Ann. Rev. Nutr. 1985. 5:341–63

CLINICAL MANIFESTATIONS OF ZINC DEFICIENCY

Ananda S. Prasad[1]

Department of Medicine, Wayne State University School of Medicine, Detroit, Michigan 48201; Harper-Grace Hospitals, 3990 John R Street, Detroit, Michigan 48201; and the Veterans Administration Medical Center, Allen Park, Michigan 48101

CONTENTS

[1]Supported in part by grants from the National Heart, Lung, and Blood Institute, Sickle Cell Center grant, a grant from the National Institute of Arthritis, Metabolic, and Digestive Diseases, NIH, and a grant from Veterans Administration Research Service.

0199-9885/85/0715-0341$02.00

INTRODUCTION

Although the essentiality of zinc for plants and animals has been known for many years, its ubiquity made it seem unlikely that deficiency of zinc could lead to significant problems in human nutrition or clinical medicine. A clinical syndrome consisting of growth retardation, male hypogonadism, skin changes, mental lethargy, hepatosplenomegaly, iron deficiency anemia, and geophagia was reported from Iran in 1961 and was suspected to be related to zinc deficiency (55). The Iranian dwarfs ate only bread made of wheat flour and their intake of animal protein was negligible. There was no evidence for blood loss. Additionally, they consumed nearly one pound of clay daily.

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 zinc deficiency was considered because growth retardation and testicular atrophy were known to occur in zinc-deficient animals. Inasmuch as heavy metals may form insoluble complexes with phosphate, we speculated that some factors responsible for decreased availability of iron in these patients with geophagia may have also decreased the availability of zinc.

Similar patients were later studied in Egypt (56, 57). The clinical features were remarkably similar, except for the following: the Iranian patients had more pronounced hepatosplenomegaly, they gave a history of geophagia, and none had any parasitic infections, in contrast to Egyptian subjects who had both schistosomaisis and hookworm infections and none of whom gave a history of geophagia.

The dietary history of the Egyptian subjects was similar to that of the Iranians. The intake of animal protein was negligible, and their diet consisted of bread and beans *(Vicia fava)* only. These subjects were demonstrated to have a zinc deficiency. This conclusion was based on the following: the zinc concentrations in plasma, red cells, and hair were decreased and radioactive zinc-65 studies revealed that the plasma zinc turnover 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 controls (56, 57).

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 an adequate animal protein diet (47, 48). 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 patients 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 only an animal protein diet. Thus,

the growth retardation and gonadal hypofunction in these subjects were related to a deficiency of zinc. The anemia was due to iron deficiency and responded to oral iron treatment.

During the past two decades, zinc deficiency in human subjects throughout the world has been reported to occur in conditions where there is an increased requirement of zinc. It is believed that zinc deficiency should be present in countries where primarily cereal proteins are consumed by the population; one would also expect to see a spectrum of zinc deficiency, ranging from severe cases to marginally deficient examples, in any given population. It is also becoming evident that nutritional, as well as conditioned, deficiency of zinc may complicate many disease states in the humans.

A detailed account of the discovery and importance of zinc in human subjects was recently published (52). In this paper, etiology, clinical manifestations, laboratory diagnosis, and treatment of zinc deficiency are presented.

ETIOLOGY

Nutritional Deficiency

Nutritional deficiency of zinc in humans is fairly prevalent throughout the world. Besides Iran and Egypt, it has now been reported from Turkey, Portugal, Morocco, Yugoslavia, and other developing countries, and from Australia (15–17, 51). These reports represent only awareness in diagnosing overt cases, rather than the actual worldwide incidence of zinc deficiency.

Nutritional deficiency of zinc occurring in children and infants has been recognized also in the United States. A study from Denver in 1972 identified a number of children from middle- and upper-income families whose zinc levels in the hair were significantly decreased and whose growth was retarded (31). A later study showed that hair and plasma zinc levels were exceptionally low in infants in the United States (69). 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 have contributed to zinc deficiency was the low concentration of this element in certain popular milk formulae. It appears that those infants 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.

Old age, pregnancy, lactation, and alcoholism are also associated with a higher incidence of poor zinc nutrition (51). Although many of these observations have been made mainly in the United States, it is likely that zinc deficiency will be recognized in other countries as well, as investigators become aware of this problem.

Dietary surveys of adult population in a number of countries, including the US, have shown that mean zinc intakes are frequently less than the recommended dietary allowances (Food and Nutrition Board, United States National Academy of Sciences). However, caution must be exercised in using zinc dietary intake as an indicator of risk of deficiency; other dietary and environmental factors may very significantly affect zinc status. As an example, zinc deficiency occurs in rural Iran despite relatively high dietary intakes because the major source of protein is refined cereals containing high levels of phytate, consumption of which renders zinc unavailable for absorption. Conversely, Tokelau Islanders, whose diet consists of coconut and fish, have a very low dietary zinc intake (4.5 mg/day), but their zinc status as assessed by zinc levels in hair, plasma, and urine appears to be within the acceptable normal range. Thus, zinc deficiency may be expected to occur in areas where the consumption of unrefined cereal is predominant. It has been estimated that 3% of adolescents in rural areas of the Middle East and North Africa show signs of moderate zinc deficiency, and mild deficiency of zinc is probably very prevalent in such areas, particularly in infants, growing children, and pregnant and lactating women.

Recently, the relationship between nutritional status of zinc and growth and development was studied in a group of school children between the ages of 9 and 12 in Yugoslavia (14). This study showed an increased incidence of retarded growth in the children who also had low hair and plasma zinc levels. The occurrence of moderate to severe hypogeusia was associated in a statistically significant way with the reduction of hair zinc level. Thus, their results were in agreement with the hypothesis that zinc deficiency may have caused poorer growth and hypogeusia in children of Yugoslavia.

In Turkey, geophagia is a common problem and a majority of the adolescents with geophagia exhibit both iron and zinc deficiencies. Growth retardation and hypogonadism are related to zinc deficiency in such cases, and zinc supplementation completely corrects these problems (16). Poor dietary habits of Turkish villagers appear to be the major etiologic factor responsible for these deficiencies.

A decreased zinc level in almost 30% of pregnant women, all of whom were in a low socioeconomic group, has been reported. Their diet consisted mainly of cereals. Maternal zinc deficiency has severe teratogenic consequences in experimental animals, and there is epidemiological evidence that it could be a factor responsible for severe congenital malformation of the central nervous system in humans. In light of this, more studies and correction of this nutritional problem are urgently needed.

Alcohol

Alcohol induces hyperzincuria (3, 28). The mechanism is not well understood, but a direct effect of alcohol on renal tubules may be responsible.

The serum zinc level of the alcoholic subject tends to be lower in comparison with controls. An absolute increase in renal clearance of zinc in alcoholics, demonstrable at both normal level and low serum zinc concentration, has been observed. Thus, the measurement of renal clearance of zinc may be clinically useful for etiological classification of chronic liver disease attributable to alcohol in different cases. Excessive ingestion of alcohol may lead to a severe deficiency of zinc, as reported by Weismann et al (72).

Liver Disease

Vallee et al (68) demonstrated that patients with cirrhosis of the liver had low serum and hepatic zinc, and paradoxically hyperzincuria. These observations suggested that zinc deficiency in the alcoholic cirrhotic patient may be a conditioned deficiency related somehow to alcohol ingestion. These observations have been now confirmed by several investigators.

Gastrointestinal Disorders

Zinc deficiency has been reported in patients with steatorrhea (64). In an alkaline environment, zinc would be expected to form insoluble complexes with fat and phosphates. Thus, fat malabsorption from 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 concentration that occurs in patients with inflammatory disease of the bowel. It seems likely that protein-losing enteropathy may impair zinc homeostasis. Another potential cause of negative zinc balance is a massive loss of intestinal secretions.

In one study, 21 of 52 patients with regional enteritis had low serum zinc concentrations (43). These authors also concluded that certain complications of regional enteritis may be due to zinc deficiency.

Neoplastic Diseases

The occurrence of conditioned deficiency of zinc in patients with neoplastic diseases obviously depends upon the nature of the neoplasm. Anorexia and starvation, plus lack of abundance of foods rich in available zinc, are probably important conditioning factors.

Hypozincemia has now been reported in many malignant conditions (9). Frequently, this is associated with hypercupremia, thus presenting with an increased ratio of copper to zinc. In a recent study, a significant survival advantage was demonstrated for those patients with extensive squamous cell lung cancer who had higher plasma zinc concentration (>45 μg per 100 ml) (35). Decreased serum zinc level correlated with decreased neutrophil migration as measured by the skin window technique and with decreased triceps skin-fold thickness.

Zinc levels in serum, red cells, and hair were observed to be decreased in patients with Hodgkin's disease in Turkey. Whether or not a preexisting zinc deficiency affecting thymic functions adversely had any role to play in the development of this lymphoma cannot be determined.

It is obvious that there is a great need to investigate further the changes in trace elements in malignancy. Their precise roles in tumor development and as prognostic indicators remain to be documented.

Burns and Skin Disorders

Among the causes of zinc deficiency in patients with burns is loss of exudates. Starvation of burn patients 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 can 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.

Renal Disease

In recent studies Mahajan et al (40-42) documented that patients with chronic renal failure have a low concentration of zinc in plasma, leukocytes, and hair as well as increased plasma ammonia level and increased activity of plasma ribonuclease. Patients with uremia, whether or not they were on dialysis, had a mean plasma zinc level significantly less than that in controls. Patients undergoing maintenance hemodialysis and peritoneal dialysis had plasma zinc levels similar to those not on dialysis. The concentration of zinc in hair and leukocytes was also significantly decreased in all groups with chronic renal failure compared to the controls. Neither the prescribed amount of protein in the diets nor the albumin levels correlated significantly with plasma zinc concentration in dialyzed and nondialyzed uremic patients. Patients with chronic renal failure exhibit several clinical manifestations of zinc deficiency that are reversible with zinc supplementation (40). The pathogenesis of zinc deficiency in chronic renal diseases is not well understood at present.

Pregnancy

An additional positive zinc balance of approximately 375 mg is required during normal pregnancy. In one study, mild deficiency of zinc in pregnant women was reported to be associated with increased maternal morbidity and increased risks to the fetus (36).

Iatrogenic Causes

Possible iatrogenic causes of conditioned deficiency of zinc include use of chelating agents, antimetabolites, antianabolic drugs, and diuretics. Failure to

include zinc in fluids for total parenteral nutrition (TPN) is another example of iatrogenically induced conditioned deficiency of zinc. In some cases the deficiency may be very severe and resemble congenital type of acrodermatitis enteropathica. Severe zinc deficiency occurring in a patient following penicillamine therapy for Wilson's disease has been reported (38).

Genetic Disorders

ACRODERMATITIS ENTEROPATHICA Acrodermatitis enteropathica is a lethal, autosomal, recessive trait that usually occurs in infants of Italian, Armenian, or Iranian lineage. The disease is not present at birth, but typically develops in the early months of life, soon after weaning from breast feeding. Zinc supplementation results in complete cure (7). The underlying mechanism of zinc deficiency in these patients is malabsorption. The genetic basis of zinc malabsorption remains to be elucidated.

SICKLE CELL ANEMIA Certain clinical features are common to some sickle cell anemia patients and zinc-deficient subjects, the latter as reported from the Middle East. The results of assays of zinc in plasma, erythrocytes, hair, and leukocytes, and assays of zinc-dependent enzymes such as plasma RNAse, leukocyte alkaline phosphatase, deoxythymidine kinase in newly synthesized connective tissue, and carbonic anhydrase in red cells, both supported our conclusion that zinc deficiency existed in adults with sickle cell anemia (50). Hyperzincuria was also documented in patients with sickle cell anemia. Increased requirement of zinc due to hyperzincuria and possibly decreased intake of zinc may have contributed to a zinc-deficient state in these subjects.

Zinc supplementation to sickle cell anemia subjects significantly increased weight gain, growth of pubic hair, serum testosterone level, plasma zinc, neutrophil zinc, and neutrophil alkaline phosphatase activity. The maximum level of serum testosterone and mean serum testosterone level following gonadotropin-releasing hormone (GnRH) also showed a significant increase following zinc supplementation.

CLINICAL MANIFESTATIONS

Growth and Development

Growth retardation, hypogonadism in males, poor appetite, mental lethargy, and skin changes were the classical clinical features of chronically zinc-deficient subjects from the Middle East as reported by the author in the early 1960s. All the above-mentioned features were corrected by zinc supplementation. Liver and spleen were also found to be enlarged in the zinc-deficient dwarfs, a condition that improved following zinc supplementation. The mechanism of spleen and liver enlargement in this syndrome is not well understood at present.

Later studies by Halsted et al (29) showed that development was slow in 19- or 20-year-old subjects receiving a well-balanced diet alone. The effect on height increment and onset of sexual function was strikingly enhanced in those receiving zinc. The two women described in their report were from a hospital clinic and represented the first cases in females of dwarfism due to zinc deficiency.

Hambidge and co-workers (30) in 1972 proposed that marginal zinc deficiency could occur among children in the United States. While surveying zinc concentrations in hair of normal children in the Denver area, they found that 10 subjects between the ages of 4–6 years had poor appetite, impaired taste acuity, mild retardation in growth, and decreased concentration of zinc in the hair. On supplementation with zinc, the above abnormalities were corrected.

Butrimovitz & Purdy (13) measured plasma zinc of inner-city children in Baltimore and found them to be lower than the levels of Denver children of middle-income families. In their cross-sectional study of plasma zinc levels of Baltimore children, they showed that the lowest levels of plasma zinc occurred during infancy and puberty, two periods characterized by rapid growth. After curve-fitting of the plasma zinc concentration data, they demonstrated a mirror relationship between plasma zinc levels and the growth index, an indicator of relative growth velocity. The authors suggested that depletion of body stores of zinc may occur during periods of rapid growth and that subjects subsisting on low animal protein intake would benefit from higher intake of zinc during such periods.

In Turkey, geophagia is a common problem and the majority of the adolescents with geophagia exhibit both iron and zinc deficiencies. Growth retardation and hypogonadism have been related to zinc deficiency in such cases and zinc supplementation has resulted in complete correction of these problems (16). The average increase in height was 4.4 cm in three months and 10.4 cm six months after zinc supplementation. Poor dietary habits of Turkish villagers appear to be the major etiologic factor responsible for these deficiencies. The village diet consists of wheat bread that is rich in phytate, thus the availability of iron and zinc are less than optional. The authors have concluded that indeed zinc deficiency is one of the major nutritional problems in Turkey.

Ghavami-Maibodi et al (24) studied 13 short children from Nassau County (New York) who had retarded bone age and low concentrations of zinc in their hair. They were treated with oral zinc supplements for one year. Following zinc supplementation, the growth rate and the levels of serum growth hormone, testosterone, and somatomedin C increased along with an increase in hair zinc concentration.

In order to determine the effect of zinc on growth in sickle cell anemia patients a controlled trial with zinc supplementation was carried out in 14- to 18-year-old patients who were retarded in growth (54). In the first zinc

supplementation experiment, 10 male patients between the ages of 14 and 17 with retarded growth were subdivided randomly into two groups. Five patients received placebo twice a day and the other five received 15 mg of zinc supplementation as acetate twice a day for one year. Height and body weight were carefully recorded initially and at three-month intervals throughout the study by a single observer. Bone age was determined radiographically twice, initially and at the end of the one-year treatment period. Zinc was measured in the plasma and erythrocytes once a month. Two zinc-dependent enzymes, neutrophil alkaline phosphatase and nucleoside phosphorylase in erythrocytes, were also assayed simultaneously once a month. The data obtained during the last three months of each treatment phase were assayed and analyzed statistically. Basal serum testosterone was measured in duplicate every six months.

In the second zinc supplementation experiment, five male patients between the ages of 14 and 18 years with retarded growth were supplemented with placebo the first year and zinc (15 mg twice a day as acetate) the second year. Thus, the patients on zinc supplementation served as their own controls in this study.

Patients who received zinc supplementation showed a greater increase in height, body weight, and serum testosterone levels than did the placebo group. In the first experiment changes in height and body weight in zinc-supplemented and placebo groups on a yearly basis were as follows: 6.4 ± 1.0 vs 2.2 ± 0.3 cm and 4.4 ± 1.4 vs 0.9 ± 0.7 kg, ($P < 0.001$) respectively. In the second experiment, changes in height and body weight in zinc-supplemented and placebo groups on a yearly basis were as follows: 4.9 ± 0.7 vs 2.0 ± 0.9 cm and 4.0 ± 0.6 vs 1.7 ± 0.9 kg, ($P < 0.001$) respectively. Zinc levels in the plasma, erythrocytes, and neutrophils and activities of neutrophil alkaline phosphatase and nucleoside phosphorylase in erythrocytes were also higher in the group receiving zinc supplements as compared with those in the group on placebo in both experiments. Thus, in our subjects with sickle cell anemia, the abnormally decreased growth rate was correctable with zinc supplementation.

The administration of bovine growth hormone to zinc-deficient nonhypophysectomized rats in the studies by Prasad et al (58) failed to enhance growth, whereas the growth rate increased after zinc supplementation. The growth rate of hypophysectomized rats, however, responded to both hormone and zinc supplementation, regardless of zinc status. It then appears that the effects of growth hormone and zinc on the growth of rats were additive but independent of each other. One report in animal studies suggests that somatomedin may be zinc dependent (45). Undoubtedly, more studies are required for the understanding of the interrelationship of somatomedin and zinc in humans.

Zinc is needed for many enzymatic functions (49, 51, 52). It is needed for DNA synthesis, cell division, and protein synthesis. Recently its role in gene

expression was recognized (67). It is likely that the most important effect of zinc on growth is at the peripheral tissue level as related to its role in cell division.

Gonadal Effects

Genital and secondary sexual development were retarded in all zinc-deficient male dwarfs from Iran and Egypt (55–57). Pubic hair was limited to a few fine, darkly pigmented hairs at the base of the penis. Facial hair of the same character was present on the upper lip and axillary hair was absent. Treatment with zinc was followed by surprisingly accelerated sexual maturation. In some individuals pigmented pubic, extremity, and facial hair appeared within three weeks of beginning zinc treatment and maturational changes in the penis and scrotum.

Thirty-two adult patients with sickle cell anemia were investigated for relationship between zinc deficiency and hypogonadism in males (53). Secondary sex characteristics were abnormal in 29, and eunuchoidal skeletal proportions were present in all except one. The age at which different stages of pubic hair growth was attained in these patients was delayed in comparison to normals. Hormonal assays were carried out in 14 patients.

Basal serum testosterone, dihydrotestosterone and androstenedione values were lower in sickle cell anemia patients than controls. Serum LH and FSH levels before and after stimulation with gonadotropin-releasing hormone were consistent with primary testicular failure. Erythrocyte and hair concentrations were significantly decreased, and there was positive correlation between erythrocyte zinc and serum testosterone ($r = 0.61$, $P < 0.001$) in sickle cell anemia. These studies showed that androgen deficiency in sickle cell anemia is a result of primary rather than secondary hypogonadism.

Studies of zinc supplementation to patients with sickle cell anemia showed that androgen deficiency in those patients was correctable with zinc (53). In the first experiment, four subjects received placebo for 12 months, following which zinc (15 mg tid as zinc acetate) was administered orally for 12 months. In the second experiment, 14 subjects were divided into two groups. Seven subjects received placebo orally, and seven received 15 mg of zinc orally three times a day as acetate. Both groups were age matched. The first three pairs received supplementation for 12 months. The next three pairs were supplemented for six months following which zinc supplementation was discontinued and all the subjects were observed for an additional six months. One pair received the supplementation for 18 months. The periods of supplementation were varied to determine the length of time necessary for the supplementation to show its maximum effects on serum testosterone levels. The controls for these studies were healthy age-matched subjects.

In the first experiment, zinc supplementation significantly increased plasma

zinc, neutrophil zinc, neutrophil alkaline phosphatase activity, and basal serum testosterone levels in each subject in comparison to placebo treatment ($P < 0.01$). The maximum level of serum testosterone and the mean serum testosterone level following administration of GnRH also increased significantly following supplementation with zinc ($P < 0.05$).

In the second experiment, the effect of zinc supplementation appeared to be maximal at the end of six months with respect to basal serum testosterone, dihydrotestosterone, plasma zinc, neutrophil zinc, and neutrophil alkaline phosphatase activity; the effect was significantly greater than that of the placebo treatment ($P < 0.01$). In three subjects, zinc supplementation was discontinued for six months, which resulted in a significant decline in serum testosterone, neutrophil zinc, and neutrophil alkaline phosphatase activity ($P < 0.01$).

Zinc deficiency may account for the persistence of gonadal dysfunction in a majority of uremic men despite adequate dialysis (40). Twenty stable patients undergoing hemodialysis three times a week completed a double-blind therapeutic trial using either 50 mg of elemental zinc acetate (10 patients) or placebo (10 patients), orally. At the end of the six months, a significant increase in the mean (± SE) plasma zinc level (75 ± 2 to 100 ± 2 μg/dl, $P < 0.001$), serum testosterone level (2.8 ± 0.3 to 5.2 ± 0.5 ng/ml, $P < 0.001$), and sperm count (30 ± 3 to 63 ± 5 million/ml, $P < 0.001$) occurred in the zinc-treated group, but not in those receiving the placebo. The zinc-treated group also had a significant fall in serum luteinizing hormone (LH) (92 ± 10 to 49 ± 26 mIU/ml, $P < 0.005$) and follicle-stimulating hormone (FSH) (45 ± 9 to 25 ± 7 mIU/ml, $P < 0.05$), which was not seen in the placebo group. Patients receiving zinc showed an improvement in potency, libido, and frequency of intercourse not observed in the placebo group. These results suggest that zinc deficiency is a reversible cause of gonadal dysfunction in patients having regular hemodialysis.

The role of zinc in gonadal function was also investigated in rats (39). The increases in LH, FSH, and testosterone were assayed after intravenous administration of synthetic luteinizing-hormone-releasing hormone (LHRH) to zinc-deficient and restricted-fed control rats. Body weight gain, zinc content of testes, and weight of testes were significantly lower in the zinc-deficient rats compared with the controls. The serum LH and FSH response to LHRH administration was higher in the zinc-deficient rats but serum testosterone response was lower in comparison with the restricted-fed controls. These studies indicated a specific effect of zinc on testes and suggested that gonadal function in zinc-deficient rats is affected through some alteration of testicular steroidogenesis.

The mechanism by which zinc affects the testosterone level in zinc-deficient subjects is not well understood. Zinc is essential for the function of many

enzymes. It is possible that a zinc-dependent enzyme or enzymes may be involved in sex hormone steroidogenesis. The other possibility is that the main effect of zinc is on the testicular size, and inasmuch as testicular size is known to decrease with zinc deficiency, testosterone production may also be affected adversely. Zinc is required for cell division in general, but the testis appears to be a very sensitive organ and is known to atrophy because of a lack of zinc.

Although hypogonadism due to zinc deficiency in males appears to be more common than menstrual abnormalities in females, zinc does play an important role in pregnancy and lactation. Recently, cytogenetic effect of dietary zinc deficiency on oogenesis and spermatogenesis were studied in mice (71). Spontaneously ovulated metaphase II oocytes were examined morphologically and cytogenetically. For male mice, chromosome analysis was performed on spermatological metaphase and primary and secondary metaphases of spermatocytes after 28 days of dietary treatment. The incidence of degenerated oocytes was very high in the moderately severe zinc-deficient group, and even in the marginally zinc-deficient group hypohaploidy and hyperhaploidy in metaphase II oocytes were significantly increased (71). There were no increases in the incidence of chromosome aberrations in spermatogenesis or spermatocytes in the zinc-deficient groups. If indeed these results are applicable to humans, it really becomes very important to maintain adequate zinc status in preconceptual women.

Liver and Gastrointestinal Disorders

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 cirrhosis of the liver. In the future, careful clinical trials with zinc supplementation need to be carried out in order to establish the effects of zinc in patients with chronic liver disease.

We have previously demonstrated an increased level of plasma ammonia and a decreased activity of the hepatic ornithine carbamoyltransferase (OCT), an enzyme required for urea synthesis, in zinc-deficient rats (61). An increased plasma ammonia level has also been observed in human volunteers who became mildly zinc deficient as a result of restricted zinc intake and in adult sickle cell anemia zinc-deficient patients (60). Zinc supplementation corrected this metabolic abnormality in every case.

Ammonia is highly neurotoxic and, along with other factors, undoubtedly contributes to the development of encephalopathy and coma, a terminal event in patients with severe liver disease. In a recent study, 22 cirrhotic patients with chronic encephalopathy were given oral zinc supplementation (600 mg a day of zinc acetate) or placebo in a double-blind randomized trial for seven days. The zinc supplemental group showed increased serum zinc level and marked im-

provement in the encephalopathy (62). The authors concluded that short-term oral zinc supplementation probably improved hepatic encephalopathy by correcting zinc deficiency.

Some patients with celiac disease who failed to respond to diet, steroids, and nutritional supplements made remarkable recovery when zinc was administered. They gained weight, and *d*-xylose absorption test and steatorrhea improved following zinc therapy (64). Zinc supplementation in a few subjects with malabsorption syndrome (other than celiac disease) seemed to have produced beneficial results with respect to growth retardation, hypogonadism in the males, mental lethargy, skin changes, and loss of hair (43). One should, therefore, be aware of zinc deficiency as a possible complication of malabsorption syndrome, since this is easily correctable.

Impaired Wound Healing

Pories & Strain (46) 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 promotes wound healing remained controversial for several years. Studies in experimental animals have demonstrated that (*a*) healing of incised wounds is impaired in rats with zinc deficiency; (*b*) collagen and noncollagen proteins are reduced in skin and connective tissues of rats with dietary zinc deficiency; and (*c*) zinc supplementation does not augment wound healing in normal rats. Many studies have now shown that zinc supplementation is beneficial for wound healing in zinc-deficient patients. It is becoming evident that many hospitalized and elderly subjects are marginally zinc deficient. As such, one may consider zinc supplementation to those individuals if wound healing becomes a problem.

Neurosensory and Psychiatric Manifestations

Abnormalities of taste have been related to a deficiency of zinc in many cases by some investigators. Decreased taste acuity (hypogeusia) has been observed in zinc-deficient subjects, such as patients with liver disease, malabsorption syndrome, thermal burns, chronic uremia, and those who have taken oral penicillamine or parenteral histidine (32, 34, 41). A double-blind study, however, failed to show the effectiveness of zinc in the treatment of hypogeusia in various diseases (34).

Diminished taste acuity may account for the persistence of protein and calorie malnutrition observed in a majority of hemodialysis patients in spite of liberalization of the prescribed amount of dietary protein (41). Twenty-two patients undergoing thrice weekly hemodialysis for more than six months were tested for taste acuity and plasma zinc concentration, after which a double-blind study was instituted using a zinc supplement (50 mg of elemental zinc as

zinc acetate per day) or a placebo. The threshold of taste detection and recognition for salt (NaCl), sweet (sucrose), and bitter (urea), but not for sour (HCl), improved significantly in all patients on zinc supplementation. None of these parameters improved in those taking placebo. During the study period, the mean plasma zinc level increased from 75 ± 8 to 97 ± 10 μg/dl ($P < 0.001$) in patients receiving zinc acetate. There was no significant change in plasma zinc level in the placebo group (75 ± 15 to 80 ± 15). The results of this study showed that uremic hypogeusia improved in association with zinc supplementation and elevation of plasma zinc concentration. This may suggest that depletion of zinc may lead to decreased taste acuity but not all cases of hypogeusia are due to zinc deficiency. The role of zinc in hypogeusia needs to be delineated further.

Recently, abnormal dark adaptation in alcoholic cirrhotics has been related to a deficiency of zinc (44). Zinc administration to these patients improved their dark adaptation. Similar clinical observations have now been made in some zinc-deficient sickle cell anemia patients and patients with malabsorption (70). It was proposed that the effect of zinc on the retina is mediated by an enzyme retinene reductase, which is known to be zinc dependent (18). This interesting clinical observation needs further documentation.

Zinc deficiency occurring in a patient following penicillamine therapy for Wilson's disease has been reported (38). The manifestations included parakeratosis, alopecia, keratitis, and central scotoma, all of which were reversed with zinc supplementation. Treatment with zinc sulfate significantly improved visual capacity of the central scotoma area in patients who were heavy smokers and alcoholics, according to another study (8).

Neuropsychiatric signs in humans who developed severe deficiency of zinc as a result of oral administration of histidine included irritability, emotional disorders, tremors, and occasional cerebellar ataxia (33). Similar manifestations have been also reported in patients with acrodermatitis enteropathica. Zinc therapy produces remarkable improvements in these patients and is considered to be a life-saving measure.

Dermatological Manifestations

The dermatological manifestations of severe zinc deficiency include progressive bullous-pustular dermatitis of the extremities and the oral, anal, and genital areas, combined with paronychia and generalized alopecia such as seen in acrodermatitis enteropathica. Infection with *Candida albicans* is a frequent complication. These manifestations are also seen in cases with severe deficiency of zinc following total parenteral nutrition (without zinc supplementation), following penicillamine or histidine administration, and in severe cases of alcoholism and malabsorption syndrome.

Effect on Pregnancy, Fetus, and Infants

According to Jameson (36), zinc deficiency syndrome in pregnancy is characterized by increased maternal morbidity, abnormal taste sensations, prolonged gestation, inefficient labor, atonic bleeding, and increased risks to the fetus, especially postmaturity. A possible correlation between maternal zinc deficiency and congenital malformations, especially of the central nervous system, has been postulated. A high incidence of congenital malformations is observed in fetuses born of adult women suffering from acrodermatitis enteropathica.

"Fetal alcohol syndrome" is characterized by prenatal and postnatal growth deficiency, microcephaly, short palpebral fissures, epicanthal folds, cleft palate, small jaw, joint deformities, and cardiac, renal, and genitalia anomalies. 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. Whether or not the "fetal alcohol syndrome" is related to maternal zinc deficiency induced by alcoholism remains to be proven.

In young children recovering from severe malnutrition, limitation of lean tissue synthesis, with resultant obesity and a propensity to infection, are the major features of a mild zinc deficiency.

Immunological Effects

Zinc is required for phytohemagglutinin-induced transformation of human peripheral blood lymphocytes (2). Zinc appears to act as a mitogen, and the kinetics of its influences most closely approximates the effects of antigen stimulation on lymphocyte culture. Assessment of the role of zinc in the development and functions of different lymphoid cell populations strongly indicates that this element predominantly affects thymic-dependent lymphocytes (T cells). Fraker and associates (21) were the first to show that severely and marginally zinc-deficient young adult A/Jax mice have involuted thymuses and reduced ability to form antibodies to sheep red blood cells. Additional studies were carried out and results were interpreted to mean that the deficit in antibody formation was not primarily in the B-cell response, but rather T-helper function was affected adversely in zinc deficiency.

Later studies have shown that not only is T-helper function affected adversely as a result of zinc deficiency but other functions such as T-suppressor and natural-killer (NK) cell activity may also be zinc dependent (20, 23, 26). Recent studies indicate that a monocyte factor may be required for the zinc-induced mitogenic response of T lymphocytes (63). In experimental models, zinc deficiency results in a sharp drop in levels of the thymic hormone facteur thymique serique (FTS) (27). This hormone is required by precursor thymocytes to differentiate into theta-positive lymphocytes.

Deficiency of the purine enzyme nucleoside phosphorylase is associated

with a severe T-cell immune deficiency (25). Our recent studies in experimental animals indicate that nucleoside phosphorylase may be zinc dependent (59). Thus a decreased activity of nucleoside phosphorylase may additionally account for T-cell dysfunction in zinc deficiency.

Currently available data suggest a direct stimulatory influence of zinc upon DNA metabolism, either by enzyme activation or by altering the binding of F_1 and F_3 histones to DNA so as to effect RNA synthesis. Direct cell surface effects of zinc cannot be ruled out, however. It is conceivable that zinc could be operating at several different levels in influencing lymphocyte functions.

Patients with acrodermatitis enteropathica have thymic aplasia and T-cell dysfunction; they frequently die of serious infections due to cell-mediated immune disorders. The disease is completely cured by administration of zinc (7). T-cell-mediated immune disorder in some cancer patients and several subjects with common variable immunodeficiency has been related to deficiency of zinc (27).

Lymphopenia, depressed T-cell mitogen response, and decreased T-helper and NK-cell activity have been reported in zinc-deficient human subjects (4). All the above manifestations were corrected by zinc supplementation. Anergy and decreased NK-cell activity have been observed in zinc-deficient SCA subjects (6, 66). These manifestations were also correctable with zinc supplementation (6, 66).

Anergy, responsive to zinc supplementation, was reported in elderly subjects (19), and in patients with chronic renal failure (5). The above reports thus clearly indicate that zinc deficiency in humans may impair cell-mediated immune functions.

Abnormal chemotactic function of neutrophils was reported in zinc-deficient patients with acrodermatitis enteropathica and chronic renal disease (12, 73). Zinc supplementation reversed this cellular defect.

Spectrum of Clinical Effects

It is now apparent that the clinical severity of zinc deficiency exists as a spectrum: on the one end the manifestations may be mild or marginal and on the other end the symptoms may be severe. Tables 1–3 summarize the clinical manifestations of zinc deficiency in humans.

Table 1 Clinical manifestations of mild zinc deficiency

Causes	Manifestations
1. Experimentally Induced	Oligospermia Weight loss Hyperammonemia

Table 2 Clinical manifestations of moderate zinc deficiency

Causes	Manifestations
1. Dietary	Growth retardation
2. Alcohol	Hypogonadism (males)
3. Malabsorption	Skin changes
4. Chronic renal disease	Poor appetite
5. Sickle cell disease	Mental lethargy
6. Chronic debilitation	Delayed would healing
	Taste abnormalities
	Abnormal dark adaptation
	Anergy

Severe zinc deficiency is characterized by bullous-pustular dermatitis, alopecia, neuropsychiatric disorders, weight loss, diarrhea, cell-mediated immune disorder manifested as severe intercurrent infections and, if unrecognized and untreated, the condition becomes fatal. The etiological factors responsible for severe deficiency of zinc are acrodermatitis enteropathica, following total parenteral nutrition (without zinc), following extensive alcohol ingestion, severe malabsorption, and iatrogenic causes such as treatment with histidine or penicillamine.

A moderate deficiency of zinc in humans is characterized by growth retardation (in growing children and adolescents), hypogonadism (particularly in males), rough skin, poor appetite, mental lethargy, impaired wound healing, taste abnormalities, abnormal dark adaptation, and cell-mediated immune disorder as evidenced by anergy and susceptibility to infections. These manifestations have been observed in zinc-deficient dwarfs from the Middle East and other parts of the world and are due to poor availability of zinc in the diet. A moderate deficiency of zinc has been reported in several other conditions such

Table 3 Clinical manifestations of severe zinc deficiency

Causes	Manifestations
1. Acrodermatitis enteropathica	Bullous pustular dermatitis
2. Following total parenteral nutrition	Alopecia
3. Following excessive alcohol ingestion	Diarrhea
4. Following penicillamine therapy	Emotional
5. Severe malabsorption syndrome	Weight loss
	Intercurrent infection
	Hypogonadism in males
	Death

as malabsorption, chronic renal disease, sickle cell anemia, and chronic debilitation.

Recently, we induced a marginal deficiency of zinc in human volunteers by dietary means (1, 60). In such subjects, we observed weight loss, oligospermia, decreased serum testosterone level, and hyperammonemia, which were all reversed with zinc supplementation.

LABORATORY DIAGNOSIS

Measurement of zinc level in plasma is useful provided the sample is not hemolyzed or contaminated. In conditions of acute stress, following myocardial infarction or acute infections, zinc from the plasma compartment may redistribute to other tissues; this makes an assessment of zinc status in the body a difficult task (49, 51). Intravascular hemolysis would also increase the plasma zinc level inasmuch as the content of red cell zinc is much higher than the plasma.

Many investigators use the plasma copper: zinc ratio to clinically assess certain diseases (9). It has been suggested that an increase in this ratio in patients with malignancy may indicate activity of the disease and poor prognosis. A plasma copper: zinc ratio of greater than two in chronic alcoholics has been associated with greater incidence of alcoholic hepatitis and cirrhosis of the liver. In another study, a plasma copper: zinc ratio of less than two was seen in patients who had uncomplicated alcohol withdrawal. Those subjects who had a ratio of greater than two showed delirium tremens and a prolonged, severe hallucinatory state. Plasma copper: zinc ratio also has been used to monitor therapeutic response in pulmonary tuberculosis. Although this is an interesting observation, the mechanism of the alteration in plasma copper: zinc ratio in various diseased states is not clear. Further investigations are needed to properly understand and use this ratio as a diagnostic clinical tool.

Zinc in the red cells and hair may also be used to assess body zinc status. Because these tissues turn over slowly, the zinc levels do not reflect recent changes with respect to body zinc stores. Neutrophil zinc determination, on the other hand, appears to reflect the body zinc status more accurately and is thus a very useful parameter (54, 74). Quantitative assay of alkaline phosphatase activity in the neutrophils is also a very useful tool in our experience. Additionally, studies are needed to determine whether or not zinc determination in lymphocytes and/or platelets may be useful in diagnosing zinc deficiency in humans.

Urinary excretion of zinc is decreased as a result of zinc deficiency. Thus,

determination of zinc in 24-hour urine may be of additional help in diagnosing zinc deficiency, provided cirrhosis of the liver, sickle cell disease, chronic renal disease, and other conditions known to cause hyperzincuria are ruled out. Hyperzincuria may be associated with zinc deficiency in the above-mentioned disorders.

A metabolic balance study may clearly distinguish zinc-deficient from zinc-sufficient subjects (60). Recently, an oral zinc tolerance test was utilized for diagnostic purposes (65). In one study an increased plasma response to a zinc load and a decrease in salivary sediment zinc level were noted after institution of a vegetarian diet, which suggests that this diet may have affected the body zinc status adversely (22). This test may provide useful information with respect to zinc status. Further studies are needed to document the usefulness of this test in clinical medicine.

The activities of many zinc-dependent enzymes have been shown to be affected adversely in zinc-deficient tissues. Three enzymes—alkaline phosphatase, carboxypeptidase and thymidine kinase—appear to be most sensitive to zinc restriction in that their activities are affected adversely within three to six days of institution of a zinc-deficient diet to experimental animals. In human studies, the activities of deoxythymidine kinase in proliferating skin collagen and alkaline phosphatase activity in neutrophils were shown to be sensitive to dietary zinc intake. As a practical test, quantitative measurement of alkaline phosphatase activity in neutrophils may be a useful adjunct to neutrophil zinc level determination in order to assess body zinc status in man. Following supplementation with zinc to deficient subjects, a prompt response in the activities of sensitive enzymes is observed.

In one study, the ratio of the maximal posttreatment to pretreatment serum alkaline phosphatase activity correlated inversely with pretreatment serum zinc level in subjects who received a zinc-restricted diet initially and later were supplemented with zinc (37). The authors suggested that a serial determination of serum alkaline phosphatase and calculation of alkaline phosphatase ratio during a trial of zinc therapy may provide biochemical confirmation of the adequacy of zinc replacement and may be useful in detection of mild zinc deficiency.

Cell-mediated immune functions are affected adversely as a result of zinc deficiency. Skin tests for anergy, natural-killer cell activity, proliferative responses of peripheral blood lymphocytes, T-helper and suppressor functions, serum thymic hormone (facteur thymique serique) assay, chemotaxis, and a correlation of the above tests with zinc concentration of lymphocytes and neutrophils may provide valuable tools for the diagnosis of zinc deficiency in humans in the future. Obviously much more scientific work is needed in this area.

TREATMENT

Deficiency of zinc can be corrected easily by oral supplementation of zinc. The amount of supplement required depends upon the nature of the diet consumed by the subjects. If the intake of animal protein is adequate, 15 to 30 mg of daily oral zinc supplementation taken in fasting state should be sufficient to correct the deficiency state. It may be necessary to give much more supplement, if the dietary intake is predominantly cereal protein. In patients with malabsorption syndrome and hyperzincuria, the dietary requirement of zinc may be increased; hence it is advisable to assess these losses prior to replacement therapy.

Zinc has been used therapeutically in larger doses (25 mg every four hours) to suppress irreversible sickle cells (10) and decrease copper load in patients with Wilson's disease (11). It is likely that more therapeutic uses of zinc will emerge in the future, as we begin to understand the various functions of zinc in the body.

SUMMARY

The essentiality of zinc for humans was recognized in the early 1960s. The causes of zinc deficiency include malnutrition, alcoholism, malabsorption, extensive burns, chronic debilitating disorders, chronic renal diseases, following uses of certain drugs such as penicillamine for Wilson's disease and diuretics in some cases, and genetic disorders such as acrodermatitis enteropathica and sickle cell disease. In pregnancy and during periods of growth the requirement of zinc is increased. The clinical manifestations in severe cases of zinc deficiency include bullous-pustular dermatitis, alopecia, diarrhea, emotional disorder, weight loss, intercurrent infections, hypogonadism in males; it is fatal if unrecognized and untreated. A moderate deficiency of zinc is characterized by growth retardation and delayed puberty in adolescents, hypogonadism in males, rough skin, poor appetite, mental lethargy, delayed wound healing, taste abnormalities, and abnormal dark adaptation. In mild cases of zinc deficiency in human subjects, we have observed oligospermia, slight weight loss, and hyperammonemia.

Zinc is a growth factor. Its deficiency adversely affects growth in many animal species and humans. Inasmuch as zinc is needed for protein and DNA synthesis and for cell division, it is believed that the growth effect of zinc is related to its effect on protein synthesis. Whether or not zinc is required for the metabolism of somatomedin needs to be investigated in the future.

Testicular functions are affected adversely as a result of zinc deficiency in both humans and experimental animals. This effect of zinc is at the end organ level; the hypothalamic-pituitary axis is intact in zinc-deficient subjects. Inasmuch as zinc is intimately involved in cell division, its deficiency

may adversely affect testicular size and thus affect its functions. Zinc is required for the functions of several enzymes and whether or not it has an enzymatic role in steroidogenesis is not known at present.

Thymopoeitin, a hormone needed for T-cell maturation, has also been shown to be zinc dependent. Zinc deficiency affects T-cell functions and chemotaxis adversely. Disorders of cell-mediated immune functions are commonly observed in patients with zinc deficiency.

Zinc is beneficial for wound healing in zinc-deficient subjects. In certain zinc-deficient subjects, abnormal taste and abnormal dark adaptation have been noted to reverse with zinc supplementation.

Literature Cited

1. Abbasi, A. A., Prasad, A. S., Rabbani, P., DuMouchelle, E. 1980. Experimental zinc deficiency in man: Effect on testicular function. *J. Lab. Clin. Med.* 96:544–50
2. Alford, M. 1970. Metal cation requirements for phytohemagglutinin-induced transformation of human peripheral blood leukocytes. *J. Immunol.* 104:698–703
3. Allan, J. G., Fell, G. S., Russell, R. I. 1975. Urinary zinc in hepatic cirrhosis. *Scot. Med. J.* 20:109–11
4. Allen, J. I., Perri, R. T., McClain, C. J., Kay, N. E. 1983. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J. Lab. Clin. Med.* 102:577–89
5. Antoniou, L. D., Shalhoub, R. J., Schechter, G. P. 1981. The effect of zinc on cellular immunity in chronic uremia. *Am. J. Clin. Nutr.* 34:1912–17
6. Ballester, O. B., Prasad, A. S. 1983. Anergy, zinc deficiency and decreased nucleoside phosphorylase activity in patients with sickle cell anemia. *Ann. Intern. Med.* 98:180–82
7. Barnes, P. M., Moynahan, E. J. 1973. Zinc deficiency in acrodermatitis enteropathica: Multiple dietary intolerance treated with synthetic diet. *Proc. R. Soc. Med.* 66:327–29
8. Bechetoille, A., Ebran, J. M., Allain, P., Mauras, Y. 1983. Effect therapeutique du sulfate de zinc sur le scotome central des neuropathies optiques alcoholtabagiques. *J. Fr. Ophthalmol.* 6:237–42
9. Bogden, J. D. 1980. Blood zinc in health and disease. In *Zinc in the Environment*, ed. J. O. Nriagu, pp. 138–69. New York: Wiley
10. Brewer, G. J., Brewer, L. F., Prasad, A. S. 1977. Suppression of irreversibly sickled erythrocytes by zinc therapy in sickle cell anemia. *J. Lab. Clin. Med.* 90:549–54
11. Brewer, G. J., Hill, G. M., Prasad, A. S., Cossack, Z. T., Rabbani, P. I. 1981. Oral zinc therapy for Wilson's disease. *Ann. Intern. Med.* 99:314–20
12. Briggs, W. A., Pedersen, M. M., Mahajan, S. K., Sillix, D. H., Prasad, A. S., McDonald, F. D. 1982. Lymphocyte and granulocyte function in zinc-treated and zinc-deficient hemodialysis patients. *Kidney Int.* 21:827–32
13. Butrimovitz, G. P., Purdy, W. C. 1978. Zinc nutrition and growth in a childhood population. *Am. J. Clin. Nutr.* 31:1409–12
14. Buzina, R., Jusic, M., Sapunar, J., Milanovic, N. 1980. Zinc nutrition and taste acuity in school children with impaired growth. *Am. J. Clin. Nutr.* 33:2262–67
15. Casey, C. E., Hambidge, K. M. 1980. Epidemiological aspects of human zinc deficiency. See Ref. 9, pp. 1–27
16. Cavdar, A. O., Arcasoy, A., Cin, S., Gumus, H. 1980. Zinc deficiency in geophagia in Turkish children and response to treatment with zinc sulphate. *Haematologica* 65:403–8
17. Cheek, D. B., Spargo, R. M., Hay, H. J., Smith, R. M. 1982. Zinc deficiency in the aboriginal people of the Northwest of Australia. In *Clinical, Biochemical, and Nutritional Aspects of Trace Elements*, ed. A. S. Prasad, pp. 63–82. New York: Liss
18. Cossack, Z. T., Prasad, A. S., Rabbani, P., Briggs, W. A., McDonald, F. D. 1982. Effect of zinc supplementation on retinal reductase in zinc deficient rats. *Nutr. Rep. Int.* 26:841–48
19. Duchateau, J., Delepesse, G., Vrijens,

R., Collet, H. 1981. Beneficial effects of oral zinc supplementation on the immune response of old people. *Am. J. Med.* 70:1001-4

20. Fernandes, G., Nair, M., Onoe, K., Tanaka, T., Floyd, R., Good, R. A. 1979. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. *Proc. Natl. Acad. Sci. USA* 76:457–61
21. Fraker, P. J., Haas, S., Luecke, R. W. 1977. Effect of zinc deficiency on the immune response of the young adult A/Jax mouse. *J. Nutr.* 107:1889–95
22. Freeland-Graves, J. H., Ebangit, M. L., Hendrikson, P. J. 1980. Alterations in zinc absorption and salivary sediment zinc after a lacto-ovo-vegetarian diet. *Am. J. Clin. Nutr.* 33:1757–66
23. Frost, P., Rabbani, P., Smith, J., Prasad, A. S. 1981. Cell-mediated cytotoxicity and tumor growth in zinc-deficient mice. *Proc. Soc. Exp. Biol. Med.* 167:333–37
24. Ghavami-Maibodi, S. Z., Collipp, P. J., Castro-Magana, M., Stewart, C., Chen, S. Y. 1983. Effect of oral zinc supplements on growth, hormonal levels, and zinc in healthy short children. *Ann. Nutr. Metab.* 27:214–19
25. Giblett, E. R., Ammann, A. J., Wara, D. W., Sandman, R., Diamond, L. K. 1975. Nucleoside phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet* 1:1010–13
26. Good, R. A., Fernandes, G. 1979. Nutrition, immunity, and cancer—A review. *Clin. Bull.* 9:3–12
27. Good, R. A., Fernandes, G., Garofalo, J. A., Cunningham-Rundles, C., Iwata, T., et al. 1982. Zinc and immunity. See Ref. 17, pp. 189–202
28. Gudbjarnason, A., Prasad, A. S. 1969. Cardiac metabolism in experimental alcoholism. In *Biochemical and Clinical Aspects of Alcohol Metabolism,* ed. V. Sardesai, pp. 266–72. Springfield, Ill: Thomas
29. Halsted, J. A., Ronaghy, H. A., Abadi, P., Haghshenass, M., Amirhakimi, G. H., et al. 1972. Zinc deficiency in man: the Shiraz experiment. *Am. J. Med.* 53:277–84
30. Hambidge, K. M., Hambidge, C., Jacobs, M., Baum, J. D. 1972. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. *Pediatr. Res.* 6:868–74
31. Hambidge, K. M., Walravens, P. A. 1976. Zinc deficiency in infants and preadolescent children. In *Trace Elements in Human Health and Disease,* ed. A. S. Prasad, 1:21–32. New York: Academic
32. Henkin, R. I., Bradley, D. F. 1969. Regulation of taste acuity by thiols and metal ions. *Proc. Natl. Acad. Sci.* 62:30–37
33. Henkin, R. I., Patten, B. M., Peter, K., Bronzert, D. A. 1975. A syndrome of acute zinc loss: cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. *Arch. Neurol.* 32: 745–51
34. Henkin, R. I., Scheichter, P. J., Friedewald, W. T., Demets, D. L., Raff, M. 1976. A double-blind study of the effects of zinc sulfate on taste and smell function. *Am. J. Med. Sci.* 272:285–99
35. Issell, B. F., MacFadyen, B. V., Gum, E. T., Valdivieso, M., Dudrick, S. J., Bodey, G. P. 1981. Serum zinc levels in lung cancer patients. *Cancer* 47:1845–48
36. Jameson, S. 1980. Zinc and pregnancy. In *Zinc in the Environment, Part II: Health Effects,* ed. J. O. Nriagu, pp. 183–97. New York: Wiley
37. Kasarskis, E. J., Schuna, A. 1980. Serum alkaline phosphatase after treatment of zinc deficiency in humans. *Am. J. Clin. Nutr.* 33:2609–12
38. Klingberg, W. G., Prasad, A. S., Oberleas, D. 1976. Zinc deficiency following penicillamine therapy. See Ref. 31, pp. 51–65
39. Lei, K. Y., Abbasi, A., Prasad, A. S. 1976. Function of the pituitary-gonadal axis in zinc-deficient rats. *Am. J. Physiol.* 230:1730–32
40. Mahajan, S. K., Abbasi, A. A., Prasad, A. S., Rabbani, P., Briggs, W. A., et al. 1982. Effect of oral zinc therapy on gonadal function in hemodialysis patients. *Ann. Intern. Med.* 97:357–61
41. Mahajan, S. K., Prasad, A. S., Lambujon, J., Abbasi, A. A., Briggs, W. A., McDonald, F. D. 1980. Improvement of uremic hypogeusia by zinc: A double-blind study. *Am. J. Clin. Nutr.* 33:1517–21
42. Mahajan, S. K., Prasad, A. S., Rabbani, P., Briggs, W. A., McDonald, F. D. 1979. Zinc metabolism in uremia. *J. Lab. Clin. Med.* 94(5):693–98
43. McClain, C., Soutor, C., Zierge, L. 1980. Zinc deficiency: A complication of Crohn's disease. *Gastroenterology* 78: 272–79
44. Morrison, S. A., Russell, R. M., Carney, E. A., Oaks, E. V. 1978. Zinc deficiency: A cause of abnormal dark adaptation in cirrhotics. *Am. J. Clin. Nutr.* 31:276–81
45. Oner, G., Bhaumick, B., Bala, R. M. 1984. Effect of zinc deficiency on serum somatomedin levels and skeletal growth in young rats. *Endocrinology* 114:1860–63

46. Pories, W. J., Strain, W. H. 1966. Zinc and wound healing. In *Zinc Metabolism,* ed. A. S. Prasad, pp. 378–91. Springfield, Ill: Thomas
47. Prasad, A. S. 1966. Metabolism of zinc and its deficiency in human subjects. In *Zinc Metabolism,* ed. A. S. Prasad, pp. 250–303. Springfield, Ill: Thomas
48. Prasad, A. S. 1976. Deficiency of zinc in man and its toxicity. See Ref. 31, pp. 1–20
49. Prasad, A. S. 1978. Zinc. In *Trace Elements and Iron in Human Metabolism,* ed. A. S. Prasad, pp. 250–346. New York: Plenum
50. Prasad, A. S. 1981. Zinc deficiency and effects of zinc supplementation on sickle cell anemia subjects. In *The Red Cell: Fifth Ann Arbor Conference,* ed. G. J. Brewer, pp. 99–117. New York: Liss
51. Prasad, A. S. 1982. Clinical and biochemical spectrum of zinc deficiency in human subjects. See Ref. 17, pp. 3–62
52. Prasad, A. S. 1984. Discovery and importance of zinc in human nutrition. *Fed. Proc.* 43:2829–34
53. Prasad, A. S., Abbasi, A. A., Rabbani, P., DuMouchelle, E. 1981. Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects. *Am. J. Hematol.* 10:119–27
54. Prasad, A. S., Cossack, Z. T. 1984. Zinc supplementation and growth in sickle cell disease. *Ann. Intern. Med.* 100:367–71
55. Prasad, A. S., Halsted, J. A., Nadimi, M. 1961. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am. J. Med.* 31:532–46
56. Prasad, A. S., Miale, A., Farid, Z., Sandstead, H. H., Darby, W. J. 1963. Biochemical studies on dwarfism, hypogonadism and anemia. *Arch. Intern. Med.* 111:407–28
57. Prasad, A. S., Miale, A., Farid, Z., Schulert, A., Sandstead, H. H. 1963. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hypogonadism and dwarfism. *J. Lab. Clin. Med.* 61:537–49
58. Prasad, A. S., Oberleas, D., Wolf, P., Horwitz, J. P. 1969. Effect of growth hormone on nonhypophysectomized zinc-deficient rats and zinc on hypophysectomized rats. *J. Lab. Clin. Med.* 73:486–94
59. Prasad, A. S., Rabbani, P. 1981. Nucleoside phosphorylase in zinc deficiency. *Trans. Assoc. Am. Physicians* 94: 314–21
60. Prasad, A. S., Rabbani, P., Abbasi, A., Bowersox, E., Fox, M. R. S. 1978. Experimental zinc deficiency in humans. *Ann. Intern. Med.* 89:483–90
61. Rabbani, P., Prasad, A. S. 1978. Plasma ammonia and liver ornithine transcarbamoylase activity in zinc deficient rats. *Am. J. Physiol.* 235:E203–6
62. Reding, P., Duchateau, J., Bataille, C. 1984. Oral zinc supplementation improves hepatic encephalopathy. *Lancet* 2:493–94
63. Ruhl, H., Kirchner, H. 1978. Monocyte-dependent stimulation of human T cells by zinc. *Clin. Exp. Immunol.* 32:484–88
64. Sandstead, H. H., Vo-Khactu, K. P., Solomons, N. 1976. Conditioned zinc deficiencies. See Ref. 31, pp. 33–49
65. Sullivan, J. F., Jetton, M. M., Burch, R. E. 1979. A zinc tolerance test. *J. Lab. Clin. Med.* 93:485–92
66. Tapazoglou, E., Prasad, A. S., Hill, G., Brewer, G. J., Kaplan, J. 1985. Decreased natural killer cell activity in zinc deficient subjects with sickle cell disease. *J. Lab. Clin. Med.* 105:19–22
67. Vallee, B. L. 1983. A role for zinc in gene expression. *J. Inherit. Metab. Dis.* 6(Suppl. 1):31–33
68. Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F., Robin, E. D. 1956. Zinc metabolism in hepatic dysfunction. I. Serum zinc concentrations in Laennec's cirrhosis and their validation by sequential analysis. *N. Engl. J. Med.* 255:403–8
69. Walravens, P. A., Hambidge, K. M. 1977. Nutritional zinc deficiency in infants and children. In *Zinc Metabolism: Current Aspects in Health and Disease,* ed. G. J. Brewer, A. S. Prasad, pp. 61–70. New York: Liss
70. Warth, J. A., Prasad, A. S., Zwas, F., Frank, R. N. 1981. Abnormal dark adaptation in sickle cell anemia. *J. Lab. Clin. Med.* 98:189–94
71. Watanabe, T., Salo, F., Endo, A. 1983. Cytogenetic effects of zinc deficiency on oogenesis and spermatogenesis in mice. *Yamagata Med. J.* 1:13–19
72. Weismann, K., Roed-Petersen, J., Hjorth, N., Kopp, H. 1976. Chronic zinc deficiency syndrome in a beer drinker with Billroth II resection. *Int. J. Dermatol.* 15:757–61
73. Weston, W. L., Huff, J. C., Humbert, J. R., Hambidge, K. M., Neldner, K. H., Walravens, P. A. 1977. Zinc correction of defective chemotaxis in acrodermatitis enteropathica. *Arch. Dermatol.* 113: 422–25
74. Whitehouse, R. C., Prasad, A. S., Rabbani, P. I., Cossack, Z. T. 1982. Zinc in plasma, neutrophils, lymphocytes, and erythrocytes as determined by flameless atomic absorption spectrophotometry. *Clin. Chem.* 28:475–80