

## Recent studies of antagonistic interactions in the aetiology of trace element deficiency and excess

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In recent years, evidence from both field and laboratory studies has shown that a number of trace element deficiencies arise or can be induced as a result of biological interactions between these nutrients. Similarly, some of the toxic effects resulting from the ingestion of excessive amounts of both essential and non-essential elements may be ascribed to interactions with biological processes directly or indirectly involving other trace elements. Some appreciation of the range and complexity of the trace element interactions that may occur under practical situations can be obtained from Fig. 1.

In this paper a positive interaction is defined as one in which the presence of one element is essential for the normal metabolic action of a second element and a negative interaction as one in which the presence of an excess of one (or more) element antagonizes the normal actions of a second. It is clear that a complete discussion of all these complex interactions is beyond the scope of this review and thus a selected few will be considered in order to highlight some of the important features characteristic of these interactions.

### *Competitive interactions*

Some of these interactions were first rationalized by Hill & Matrone (1970) who advanced the thesis that 'those elements whose physical and chemical properties are similar will act antagonistically to each other biologically'. The mutual interactions occurring in biological systems between copper, zinc and cadmium amply illustrate this hypothesis. The  $Zn^{2+}$ ,  $Cd^{2+}$ ,  $Cu^{+}$  (as well as  $Hg^{2+}$ ) ions all have the same electronic structure of their valence shells and have similar tendencies to form complexes with a co-ordination number 4 and a tetrahedral disposition of ligands around the metal. Mutual biological antagonisms between these elements should therefore occur as a consequence of the isomorphous replacement of one element by another at some functional site. Indeed, results of the nutritional studies on chicks described by Hill & Matrone (1970) which demonstrated that both Zn and Cd acted as Cu antagonists and that Cd acted as a Zn antagonist are consistent with their hypothesis.

For the purposes of this paper biological interactions of this nature will be referred to as 'competitive interactions'. In addition to the Cu-Zn-Cd interrelations the nutritional interactions between arsenic and selenium, sulphur and Se, tungsten and molybdenum, manganese and iron, and vanadium and chromium (see Fig. 1) can all be shown to fall into this category.

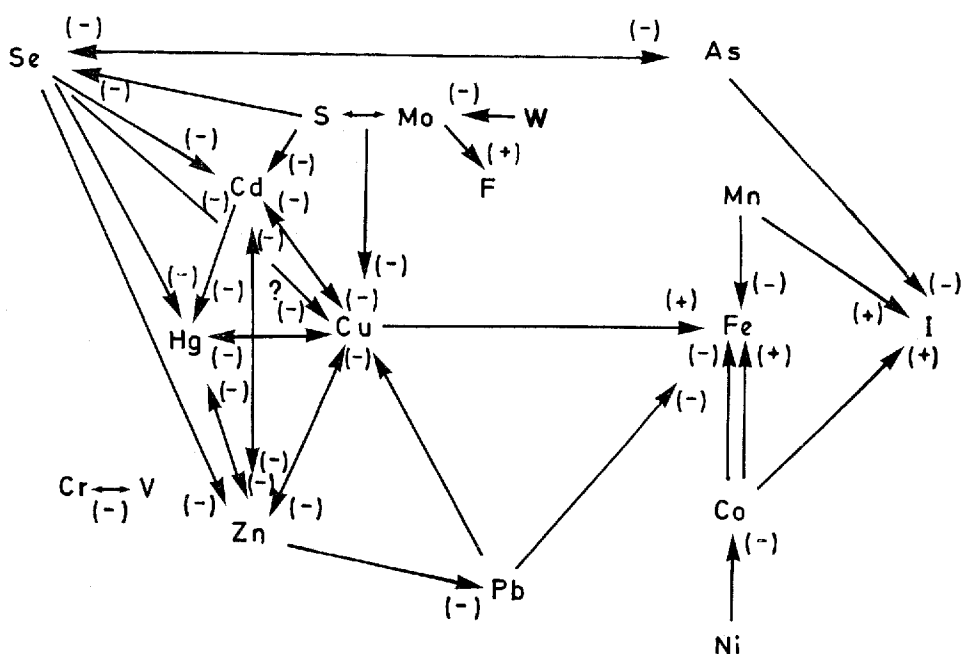


Fig. 1. Schematic representation of biological interactions between trace elements selenium, arsenic, sulphur, molybdenum, tungsten, cadmium, fluorine, manganese, mercury, copper, iron, iodine, chromium, vanadium, zinc, lead, cobalt and nickel. The direction of an arrow between two elements indicates the direction of the interaction from antagonist to agonist: (-) represents a negative interaction and (+) a positive interaction. For definitions, see p. 293.

Two features are common to all competitive interactions. First they are 'negative' and secondly, the effects of one element on another are mutual. These features are evident in the mutually antagonistic effects of excessive dietary intake of Cu or Zn. Campbell & Mills (1974) have demonstrated that rats maintained on a diet providing marginally adequate amounts of Cu exhibit a range of signs indicative of Cu deficiency when the diets were supplemented with high levels of Zn. On the other hand pigs fed on high-Cu rations suffer parakeratotic lesions which are symptomatic of uncomplicated Zn deficiency (Ritchie, Luecke, Baltzer, Miller, Ullrey & Hofer, 1963).

The syndrome resulting from the effects of excessive dietary intake of an antagonistic element on the metabolism of another should be reversible if the intake of the agonist is increased. This is exemplified by the protective effect of Cu supplementation on the toxicity syndrome in chicks resulting from high dietary intakes of Zn (Hill & Matrone, 1970). Conversely, Suttle & Mills (1966) found that increased dietary intakes of Zn increased the tolerance of pigs to high dietary concentrations of Cu.

One absolute requirement of this thesis is that the chemical forms of respective agonist and antagonist at the site of interaction are similar. Thus the competitive interaction of dietary S on Se metabolism is conditioned by the chemical forms of the

ingested S and Se compounds. Dietary sulphate, for instance, is very much more effective in protecting against Se toxicity resulting from excessive intake of dietary selenate than of either selenite or the organic Se compounds present in seleniferous grains (Halverson & Monty, 1960; Halverson, Guss & Olson, 1962).

One further point which needs to be emphasized is that if the chemical forms of essential elements both in the diet and at the functional sites are known it should be possible to predict which other elements may give rise to biologically significant interactions. These interactions may be either deleterious and toxic antagonisms inducing deficiency states, or beneficial antagonisms conferring protection against toxicity syndromes. The relevance of such interactions to the utilization of the newly discovered essential trace elements (Mertz, 1974) remains to be determined.

#### *Non-competitive interactions*

While this chemically described model accounts for many of the interactions encountered in trace element metabolism there are others such as the effects of Cu on Fe metabolism, and Mo and S on Cu status, which do not fit into this general classification. In this paper, these will be referred to as 'non-competitive' interactions. They arise when deficiency or excess of one (or more) element influences the metabolic fate of a second element or interferes with some biological process(es) essential for the full expression of its biological activity. One of the first trace metal interactions reported, namely the requirement of dietary Cu for promotion of haematopoiesis (Hart, Steenbock, Waddell & Elvehjem, 1928) may be described as non-competitive. Recent experiments have shown that this 'positive' interaction arises because the Cu-containing protein ferroxidase I, whose activity is markedly depressed in Cu deficiency, is essential for mobilization of Fe from Fe stores prior to its incorporation into haemoglobin (Ragan, Nacht, Lee, Bishop & Cartwright, 1969; Roeser, Lee, Nacht & Cartwright, 1970).

An example of a 'negative non-competitive interaction' commonly encountered in ruminant nutrition is the Mo, S and Cu interrelationship. The clinical signs induced by a dietary excess of Mo resemble those of Cu deficiency and may be reversed by increasing the intake of Cu. Two possible mechanisms have been proposed to account for this; first an interference with ruminal S metabolism (Mills, 1960) and secondly the direct formation of an insoluble, non-available Cu-Mo complex (Dowdy & Matrone, 1968*a, b*). Which, if either, of these mechanisms accounts for the disturbance in Cu metabolism is not yet clear, but nevertheless it is evident that this interaction does not arise due to similarities in the chemical properties of the agonist and antagonists.

Considering the many different ways in which 'non-competitive' interactions may arise it is obvious that no set of rules concerning reversibility, mutuality or the negative or positive nature of the effects can be drawn up as for competitive interactions. Similarly, interactions of this type are not predictable from a knowledge of the chemistry of the elements in question and their discovery usually results from empirical observation.

*Multi-element interactions*

A further point of significance relevant to both competitive and non-competitive interactions is that the effect of an antagonistic element on another may occur as a consequence of its effects on a third element. This can be illustrated most clearly by the interaction between Zn, Cu and Fe. High dietary intakes of Zn cause an anaemia which can be largely overcome by dietary Cu supplementation (Hill & Matrone, 1970). In these circumstances Zn induces or exacerbates a conditioned Cu deficiency which in turn restricts the utilization of Fe. Similar secondary effects upon Fe utilization occur during the interactions of Cu and Cd, and Zn and Cd (Hill, Matrone, Payne & Barber, 1963).

From these examples it is evident that due account must be taken of the possible influence of multi-element interactions both in the design of experimental studies of the effects of deficiency and toxicity and in the interpretation of field survey data on the composition of diets or of animal tissues.

*Recent studies on Zn-Cu interactions*

A trace-element deficiency syndrome results from a failure in the supply of the essential nutrient to at least one of its functional sites. When the deficiency is 'conditioned', i.e. induced by the presence of antagonists in the diet, the interaction may occur at any one (or more) of these essential loci. This may occur during gastrointestinal or cellular absorption and during incorporation into binding sites on enzymes, transport proteins, storage compounds, and cellular structural proteins. The mutually antagonistic effects of Zn and Cu in biological systems illustrate the many sites at which such interactions can arise. Kinetic studies indicate that both Zn (Davies, 1973) and Cu (Gitlin, Hughes & Janeway, 1960) are absorbed by mechanisms involving binding to 'carrier' proteins. The possibility that competition for a common carrier could be significant in Zn-Cu interactions is supported by findings of inhibitory effects of Zn on Cu absorption (Van Campen & Scaife, 1967; Starcher, 1969) and of Cu on Zn absorption (Van Campen, 1969). Chvapil, Ryan & Brada (1972) have reported that Cu can decrease the stability of lysosomal membranes *in vitro* and that Zn exerts opposite and antagonistic effects. The activity of the Zn metallo-enzyme  $\alpha$ -mannosidase (*EC* 3.2.1.24) from rat epididymis is sensitive to Cu inhibition *in vitro* (Snaith & Levvy, 1969). Whether or not these or other directly competitive effects at membrane or enzyme levels are of significance in the dietary antagonism between these elements has yet to be determined; nevertheless they do point to possible operative sites of interaction.

*Cu-Zn interaction in metal-binding proteins*

Excessive concentrations of one element even within the cell may prevent the effective utilization of another element in essential biochemical processes. It is possible therefore for a deficiency to arise when the tissue content of the essential element in question may appear normal or even elevated, as the metal may be either

in a form, or be retained at a site, which prevents it from fulfilling its normal metabolic role.

In this context the recent findings of Bremner & Marshall (1974*a, b*) on the occurrence and nature of low-molecular-weight Zn- and Cu-binding proteins (molecular weight 12 000) in ruminant liver may be of special relevance. They established that the pattern of distribution of Zn and Cu between three major metal-binding fractions in liver cytosol depended upon (1) the content of Zn in the liver and (2) the total amount of Zn and Cu in this low-molecular-weight fraction. The Zn-binding component in this fraction was identified as metallothionein. An increase in the liver Zn concentration above about 20 µg/g fresh weight was associated with an increase in the amount of metal appearing in this fraction. No similar relationship was observed with liver Cu concentration, although the ratio of Cu:Zn concentrations in the liver cytosol determined the Cu:Zn ratio in this fraction.

In subsequent studies with rats (Bremner, Davies & Mills, 1973; Davies, Bremner & Mills, 1973), it was noted that procedures which lead to increases in liver Zn content, including intraperitoneal (IP) injection of ZnSO<sub>4</sub> or food restriction, similarly promote the appearance of a Zn-binding protein of low molecular weight probably by an inductive mechanism involving protein synthesis *de novo*. These Zn-binding proteins have been characterized as metallothioneins (I. Bremner & N. T. Davies, unpublished results).

IP injection of CuSO<sub>4</sub> leads to the appearance of similar but as yet uncharacterized hepatic Zn- and Cu-binding components and, from experiments with Zn-deficient rats, it is clear that increases in liver Cu content as well as Zn content mediate this response (Bremner & Davies, 1974).

These relationships between changes in liver Zn or Cu contents and the occurrence of metal-binding proteins with high affinity for several metals are of considerable interest in studies of trace element interactions. It is now obvious that they are one site at which competitive interactions can occur; however, their precise metabolic significance is not yet clear. At present most workers consider the metallothioneins, originally isolated as a protein containing Cd and Zn, are involved in the detoxication of Cd (Nordberg, 1971) and mercury (Wiśniewska, Trojanowska, Piotrowski & Jakubowski, 1970). In view of these recent findings it seems more likely that this type of metal-binding component may play a fundamental role in the metabolism of Zn and Cu. The influence that the non-essential heavy metals Cd and Hg have on the synthesis or metal content of these proteins may well result from their chemical similarities to Zn and Cu. Bremner & Davies (1973) have speculated on possible roles for the metallothioneins in cellular detoxication mechanisms offering protection against excessive accumulation of Zn and Cu, and their roles in the temporary storage of these elements. Further investigations on their synthesis, stability and turnover in relation to trace-element status should provide insight into the molecular mechanisms involved in trace element interactions.

This review has attempted to show the wide range of interactions which may influence the metabolism of the essential trace elements. Clearer understanding of these relationships will do much to improve the precision of statements of require-

ments for the trace elements and will facilitate the prediction of circumstances under which deficiency and toxicity states are likely to occur.

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