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Assessment of liver copper status in cattle from plasma copper and plasma copper enzymes

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Summary — Copper status determined by liver biopsy was also assessed by 3 plasma parameters: total plasma copper (Cu) as well as ceruloplasmin (Cp) and amine oxidase E.C.1.4.3.6. (AO) activities. While 14 out of the 98 cattle, all kept in field conditions, had liver copper levels of less than 20 mg/kg only 5 of these could be distinguished by low plasma Cu and 3 by low Cp activity. In addition, some animals with apparently adequate liver copper had relatively low plasma Cu values, ca 0.5–0.6 mg/l. None of the cattle had decreased AO activity. When all 3 plasma parameters were combined in the form $[AO] + ([Cp] \times [Cu])$, detection of the low liver copper was improved in that 9 out of 14 could be distinguished, whereas animals with more than 20 mg/kg DM liver Cu could not. It is suggested that this relationship, tentatively called an index, would merit examination in other situations, since it might provide a better guide to copper status and indicate whether copper repletion therapy was worthwhile.

bovine / liver / plasma / copper

Résumé — Évaluation du statut en cuivre hépatique à partir du cuivre plasmatique et des enzymes à cuivre du plasma chez les bovins. Le statut en cuivre déterminé par biopsie du foie a été également évalué à l'aide de 3 paramètres plasmatiques : la concentration en cuivre plasmatique total ainsi que les activités céruloplasmine (Cp) et amine oxydase E.C. 1.4.3.6. (AO). Alors que 14 des 98 bovins (tous maintenus dans les conditions du terrain) avaient des taux en cuivre hépatique inférieur à 20 mg/kg (MS) seulement 5 d'entre eux ont pu être différenciés par un niveau faible en cuivre plasmatique et 3 par une faible activité de céruloplasmine. De plus, quelques animaux ayant un taux en cuivre hépatique apparemment satisfaisant avaient des taux plasmatiques relativement faibles (environ 0,5-0,6 mg/l). Aucun bovin n'avait une activité amine oxydase réduite. Quand les 3 paramètres plasmatiques ont été combinés sous la forme $[AO] / ([Cp] \times [Cu])$ la détection a été améliorée. En effet, 9 bovins sur 14 ont pu être différenciés, tandis que les animaux ayant plus de 20 mg/kg MS de cuivre hépatique n'ont pas pu l'être. Ces résultats suggèrent que cette relation qui a été appelée «index» mériterait d'être examinée dans d'autres situations puisqu'il pourrait être un meilleur indicateur du statut en cuivre. Cet index pourrait permettre d'indiquer si un apport en cuivre est valable.

bovin / foie / plasma / cuivre

* Correspondence and reprints

INTRODUCTION

The assessment of copper status in ruminants is fraught with problems (Mills, 1987; Paynter, 1987). It has been difficult to relate the precise biochemical data obtained in the laboratory to symptoms, even to overall growth rate, and in addition some indicators of clinical status proposed, such as neutrophil function (Arthur and Boyne, 1985) or erythrocyte superoxide dismutase (ESOD) (Suttle and McMurray, 1983) necessitate complex assays difficult for routine usage (Herbert *et al*, 1991; for ESOD). Moreover, ESOD depression is indicative of severe and prolonged deficiency (Paynter, 1987).

The relationship of the body Cu reserves estimated from liver biopsies to plasma Cu concentrations or to ceruloplasmin (Cp) activity has been frequently examined and best fit curves have been derived from large numbers of samples to describe the asymptotic relationship (Claypool *et al*, 1975). It is generally considered (Mills, 1987) that normal plasma Cu levels or Cp can be maintained until liver concentrations fall below 30 mg/kg DM, but are consistently low only when hepatic reserves fall below 20. However there are exceptions, and young rapidly growing cattle with apparently adequate liver reserves (> 100) may fail to maintain plasma Cu concentrations (or Cp) when exposed to diets low in available Cu (Mills, 1987). Thus the plasma parameters by themselves are not reliable guides to clinical Cu status and many hypocupremic animals do not show a response to supplementation (Suttle *et al*, 1980; Mills, 1987). This is a common experience in Ireland (Todd *et al*, 1967; P Rogers, personal communication). By contrast, it is also important to recognise that Cp is an acute phase protein and its levels (and thus plasma Cu) may reflect infection, stress or parasitism with *Trichos-*

trongylus for example (Paynter, 1987) as well as Cu status. In addition, in ruminants exposed to Mo a new Cu fraction, associated with albumin, appears. This copper is probably of hepatic origin (Mason *et al*, 1988) and Wang *et al* (1988) showed that in cattle fed Mo-supplemented diets (35 mg Mo/kg) its levels correlated well with the labile "nuclear" fraction of liver Cu. Albumin-bound thiomolybdates act as powerful new ligands (Mason, 1986, 1990) removing Cu from copper proteins including liver metallothionein (McQuaid and Mason, 1990). However, while dietary Mo: Cu imbalance is a very common cause of bovine hypocuprosis it would be difficult to use this plasma fraction (broadly the TCA-insoluble Cu) diagnostically, since levels must depend not only on "nuclear" reserves but on Mo intake and rumen generation of trithiomolybdate. These factors would generally be unknown. Nor is it certain that thiomolybdates would be stable during the transport of blood samples to the laboratory.

In the field, the identification of a key Cu enzyme or the determination of the precise levels of liver copper at which symptoms appear is of lesser importance than the provision of an easy test from a reasonable number of samples which would indicate the level of reserves and/or status of a herd. In any event, the precise point at which deficiency affects performance must also depend upon the many challenges to which the animal is subjected. Liver biopsy is an option but is not always practicable since it requires an expertise which is not always available and which is not without risk; it is in any event far more time-consuming than blood sampling. Most previous reports have been based upon experiments carried out under carefully controlled conditions, often with housed animals. This paper, by contrast, examines the relationship between liver Cu concen-

trations and 3 plasma parameters in beef cattle in field conditions such as those that would be encountered by clinicians. As well as total plasma Cu concentrations and Cp activity the authors have also examined the activity of diamine oxidase (AO). This enzyme containing Cu, also known as bovine serum amine oxidase (EC.1.4.3.6.) has been shown, when measured as benzylamine oxidase, to be reduced in experimentally induced primary hypocuprosis in cattle (Mills *et al*, 1976).

MATERIALS AND METHODS

Amine oxidase (AO)

AO activity was measured using *p*-dimethylaminobenzylamine rather than benzylamine as a substrate (Mulryan, 1990) in a cocktail (1 ml final vol) containing 3.6 mM *p*-dimethylaminobenzylamine in 0.1 M potassium phosphate buffer, pH 8.0 at 25 °C. The reaction was started by the addition of plasma (0.1 ml) and the product *p*-dimethylaminobenzaldehyde (λ_{\max} 355 nm) measured continuously in a Gilford 2000 single beam spectrophotometer coupled to a Philips 8251a single pen recorder. The reaction rates were linear for at least 20 min and a unit of AO activity was defined as the amount of enzyme required to produce an absorbance change of 1×10^{-3} OD (355 nm) per min. The enzyme is not inhibited at concentrations of thiomolybdate likely to be encountered *in vivo* (Mulryan, 1990).

Ceruloplasmin, plasma and liver copper were measured by standard techniques which have already been described, for example by Poole (1973).

Field study

The relationship between liver copper levels and plasma parameters under field conditions was examined in liver biopsy and plasma samples obtained from 98 animals by an Foras Taluntais (now Teagasc) during the period 1985–1988.

The samples came from animals kept under a wide range of conditions; from cattle in Teagasc Field Stations to commercial farm animals. There was a general background of suspected hypocuprosis. Clinical data on the degree of parasitism or other stress factors was not on record. The animals would be the kind of population encountered by the veterinary practitioner in the field; the survey contrasts with experiments carried out under carefully controlled conditions with increasing or decreasing planes of copper nutrition whose results would be applicable only to those narrow conditions.

RESULTS

The liver biopsy samples revealed that the 98 cattle ranged widely from near 300 mg Cu/kg DM liver to 14 animals with liver Cu levels of less than 20 (fig 1). Amine oxidase activity ranged from 1–7 units and did not decline with lower copper concentrations (fig 1). By contrast, Cp activity tended to decrease (results not shown). However, only 3 out of the 14 animals with liver copper concentrations of less than 20 mg/kg DM had Cp activities of less than 0.05 units/l and 6 of the 14 animals had normal or near normal Cp levels (> 0.09 units/l). In addition, a number of animals of apparently adequate status had plasma levels of < 0.07 , including 2 with liver Cu at 150 mg/kg. Plasma copper provided a slightly better guide to status in that 5 out of the 14 animals could be distinguished (fig 1) with Cu concentrations of less than 0.5 mg/l. But again, the majority could not be detected (Cu levels > 0.7 mg/l) and a number of animals of apparently adequate status (liver copper > 30 mg/kg) had plasma copper levels between 0.5 and 0.7 mg/l (fig 1). Attempts were made to combine all 3 plasma parameters, and relating AO activity to the product of Cp and plasma Cu ($[AO] + ([Cp] \times [Cu])$) was particularly revealing (fig 1). This gave a narrow band for animals of adequate liver copper status, thus there were

Plasma parameters

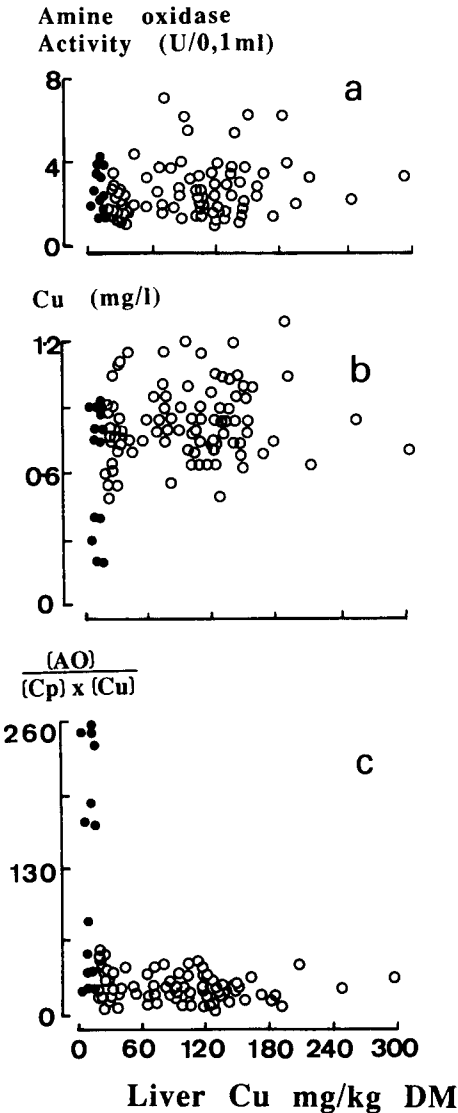


Fig 1. The relationship of plasma parameter to liver copper concentrations in 98 cattle. ^aAmine oxidase (AO) activity; ^bplasma copper (Cu); ^call 3 plasma parameters combined, $\text{AO} \div (\text{Cp} \times \text{Cu})$ (Cp represents ceruloplasmin activity); (●) the 14 cattle with liver copper of less than 20 mg/kg DM.

not "false positives" but 8 out of the 14 low status (< 20 mg/kg) could be clearly distinguished (fig 1), as being well outside this band. If a figure of 50 is taken as the significant limit then 9 out of 14 are detectable together with 4 animals of marginal status (liver copper ca 20–25). If a plot solely of the reciprocal of Cp x plasma copper (as compared to liver copper) is used, only 6 animals out of 14 can be detected (not shown).

DISCUSSION

The studies indicate that plasma AO activities are relatively resistant to hypocuprosis and/or hypocupremia and appear to confirm the observations made by Mills *et al* (1976) with experimental bovine primary deficiency, that is that activities decline at a relatively late phase. The results also confirm that while correlations and equations linking plasma Cu to liver Cu can be obtained from large numbers of samples, at lower liver copper levels the plasma levels are, in reality, extremely variable (Claypool *et al*, 1975; Stoszek *et al*, 1986). Therefore with smaller numbers the relationship is unclear. Thus in the data of Stoszek *et al* (1986), only 2 out of 5 cattle with liver Cu of less than 20 mg/kg DM were detectable (at < 0.2 mg/l plasma), that is outside the broad range of values observed, which was 0.6–1.2 mg/l plasma. In Ireland 0.5 mg/l is taken as a definitive indication of deficiency. The wide variations at higher levels may partially explain the observation commonly made in Ireland (P Rogers, personal communication; Todd *et al*, 1967) that hypocupremic cattle which fail to respond to supplementation by increased growth rate may not be clinically Cu-deficient or even of low copper status as defined by liver levels. However, in other situations, clinically silent hypocupremia

has responded to treatment (Bingley and Anderson, 1972). What is perhaps more surprising is the number of cattle with low and marginal (less than 25–30 mg/kg DM liver as defined by Paynter, 1987) reserves which were undetected by falls in plasma Cu or Cp activity alone. In Irish conditions, animals with under 20 mg/kg DM liver Cu generally show a response (P Rogers, personal communication) and in any event would generally (Mills, 1987; Paynter, 1987) be considered to have low reserves worthy of a precautionary boost. Scrutiny of previous data (eg Claypool *et al*, 1975) revealed many animals in this category. When all 3 plasma parameters measured were used, in the form $[AO] \div ([Cp] \times [\text{plasma Cu}])$, the detection of low copper reserves in the group of cattle examined was much improved; used as an index it may be of interest in providing a guide as to whether preventive copper therapy is worthwhile. Overt clinical deficiency with the obvious symptoms (with low blood copper levels) is generally readily recognized and treated and responsive sub-clinical problems with hypocupremia also occur (Bingley and Anderson, 1972). However, our data and the other data cited show that many normocupremic animals have marginal reserves. Sub-clinical situations are generally more common and the index, if confirmed elsewhere, would be most useful in the marginal context.

The index is based on parameters which depend upon many factors. Bovine AO, while much studied, has not been related convincingly to any metabolic pathway although the ruminant type of AO is a polyamine oxidase (Mulryan, 1990), nor is its tissue of known origin. We consider that its relevance to the index is probably its relative resistance to hypocupraemia and hypocuprosis, and that it may thus provide a stable internal standard. The assay could easily be adapted for routine usage. By

contrast, while Cp and plasma Cu tend to decline at variable thresholds of liver Cu, they may also be affected by other factors such as stress and parasitism (Paynter, 1987) and in the case of plasma Cu by the dietary Mo and the absorption of thiomolybdates. Since thiomolybdates and parasitism (Paynter, 1987) can both increase and decrease plasma Cu concentrations, the relationships must be very complex, particularly in animals in the field as opposed to experimental animals. For these reasons, at this stage the index should be considered essentially as an empirical guide. Nevertheless the use of all 3 plasma parameters, which are all simple to assay, appeared to improve the accuracy of detection of low liver copper reserves. It will of course be necessary to confirm that the same relationship occurs in other situations and in other populations of cattle outside the limited population available to the authors in Ireland and we invite other laboratories to examine the relationship, particularly in France where Mo-induced hypocuprosis is less common than primary copper deficiency (Lamand, Unité des Maladies Nutritionnelles, INRA; France; personal communication).

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