Ultra Trace Elements and Selenium

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During the past decade there has been a large upsurge in interest in nutritionally essential trace elements. In the clinical field the recognition that deficiency can occur in human subjects in many practical dietary situations has been of crucial importance from the public health viewpoint worldwide. In this chapter we review briefly our knowledge of the ultra trace elements chromium, manganese, nickel, and silicon; the remaining space is devoted to selenium because recent findings in the People's Republic of China are of particular interest in medicine. A full understanding of the role of a given trace element in human subjects can be achieved only when the detailed biochemical role of the element is elucidated; therefore the biochemistry of the various elements is also considered insofar as it is understood.

CHROMIUM

The specific biochemical function of chromium appears to be uniquely as a cofactor of insulin (1). In the form of the naturally occurring dinicotinic acid-glutathione complex, called the glucose tolerance factor (GTF), chromium significantly increases the effect of exogenous insulin on glucose oxidation in epididymal adipose tissue in vitro and has a similar effect in several systems that are responsive to insulin in vitro (2). The effect of chromium is a true potentiation of the effect of the hormone, and GTF has no effect in the absence of insulin. The role of chromium appears to extend to all the known functions of insulin in vivo, e.g., glucose tolerance, insulin-induced hypoglycemia, utilization of glucose in glycogenesis, plasma membrane transport of amino acids and amino acid analogs, and utilization of amino acids in protein synthesis (3).

Studies of chromium deficiency in animals have demonstrated three phases in the development of the resulting disease state, which are also seen in human studies. First, there is a rise in the circulating level of insulin and a greater-than-normal insulin response in the glucose tolerance test, as greater hormone production compensates for the insulin resistance caused by chromium insufficiency. In the second phase, metabolic disorders such as abnormal glucose tolerance and disturbed lipid metabolism develop in the presence of a further increase in circulating insulin,

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1For a discussion of this chapter, see Discussion in the chapter by G. Q. Yang (this volume).
and in the third phase there is marked insulin resistance and the development of a syndrome akin to insulin-dependent diabetes mellitus, when pancreatic insulin production is exhausted. In this context, therefore, it should be noted that chromium deficiency should be considered in clinical cases where there is unexplained insulin resistance.

No simple answer can be given to the question of whether chromium deficiency is of significance as a major world health problem. Certainly the occurrence of chromium deficiency with its metabolic consequences is established in human populations around the world. There is a considerable risk that patients given long-term total parenteral nutrition may become chromium-deficient because the chromium content of intravenous fluids was not well controlled. In one study in Scotland a highly negative balance was seen in three patients on total parenteral nutrition (4), and chromium deficiency was reported in the United States by Jeejeebhoy et al. (5). On the other hand, in West Germany high chromium concentrations observed in parenteral fluids were well above the level needed to maintain an adequate chromium intake (6). The identification of the possibility of a problem in this area should ensure that users of total parenteral programs around the world will take steps to ensure adequate chromium content in the intravenous fluids employed. The extension of this concern to special infant formula feeding may define the need for supplementary chromium here too, but at the present time there appears to be little information available on the chromium requirements of infants.

In the general population, the risk of chromium deficiency increases under all situations where highly purified foods replace a more natural varied diet. However, it is in developing countries that the greatest risk arises, particularly among malnourished infants and children, and reports of chromium deficiency are available from Jordan (7), Nigeria (7), Turkey (8,9), and India (10). The hypothesis that chromium deficiency may be of significance in certain forms of ischemic heart disease has given rise to widespread interest. The topic was reviewed briefly by Mertz (11), who concluded that "the known effect of chromium on three risk factors for ischaemic heart disease (circulating insulin, glucose tolerance and serum lipoproteins) should provide the stimulus for further studies in man."

**MANGANESE**

In contrast to many other trace elements, the concentration of manganese does not vary much from tissue to tissue, although bone and liver appear to have a fairly high concentration (about 2 μg/g fresh weight) (12), and the serum level is very low (1–15 μg/liter) (13). Homeostatic regulation of manganese levels in vivo is principally brought about through excretion via the bile, although in situations of overload a further gastrointestinal route is also employed. Minor regulation may, however, be applied in ruminants at the absorptive stage (14).

The biochemical function of manganese is diffuse in the sense that many enzymes use the element as a cofactor, but few of them show lowered activity in manganese deficiency, probably because another divalent cation such as magnesium can sub-
stitute for manganese when the availability of manganese is limited. The glycosyl transferases do, however, show lowered activity in manganese deficiency (15), and there is a need to establish whether the resultant impairment of glycoprotein and glycosaminoglycan synthesis is of physiological significance. Pyruvate carboxylase, on the other hand, is a metalloenzyme that normally depends on manganese for its catalytic activity, but in manganese deficiency the enzyme continues to function by replacing the manganese with magnesium; indeed, in some species pyruvate carboxylase appears to contain both manganese and magnesium under normal conditions (16).

Of particular current interest is the fact that one form of the superoxide dismutase (SOD) enzymes contains manganese; the mammalian cytosolic SODs are copper/zinc enzymes, and the mitochondrial (and bacterial) enzyme is a manganese-containing metalloenzyme (17). Ultrastructural changes in several tissues of manganese-deficient animals are consistent with damage caused by oxygen radicals, and it is reasonable to suppose that, in the absence of manganese, deficiency of the mitochondrial Mn SOD leads to damage caused by superoxide anion radicals or by other oxygen-derived radical species. This may be of particular importance when considering selenium deficiency, where another enzyme, glutathione peroxidase, that is involved in protection against oxygen radicals may be impaired when the element is deficient (Fig. 1) (18).

Although much of the evidence which indicates that manganese is an essential element for man is indirect, the study of Doisy (19) appears to indicate the truth of this conclusion. At the present time, interest is focused on the role of manganese in brain metabolism, as it has been known for many years that the element plays a part in the normal function of the brain. Both manganese deficiency (20) and manganese toxicity affect brain metabolism, and human subjects with manganism show profound neurological disturbance similar to that of Parkinson's disease (21). It is now clear that there is a close relationship between manganese and catechol-

**FIG. 1.** Interaction of several nutrients in the prevention of oxygen radical-induced cellular injury.
amine metabolism, and the earlier experiments in manganese-deficient rats (20) have now been related to a study in which it was shown that the blood manganese level of 25 children with convulsive disorders was significantly lower than in controls (22,23). The significance of these findings in the etiology and management of epilepsy and convulsive disorders in man is not clear, and further work is needed in this important area.

NICKEL

Signs of nickel deficiency have been demonstrated to date in at least six species, but no adequate similar demonstration has been made for man (24). The signs of deficiency in different animal species are bewilderingly different, but Nielsen (24) has suggested that, if it is recognized that there is a close interaction between nickel and iron metabolism, some of the differences may be resolved by explanations that involve taking into account the differing iron status of the animals used in the various studies, and that it can then be established that nickel fulfills the requirements for essentiality defined by Mertz (1). The evidence showing that nickel is essential does not, however, extend at present to an unequivocal identification of the functional site of its activity in biochemical terms. The most promising possibilities are either that nickel may function as a cofactor or structural component in a specific metalloenzyme or metalloprotein, or that it is a cofactor which facilitates the intestinal absorption of ferric iron. A nickel-containing macroglobulin called nickelplasmin was isolated from rabbit and human serum (25), but there is no indication to date as to the physiological significance of nickelplasmin. The discovery that in plants and bacteria urease is a nickel metalloenzyme (26) has stimulated the search for the element in eukaryotic metabolism, so far without success. Studies by Nielsen (27,28) have demonstrated that, depending on the form of dietary iron, nickel is of importance in the absorption of iron, and it was concluded (24) that nickel is essential for the enzymatic formation or structural integrity of a molecule involved in ferric iron absorption.

The implication that nickel may be of importance in human metabolism rests on a number of observations of a somewhat circumstantial nature (24), and although there are a number of situations where nickel may be of importance in medicine, e.g., nickel allergy, there is no obvious area to which research could particularly be directed.

SILICON

Most early studies which indicated the essentiality of silicon involved the use of purified amino acid diets that gave suboptimal growth rates in the control animals (29). More recently, the semisynthetic diets used by Carlisle (30,31) gave near-optimal growth in controls; here silicon deprivation gave little outward sign of deficiency, but bone deformities were found to be consisent with depressed contents of articular and other cartilage, water, and hexosamine and collagen, indicating clearly that silicon is essential to the chick and the rat.
The precise biochemical function of silicon is not known, but the tissue distribution of the element and the effect of its deficiency on connective tissue support the hypothesis that silicon functions as a biological cross-linking agent which thereby gives resilience to the structure of collagen and possibly of elastin. Although there has been some speculation about the possible structural contribution of silicon, none of the proposed structures have been unequivocally identified in biological material. The possibility that silicon is involved in calcification directly, rather than by its role in the formation of the collagen matrix, has been considered. The high level of silicon in bone mitochondria tends to suggest a direct role of silicon in the calcification process.

The estimated requirement of silicon by the chick is in the range of 100 to 200 ppm. One balance study in man has been carried out which indicated that on a low-fiber diet young men excreted about equal amounts of silicon in urine and feces, whereas on a high-fiber diet which contained more silicon three times as much silicon was excreted in the feces as in the urine; it was estimated that the oral intake of silicon was in the range of 21 to 46 mg/day. The question as to whether silicon is an essential trace element for man remains an open one, although there has been speculation that the element may be involved in several human disorders, notably atherosclerosis, osteoarthritis, and hypertension, because of the involvement or possible involvement of collagen and elastin in the etiology of these conditions. There is a great need therefore for further detailed investigation here of the possible role of silicon.

**SELENIUM**

The biochemical role of selenium in animals was elucidated in 1973 when Hoekstra and his co-workers showed that the element is an integral functional part of the active center of the enzyme glutathione peroxidase. There still exists, however, the possibility that other mammalian functions for selenium unrelated to glutathione peroxidase may come to light. The past decade has seen the elucidation of the role of selenium as one factor in the body's armory of protection against the toxicity of oxygen and the clear establishment of selenium as an essential dietary factor for man. The detail of the work that has led to these important conclusions has been reviewed, and only the key salient features are repeated here.

Oxygen is usually regarded as having a benign role in biology by virtue of its function as the terminal electron acceptor in respiratory and other processes which, in general, protect the constancy of the internal cellular environment and ensure the availability of energy-rich substrates. However, during the four-electron reduction of oxygen in water, oxygen-derived radicals and activated forms of oxygen may arise which are potentially damaging to key biological macromolecules such as DNA and proteins, as well as to the unsaturated phospholipids of biological membrane phospholipids on whose integrity so much of the functional complexity of cells depends. The reduction of molecular dioxygen involves four single electron steps, which can be summarized thus:
O₂ → O₂⁻ (Superoxide anion radical)

O₂⁻ → O₂⁻ (Peroxyl anion)

O₂⁻ + H⁺ → HO₂⁻ → H₂O₂ (Hydrogen peroxide)

H₂O₂ → OH⁻ + OH⁻ (Hydroxyl radical)

Net: O₂ + 4H⁺ → 2H₂O

Although the reactivity of the superoxide anion radical and the peroxyl anion must be considered to be of importance in the biological context, the reactivity of the hydroxyl radical is many orders of magnitude greater and must be regarded as of primary importance in the initiation of oxygen metabolite-induced tissue damage. At neutral pH in a buffered biological system, the disproportionation of O₂⁻ to H₂O₂ and O₂, and of H₂O₂ to H₂O and O₂, is very slow. It is possible that if O₂⁻ and H₂O₂ accumulate reactions of the type first described by Haber and Weiss (37) may take place:

O₂⁻ + H₂O₂ → O₂ + OH⁻ + OH⁻

but this reaction is likely to be insignificant at neutral pH. However, if it is appreciated that it can be catalyzed by divalent cations such as iron, it may be an important means of generating hydroxyl radicals in vivo (38):

O₂⁻ + Fe^{III} → O₂ + Fe^{II}

H₂O₂ + Fe^{II} → OH⁻ + OH⁻ + Fe^{III}

The question of whether enough free iron is present in tissues to carry out this function is a vexatious one, but in view of the fact that the role of iron here is catalytic it is probable that the small amounts of free iron present are sufficient (39). Fortunately, in living organisms the concentrations of O₂⁻ and H₂O₂ are unlikely to reach high levels because their removal is catalyzed in the case of O₂⁻ by the superoxide dismutases, and in the case of H₂O₂ by peroxysomal catalase and glutathione peroxidase located in the mitochondria and in the cytoplasm. Superoxide dismutase enzymes are of two main types: the mitochondrial manganese-containing enzyme mentioned earlier and the cytosolic enzyme that contains copper and zinc. The cytosolic enzyme appears to depend principally for its catalytic activity on copper, and the dependence of the enzyme on zinc is at present less certain. Thus imbalance in the nutritional supply of manganese, or copper and zinc, may lead to unfortunate consequences, and deficiency of selenium also gives rise to severe oxygen radical-induced pathology due to the absence or inadequacy of glutathione peroxidase. These interactions are summarized in Fig. 1.

Despite the fact that selenium deficiency in farm animals has for many years been well recognized and indeed has been of considerable economic significance in parts of the world such as the South Island of New Zealand and Oregon in the
United States, where soil levels of selenium are very low, no comparable selenium deficiency disease was recognized in man until quite recently. Attention has now been focused on the People's Republic of China, where a cardiomyopathy of children and young women, called Keshan disease, has been shown to be selenium-responsive, and where it appears that the condition is caused by an exceedingly low level of dietary intake of selenium (40). Keshan disease is characterized by multiple focal myocardial necrosis throughout the heart muscle, and the focal nature of the pathology distinguishes it from viral myocarditis with which it was at first confused. The ultrastructural pathology is of particular interest in that early mitochondrial lesions occur that are very reminiscent of the mitochondrial lesions seen in the livers of selenium-deficient rats (K. Ge, personal communication). The incidence of the disease is restricted to certain strongly leached hill and mountain soil regions in a broad band that extends from the northeast to the southwest of China. The similarity of the disease to mulberry heart disease in pigs, the fact that the degeneration of the leg muscles of certain Keshan disease patients is similar to white muscle disease in lambs, and the incidence in the endemic Keshan disease areas of selenium deficiency myopathies led to the discovery that Keshan disease was a selenium-responsive condition (C. G. Xu, personal communication). Studies of selenium contents of soils, pastures, grains, and food constituents derived from the affected areas, together with measurement of selenium in blood and other body fluids, hair, and pathological material, were carried out by the Chinese workers with meticulous care (18, 40).

When assessing the importance of Keshan disease in a world context, it is necessary to evaluate the blood levels of selenium seen in deficient populations in China and compare them with blood selenium levels in other populations around the world. Unfortunately, different units of measurement have been used by different workers in reporting blood selenium levels, and it is necessary to make some assumptions when calculating common values that can be compared. Table 1 shows values that were obtained as a result.

<table>
<thead>
<tr>
<th>Location</th>
<th>Selenium (ng/ml whole blood)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>New Zealand</td>
<td>20–140</td>
<td>95</td>
</tr>
<tr>
<td>West Germany</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal children</td>
<td>53–130</td>
<td>90</td>
</tr>
<tr>
<td>Dietetically treated children</td>
<td>6–52</td>
<td>42</td>
</tr>
<tr>
<td>United States</td>
<td>145–210</td>
<td>200</td>
</tr>
<tr>
<td>London*</td>
<td>112–157</td>
<td>120</td>
</tr>
<tr>
<td>China</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keshan disease area</td>
<td>5–10</td>
<td>7</td>
</tr>
<tr>
<td>Nearby &quot;safe&quot; area</td>
<td>20–50</td>
<td>40</td>
</tr>
</tbody>
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*Published here for the first time.
It is clear from these figures that few if any of the subjects in New Zealand approached the remarkably low levels of selenium found in many Keshan disease patients. It is, however, noteworthy that the dietetically treated children in Dusseldorf, West Germany, who were given special diets because they had phenylketonuria or maple syrup urine disease, often had blood selenium levels within the Keshan disease range. The patients with Keshan disease clearly had very low levels of selenium in their blood. It seems that very low levels of selenium can be tolerated by man and that only when the blood level falls below about 10 ng/ml is it likely that a true deficiency syndrome may be seen. This explains the fact that in the South Island of New Zealand even though the selenium intake may be very low there is still marginally sufficient dietary selenium available to prevent a selenium-responsive lesion from developing. An immediate question arises as to whether it is necessary to supplement special diets given to genetically disordered children and special infant formula feeding regimens with selenium. It is now certain that selenium is an essential trace element for man, and that blood selenium levels have been recorded in children given these diets that are severely low. This renders inescapable the conclusion that a modest level of supplementary selenium should now be included in such infant feeds.

REFERENCES


