in animals and man as reported earlier (57) and subjects with acrodermatitis enteropathica existed particularly with respect to skin lesions, growth pattern, and gastrointestinal symptoms.

Zinc supplementation to these patients led to complete clearance of skin lesions and restoration of normal bowel function, which had previously resisted various dietary and drug regimens. This original observation was quickly confirmed in other cases with equally good results. The underlying mechanism of the zinc deficiency in these patients is most likely due to malabsorption. The cause of poor absorption is obscure, but an abnormality of Paneth's cells may be involved.

MISCELLANEOUS GENETIC DISORDERS Low levels of plasma zinc have been noted in patients with mongolism (24). The mechanism is unknown. Congenital hypoplasia of the thymus gland in cattle may be an example of zinc deficiency on genetic basis (58). It is unknown whether thymus hypoplasia in man is in some way related to zinc deficiency.

CLINICAL MANIFESTATIONS OF ZINC DEFICIENCY

Growth retardation, hypogonadism in the males, poor appetite, mental lethargy, and skin changes were the classical clinical features of chronic zinc deficiency as reported from the Middle East by Prasad et al (3–6). Hepatosplenomegaly, which was consistently present in the zinc-deficient dwarfs, improved following zinc supplementation. The mechanism of spleen and liver enlargement in this syndrome, however, is not well understood at present. All the above-mentioned features were corrected by zinc supplementation.

Recently, Morrison et al (59) reported abnormal dark adaptation in six stable alcoholic cirrhotics who also had low serum zinc level. Zinc administration to these patients resulted in improvement of dark adaptation. This is an interesting clinical observation that needs further confirmation. The

effect of zinc on the retina may be mediated by retinene reductase, which is a zinc-dependent enzyme.

It is likely that some of the clinical features of cirrhosis of the liver such as loss of body hair, testicular hypofunction, poor appetite, mental lethargy, difficulty in wound healing, and night blindness may indeed be related to the secondary zinc-deficient state in this disease. In the future, careful clinical trials with zinc supplementation must be carried out in order to determine whether or not zinc is beneficial to patients with chronic liver disease.

Several publications have focused attention on a distinctive pattern of abnormalities occurring in the offspring of alcoholic mothers, known as "fetal alcohol syndrome." It is characterized by prenatal and postnatal growth deficiency, microcephaly, short palpebral fissures, epicanthal folds, cleft palate, micrognathia, joint anomalies, cardiac and renal anomalies, and anomalies of external genitalia (60, 61). Many of these features are similar to those reported in rat fetuses when zinc intake was restricted in the mothers during the crucial stage of gestation (41). In view of the fact that excessive alcohol intake may deplete body zinc store and inasmuch as zinc plays a vital role in DNA synthesis and cell division, one may speculate that a maternal zinc deficiency may indeed be responsible for the "fetal alcohol syndrome." A careful clinical study is needed to test this hypothesis.

In sickle cell disease, delayed onset of puberty and hypogonadism in the males, characterized by decreased facial, pubic, and axillary hair, short stature and low body weight, rough skin, and poor appetite have been noted and related to a secondary zinc-deficient state (49, 50). Many patients with sickle cell anemia develop chronic leg ulcers that do not heal, and a beneficial effect of zinc supplementation in such cases has been reported (49, 50). In one study, zinc sulfate (660 mg/day) was administered orally to seven men and two women with sickle cell anemia (49, 50). Two 17-year-old males gained 10 cm in height during 18 months of therapy. All but one patient gained weight. Five of the males showed increased growth of pubic, axillary, facial, and body hair, and in one a leg ulcer healed in six weeks on zinc and in two others some benefits of zinc therapy on healing of ulcers was noted. Further controlled clinical trials are indicated in order to establish the effect of zinc therapy in this condition.

In limited uncontrolled studies, zinc appears to have been effective in decreasing symptoms and crisis of sickle cell anemia (62). The therapeutic rationale is based on effects of zinc on the red cell membrane by which it decreases hemoglobin and calcium binding and improves deformability which may result in decreased trapping of sickle cells in the capillaries where the pain cycle is normally initiated. Undoubtedly, more thorough evaluation of zinc therapy in sickle cell disease is needed in the future.

Zinc deficiency may play an important role in the functional activity of the enterocyte in coeliac disease, and some "non responsive" coeliac disease patients may be profoundly zinc deficient (22). In a recent report, six patients were observed who failed to respond to diet, steroids, and nutritional supplements but made remarkable recovery when zinc was administered (22). They gained weight, and *d*-xylose absorption test and steatorrhea improved following zinc therapy.

Zinc therapy in a few subjects with malabsorption syndrome (other than coeliac disease) seemed to have produced beneficial results with respect to growth retardation, hypogonadism in the males, mental lethargy, skin changes, and loss of hair (9). One should, therefore, be aware of the occurrence of zinc deficiency as a possible complication of malabsorption syndrome, since this is easily correctable.

In 1966, Pories et al (63, 64) reported that oral administration of zinc to military personnel with marsupialized pilonidal sinuses was attended by a twofold increase in the rate of reepithelialization. The authors' conclusion that zinc can promote the healing of cutaneous sores and wounds has been the subject of controversy during the past several years. Clinical investigations by Cohen (65), Husain (25), Greaves & Skillen (66), and Serjeant et al (26) have substantiated the beneficial effects of zinc on wound healing, whereas studies by Barcia (67), Myers & Cherry (69), Clayton (69), and Greaves & Ive (70) have failed to demonstrate any therapeutic benefit. Hallbook & Lanner (71) found that the reepithelialization rate of venous leg ulcers was enhanced by zinc in patients who initially had diminished concentrations of serum zinc, but they did not find any benefit in patients whose initial measurements of serum zinc were within the normal range.

Studies in experimental animals have demonstrated that (*a*) healing of incised wounds is impaired in rats with dietary zinc deficiency, (*b*) collagen and noncollagen proteins are reduced in skin and connective tissues from rats with dietary zinc deficiency, (*c*) zinc supplementation does *not* augment wound healing in normal rats, and (*d*) zinc supplementation *does* augment wound healing in chronically ill rats (72). These data provide evidence that zinc supplementation may promote wound healing in zinc-deficient patients.

Abnormalities of taste have been related to a deficiency of zinc in man and *animals* (73–75). The animal studies with various drugs seem to indicate that thiols as well as agents that deplete trace metals such as zinc cause an increased intake of certain solutions distinguished primarily by their taste, suggesting that a change in taste function may have occurred due to zinc deficiency (74). Decreased taste acuity (hypogeusia) has been observed in zinc-deficient subjects, such as patients with liver disease, malabsorption syndrome, following thermal burns and following administration of penicil-

lamine or histidine (73). A double-blind study, however, failed to show the effectiveness of zinc in the treatment of hypogeusia in various patients (75). This may suggest that depletion of zinc may lead to decreased taste acuity but not all cases of hypogeusia are related to zinc deficiency. Role of zinc in hypogeusia needs to be further delineated, and careful clinical studies are warranted in the future.

Severe and relatively acute deficiency of zinc has been observed in patients with acrodermatitis enteropathica (55, 56), following penicillamine therapy in a patient with Wilson's disease (34), following total parenteral nutrition (33), and in patients following excessive ingestion of alcohol (19).

In acrodermatitis enteropathica, the dermatological manifestations include progressive bullous-pustular dermatitis of the extremities and the oral, anal, and genital areas, combined with paronychia and generalized alopecia. Infection with *candida albicans* is a frequent complication. Ophthalmic signs may include blepharitis, conjunctivitis, photophobia, and corneal opacities. Gastrointestinal disturbances are usually severe, including chronic diarrhea, malabsorption, steatorrhea, and lactose intolerance. Neuropsychiatric signs include irritability, emotional disorders, tremor, and occasional cerebellar ataxia. The patients generally have retarded growth and males exhibit hypogonadism. Zinc therapy has been shown to produce remarkable improvements and is considered to be a life-saving measure in these subjects.

A very similar clinical picture has been reported in a patient who received penicillamine therapy for Wilson's disease (34). Following total parenteral nutrition and excessive ingestion of alcohol, clinical manifestations of zinc deficiency resemble acrodermatitis enteropathica, and this may be considered an example of acquired acrodermatitis enteropathica. Once a zinc deficiency is recognized, zinc therapy is imperative in such cases.

PHYSIOLOGICAL AND BIOCHEMICAL ROLE OF ZINC

Metabolism

The zinc content of a normal 70 kg male is approximately 1.5 to 2.0 g. Liver, kidney, bone, retina, prostate, and muscle appear to be rich in zinc. In man, zinc content of testes and skin has not been determined accurately, although clinically it appears that these tissues are sensitive to zinc depletion.

Zinc in the plasma is bound mainly to albumin, but other proteins such as α-macroglobulin, transferrin, ceruloplasmin, haptoglobin, and gamma globulins also bind significant amounts of zinc (76). Besides protein-bound fraction, a small proportion of zinc (2–3% of oral zinc) in the plasma exists as an ultrafiltrable fraction, mostly bound to amino acids but a smaller

fraction as ionic form. Histidine, glutamine, threonine, cystine, and lysine appear to have significant zinc-binding ability (76). Whereas amino acids competed effectively with albumin, haptoglobin, transferrin, and 1 gG for binding of zinc, a similar phenomenon was not observed with respect to ceruloplasmin and α_2-macroglobulin, suggesting that the latter two proteins exhibited a stronger binding affinity for zinc (76).

Approximately 20 to 30% of ingested dietary zinc is absorbed. Data on both the site(s) of absorption in man and on the mechanism(s) of absorption, whether it be active, passive, or facultative transport, are meager. Zinc absorption is variable and is highly dependent upon a variety of factors. Zinc is more available for absorption from animal proteins. Among other factors that might affect zinc absorption are body size, the level of zinc in the diet, and the presence in the diet of other potentially interfering substances, such as calcium, phytate, fiber, and the chelating agents. Recently it has been shown that prostaglandin E_2 not only binds zinc, but also facilitates its transport across the intestinal mucosa in the rat (77).

Normal zinc intake in a well-balanced American diet with animal protein is approximately 12 to 15 mg/day. Urinary zinc loss is approximately 0.5 mg/day. Loss of zinc by sweat may be considerable under certain climatic conditions. Under normal conditions approximately 0.5 mg of zinc may be lost daily by sweating. Endogenous zinc loss in the gastrointestinal tract may amount to 1 to 2 mg/day.

Daily requirement of zinc for human subjects is not established. In view of the fact that several dietary constituents may affect the availability of zinc, it is apparent that the dietary requirement must vary greatly from one region to the other depending upon the food habits of the population.

Biochemistry

Major functions of zinc in human and animal metabolism appear to be enzymatic. There are now over 70 metalloenzymes known to require zinc for their functions (78). Zinc enzymes are known to participate in wide variety of metabolic processes. The metal is present in several dehydrogenases, aldolases, peptidases, and phosphatases.

Zinc atoms in some of the enzyme molecules participate in catalysis and also appear to be essential for maintence of structures of apoenzymes (78). For instance, alcohol dehydrogenase from horse liver contains 4 g atoms of zinc per molecular weight of 80,000. Two zinc ions are essential for cataytic activity and are bound to the enzyme via two cysteinyl SH groups and the immidazole ring of a histadyl residue. The fourth metal coordination site is thought to be occupied by water. The other two zinc ions are involved in maintaining structure and are each bound to four SH groups.

A deficiency of zinc in *Euglena gracilis* has been shown to affect adversely all the phases of cell cycle (G_1, S, G_2, and mitosis), indicating that zinc is required for biochemical processes essential for cells to pass from G_2 to mitosis, from S to G_2, and from G_1 to S (78). The effect of zinc on the cell cycle is undoubtedly due to its vital role in DNA synthesis (79, 80). Many studies have shown that zinc deficiency in animals impairs the incorporation of labeled thymidine into DNA. This effect in the animals has been detected within a few days after the zinc-deficient diet is instituted (79).

Prasad & Oberleas (79) provide evidence that decreased activity of deoxythymidine kinase may be responsible for this early reduction in DNA synthesis. As early as 6 days after the animals were placed on the dietary treatment, the activity of deoxythymidine kinase was reduced in rapidly regenerating connective tissue of zinc-deficient rats, compared to pair-fed controls. These results have recently been confirmed by Dreosti & Hurley (81). The activity of deoxythymidine kinase in 12-day-old fetuses taken from females exposed to a dietary zinc deficiency during pregnancy was significantly lower than in ad libitum– and restricted-fed controls. Activity of the enzyme was not restored by in vitro addition of zinc, whereas addition of copper affected the enzyme activity adversely.

Zinc has been shown to be an essential constituent for the DNA polymerase of *Escherichia coli* (82). Whether or not this enzyme is affected adversely in an animal model, due to deficiency of zinc, is not known.

Livers from zinc-deficient rats incorporated less phosphorus-32 into the nucleotides of RNA than livers from pair-fed controls, and DNA-dependent RNA polymerase has been shown to be a zinc-dependent enzyme (83, 84). The activity of RNase is increased in zinc-deficient tissues (85). This suggests that the catabolism of RNA may be regulated by zinc.

From the above discussion, it appears that zinc may have its primary effect on zinc-dependent enzymes that regulate the biosynthesis and catabolic rate of RNA and DNA. In addition, zinc may also play a role in the maintenance of polynucleotide conformation. Sandstead et al (86) observed abnormal polysome profiles in the liver of zinc-deficient rats and mice. Acute administration of zinc appeared to stimulate polysome formation both in vivo and in vitro. This finding is supported by the data of Fernandez-Madrid, Prasad & Oberleas (28) who noted a decrease in the polyribosome content of zinc-deficient connective tissue from rats and a concomitant increase in inactive monosomes.

Enzyme Changes in Zinc Deficiency

Since zinc is required for many enzymes, it is reasonable to speculate that the level of zinc in cells controls the physiological processes through the

formation and/or regulation of activity of zinc-dependent enzymes. Until 1965, there had been no evidence to support this concept. During the past decade it has been shown that the activity of various zinc-dependent enzymes was reduced in the testes, bones, esophagus, and kidneys of zinc-deficient rats in comparison to their pair-fed controls (79, 80, 87, 88). These results correlated with the decreased zinc content in the above tissues of the zinc-deficient rats, suggesting that the likelihood of detecting any biochemical changes is greatest in tissues that are sensitive to zinc depletion.

In several studies, the activity of the alkaline phosphatase was found to be reduced in bones from zinc-deficient rats, pigs, cows, chicks, turkey poults, and quails (80). The activity of alkaline phosphatase may be reduced also in the intestine, kidneys, and stomach in experimental animals due to zinc deficiency. There may not only be a loss of activity due to a lack of sufficient zinc for maintaining the enzyme activity, but the amount of apoenzyme present appears to be diminished because of either a decreased synthesis or an increased degradation. Inasmuch as a lowering in the activity of this enzyme in intestinal tissue and the plasma is observed before any sign of a lowered food intake is evident, it is concluded that the loss of enzyme activity is directly attributable to zinc deficiency.

Two important zinc metalloenzymes in protein digestion are the pancreatic carboxypeptidase A and B. A loss of activity of the pancreatic carboxypeptidase A in zinc deficiency is a consistent finding (80). According to some investigators, within two days of instituting a zinc-deficient diet in rats the enzyme lost 24% of its activity and within three days of dietary zinc repletion, the activity of pancreatic carboxypeptidase was restored to normal levels (80). These results indicate that the level of food intake has no influence and that a decreased activity of carboxypeptidase A in the pancreas was related specifically to a dietary lack of zinc. As in the case of the alkaline phosphatase, the amount of carboxypeptidase A apoenzyme appears to be diminished in zinc-deficient pancreas.

Reduced activity of carbonic anhydrase, another zinc metalloenzyme, has been reported in gastric and intestinal tissues and in erythrocytes when the activity of the enzyme was expressed per unit of erythrocytes (80). Recently in patients with sickle cell disease, which is a conditioned zinc-deficient state, the content of carbonic anhydrase in the red cells was found to be decreased, correlating with the zinc content of the red cells (49, 50). Inasmuch as the technique measured the apoenzyme content, it appears that zinc may have a specific effect on the synthesis of this protein by some mechanism, yet to be understood.

Several investigators have shown that zinc deficiency lowers the activity of alcohol dehydrogenase in the liver, bones, testes, kidneys, and esophagus of rats and pigs (87, 89, 90). In another study, alcohol dehydrogenase was

assayed in subcellular fractions of liver and retina from zinc-deficient and control rats using retinol and ethanol as substrates (90). The activity of alcohol dehydrogenase was significantly decreased as a result of zinc deficiency in growing animals. In older rats, although no changes in liver zinc and activity of alcohol dehydrogenase were found, the retina was shown to be sensitive to the lack of zinc. These data show that zinc is required for the metabolism of vitamin A as well as the catabolism of ethanol. An attempt to demonstrate accumulation of apoenzyme of alcohol dehydrogenase in the zinc-deficient tissues was not successful.

Numerous metalloenzymes have the ability to remain functional even after the metal, which presumably is present at their active center, has been replaced by another metal (78). Thus in zinc deficiency, if the apoenzyme is synthesized, as has been observed in the case of *E. coli* alkaline phosphatase (78), then other metals that might have accumulated or are normally within the cell could substitute for zinc and generate an active enzyme. Although this is a possibility in the case of microorganisms, in experimental animals, and man, the apoenzymes of alkaline phosphatase, carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, and deoxythymidine kinase do not accumulate in zinc-deficient tissues. Thus, one may conclude that a deficiency of zinc does specifically affect the activities of zinc-dependent enzymes in sensitive tissues.

One should not expect that zinc-dependent enzymes are affected to the same extent in all tissues of a zinc-deficient animal. Differences in the sensitivity of enzymes are evidently the result of differences in both the zinc-ligand affinity of the various zinc metalloenzymes and in their turnover rates in the cells of the affected tissues. Thus it is to be expected that those zinc metalloenzymes that bind zinc with a very high affinity are still fully active even in extreme stages of zinc deficiency. The extent to which the metalloenzymes lose their activity also depends on the functional role that zinc plays in maintaining the enzyme structure. In some zinc-dependent enzymes, zinc deficiency may induce structural changes that increase the chance of degradation. The consequence is an increased turnover rate and a lower activity of the enzymes in the tissues. It has been suggested that the rapidity with which biochemical changes arise in response to zinc depletion and then disappear upon repletion helps to identify the primary site of metabolic functions of zinc.

Role of Zinc in Endocrine Functions

Recently, the role of zinc in gonadal function was investigated in rats (91). The increase in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone was assayed following intravenous administration of synthetic LH-releasing hormone (LH-RH), to zinc deficient and re-

stricted fed control rats. Body weight gain, zinc content of testes, and their weights were significantly lower in the zinc-deficient rats as compared to the controls. The serum LH and FSH responses to LH-RH administration was higher in the zinc-deficient rats, but serum testosterone response was lower in comparison to the restricted fed controls. These data indicate a specific effect of zinc on testes and suggest that gonadal function in the zinc-deficient state is affected through some alteration of testicular steroidogenesis.

Effect of Zinc on Cells and Membranes

Zinc prevents induced histamine release from mast cell (92). It is believed that this effect of zinc is due to its action on the cell membrane.

Platelets are also affected by zinc ions. Collagen-induced aggregation of dog platelets and collagen or epinephrine-induced release of ^{14}C-serotonin, were significantly inhibited by zinc (92). Supplementation of zinc in dogs effectively decreased aggragability of platelets as well as the magnitude of ^{14}C-serotonin release.

Zinc supplementation inhibits migration and other activities of macrophages and eventually of polymorphonuclear leukocytes; thus an induction of sterile inflammatory reaction, for instance by intraperitoneal injection of mineral oil to animals treated with zinc, results in less cellular infiltration of peritoneal cavity by either type of inflammatory cell.

It has been speculated that zinc may form mercaptides with thiol groups of proteins, possibly linking to the phosphate moiety of phospholipids or interaction with carboxyl groups of sialic acid or proteins on plasma membranes resulting in change of fluidity and stabilization of membranes (92).

There are also several enzymes attached to the plasma membrane that control the structure and function of the membrane and the activities of these enzymes may be controlled by zinc. Adenosinetriphosphatase (ATPase) and phospholipase A_2 are known to be inhibited by zinc, and this effect may explain immobilization of energy-dependent activity of plasma membrane or increase integrity of the membrane structure (92).

Several receptors at the plasmatic membrane presumably function as a gate for transmitting information to intracellular space. In the case of mast cells, histamine-releasing agents seem to work through specific receptors at the membrane. Masking of such receptor sites by membrane-impermeable Zn-8-hydroxyquinolone would thus explain the inhibition of the release reaction.

The role of Ca^{2+} in the function of cell microskeleton, represented by microtubules and by microfilament, has been well documented (92–94). The contractile elements of this system are in some way responsible for the mobility of microorganelles, transport of granules to the membrane, as well

as excitability of the plasma membrane itself. Zinc may compete with calcium and thereby inhibit the calcium effect.

Zinc has been shown to improve filterability through 3.0 nm nucleopore filter of sickle cells in vitro (93). Improvement in filterability at low concentration of zinc suggests that the process of formation of irreversibly sickled cells involves the cell membrane. Calcium and/or hemoglobin binding may promote the formation of irreversibly sickled cells, thus hindering the filterability of such cells. Zinc may act favorably on the filterability of sickled cells by blocking the proposed calcium and/or hemoglobin binding to the membrane. The beneficial effect of zinc on the sickling process has been demonstrated both in vitro and in vivo (95). It has been suggested recently that irreversibly sickled cells are stabilized by abnormal interactions between the spectrin and/or actin components of the membrane skeleton and that disruption of these bonds by zinc may allow the skeleton to resume a normal shape (94).

Interaction of Zinc with Other Metals

Zinc is known to compete with cadmium, lead, copper, iron, and calcium for similar binding sites (96). In the future, a potential use of zinc may be to alleviate toxic effects of cadmium and lead in human subjects. Use of zinc as an antisickling agent is an example of its antagonistic effect on calcium, which is known to produce irreversible sickle cells by its action on red cell membrane. Therapeutic use of zinc is known to produce hypocupremia in human subjects (97). Whether or not zinc could be utilized to decrease copper load in Wilson's disease, remains to be demonstrated.

Zinc in Free-Radical Reactions

Zinc may also intervene in nonenzymic, free radical reactions (98). In particular, zinc is known to protect against iron-catalyzed free-radical damage. It has been known that the free-radical oxidation (autooxidation) of polyunsaturated lipids is most effectively induced by the interaction of inorganic iron, oxygen, and various redox couples, and recent work suggests that this interaction underlies the pathological changes and clinical manifestations of iron toxicity. Iron-catalyzed free-radical oxidation is known to be inhibited by zinc, ceruloplasmin, metalloenzymes (catalase, peroxidases, superoxide dismutase), and free-radical scavenging antioxidants such as Vitamin E.

Carbon tetrachloride–induced liver injury is another animal model for studying free-radical injury to tissues. Animals maintained on a high zinc regimen are resistant to this type of biochemical injury, suggesting that zinc may be protective against free-radical injury. More recent studies have

shown that zinc also inhibits the analogous metromidazole-dependent free-radical sequence.

Role of Zinc in Infection and Immunity

Zinc-deficient animals are known to be susceptible to infections (99–101). A reduced resistance to infections is seen in severely deficient calves with lethal trait, A 46, commonly known as Adema disease, as well as in man with acrodermatitis enteropathica (100). The primary cause of death in both situations is sepsis or pneumonia. Pneumotropic viral infections and secondary bacterial infections (e.g. *Salmonella, Pasteurella,* and *Pyrogenic* bacteria) have been noted to occur particularly. Hypoplasia of thymus, Peyer's patches, and lymphatic nodes have been found in Adema disease of calves, in zinc-deficient rats, and at autopsy in patients with acrodermatitis enteropathica (100). Following zinc replacement, the calves with Adema disease and patients with acrodermatitis enteropathica recover, and the reduced resistance to infections normalizes.

Absolute lymphopenia has been noted in patients with cirrhosis of the liver (an example of conditioned zinc-deficient state) and in some species of animals made zinc deficient (6, 57, 101). Frost et al (102) have reported that patients with sickle cell anemia (also an example of a conditioned zinc-deficient state) have a considerable suppression in peripheral T-cell numbers and an increased number of null cells. In addition, these patients, who show normal mitogen responsiveness in vitro, demonstrate an impaired cellular immune response in vivo as measured by skin tests.

Administration of zinc in incubation media stimulates DNA synthesis of lymphocytes within 6 to 7 days (100, 101, 103). Zinc must be present in the media for the entire culture period in order to produce maximal stimulation of H^3-thymidine incorporation in DNA of lymphocytes.

Decreased ability to develop cell-mediated immune response in vitro has been demonstrated in calves and rats (100). Fraker et al (104) reported that animals immunized with Keyhole Limpet Hemocyanin-P-azophenylarsonate on the first day of a zinc-deficient diet and subsequently at ten-day intervals up to 30 days showed a severe depression in their antibody response to this antigen. These findings implied that both the primary immune response and the memory immune response are impaired in zinc-deficient mouse. Recent studies in rats indicate that zinc deficiency causes a selective suppression of lymphoid organ weight and abnormalities in the immune response to sheep erythrocytes (105). The overall response to this antigen is markedly depressed and delayed. Thus although it appears that zinc is involved in immune responses, further studies are required in order to define its role more precisely.

Serum zinc is known to decline during infection in man or after endotoxin administration to experimental animals (99). A decrease in serum zinc may be accounted for to a large extent by an accelerated flux of zinc from plasma to liver mediated by leukocyte endogenous mediator (LEM) which is liberated by phagocytizing cells (99). LEM is a heat labile trace protein of low molecular weight and acts on liver to stimulate and accelerate flux of iron and zinc into hepatic cells and cause an accelerated synthesis of ceruloplasmin (99). Possible purposes or value to the host of these changes in the plasma concentrations of trace elements remains to be elucidated.

A recent report implicates zinc as an antiviral agent (106–108). The rhinoviruses (common cold viruses) are a large group of very small, simple human parasites. These viruses are composed of a single strand of RNA enclosed in a protein coat or capsid. They are able to form visible sites of infection on the host cells unless an inhibitor is present. Eight out of nine human rhinoviruses were studied and found to be susceptible to 10^{-4} M zinc ion (106–108). One rhinovirus (type 5) as well as two serotypes of poliovirus were resistant (106–108).

There are two probable ways by which zinc may block cleavage of rhinovirus proteins (106–108). One is by activation of one or more proteases; the other is by binding to and altering the substrate so that it cannot be cleaved. The latter model is preferred for the following reasons (106–108): 1. Zinc almost immediately blocked virus production, suggesting that one of the components of the virion is affected directly by zinc. 2. Zinc interacted with thinovirus capsids. 3. Whereas sufficient amounts of purified virus produced crystals, amorphous precipitates formed in the presence of a small amount of zinc. 4. The results of cleavage inhibition indicated that the sensitive proteolytic reactions invariably involved precursors containing capsid protein sequences.

These data suggest that zinc ions bind to rhinovirus capsid polypeptides, prevent their successful combination with viral RNA, and block their nascent cleavage. It remains to be elucidated whether or not these observations have any significance with respect to viral infections in man.

Zinc and Neoplasia

Tumor inhibition is a general effect of zinc deficiency, irrespective of cell type, cell growth rate, species, or site of growth (109). This is not surprising, in view of the fact that zinc is required for cell division and protein synthesis. In a recent paper, it was reported that a high zinc diet initially protected against the development of oral cancer induced by a chemical carcinogen in rats (110). Once the protective barrier was overcome, a high zinc diet accelerated the tumor growth. It is obviously important to understand the

role of zinc in neoplasia, particularly because reverse transcriptase from viruses has been shown to be a zinc enzyme and zinc is essential for cell division. Future studies are needed in this area.

EXPERIMENTAL PRODUCTION OF ZINC DEFICIENCY IN MAN

Although the role of zinc in human subjects has been defined and its deficiency recognized in several clinical conditions, these examples are not representative of a purely zinc-deficient state in man. It was therefore considered desirable to develop a human model that would facilitate the study of the effects of a mild zinc-deficient state in man and also provide sensitive parameters that could be used clinically for diagnosing zinc deficiency. Recently, marginal deficiency of zinc by dietary means has been successfully produced in human volunteers, and changes in several zinc-dependent parameters have been documented (111).

Four male volunteers (1–4) participated in this study. Their ages ranged from 55 to 65 years. The physical examination revealed no abnormal features and their zinc status was within normal limits.

A semipurified diet based on texturized soy protein purchased from General Mills Co., Minneapolis, Minnesota (Bontrae Products) and Worthington Foods Co., Division of Miles Laboratory, Elkhart, Indiana, was developed for this study. Soy protein isolate, which was used as soy flour in the baked goods, was purchased from General-Biochemicals (Teklad Mills, Chagrin Falls, Ohio). The texturized soy meals utilized were hamburger granules, chicken slices, turkey slices, and chicken chunks. The texturized soy protein and soy protein isolate were washed twice with ethylenediaminetetraacetate (EDTA), then rinsed three times with deionized water, boiled for 30 minutes, and kept frozen until ready to be used. The diet was supplemented with vitamins, mineral mix (except zinc), and protein supplement in order to meet the recommended dietary requirements. The daily intake of zinc was 2.7 mg for patients 1 and 2 and 3.5 mg for patients 3 and 4.

The patients were kept under strict metabolic conditions. The first group of subjects (1 and 2) received hospital diet for 2 weeks, and then the experimental diet with 10 mg of supplemental zinc (as zinc acetate) orally for 6 weeks. Following this, they were given only the experimental diet (daily zinc intake of 2.7 mg) for 24 weeks. At the end of this phase, while continuing the experimental diet, the two subjects daily received 30 mg of zinc (as zinc acetate) supplement orally for 12 weeks. Finally they were maintained on the hospital diet with total daily intake of 10 mg zinc plus 30 mg of oral zinc (as zinc acetate supplement for 8 weeks). The hospital

diet provided the same amount of calories and protein as the experimental diet. Thus these two subjects were observed for a total period of 52 weeks.

The second group of subjects (3 and 4) received the hospital diet (10 mg of zinc intake daily) for 3 weeks, followed by the experimental diet with 30 mg of oral zinc supplement (as zinc acetate) for 5 weeks. Following this they were given only the experimental diet (3.5 mg of zinc intake daily) for 40 weeks. The repletion phase was begun by giving 30 mg of zinc (as zinc acetate) orally while maintaining the same experimental diet and continued for a total period of 8 weeks, at the end of which the hospital diet replaced the experimental diet. Oral zinc supplement (30 mg as zinc acetate) was continued along with the hospital diet for a period of 8 weeks. Altogether these two subjects were observed for 64 weeks.

Body weight decreased in all four subjects as a result of dietary zinc restriction and weight loss was more pronounced in the first group of subjects than in the second group. Body weight correlated highly with the subscapular thickness in the two subjects in whom these data were obtained. An approximate calculation revealed that the weight loss could be accounted for as follows: 50% fat, 30% water, and 20% other.

In the first group of two subjects, during the zinc restriction phase (2.7 mg zinc daily intake), the apparent negative balance for zinc ranged from 1 to 4 mg per day whereas in the second group of subjects the apparent negative balance for zinc was 1 to 2 mg per day. Following supplementation with 30 mg of zinc, the positive zinc balance ranged from 11 mg to 22 mg per day in the first group of two subjects. In the second group of subjects (3 and 4), during the baseline period when the daily zinc intake was 33.5 mg daily, the positive balance for zinc was 3–4 mg daily. On the other hand these subjects, on the same level of zinc intake (33.5 mg daily) following zinc depletion phase, showed a positive zinc balance of 14–16 mg daily.

The plasma zinc decreased significantly in all four subjects as a result of zinc restriction and increased following supplementation with zinc. The changes were more marked for patients on 2.7 mg of daily zinc intake as compared to those on 3.5 mg daily zinc intake. The red cell zinc decreased significantly in the first group of two subjects (1 and 2) although the decrease was not evident until 12 weeks on restricted zinc intake. In the second group of subjects, although the red cell zinc did not decrease significantly during the zinc-restricted period, it showed a marked increase following zinc supplementation. The leucocyte zinc decreased significantly in the second group of subjects as a result of zinc restriction in whom this parameter was measured.

Plasma alkaline phosphatase was monitored carefully in the second group of subjects. In both cases, the activity slowly declined as a result of zinc restriction, and following supplementation with zinc, the activity

nearly doubled in 8 weeks. In all four subjects, the activity of plasma ribonuclease was almost twice as great during the zinc-restricted period as compared to the zinc-supplemented phase. Surprisingly, it was observed that plasma ammonia levels increased during zinc restriction and decreased following zinc supplementation in the second group of two subjects in whom this was monitored.

Urinary excretion of zinc decreased in three out of four subjects as a result of zinc restriction. In one subject, the decrease in urinary zinc excretion was not seen, as a result of the diuretic therapy which he received for mild hypertension during the study.

Total protein, total collagen, ribonucleic acid (RNA), DNA, and deoxythymidine kinase activity were measured in the connective tissue obtained after implantation subcutaneously of a small sponge, during zinc restriction and zinc supplementation phases in the first group of two subjects. A marked increase in the total protein, total collagen, and RNA/DNA was observed in the sponge connective tissue as a result of zinc supplementation. Whereas the activity of deoxythymidine kinase was not measurable in the connective tissue during zinc restriction phase, it became near normal following zinc supplementation.

Thus changes in the zinc concentration of plasma, erythrocytes, leucocytes, and urine, and changes in the activities of zinc-dependent enzymes such as alkaline phosphatase, and RNase in the plasma and deoxythymidine kinase in the tissue during the zinc restriction phase, appear to have been induced specifically by a mild deficiency of zinc in the volunteers. One unexpected finding was with respect to the plasma ammonia level which appeared to increase during the zinc-restricted period. We have recently reported a similar finding in zinc-deficient rats (112). This may have important health implications insofar as zinc deficiency in man is concerned, inasmuch as in the liver disease hyperammonemia is believed to affect the central nervous system adversely.

The changes observed with respect to body weight were related to dietary zinc intake. An increased loss of fat, as determined by subscapular thickness, and normal absorption of fat during the zinc restriction phase, suggests that deficiency of zinc may have led to hypercatabolism of fat in our subjects. In experimental animals an increase in free fatty acids has been observed as a result of zinc deficiency (101). Indeed more studies are required in human subjects in order to document increased fat catabolism due to zinc restriction.

Direct measurements of DNA and protein synthesis in experimental animals suggest that in zinc deficiency, protein synthesis is adversely affected (79, 80). Our data in human subjects revealed that the total protein, total collagen, and RNA/DNA increased as a result of zinc supplementa-

tion following depletion of zinc. The activity of deoxythymidine kinase was not measurable during zinc restriction phase but reached 70% of normal level following zinc supplementation for 3 months. Similar data have been reported for experimental animals. Thus it appears that deoxythymidine kinase in human subjects is also a zinc-dependent enzyme and an adverse effect of zinc deficiency on this enzyme may adversely affect protein synthesis. Our studies do not rule out an adverse effect of zinc deficiency on protein catabolism. Further studies are required in order to establish the effect of zinc restriction on protein catabolism.

These data indicate that plasma alkaline phosphatase, and deoxythymidine kinase in sponge connective tissue in human subjects, are zinc-dependent enzymes inasmuch as changes in their activities are related to only one dietary manipulation, namely zinc intake. Changes in the activities of plasma RNase also appear to be related to zinc intake under the conditions of our experiments. Thus the determination of the activities of these enzymes may be helpful in correlating uncomplicated zinc status in man, particularly if the changes are observed following zinc supplementation for a short period of time.

Changes in the plasma zinc concentration were observed early (within 4 to 6 weeks) and correlated with the severity of dietary zinc restriction. Thus plasma zinc may be very useful in assessment of zinc status in man provided that infections, myocardial infarction, intravascular hemolysis, and acute stress are ruled out (111). As a result of infections, myocardial infarction, and acute stress, zinc from the plasma compartment may redistribute to other tissues, thus making an assessment of zinc status in the body a difficult task. Intravascular hemolysis would also spuriously increase the plasma zinc level inasmuch as the content of red cell zinc is much higher than the plasma.

Changes in the red cell zinc were slow to appear as expected; on the other hand, changes in the leucocyte zinc appeared more sensitive to changes in zinc intake. Urinary excretion of zinc decreased as a result of dietary zinc restriction, suggesting that renal conservation of zinc may be important for homeostatic control mechanism in man. Thus determination of zinc in 24-hr urine may be of additional help in diagnosing zinc deficiency provided that cirrhosis of the liver, sickle cell disease, and chronic renal disease are ruled out (111). These conditions are known to have hyperzincuria and associated zinc deficiency.

Our data indicate that following the zinc-deficient state, the subjects showed a greater positive balance for zinc. This would suggest that perhaps a test based on oral challenge of zinc and subsequent plasma zinc determination may be able to distinguish between zinc-deficient and zinc-sufficient states in human subjects.

The concentration of zinc in hair appears to reflect chronic zinc nutriture. Thus if the hair has been growing at a reasonable rate, hair zinc is a useful index of chronic zinc status in the body. Hair zinc, however, does not reflect changes in the status of zinc on an acute basis. Similar remarks apply to zinc in the red cells. Ultimately the response to therapy with zinc is probably the most reliable index for making a diagnosis of the zinc-deficient state in man.

THERAPEUTIC USES OF ZINC

Zinc deficiency symptoms are easily corrected by oral administration of 15 to 25 mg of zinc daily, provided the diet contains adequate animal protein. Many dietary factors such as phytate, fiber, and phosphates may affect the availability of zinc adversely and as such the daily requirement of zinc may be increased. Although zinc sulfate is commonly available in the pharmacies, some patients do not tolerate this compound. In our experience, zinc acetate and zinc gluconate are much better tolerated and as such, we recommend these for general oral use.

Some investigators have used larger amounts of oral zinc for therapeutic purposes. For the treatment of pain crisis in sickle cell anemia patients, and for wound healing, 660 mg of zinc sulfate orally daily in divided doses has been used (62, 95). Although no serious toxic effects have been observed, except for hypocupremia, even up to two years of daily oral administration, the physician must remain alert and watch the patients carefully for any unusual side effects.

There is a need for an intravenous preparation of zinc for use in patients who receive total parenteral nutrition. In future, availability of a proper depot zinc preparation may simplify the treatment schedule of patients who must receive zinc indefinitely on a regular basis.

HYPERZINCEMIA

Recently, an example of familial hyperzincemia has been reported (113). In five out of seven members of one family and two out of three second generation individuals, the plasma zinc ranged from 250 to 435 μg%. Zinc levels in the red cells, hair, and bone were unremarkable, and no apparent clinical effects of hyperzincemia were observed.

TOXICITY

Three types of toxic reactions to zinc have been reported in man (6, 57, 101). First, the "metal fume fever," characterized by pulmonary manifestations,

fever, chills, and gastroenteritis, has been reported to occur in industrial workers who are exposed to fumes. In the second type, toxicity was observed in a 16-year-old male who ingested 12 g of zinc sulfate over a period of two days. The third type of acute zinc toxicity has been observed in a patient with renal failure following hemodialysis. (The water for hemodialysis was stored in a galvanized tank.) The patient suffered from nausea, vomiting, and fever, and severe anemia.

Many of the toxic effects attributed to zinc in the past may actually be due to other contaminating elements such as lead, cadmium, or arsenic. Zinc is noncumulative, and the proportion absorbed is thought to be inversely related to the amount ingested. Vomiting, a protective phenomenon, occurs after ingestion of large quantities of zinc. In fact, 2 g of zinc sulfate has been recommended as an emetic (57).

The symptoms of zinc toxicity in humans include dehydration, electrolyte imbalance, abdominal pain, nausea, vomiting, lethargy, dizziness, and muscular incoordination. Acute renal failure caused by zinc chloride poisoning has been reported. The symptoms occurred within hours after large quantities of zinc were ingested. Death is reported to have occurred after ingestion of 45 g of zinc sulfate. This dose would be considered massive, in view of the fact that the daily requirement of zinc for man is considered to be in the range of 15 to 20 mg/day.

Gastrointestinal bleeding following ingestion of zinc sulfate, 220 mg twice daily for treatment of acne, was recently observed in one patient (114). Zinc level in the plasma was not reported. In our experience this has not been observed. With prolonged use of zinc we have observed hypocupremia in sickle cell anemia patients (97). Zinc acetate or zinc gluconate may be preferable for oral use, inasmuch as gastric discomfort has not been a problem in our patients with their use.

In rats, ingestion of 0.5 to 1.0% of diet as zinc results in reduced growth, anemia, poor reproduction, and decreased activity of liver catalase and cytochrome oxidase (57, 101). The latter are reversed by copper administration, thus indicating that excessive intake of zinc may induce copper deficiency. In a study in which 5 to 6 times higher than normal levels of dietary zinc intake was provided to pregnant rats, a higher rate of resorption of fetuses was observed (115). The same author also observed that supplements of 100 mg of zinc sulfate given during the third trimester of pregnancy in human subjects resulted in three premature births and one still birth in four consecutive subjects (115). Unfortunately, critical dietary and biochemical information with respect to zinc studies was not provided in either the rat or the human study by the investigator; as such a proper evaluation of these results cannot be made. In contrast, other investigators have not observed this effect with excess zinc supplementation to pregnant

rats (116) and during the past few years in the United States many vitamin supplements containing comparable amounts of zinc have been used by pregnant women without any untoward effects.

In view of long-term clinical usage of zinc in therapeutic dosages for several clinical conditions in humans, one must remain alert for possible toxic effects. There may still be other toxic effects of high-dosage zinc administration for long periods of time that have not yet been recognized. In general, however, zinc appears to be much less toxic in comparison to other trace elements.

SUMMARY

Zinc is essential for many biological functions in man and animals. Growth retardation, hypogonadism in males, skin changes, poor appetite, mental lethargy, and delayed wound healing are some of the recognized clinical manifestations of chronic zinc deficiency in man. A severe deficiency of zinc, such as is seen in acrodermatitis enteropathica and following total parenteral nutrition, is characterized by progressive bullous-pustular dermatitis, alopecia, severe diarrhea, emotional disorders, and if untreated it may be fatal.

Major functions of zinc in the metabolism of humans and animals appear to be enzymatic. There are now over 70 metalloenzymes known to require zinc for their functions. Many enzymes involved in nucleic acid metabolism, such as thymidine kinase, DNA polymerase, RNA polymerase, and reverse transcriptase require zinc for their activities. Zinc is an inhibitor of RNase, and its activity is known to increase in zinc-deficient tissues. Zinc is required in all stages of the cell cycle and as a result of zinc deficiency, DNA and protein synthesis are affected adversely. Activities of many enzymes are known to be decreased in zinc-deficient tissues of experimental animals. Thymidine kinase, alkaline phosphatase, and carboxypeptidase are affected adversely within six days of institution of a zinc-deficient diet to experimental animals.

Zinc atoms in some of the enzyme molecules participate in catalysis and also appear to be essential for maintenance of structures of apoenzymes. There is evidence to suggest that zinc may play a significant role in stabilization of biomembrane structure and polynucleotide conformation. Inasmuch as zinc appears to have a protective influence on hepatic cellular damage induced by carbon tetrachloride poisoning, it is reasonable to suggest that zinc may have a direct effect on free radicals.

Zinc deficiency is known to affect testicular functions adversely in man and animals. This effect of zinc is at the end organ level, and it appears that zinc is essential for spermatogenesis and testosterone steroidogenesis.

Zinc is known to compete with cadmium, lead, copper, iron, and calcium for similar binding sites. In future, a potential use of zinc may be to alleviate toxic effects of cadmium, and lead in human subjects. Use of zinc as an antisickling agent is an example of its antagonistic effect on calcium which is known to produce irreversible sickle cells by its action on red cell membranes.

Therapeutic use of zinc is known to produce hypocupremia in human subjects. Whether or not zinc could be utilized to decrease copper load in Wilson's disease remains to be demonstrated.

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