THE UNIVERSITY OF MANITOBA

INVESTIGATIONS OF THE SEVERITY, EXTENT, TYPE AND TREATMENT OF COPPER DEFICIENCY IN BEEF CATTLE IN NORTHWESTERN MANITOBA

BY

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Drysdale, Robert, Andrew, M.Sc., The University of Manitoba, May, 1979. Investigations of the Severity, Extent, Type and Treatment of Copper Deficiency in Beef Cattle in Northwestern Manitoba. Major Prof; Thomas J. Devlin.

Blood serum, forage and soil samples were collected from beef cattle of forty-four herds in the North West agricultural region of Manitoba. Copper deficiency was identified in all herds and related to low copper and/or excessive molybdenum in the pasture forages. The forages also indicated a potential zinc and manganese deficiency in cattle at these locations. The low concentrations of copper, zinc and manganese in the forages were attributed to low content of copper and zinc and to low availability of copper, zinc and manganese in the soil. Parenteral administration of copper glycinate at the start of the pasture season was insufficient to affect weight gains or to maintain normal serum copper values during the grazing period.

A soil and forage survey was undertaken in the agriculturally productive area of the North West region. Differences in nutrient concentrations were identified among forage types. The forage analyses confirmed potential primary deficiencies of copper, zinc and manganese in relation to cattle production. Elevated molybdenum levels were identified in legumes throughout the region and in grasses confined to the soils derived from the shale bearing Ashville, Favel, Vermillion River and Riding Mountain Geologic Formations.

Supplementation of copper, zinc and manganese is indicated for cattle in northwestern Manitoba and other areas of the province with similar geologic factors.

I. INTRODUCTION

Prior to 1910, occurrences of an ailment of grazing cattle were reported in the Thunder Hill district of northwestern Manitoba. The symptoms were similar to those attributed to molybdenosis, or secondary copper deficiency reported by Ferguson et al (87) in England, Barshad (16), Britton and Gross (30) in the United States, and Alcroft (2) in New Zealand. In 1950, Cunningham (53,54) confirmed the ailment reported in Manitoba to be molybdenosis, responsive to copper therapy. His findings were substantiated by Smith (182) and Findlay (89).

The severity of copper deficiency in the affected areas of this region of Manitoba increased during wet pasture seasons. The summer of 1973 was typical of this phenomenon, but the incidence of copper deficiency appeared to have spread to new areas within the region. This was attributed to increased cognizance of the disorder. A survey by rural veterinary practitioners and staff members of the Manitoba Department of Agriculture located copper deficient cattle in many areas within the region. In addition, results from a preliminary geochemical survey of the region, by the University of British Columbia became available. Results from this and subsequent studies (76) indicated that conditions of potential copper deficiency of cattle existed throughout a much larger area than was previously anticipated.

In the spring of 1974, staff members of the Manitoba Department of Agriculture and the University of British Columbia met with rural veterinary practitioners from the North West Region. Funding became available through the Manitoba Department of Agriculture Animal Industry

Branch, Veterinary Services Branch and North West Regional Division, the Manitoba Youth Secretariat and the University of British Columbia, Geological Sciences Department. In May of 1974, an investigation of the severity, extent and treatment of copper deficiency of cattle in the region was initiated. Subsequent investigations funded by the Manitoba Department of Agriculture North West Regional Division and Veterinary Services Branch, and the Manitoba Youth Secretariat were instituted during 1975, 1976, 1977 and 1978. The results of these studies, in part, are documented in this dissertation.

II. LITERATURE REVIEW

1. Definitions of the Disease

Primary or simple copper deficiency is caused by uncomplicated deficiency of copper in the diet. Indicators in cattle (57) are anemia, unthriftyness, poor growth, lameness and reduced reproductive and milk producing capacities. The hair coat is rough and staring and may show loss of coloration. Calf growth is impaired with increased incidence of bone abnormalities and fracture. Ataxia, seen as a loss of muscular control, has been observed after exercise.

Secondary copper deficiency is caused by the involvement of molybdenum and/or sulfate causing reduced availability or retention of dietary copper. The only objective sign distinguishing simple and complicated copper deficiency in cattle is an acute seasonal scouring condition (57). The animals scour profusely while on lush spring and fall pasture and may become extremely debilitated. During midsummer, when pasture growth is less rapid, scouring ceases and body condition is regained to some extent.

In sheep, copper deficiency is also observed as "stringy" or "steely" wool as described in Australia (7). The effects of copper deficiency of the ewe are usually seen in the lamb, which may be affected by osteoporosis or ataxia associated with regional myelin degeneration or hypomyelination. Osteoporosis seems to occur in cases less depleted than those involving ataxia. Hypomyelinating ataxia occurs as enzootic neonatal ataxia (21, 116) or as delayed swayback (57)

which develops between 3 weeks to 4 months of age. The signs of copper deficiency of sheep are usually more severe in cases of secondary deficiency.

2. Geographic Occurence of the Disease

The first citing of naturally occurring copper deficiency was that of "salt sick" cattle of Florida by Neal et al (154) in 1931. The condition was identified in Holland in 1933 by Sjollema (177) and in Western Australia by Bennetts and Chapman (22) in 1937. In 1938, Ferguson, Lewis and Watson (86) identified the "teartness" of pastures in Sommerset, England, to be due to their molybdenum content and thus initiated copper therapy as a cure for molybdenosis (88).

Other investigators have identified the incidence of bovine copper deficiency in New Zealand (57), Sweden (100), Ireland (155), Wales (63), France (127); in parts of California (16, 30), Oregon (160), Nevada (79), and Hawaii (195) in the United States, and in the province of Ankavan, Armenia in the United Soviet Socialist Republics (124).

In Canada, primary and secondary copper deficiencies have been found in Manitoba (182, 89, 54, 76), Ontario (28), British Columbia (147) and very recently in eastern Saskatchewan (186, 31). Conditions similar to those in Manitoba have been found in North Dakota (41). Areas of South Dakota and Minnesota are experiencing a deficiency situation but the causal conditions are only now under investigation (132, 161).

3. The Essentiality and Requirement of Molybdenum

Molybdenum has been found to be a constituent of the metaloflavoprotein enzymes xanthine oxidase (214, 68), aldehyde oxidase (123), and sulfite oxidase (49). The first two of these enzymes are involved in the electron transport chain by interacting with cytochrome c (133, 93) as the electron acceptor. Molybdenum is present at the substrate binding site of these enzymes (164). Xanthine oxidase is required for the oxidation of the purines xanthine and hypoxanthine to uric acid in the rat and chick (105, 129). Aldehyde oxidase catalyses the oxidation of aldehyde and various nitrogen containing heterocyclic aromatic compounds (123) and hypoxanthine (164). Sulfite oxidase is involved in the oxidation of sulfite to sulfate in the mammalian metabolism of sulfur amino acids and sulfur-containing compounds.

The essentiality of dietary molybdenum has been demonstrated only in the chick and rat (105, 48, 120). No required dietary level has been established for these species due to the use of tungsten to establish a deficiency state. The requirement of molybdenum by sheep has been studied (81, 175). Sheriha et al, 1962 (175) concluded that if required, the dietary requirement was less than 0.01 ppm for sheep. A dietary requirement for molybdenum has not been established for ruminants but is required for the healthy growth of pasture legume forages.

4. The Essentiality and Requirement for Copper

Copper has been known as an essential element since the works of Neal et al (154) in 1931 and Sjollema (177) in 1933. Copper is a constituent of the enzymes tyrosinase, ascorbic acid oxidase, phenol

oxidase, uricase and cytochrome oxidase. The functional changes of these enzymes under deficiency situations have been reviewed by Adelstein and Vallee (1). Research on the requirements and metabolism of copper has been reviewed by Underwood (202).

Values for the requirement of available copper for the ruminant animal are difficult to assign due to interfering dietary factors. The basic requirement of copper for cattle is accepted at 10 parts per million (ppm) in the dry ration (145, 188, 57, 45, 210) when dietary molybdenum and sulfur are less than 1.0 ppm and 0.10% respectively. Whenever molybdenum levels exceed 1 ppm, the absolute levels of copper and molybdenum, and the copper to molybdenum ratio, must be considered. In such cases, a dietary ratio of 4 parts copper to 1 part molybdenum is desirable (210). Thus the requirement of cattle for copper is accepted as follows in Manitoba:

TABLE 1. Required level of copper in relation to molybdenum in the diet.

Dietary level of Mo, ppm D.W.	Required level of Cu, ppm D.W.
0-1	10
1-3	12
4	16
5(a)	20

(a) for levels of dietary molybdenum greater than 5 ppm, refer to the literature review on supplementation of copper.

For sheep, the dietary requirement for copper is accepted as 4 to 6 ppm for British breeds and 6 ppm for Merino sheep (202). Under moderate intakes of calcium carbonate, molybdenum, or sulfur, the dietary

requirement is increased as high as 10 milligrams (mg) per day or 10 ppm in the diet.

In summary, the dietary requirement of cattle and sheep for copper, under Manitoba conditions, may only be adequately estimated when both copper and molybdenum analyses are available. This is in agreement with Miltimore and Mason (146).

5. Body Levels of Molybdenum and Copper

a). Molybdenum

Molybdenum levels in the body are higher in the liver and kidneys than in other organs (196, 105) but accumulation in the liver is not excessively high (202). Levels do not change appreciably with age, and species differences are small (204). Molybdenum levels in the hair (59), bones and liver (66) are influenced by dietary molybdenum levels. Normal liver molybdenum levels of 2 to 4 ppm may increase to 25 to 30 ppm in cattle and sheep on high molybdenum rations (203). Tissue molybdenum levels are decreased by dietary inorganic sulfate and sulfur-containing amino acids (60, 61, 142). Tungsten may also decrease tissue molybdenum levels (9, 64, 65).

Normal values for whole blood molybdenum for cattle and sheep grazing pastures normal in copper and low in molybdenum are 2 to 6 Ug/dL (57, 72) or 0.02 to 0.06 Ug/ml. This level rose to 60 to 80 Ug/dL in young cattle and to 240 to 340 Ug/dL ml in ewes when fed a ration of 30 ppm molybdenum. Dick (7) noted that over 70% of the blood molybdenum was present in the erythrocytes under normal conditions. Increases were due to increases of the plasma fraction. Scaife (171)

showed that molybdenum from both fractions of the blood was a readily dialyzeable anion, probably molybdate.

The molybdenum content of milk is normally 10 Ug/l or 0.01 ppm (108). The content of cows milk has been raised from 73 Ug/l to 371 Ug/l by adding 500 milligrams molybdenum daily to the diet (9). Ewes grazing pastures of 13 ppm molybdenum showed a value of 980 Ug/l molybdenum in their milk (110). These researchers found a reduction from 1,043 Ug/l to 137 Ug/l molybdenum after 3 days, in ewes grazing pastures of 25 ppm molybdenum when dosed with 23 grams of sulfate per day. The molybdenum in milk of cows grazing normal pastures is bound to xanthine oxidase (102) and is proportional to the xanthine oxidase activity. Rapid increases in molybdenum values of milk do not show a corresponding increase in xanthine oxidase activity (101).

b). Copper

The distribution of copper in tissues varies with species, age, and copper intake. Under normal conditions, copper concentrations tend to be correspondingly higher in the liver and brain than in other tissues (40, 150). In ruminants, the capacity for storage of hepatic copper can be very high (55), and closely reflects the dietary copper levels, with and without supplementation (83).

The generally accepted values for liver copper concentrations of cattle and sheep are given in TABLE 2. Under Manitoba conditions, the deficient liver copper level was defined as 40 ppm dry weight for adult cattle, in agreement with Claypool et al, 1975 (47). The deficiency level for newborn calves was defined as 55 ppm dry weight as set by Cunningham (56).

TABLE 2. Liver copper level in relation to dietary copper status (56).

Species	Age & Cu Treatment	No. of Animals	Cu ppm dry weight Average	Range
Sheep	newborn, normal diet	27	168	74-430
	newborn, deficient diet	29	14	4- 34
	mature, normal diet	44	599	186-1374
	mature, deficient diet	35	27	7-106
Cattle	newborn, normal diet	41	381	143-655
	newborn, deficient diet	20	55	8-109
	mature, normal diet	23	200	23-409
	mature, deficient diet	41	11.5	3- 32

Copper values in the hair range from 10 to 47 ppm. There appears to be no relationship between species or color to copper content (96, 8). Van Koetsveld (207) reported an average value of 10 ppm copper with values below 8 ppm having been associated with deficiency symptoms. O'Mary et al (159) found that hair of Hereford cattle ranged from 10 to 31 ppm copper and that differences were not attributable to color but to season and time of sampling. In Manitoba, the normal value of copper in the hair of cattle and wool of sheep has been accepted as 7 to 10 ppm.

Copper in the blood of cattle is present in three basic forms. Firstly, erythrocyte hemocuprein is a blue low-molecular weight (35,000) protein with 2 atoms of copper per molecule (0.34% Cu) in the cupric form (135). The plasma copper fractions are the ceruloplasmin and "direct reading copper" protein-bound forms. Ceruloplasmin is an $\alpha 2$-globulin of molecular weight of 151,000 with 8 atoms of copper per molecule (111). It is an oxidase enzyme catalyzing the oxidation of various polyphenols (111) and biological compounds (139, 97). The amount of ceruloplasmin copper exchanged per day is relatively small

compared to intake. In mammalian species ceruloplasmin copper represents approximately 80% of the plasma copper while representing only a negligible portion in avian species (185, 217). Highly significant correlations between plasma, serum and whole blood copper levels and ceruloplasmin activity have been drawn (151, 200). Direct reading copper is named for its direct reaction with dithizone. It is nondialyziable and loosely bound to a serum protein, probably albumin (35, 217). The balance of the plasma copper fraction is comprised of the copper containing enzymes: trosinase, laccase, ascorbic acid oxidase, cytochrome oxidase, monoamine oxidase, δ-aminolevulinic acid dehydrase and dopamine-β-hydroxylase (90).

Generally, the normal range for serum copper has been accepted as 0.8 to 1.2 Ug/ml (181, 20). Values below 0.6 Ug/ml have indicated a deficiency for cattle and sheep (202, 63) with values below 0.5 Ug/ml demonstrating severely deficient liver copper levels in cattle (47).

The blood copper levels of sheep are greatly influenced by pregnancy, parturition and disease. Blood copper parameters have been shown to decline during pregnancy in housed (36) and grazing (4, 37) ewes and increase to premating levels approximately one month post partum. Howell et al (112) found that blood copper values rose to very high levels approximately one week post partum and then fell to normal. They found the blood copper values of lambs to be low at birth with an increase to normal adult levels within one week. This finding was confirmed by McDougall (153). In the case of calves, Bingley and Dufty (24) reported whole blood and plasma copper levels of newborn calves to be significantly lower and erythrocyte copper levels to be significantly

higher than their mothers.

Variations in blood copper parameters have arisen with the incidence of disease (152, 19, 168) in cattle and sheep. Infestation with internal parasites have been shown to cause depressed copper values (152, 29). Low blood copper values had been attributed to (162) but were later found to be coincidental with Border disease in sheep (14).

The normal copper level in milk is thought to be approximately 0.6 Ug/ml (52) for cattle. For sheep, values of 0.2 to 0.6 Ug/ml fall to 0.04 to 0.16 Ug/ml several months post partum (18). For both species copper values of colostral milk are substantially higher than in later milk (202). Cattle grazing copper deficient pastures have shown milk copper levels of 0.01 to 0.02 Ug/ml (18). Very little response has been shown in milk copper levels to supplementation of ewes (191), cows and goats (82) on already adequate diets. However, Dunkley et al (78) showed a four week elevation of milk copper levels in cows parenterally injected with 300 milligrams copper as glycinate. There was no mention of dietary copper levels in this study.

6. The Diagnostic Reliability of Copper Parameters in Serum

The parameter of total serum copper may not be adequate for the diagnosis and treatment evaluation of copper deficient animals in terms of potentially available copper in the serum. Investigators with sheep (74, 75) and cattle (46) have hypothesized the presence of a copper-molybdenum complex at the serum level similar to that found at the rumen level. Dowdy and Matrone (74, 75) observed that this copper-molybdenum complex had a Cu:Mo ratio of 4:3 and could exist in vivo.

The copper in this complex was biologically unavailable to pigs and sheep. Marcilese et al (136) compared the effects of dietary sulfate alone with molybdenum plus sulfate in the diets of sheep. They found that when molybdenum plus sulfate was added to the diet, the uptake of injected radiocopper by the liver was reduced and that ceruloplasmin synthesis was impaired. This postulated a copper-molybdenum-sulfate complex at the serum level. Recent electron paramagnetic resonance studies by Huisingh and Matrone (114) have shown, however, that the serum Cu^{+2} cation becomes bound to serum proteins and the molybdate remains as a free anion in proximity to that protein. They have proposed that dietary molybdate and sulfate together affect copper transport into and out of the tissues, particularly the liver, by mobilizing copper stores. This increases the total serum copper level. They have also proposed that molybdate plus sulfate inhibits synthesis of copper storage complexes and ceruloplasmin (136). This means that this mobilized copper, shown as an increase in total serum copper level, may be only slightly available to the animal.

In summary, under conditions of molybdate plus sulfate inter-action on copper metabolism, there is merit to the use of the total serum copper plus ceruloplasmin activity criteria in diagnosing copper deficiency. This may explain why deficiency symptoms persist in a herd when the total serum copper levels are apparently adequate, especially when sulfate and molybdate analyses are unavailable. Unfortunately, rapid post-sampling oxidation of ceruloplasmin makes this serum parameter unreliable under field conditions, unless special precautions are taken.

7. Metabolism of Molybdenum and Copper and the Effects of Interrelated Minerals

a). Molybdenum

Under normal conditions, molybdenum is well absorbed from the diet. The hexavalent water soluble forms of sodium and ammonium molybdate, and the water soluble molybdenum of fresh herbage are well absorbed by cattle (88). When fed in large amounts, insoluble compounds such as molybdenum trioxide and calcium molybdate, but not molybdenum disulfide, are well absorbed (84). The active site and mechanism of molybdenum absorption are unknown (204).

On low dietary sulfate, the major route of molybdenum excretion is via the urine in monogastrics (176, 156, 169) but not in cattle (208) or sheep (72, 171). By increasing the dietary level of sulfate from 0.1% to 0.3% in sheep, the amount of molybdenum excreted via the urine increased from 3.0 to 4.6% to 50 to 54% of intake. This effect of sulfate on molybdenum metabolism is very specific in sheep and has been shown to be as effective from endogenous as from dietary sources of sulfate (72, 171, 51).

Dick (72) explains the influence of sulfate on molybdenum absorption and excretion by the interference of inorganic sulfate with, and when concentrations are high enough, the prevention of molybdenum transport across membranes. This is hypothesized to increase molybdenum excretion through the rise in the sulfate concentration of the ultra filtrate of the renal glomerulus following high sulfate intakes. This in turn impedes or blocks reabsorption of molybdenum through the renal tubule. The mechanism of this postulated interference may be due to the similarities between the molybdate and sulfate anions (141).

Very high molybdenum intakes have been reported to give rise to increased phosphorus excretion with accompanying lameness, abnormal joints, osteoporosis and high serum phosphatase levels (66). This may lead to increased incidence of calving difficulties and reduced or absent libido in young bulls. Thomas and Moss (192) have demonstrated damage of the interstitial cells and germinal epithelium of the testes of such animals.

b). Copper

The site of copper absorption has not been demonstrated but is thought to be in the small intestine (45). Ingested copper is rather poorly absorbed in most species (27, 50) and very little is known about the mechanism of copper absorption. A copper-binding protein has been demonstrated in the duodenal mucosal cells of the chick (184) but not in other species.

Absorbed copper is stored in liver and may reach very high levels in ruminants. Copper is excreted via the urine and actively from the liver via the bile (202). The absorption and retention of copper by cattle and sheep is greatly influenced by interrelated dietary factors.

The absorption of dietary copper is reduced under conditions of high dietary intakes of calcium carbonate and sulfide (71). The calcium carbonate reduces the absorption of copper by raising the intestinal pH (71, 134). Dietary sulfur above 0.1% has been found to increase the formation of an insoluble copper sulfide complex in the rumen and intestines (188). The latter finding has also been confirmed in swine (27).

The copper of fresh herbage has been shown to be less available than the equivalent dry herbage or hay (103). Mills (143) has shown the

greater part of the copper component in fresh herbage to be in the form of as neutral or negatively charged complexes. Mills has postulated that copper may be transported through the intestinal mucosa both as an ion and in the form of complexes as found in herbage. This postulation is supported by Kirchgessner et al (122) who have shown that the affinity of copper ions for inorganic and organic ligands in food can reduce the rate of absorption, depending upon the size and stability of the resultant complexes.

Several organic and inorganic factors have been shown to decrease the utilization of dietary copper. Davis et al (67) have demonstrated a reduction in the assimilation of copper by phytate. Van Campen and Gross (206) showed that high dietary levels of ascorbic acid significantly reduced the retention of copper by depressing intestinal absorption, rather than by increasing excretion. Zinc and cadmium both decrease the utilization of copper as summarized by Mills (144) and Starcher (184). Zinc, cadmium, copper and mercury are mutually antagonistic elements which are competitively absorbed at binding sites on a protein, believed to be of the metallothionein type located in the liver, kidney and duodenal mucosa (144). Thus increases in dietary levels of zinc, cadmium and mercury may directly influence the absorption and excretion of copper. Iron and manganese have been shown to be competitively absorbed with copper in the intestinal mucosa, thus reducing its availability to the animal (201).

The two dietary factors which most influence the absorption and utilization of dietary copper are molybdenum and sulfur. Sulfur reduces the absorption of copper by formation of insoluble copper sulfide (188),

as previously discussed. Molybdenum interferes with both the absorption of copper and its retention in the liver (202). Matrone (140) has suggested that molybdenum reduces copper absorption by the formation of a copper-molybdenum complex referred to as cupric molybdate ($CuMoO_4$). Cunningham (57) and Dick (71) have shown that the deleterious effects of molybdenum and sulfate upon copper utilization are increased when both are administered together. This has suggested the formation of a cupric thiomolybdate complex. Molybdenum, sulfur and molybdenum plus sulfur all decrease the utilization of copper by the animal. The present hypotheses concerning the interactions of these nutrients are complex and are discussed in section 8 below.

8. The Copper-Molybdenum-Sulfate Interaction and its Influence on Absorption, Metabolism and Excretion of Copper in Ruminants

Dowdy and Matrone, 1968, studied the copper-molybdenum-sulfate reaction in ruminants (74, 75). They observed the formation of an insoluble precipitate at neutral pH. Dowdy et al (73) later showed this precipitate to be lindgenite, $2CuMoO_4 \cdot Cu(OH)_2$, which previous workers had referred to as cupric molybdate or the Cu-Mo complex. Dowdy and Matrone (74, 75) were unable to explain the role of sulfate in the copper-molybdenum interaction. They found that the resultant complex was absorbed and transported by the body but was unavailable for ceruloplasmin synthesis. Huisingh and Matrone (114) have postulated models of the action of sulfur in the Cu-Mo interaction.

One model has suggested the unavailability of copper at the rumen level to be due to (a) the formation of cupric molybdate and (b) the precipitation of cupric sulfide (113). As a result of in vitro studies

(94) which showed an inhibition of the sulfate to sulfide reaction by molybdenum in rumen cells, Huisingh and Matrone proposed that molybdenum could alleviate high dietary sulfate induced copper deficiency by blocking the formation of cupric sulfide from copper plus sulfate. Using a dietary level of 50 ppm molybdenum and either sodium sulfate or methionine as a sulfur source, they showed that molybdenum inhibited sulfide formation from sulfate but increased the production of sulfide from methionine.

A more current model of the Cu-Mo-S interrelationship by Huising and Matrone (141) is based on in vivo intestinal loop studies. Molybdenum and sulfate have been observed to interact at (a) the site of enzyme activity, such as molybdate inhibition of sulfate reduction by inhibiting the enzyme ATP-sulfurylase, and at (b) the site of membrane transport, such as intestinal absorption and renal tubule reabsorption, as postulated by Dick (69, 72). Due to the high degree of similarity of the molybdate (MoO_4^{-2}) and sulfate (SO_4^{-2}) oxy-anion (both have $2\pi d$ bonds and sp^3 configurations) chemical parameters, Huising and Matrone have hypothesized:

a) both molybdenum and sulfate are transported across membranes by a carrier.

b) both anions use the same carrier.

c) sulfate can replace molybdate on the carrier.

d) when sulfate replaces molybdate on the carrier at the site of reabsorption in the renal tubule, then molybdate excretion via the urine is increased.

Using the intestinal loop technique, they found that not only sulfate but other Group VI oxy-anions, such as selenate and chromate interfered with molybdate absorption, but Group V phosphate did not. The proposal of a competitive absorption-transport mechanism is supported by the findings of Cardon and Mason (39). This interaction of similar Group VI oxy-anions may offer an explanation to the study by Sheriff and Rankin (174) of a precipitant selenium deficiency, believed to be due to high dietary sulfate.

A brief summary of the effects of dietary molybdenum and sulfate on body copper levels, as found by Huising and Matrone (114), is given in Figure 1. When sheep are fed a copper deficient diet, molybdate or sulfate alone always decreases the plasma, liver and kidney copper level and serum ceruloplasmin activity. When both molybdate and sulfate are added to a copper deficient diet, plasma copper is increased without a corresponding increase in ceruloplasmin activity. There is mobilization and subsequent depletion of body copper stores with an increased urinary excretion of copper (137). In respect to liver copper status, if the animals are receiving adequate dietary copper, then molybdate plus sulfate appears to decrease the level of copper in the liver, but at a reduced rate, leading to a higher retention of liver copper than in comparably deficient animals (114). This supports the hypothesis that molybdate plus sulfate impairs the transport of copper into or out of the liver by impairing the membrane transport system.