ZINC DEFICIENCY IN HUMAN SUBJECTS

Proceedings of an International Symposium held in Ankara, Turkey, April 29–30, 1982

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Introduction

Although the essentiality of zinc for animals was known, prior to 1961 its ubiquity made it unlikely that zinc deficiency in man could pose a significant problem. Reports from Iran in 1961 and later studies from Egypt between 1961 and 1963, however, established that nutritional deficiency of zinc could occur in human subjects under practical dietary conditions.

For the next decade the question of zinc deficiency remained somewhat controversial. By the mid-seventies, however, deficiency of zinc was recognized as occurring under a variety of clinical conditions in various developing and developed countries. Today, zinc deficiency is a recognized aspect of several disease states. The index of suspicion in such cases could lead to a significant increase in the diagnosis of clinical zinc deficiency.

In some parts of the world, because of dietary conditions, the high incidence of zinc deficiency appears to be a public health problem. Because considerable progress has been made during the past two decades in the field of zinc metabolism, and because the clinical and biochemical features of zinc deficiency are now well understood, it is possible to correct this form of malnutrition. Considerable progress has also been made in the overall understanding of the bioavailability of zinc, its role in immunological functions, the interactions of trace elements, the role of zinc in bio-membranes, and the enzymatic functions of this mineral. It is these aspects of zinc deficiency in human subjects that are described in this book.

As Chairman of the International Union of Nutritional Sciences Committee on Trace Elements, the editor (A.S.P.) was particularly interested in holding this symposium in a country where the problem of zinc deficiency is widespread. It was hoped that such a meeting would stimulate not only research interest but also the implementation of corrective measures to minimize the effects of zinc malnutrition in Turkey. We are grateful to Dr. Çavdar and other members of the faculty of Ankara University and Hacettepe University who agreed to host the meeting. We thank them for their extreme hospitality and efficient arrangements.

The Editors

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We wish to thank all the speakers and other participants for making this conference a success.

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ZINC DEFICIENCY IN HUMAN SUBJECTS*

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ABSTRACT

During the past two decades, the essentiality of zinc for man has been established. Deficiency of zinc in man due to nutritional factors and several diseased states has been recognized. High phytate content of cereal proteins decreases availability of zinc; thus the prevalence of zinc deficiency is likely to be high in a population subsisting mainly on cereal proteins. Alcoholism is known to cause hyperzincuria and thus may play a role in producing zinc deficiency in man. Malabsorption, cirrhosis of the liver, chronic renal disease and other chronically debilitating diseases may similarly induce zinc deficiency in human subjects. A severe deficiency of zinc has recently been recognized to occur in patients with sickle cell anemia and a beneficial effect of zinc therapy in such patients has been reported.

Growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy and delayed wound healing are some of the manifestations of chronically zinc-deficient human subjects. Taste abnormalities, correctable with zinc supplementation, have been observed in uremic subjects. Recently, abnormal dark adaptation related to zinc deficiency in patients with cirrhosis of the liver and sickle cell

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disease has been reported. In severely zinc-deficient patients, dermatological manifestations, diarrhea, alopecia, mental disturbances and intercurrent infections predominate and if untreated the condition becomes fatal.

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 involved in many biochemical functions. Several zinc metalloenzymes have been recognized in the past decade. Zinc is required for each step of cell cycle in microorganisms and is essential for DNA synthesis. Thymidine kinase, RNA polymerase, DNA-polymerase from various sources and RNA-dependent DNA polymerase from viruses have been shown to be zinc-dependent enzymes. Zinc also regulates the activity of RNase; thus the catabolism of RNA appears to be zinc-dependent. The effect of zinc on protein synthesis may be attributable to its vital role in nucleic acid metabolism.

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.

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

Zinc is known to compete with cadmium, lead, copper, iron and calcium for similar binding sites. 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 anti-sickling 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. Whether

or not zinc could be used to decrease copper load in Wilson's disease remains to be demonstrated.

INTRODUCTION

In biological systems, it was not until 1869 when Raulin showed that zinc was essential for the growth of *Aspergillus niger*. Its essentiality for higher plant life was established in 1926 (Sommer and Lipman 1926). Zinc was reported to be necessary for the growth and well-being of the rat in 1934 (Todd et al. 1934). Tucker and Salmon (1955) observed that zinc cured and prevented parakeratosis in swine. Subsequently zinc deficiency was either observed to occur spontaneously under natural conditions or experimentally induced in many animal species. The manifestations of zinc deficiency in animals included growth retardation, loss of hair, thickening and hyperkeratinization of the epidermis and testicular atrophy. Deficiency of zinc in the diet of breeding hens resulted in lower hatchability, gross embryonic anomalies characterized by impaired skeletal development and varying degrees of weakness in chicks that hatched (Blamberg et al. 1960).

Although essentiality of zinc for animals was recognized, its ubiquity made it seem unlikely that alterations in zinc metabolism could lead to significant problems in human nutrition or clinical medicine. This attitude has now changed.

A strange turn of events took me to Shiraz, Iran, in 1958. I went there to help the American team engaged in setting up a modern medical curriculum and postgraduate training program in medicine at the Nemazee Hospital and Shiraz Medical School. In the fall of the same year, Dr James A. Halsted brought to my attention a 21-year-old male at Saadi Hospital, Shiraz, who looked like a 10-year-old boy. In addition to severe growth retardation and anemia he had hypogonadism, hepatosplenomegaly, rough and dry skin, mental lethargy and geophagia. The patient ate only bread made from wheat flour and the intake of animal protein was negligible. He consumed nearly 1 lb (0.5 kg) of clay daily. Later we discovered that the habit of geophagia (clay-eating) was fairly common in the villages around Shiraz. Further studies documented the existence of iron-deficiency anemia in our patient. There was no evidence for blood loss. Inasmuch as

10 additional similar cases were brought to the hospital under my care within a short period of time, hypopituitarism as an explanation for growth retardation and hypogonadism was considered to be very unlikely. The probable factors responsible for anemia in these patients were:

1. The total amount of available iron in the diet was insufficient.
2. Excessive sweating probably caused greater iron loss from the skin than would occur in a temperate climate.
3. Geophagia may have further decreased iron absorption as was observed later by Minnich et al. (1968).

The anemia was corrected by administration of oral iron in every case.

Lemann (1910) previously observed this clinical syndrome in patients who had hookworm infection. He did not relate this to a nutritional deficiency. Similar cases from Turkey were reported by Reimann (1955). Detailed descriptions were not provided and the author considered a genetic defect to be a possible explanation for certain aspects of the clinical syndrome. Our detailed clinical description of this syndrome from Iran was published in 1961 (Prasad et al. 1961). Although no data were available at that time to document zinc deficiency in those patients, the possibility of this accounting for growth retardation, gonadal failure, skin changes and mental lethargy was considered (Prasad et al. 1961).

The anemia promptly responded to oral administration of pharmaceutical iron. Following therapy with orally administered pharmaceutical ferrous sulfate (1 g daily) and a good hospital diet containing adequate animal protein, the anemia was corrected, hepatosplenomegaly improved, they grew pubic hair, and their genitalia size increased (Prasad et al. 1961). Liver function tests were unremarkable except for the serum alkaline phosphatase which increased following treatment. Retrospectively one might explain this observation on two bases: one is that ordinary pharmaceutical preparations of iron may contain appreciable quantities of zinc as a contaminant and the other is that animal protein most likely did supply available zinc, thus inducing the activity of alkaline phosphatase, a known zinc metalloenzyme.

It was difficult to explain all 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 that zinc deficiency may have been present was considered. As noted earlier, zinc deficiency was known to produce retardation of growth and testicular atrophy in 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 also have decreased the availability of zinc. O'Dell and Savage (1960) first observed that phytate (inositol hexaphosphate), which is present in cereal grains, markedly impaired the absorption of zinc. Changes in the activity of alkaline phosphatase following zinc supplementation to deficient animals were also similar to those observed in our subjects fed adequate diet. Thus, in these subjects dwarfism, testicular atrophy, retardation of skeletal maturation and changes in serum alkaline phosphatase could have been explained on the basis of zinc deficiency.

Subsequently I moved to Egypt. Similar patients were encountered in the villages around Cairo, Egypt. 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 hookworm infection, in contrast to Egyptian subjects who had both schistosomiasis and hookworm infestations, and none gave a history of geophagia.

We were able to carry out a detailed investigation of the Egyptian cases at the U.S. Naval Medical Research Unit No. 3, Cairo. 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 mainly of bread and beans (*Vicia fava*). 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 control subjects (Prasad et al. 1963a, b).

Hypozincemia in humans, in the absence of advanced cirrhosis of the liver, had not been described before. Liver function tests and biopsy failed to reveal evidence of

cirrhosis of the liver in these subjects (Prasad et al. 1963c). Furthermore, in contrast to cirrhosis patients who excrete abnormally high quantities of zinc in urine, our patients excreted less stable zinc in urine, as compared to control subjects. Detailed examination of these patients ruled out other chronic debilitating diseases which might affect the serum zinc levels.

Investigations for deficiency of other metals were also conducted. Serum iron was decreased, unsaturated iron-binding capacity was increased, serum copper was slightly increased and serum magnesium was normal. Hair analysis for manganese, cobalt, molybdenum and other elements revealed no significant decrease as compared to the normal subjects.

Investigation for vitamin deficiency in these patients were also unrevealing. Serum B12, ascorbic acid, vitamin A and carotene levels were not abnormally low. Formimino-glutamic acid excretion following histidine loading and xan-thurenic acid excretion following tryptophan loading were also normal, thus indicating that folic acid and vitamin B12 deficiences were not implicated.

It was a common belief among medical practitioners in Iran that severe growth retardation and sexual hypofunction, as noted above, were the results of visceral leishmaniasis and geophagia. In our detailed investigations, no evidence of visceral leishmaniasis was found. The role of geophagia was not entirely clear; however, it was suspected that the excess amount of phosphate in the clay may have prevented absorption of both dietary iron and zinc. The predominantly wheat diet in the Middle East, which is known to contain high quantities of phytate and fiber, may also have reduced the availability of zinc.

In Egypt, the cause of dwarfism was commonly considered to be schistosomiasis. Chinese investigators have also implicated schistosomiasis as a causative factor for growth retardation (Huang et al. 1957).

Since the Iranian subjects exhibited dwarfism but did not have schistosomiasis or hookworm infections, the question arose as to whether or not schistosomiasis was the fundamental cause of dwarfism in Egypt. An investigation was undertaken by us to answer this question (Prasad et al. 1963d). It was known that there was no schistosomiasis or

hookworm infection in the villages of Kharga, a desert oasis that is 500 km south-west of Cairo. Culturally and nutritionally speaking, however, the people of Kharga are similar to those in the delta region. Therefore, a field study was conducted in this oasis. Sixteen patients with hypogonadism and dwarfism but only mild anemia were studied. None of them had schistosomiasis or hookworm infections and serum concentrations of iron and zinc were low in the group.

Patients in the delta region of Egypt had both hookworm and schistosomiasis infections, which caused blood loss. Since red blood cells are rich in both iron and zinc, these infections were important factors in the production of iron and zinc deficiencies. In Kharga, parasitic infections did not seem to be responsible for these deficiencies. An analysis of a water sample from an artesian spring, which was the principal source of water for the Kharga villages, revealed iron and zinc concentrations of 317 μg% and 1.8 μg% respectively. In Cairo, the iron and zinc concentrations of drinking water were 7 and 40 μg% respectively. Although the food consumed by the subjects in both the delta region and the oasis villages was similar, the latter probably derived a significant amount of iron, but not zinc, from their water source.

In Egypt and China, dwarfism and hypogonadism have been attributed to liver dysfunction due to schistosomiasis. However, the existence of such patients in Kharga and Iran, where schistosomiasis was absent, indicated that this parasitic infection was not responsible for these clinical findings. The study also indicated that severe anemia and iron deficiency were not necessary factors for growth retardation and hypogonadism. Furthermore, as noted earlier, iron deficiency in animals and human subjects does not cause growth retardation and hypogonadism. In view of the above findings and the similarity between the clinical features of dwarfish and hypogonadism and those seen in several species with zinc deficiency, it was a reasonable hypothesis to attribute the dwarfism and hypogonadism in this syndrome to a deficiency of zinc.

It must be re-emphasized that the anemia in all cases was hypochromic and microcytic due to iron deficiency. It was completely corrected by oral administration of iron salts.

The hepatosplenomegaly in this syndrome deserves brief comment. We were unable to account for this on the basis of liver disease. This left three possibilities: anemia, zinc deficiency or a combination of these. Although our studies were inconclusive in this regard, in each case the size of the liver and spleen decreased significantly following zinc supplementation.

Our studies in the Middle East included only males. Female subjects refused to participate in our studies. Later studies from Iran by Halsted et al. (1972) showed that zinc deficiency in females manifesting growth retardation was probably prevalent.

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. 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 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.

Halsted et al. (1972) published the results of their study involving a group of 15 men who were rejected at the Iranian Army Induction Center because of 'malnutrition'. Two women, 19 and 20 years old, were also included. A unique feature was that all were 19 or 20 years old. Their clinical features were similar to those reported by Prasad et al. (1961, 1963). They were studied for 6 to 12 months. One group was given a well-balanced diet containing ample animal protein plus a placebo capsule. A second group was given the same diet plus a capsule of zinc sulfate containing 27 mg of zinc. A third group received the diet without additional supplement for 6 months, followed by the diet plus zinc for another 6-month period. The two women lived in the house of Dr Ronaghy and received the same treatment and observations program.

The development in subjects receiving the diet alone