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SYMPOSIUM ON 'TRACE ELEMENTS AND NUTRITION'

The clinical significance of trace element deficiencies in man

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Trace elements represent a new area of human nutritional research. With the exceptions of iron and iodine, deficiency states were not recognized prior to the 1960's. Indeed, the risk of other trace element deficiencies in man was generally discounted, despite the recognized economic importance of dietary insufficiencies of zinc, copper, manganese, cobalt and selenium in animal husbandry (Underwood, 1971). Since then, however, perspectives in human trace element nutrition have been changing rapidly. In particular, Zn, Cu and chromium deficiency states are now recognized in man, and it is the clinical significance of these deficiencies that will be considered in this paper.

Zn

The occurrence of Zn deficiency was postulated first in 1961 (Prasad, Halsted & Nadimi, 1961). Since then, an increasing number of Zn-responsive syndromes have been recognized. The earliest and most extensive investigations pertained to the syndrome of 'nutritional dwarfism'. It has been estimated that approximately 3% of adolescents in rural areas of Iran and Egypt suffer this syndrome, which also occurs in many other countries (Halsted, Ronaghy, Abadi, Haghshenass, Amirhakemi, Barakat & Reinhold, 1972). On the basis of available evidence it is reasonable to conclude that Zn deficiency is a major aetiological factor in the retarded growth and delayed sexual maturation which are important clinical components of this syndrome. Other features that have been attributed to Zn deficiency include: delayed closure of the epiphyses; roughened, hyperpigmented skin; lethargy; poor appetite; and possibly hepatosplenomegaly.

Geophagia, once thought to be an aetiological factor in Zn depletion, is probably another manifestation of the deficiency state. Other forms of pica may also result from a deficiency of this element (Hambidge & Silverman, 1973). Laboratory

confirmation of Zn depletion in these patients included low levels of Zn in plasma, erythrocytes, hair and urine, and an abnormally low 24 h exchangeable pool of body Zn. Though most studies have focused on teenagers and young adults, a similar syndrome has been observed in younger Iranian children, and Zn depletion may commence in infancy in association with protein-energy malnutrition (Sandstead, Shukry, Prasad, Gabr, El Hifney, Mokhtar & Darby, 1965).

The role of Zn deficiency in the aetiology of 'nutritional dwarfism' has been the subject of considerable controversy, resulting, in part, from the concomitant deficiency of other nutrients, e.g. Fe, and in some instances protein. Moreover, beneficial effects of Zn supplementation on growth have not been observed consistently. However, recent controlled studies have demonstrated an increased growth velocity resulting from dietary Zn supplementation. The failure of some studies to achieve this effect can be attributed to the chemical and physical nature of the local diet, which contains large quantities of phytate and fibre that inhibit the absorption of both supplemental Zn salts and the Zn present in the basic diet (Ronaghy, Reinhold, Mahloudji, Ghavami, Spivey Fox & Halsted, 1974).

Typical diets in Western countries do not have these same characteristics, and, for several years, interest in human Zn deficiency was confined to the Middle East. However, in the United States and elsewhere, symptomatic Zn deficiency has been reported in association with intestinal malabsorption syndromes (Sandstead, 1973), and as a result of excessive urinary excretion of this metal. The medical use of oral and intravenous synthetic diets can also lead to Zn depletion (Hambidge, 1974*a*). Disorders which have been reported to respond favourably to Zn therapy include delayed wound healing and abnormalities of the special senses of taste and smell. Zn supplements have been used to promote rapid healing of surgical wounds (Pories, Henzel, Rob & Strain, 1967), but at considerably higher doses than the normal dietary intake. However, animal studies have shown that a beneficial effect of Zn on the rate of tissue regeneration is demonstrable only if the latter is delayed by Zn deficiency; and, in man, only patients with hypozincaemia have shown a favourable response. Thus, it is concluded that some surgical patients do not have an optimal rate of wound healing because of Zn deficiency.

Idiopathic hypogeusia (diminished taste acuity) and dysgeusia (distaste for food) are now recognized as common conditions in adult Americans, particularly following even mild infections. In a single-blind controlled study, Zn therapy has been effective in correcting these disorders in a large percentage of patients (Schechter, Friedewald, Bronzert, Raff & Henkin, 1972). Alterations in Zn metabolism, including a depression of plasma Zn levels, occur in association with acute infections (Beisel & Pekarek, 1972), but it is not known if the patients with hypogeusia had a total body depletion of Zn. Hypogeusia, anorexia, hyperzincuria, hypozincaemia, and a decline in hair Zn levels have all been noted following thermal burns and are probably interrelated (Cohen, Schechter & Henkin, 1973). Dietary Zn supplementation corrects the hypogeusia manifested by normal subjects who have developed hypogeusia, hyperzincuria and hypozincaemia following the administration of large quantities of histidine (Henkin, Keiser & Bronzert, 1972).

More direct evidence that hypogeusia can result from Zn deficiency has been derived from studies on Denver schoolchildren (Hambidge, Hambidge, Jacobs & Baum, 1972). These studies commenced with a survey of hair trace element concentrations in apparently normal subjects from middle- and upper-income families. Approximately 5% of children more than 4 years of age were found to have hair Zn levels below 70 μg/g, or more than 3 SD below the mean for normal adults. Repeated objective measurements of taste acuity were performed on six of these children with low hair Zn levels, and five had consistent evidence of objective hypogeusia. Tests were repeated 1–3 months after commencing dietary Zn supplementation (0·2–0·4 mg Zn/kg body-weight per d) and taste acuity was then normal in each case. This improvement could not be attributed to a placebo effect. Hair Zn levels increased concurrently with the improvement in taste acuity.

In this study, low hair Zn levels were also associated with poor appetite and growth, both prominent features of Zn deficiency in young animals. Unfortunately, these symptoms are very common, and are not specific for Zn depletion. Definitive investigations are needed to ascertain if the latter is causally related to the poor growth and appetite manifested by these children, who were not preselected with respect to height or weight, and whose low height percentiles were not explicable on a familial basis. Incidentally, low hair Zn levels are not a characteristic feature of short stature per se; children with short stature, attributable to other recognized causes, do not have unusually low hair Zn levels.

In the absence of any other predisposing factors, it appears probable that these children were suffering from an inadequate dietary intake of Zn. Although it has long been considered impossible, recent evidence suggests that substantial sections of the population of the United States are at risk from suboptimal Zn nutrition (Sandstead, 1973), especially at times of increased requirements and among those living on low-income diets. The original Denver study did not include children from low-income families; subsequently, the Zn status of twenty-nine young children enrolled in the Denver 'Head Start' program has been investigated. The mean hair Zn level of these children, aged 3·5–6 years, whose heights were below the 3rd percentile, was significantly lower than that of children from middle- and upper-income families. Of these children 40% had hair Zn levels of less than 70 μg/g. In addition, the mean plasma Zn concentration and the mean rate of Zn secretion in parotid saliva were significantly lower than those of normal children.

Hair Zn levels are particularly low during infancy in the United States (Hambidge *et al.* 1972). It is not certain to what extent these low levels may reflect some degree of body depletion of Zn, but results from other countries, e.g. Thailand (Hambidge, Walravens, Kumar & Tuchinda, 1974), indicate that this marked decline in hair Zn concentrations during infancy cannot necessarily be accepted as normal. One factor predisposing to Zn depletion may be the low Zn content of some infant milk formulas that are used widely in the United States. The mean hair Zn level (124 ± 12 μg/g) of a small group of English infants, though lower than that of adults, was found to be 68% higher than that of the Denver infants. The Zn content of the milk formulas consumed by the English infants was comparable to that of cow's milk and of

human milk during the first 2 months of lactation. Plasma Zn levels of infants in the United States are also low compared with those of other age groups (Henkin, Schulman, Schulman & Bronzert, 1973), and can be increased significantly at 3 months of age by Zn supplementation of infant milk formulas (P. Walravens & K. M. Hambidge, unpublished results).

In most instances, these low Zn levels are not associated with any detectable, clinical sequelae, but this does not apply to all infants. Experience with individual patients who have had low plasma and hair Zn concentrations, and who respond favourably to Zn supplementation, indicates that Zn deficiency has to be considered in the differential diagnosis of failure to thrive in infancy, especially when anorexia is a prominent feature. It is interesting to note that the growth percentiles of the older children with low hair Zn levels, who were included in the original Denver study, first began to decline during infancy.

The clinical importance of some of the documented sequelae of 'marginal' Zn deficiency, for example, minor deviations from optimal growth rates, may be debatable. However, studies of Zn-deficient animals, which have been more extensive than in man, give reason for concern that the extent of human pathology resulting from an insufficiency of dietary Zn has not yet been realized. For example, in the rat, Zn depletion in early postnatal life can impair learning ability, and maternal Zn deficiency has severe teratogenic effects (Hurley, 1969). Some degree of human Zn depletion may be common during pregnancy (Hambidge & Droegemueller, 1974), but it is not known if this can be detrimental to the foetus (Sever, 1973). Though information on the clinical significance of human Zn deficiency is limited, there are sufficient indications of its importance to necessitate more extensive research.

Cu

For many years human Cu deficiency had been considered impossible because of the small nutritional requirement and the presence of this metal in water and all foods. This concept received some support in 1960 when a deliberate attempt to induce Cu deficiency in premature infants was unsuccessful. However, since 1964 there have been numerous reports of Cu deficiency. In most cases the deficiency has been associated with more generalized malnutrition, or has been secondary to intestinal malabsorption states and prolonged diarrhoea, followed by rehabilitation with milk-based diets (Graham & Cordano, 1969). Milk is one of the poorest sources of dietary Cu, and a syndrome of pure Cu deficiency has been reported (Ashkenazi, Levin, Djaldetti, Fishel & Benvenisti, 1973) in premature infants who had been fed with low-Cu milk preparations. Another example of a poor dietary source of Cu is provided by some solutions used for total parenteral feeding, which have been implicated in the aetiology of symptomatic Cu deficiency (Karpel & Peden, 1972). Although mainly a disease of infants, Cu deficiency has been recognized in older children and adults (Dunlap, James & Hume, 1974).

In comparison with Zn deficiency, the incidence of Cu deficiency is probably small and limited to special circumstances. However, the clinical sequelae, which

are relatively dramatic and acute, have been more clearly defined and are better understood in terms of aberrations of normal biochemistry.

Neutropaenia and hypochromic anaemia, which does not respond to oral Fe therapy, are early manifestations of the Cu-deficiency syndrome. The anaemia results, in part, from a reduction in Cu-containing ferroxidases, including caeruloplasmin, which are involved in the release of Fe from body stores. Cu is also required for the intracellular metabolism and transport of Fe within the normoblast, and, in later stages of Cu deficiency, anaemia does not respond to parenteral Fe.

Skeletal lesions are another dramatic feature of this disease. Osteoporosis is an early finding, followed by enlargement of the costochondral cartilages. Later changes include cupping and flaring of long bone metaphyses with spur formation and submetaphyseal fracture, epiphyseal separations, periosteal reactions, and spontaneous fractures of the ribs. The radiological findings may suggest a diagnosis of battering. Lack of Cu-containing amine oxidases, required for the cross-linking of bone collagen, and of the Cu-enzyme ascorbic acid oxidase (*EC* 1.10.3.3), have both been implicated in the pathogenesis of the bone lesions.

Decreased pigmentation of the skin and hair in Cu deficiency is secondary to impairment of tyrosinase (*o*-diphenol oxidase; *EC* 1.10.3.1) activity, which is necessary for the production of melanin. Dilated superficial veins, seborrhoeic dermatitis, anorexia, failure to thrive, diarrhoea and hepatosplenomegaly are other clinical features which have been associated with Cu deficiency. Neurological abnormalities described in Cu-depleted premature infants include hypotonia, possible psychomotor retardation, and apnoeic episodes, with marked improvement following Cu supplementation. Very severe central nervous system disease is present in Menkes' 'Steely Hair' syndrome, in which a profound Cu deficiency state results from a specific inherited defect in the intestinal absorption of this element (Danks, Cartwright, Stevens & Townley, 1973). It is probable that a deficiency of several Cu-containing enzymes, including cytochrome oxidase (*EC* 1.9.3.1), is involved in the pathogenesis of the central nervous system disease.

Recognition of the clinical importance of human Cu deficiency has been followed by certain prophylactic measures, including Cu supplementation of one 'low-Cu' infant milk formula in the United States, and the addition of Cu to parenteral hyperalimentation solutions in some centres. However, it is necessary to maintain a high index of awareness of the possibility of this nutritional deficiency in sections of the population at risk, particularly in the presence of neutropaenia, anaemia, or radiological abnormalities of the skeleton. Treatment of the severe morbidity resulting from nutritional Cu deficiency is simple and very satisfactory once the diagnosis has been made.

Cr

In contrast to the situation for Zn and Cu, it is only recently that a mammalian nutritional requirement for Cr has been recognized (Mertz, 1969). A main physiological role of this element is as a cofactor for insulin at the insulin-responsive cell membrane, and the earliest detectable defect resulting from Cr deficiency is an

impairment of glucose tolerance. Other reported sequelae of Cr deficiency in animals include retarded growth, high circulating cholesterol levels, and increased atherosclerosis.

Cr does not have any pharmacological affect on glucose tolerance, and, therefore, any improvement in glucose utilization following human Cr supplementation, under adequately controlled conditions, provides prima facie evidence for pre-existing Cr deficiency. By this means, Cr deficiency has been shown to be one of the aetiological factors responsible for the impairment of glucose tolerance associated with protein-energy malnutrition in Jordan, Nigeria and Turkey. In Turkey the weight gain of Cr-supplemented infants was significantly greater than that of control infants over the subsequent 30 d (Gürson & Saner, 1973).

Dietary supplementation with inorganic trivalent Cr has also corrected the mild impairment of glucose tolerance of some middle-aged and elderly subjects in the United States. However, in contrast to the situation in malnourished infants, many weeks or months of supplementation have been necessary before any improvement is noted. This can be attributed to the poor absorption and relatively low biological activity of inorganic Cr. The naturally-occurring organic Cr complex, in which Cr is liganded to nicotinic acid and certain amino acids (Mertz, 1974), is better absorbed and has much greater biological activity. This 'glucose tolerance factor Cr' (GTF-Cr) is not yet available in a purified form. The best natural source of GTF-Cr is brewer's yeast, and this is now being used in clinical trials. Initial results have been encouraging with respect to improvement in glucose tolerance and hyperlipidaemia (R. J. Doisy, personal communication).

Laboratory markers for the diagnosis of Cr deficiency have not yet been refined to the extent needed to achieve a confident diagnosis. However, results derived from analyses of plasma and hair samples indicate that Cr depletion is common during pregnancy (Hambidge, 1974*b*). Excessive urinary loss of Cr may contribute to an increased risk of Cr deficiency in diabetic patients.

The importance of human Cr deficiency must be assessed not only on the basis of its clinical importance to the individual, but also on the incidence of this nutritional deficit. A large percentage of dietary Cr is lost from some food items as a result of modern methods of food processing, and high-carbohydrate diets may increase the risk of deficiency by enhancing urinary Cr excretion. Suboptimal Cr nutrition may be common in adults in some areas of the world, including the United States (Schroeder, 1968). It is not known to what extent a deficiency of this nutrient may contribute to the high incidence of glucose intolerance, hypercholesterolaemia and cardiovascular disease in that country.

Conclusions

Despite substantial progress within the last few years, it is apparent that the full clinical significance of these trace element deficiencies in man is not known. Moreover, in the light of recent experience with Zn, Cu and Cr, there can be no reassurance that man is not at risk from other trace element deficiencies. For example, in 1973 a case of probable Mn deficiency was described in a volunteer who had been

fed on an artificial diet fortuitously low in this element (Doisy, 1974). The list of essential trace elements for animals continues to grow, and each of the 'new' elements presents an additional challenge to those engaged in human trace element nutritional research.

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33 (3) 5