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The detection of trace element deficiency and excess in man and farm animals

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The certainty and speed with which a disorder arising from deficiency or excess of a dietary component can be recognized depends greatly upon the clarity with which the clinical consequences of this disorder have been characterized in experimental studies. Among the many disorders attributable to deficiency or excess of the trace elements only one, 'swayback' in the copper-deficient lamb, can be clearly recognized by its gross signs. With the others the signs are insufficiently specific to achieve a satisfactory diagnosis by consideration of a single gross lesion. In these circumstances, progress towards identification of the disorder must then be achieved by more detailed consideration of the variety of clinical and metabolic lesions, by determination of the trace element content of animal tissues obtained by biopsy or post mortem or, finally, by determination of the trace element content of the diet or other vehicle whereby these elements gain access to the subject.

The purpose of this paper is to examine some of these supporting diagnostic procedures and particularly to consider their limitations, where these may originate from inadequacies in experimental studies of the clinical and metabolic consequences of trace element deficiency and excess in animals.

The criterion of normality with respect to trace element status

The definition of 'normality' with respect to trace element status differs greatly in different circumstances. When considering the impact of trace element supply upon the health of human subjects or its effects upon the modification of interspecies relationships in ecological systems, the definition must apply to all aspects of physiological function involved during growth, development and reproduction, and to the maintenance of health during later phases of the individual's existence. In its agricultural context a less rigorous definition becomes acceptable. Thus, although commercial diets used for calves in veal production systems are extremely deficient in Cu as well as in iron, this fact is largely irrelevant, as hepatic stores of Cu in the newborn calf are sufficient to meet demands for this element at least up to the normal time of slaughter for veal (Bremner & Dalgarno, 1973). Similarly, the development of lesions in connective tissue and rarefaction of the skeletal matrix as a consequence of Cu deficiency may well be considered irrelevant in a fattening animal if these lesions do not proceed to the point that they adversely affect the ultimate yield of edible meat.

The speed and extent with which tissue reserves of individual trace elements can be mobilized at times of dietary inadequacy are important determinants of the

time that must elapse between the onset of a deficient intake and the development of the clinical manifestations of deficiency. Thus the onset of the effects of zinc deficiency is extremely rapid, particularly in young subjects; the effects of cobalt, selenium and Cu deficiencies take longer to appear, while the full consequences of a deficient manganese intake may not become manifest until the second generation of exposure. Such aspects are often inadequately considered, both when attempting to assess the practical significance of individual deficiencies and when attempting to describe the nature of the functional disturbances that ensue. They have been almost entirely neglected in studies of chronic low-level toxicity of many of the heavy metals in situations where these elements, by virtue of antagonistic interactions, influence the metabolism of the nutritionally essential elements. For example, until recently no study of the chronic low-level effects of Zn toxicity exceeded a duration of 12 weeks. It is now appreciated that an important component of the toxic action of Zn is its adverse affect upon Cu metabolism and, even when deficiency is induced by the use of extremely Cu-deficient diets, it may take considerably longer than 12 weeks for clinical signs to appear in some species.

The importance of selecting the appropriate duration for an experimental study aimed at defining the effects of deficiency or toxicity is greatest when attempting to assess the possible effects of the deficiency or excess upon human health, upon the reproductive performance of domesticated livestock throughout several generations, or when assessing the toxic effects of heavy metals upon individual species in an ecosystem. The short-term study employing severely deficient diets or high dietary concentrations of a potentially toxic element has a more limited relevance than is generally recognized, in that the sequence of metabolic consequences becomes foreshortened in time, sometimes with quite different clinical signs from those resulting from less severe treatments imposed over a longer period. Typical of this situation was the writer's early work on the induction of Cu deficiency in lambs using abnormally high dietary concentrations of molybdenum. The consequence of this treatment was that although the deficiency induced lesions of central nervous tissue that were identical to those of delayed swayback, these lambs also suffered a severe anaemia, a situation that hardly ever accompanies swayback in the lamb under field conditions.

The sequential development of lesions attributable to trace element deficiency or excess

Inadequate examination of the pathological changes that arise as a consequence of the deficiency or excess of an individual trace element has frequently hindered the practical exploitation of many sound nutritional studies. Such studies are essential to reveal the full extent of the consequences of these insults, to reveal differing sensitivities among trace element-dependent metabolic systems at differing stages of physiological development and, finally, to assist with the task of developing improved methods of detecting deficiency or excess.

Undue over-emphasis of the fact that Cu deficiency causes anaemia, without recognition of the point that it is a comparatively late consequence of the disorder,

certainly hindered the discovery of Cu deficiency in ruminants and led to the erroneous conclusion that it is unlikely to arise in human subjects. More complete description of the pathology of Cu deficiency has led to recognition that Cu is involved in oxidative function, in lipogenesis and in the synthesis of collagen, elastin and keratin and, in direct consequence, has led to the discovery of Cu-responsive syndromes in the human infant almost 40 years after the first suggestion that it was an essential trace element (Graham & Cordano, 1969).

Also emerging from detailed pathological studies is evidence that certain phases of development are particularly sensitive to modifications in trace element supply. Thus, foetal death occurs in the rat if the dam is briefly subjected to a low intake of Cu (Dutt & Mills, 1960; Howell & Hall, 1969), and similar studies have indicated that brief exposure of the dam to low intakes of Mn or Zn (Hurley & Schrader, 1972), or to excessive cadmium (Ferm & Carpenter, 1967), adversely and permanently affects foetal development without permanent consequences to the dam herself. Work with several species has shown the sensitivity of the young growing animal to a suboptimal Zn supply, and has illustrated that demands to meet specific metabolic functions may differ greatly, as is exemplified by the finding that spermatogenesis is depressed by even mild Zn deficiency, in the absence of other detectable lesions (Underwood & Somers, 1969).

Such observations contribute extensively to our appreciation that the spectrum of lesions may change with age and with the extent of deficiency or excess even within one species. They undoubtedly merit wider consideration in experimental work aimed at determining the requirement for an essential element or in determining the tolerance to a potentially toxic element. As nutritionists, it is chastening for us to recognize that nearly all our present, somewhat limited, knowledge of the influence of genetic characteristics upon trace metal homeostasis and requirements originated from detailed studies of morphological abnormalities of tissues in mice, sheep and human subjects and the inference, from often unrelated biochemical studies, that these lesions might be attributable to deficiencies or excesses of the elements Cu, Zn, Mn or Cd (e.g. Hunt, 1974; Hurley, 1974).

Lastly, it must be emphasized that failure to achieve an adequate description of the relationships between changes in trace element supply and the response of the animal is responsible for many of the difficulties in assessing the impact of the trace elements upon human and animal health. Among the current arguments originating from this situation are those relating to the significance of Zn deficiency in human subjects (WHO, 1973) and in ruminants, those concerning the significance of survey work suggesting from blood analysis that Cu deficiency in ruminants may be more widespread than hitherto believed (Davies & Baker, 1974) and, of even greater possible significance, those suggesting that deficiencies of Cu, Mn or Zn may be a cause of poor reproductive performance in farm livestock. That current differences of opinion on the significance of environmental lead, Cd, mercury and Zn again originate from the dearth of pathological and biochemical studies on subjects subjected to chronic, low-level exposure requires no further amplification in this paper.

The trace element content of tissues

In the absence of a specifically diagnostic clinical lesion, possible anomalies in the trace element content of the diet or of animal tissues are frequently explored. Although very many instances of deficiency or excess of individual trace elements have been successfully detected by such approaches it is nevertheless important to recognize their limitations. Those aspects which influence the interpretation of dietary analysis arising from the problem of defining trace element availability were recently reviewed in this journal and will not be reiterated (Mills & Williams, 1971).

When employing the inorganic analysis of animal tissues as a diagnostic aid, at least three assumptions are frequently made. It is tacitly assumed that the content of the element in the tissue sampled accurately reflects the content of that element in its active form at the metabolic sites involved in the genesis of lesions. Secondly, it is assumed that the analytical result in some way presents an integrated picture of the trace element status of the subject during the period in which these lesions have developed. Finally it is frequently assumed that widely applicable standards can be derived to distinguish between normal subjects and those adversely affected by deficiency or excess. The extent to which these assumptions are justifiable will be considered briefly.

Criteria for the detection of Cu deficiency by determination of Cu in whole blood or plasma have become increasingly sophisticated with, for example, recognition that in human subjects normal blood Cu is greatly influenced by age and pregnancy (Mills, 1973) and, further, the recognition by Dutch workers that what constitutes an acceptable blood Cu concentration in cattle depends upon the management of the animal and its rate of growth. The relationship between changes in blood Cu concentration and that in other tissues may well differ appreciably between species. Recent work at this Institute suggests that, in cattle, a marked decline in blood Cu concentration may precede by several months the development of those lesions which lead to an unacceptably low rate of weight gain, but that it may be more closely related in time to the development of lesions in connective tissue and cardiac muscle that are nevertheless of no economic significance. In human subjects, with their less extensive hepatic stores of Cu and more rigorous demands for the maintenance of unimpaired function of all tissues, it may well be that blood Cu concentration provides a more acceptable index of Cu status than it does with cattle, where its limitations have been commented upon by several investigators.

The labile nature of plasma Zn, influenced rapidly by daily changes in Zn intake and even by the protein content of the diet (Mills, 1973), neither reflects changes in the Zn content of other tissues nor gives an indication of long-established changes in the over-all Zn status of the subject. Its value as a diagnostic aid is thus strictly limited and subject to difficulties of interpretation unless the history of the subject is closely defined.

With Co and iodine, the identification of the roles of these elements, followed by determination of their 'active' forms, in cyanocobalamin and the iodothyronines in serum, greatly increased the value of blood analysis for the detection of Co- and

I-responsive syndromes, compared with that of the determination of total Co or I. It appears probable that recent progress in studies of chromium and Se metabolism may similarly remove present difficulties in interpreting results for the concentrations of these elements in blood.

Relationships between erythrocyte Pb content and the development of clinical lesions of Pb intoxication are very inadequately defined. In contrast, elevated plasma Zn concentration, particularly when associated with low plasma Cu, are reliably indicative of chronic Zn intoxication. Serious metabolic defects become established as a consequence of chronic exposure to dietary Cd long before any elevation of plasma Cd concentration is apparent (Mills & Dalgarno, 1972) and, in ruminant species, plasma Cu concentration is also an inadequate indicator of incipient Cu intoxication.

These few examples illustrate that the reliability with which blood composition may be used as an index of trace element inadequacy or intoxication is greatest when direct determination of a metabolically active component bearing the element is possible, and that its value decreases as relationships between blood and tissue concentrations of that element become more remote or are less well defined. Virtually identical considerations influence the interpretation of results for liver composition. Again, with several elements much has been achieved and some difficulty encountered, particularly with the task of setting universally applicable standards for 'normality' of trace element content. Thus Australian experience regarding the lower acceptable limits for hepatic Cu in the healthy lamb are not applicable in north-east Scotland, and similar difficulty has been encountered in attempting to apply UK levels of hepatic Cu in cattle to surveys of Cu deficiency in this species in Malaysia (Hill, Thambya, Wan & Shanta, 1962). Hepatic Zn, Se, I and Cr contents are relatively poor indicators of 'normal' or deficient conditions, while the situation with respect to Mn has been inadequately explored.

The accumulation of an element within a tissue may, in some circumstances, lead to the erroneous assumption that the concentration of that element is sufficient to maintain its normal metabolic functions. Examples worthy of note are the hepatic accumulation of Fe (but as relatively immobile haemosiderin) during Cu deficiency and the hepatic accumulation of Cu (despite the development of a clinical syndrome at least partially responsive to extra dietary Cu) during the development of molybdenosis in the rat. Rapid rates of tissue repair appear to be associated with an increased tissue uptake of Zn and, in the liver of the Zn-deficient rat, this can continue to the extent that the liver content of Zn becomes undistinguishable from that of the normal animal (J. K. Chesters, personal communication). Lastly, as studies of Se metabolism have clearly indicated, the form in which the element is present in the diet can have a marked influence on the relationship between the dietary and tissue contents of that element and, ultimately, its metabolic fate.

The detection of anomalous relationships between several elements within a tissue can prove particularly informative (Mills, 1974). Severe Cu deficiency frequently leads to an increase in tissue Fe content while, conversely, a depression of hepatic Fe with an increase in Zn is a frequent consequence of Cu intoxication in

the pig. Several workers have now established that a significant consequence of chronic exposure to high intakes of Cd or Zn is a decline in hepatic Cu content (Bunn & Matrone, 1966; Campbell & Mills, 1974), and there are indications from work with the rat that low hepatic Fe content both promotes the retention of Pb by the liver and may decrease tolerance to Pb (Six & Goyer, 1972).

Complex though many of these relationships may appear, an understanding of their extent and nature is essential when attempting to interpret analytical data on the trace element content of tissues. Failure to achieve this has, for example, resulted in some sensational and unsustainable claims regarding the relevance of trace elements to human disease. In less dramatic circumstances it has merely delayed recognition of the existence of trace element-responsive syndromes.

Metabolic lesions attributable to trace element deficiency and excess

The identification of metabolic defects arising during trace element deficiency or excess provides information which may well be applied to the task of improving techniques for the detection of such disorders. The extent to which such an approach can supplement or replace the inorganic analysis of tissues in this role is, as yet, uncertain, but substantial progress has already been achieved with the detection of Cu, Se and Mo deficiencies and toxicities of Pb, Zn and Cd. Such techniques, based usually upon assessment of changes in the activity of trace metal-dependent enzyme systems in accessible tissues, virtually provide an indication of whether sufficient of the element is available in that tissue to maintain an essential metabolic process. They thus ignore tissue compartments of that element that may be present in a relatively unavailable form and so circumvent some of the interpretative difficulties arising from the determination of total trace element content.

The success of this approach depends upon: (1) the certainty with which an important pathological lesion can be identified and its causative metabolic defect established, (2) the ease with which the assay of the sensitive enzyme system can be conducted as a routine, and (3), as with other diagnostic approaches, the clarity with which changes in such systems reflect the progressive development of a clinically significant disorder.

Despite these stringent limitations, assays of tissue caeruloplasmin (ferroxidase I) (Todd, 1970) and cytochrome oxidase (*EC* 1.9.3.1) activity (Poole, 1973) are now being effectively applied in the detection of Cu deficiency (and consequently in the detection of Zn and Cd intoxication) and the validity of plasma monoamine oxidase (*EC* 1.4.3.4) for this purpose is being assessed. Similarly there are indications that erythrocyte glutathione peroxidase (*EC* 1.11.1.9) may provide a useful guide to Se status (Hafeman, Sunde & Hoekstra, 1974). In contrast, efforts to find a suitable indicator of suboptimal Zn status have so far proved disappointing.

Dietary and environmental considerations influencing the diagnostic approach

Scrutiny of existing values for the trace element content of foods and feeding-stuffs clearly suggests that diets or rations adequately formulated to meet protein and energy requirements may in some circumstances be low in trace element

content. Such was the situation in the first clear demonstration of Cu deficiency in human infants undergoing rehabilitation from kwashiorkor or marasmus on diets based on dairy products and refined cereals. Similarly, the low content of Zn in cereal straws, 'feed grade' urea and some root crops offered to cattle suggests that the Zn status of such animals may well be open to question.

It is now recognized that geochemical anomalies may influence crop trace element content or the balance between individual elements, and that, once recognized, this information can be of value in narrowing the diagnostic approach. Soil survey work and stream sediment analysis have already proved their value in the detection of Mo excess, Co deficiency and Se deficiency and excess in farm animals, and may possibly be of value in the detection of Cr deficiency in man.

Practical experience using both these approaches has indicated that two important provisos must be borne in mind; first, that soil conditions and crop management frequently have very significant effects in modifying trace element relationships between soil and the plant used as food and, secondly, that many compositional variables (e.g. Mo, phytate, sulphate and sulphur amino acids) markedly influence trace element availability from the diet.

Incomplete though our knowledge of these aspects may be, existing information made available to those concerned with the task of achieving an unequivocal diagnosis can considerably narrow the field of enquiry. In surprisingly many instances, there is nevertheless evidence that such a co-ordinated approach to a problem has been a last, rather than an initial, resort.

Conclusions

Economic necessity is imposing unexpected changes in the animal industries of many developed countries. There are already indications that these changes, probably by influencing fertilizer and feeding-stuffs usage, are in turn modifying the incidence and regional distribution of some trace element deficiency disorders (notably those of Cu and Co) and placing greater demands upon the diagnostic services. Even before this, there existed evidence of widespread need for more effective recognition of such disorders. Thus, of 157 countries reporting on the incidence of animal disease in the current FAO/WHO (1974) Animal Health Yearbook, only thirty provide evidence suggesting adequate appraisal of the significance of trace element disorders.

It is perhaps realistic to anticipate that changes in living standards, and thus of dietary habits, may increase uncertainties as to the significance of the trace elements in human disease.

This paper has considered the advantages and limitations of several different approaches to the task of identifying such disorders. The existing limitations of each approach are such that effective progress can best be achieved by an interdisciplinary attack. Where this has been achieved, notably in the agricultural advisory and research services in the Netherlands (Committee on Mineral Nutrition, TNO, 1973) and, in a more restricted sphere, in the resolution of the role of Cu in Menkes' disease in man (Danks, Campbell, Walker-Smith, Stevens, Gillespie, Blomfield &

Turner, 1972), progress has been rapid. This fact merits wider recognition in the training of those nutritionists, veterinarians and clinicians who may ultimately have to undertake such work.

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