BIOCHEMICAL AND PHYSIOLOGICAL INDICATORS OF MINERAL STATUS IN ANIMALS: COPPER, COBALT AND ZINC

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ABSTRACT

The value of estimates of trace element requirements or of data on tissue trace element content for identifying and controlling trace element-related disorders is often limited by inadequate data on the relationships of such criteria to physiological performance. Investigations of metabolic events initiating early pathological responses to deficiency are beginning to suggest more effective indicators of physiologically relevant abnormalities in trace element intake or status. Progress in studies of metabolic responses to deficiencies of copper, cobalt and zinc is reviewed.

(Key Words: Copper, Cobalt, Zinc, Deficiency, Diagnosis, Metabolic Disorders.)

Introduction

Techniques for detecting and controlling trace element problems in livestock differ widely in character and effectiveness. In many situations, previous experience of such problems arising from a particular management system or usage of indigenous feeds with specific anomalies in trace element composition is sufficient to justify preventive action. However, as cultural practices and animal management systems change, these often modify trace element flow through the food chain sufficiently to introduce a new range of problems. Typical examples are copper (Cu) deficiency and, sometimes, molybdenum (Mo) toxicity in ruminants maintained on newly-irrigated or limed areas, selenium (Se) vitamin E deficiency following introduction of techniques for moist grain storage and zinc (Zn) deficiency in young ruminants offered milk-replacers rich in constituents capable of inhibiting Zn absorption, such as phytic acid. These and other examples have been reviewed by Mills et al. (1982).

Investigative measures appropriate for extensive or intensive systems of animal management also differ. Under extensive conditions, the principal problem is to identify those areas in which it is feasible to deploy relatively costly and labor-intensive investigations needed to define the trace element status of diets and animals. Recent advances in methods for surveying abnormalities in the inorganic composition of soils and their parent materials and better understanding of the factors influencing trace element flow from soils to staple crops are beginning to contribute appreciably to the preliminary identification of such areas (Bowie and Thornton, 1984).

For intensive management systems based on bulk supplies of staple feeds relatively uniform in composition, an approach based initially upon dietary analysis and its interpretation is often feasible. Here, however, the value of this approach is conditioned not merely by the adequacy with which dietary trace element content and requirements are defined, but also by the availability of information on those dietary variables that modify physiological responses to changes in essential or toxic element intake. The past experience of advisory laboratories has revealed very few instances in which it is possible to predict animal response merely from analytical data on a single element and comparison of these data with tabulated estimates of requirements or tolerance. The significance of this problem created by metabolic antagonisms arising from unbalanced


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inorganic element supply is certainly declining as advisory laboratories introduce new, automated, multi-element analytical techniques. Such developments are steadily improving our ability to predict and anticipate changes in the trace element status of livestock. However, they are also revealing, often dramatically, that our ability to predict production from changes in the trace element status of diets or tissues is inadequate. Specific examples of these difficulties and of progress towards their resolution are considered in following sections of this paper.

The Need for Metabolic Indicators of Trace Element Status

Fundamental to the interpretation of data on the trace element content of diets or animal tissues is the assumption that compositional anomalies are reflected, ultimately, by changes in animal performance. Advances in analytical methodology have far outstripped the progress of studies attempting to relate changes in tissue trace element status to the development of metabolic defects and their pathological consequences. With few exceptions, our current estimates of minimal requirements for trace elements are based upon balance data or tissue Cu retention data for these elements rather than on physiological criteria. The validity of many estimates derived by such approaches is questionable. For example, in cattle the close positive relationship between the magnitude of hepatic Cu stores and endogenous losses of Cu (Bremner and Mills, 1981) implies that minimal estimates of requirement can only be derived from balance or tissue Cu retention data if the animals used in such studies had hepatic Cu stores that initially were marginal. In turn, the concept of "marginality" of such stores implies an understanding of relationships between hepatic Cu and functional normality, which, at present, is inadequate. Other considerations influence the validity of estimates of Zn requirement based upon the analysis of tissues or estimates of intake and excretion. Evidence with a variety of species points to the probability that physiological normality with respect to Zn status reflects the availability of Zn from a small, labile, but as yet unidentified tissue pool of this element, the magnitude of which is more strongly governed by the balance between anabolic and catabolic activity in tissues than by total tissue Zn content (Mills, 1981a).

In the past, the validity of balance and tissue compositional data has been virtually ignored in many attempts to estimate trace element requirements. Accordingly, the estimates so derived are of greater value for predicting long-term changes in trace element body content than for predicting physiological responses or for retrospective diagnosis. It is in the diagnostic context that the need for physiologically realistic criteria of trace element status is most pressing. This need has been accentuated by growing demands for techniques to identify the pathological consequences of sub- or supra-optimal trace element intake before productivity declines or diagnostically characteristic clinical signs of severe deficiency or toxicity become evident. An essential feature of the research programs needed to develop such indicators is that the metabolic events leading to both pre-clinical and clinical manifestations of deficiency or toxicity should be examined in detail. Aspects of such studies of Cu, Co and Zn deficiencies are considered in the remainder of this paper.

Metabolic Consequence of Copper Deficiency

Low intakes of available Cu by ruminants are usually accompanied by an exponential decline in hepatic Cu reserves. Under conditions of severe Cu depletion, total liver Cu declines by approximately 50% every 25 to 30 d (Bremner and Mills, 1981). While the rate of release of hepatic Cu is frequently sufficient to maintain plasma Cu within the normal range until liver Cu falls below 30 mg Cu/kg dry matter, exceptions to this have been noted. Thus, young rapidly-growing cattle with ostensibly adequate hepatic Cu reserves (e.g., 100 to 150 ppm) frequently appear unable to release sufficient Cu to maintain normal plasma Cu concentrations if maintained on severely deficient diets (A. C. Dalgarno and C. F. Mills, unpublished). Because such situations are rarely accompanied by a decline in growth rate or other manifestations of deficiency, the pathological significance of a low plasma Cu in this instance is unclear.

The more consistently low plasma Cu established once hepatic Cu is less than 20 to 30 ppm is accompanied by a corre-
ponding loss of Cu-dependent ceruloplasmin activity. On grounds both of the operational simplicity of ceruloplasmin determination and claims that a decline in its activity may be responsible for defects in iron (Fe) metabolism that develop during Cu deficiency, it has been suggested that this enzyme may be a suitable metabolic marker of Cu deficiency. However, experience has shown that a low plasma Cu and low ceruloplasmin activity may become established for many months before clinical signs of Cu deficiency or indications of deranged Fe metabolism develop (Mills et al., 1976). One review has also emphasized that many cattle aged 12 mo or more with low plasma Cu and ceruloplasmin fail to show a positive growth response to provision of supplementary Cu (Phillippo, 1983). Use of either plasma Cu or ceruloplasmin as indicators of Cu status is further complicated by the fact that both are enhanced during the acute phase of many infections.

The most serious limitation to use of plasma Cu and ceruloplasmin as indicators of Cu status is the variable interval between onset of low values and development of pathological changes. Although indications are that the interval is shorter in young animals, the oversensitivity of plasma Cu (Todd et al., 1967) and ceruloplasmin has stimulated the search for other indicators.

Because activity of the erythrocyte enzyme (CuZn) superoxide dismutase (SOD) is principally dictated by Cu status at the time of erythrocyte synthesis, it has been claimed to have the advantage of providing a retrospective, and probably more reliable, indicator of Cu status (Suttle and McMurray, 1983). Copper depletion and subsequent repletion studies with both sheep and cattle indicate that erythrocyte SOD declines and recovers more slowly than plasma Cu; these studies further suggest that differences in the rate of decline of both indices during depletion directly reflect its severity. Thus, such a dual approach may well be capable of providing information both on the period for which Cu supplies have been inadequate and on the extent of depletion (table 1).

Although the role of (CuZn) SOD in converting potentially toxic superoxide free radicals to \( \text{H}_2\text{O}_2 \) is well established, the pathological significance of the decline in SOD activity of many tissues during Cu deficiency is not yet clear.

One of the earliest functional defects in cattle undergoing Cu depletion is failure of microbicidal defense mechanisms. Circulating neutrophils retain their capacity for phagocytosis of foreign organisms, but microbicidal activity declines steadily (Boyne and Arthur, 1981). Because microbicidal activity is contingent upon a free radical-mediated process, it was suspected initially that this defect was perhaps one manifestation of a generalized decline in SOD activity. More recent studies indicate, however, that loss of microbicidal function precedes SOD loss and appears more closely related to a failure to produce superoxide within the neutrophil phagosome (Arthur and Boyne, 1985). Although total oxygen consumption by the Cu-deficient neutrophil is not impaired at the stage that microbicidal activity fails, histochemically demonstrable decreases in

| TABLE 1. TENTATIVE CRITERIA FOR DETECTION OF PRE-CLINICAL AND CLINICAL PHASES OF Cu DEFICIENCY\(^a\) |
|--------------------------------------------------|------------------|------------------|------------------|
| Species  | Erythrocyte SOD\(^b\) (mg/g hemoglobin) | Plasma Cu, mg/liter | Interpretation           |
|          | Young | Adult |                      |                      |
| Cattle   | >.7   | >.3   | <.6                 | Deficiency of short duration; response to Cu unlikely |
| Sheep    | >.5   | >.3   | <.6                 |                      |
| Cattle   | <.5   | <.3   | <.6                 | Prolonged deficiency; Cu response likely |
| Sheep    | <.3   | <.2   | <.6                 |                      |

\(^a\)Adapted from Suttle and McMurray (1983).

\(^b\)CuZn superoxide dismutase. Low SOD with plasma Cu >.6 indicative either of infection or recent increase in Cu supply.
cytochrome oxidase activity are often evident in Cu deficient neutrophils (Boyne, 1978). This occurs several weeks before the activity of cytochrome oxidase falls in other tissues. Thus, cytochrome oxidase activity appears particularly sensitive to changes in Cu status. In the light of additional evidence of a closer temporal relationship of such changes to the development of pathological lesions in other tissues than for ceruloplasmin or plasma Cu, it is surprising that the potential diagnostic value of such findings has not been more closely investigated.

Other significant pathological responses to Cu deficiency are summarized in Table 2. The studies upon which these findings are based have shown that a wide range of pathological defects can develop before prominent and characteristic, clinical deficiency features appear. Covert lesions such as skeletal rarefaction and degenerative changes in connective tissue developing close to the time at which an animal is marketed may have little economic significance. However, for stock being maintained for several productive cycles under conditions provoking even marginal Cu deficiency, there are much stronger pressures for measures suitable for identifying animals at risk. While the biochemical origins of many of the defects considered in Table 2 are known, the highly invasive procedures needed to monitor their development precludes their use for routine diagnostic purposes. Possible alternatives such as monitoring of characteristic abnormalities in plasma catecholamine patterns or the presence in plasma or urine of characteristic degradation products of inadequately cross-linked connective tissue proteins are being investigated. For the immediate future, however, the need is to resolve the problem of the hypersensitivity of plasma Cu or ceruloplasmin as markers and, as a temporary expedient, to find indices that (although perhaps linked only indirectly

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Pathological response</th>
<th>Metabolic/enzymic response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Neutrophil function</td>
<td>Ceruloplasmin ↓</td>
</tr>
<tr>
<td>2</td>
<td>Cardiac enlargement and fragility</td>
<td>Cytochrome oxidase (neutrophil) ↓</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>( O_2^- ) generation (neutrophil) ↓</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Defective maturation of keratin</td>
<td>Conversion Cys (SH) to Cys (S-S) Cys ↓</td>
</tr>
<tr>
<td>5a</td>
<td>Postural changes</td>
<td>Melanin synthesis ↓</td>
</tr>
<tr>
<td>5b</td>
<td>Skeletal abnormalities</td>
<td>Monophenol monooxygenase ↓</td>
</tr>
<tr>
<td>5c</td>
<td>Hepatic Fe accumulation</td>
<td>Elastin cross-linking ↓</td>
</tr>
<tr>
<td>5d</td>
<td>Vascular degeneration/ECG anomalies</td>
<td>Lysyl oxidase ↓</td>
</tr>
<tr>
<td>5e</td>
<td>Growth failure</td>
<td>Pyridinium cross-linking ↓</td>
</tr>
<tr>
<td><strong>Late</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Anemia</td>
<td>Respiratory function ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleotide translocase ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytochrome oxidase ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cu/Zn superoxide dismutase ↓</td>
</tr>
</tbody>
</table>
to the metabolic defects provoking significant pathological change), only respond to Cu deficiency when such changes are imminent.

The potential of neutrophil studies in this context has been indicated already. A further possibility is offered by the dramatic change in Fe metabolism, both in ruminant and nonruminant species, that characteristically precedes the first overt signs of Cu deficiency. The direct pathological relevance of the rapid accumulation of hepatic ferritin-Fe and the accompanying decrease of plasma Fe that appears at this stage is obscure (Mills et al., 1976). However, when other chemical or biochemical indications of a low Cu status exist, the finding of a low plasma Fe, or (from biopsy or postmortem sampling), a high hepatic Fe, invariably indicates the imminence of a pathological response to deficiency, which usually includes growth failure (Mills et al., 1976; Mills, 1980).

Recent studies with cattle suggest that beneficial responses to Cu supplementation are probably not confined to situations in which plasma Cu or ceruloplasmin are low. Abnormally high dietary intakes of Mo are principally of interest in the context of their adverse effect on Cu absorption when dietary sulphur is also high. However, situations are now recognized in which high Mo anomalies rapidly provoke muscular weakness and leg stiffness in grazing cattle without concurrent evidence of a low plasma Cu (S. Garden and W. R. Humphries, personal communication). Nevertheless, Cu prophylaxis prevents the development of this disorder, possibly by limiting Mo absorption and tissue redistribution. Such findings, and evidence that diets high in Mo content delay estrus and conception in cattle of low Cu status (Phillippo et al., 1985), point to the additional need for metabolic indices of Mo status.

**Metabolic Indices of Cobalt Deficiency**

The difficulty of measuring the low concentrations of cobalt (Co) in body fluids of normal and Co-deficient ruminants, and the frequently poor correlation between hepatic Co concentration and physiological performance in deficient sheep, provided an early stimulus to the quest for alternative indicators of Co status. Initially, this search was directed solely towards measurements in serum or tissues of the physiologically active Co-containing cofactor, vitamin $\text{B}_{12}$ (cobalamin), which, as indicated later, constitutes the essential functional core of enzymes involved in propionate utilization and methyltransfer processes. While such an approach does not directly address the question of whether Co supply is or is not adequate to maintain the full functional activity of these cobalamin-dependent processes, the assumption that such data reflect the total cofactor pool probably available for enzyme synthesis provided the basis of its use for diagnosis of Co deficiency.

Fundamental to the selection of serum as the substrate for diagnostic analysis was the assumption that serum cobalamin concentrations reflected the concentration of this cofactor in tissue stores such as the liver. Early studies with sheep offered experimental diets differing in Co content suggested that such relationships were close (Marston, 1970). However, the validity of this conclusion has been seriously questioned in several recent studies (e.g., Millar and Penrose, 1980). Among the factors contributing to this controversy are the application in different investigations of cobalamin assay techniques differing in their specificity towards physiologically active and inactive analogues of cobalamin. The intraruminal synthesis and partition of these forms between serum and liver is probably influenced not only by cobalt intake but also by other dietary attributes (Gawthorne, 1970; Bigger et al., 1976). The latter possibility is suggested by the markedly closer relationship between serum and liver cobalamin in sheep offered conserved diets rather than fresh herbage or rations high in metabolizable energy content (for review see Mills, 1981b).

Despite these difficulties in interpreting serum cobalamin data, the approach has proved reasonably satisfactory for discriminating between normal and severely Co-deficient sheep on the basis of threshold cobalamin values of approximately 200 to 300 $\mu$g cobalamin/liter serum (Russel et al., 1975). It is proving less satisfactory for the diagnosis of a mild Co deficiency. Furthermore the outcome of attempts to diagnose Co deficiency in cattle by this approach is frankly chaotic, a situation probably related to the variable, but frequently high, proportion of low-potency cobalamin analogues present in bovine serum (Halpin et al., 1984). Such problems
emphasize the need for more direct metabolic indicators of cobalamin status and the adequacy of dietary Co supply.

Both approaches being investigated currently exploit evidence of the involvement of cobalamin in the metabolism of propionate (Marston et al., 1961; figure 1) and in metabolic sequences involving transfer of reactive methyl groups (Gawthorne, 1968; figure 2). Both are based on the appearance in blood or urine of abnormal concentrations of metabolic intermediates accumulating from enzymic processes limited because of a deficiency of cobalamin-cofactors.

Conversion of propionate to succinate is contingent upon enzymic action of methylmalonyl CoA mutase, for which adenosylcobalamin serves as cofactor. Thus, a physiologically significant deficiency of Co (and thus of cobalamin) is reflected, initially, by accumulation of methylmalonate (figure 1) and, later, by increases in urinary methylmalonate (MMA) output. Relationships between urinary MMA, Co intake and the

Figure 1. Metabolic steps related to enhanced plasma and urinary methylmalonate in Co-deficient ruminants. Step interrupted by Co-deficiency (and thus depletion of cobalamin pool) is indicated by open arrow.

Figure 2. Indirect relationship of increased urinary formiminoglutamate (FIGLU) output arising during Co deficiency to reduced activity of cobalamin containing tetrahydrofolate methyltransferase. Steps inhibited by deficiency indicated by open arrows. The increase in FIGLU output is believed to reflect a depletion of the tetrahydrofolate (FH4) pool in tissues.
TABLE 3. DIAGNOSTIC IMPLICATIONS OF PLASMA METHYLMALONATE (MMA) AND COBALAMIN DATA (SHEEP)\(^a\)

<table>
<thead>
<tr>
<th>MMA, (\mu)mol/liter</th>
<th>Cobalamin, nmol/liter</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4.6</td>
<td>&gt;.2</td>
<td>Normal Co status</td>
</tr>
<tr>
<td>&lt;4.6</td>
<td>&lt;.2</td>
<td>Marginal Co status</td>
</tr>
<tr>
<td>4.6–15</td>
<td>&lt;.2</td>
<td>Pre-clinical Co deficiency</td>
</tr>
<tr>
<td>&gt;15</td>
<td>&lt;.2</td>
<td>Clinical phase; response to Co likely</td>
</tr>
</tbody>
</table>

\(^a\)Based on data of McMurray et al. (1985).

cobalamin-status of serum or liver of sheep have been examined in detail (Gawthorne, 1968; Millar and Lorenz, 1979). Although the consensus is that Co deficiency ultimately provokes a rise in urinary MMA output, there is disagreement whether this precedes the clinical phase of Co deficiency or is a late development. In contrast, a steady rise in plasma MMA clearly signals the imminent appearance of clinical signs of deficiency and, with minor reservations, appears to be a particularly useful indicator of a physiologically marginal Co intake (table 3) (McMurray et al., 1985).

An alternative biochemical marker of Co deficiency is provided by the metabolic responses occurring when cobalamin status becomes insufficient to maintain the activity of the enzyme, tetrahydrofolate methyltransferase (or methionine synthase) involved in methyl group-transfer reactions with methyltetrahydrofolate and homocysteine as substrates (figure 2). The procedural approach currently employed is based upon a secondary consequence of this lesion; a depletion of tissue tetrahydrofolate, which, in turn, inhibits the breakdown of formimino glutamic acid (FIGLU) produced as a normal metabolite of histidine. Use of an increased urinary FIGLU output as an early marker of Co deficiency in sheep has been validated both experimentally (Gawthorne, 1968) and under field conditions (Russel et al., 1975). In the latter studies a closer correlation was found between impaired growth rate and elevated MMA output than with a decline in serum cobalamin.

Despite this progress, not all problems in the diagnosis of Co deficiency are solved. Thus, neither the MMA or FIGLU procedure has been evaluated adequately for cattle for which the diagnostic limitations of cobalamin assay are most severe. Furthermore, procedures based upon analysis of metabolites present in urine are difficult to apply in routine investigative work. While plasma MMA estimation is feasible, an alternative to urinary FIGLU is required that may well arise from recent developments in the micro-analysis of substituted tetrahydrofolate derivatives, which as evident from figure 2, are influenced directly by cobalamin deficiency.

Finally, it must be emphasized that the reproducibility of biochemical diagnostic procedures based upon the production of abnormal metabolite concentrations in tissues or fluids is greatest when substrate supply is in excess. That normal variations in propionate supply from the rumen influence plasma MMA, even in cobalt-adequate animals, is evident from a higher MMA concentration found in sheep offered high grain diets and the lower values found when food intake is depressed (McMurray et al., 1985). The availability of the endogenous substrates, homocysteine and histidine, required for FIGLU production must also be governed by the balance of protein synthesis and degradation in normal and Co-deficient animals, and must thus influence the outcome of a FIGLU test. For both approaches a case exists for investigation of the possibilities of improving precision by infusion of a known excess of appropriate substrates before sampling blood or urine for metabolite assays. Such precautions would reproduce, in vivo, the most fundamental requirement for a successful in vitro assay of enzyme activity (i.e., that activity, and not substrate supply, should influence product yield). Fully automated techniques for substrate infusion and for serum sampling after a defined time interval are becoming available (H. Wolf, personal communication).

**Physiologically Relevant Indices of Zinc Status**

Susceptibility to Zn deficiency is high at periods of rapid growth, can be influenced markedly by variations in the dietary content of inhibitors of Zn absorption and is enhanced when chronic infection or tissue damage accelerates urinary loss of Zn (Mills, 1981a). Detection of sub-optimal Zn status is complicated by several unique features reflecting the biological behaviour of Zn. Important
among these are: 1) pathological expression of deficiency usually occurs long before major depletion of total body Zn is detectable, 2) the nature and location of vital but small pool(s) of tissue Zn required to maintain growth and other functions is unknown and 3) the size of this pool must be influenced not only by the dietary supply of Zn but also by the bidirectional flux of the element associated with its incorporation or release from organs such as muscle and the skeleton when the balance between tissue synthesis and catabolism changes.

When, as under the conditions of a controlled experiment, dietary composition is constant, Zn intake is low and is regulated, and monitoring of infectious disease and other important variables modifying Zn metabolism is undertaken, then a decline in plasma Zn can provide an adequate indication of the likely development of pathological responses to Zn deficiency. While it is apparent for most farm animals that plasma Zn values within the range .6 to .4 mg/liter can be regarded as marginal and <.4 deficient, these criteria are subject to qualification (Mills et al., 1967). The appearance of clinical signs of deficiency once plasma Zn falls below <.4 mg/liter is usually rapid in young growing stock. At other stages of development or when growth is limited because of a deficiency of other nutrients, clinical manifestations can be delayed or not appear, even though a low plasma Zn is maintained (Mills et al., 1967). An equally paradoxical situation has also been encountered in the study of the bovine response to Zn therapy when a genetic defect in Zn absorption provoked clinical signs of deficiency. Oral administration of Zn re-initiated growth at a normal rate but failed to restore plasma Zn to normal (Price and Wood, 1982). These circumstances suggest that the tissue pool of Zn essential for the maintenance of Zn-dependent growth processes can be saturated before "normality" of plasma Zn is achieved.

Such complications to the interpretation of plasma Zn restrict its practical diagnostic value. They would be surmountable if alternative plasma indices reflecting the development of metabolic defects attributable to Zn deficiency were available. Although more is known about the roles of Zn than of any other trace element (for review see Chesters, 1978), the nature of biochemical lesions responsible for failure of growth, appetite and immunocompetence, for the development of parakeratosis and other pathological changes is poorly understood. At present, we lack a biochemical marker of Zn deficiency that is related to its pathological effects.

This limitation is being partially overcome by a technique that monitors the level of a cysteine-rich, low molecular weight, Zn-protein, metallothionein (MT), in tissues and body fluids (Mehra and Bremner, 1983). Increases in tissue MT are believed to indicate that Zn is entering cells in sufficient quantity to meet physiological requirements, and the excess is initiating MT synthesis to sequester surplus cellular Zn as its ZnMT complex. Ultra-sensitive immunochemical techniques have shown that small quantities of this protein diffuse from tissues into plasma. Furthermore, studies with rats indicate that plasma MT assay provides an index of Zn status much less susceptible than plasma Zn to difficulties of interpretation introduced by the depressive effect upon the latter caused by infection or stress (Sato et al., 1984). The imminent development of MT antisera suitable for MT assay in the plasma of domesticated livestock will facilitate a more reliable appraisal of their Zn status than the only alternative available at present — a careful interpretation of evidence derived from a sequence of samples in which plasma Zn is found to be subnormal.

**Conclusions**

This selective review of progress in the development of metabolic indicators of trace element status has concentrated on aspects that hold promise of routine diagnostic application. A number of alternative approaches whose applicability is confined to experimental studies supported by facilities for complex biochemical assays have been given less attention. The value of these for routine use could change rapidly as progress in microanalytical methodology is made. Typical of the possibilities are improved techniques for proton nuclear magnetic resonance and high-performance liquid chromatography suitable for rapid routine "fingerprinting" of abnormal metabolites in body fluids. Progress will be contingent upon better understanding of the precise metabolic and pathological consequences of trace element deficiency and how these are reflected in the composition of accessible tissues and fluids.
The research programs supporting these developments are already clarifying some puzzling aspects of the etiology of trace element responsive disorders. Typical of such are the probably interrelated consequences of Cu and Co deficiencies, and of Cu and Se deficiencies, and suggestions that localized rather than generalized deficiencies of tissue Zn can influence physiological response. Thus, hair depigmentation is regarded as a classical feature of Cu deficiency, and yet instances are known in which concurrent administration of Co is essential to restore normal pigmentation (Judson et al., 1981; A. MacPherson and A. C. Dalgarno, personal communications). Consideration of the metabolic sequences involved in melanin production from tyrosine indicate that, while Cu-dependent processes predominate, initial events are also mediated by a tetrahydrofolate-dependent enzyme, which may well be influenced by Co status. Studies with rats show that the efficiency of utilization of Se for synthesis of the Se-containing enzyme, glutathione peroxidase, is markedly decreased as Cu deficiency develops (Jenkins et al., 1982); a finding that may well be relevant to growing evidence that concurrent Cu and Se therapy sometimes accelerates recovery in cattle previously suspected on the basis of clinical evidence to be solely deficient in Cu (Gleed et al., 1983). Finally, growing appreciation of the very localized enzymic responses that can occur as tissue Zn declines (Westmoreland and Hoekstra, 1969) is providing a basis for understanding why Zn administration can accelerate wound healing in animals exhibiting no other clinical manifestations of Zn deficiency (Demertzis and Mills, 1973).

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