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THE INTERACTIONS BETWEEN COPPER, MOLYBDENUM, AND SULPHUR IN RUMINANT NUTRITION

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INTRODUCTION

The interaction between copper (Cu), molybdenum (Mo), and sulphur (S) in ruminant nutrition is probably unique in its effects on health and production. No other interaction in ruminants is known to have the capacity to swing the nutritional status of its unsuspecting host from deficiency to toxicity while wholly natural foodstuffs are consumed. Sheep consuming a complete diet, low in S and Mo and with a modest 12–20 mg Cu/kg dry matter (DM), can succumb to Cu toxicity (80) while others grazing pasture of similar Cu content but high in Mo and S can give birth to lambs suffering from the Cu deficiency disease swayback (2). Bovine Cu deficiency is endemic in regions throughout the world, and a frequent feature is the presence of high Mo concentrations in the pastures (47). Affected animals may show only mild symptoms such as loss of coat condition and poor growth, but under more extreme conditions stiffness of gait, infertility, diarrhoea, and emaciation are seen (15, 50). The biochemical explanations for the diverse clinical signs are by no means clear, and no other nutrient interaction in ruminants needs to be understood and managed with quite the same urgency as that between Cu, Mo, and S.

The complex history of the interaction in ruminants can be summarized by referring to three earlier reviews. By the mid-1970s it was clear that events in the rumen explained why the interaction had marked effects on ruminant species (56). Cu, Mo, and S (from organic or inorganic sources) could combine in the rumen to form an unabsorbable triple complex, possibly Cu tetrathiomolybdate ($CuMoS_4$), thus depleting the tissues of Cu. By the end of the decade (58), considerable support for the "copper thiomolybdate" hypothesis had accrued and a systemic dimension had been added to it: Cu could be rendered unavailable post- as well as pre-absorptively by the highly reactive metabolites thiomolybdates (TMs), formed in the sulphide-rich environment of the rumen. Recently an unexpected twist has been added by the suggestion that Mo rather than Cu may be the dominant interactant, exerting toxic effects on metabolism that Cu can negate (9, 24, 48, 49). That is, the problem may be one of Mo excess rather than Cu deficiency: molybdenosis not hypocuprosis. The third and most recent review found that the case for the alternative "TM toxicity hypothesis" was unproven (63), but the issue is so important that it will provide the framework for this review. Nutritionally valid hypotheses can and must be established before the outstanding practical problems and possibilities presented by the Cu-Mo-S antagonism can be addressed.

THIOMOLYBDATES: NUTRITIONAL DOUBTS AND CERTAINTIES

Qualitative Evidence for Thiomolybdate Formation in vivo

Thirteen years passed before the "copper thiomolybdate hypothesis," first proposed in 1974 (56), gained the necessary experimental corroboration with the demonstration that TMs could be formed in the rumen under nutritionally relevant dietary conditions and that they remain stable during transit to the ileum. Using displacement chemistry and characteristic elution profiles of the radio-labelled species, Price et al (52) showed that tri- ($MoOS_3^{2-}$ or TM_3) and tetrathiomolybdate (MoS_4^{2-} or TM_4) accounted for approximately 41 and 34%, respectively, of the displaceable ^{99}Mo bound to the solid phase of the rumen contents of sheep, 16 h after placing $^{99}MoO_4^{2-}$ in the rumen; little mono- (MoO_3S^{2-} or TM_1) or dithiomolybdate ($MoO_2S_2^{2-}$ or TM_2) was found. The diet used was grass pellets containing Cu and Mo in a 1 : 1 ratio at commonly encountered concentrations (6 mg/kg DM). Within individual sheep the proportion of displaceable TM_4 found on the solid phase in the duodenal and ileal digesta was similar to that found in the rumen. This observation confirmed the suggestion that, while TMs per se were unstable in acid solution and unlikely to escape hydrolysis in the abomasum, association with the solid phase in the rumen conferred stability upon them (3). Furthermore, association with the solid phase would draw the reaction

$$\begin{array}{ccccccc} S^{2-} & & S^{2-} & & S^{2-} & & S^{2-} \\ + & & + & & + & & + \\ MoO_4^{2-} & \rightarrow & MoO_3S^{2-} & \rightarrow & MoO_2S_2^{2-} & \rightarrow & MoOS_3^{2-} \rightarrow MoS_4^{2-} \end{array}$$

to the right (66) and give more time for it to occur by delaying outflow from the rumen (39). What then are the consequences (in terms of the antagonism of Cu metabolism) of this stable association between TMs and the solid phase of rumen digesta?

Qualitative Evidence for Thiomolybdate Inhibition of Copper Absorption

Allen & Gawthorne (3) showed that addition of TM_4 to the rumen increased the binding of Cu to protozoa, bacteria, and particularly to undigested feed particles; the addition of MoO_4^{2-} had the same effect. Using neutral detergent to break down complexes, they found that the bound Cu was associated with high molecular weight proteins. Others found that preformed TM_4 reduced the absorption of Cu when added to the diet of sheep (66). Furthermore, when preformed $CuTM_3$ and $CuTM_4$ were placed in the rumen they were quantita-

tively recovered from the feces with their characteristic spectra (seen after KCN extraction) intact (72). Digesta solids from sheep given dietary molybdenum have a poor capacity to replete cytochrome oxidase activity in the intestinal mucosa of Cu-depleted rats (51). These findings together suggest strongly that the "higher" TMs (TM_3 and TM_4) cause Cu to be irreversibly bound to high molecular weight proteins and thus reduce Cu absorption. The lesser substituted oxythioanions are probably not involved because TM_2 did not impair Cu absorption when added to a low S diet; it only impaired Cu absorption with a high S diet, which would presumably convert TM_2 to TM_3 and TM_4 (66).

Qualitative Evidence for Absorption of Thiomolybdates

Before TMs can either add a systemic dimension to their antagonism or exert direct toxic effects of their own on tissue metabolism, some TMs must first be absorbed. Price et al (52) showed that small amounts of TM_3 and larger amounts of what was probably TM_1 were detectable in the duodenal supernatant 16 h after dosing with ^{99}Mo. Release of TMs probably occurred in the abomasum, since acidification of rumen solids in vitro (simulating passage through the abomasum) released TM_3 (but not TM_4). TM_3 alone was detectable in the plasma after 16 h, suggesting that TM_1 (i.e. most of the absorbable TM) was probably broken down prior to or during absorption. Thus, TM_3 alone remained both stable and absorbable, although the proportion of the dose that finally entered the hepatic portal vein as TM_3 and was recovered in the peripheral blood was not calculated. Presumably, the absorbed TM_3 had been reversibly bound to the solid phase independently of Cu.

Mason and his associates (for reviews see 38, 39) have shown that in sheep, cattle, and deer, labelled TM_3 and TM_2 were detectable in plasma after large single doses of $^{99}MoO_4$ were placed in the sheep's rumen. The same group detected TM_4 in plasma after ruminal administration, but they concluded that under normal conditions TM_3 was the more likely mediator of any systemic effects of TM on Cu metabolism (40). The appearance of labelled TM_3 in the bloodstream after its administration via the duodenum confirmed their view (29). However, the extent of such systemic effects is probably much smaller when Mo is presented at comparable concentrations to Cu via the diet. A better perspective of the quantitative importance of TM absorption can be gained from data for TM excretion in feces.

Quantitative Evidence for Thiomolybdate Excretion in Feces

Since Cu and Mo can be concomitantly and irreversibly bound in reactions with protein, the inhibition of Mo absorption should be no less than that of Cu absorption following CuTM complex formation. The complexed elements

would be equally unavailable to function or antagonize systemically. Raising the Mo concentration of semi-purified diets or fresh grass to a 1:1 ratio with Cu reduces Cu absorption by a factor of three (59); a stoichiometric reduction in the absorption of Mo (i.e. fourfold in terms of atomic mass) would be expected. Experiments indicate a smaller though still substantial inhibitory effect of TM formation on Mo absorption. Addition of S to diets containing MoO_4^{2-} or TM_2 reduced the absorption of Mo from 60 to 30%, probably through the formation of unabsorbable TM_3 and TM_4 (66). Price et al (52) found that half of the ^{99}Mo in rumen (strained), duodenal, and ileal digesta was nondisplaceable and presumably nonabsorbable. Thus evidence exists for substantial though incomplete formation of nonabsorbable TMs under suitable rumen conditions. Where it is not present in excess of Cu [i.e. the norm for most Mo-enriched pastures (47)], most of the dietary Mo is probably excreted in the feces and is not absorbed. If and when TM_3 enters the bloodstream, what are the likely consequences for Cu and Mo metabolism?

Systemic Effects of Thiomolybdates

A vast amount of information on the systemic effects of preformed TMs in ruminants has been generated, mostly by Mason and his co-workers (for reviews see 38, 39). These effects mainly involve the inhibition of Cu metabolism and support the concept, though not necessarily the reality, of a systemic component to the "copper thiomolybdate hypothesis."

PLASMA COPPER When free TM_3 (or TM_4) is mixed with plasma, it reacts with albumin and causes Cu to be bound at a site other than the normal N-terminal locus; at the same time, Cu increases the affinity of the site for TMs (79). The complex with albumin is so strong that it precipitates with the protein upon acidification, which explains earlier findings of an abnormal trichloracetic acid (TCA) insoluble fraction in the plasma of sheep exposed to Mo and S (cf 58). The Cu-TM-albumin complex prepared in vitro has a longer biological half-life than does copper-albumin when administered to cattle (25). The delayed clearance of intravenously administered ^{64}Cu from plasma following administration of excess Mo (25 mg/kg diet DM; 55) and of stable Cu following the intravenous administration of TM_3 (44) suggests strongly that the Cu:TM:albumin complex can form in vivo; however, it has only been detected following an excessive intake of Mo or following parenteral administration of TM_3 or TM_4.

The important physiological effects of the formation of the Cu-TM-albumin complex in the bloodstream could be twofold: first, to restrict the availability of Cu for ceruloplasmin synthesis from absorbed Cu delivered to the liver via the hepatic portal vein; secondly, to restrict the availability of the absorbed TM in an effective detoxification mechanism. While the TMs (TM_2, TM_3,

and TM_4) can reversibly inhibit the diamine oxidase activity of ceruloplasmin in vitro (30, 35), even TM_4 does not do so in vivo (19).

BILIARY COPPER EXCRETION TMs can deplete body reserves of Cu by interfering with the enterohepatic circulation of Cu via biliary secretion. Studies with cannulated sheep showed that the intravenous administration of TM_3 or TM_4 enhanced biliary Cu secretion (18, 28) and fecal Cu excretion (41). The removal of Cu from liver can be so swift and substantial after parenteral administration of TM_4 that sheep can be saved from Cu poisoning when on the brink of a hemolytic crisis (19, 23). The extent to which endogenous Cu losses are enhanced following the addition of Mo to the diet is, however, debatable; Smith et al (55) found no such enhancement, whereas it has been implied by others (66). It should be noted that increased endogenous losses of Cu could arise through complex formation with biliary Cu within the intestines as well as systemically.

URINARY COPPER EXCRETION The urinary route of excretion for Cu has always been regarded as of minor importance when compared with the biliary route, but this perspective has been formed in the context of excess rather than deficient supplies of Cu. In terms of unavoidable losses, urinary Cu excretion may constitute 25% of the total loss and is higher in young than in old animals (62). Addition of Mo and S to diets with adequate Cu increased urinary Cu excretion (37) and did so independently of the margin of Mo excess (77). Diuretic effects of the sulphate supplement may contribute to the enhanced urinary Cu excretion, and the modest increase ($<$ 1 mg Cu per day) may disappear if dietary Cu status is reduced (37). Injection of TM_3 did not increase urinary Cu excretion in short-term studies (28, 41), but this finding would be expected if Cu was initially bound in nonexchangeable forms to albumin. Mason et al (42) have suggested, by analogy with the metabolism of thiotungstates, that Cu:TM:albumin complexes will eventually be hydrolyzed and their constituent Mo excreted in urine; previously complexed Cu may be simultaneously released. Long-term feeding of Mo increased the loosely bound fraction of plasma Cu (65) and may thus have increased urinary Cu losses.

COPPER AND MOLYBDENUM IN TISSUES Gawthorne (17) suggested that TMs profoundly altered the equilibria between free Cu and cupro-proteins, including those with enzyme activity, throughout the body. Under in vitro conditions, TMs removed Cu from metallothionein, which has a very high binding constant (10^{15}) for Cu (4). The intravenous administration of ^{35}S-labelled TM_3 to cattle enabled these effects to be demonstrated in vivo. The oxythioanion was traced to the liver where it arrived intact and shifted Cu

from metallothionein to a high-molecular weight ^{35}S-labelled protein (75). Histochemical studies showed that intravenous TM_4 removed Cu from both the lysosomes and cytosol of hepatocytes (33); these effects probably explain the increased biliary Cu secretion seen in TM_4-treated sheep. Wang et al have suggested that the initial increase in plasma TCA-insoluble Cu following the parenteral administration of TM_3 represented Cu removed from liver stores, since less was found as liver Cu stores were progressively depleted (76). However, in ruminants receiving Mo in pasture or forage the most vulnerable tissue would be the intestinal mucosa, exposed to TMs liberated during digestion, rather than the liver. No studies of placental permeability to TMs have been carried out. Mo crosses the placenta, but it seems highly unlikely that TM-protein complexes will do so, although they may accumulate there with harmful effects on placental metabolism.

The turnover of CuTM- or TM-tissue protein complexes has not been studied but it is probably slow, contributing to a retention of body Mo. Dietary molybdate increased Cu and Mo concentrations in kidney in sheep (58) while addition of S to the diet increased whole body retention of Mo despite a reduction in Mo absorption (21). The presence of abnormal, unreactive Cu-TM-protein complexes in tissues would complicate the assessment of tissue as well as plasma Cu status when vast excesses of Mo are ingested, giving falsely high estimates of available tissue Cu.

COPPER-DEPENDENT FUNCTIONS When molybdenum is presented via the diet, the effects of TM_4 and insoluble Cu:TM_4 complex formation should be confined to the gut and to the reduction of Cu absorption; and they should not differ in any way from the effects of straightforward Cu deficiency lucidly described by Fell (13). Systemic effects of TM_3 are possible and gain interest because of their uncertainty. In the course of absorption across the intestinal epithelium, this powerful sequestrating agent may remove Cu from superoxide dismutase and from cytochrome oxidase (67), which may lead to impaired local mitochondrial integrity and cell function that manifests itself as diarrhoea. Addition of only 3 mg Mo/kg DM to the diet of rats as TM_4 produced gross changes in gut morphology with mitochondrial lesions (14). Elsewhere, Wang et al (75) estimated that up to 10–15% of an intravenous dose of TM_3 was found in bovine liver: Distribution of Cu within subcellular fractions was altered, but functional consequences were not studied.

The distribution of TM effects on Cu-dependent functions within the body may not be the same as that caused by straightforward dietary Cu depletion because of the abnormal binding of Cu to what some believe is its principal carrier protein, albumin. When excess Mo was added to silage and fed to cattle, coat changes and diarrhoea were seen even though circulating Cu and ceruloplasmin concentrations remained high (76). Repeated injections of TM_4

into sheep reduced wool crimp and strength, similar effects to those seen in Cu deficiency, even though liver and blood Cu concentrations were normal (20). One possible explanation is that TMs accumulate in the skin and produce local Cu-depletion in developing hair or fleece. Connective tissue may be another vulnerable site, given the prominence of periosteal and cartilagenous tissue changes in lambs exposed to Mo (22). Physiological barriers may partially protect the fetus and organs such as the brain and spinal cord from the harmful effects of any circulating TMs.

MOLYBDENUM METABOLISM AND FUNCTION Effects of TM formation on Mo metabolism and function have been largely ignored, but three areas are of particular interest. The first concerns xanthine oxidoreductase, which can act as an oxidase (type O) or dehydrogenase (type D). Is Mo that is absorbed as TMs available for oxidoreductase synthesis? Does it form complexes that affect specific activity of the enzyme or its conversion from one mode of action to another? The conversion from type D to type O is stimulated by O_2-deprivation and is believed to occur in the joints of rheumatoid arthritis sufferers (5). Stiffness of gait has long been recognized as a feature of cattle introduced to Mo-rich pasture (15), but sheep are not affected. Far higher levels of type O activity are found in bovine than in ovine tissues (10), and it is possible that arthritic changes occur in the joints of grazing cattle but not in the joints of sheep. Inhibition of the cupro-enzyme cytochrome oxidase could initiate or increase conversion to type O by impairing cell respiration, thus giving the Cu × Mo antagonism yet another intriguing dimension. The greater susceptibility of cattle than sheep to Mo-rich pasture, first noted in the 1940s, has yet to be explained, and the answer may be found in Mo rather than Cu metabolism.

The second area of interest concerns the affinity of Mo for bi-hydroxy groups (78) such as those on catechol estrogens. Impairment of fertility in heifers exposed to Mo is believed to involve interference with estrogen metabolism (48). Interactions of Mo, whether as MoO_4 or TM_3, with bi-hydroxy groups on other molecules such as catecholamines and dihydroxy vitamin D seem possible. Increases in catecholamine concentrations in the intestinal mucosa have been reported in cattle given Mo. However, a Cu-containing enzyme, dopamine B hydroxylase, is also involved in catecholamine metabolism, and such effects could not be attributed to TM toxicity rather than Cu deficiency without further study.

The third area concerns proteinases, which are widely distributed in mammalian tissues and have a variety of functions including immune defense and blood clotting. Observations that dietary Mo enhanced the pathogenicity of nematode infections of the gut in lambs while reducing worm burdens (67, 68) led to the study of proteinase activity in the parasite. Proteinases are

important for parasite entry and migration through the mucosa and for the digestion of nutrient protein. Proteinase activity was reduced in *Trichostrongylus vitrinus* recovered from lambs fed molybdate, and the effect was reproduced by exposing cultured worms to MoO_4^{2-} (with no sulphide source) in vitro (31). Since other indicators of metabolic activity were not impaired, specific effects of Mo on proteinases may exist that are independent of TM formation. Proteinase activities in the host's intestinal mucosa were also affected by exposure to dietary Mo (D. Knox and N. Suttle, unpublished data).

Thus, many more pathways whereby Cu × Mo × S interactions mediate their effects on ruminants may possibly exist than have so far been considered. The involvement of a Mo-sensitive pathway in the Cu × Mo interaction could influence the biochemical methods used in the detection and prevention of ill health in stock grazing molybdeniferous pastures, as is discussed in the latter part of this review.

Conclusions on Physiological Importance of Thiomolybdates

Formation of TMs, particularly TM_3 and TM_4, in the rumen reduces Cu absorption in ruminants. Furthermore, as the Mo:Cu ratio in the diet approaches and exceeds unity, TMs, notably TM_3, can be absorbed in sufficient quantities to change the binding of Cu to albumin. Whether other changes seen in vitro (e.g. the removal of Cu from metallothionein) or following the intravenous administration of TM_3 occur even under extreme dietary conditions remains questionable. Responses to Cu supplementation have usually been obtained on pastures containing only a slight excess of Cu over Mo and rarely > 8 mg Mo/kg DM (47). Under these conditions, Mo intakes for a cow might not exceed 80–160 mg per day of which only a small fraction (< 10%?) would be slowly absorbed as TM_3 over 24 h. It is against such figures that the "physiological" relevance of apparently modest intravenous doses of TM_3 [e.g. 26 mg Mo for a 400-kg steer (75)] must be judged. Estimates of the amounts of TM_3 arriving at the liver under different dietary conditions would help to place experiments employing parenteral TMs in physiological and nutritional perspective.

The new hypothesis that harmful effects of Mo may arise chiefly from Mo or TM toxicity is founded on the poor correlation between conventional measurements of low blood and tissue Cu status and the occurrence of clinical symptoms of Cu deficiency in cattle (9, 24, 48, 49). It is clear, however, that TMs associate extensively with Cu in unreactive forms, wherever they appear. Under such circumstances, measurements of total blood, plasma, or tissue Cu will not reflect the ability of Cu enzymes to perform their essential functions. Most of the clinical signs attributed to "thiomolybdate toxicity" are the same as those produced by simple Cu deficiency and therefore are likely to

have arisen from impaired Cu metabolism (63). It is possible that fertility is uniquely vulnerable to the effects of TMs on estrogen metabolism and alone responds indirectly to Cu acting as an antidote. Much more work needs to be done, however, to differentiate putative effects of TM toxicity from the well-established, predominant effects of induced Cu deficiency.

NUTRITIONAL VARIABLES AFFECTING THE RUMEN INTERACTION

The vast differences in Cu availability that have been recorded for various foodstuffs of low Mo content probably owe much to the effects of diet type on S metabolism (61); these effects in turn are amplified by Mo and fiber, which affect, respectively, the formation and stability of TMs. Whether or not the effects of TMs are mediated through Cu, an understanding of the dietary factors likely to influence TM formation and absorption is critical to understanding and manipulating the Cu × Mo × S interaction.

Sulphur

Of the three principal components in the interaction, S provides the most opportunities for variation in outcome because of alternative metabolic pathways from the rumen. S leaves the rumen extensively by absorption as sulphide (S^{2-}) but also by outflow as undegraded protein S or in microbial protein. Only degraded protein S and inorganic S from the diet or saliva are available for interaction with Mo and Cu in the rumen. Partition of degradable S depends on such factors as supply of degradable nitrogen, rate of eating, rate of S degradation by rumen microbes, and the rate of arrival of readily fermentable carbohydrate, which influences rumen pH and hence S^{2-} absorption. Those factors that maximize the area under the rumen sulphide concentration-versus-time curve are likely to increase the formation of higher TMs if the diet is rich in Mo. For example, continuous feeding of a readily fermented, semi-purified diet, high in S but low in Mo, increased rumen sulphide concentrations and decreased Cu availability in sheep more than did a once daily feeding (71). In addition to levelling the rate at which S entered the rumen, continuous feeding may have increased the numbers of protozoa in the rumen, thus lowering Cu availability by increasing rumen sulphide formation (26). Weak expression of the Cu × Mo × S antagonism in sheep without rumen protozoa (53) may be attributable to reduced "sulphide profiles" and the decreased opportunities for TM_3 and TM_4 formation. Differences in the outcome of the Cu × Mo × S interaction in terms of Cu availability for sheep continuously grazing grass or discontinuously fed conserved roughages and semi-purified diets (59) may also involve the kinetics of S metabolism in the rumen.

Molybdenum

Fewer alternative pathways to leave the rumen are known for Mo than for S. Mo is not absorbed from the rumen and little is known about its incorporation into microbial protein. Mo generally occurs in readily soluble and releasable forms in feeds and is unlikely to occur in undegradable forms that bypass the Cu × Mo × S interaction in the rumen. The predominant sources of variation in Mo metabolism are therefore likely to be Cu and S that bind Mo to the solid phase.

Copper

Cu is extensively bound to particulate matter in the rumen and is not absorbed from the rumen; the bonds may not be completely broken down by digestion further down the gastrointestinal tract. Some Cu may thus be protected from interaction with Mo in the rumen, but may remain unavailable for absorption. Cu protected via association with protein that escapes rumen degradation may, however, have enhanced availability when it is released in the intestine. The vulnerability of the ruminant to Cu deficiency is chiefly determined by these equilibria and by the readiness with which the small fraction of Cu ingested in feeds and potentially available is then complexed via the interaction with Mo × S or S per se.

Undigested Organic Matter

Diets that allow significant amounts of organic matter to pass undigested from the rumen should theoretically provide plentiful binding sites for products of the Cu × Mo × S interaction; readily digested feeds such as cereals and brassicas should not, and therefore they yield their Cu more readily for absorption (11). Furthermore, the high fermentable carbohydrate content of digestible feeds may lower rumen pH and enhance Cu availability by increasing absorption of S^{2-} and breakdown of TMs; this would explain why Mo (2.5–5.0 mg/kg DM) did not accelerate the rate of depletion of liver Cu in sheep given a whole grain diet (N. F. Suttle, unpublished data). Rumen pH is also relatively low in animals on silage diets, and the high availability of Cu in Mo-rich silage (60) may be attributable to a combination of rapid digestibility and low rumen pH. Further evidence of reduced potency of the Cu × Mo × S interaction on silage diets comes from the study of Wang et al (76) in which it took 13–14 weeks for diarrhoea to be induced in steers given silage containing 35 mg Mo/kg DM. Similar Mo concentrations in pasture cause immediate diarrhoea or "teartness" (15).

Sulphide-Trapping Agents

Any agent that competes with molybdate for sulphide in the rumen is likely to influence the course of the Cu × Mo × S interaction. Iron (Fe) is potentially

the most important example for grazing animals because large quantities of Fe are ingested in soil though only a small fraction is likely to be available for sulphide trapping. Inhibition of Cu metabolism by soluble Fe supplements in sheep has been partly attributed to trapping of S_2^{2-} as FeS in the rumen, followed by release of S^{2-} in the acid abomasum to form CuS (64). Brebner (8) found that the inhibitory effects of soil ingestion on Cu availability in sheep were correlated with the pyrophosphate-extractable Fe content of the soil. In the presence of Mo, rumen-soluble Fe may thus reduce TM formation while continuing to deplete the ruminant of Cu by a different mechanism (CuS formation). The two antagonists should therefore have less than an additive effect when combined, as has indeed been found in calves (9, 24). The formation of polymeric complexes involving Fe, Mo, and S has been suggested (54), but this would only lead to less than additive Cu-depleting effects if the polymeric complexes had an affinity for Cu that was less than the combined affinities of sulphide (released from FeS) and TMs. Other metals that form acid-labile sulphides could have similar effects to Fe (e.g. Mn), but those that form acid-insoluble sulphides (such as lead and cadmium) could be protective by restricting both TM and CuS formation.

Copper-Trapping Agents

In theory, agents other than S^{2-}, which prevent Cu from reacting with TMs in the rumen, could alter the course of the Cu × Mo × S antagonism; the net outcome in terms of Cu status would thus depend on the reversibility of any bonds formed. Fe as Fe_2O_3 inhibits Cu absorption independently of any involvement of sulphide (71). This Fe × Cu antagonism may be explained by the fact that Fe_2O_3 adsorbs Cu even under mildly acid conditions (N. Suttle, unpublished data); other oxides, for example, of Mn, may do the same. Since the metal oxides do not compete for sulphide, they would not reduce TM formation. The effects of Fe_2O_3 and Mo as inhibitors of Cu absorption may still not be fully additive because they compete for a small pool of absorbable Cu in the rumen. By binding Cu, however, Fe_2O_3 may lessen the strength of binding between TM_3 or TM_4 and the solid phase and may enhance the prospects for systemic effects of TMs.

Post-Ruminal Interactions

To confine consideration of the important interactions between Cu, Mo, and S to the rumen would be a mistake. The cecum, for example, has been shown to play an important role in S metabolism in ruminants, and it would be surprising if there was not some reformation of thiomolybdates in the alkaline, S^{2-}-rich environment provided in this organ. Reactions with the more concentrated but much changed cecal digesta are hard to predict, but continuation of Cu × Mo × S interactions in the cecum is clearly possible and

merits study. Indeed these interactions may be responsible for the most debilitating consequence of the antagonism, i.e. the onset of diarrhoea.

ASSESSING RISKS OF MOLYBDENUM-INDUCED DISORDERS

A variety of approaches attempt to confirm or predict the extent to which excess Mo affects the health and productivity of grazing livestock. Some have focused on the soil, some on herbage, others on the animal; some restrict analysis to Mo while others include the principal interactants, Cu and S. New knowledge about the pivotal role of TMs in the Cu × Mo × S should now influence the approaches taken.

Analysis of Soils

Analysis of soils for Mo represents one of the most successful applications of geochemistry to the improvement of animal health. Thornton (73) used stream sediment reconaissance in the UK to predict soil Mo and delineate large areas underlain by molybdeniferous shales where grazing animals were likely to become hypocupremic. Soil scientists in Canada (12) and Scotland (11) have begun to map areas of risk using extractable Mo in the soil to predict herbage Mo, with levels above 5 mg Mo/kg DM in herbage as the threshold. The well-known effects of soil pH, herbage species, and season on Mo uptake (e.g. 11, 36, 69) impose limits on the predictability of herbage Mo from soil Mo. Furthermore, the use of extractable soil Mo cannot resolve these problems when the procedure standardizes pH (e.g. neutral ammonium acetate) and ignores plant factors. Herbage Mo did not correlate with extractable soil Mo in the acid soils of western Kenya (pH range 4.8–5.6; r^2 = 11.3%); although the range of herbage Mo was small (0.1 to 4.2 mg Mo/kg DM), the higher levels could induce Cu deficiency (27). As a first line of attack, analysis of the soil for Mo remains a useful approach; it predicts the approximate capacity of the overlying herbage to produce TMs, however they may act in the body. Whether extraction of soil Mo can and does improve prediction of ill health remains to be seen.

Analysis of Herbage and Drinking Water

An early attempt to use herbage analysis to predict health problems was made by Kubota (32), who used legume Mo concentrations to map the soils of the USA in terms of their Mo status. The ratio of agonist to antagonist should be important and Cu:Mo ratios in herbage have been used for predictive purposes (e.g. 6, 45). However, a recent survey indicates that the risk threshold may not be a fixed ratio (e.g. 3:1) and that the tolerable ratio declines from 5:1 to

2:1 as pasture Mo concentrations increase from 2 to 10 mg Mo/kg DM (69). The Agricultural Research Council (1) went one step further by using the equations of Suttle & McLauchlan (70) for semi-purified diets to predict the outcome of the full Cu × Mo × S interaction in terms of available Cu. However, when the approach was tested under grazing conditions in Canada for prediction of hypocupremia, it was unsuccessful (7).

Since then, studies have shown that different equations are needed not only for pasture but for various types of conserved pasture that differed by several orders of magnitude in Cu availability at the same Mo and S concentrations (60, 61). The new "grass equation" has been applied to field problems in the UK, although it could not accommodate particularly high Mo or S pastures (36). Further difficulties of applying the latest equations in the field were illustrated by Jumba (27), who sought to predict available Cu concentrations for different grass species in western Kenya. Most species were sampled at the mature hay stage while a few, such as Kikuyu grass, remained lush and green. The fresh grass equation predicted 2–3 times lower available Cu concentrations than the hay equation for Kikuyu but was considered inappropriate for other grass species. The small effects of Mo and S on liver Cu in grazing Merinos in Armidale, NSW (34), compared with those expected for a temperate grazed sward (59), would probably be more appropriately predicted by the equation for hay. In two studies, one in lambs (69) and the other in cattle (7), inclusion of herbage S in prediction equations did not improve relationships with growth or plasma Cu, but the failure may have been due to the absence of pastures low in S. Boila et al (7) also pointed out that in some regions the drinking water was a significant source of S.

Predicting the outcome of Cu × Mo (± S) interactions in the above manner makes two important assumptions: First, that factors such as Fe can be ignored, and secondly that the impairment of health is due solely to induced Cu deficiency. In the case of Fe the assumption is necessary because only limited quantitative information on the effects of Fe on Cu absorption has been published (64, 71) and none relates to Mo-supplemented diets in which the effect of Fe may be diminished (9, 24). This assumption may only lead to error during periods of limited pasture supply and high soil ingestion when Fe intakes are maximal. The second assumption may be more serious. If the TM toxicity hypothesis is valid in some circumstances, prediction of health risks will have to be based on estimates of Mo excess or capacity for TM formation. The need for alternative models for prediction will be best demonstrated by any failure of the appropriate available Cu model (i.e. forage equation) to predict risks of ill health.

The greater sensitivity of cattle than sheep to the Cu × Mo antagonism has long been recognized, but recent work has revealed further differences between species of grazing ruminants that imply a need for yet more specific

equations for predicting risks of ill health. Mason et al (43) have shown that the conversion of Mo to TM_4 in the rumen is less marked in deer than in sheep or cattle. Others have suggested that addition of Mo to silage depleted liver Cu more in sheep than in deer (16), but interpretation was complicated by species differences in initial Cu stores. Zervas et al (81) have shown that goats accumulate far less Cu in their livers than do sheep on the same low Mo diet. It would be surprising if the Cu × Mo × S antagonism did not also vary between the two species. Species differences may reflect differences in the extent of enterohepatic recycling of Cu as well as differences in rumen S metabolism.

Analysis of Blood, Tissue, and Excreta

The limitations of blood and/or liver Cu concentration as the sole arbiter of Cu responsiveness in grazing ruminants have long been recognized. While interpretation can be improved and additional criteria of Cu status used (60), further more drastic changes may be necessary. During the assessment of Cu responsiveness in lambs on improved Scottish hill pastures, high in Mo, it was noticed that some growth responses occurred in normocupremic lambs. Furthermore, the responses waned on the pastures highest in Mo following the oral administration of CuO needles even though normocupremia was maintained (69). Whether or not these effects were related to the direct or Cu-mediated effects of TM, their existence suggests that conventional standards (i.e. normal plasma Cu is > 9 μmol per liter) do not always predict the outcome of the Cu × Mo × S interaction.

A novel approach, stemming from the study of Tangdilintin (72), might be to extract and measure the CuTM complexes from feces to give an integrated measure of TM formation on a particular diet. Measures of TMs in the circulation may only be needed at extremely high Mo intakes, and then it may prove necessary to distinguish "reactive" from "nonreactive" Cu in the bloodstream. Ceruloplasmin concentration, or rather its ferroxidase activity, has long been advocated as an alternative to plasma Cu concentration for assessing Cu status in grazing animals; however, ceruloplasmin is an acute phase protein and is greatly influenced by infection and even vaccination (Irene Wadsworth, personal communication). If analytical difficulties can be overcome, assay of SOD activity may be more stable and representative of functional Cu status (69).

The need for alternative measures of blood Cu status may vary between species. For example, TM formation is less likely to proceed beyond the TM_3 stage in deer (43), and systemic effects may be more important in this species than in those where the reaction proceeds readily to the TM_4 (i.e. nonabsorbable) stage. When dietary Mo intakes were increased, TCA-insoluble Cu increased more markedly in deer than in sheep on a high S diet (46).

TREATING MOLYBDENUM-INDUCED DISORDERS

The approach to and successful treatment of Mo-induced disorders in grazing ruminants will be influenced by the mechanisms for induction of the disorder and the sites affected. Chronically Cu-depleted animals with characteristic hair, fleece, and bone abnormalities and impaired growth should respond equally well to dietary Cu supplements, oral Cu boluses, and Cu injections. If TMs have localized effects on the gut mucosa, these may be countered more effectively by a continuous supply of Cu in the digesta than by a pulse of Cu from an injection that is then slowly dispensed from the liver. If TMs have widespread sytemic (and toxic) effects on reproduction, it is questionable whether either oral or parenteral Cu would be capable of fully and instantaneously overcoming them.

The whole strategy of treating and preventing Mo-induced disorders by Cu supplementation is therefore called into question by the TM toxicity hypothesis, and—if it is proven—radical alternatives such as lowering Mo intakes may have to be considered. Besides the obvious tactic of keeping soil pH as low as possible to restrict Mo uptake by pasture, more use might be made of sulphate as fertilizer and antagonist of Mo uptake by the plant. Cereals take up Mo less avidly than grasses, and analyses of maize silage grown on the infamous "teart" pastures of Somerset show remarkably low Mo concentrations (Alan Adamson, personal communication). Thus by matching cropping policy to soil conditions and making more use of grazed or conserved cereals, the harmful effects of Mo-rich soil types might be lessened.

TREATING DISORDERS OF COPPER EXCESS

The Cu × Mo × S interaction has been successfully exploited in the treatment and prevention of Cu poisoning in sheep. In addition to the remarkable rates of Cu depletion achieved by parenteral injections of TM_4 (19, 23) already referred to, the dietary antagonism has been used to prevent excessive Cu accumulation in the liver. Effective concentrations of Mo are far lower than those investigated in Scandinavia (74) because the protective effect soon reaches a plateau with increasing concentration (57). This is fortunate because Mo is still widely regarded as foe rather than friend and, in Europe, additions to the diet are restricted by law to 2.5 mg Mo/kg DM. Even at this low level, risk of toxicity is reduced (57) and many feed compounders employ Mo routinely as a Cu antagonist. The more that becomes known about the systemic and toxic effects of TMs, the wiser that policy might prove. It is important that minimal use is made of parenteral TMs or dietary Mo in combatting Cu toxicity, because overuse of Mo by either route may have prolonged adverse effects on fertility.

CONCLUSIONS

Considerable progress has been made in the last decade in unravelling the complexities of the Cu × Mo × S interaction in ruminants. The principal mechanism by which the interaction depletes the grazing animal of Cu is the formation of unabsorbable complexes with TM_3 and TM_4 in the rumen and their irreversible binding to the solid phase of the digesta. When Mo is present in excess of Cu in S-rich diets, sufficient TM_3 may be absorbed to inhibit cupro-enzyme activity in the gut and peripherally. TM_3 may also exert toxic effects on estrogen metabolism, resulting in impaired fertility, and on activity for xanthine oxidoreductase, causing lameness; such effects might respond to Cu as an antidote. Resolution of the relative importance of gut and systemic effects of the Cu × Mo × S interaction and of Cu- and Mo-mediated effects will determine the success of monitoring, treating, and preventing the widespread adverse effects that the Cu × Mo × S interaction currently has on the health of grazing livestock throughout the world.

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