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Trace elements in animals

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Trace element deficiency and toxicity in animals induces a wide variety of clinical effects although few are sufficiently specific to permit diagnosis without supporting investigation of changes in tissue trace element content or of the activity of metabolic processes influenced by trace element supply. Study of such trace element dependent processes has shown that extensive changes often arise before overt signs of disease appear. Some of these subclinical effects have pathological consequences and thus cannot be ignored when seeking correlations between geochemical anomalies and disease incidence.

Many past estimates of the quantitative requirements of animals for the essential trace elements are imprecise. Although recent work is providing clearer definition of requirements, many common dietary components have a marked influence upon the efficiency with which such elements can be utilized from the diet. Recent evidence indicates that such antagonists influence both the absorption and the subsequent fate of essential and toxic elements in body tissues and these processes have to be taken into account when investigating the aetiology of disorders believed to be attributable to anomalies in trace element supply. Their existence is not always detectable if attention is confined to the trace element analysis of body tissues or to the nature of clinical lesions.

Provided the complexity of soil–plant–animal relations with respect to trace element supply is fully recognized in the interpretation of data, the geochemical approach to the initial recognition of areas associated with a high risk of anomalies in trace element supply to animals and man has considerable potential value. This is already apparent from investigations upon the incidence of trace element problems in animals. As yet, its validity for similar purposes in man is less fully established.

Investigation of the influence of environmental geochemistry upon health depends greatly upon the clear recognition of adverse responses in animals. In some instances this search is based solely upon the incidence of overt disease. In others, it depends upon appraisal of the effect of anomalies in soil or parent material composition upon the trace element contents of the food or body tissues of animals.

This paper considers some inherent limitations of each of these approaches that influence the interpretation of data describing trace element relations between the animal and its environment.

The nature of trace element deficiency disorders

The most significant consequences of essential trace element deficiency in animals are summarized in table 1. This illustrates the wide variety of adverse effects upon health that can arise and also emphasizes the infrequency with which clinical signs are sufficiently specific to permit unequivocal diagnosis. This situation limits the value of an epidemiological approach based primarily upon overt clinical signs unless supporting biochemical or pathological procedures of adequate specificity are available.

Table 1. Major clinical, pathological and metabolic defects in essential trace element deficiencies

deficiency	gross pathological responses	species†	associated metabolic defect‡
copper	defective melanin production: hair, wool	ro, ru, pr, m	tyrosinase (−)
	defective keratinization: hair, wool	b, ro, ru, m	−SH oxidation to S–S (−)
	cardiac hypertrophy	ro, p, ru	cytochrome oxidase (−) lysyl oxidase (−)?
	skeletal and vascular defects	all	lysyl oxidase (−)
	ataxia, myelin aplasia	ru (sheep)	cytochrome oxidase (−)?
	anaemia	all	ferroxidase I (−)
	foetal resorption	ro	?
cobalt	anorexia	ru	?
	anaemia	ru	
selenium	myopathy, cardiac and skeletal	ru	glutathione peroxidase (−)
	myoglobinuria	ru	singlet oxygen (+)?
	liver necrosis	ro, b, p	lipid hydroperoxides (+)?
zinc	anorexia	all	?
	parakeratosis/hyperkeratosis	all	?
	foetal malformation	ro	?
	perinatal mortality	ro	?
manganese	skeletal and cartilage defects	b, ro, ru	chondroitin sulphate synthesis (−)
	ataxia	ro	otolith mucopolysaccharide synthesis (−)
	reproductive failure	ro, ru	
silicon	skeletal and cartilage defects	b, ro	Si in mucopolysaccharide cross links (−)?
iodine	thyroid hyperplasia, reproductive failure, hair, wool loss	all	thyroid hormone synthesis (−)
chromium	corneal opacity	pr	?
nickel	perinatal mortality	ro, p	?
molybdenum	defective keratinization	b	?
fluorine	perinatal mortality, anaemia	b	?
vanadium	skeletal defects	b	?
	reproductive failure	ro	?

† Key to species: ro, laboratory rodents; b, bird; p, pig; ru, ruminant; pr, laboratory primate; m, man.

‡ Responses indicated thus: (−) depression of enzyme activity or of synthetic process; (+) increased concentration of product; (?) lesion not established or direct relevance not confirmed.

Note: with the exception of chromium, deficiency of all of the above elements ultimately results in growth failure. Growth inhibition has also been reported for arsenic and cadmium deficiencies but no gross lesions have yet been described.

It is also evident from table 1 that some species-specific responses occur. However, these are more often a reflexion of species differences in the rate of growth or metabolic activity of sensitive tissues at the time deficiency arises rather than an indication that the functional roles of the elements differ between species. Failure to recognize this has often resulted in an incomplete exploration of the full consequences of deficiency. Thus, for example, there has been a long delay in recognizing that ataxia and deterioration of wool quality are not the only important practical consequences of Cu deficiency in sheep but that, as in all other species, growth of the young can be retarded (Whitelaw *et al.* 1977). A 30 year delay in recognizing the existence of nutritional Cu deficiency in human infants had a similar origin, in this instance because of

failure to recognize from studies with rats and chicks that many other metabolic defects precede the development of the Cu deficiency anaemia that was being sought without success.

The speed of response to sub-optimal trace element intake differs markedly between elements. The response to a low intake of Zn is particularly rapid in young growing animals (Mills *et al.* 1969) and although growth failure, loss of appetite and the appearance of lesions of Zn deficiency are invariably preceded by a marked decline in plasma Zn, no decrease in the Zn content of other tissues may be evident at this stage (Mills *et al.* 1967; Williams & Mills 1970). In contrast, the clinical effects of Cu, Se and Co deficiencies are often slow to appear if previous nutritional history has permitted the deposition of hepatic reserves that can be utilized. The supply of these elements during the terminal stages of pregnancy markedly influences the hepatic reserves of the young and thus its protection against subsequent exposure to a low dietary intake. Instances are known with young ruminants where such reserves have delayed the appearance of clinical signs of deficiency by up to 3 months.

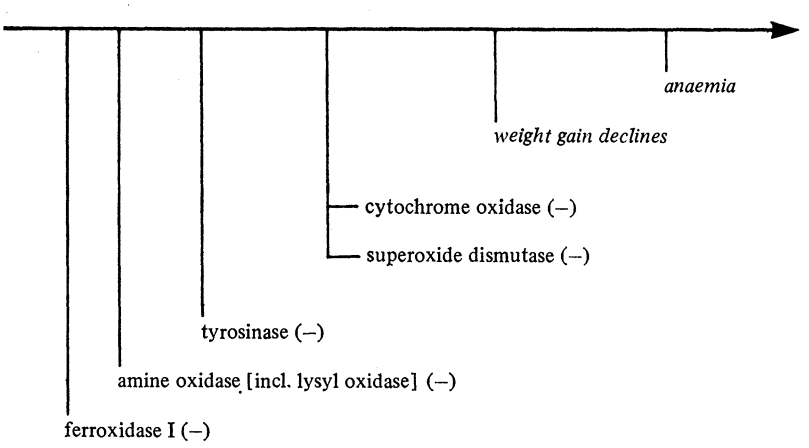

FIGURE 1. Metabolic responses to the progressive development of Cu deficiency in animals. Changes in the activity of Cu-dependent enzymes occurring before overt manifestations of deficiency arise are indicated (–). The implications of changes in the activity of ferroxidase I, lysyl oxidase and cytochrome oxidase are considered in the text. Note: in species such as sheep, in which neurological sequelae are common in the neonate, such lesions occur subsequent to a decline in nervous tissue cytochrome oxidase activity and precede adverse effects upon growth rate.

The importance of subclinical effects of deficiency or toxicity is often questioned. In farm animals they have received much less attention than overt clinical effects because of doubt about their immediate economic significance, and their long-term significance with respect to health or longevity has rarely been investigated. The following examples drawn from recent studies illustrate that such covert effects must be considered if the consequences of changes in trace element supply upon health are to be anticipated.

Figure 1 illustrates the sequence of metabolic changes believed to precede the appearance of overt signs of Cu deficiency in the rat and probably in most other animal species. Perhaps surprisingly, the first detectable response, a decline in the plasma and tissue activity of the copper-containing enzyme ferroxidase I (caeruloplasmin), has little immediate consequence. Later, when its activity becomes rate-limiting for the obligatory oxidation of Fe^{2+} to Fe^{3+} before liver ferritin iron can be mobilized by incorporation into plasma transferrin (Frieden 1974), a secondary deficiency of Fe develops and contributes to the anaemia often arising at later stages of the copper deficiency syndrome.

Of greater interest and significance in the present context is the early decline in the activity of lysyl oxidase and other Cu dependent amine oxidases. It is becoming increasingly apparent that the obligatory involvement of lysyl oxidase in the crosslinking of connective tissue proteins by oxidative deamination of lysyl ε-amino groups of their precursors is reflected by the defective 'maturation' of such proteins following even brief periods of Cu depletion and in animals showing no overt signs of deficiency. Evidence that this metabolic defect adversely affects the structure and integrity of the proteins elastin and collagen of connective tissue in ligaments, tendons, the vascular system and skeleton has been obtained from experimental studies with a variety of species (see, for example, O'Dell *et al.* 1966; Whiting *et al.* 1974; Mills *et al.* 1976).

The aetiology of the cardiac hypertrophy frequently found in Cu deficient animals (Leigh 1975) is now being investigated, and studies with rats are providing convincing evidence that the maturation of cardiac collagen is impaired before overt signs of Cu deficiency develop (R. B. Williams & R. Dawson, personal communication). It is also noteworthy that similar connective tissue defects have been found in children with genetic defects in Cu utilization (Danks *et al.* 1972). They also develop during chronic exposure of rats to relatively low dietary concentrations of Cd, unless Cu intake is simultaneously increased, thus providing convincing evidence of antagonistic metabolic interactions between these two elements (see later).

As indicated in figure 1, a fall in tissue cytochrome oxidase activity precedes the development of most overt signs of Cu deficiency. Although it has been suggested that a resulting decline in respiratory activity may be the cause of nervous tissue lesions in sheep (Howell & Davison 1959; Fell *et al.* 1965) and mitochondrial damage in the small intestine (Fell *et al.* 1975), the primary advantage of monitoring the activity of this enzyme lies in the fact that it provides a highly specific indication that Cu depletion proceeded to the point that overt manifestations of deficiency are imminent.

In the instance of Se, similar specificity for the detection of deficiency is readily achieved by monitoring changes in the tissue activity of the seleno-enzyme glutathione peroxidase (Rotruck *et al.* 1973). Measurement of the activity of this enzyme in whole blood or erythrocytes directly reflects changes in Se status and is now being used as the basis for extensive surveys of the regional distribution of Se deficiency in animals (see, for example, Anderson & Paterson 1976). Current surveys based on this approach are confirming the existence of a low Se status in ruminants in areas where growth responses had previously resulted from the administration of Se even though overt signs of deficiency were not apparent (Blaxter 1963). They are also revealing that the incidence of animals with a low Se status is much higher in Scotland than in other areas of Britain. The reasons for this have yet to be determined.

Although there are good grounds for the belief that this enzyme could have a broadly defined role in protecting tissues from oxidative damage arising from peroxide and its reactive metabolite, singlet oxygen, it is not yet clear whether a depression in glutathione peroxidase activity is the primary lesion resulting in the myopathy or liver necrosis often arising from Se deficiency. Other manifestations of a low glutathione peroxidase activity may be of greater relevance, again with respect to effects preceding overt signs of deficiency. Thus Serfass & Ganther (1975), using rats, and R. Boyne & J. R. Arthur (personal communication), using cattle, have found that although the phagocytic activity of polymorphonuclear leucocytes remained unimpaired in the clinically normal but Se deficient animal, a marked decline occurred in the microbicidal activity of such leucocytes in peripheral blood or peritoneal fluid. This response, believed to arise from peroxidative damage to lipoprotein membranes in the leucocyte, may well be relevant

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to circumstantial evidence that correction of Se deficiency improves resistance to respiratory disease even when the incidence of muscular dystrophy is low or other overt manifestations of deficiency are absent.

Even these few examples illustrate the importance of considering subclinical effects when investigating the influence of the geochemical environment upon disease incidence. Nevertheless arguments invoking the existence of subclinical disease only carry conviction if supported by biochemical or pathological evidence suggesting the initiation of a progressive sequence of changes which, if not interrupted, would ultimately provoke overt manifestations of disease. Current interest in defining the early metabolic consequences of trace element deficiency and toxicity should ultimately facilitate the more effective appraisal of subclinical responses to geochemical anomalies – and, by so doing, resolve a number of controversies.

Aetiology and detection of trace element deficiency and excess in animals

Essential trace element requirement and supply

In situations where geochemical anomalies or changes in soil composition are known to influence the trace element content of crops, a demand arises for comparison of data on crop composition with estimates of the trace element requirements of animals.

The reliability of such estimates differs greatly between individual elements. Where, as for Co, the primary metabolic role of the element is known and has been monitored in a study of intake-response relations (Marston 1970) the precision of estimates is good and accuracy appears to be high. Requirements for Cu, Zn, Mn and Se are less precisely known and a threefold discrepancy in estimates is not uncommon. Most estimates have been based upon a subjective appraisal of the results of experimental studies or, in the instance of farm animals, upon advisory investigations in which deficiency disease incidence has been related to the dietary content of the element presumed to be deficient. Few reports provide sufficient information to determine whether the presence in the diet of antagonists known to influence trace element utilization may have influenced results and thus the applicability of the conclusions. Such limitations to the validity of estimates derived in this way have been discussed elsewhere (Agricultural Research Council 1967, 1978).

A more satisfactory approach, recently explored for the estimation of Cu and Zn requirements of ruminants (Agricultural Research Council 1978) is based on a factorial analysis of the requirement to meet identified demands for specific body processes. In this approach, net requirement, N, is defined by

$$N = F_{\min} + U_{\min} + D_{\min} + A,$$

where $F_{\min}$, $U_{\min}$ and $D_{\min}$, collectively described as minimal endogenous losses ($E_{\min}$), represent inevitable losses of the element in faeces, urine and dermal debris when the trace element status of the animal is just sufficient to maintain all essential functions, and A represents the total demand for growth and other anabolic processes. Gross requirement, G, specifying the dietary intake of the element needed to maintain homeostasis in this strictly defined situation is derived from

$$G = (E_{\min} + A)/C,$$

where coefficient C describes the fractional efficiency of absorption of the element from the diet.

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The success of this approach for determining trace element requirements depends greatly upon the reliability of estimates of the factors E_{min} and C and care in the definition of restraints to their application. Thus, for Cu, a recent study at this Institute shows that endogenous losses (E) are directly proportional to existing tissue reserves of this element and C can vary between 0.03 and 0.01 depending upon the Mo and S content of the diet. Although delay will arise before appropriate factors have been determined experimentally for other elements, the flexibility of this approach will permit estimation of requirements for a much wider range of circumstances than has been practicable hitherto.

Despite the imprecise nature of many estimates of requirement, situations can be recognized as being associated with a high or low risk that supply may be inadequate, particularly for farm animals. Thus, the Cu and Co content of many 'improved' strains of grass is often inadequate to meet the requirements of ruminants and pasture management systems that decrease the proportion of legumes, frequently rich in these elements, increase the risk that deficiency will arise. Except where soils are high in available Se, the content of this element in barley and most other cereal grains is rarely adequate.

The influence of geochemical anomalies in these situations depends upon the proportion of the total feed consumed that is accounted for by indigenous crops. Protein and energy supplements of vegetable origin are frequently high in Cu, Co and Mn content while those of animal origin are usually rich in Zn and Se. Under some feeding systems, such supplements may account for up to 80 % of the total supply of these elements and thus frequently compensate for deficiency in the trace element content of other dietary components.

Trace element utilization

Although clinical disease often results from a dietary deficit of Cu, Co, Se, I, Fe or Zn, in many instances it originates from poor utilization of ostensibly adequate dietary supplies. In such instances of 'conditioned' deficiency the correct interpretation of data on the composition of diet or animal tissues depends upon an understanding of the processes responsible for defective utilization. Interpretation of clinical signs and analytical data in cases of trace element intoxication arising from chronic exposure at a low level can present similar difficulties if the existence of competitive biological interactions between structurally similar elements or ions is ignored.

The behaviour both of the essential and the toxic trace metals in animal tissues reflects the readiness with which they form coordination compounds in an environment rich in many reactive metal-binding ligands. Regulation of absorption, utilization and excretion of essential metals and discrimination against toxic elements must be achieved by a defined sequence of metal–ligand exchange reactions. Initially, these govern equilibrium distribution of the elements between aqueous and solid phases of the digesta and in a medium usually rich in phosphate, and sometimes in sulphide, with which many would otherwise react to yield insoluble and thus physiologically inert products. Their subsequent uptake by the gastrointestinal mucosa and transport through blood plasma and cytosol to their functional sites, is again achieved through a tissue matrix rich in reactive but relatively immobile structures that, unless the element is transported in association with a carrier, would effectively block transport. Studies *in vitro* of competitive interactions between ions in aqueous systems containing a wide range of biologically significant ligands (see, for example, May *et al.* 1977) provide the basis for a limited understanding of why the efficiency of utilization of many of the trace elements, particularly of the

transition elements, can so often be influenced by changes in either the inorganic or organic components of diets.

A summary of the antagonists known to influence trace element utilization is presented in table 2. This is restricted to observations indicating that the antagonist affected metabolism of the element in question to the extent that characteristic biochemical or pathological changes resulted or that growth rate or mortality were substantially modified by changes in the balance between the element and its antagonist.

TABLE 2. DIETARY COMPONENTS KNOWN TO INCREASE ESSENTIAL TRACE ELEMENT REQUIREMENTS OR INCREASE TOLERANCE OF TOXIC ELEMENTS†

element	antagonists
Co	no direct antagonists known‡
Cu	thio and oxythiomolybdates, organic and inorganic sources of S^{2-}, Zn, Cd (Ag), (phytate)§ (phaeophytin)
Cr	(VO_4^{3-}/CrO_4^{2-})
Cd	Cu, Zn, Ca
F	Ca
Fe	Ca, PO_4^{3-}, CO_3^{2-}, Cu, Zn, Cd, Co, (phytate)§; indirect, Cu deficiency
I	thiooxazalidones, thiocyanates, Co, (As), (F)
Mn	phytate, PO_4^{3-}
Mo	SO_4^{2-}/MoO_4^{2-}, (WO_4^{2-}/MoO^{2-})
Ni	none known
Pb	Ca, PO_4^{3-}, Fe, SO_4^{2-} (ruminants only)
Se	Cu, (As), (Hg), (Te), specific organic and inorganic S analogues of Se
Si	none known
V	(CrO_4^{2-}/VO_4^{3-})
Zn	Ca, Cu, phytate§, (Cd)

† Interactions involving high degree of specificity are indicated thus: antagonist/target. Antagonisms detected solely under experimental conditions are indicated in parentheses. Effects of acute intoxication are not included.

‡ Co requirements of ruminants are believed to increase when consumption of rapidly fermentable energy sources leads to synthesis of 2-Me adenyl cobamide rather than the biologically active form of cobalt, cyanocobalamin.

§ Evidence of interaction in non-ruminant species only but may apply to ruminants before function fully developed.

(Data sources: Underwood (1977), Mills *et al.* (1971, 1974, 1978), Hill (1976).)

The following types of interaction can be identified:

(i) reactions decreasing the solubility of the element in the gastrointestinal tract and thus preventing its absorption (e.g. phosphate–Fe, sulphide–Cu, phytate–Zn and thiomolybdate–Cu antagonisms);

(ii) antagonistic interactions at sites involved in transport, storage or excretion of the element or preventing its incorporation into functional sites (e.g. sulphate–molybdate, sulphite–selenite, tungstate–molybdate);

(iii) reactions of the antagonist with tissue proteins which increase their affinity for the element (e.g. thiomolybdate–protein complex–Cu antagonism);

(iv) synthesis *de novo*, induced by high concentrations of antagonist, of sulphydryl-rich proteins having relatively non-selective affinity for heavy metals (e.g. Cd–Zn, Cd–Cu, Cu–Zn antagonisms):

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(v) secondary effects arising from interdependent involvement of one element in metabolism of a second (e.g. secondary iron deficiency resulting from loss of (Cu-dependent) ferroxidase I activity and subsequent failure to mobilize stored ferritin Fe).

Typical examples of the more important of these interactions will now be considered.

Antagonistic action of Mo *upon* Cu

In ruminants, the antagonistic action of molybdenum upon the utilization of dietary Cu, an effect synergized by both inorganic and organic dietary sources of S, is frequently implicated in the aetiology of 'conditioned' Cu deficiency. As little as 2 mg Mo/kg diet restricts the hepatic retention of Cu (Suttle 1977).

Although this interaction is involved in the aetiology of Cu deficiency in many parts of the world, it is often difficult to assess the extent of its influence. This arises because of inadequate understanding of its mechanism, poor definition of the quantitative relations between dietary Mo, S and Cu availability and because of the rapidity with which the Mo content of the diet of ruminants is influenced by seasonal effects and by modified soil conditions (see Mitchell & Burridge, this symposium).

Although many details still require clarification, present views are that Mo only exerts a marked effect upon utilization in situations which favour the formation of oxythiomolybdates $[MoO_{4-n}S_n]^{2-}$ or tetrathiomolybdates $[MoS_4]^{2-}$ within the digestive tract (Dick *et al.* 1975; Mills *et al.* 1978). As indicated in figure 2 this arises by the reaction of molybdate with free sulphide either generated in the rumen by reduction of dietary sulphate or by degradation of sulphur amino acids (reaction 1). Studies with both rats and sheep (Mills *et al.* 1977) indicate that the subsequent fate of thiomolybdate depends greatly upon the Cu content of the diet and thus of the gastrointestinal lumen. If this is sufficient to permit stoichiometric reaction with thiomolybdate to form its Cu^+ derivative, absorption of both Cu and Mo is inhibited substantially (see table 3). If thiomolybdate absorption is not so prevented, it reacts selectively with plasma albumin or a protein of similar molecular mass and possibly with other tissue proteins to form a stable complex. Studies *in vitro* of the properties of the reaction product of thiomolybdate with bovine plasma albumin indicate that this material has a substantially enhanced affinity for Cu compared with either untreated albumin or albumin previously treated with equimolar concentrations of molybdate rather than thiomolybdate. This conclusion is borne out by finding that when this Mo-containing fraction is present in the bloodstream, blood Cu content invariably rises but biochemical and clinical indications of Cu deficiency eventually develop in the animal. The parenteral injection of Cu prevents the appearance of Cu deficiency signs but inhibits neither the absorption of thiomolybdate nor the increase in tissue Mo and bound Cu content that results from its presence.

The probability that Mo can interfere with Cu metabolism either before or after absorption of its oxythio- or tetrathio-derivatives, accounts for much of the difficulty encountered in attempting to assess the extent of involvement of Mo in the aetiology of Cu deficiency. When, as at a dietary Cu : Mo ratio of 1 or greater the reaction between thiomolybdates and Cu inhibits absorption of either, the subsequent decline of plasma and tissue Cu content is not accompanied by accumulation of Mo in tissues. This is the situation resulting most frequently in Mo-induced Cu deficiency and, obviously, no association exists between the Mo content of the diet or environment and that of animal tissues. In contrast, at the substantially higher Cu : Mo dietary ratios often used in earlier experimental work (but relatively uncommon under practical

TABLE 3. TISSUE RETENTION OF Cu AND Mo BY RATS OFFERED DIETS DIFFERING IN Cu AND AMMONIUM TETRATHIOMOLYBDATE CONTENT

(*a*) Influence of varying dietary Cu on retention of dietary Mo (6 mg Mo as MoS_4^{2-}/kg diet)

dietary Cu/(mg/kg)	3	8	16
carcass retention of ^{99}Mo (fraction of oral intake)	0.40 ± 0.02	0.22 ± 0.01	0.15 ± 0.02
total liver Mo/(mg/kg dry matter)	1.2 ± 0.1	0.8 ± 0.01	0.4 ± 0.07

(*b*) Influence of varying dietary Mo (as MoS_4^{2-}) on retention of dietary Cu (3 mg Cu/kg diet)

dietary Mo/(mg/kg)	0	12
carcass retention of ^{64}Cu (fraction of oral intake)	0.34 ± 0.02	0.04 ± 0.01
total liver Cu/(mg/kg dry matter)	23 ± 1.3	12 ± 1.5

Rats were given either ^{99}Mo (as $(NH_4)_2\,^{99}MoS_4$) or ^{64}Cu (as $^{64}CuCl_2$) after receiving semi-synthetic diets supplemented with Cu or Mo as indicated in the table for at least one week. Isotopes were given in a 2 g portion of food; rats were killed 3 h later and their gastrointestinal tracts removed before isotope retention was determined.

conditions), such an association exists if conditions favour the absorption of Mo as thiomolybdate. In this instance, abnormally high plasma Cu contents may be detected even in animals showing early clinical signs of Cu deficiency.

The systemic action of absorbed thiomolybdate appears to be consistent with earlier suspicions that Mo exerts an adverse effect upon sulphide oxidation (Mills *et al.* 1958; Mills 1960). Recent studies at this Institute, with rats, suggest that traces of dietary sulphide, normally well tolerated, are toxic if the diet contains as little as 3 mg Mo/kg in this form. Further evidence is consistent with the view that when Mo and sulphide of thiomolybdate associates with specific proteins, the

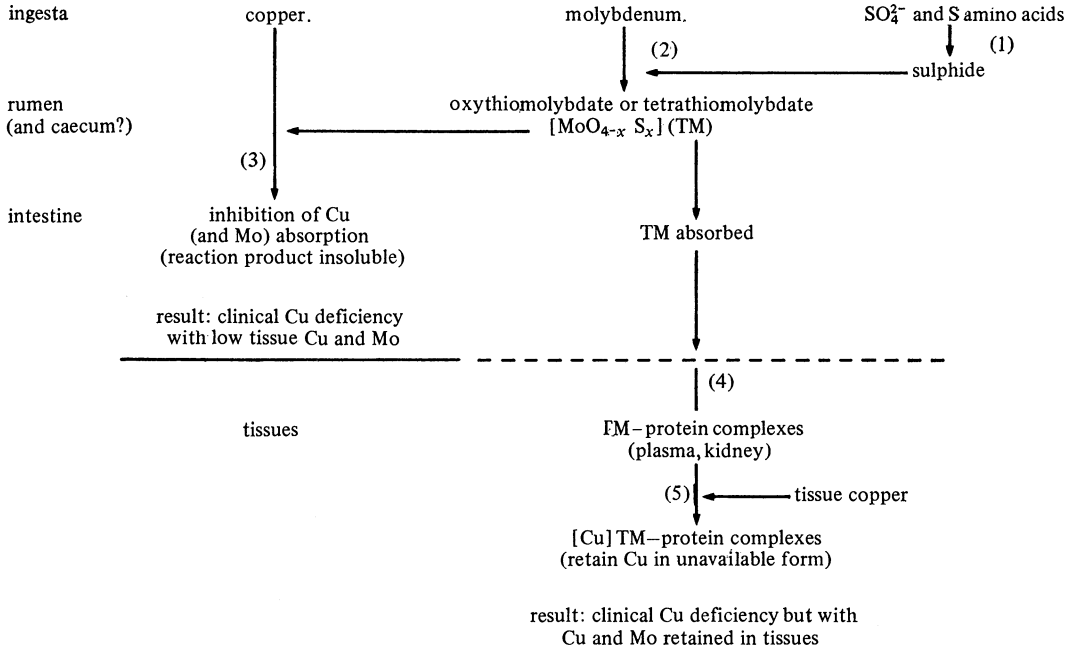

FIGURE 2. Mechanisms involved in the inhibitory action of dietary molybdenum and S upon Cu utilization by ruminants. Sulphide generation (1) in the rumen and the subsequent reaction of this with molybdenum (2) yields oxythiomolybdate or tetrathiomolybdate (TM). Reaction of TM with Cu in the gastrointestinal tract (3) prevents Cu (and Mo) absorption. Alternatively, if TM is present in excess it is absorbed (4) and in association with specific proteins (not yet characterized) reacts with Cu to induce a systemic deficiency of Cu.

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sulphide moiety is protected from oxidation to sulphate. It remains to be determined whether it is the reaction of Cu at this site that results in its conversion to a physiologically unavailable form. If the interpretation of these observations is correct, it is possible that elements structurally related to Mo may exert a similar effect upon Cu metabolism. The possibility that W, as thiotungstate, may so act is now being investigated.

Antagonistic interactions between Cd, Cu *and* Zn

Although isomorphous substitution of the metal component of metalloenzymes has long been used as an in-vitro technique for investigation of the nature of metal involvement in enzyme action, there has been slower appreciation that such reactions could also be involved in the toxic action of several of the transition elements and in the development of conditioned trace element deficiency diseases. As indication of the importance of such interactions is given by the following examples: (i) the development of conditioned Zn deficiency, despite an increase in liver Zn content, in pigs receiving high intakes of Cu (Suttle & Mills 1966), (ii) induction of

TABLE 4. INFLUENCE OF DIETARY Cd AND Zn UPON ACTIVITY OF THE Cu-CONTAINING ENZYME, FERROXIDASE I, IN RAT BLOOD PLASMA

[Cd]/(mg/kg diet) ...		0.16	1.5	6.1	18
		ferroxidase I activity (units/l plasma)			
[Zn]/(mg/kg diet)	30	43.5	18.2**	9.3**	6.5**
	300	27.4*	17.0**	5.2**	4.9**
	1000	9.5**	5.0**	5.1**	3.7**

Rats were maintained for 9 weeks on a semi-synthetic basal diet containing 2.5 mg Cu/kg sup
Cd or Zn as indicated. All supplemented groups show a significant ($p < 0.01$(*) or $p < 0.001$(**)) depression in enzyme activity compared with that in control (0.16 Cd, 30 Zn) animals. A similar inverse relation between the level of Cd or Zn supplementation and liver Cu or cortical bone thickness in femur was evident. All effects were abolished by increased dietary Cu. (From Campbell & Mills 1974.)

biochemical defects indicative of Cu deficiency in ruminants and rats exposed chronically to small increases in dietary Cd content or high intakes of Zn (see table 4), (iii) improved tolerance of Cd and Zn resulting from increased Cu intake and (iv) prevention of Cu intoxication in ruminants by increasing Zn intake (Bremner *et al.* 1976).

Many other examples of competitive antagonism involving both cationic and anionic species of the essential and toxic trace elements have been described by Hill (1976). Consideration of the specificity of these interactions led Matrone (1974) to suggest that the basis for the interactions quoted above arises from similarities in electron distribution in the outer orbitals of the ions Cu , Cd^{2+} and Zn^{2+}, each having one 4s and three 4p orbitals vacant for the formation of coordinate bonds in a tetrahedral (sp^3) array. The concept that competitive trace element antagonism may be exhibited when cations or anions have similar electron distributions in their outer orbitals and when ionic radii are not greatly dissimilar has, in many instances, been validated by direct experiment.

The value of this approach does not extend to prediction of the effect of an antagonistic interaction upon tissue concentrations of the competing elements. This is influenced by the site at which competitive interaction first becomes evident, and by the effectiveness and specificity of homeostatic responses within tissues for the two competing elements. Thus, an important step in

homeostatic control of the elements Cd, Zn and Cu appears to be the synthesis *de novo* in intestinal mucosa, liver, kidney and pancreas of the metallothioneins, a group of closely related sulphydryl-rich proteins of low molecular mass. The initiated synthesis of metallothioneins with their strong affinity for Cd, Cu and Zn provides an effective 'sink' for the metal present in physiological excess. However, they exhibit only a low order of metal-binding selectivity. Thus, induction of the protein by, say, an excess of Zn results in formation of both Zn and Cu (or mixed metal) metallothioneins. A similar situation can result from a tissue excess of Cd. The appearance in the cytosol of a 'new' metal-binding component (which may, for example, account for up to 80 % of the total Zn or Cu) markedly affects tissue metal distribution within the cell and the effectiveness with which essential metals reach their functional sites. Depending upon the flux of the most limiting essential metal, consequences may range from an inhibition of functional activity of an essential element despite an increase in its tissue concentration to substantial increases in the tissue content of mutually antagonistic elements with little evidence of functional defects (for reviews see Bremner 1974; Bremner & Davies 1973).

Detection of trace element deficiency and toxicity by tissue analysis

Analysis of blood or liver tissue has in many instances been used successfully for the detection of trace element deficiency or excess in animals and for predicting the risk that they may appear when data on dietary trace element content are unavailable or uninterpretable. The most frequent criticism of the approach lies with the difficulty in relating anomalies in blood or liver trace element content to the appearance of pathological signs of deficiency or intoxication. It is evident for example, that chronic Cd intoxication can arise even though plasma Cd concentrations show no detectable change, and that low plasma Cu (less than 0.5 mg/l), low liver Cu (less than 20 mg/kg dry matter) or low blood Se (less than 0.05 mg/l) do not always indicate the existence of pathological changes due to Cu or Se deficiency. As emphasized previously, Cu deficiency induced by a high Mo intake is rarely reflected by any increase in tissue Mo content.

That such problems of interpretation exist is often a reflexion of the fact that selection of tissues such as blood or liver for analysis is dictated solely by their relative accessibility for sampling rather than by consideration of the probable location of metabolic defects giving rise to disease. Except for research purposes, where it is both practicable and desirable to investigate the influence of changes in intracellular trace element distribution upon the activity of systems believed to be sensitive to deficiency or excess, heavy reliance upon the detection of changes in gross content of trace elements in tissues such as blood and liver will continue. Interpretation of the significance of these changes must, however, be conditioned by an appreciation that they indicate the possibility rather than the certainty that metabolic defects exist in tissues often remote from those analysed.

Conclusion

The topics considered in this paper illustrate the frequently complex relations that influence the response of animals to changes in trace element supply from their environment. They have also emphasized that progress towards a better understanding of the influence of geochemistry upon animal and human health depends upon the more effective recognition of early subclinical effects of trace element deficiency or toxicity. Without improvement of techniques for monitoring the development of characteristic metabolic lesions, marginal deficiencies and toxicities will remain difficult to detect, their significance will be difficult to appraise and the aetiology of

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disorders induced by competitive antagonistic interactions between elements will often remain obscure.

Where such techniques are already available progress has been rapid. Thus progress in the recognition of 'high risk' areas for Co, Se and I deficiencies depends more upon the speed with which geochemists and soil chemists can map and interpret analytical data rather than upon the problem of identifying a sub-optimal status of these elements in animals. Regrettably, this is not yet the situation for deficiencies of Cu, Zn, Mn and several other essential elements and for Cd toxicity. For these elements, imprecise definition of requirements or tolerance and the existence of many variables that can influence their absorption and retention will continue to necessitate a somewhat subjective appraisal of the effect of relevant geochemical anomalies upon health and productivity.

Fewer than one-fifth of the countries reporting statistics on animal disease through the joint W.H.O./F.A.O. Annual Surveys provide evidence of investigation of diseases attributable to inorganic element deficiency or excess. In contrast, almost all countries with well developed investigational and advisory services report the existence of disorders attributable to anomalies in trace element supply. If current work in Europe and the U.S.A. continues to provide evidence of regional associations between geochemistry and animal health, the application of such techniques in areas so far neglected should do much to rectify this discrepancy. With such potential value it is particularly important that the progress of geochemical approaches to the recognition of high risk areas should be continuously monitored and their advantages and limitations assessed.

With the exception of I, slower and less certain progress has been made with respect to effects upon the health of man. This is hardly surprising in view of the wide variety of dietary and other sources from which his supply of trace elements originates. As with farm animals, the effect of anomalies in the composition of man's geochemical environment will be greatest in communities where staple diets are simple and consist primarily of indigenous crops. Thus it may well be appropriate to ensure that greater emphasis be placed upon studies of the health of communities meeting these criteria in the developing countries rather than, as currently, concentrating primarily upon the quest for disease in sophisticated societies that might be related to geochemical anomalies already identified.

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