RELATIVE BIOAVAILABILITY OF COPPER AND MOLYBDENUM SOURCES AND EFFECT OF MOLYBDENUM ON MOLYBDENUM AND COPPER EXCRETION AND TISSUE ACCUMULATION IN LAMBS

By

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TO MARLENE, JUNIOR, MANO AND ALINE

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

RELATIVE BIOAVAILABILITY OF COPPER AND MOLYBDENUM SOURCES AND EFFECT OF MOLYBDENUM ON MOLYBDENUM AND COPPER EXCRETION AND TISSUE ACCUMULATION IN LAMBS

By

Edison Beno Pott

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Chairperson: Dr. Clarence B. Ammerman
Major Department: Animal Science

Five experiments were conducted, including three to estimate relative bioavailability value (RBV, %) of Cu in Cu sources, one to determine effects of feeding duration and added dietary concentrations (ADC) of Mo on daily Mo (MOEX) and Cu excretion (CUEX) and tissue uptake, and one to estimate RBV of Mo in inorganic sources, for lambs fed a corn-soybean meal-cottonseed hulls basal diet (DIET). Four to six lambs were assigned randomly to DIET or DIET supplemented for 10 days with 60, 120 or 180 ppm Cu as cupric chloride or 120 ppm Cu from cupric carbonate, cupric oxide or cupric sulfate (Exp. 1); or 60, 120 or 180 ppm Cu as chloride or 120 ppm Cu from cupric acetate, oxide or sulfate (Exp. 2); or 60, 120 or 180 ppm Cu as Cu-lysine or sulfate (Exp. 3); or with 0, 15, 30 or 45 ppm Mo from sodium molybdate (SODIUM) for 14 or 28 days (Exp. 4); or with 15,

30 or 45 ppm Mo as SODIUM or 30 ppm Mo from ammonium molybdate, molybdenum trioxide or Mo metal for 28 days. Ratios of slopes of logarithmic transformed (RATIO) hepatic Cu concentrations regressed on Cu ADC and ADC*source resulted in RBV of 100 (chloride), 47 (oxide), 123 (carbonate) and 157 (sulfate), Exp. 1; 100 (chloride), 64 (oxide), 105 (acetate) and 115 (sulfate), Exp. 2; 100 (sulfate) and 68 (Cu-lysine), Exp. 3. The RBV of acetate, carbonate, chloride and sulfate were similar and these were greater than that of oxide. The RBV of Cu-lysine was less than that for sulfate. Log MOEX or CUEX or Mo (MOCONC) or Cu concentrations (CUCONC) regressed on ADC of Mo, length of feeding time and level*time indicated linear or near-linear increases in fecal and urinary MOEX and MOCONC in kidney, muscle and serum. The ADC of Mo decreased hepatic, muscular and biliary CUCONC and increased renal and serum CUCONC. Length of time had no effect on MOEX or tissue MOCONC but affected hepatic and serum CUCONC. RATIO of MOEX or tissue MOCONC regressed on ADC of Mo and ADC*source resulted in RBV from 88 to 131 for ammonium, -15 to 85 for metal and 89 to 125 for trioxide, relative to SODIUM. The best RBV estimate seemed to be provided by MOCONC in serum: 100 (SODIUM), -15 (metal), 121 (ammonium) and 123 (trioxide).

CHAPTER 1
INTRODUCTION

Copper and molybdenum are two important minerals in animal nutrition, especially in ruminants. The importance of Cu arises from both deficiency and toxicity conditions, which may be influenced by Mo, in association with S. Molybdenum's importance stems mainly from its effect on Cu, causing either Cu deficiency or toxicity, depending on its own and on S dietary levels. Copper deficiencies in grazing ruminants have been observed worldwide (Underwood, 1981; McDowell, 1985). Concurrently, Mo-induced Cu deficiencies in cattle may be caused by environmental contamination with Mo by Mo processing factories (Hornick et al., 1977; Parada, 1981), mining activities (Vlek and Lindsay, 1977), motor oil spills (Sas, 1987), Mo originating from oil refineries (Gardner and Hall-Patch, 1962), or pelleted feed containing high concentrations of Mo (Lloyd et al., 1976). Molybdenum-induced hypocuprosis under natural conditions on pasture has been reported for sheep (Ferguson et al., 1943; Hogan et al., 1971b; Alloway, 1973) and cattle (Cunningham, 1946; Cunningham et al., 1953; Becker et al., 1965).

Copper compounds have been used worldwide as dietary supplements to overcome deficiencies caused by naturally low

Cu concentrations in feeds or forages or deficiencies induced by elevated dietary Mo levels.

Copper poisoning occurs mainly in sheep, especially in housed and intensively reared lambs fed concentrate feed (Ross, 1966, 1970; Pope, 1971; Martin et al., 1988). Exposure of sheep to feed intended for swine (Stahr et al., 1989) or feeding broiler litter to sheep worsen the problem.

Enormous economic losses occur as a result of death caused by Cu toxicosis. Molybdenum or Mo in association with S have been used to cure Cu toxicosis after it occurred. However, a better approach would be to prevent Cu toxicosis rather than treat the condition.

Gooneratne et al. (1989c,d) observed that tetrathiomolybdate administered intravenously was much less effective in removing long-term hepatic Cu storages of lambs than short-term storage, which suggests that prevention of Cu accumulation in liver is probably more efficient than its treatment. Harker (1976), Suttle (1977) and Martin et al. (1988) recommended regular addition of Mo to sheep diets where elevated Cu levels could potentially lead to Cu toxicosis and van Ryssen et al. (1986) suggested its use for limited periods of time. Until recently, addition of Mo in animal feeds was not allowed in the USA. In 1987, the Association of American Feed Control Officials (AAFCO) proposed a definition for sodium molybdate (Association of American Feed Control Officials, 1991), but apparently it

has not yet been officially accepted by the Food and Drug Administration as a GRAS (Generally Recognized as Safe) compound. According to A. R. Hanks (Purdue University, East Lafayette, IN; personal communication) sodium molybdate seems to be a case where the FDA has accepted the mineral compound by not objecting to the AAFCO definition.

Ammonium molybdate, followed by sodium molybdate, were the most frequently used Mo-containing compounds in studies involving the effect of Mo dietary supplementation on Cu metabolism or under practical conditions to cure or alleviate Cu toxicosis.

Knowledge of the bioavailability of minerals in inorganic sources is an important matter in diet formulation, especially in the case of Cu and Mo for cattle and sheep, where the range of adequacy to toxicity is very short, compared to non-ruminant animals. Little attention, however, has been given to Cu bioavailability, in part because of the lack of techniques for its determination (Anonymous, 1986). No studies of Mo bioavailability from inorganic sources or comparisons of efficacy of Mo-containing compounds on Cu status in ruminants have been published.

A bioavailability assay, using tissue mineral uptake during short-term high-level elemental supplementation as the bioassay criterion, was suggested by Black et al. (1984a,b) for inorganic Mn sources. Ledoux et al. (1989b),

working with chicks, indicated that hepatic Cu uptake could be a useful bioassay criterion for estimation of Cu bioavailability from inorganic sources. The accumulation of Cu in the liver of sheep fed high dietary Cu concentrations for 10 days was linear; therefore, Ledoux (1987) suggested a 10-day bioassay to estimate relative bioavailability of Cu sources for sheep, using slope ratio comparisons.

The association of Cu to amino acids, in the form of chelates or complexes, has been claimed to increase the availability of Cu (Kirchgessner and Grassmann, 1970; Ashmead et al., 1985; Kratzer and Vohra, 1986), but there are conflicting reports regarding their advantage compared to cupric sulfate for ruminants (Miltimore et al., 1978; Kincaid, 1988a,b; Wittenberg et al., 1990) and chicks (Baker et al., 1991). Copper-amino acid chelates or complexes have been available in the market in recent years. Since their cost is greater than the commonly used inorganic sources, the inclusion of a Cu-lysine complex in the present study was thought to be appropriate.

The present study was undertaken to determine the relative biological availability of inorganic Cu compounds and a Cu-lysine complex, based on their hepatic Cu accumulation, following short-term high-level feeding to lambs; to estimate the effect of length of time and level of Mo supplementation on Mo excretion and tissue uptake and on Cu status in lambs; and to estimate the relative

bioavailability values of Mo in inorganic sources and their effect on Cu excretion and tissue concentrations in lambs.

CHAPTER 2
LITERATURE REVIEW

Copper

History and Review

Presence of Cu in plants was reported in 1817 and its presence in animal tissues was demonstrated in 1830 (van Campen, 1971). Its importance in animal nutrition, however, was only recognized about 100 years later when McHargue (1925), based on the wide distribution of Cu in plant and animal tissues, suggested a biological function for the element in metabolism, and Hart et al. (1928) showed that Cu was required, along with Fe, for synthesis of hemoglobin in rats fed a milk diet.

Interest in Cu in nutrition was markedly increased in the 1930s, when certain disorders of sheep and cattle in various parts of the world were shown to be caused by Cu deficiency. First, a report indicated that Cu deficiency was responsible for a problem in cattle in Florida, called "salt sickness" (Becker et al., 1931; Neal et al., 1931). Next, a disorder of sheep and cattle characterized by diarrhea, loss of appetite and anemia, known as "Lecksucht" in Germany and "likzucht" in Holland, was observed in areas

where Cu content in herbage was usually small and was cured with cupric sulfate (Sjollema, 1933). A disorder of lambs known as "enzootic ataxia" in western Australia (Bennetts and Chapman, 1937) and "swayback" in England (Dunlop et al., 1939) was shown to be caused by a Cu deficiency and could be prevented by supplementary Cu to ewes during pregnancy. In Australia, Bennetts and Hall (1939) suggested that "falling disease" in cattle, characterized by sudden death, was associated with Cu deficiency. These findings were followed in the 1940s and 1950s by a great number of demonstrations of the efficacy of Cu therapy in cure and prevention of problems in sheep and cattle in different areas of the world, such as New Zealand (Cunningham, 1944, 1946), Britain (Allcroft, 1946) and the Florida Everglades (Davis et al., 1946). In all Cu-deficient areas, the situation was markedly similar: 1.) Sheep and cattle failed to thrive unless supplied with extra Cu, either directly or indirectly; 2.) blood and liver of Cu-deficient animals contained low Cu levels; and 3.) forages and soils contained low levels of Cu (Scott, 1972). An associated problem, to be presented in another section, was excessive Mo.

Numerous extensive reviews and books on various aspects of Cu have been published over the last 50 years. Some of the most recent ones are biological roles of Cu (Evered and Lawrenson, 1980); biochemistry and metabolism of Cu (Hsieh and Hsu, 1980); Cu essentiality, metabolism, toxicosis and

tissue levels (NRC, 1980); Cu deficiency and toxicity in plants, animals and man (Owen, 1981); molecular properties of cuproproteins (Spiro, 1981); Cu deficiency disorders, diagnosis and control of deficiencies, requirements, toxicity and interactions with Mo and S (Underwood, 1981); biochemical aspects of Cu (Owen, 1982a); physiological aspects of Cu in organs and systems (Owen, 1982c); Cu nutritional importance, absorption, bioavailability, interactions and therapeutics (Allen and Solomons, 1984); Cu proteins and Cu enzymes (Lontie, 1984a,b,c); absorption, bioavailability, deficiencies, functions and requirements (O'Dell, 1984b); absorption, transport and metabolism (Cousins, 1985); Cu toxicity (Osweiler et al., 1985); Cu in animal tissues, metabolism, deficiency, functions and toxicity (Davis and Mertz, 1987); Cu in animals and man (Howell and Gawthorne, 1987a,b); biochemical functions of Cu (Prohaska, 1988); Cu deficiency and metabolism in ruminants (Gooneratne et al., 1989a); metabolism, functions and deficiencies (O'Dell, 1990a); and interactions between Cu, Mo and S in ruminants (Suttle, 1991).

Copper Chemistry

Copper's atomic number is 29 and its molecular weight, 63.546. In the periodic table of elements it occupies a position in group I-B, between nickel and zinc. In addition to the metallic form, Cu can exist in +1, +2 or +3 valence

states, but more commonly occupies the +2 state (Miller, 1979). In biological systems, cuprous (Cu^{+}) and cupric (Cu^{++}) forms predominate (Miller et al., 1979). Free ionic Cu is present in physiological fluids at extremely small concentrations and the majority of Cu is associated with specific ligands that are predominantly proteins, peptides and amino acids (Prohaska, 1988).

As a member of subgroup I-B metals, Cu has great affinity for S- and N-containing ligands and, as such, it is present in a number of enzymes involved in oxidation and reduction (Luckey and Venupogal, 1977).

Copper Absorption

Anatomical sites of Cu absorption in laboratory animals are stomach, duodenum, jejunum and, to a lesser extent, ileum (Bremner and Davies, 1980; Allen and Solomons, 1984). Studies with ruminants have been less consistent and it seems that the reticulo-rumen is not an important site of Cu absorption (Gooneratne et al., 1989a).

Determinations of Cu absorption in ruminants were done using a Cu repletion technique (Suttle, 1974a), Cu isotopes (Suttle, 1975a; Buckley et al., 1985; Turner et al., 1987), or ruminal and(or) intestinal cannulated animals (Grace, 1975; Bertoni et al., 1976; Stevenson and Unsworth, 1978; Golfman and Boila, 1990), but only in a few of those studies was site of absorption investigated. Metabolic processes in

the rumen are important in determining the availability of Cu, and particular areas of the gastrointestinal tract where Cu is absorbed have not been defined with certainty (Turner et al., 1987). Large intestine seems to play an important role (Grace, 1975; Bertoni et al., 1976; Golfman and Boila, 1990), but Turner et al. (1987) reported that a wide area of the gastrointestinal tract of sheep was capable of absorbing Cu. Bertoni et al. (1976) reported an appreciable net absorption of Cu in colon and cecum in dairy cows. In steers, more than 80% of Cu absorption distal to the proximal duodenum occurred in the large intestine (Golfman and Boila, 1990). In calves fed a high-concentrate diet supplemented with Cu, Mn and Zn, alone or in combination, for 10 weeks, Ivan and Grieve (1976) observed a net secretion of Cu in the abomasum but a net absorption from the rest of the gastrointestinal tract. Sheep may secrete Cu into salivary and gastric juices, because concentration of Cu was greater in duodenum than in ingested food (Grace, 1975; Bertoni et al., 1976; Stevenson and Unsworth, 1978).

Preruminant lambs and calves absorb Cu as efficiently as monogastric species and much more efficiently than mature animals (ARC, 1980). Only a small proportion of orally administered Cu was absorbed by bovine (Comar et al., 1948) and sheep (Dick, 1954b, Suttle, 1983b).

In intestinal lumen, Cu was bound to ligands, probably amino acids, before entering intestinal epithelial cells (Evans, 1977). Copper was also equally well absorbed in rats as ionic Cu, as a complex with histidine or bound to ceruloplasmin (Marceau et al., 1970).

No studies have been reported on the mechanism of Cu absorption in ruminants but it is known that Cu homeostasis in ruminants, particularly sheep, is considerably less effective than in most non-ruminants (Bremner and Davies, 1980). Mechanisms that control Cu absorption are not fully understood (McArdle et al., 1990). It is suggested that it proceeds via two distinct steps (Crampton et al., 1965; Bremner, 1980). Crampton et al. (1965), using everted sacs of hamster intestines, suggested that Cu, either as free Cu^{++} or bound to absorbable ligands, binds to sites on cell surfaces or within cells and then a specific mechanism operates for transfer of Cu from cell to blood stream. Passage across mucosal membrane and transport across cells are concentration-dependent and saturable (Crampton et al., 1965). Uptake of Cu into mucosal cells is not energy-dependent (Crampton et al., 1965). In contrast, Cu transport out of cells does seem to be energy-dependent and may limit rate of Cu transfer (Crampton et al., 1965). The point of maximal control of Cu absorption into the body is the serosal transfer into blood (Allen and Solomons, 1984). Little evidence has appeared to contradict these suggestions

and the discovery within intestinal mucosal cells of metallothionein, a metal-binding protein has led to the suggestion that it may be involved in homeostatic regulation of Cu absorption (Turner et al., 1987).

Turner et al. (1987), however, working with everted sacs of sheep jejunum, found that Cu uptake was linearly related to concentration of Cu in medium over a range of concentrations that extended to 6.4 ppm, and the authors suggested that mucosal uptake does not seem to be a saturable process. Metabolic inhibitors had no significant effect on Cu uptake and this is an indication that no important energy-dependent processes are directly involved in Cu uptake (Turner et al., 1987). Gitlin et al. (1960) stated that in addition to energy-dependent mechanisms, some Cu is probably absorbed by simple diffusion in mice, while Crampton et al. (1965), using everted sacs of hamster intestines, concluded that Cu absorption was not the result of simple diffusion. In duodenum of mice, Cu absorption involved both saturable and first order kinetics (Bronner and Yost, 1985). On the other hand, Turner et al. (1987), using isolated segments of sheep intestines, inferred that uptake of Cu from lumen to cells was a process which was neither saturable nor energy-dependent but whose kinetics reflected that of simple diffusion. Kinetics of ^{64}Cu absorption from small doses indicate a saturable process whereas at greater doses the amount absorbed is more

directly proportional to dose, indicating a passive process of diffusion (Bremner and Davies, 1980).

A Cu-binding protein has been detected in duodenal cells of chicks (Starcher, 1969) and rats (Evans and Hahn, 1974). This protein has a molecular weight of about 10,000 daltons. Evans et al. (1970) isolated a Cu-binding protein from bovine intestine and suggested that this protein was metallothionein. Evidence also exists for a different Cu-binding protein in rat intestine, which lacks the high cysteine content of metallothionein and functions as a regulator of Cu absorption, rather than a transport protein (Evans and LeBlanc, 1976), but Evans and Johnson (1978) indicated that this Cu-binding protein was probably an artifact resulting from uncontrolled oxidation of Cu-thionein; when the Cu-binding protein from rat intestine was purified by use of completely anaerobic conditions, Cu was associated with a cysteine-rich thionein. Binding of Cu to the 10,000 dalton fraction is not energy-dependent; almost all Cu in cytosol is bound to this component and is therefore nondialysable (Allen and Solomons, 1984). According to Allen and Solomons (1984), it seems that the function of the cytosol protein may be to block transfer of Cu into blood, rather than acting as a necessary step in transfer.

Bremner (1980) and Cousins (1985) have discussed possible functions of metallothionein in Cu homeostasis and

both authors agree that its exact role remains to be defined. Since metallothionein has a stronger affinity for Cu than for Zn, the protein greatly influences Cu absorption in intestinal epithelial cells (Cousins, 1985),

There is no direct relationship between plasma ceruloplasmin levels and rate of Cu absorption (Allen and Solomons, 1984). In humans, quantity of ceruloplasmin Cu exchanged daily was negligible compared to the amount of Cu absorbed from intestine (Sternlieb, 1967). Copper may also bind to transferrin in mucosal cells (Allen and Solomons, 1984).

Portal Transport of Copper

Uptake of Cu is considerably faster than transport of Cu through the intestinal wall (Crampton et al., 1965). Passage of Cu through intestinal cells to blood is apparently regulated by a low-molecular weight Cu-binding protein, synthesis of which is induced by Cu (Evans and LeBlanc, 1976). Hoadley and Cousins (1988) suggested that during transfer across intestinal epithelial cells Cu replaces Zn in metallothionein.

After passing through intestinal epithelial cells, Cu is transported through portal blood as a histidine-copper-albumin complex (Lau and Sarkar, 1971). Egress of Cu from intestinal mucosal cells into portal blood is not

understood, but ^{64}Cu appears rapidly in peripheral blood bound to albumin (Danks, 1988).

It has been suggested that portal transport of Cu involves initial binding to a high molecular weight protein, called transcuprein (Weiss and Linder, 1985). Recent experiments did not confirm the existence of a high molecular weight plasma transport molecule for Cu (Gordon et al., 1987) and demonstrated that absorbed Cu is initially bound to albumin but transferred from albumin to ceruloplasmin with time. These data support earlier observations by Marceau and Aspin (1973), which documented the time dependent redistribution of ^{67}Cu from albumin to ceruloplasmin.

Copper Uptake by Liver

It has long been known that liver is very high in Cu, especially in young animals (McHargue, 1925). The liver serves as the chief storage organ of Cu (Comar et al., 1948; Dick, 1954b) and it is the key organ in metabolism of Cu (Evans, 1977; Bremner, 1980; Prohaska, 1988; Gooneratne et al., 1989a). Liver of very young animals is especially high in Cu and the calf is born with a hepatic Cu concentration that is 4 to 8 times greater than that of adult cattle (Comar et al., 1948). Hepatic Cu concentrations in sheep, however, increase with age (Underwood, 1977). Concentration

of Cu in liver was smaller ($P < .05$) in newborn lambs than in 30- and 60-day-old lambs (Saylor and Leach, 1980).

Hepatocytes take up Cu from albumin and amino acids (Prohaska, 1988). Hepatic Cu is then incorporated into ceruloplasmin and released into plasma or into bile or stored temporarily in liver (Bremner, 1980).

Hepatocuprein (super oxide dismutase), metallothionein and mitochondrocuprein are the suggested Cu storage proteins in liver (Bremner, 1980). The rapid incorporation of injected Cu into metallothionein could indicate that the protein is involved in initial hepatic uptake of Cu, however, inhibition of metallothionein synthesis (Bremner and Davies, 1976) does not prevent accumulation of injected Cu by liver (Bremner, 1980).

Metallothionein concentration is dependent on Zn status (Zn induces synthesis of metallothionein), however, synthesis of metallothionein can also be induced by administration of Cu (Bremner, 1980).

Bremner and Marshall (1974a) reported finding three main Cu- and Zn-containing fractions with molecular weights of approximately > 75,000, 35,000 and 12,000 in cytosol of sheep and calf liver. Amino acid analysis of the low-molecular-weight protein from calf liver revealed that it was similar to metallothionein (Bremner and Marshall, 1974b).

Bremner and Marshall (1974b) proposed that Cu content in the metallothionein fraction is directly related to Zn concentration of liver. However, Zn concentration of liver of lambs did not increase in response to Zn intake, yet Cu content of the metallothionein fraction increased with increasing Zn content in diet (Saylor et al., 1980).

Freedman et al. (1989), using a wild-type hepatoma cell line and a Cu-resistant cell line that accumulates Cu and has a greatly elevated level of metallothionein, proposed a model of Cu metabolism in which the metal is complexed by glutathione soon after entering the cell. The complexed metal is then transferred to metallothionein where it is stored. The study also indicated that resistance to metal toxicosis in Cu-resistant hepatoma cells is due to increase in both cellular glutathione and metallothionein.

Incorporation of Cu into ceruloplasmin is a vital function of liver, because Cu is transported to extrahepatic tissues in the form of ceruloplasmin (Evans, 1977). Ceruloplasmin is a serum glyco-protein with ferroxidase activity and carries either six or seven Cu atoms per molecule (McArdle et al., 1990). Ceruloplasmin is not involved in uptake of Cu from intestine but may function in transport of Cu from liver and act as donor of Cu for certain Cu enzymes (Bremner, 1980). The amount of ^{64}Cu accumulated in cultured mouse hepatocytes was the same in control and Cu-depleted cells, which suggests that neither

ceruloplasmin production nor Cu uptake is regulated by intracellular Cu levels (McArdle et al., 1990).

Gooneratne et al. (1979) indicated the predominant role of the nuclear fraction of sheep liver in accumulating Cu and the role of lysosomes in storing excess Cu. In control sheep, the greatest concentration of Cu was in nuclear fraction of hepatocytes; Cu-loading increased concentration of Cu in all fractions: heavy mitochondrial fraction (mainly mitochondria), light mitochondrial fraction (mainly lysosomes), microsomal fraction and cytosol (Gooneratne et al., 1979).

Metallothionein

The involvement of metallothionein in absorption and metabolism of Cu has been reviewed by Bremner (1980), Cousins (1985), Bremner (1987), Richards (1989) and Bremner and Beattie (1990).

Metallothionein is a Cu- and Zn-binding protein present in most, if not all, tissues of higher eukaryotic species of animals (Richards, 1989). Metallothionein may be specifically involved in control of intestinal absorption of Zn and Cu (Bremner and Beattie, 1990), but sheep may have a limited ability to synthesize metallothionein (Saylor et al., 1980).

Within intestinal mucosal cells Cu can interact with metallothionein (Danks, 1988). It is not clear whether

metallothionein plays any role in normal absorptive process or whether it can prevent excessive absorption when intake of Cu is greater (DiSilvestro and Cousins, 1983; Danks, 1988). Metallothionein is involved in the important mechanism by which excessive Zn intake can block Cu absorption (Hall et al., 1979). Zinc is a stronger inducer of metallothionein production than Cu, yet Cu can displace Zn from metallothionein (Bremner, 1987). Larger doses of Zn can trap Cu in intestinal mucosal cells bound to metallothionein (Danks, 1988). Copper incorporated into mucosal metallothionein is assumed not to be transported into plasma but eliminated on desquamated intestinal cells. However, no detailed investigation has ever been carried out on the fate of mucosal metallothionein (Bremner and Beattie, 1990).

The proportion of hepatic Cu incorporated into metallothionein in sheep and calves is much less than that in pigs. The greater susceptibility of sheep and calves to Cu poisoning could be related to their inability to accumulate large amounts of Cu as metallothionein in their liver (Bremner, 1980).

Dietary Cu supplementation of rats induces hepatic synthesis of metallothionein only after liver Cu levels exceed a threshold value of approximately 500 ppm (Bremner et al., 1986).

Metallothionein does not seem to play an obligatory role in hepatic Cu metabolism because this metabolism is normal even when no Cu-metallothionein is present, as occurs in Zn-deficient animals (Bremner and Beattie, 1990).

The main role of metallothionein in Cu metabolism is most likely to be in cellular detoxification of the metal (Bremner and Beattie, 1990). In species such as the sheep, in which only a small proportion of Cu is bound in this way, hepatotoxic effects of Cu are relatively great (Mehra and Bremner, 1984).

Copper in Plasma

Most Cu in plasma is generally present in the form of ceruloplasmin, however, the principal transport forms of Cu are its loosely bound complexes with albumin and, to a lesser extent, with selected amino acids such as histidine, threonine and glutamine (Bremner, 1980).

Ceruloplasmin is involved in Cu transport, in oxidation of Fe^{++} as it is released from hepatocytes with subsequent conversion into Fe^{+++}-transferrin and in regulation of biogenic amines (Abdel-Mageed and Oehme, 1990).

Copper Excretion

Biliary excretion

A large proportion of ingested Cu appears in feces; most of this is unabsorbed Cu but active excretion occurs

via bile (Gooneratne et al., 1989a). Copper homeostasis is achieved primarily via this route (Underwood, 1977). Very little is known about the mechanism of biliary Cu excretion in ruminants (Gooneratne et al., 1989a,c). In dogs, humans, mice, pigs, poultry and rats, the major pathway of Cu excretion is via bile through fecal excretion (Underwood, 1977; Owen, 1982b). Søli and Rambaek (1978) using intravenously injected ^{64}Cu demonstrated that in sheep urinary and biliary excretion were of the same magnitude, but total excretion of Cu was very small, since only 1.4% to 1.7% of the dose injected was excreted during a 60-hour period after administration. In dairy cows, the main endogenous loss occurs through feces and stems mainly from the stomach system, whereas contribution from bile is small and variable (Binnerts, 1978). Søli and Rambaek (1978), after intravenous administration of ^{64}Cu, detected significant concentrations of Cu in saliva of sheep, although activity in saliva was less than in bile. Pigs had biliary Cu concentrations more than 10 times greater than sheep, even when fed the same dietary Cu levels for a shorter time (Schenkel and Krehl, 1983). Copper excretion in bile in steers and pigs increased after an intravenous infusion of Cu, but the increase for up to 3 days after infusion accounted for less than 4% of Cu infused in steers, whereas in pigs it accounted for 80% to 90% (Charmley and Symonds, 1985).

In mice and rats, excretion of Cu in bile may be the principal homeostatic mechanism by which body Cu content is controlled (Gitlin et al., 1960; Owen, 1964). Biliary Cu levels in sheep, however, do not increase as dietary or hepatic Cu concentrations increase (Caple and Heath, 1978; Saylor and Leach, 1980; Gooneratne et al., 1985), liver Cu content remains elevated long after removal of Cu supplements, and only a small and variable proportion of liver Cu is bound to metallothionein (Bremner and Marshall, 1974b; Saylor et al., 1980). Biliary Cu concentrations of wethers fed for 87 days either cupric acetate, cupric chloride and cupric sulfate, to increase dietary Cu concentrations from 6.7 to 35.7 ppm, were 1.7, 1.2 and 1.4 ppm, compared to .4 ppm in control lambs, but differences were not significant (Charmley and Ivan, 1989). In cattle and sheep, biliary Cu concentration sampled from gall bladder was less than 1% of hepatic Cu concentration, whereas in pigs it was greater than 30% (Schenkel and Krehl, 1983). In cattle, biliary Cu excretion seems to increase in response to elevated Cu levels. Biliary concentration of Cu in steers was .12 ppm in control animals and it increased to .20 ppm during intravenous infusion of Cu and .23 ppm following intravenous infusion (Charmley and Symonds, 1985). In cattle, biliary Cu excretion increased when dietary Cu and S were elevated from 5 to 40 ppm and .2% to .5%, respectively (Gooneratne et al., 1987). In the bovine,

amounts of Cu secreted in bile fell dramatically as liver Cu concentration decreased (Phillippo and Graça, 1983). In calves, rate of excretion of Cu in bile varied from week to week during a 24 to 30-week study, but it increased when the calf pellets being fed were replaced by a dairy concentrate containing more Cu (Symonds et al., 1983). These reports suggest that sheep seem to have very little ability to excrete Cu in bile, and Søli and Rambaek (1978) and Gooneratne et al. (1989c) concluded that the low excretion capacity of Cu through bile may be a factor of importance for the high susceptibility of sheep to excess of Cu.

Evans (1973) proposed that biliary Cu originates from protein-bound Cu that is deposited in bile as a result of protein catabolism and pinocytosis by hepatic lysosomes. The limited ability of sheep to synthesize metallothionein may explain the susceptibility of this species to Cu poisoning, because less metallothionein-bound Cu is present for sequestration by lysosomes (Saylor et al., 1980). These authors stated that their proposal was supported by results obtained by Saylor and Leach (1980), who found no significant increase in Cu concentration of bile of lambs as dietary Cu content increased from 2.2 to 47.0 ppm. In fact, biliary Cu concentrations were .2, .5 and 1.5 ppm, for 2.2, 11.3 and 47 ppm dietary Cu and differences may have not been significant because of the small number of animals. Biliary Cu excretion in sheep and cattle was increased by duodenal

(Gooneratne et al., 1989c) or intravenous administration of tetrathiomolybdate (Gooneratne et al., 1985; Ke and Symonds, 1989; Gooneratne et al., 1989b,c,d).

Copper apparently is excreted into bile in the form of amino acid complexes (Evans and Cornatzer, 1971) that combine with macromolecules, rendering Cu unavailable for reabsorption (Mistilis and Farrer, 1968). Bile salts and pigments have been implicated as Cu-binding agents in bile (Allen and Solomons, 1984).

Losses via urine

According to Suttle (1987), urinary Cu excretion is not influenced by Cu intake, presumably because the ultrafilterable Cu concentration in plasma is held remarkably constant. Urinary Cu losses in sheep, however, increased while losses of Cu via bile and pancreatic juice decreased as Cu intake increased from 6.8 to 12.3 and 17.8 mg/day, but quantities of Cu transported from body pool in digestive secretions (bile and pancreatic juice) were much greater than urinary losses (Grace and Gooden, 1980).

In faunated and defaunated wethers fed semipurified diets containing 4.2 ppm Cu, .26 ppm Mo and .14% S, urinary excretion of Cu only accounted for 4.4% of total Cu excreted (Kreuzer and Kirchgessner, 1990). Urinary Cu excretion in adult wethers administered a single intra-abomasal or intraruminal dose of 1 mg Cu, however, was greater than biliary excretion (Neethling et al., 1968).

Urinary Cu excretion increased in sheep when dietary Mo levels were elevated (Marcilese et al., 1970; Weber et al., 1983). Elevated dietary Cu (40 ppm), Mo (10 ppm) and S (.5%) increased urinary Cu excretion in heifers, relative to those fed 5 ppm Cu, 1 ppm Mo and .2% S (Gooneratne et al., 1987).

Biological Roles of Copper

Copper is an essential micronutrient in mammals (Allen and Solomons, 1984). Current understanding of roles of Cu is based on the functions of the known Cu-enzymes and on its role in disulfide bonding of keratin by an unknown mechanism (Danks, 1988). The correspondence between physiological functions and biochemical reactions catalyzed by metalloenzymes is nowhere better demonstrated than with Cu (Allen and Solomons, 1984). However, knowledge of Cu requirements, absorption and transport and of its biological roles remains frustratingly incomplete (Danks, 1988).

Cuproenzymes are involved in 1.) formation of melanin pigment in hair and skin (tyrosinase); 2.) synthesis of structural subunits of collagen and elastin (lysil oxidase) and of tertiary structure of proteins such as keratin (monoamine oxidase); 3.) adrenal synthesis of catecholamine (dopamine ß-hydroxylase); 4.) killing of bacteria by white blood cells (Zn-Cu superoxide dismutase); and 5.) mitochondrial energy production via oxidative

phosphorylation (cytochrome C oxidase) (Allen and Solomons, 1984). Mineralization of growing bones seems possibly related to Cu, either in a cuproenzyme with ascorbate oxidase activity, or in its soluble, ionic form (Allen and Solomons, 1984). Copper plays a number of roles in mammalian Fe metabolism; whether or not serum ceruloplasmin has a physiologically important function in mobilization of Fe remains controversial (Allen and Solomons, 1984). Copper is also important in myelination of nerve fibers and production of neutrophils, but the mechanisms of its involvement in these two functions are not well understood (Allen and Solomons, 1984). Increasing evidence indicates that Cu exerts a key role in metabolism of catecholamines in many organs (Anonymous, 1990). Woolliams et al. (1986) obtained evidence that decreased resistance to infection is a clinical consequence of ovine Cu deficiency in the field, possible to be controlled by Cu treatment and genetic selection.

Copper Deficiency

A wide range of different clinical syndromes in different species is caused by Cu deficiency, sometimes expressed as different deficiency signs even within a single species (Scott, 1972). Most features of Cu deficiency can be explained by failure of one or more Cu-enzymes (Danks, 1988). For example, hair depigmentation is explained by

tyrosinase deficiency; lysil oxidase deficiency is responsible for defects in elastin and collagen that underlie connective tissue abnormalities (Danks, 1988). Anemia is a general sign for all species but depressed growth, bone disorders, depigmentation of hair, fur or wool, abnormal wool growth, demyelination of spinal cord, fibrosis of myocardium, gastrointestinal disturbances, all have been observed in Cu-deficient animals of one or more species, and all have been prevented or alleviated by administration of adequate amounts of Cu (Scott, 1972). Copper deficiency manifestations have been described in detail by Underwood (1977, 1981). Species differences in Cu deficiency are quite marked and the age at which an animal experiences the deficiency influences the effects (Danks, 1988). For instance, osteoporosis, anemia and "steely wool" are prominent in sheep, but vascular disease does not occur in this species; arterial rupture is a major problem in pigs and poultry. Neurological effects are seen in lambs or mice deprived of Cu in utero, but not in lambs subjected to Cu deficiency after birth or in other species (Danks, 1988).

Copper Requirements

Dietary Cu requirements for sheep as indicated by NRC (1985) are 7 to 11 ppm, along with .5 ppm Mo and .14% to .26% S. Maximal tolerable concentration for sheep is 25 ppm (NRC, 1980). For beef cattle, the NRC (1984) suggested

requirement was 8 ppm dietary Cu, with a range of 4 to 10 ppm, along with .08% to .15% S; no value for Mo was indicated. For dairy cattle, NRC (1989) recommended 10 ppm Cu for all categories, with varying concentrations of S (.16% to .29%), according to age or stage of lactation. The NRC (1980) suggested 100 ppm Cu as maximal tolerable concentration for cattle. Increased dietary Mo in presence of S can increase requirements for Cu by ruminant animals several times above "normal" levels (Ammerman and Henry, 1979).

Copper Toxicity

An early description of experimentally produced Cu poisoning in man and rabbits was given at the beginning of this century (Mallory et al., 1921; Mallory, 1925). Copper toxicity was reviewed by Todd (1969), Buck (1978), and Osweiler et al. (1985).

Numerous cases of Cu intoxication have been reported, in cattle (Mylrea and Byrne, 1974; Stogdale, 1978), goats (Belford et al., 1989) and mainly in sheep (Pierson and Aanes, 1958; Ross, 1966; Harker, 1976; Hidiroglou et al., 1984; Martin et al., 1988; Stahr et al., 1989). Ruminants seem to be more susceptible to chronic Cu toxicosis than monogastric animals (Todd, 1969), but this condition rarely occurs in goats (Søli and Frøslie, 1979; Zervas et al., 1990). Sheep are by far the most susceptible species to Cu-

poisoning and may be affected at any age; young calves are also susceptible, but cattle become less susceptible as they mature (Todd, 1976).

Only small differences in dietary intake separate Cu poisoning and Cu deficiency in sheep (Clegg et al., 1986). The relevance of this statement is shown by the fact that dietary Cu levels similar to requirements suggested by the NRC (1985) resulted in Cu intoxication in sheep (Hogan et al., 1968; Woolliams et al., 1982). Sheep are more susceptible to Cu toxicosis because of a restricted filtration mechanism of Cu and they are incapable of increasing biliary Cu excretion when excess Cu is fed (Brown, 1967; Gooneratne et al., 1985, 1989b).

Copper poisoning may be acute or chronic, but the terms are misleading because chronic refers to a long cumulative poisoning which may suddenly develop into an acute hemolytic crisis (Bostwick, 1982).

Accumulation of Cu is almost entirely located in liver, with virtually no change in concentration in any of the other tissues (Todd, 1969). According to Howell (1978), excessive Cu accumulates in nuclei and lysosomes. Kumaratilake and Howell (1989) observed, however, that the increase in concentration of Cu in liver cells of Cu-loaded sheep occurred predominantly in lysosomes and in cytosol. These authors suggested that necrosis of isolated hepatocytes observed in chronic Cu-poisoned sheep could be

due to a saturation of uptake of Cu into the lysosomal system of cells, leading to accumulation of toxic levels of Cu in cytosol. Large amounts of Cu-metallothionein were obtained from liver of pigs fed high-Cu^{++} diets or from rats injected with Cu^{++}, but no Cu-metallothionein was present in liver of Cu^{++}-loaded sheep or of rats fed Cu^{++}-supplemented diets (Mehra and Bremner, 1984).

The cellular target responsible for cell death from Cu poisoning is unknown. One mechanism by which Cu can cause toxic effects is through coordination reactions. By forming complexes with biological ligands such as proteins, DNA, membranes, or small cellular molecules, normal functions of these molecules are inhibited (Ecker et al., 1989).

The imbalance of Cu, Mo and S in cereal-rich diets, which are potentially low in S and Mo may be an important factor in the well known susceptibility of housed sheep to chronic Cu poisoning (Gooneratne et al., 1989a). Copper toxicosis is essentially a problem of housed ruminants because they are fed feedstuffs of high Cu availability; conversely, Cu deficiency is a problem of grazing animals because of poor availability of Cu in grass (Suttle, 1986). An uncomplicated Cu toxicosis in grazing animals related to an excessive concentration of Cu in pastures has seldom been confirmed (Beeson and Matrone, 1976).

Genetic Effects on Copper Metabolism

Most research which has been conducted to investigate the involvement of heredity in Cu metabolism of ruminants arose from an observation in 1964 that incidence of swayback differed greatly among three breeds of sheep and their crosses in two farms in Scotland (Wiener, 1966).

Wiener et al. (1969) reported breed differences in blood Cu concentrations of sheep, while Wiener and Field (1969) and Wiener et al. (1976) found that hepatic Cu concentrations varied among breeds. There are differences among breeds of sheep in the efficiency at which they absorb Cu from diets of low Cu content (Wiener et al., 1978) and in their ability to retain Cu in liver from diets of large Cu content (Herbert et al., 1978). Woolliams et al. (1982) and Harrison et al. (1987) observed a wide range of breed differences in hepatic accumulation of Cu. Differences in Cu concentration in liver and plasma of sheep reflected differences in efficiency of Cu absorption (Woolliams et al., 1983).

Great differences among breeds and crossbreeds of lambs in accumulation of Cu in liver at both normal (10 ppm) and high (34 ppm) Cu diets were registered by van der Berg et al. (1983). Smart and Christensen (1985) indicated a breed effect on plasma Cu levels of beef heifers on a Cu deficient diet. Breed differences in biliary Cu excretion were reported in cattle (Gooneratne et al., 1988).

Genetic selection of sheep led to a highly significant difference between lines in Cu concentration in plasma (Wiener et al., 1985) and in liver (Woolliams et al., 1985b).

Variation in uptake of Cu was extremely large for 25 sheep of the same breed; there was approximately a 10-fold difference in rates of uptake between the sheep with the greatest and smallest values (Turner et al., 1987).

Effect of Dietary Copper Levels

Several investigations have shown linear increases in hepatic Cu levels in sheep after elevated Cu intake (Dick, 1954b; Abdellatif, 1968; van Ryssen and Stielau, 1980a; Zervas et al., 1990). In poultry, pigs and rats, no changes in Cu concentrations in liver occur until dietary Cu concentrations exceed about 100 ppm (Suttle, 1987). Corbett et al. (1978), in view of this close relationship between dietary and hepatic Cu concentrations in sheep, suggested the existence of very little homeostatic control over hepatic Cu concentrations in this species. However, Woolliams et al. (1983) reported a curvilinear relationship in sheep between hepatic and dietary Cu over a range of 4 to 29 ppm dietary Cu. Also, efficiency of Cu accumulation in liver was lower in lambs fed 15.4 ppm dietary Cu than in those fed 7.3 ppm (Ivan, 1988).

Woolliams et al. (1983) suggested the existence of a saturation of the absorptive mechanism or an increase in endogenous loss in bile as Cu concentrations in liver of sheep were increased.

Effect of Other Dietary Factors

Absorption of Cu from fresh herbage seems to be less than that from concentrate diets (ARC, 1980). Hartmans and Bosman (1970) have suggested that availability of Cu increases as herbage matures and is greater in hay than in fresh herbage.

In addition to Mo and S, a number of other nutrients or dietary components can affect Cu absorption, excretion or metabolism in ruminants, including Ca (Kirchgessner and Grassmann, 1970; Suttle and Field, 1970); Fe (Abdellatif, 1968; Campbell et al., 1974; Standish et al., 1969, 1971; Humphries et al., 1983; Bremner et al., 1987; Phillippo et al., 1987b) and Zn (Campbell and Mills, 1979).

Large dietary concentrations of either Zn or Fe alone accentuated the increase in hepatic Cu caused by additional dietary Cu, but both elements together decreased liver Cu below concentrations when neither was supplemented (Rosa et al., 1986). The antagonistic effect of Zn on Cu absorption has been attributed to Zn-induced synthesis of metallothionein, followed by increased and preferential binding of Cu to the protein (Hall et al., 1979). This has

been confirmed by Fischer et al. (1983), using isolated duodenal segments from rats fed different amounts of Zn. The authors suggested that metallothionein then sequesters Cu in mucosal cells, making it unavailable for serosal transfer.

Response to Cu supplementation in sheep was improved by simultaneous Se supplementation (Hill et al., 1969; Hogan et al., 1971b). In sheep high dietary protein is associated with reduced Cu availability and retention (Ivan and Veira 1981) but this may also be related to S content.

Molybdenum

History and Review

Karantassis, in 1924, described a toxicologic study of Mo with guinea pigs. The biological importance of Mo, however, was not realized until Bortels (1930) showed that the element was highly beneficial in fixation of N_2 by N-fixing bacterium Azotobacter chroococcum. Molybdenum was later found to be necessary for all N-fixing organisms and for Aspergillus niger (Steinberg, 1936). Essentiality of Mo for higher plants, independent of nitrogen fixation, was established by Arnon and Stout (1939) in tomato plants.

Ter Meulen (1932), using a colorimetric method, determined Mo concentrations in a wide range of materials. In tissues of man and animals, the largest concentrations were found in liver.

In animal nutrition Mo was first distinguished as a toxic element, mainly because of its antagonistic effects on Cu metabolism. A disorder, known as "teart" in Great Britain, occurred in cattle grazing pastures located on certain soils high in organic matter. Ferguson et al. (1938) noted an unusually large concentration of Mo in these pastures. Based on a lead indicated by Brouwer et al. (1938) that cupric sulfate was effective in preventing and curing a chronic diarrhea in dairy cattle on pastures in Holland, Ferguson et al. (1943) suggested that excess Mo was the complicating factor in what seemed to be a poor Cu metabolism in grazing animals. Soon, in New Zealand, Cunningham (1944) reported a Cu deficiency syndrome in cattle referred to as "peat-scours," suggested to result from excess Mo in diet (Cunningham, 1946). Dick and Bull (1945) in Australia showed experimentally that storage of Cu in liver of sheep and cattle could be significantly reduced by increasing dietary intake of Mo. In the United States, chronic Mo toxicosis affected cattle in California (Britton and Goss, 1946; Barshad, 1948). Also, Davis et al. (1946) discussed a syndrome in beef cattle on organic soils of the Florida Everglades that was prevented by Cu therapy. Later, Davis (1950) related the syndrome to high Mo content of the forages. Cunningham et al. (1953) described Mo toxicosis in dairy cattle in Canada. Continuing, Dick (1954b) showed that adequate intakes of SO_4 were also necessary to allow Mo

to exert its limiting effect on Cu storage. Dick (1953a), Wynne and McClymont (1956) and Allcroft and Lewis (1957) presented information relating Cu, Mo and inorganic SO_4.

The first indication that Mo was essential for animal nutrition came in 1953, when it was shown that the flavoprotein enzyme xanthine oxidase is a Mo-containing metalloenzyme which requires Mo for its activity (de Renzo et al., 1953a,b; Richert and Westerfeld, 1953).

There are many reviews and books written about Mo and some of the most recent publications include biology of Mo (Chappell and Petersen, 1976); geochemistry, cycling and industrial uses of Mo (Chappell and Petersen, 1977); coordination and bioinorganic chemistry of Mo (Stiefel, 1977); Mo-containing enzymes (Coughlan, 1980); nutritional aspects of Mo in animals (Mills and Bremner, 1980); chemistry and uses of Mo (Barry and Mitchell, 1982); Mo-containing enzymes, biochemistry and nutritional aspects (Rajagopalan, 1984); Cu x Mo interaction (Osweiler et al., 1985); and Mo toxicity and metabolism (Ward, 1991).

Molybdenum Chemistry

Molybdenum is classified in group VI-B of the periodic table of elements, together with chromium and tungsten. Its atomic number is 42 and it has a molecular weight of 95.94. There are seven natural isotopes with mass numbers of 92,

94, 95, 96, 97, 98 and 100. Molybdenum makes up about .0015% of the earth's crust (Shamberger, 1979).

Among members of the second transition series, Mo is the only element definitely known to have specific biological functions. With its ability to exist in oxidation states from -2 to +6 and coordination ranging from 4 to 8, the metal has an extraordinarily complex chemistry (Rajagopalan, 1984).

Molybdenum Absorption, Tissue Level and Effect on Copper

Molybdenum (as ammonium molybdate) absorption in calves occurred from stomach through small intestine and no absorption was apparent from rumen or omasum (Miller et al., 1972). Intraruminally administered ^{99}Mo first appeared in plasma of calves 4 hours after dosing, with peak concentrations ranging from .2% to .7% of the dose at 24 hours (Fischer et al., 1976b). Rate of uptake of molybdate from intestinal segments of sheep, in vitro, was greatest in distal ileum, being much smaller in mid small intestine and even lower in proximal duodenum (Mason and Cardin, 1977). Duodenally administered molybdate (as ammonium molybdate) was efficiently and rapidly absorbed in sheep (Mason et al., 1978). Molybdenum isotope ^{99}Mo administered to sheep duodenally as either di- or trithiomolybdate was readily absorbed (Mason et al., 1982b). In sheep, tri- and

tetrathiomolybdate were rapidly absorbed from rumen and small intestine (Kelleher et al., 1983).

Single doses of ^{99}Mo to calves resulted in greater absorption when administered into the abomasum instead of the rumen (Miller et al., 1972). Passage of radioactive molybdate through the rumen led to chemical modification of the compound, so that, particularly at large S levels, most radioactivity remained in feces (Mason et al., 1978). Plasma Mo concentrations in lambs increased from birth to about 3 weeks of age, after which concentrations declined to 32% and 21% of peak levels for lambs of ewes fed 20 and 40 ppm Mo, respectively (Wittenberg and Devlin, 1988). The decrease was thought to be a consequence of less absorption, after the rumen of lambs became functional.

Immediately after absorption in sheep, tri- and tetrathiomolybdate were detected in plasma mainly as protein-bound and TCA-insoluble ^{99}Mo (Kelleher et al., 1983).

Molybdenum trioxide administered orally to guinea pigs in a single dose (50 mg Mo) was rapidly and extensively absorbed (Fairhall et al., 1945). Molybdenum levels were elevated in kidney, spleen, blood, bile, lung, muscle and urine.

In sheep and cattle, plasma and tissue levels of Mo reflect absorption of Mo when dietary concentrations are large (Suttle, 1983b; Ivan and Veira, 1985; Wittenberg and

Boila, 1988), but response of tissues to change in dietary Mo is greatly influenced by composition of the diet, particularly its content of sulfate, protein and tungstate (Underwood, 1976). According to Grace and Martinson (1985) and Nielsen (1990), liver retains the greatest amounts of Mo. Increments in Mo concentrations in plasma of sheep fed increasing dietary levels of Mo were linear in many studies (Dick, 1956b, 1969; Suttle, 1977; Suttle, 1983b; Weber et al., 1983), yet in liver the response was generally less evident (Wittenberg and Devlin, 1988) and non-linear (Cox et al., 1960; Vanderveen and Keener, 1964). Non-linear increases in hepatic Mo levels were also observed in rats (Yang and Yang, 1989).

In sheep, the amount of Mo absorbed depends on the amount of SO_4 in the diet (Dick, 1953b). In this species, SO_4 limits Mo retention both by reducing intestinal absorption and by increasing urinary excretion (Underwood, 1976). Inorganic sulfate inhibits intestinal absorption of molybdate in chicks (Huisingh et al., 1973), rats (Cardin and Mason, 1976) and sheep (Mason and Cardin, 1977). The inhibition is competitive; both molybdate and sulfate apparently are transferred across the intestine by a common carrier system (Cardin and Mason, 1975; Mason and Cardin, 1977).

Tissue response to Mo intake is dependent upon dietary S (Dick, 1952; Dick, 1953b; Wynne and McClymont, 1956;

Bremner and Young, 1978; van Ryssen et al., 1990). With additional S, Mo concentration increase in blood was less marked than with Mo alone (Weber et al., 1983). Large dietary SO_4 concentrations caused a decrease in Mo retention in rats (Miller et al., 1956), cattle (Cunningham et al., 1959) and sheep (Dick, 1956a; Scaife, 1956).

Dick (1953a,b; 1954b) first suggested that the form of S interacting with Cu and Mo was inorganic SO_4, but Cook et al., 1966; Suttle, 1973a; 1974b, 1975b) showed that both inorganic and organic S play similar roles in the three-way antagonism. Both S sources potentiated the inhibitory effect of Mo on Cu repletion and simultaneously decreased Mo concentration in plasma (Suttle, 1975b).

Response to Mo exposure is species-specific. Cattle reacted to Mo exposure more sensitively. They were followed by sheep and other species of wild ruminants (Anke and Risch, 1989). The greater tolerance of goats towards excessive dietary Mo does not result from insufficient Mo absorption. All organs of Mo-exposed goats and blood serum accumulated Mo (Anke et al., 1985). Bell et al. (1964) observed marked differences in absorption of ^{99}Mo between cattle and swine. Molybdenum from oral doses in swine reached a radioactivity peak in blood at 2 to 4 hours, whereas the average time in cattle was 96 hours. In swine, ^{99}Mo in blood averaged 6% at 2 and 4 hours after an oral dose and it declined rapidly to .07% at 48 hours. In

cattle, the greatest blood ^{99}Mo peak from an oral dose was observed as 2.6% of the dose at 96 hours, and it declined slowly to .9% at 168 hours.

Increments in consumption of Mo by cattle and sheep lead to a series of changes in plasma and tissue concentrations of Cu. Hepatic Cu levels in general decrease as dietary Mo increases (Dick and Bull, 1945; Dick, 1954a; Wynne and McClymont, 1956; Cox et al., 1960; Chapman and Kidder, 1963; Lesperance and Bohman, 1963; Vanderveen and Keener, 1964; Goodrich and Tillman, 1966a; Marcilese et al., 1969; Ross, 1970; Huber et al., 1971; Harker, 1976; Suttle, 1977; van Ryssen, 1979; Kincaid, 1980; Pitt et al., 1980; van Ryssen and Stielau, 1981; Hidiroglou et al., 1984; Phillippo et al., 1985; Harrison et al., 1987; van Ryssen et al., 1986; Bremner et al., 1987; Phillippo et al., 1987a,b; Humphries et al., 1988b; Kincaid, 1988b; Suttle et al., 1988; van Ryssen and Barrowman, 1988; Wang et al., 1988; Kleczkowski, 1989; Moshtaghi-Nia et al., 1989; van Niekerk and van Niekerk, 1989; White et al., 1989; van Ryssen et al., 1990). In some circumstances, however, no response of hepatic Cu to elevated Mo intake occurred (Cook et al., 1966; Kincaid, 1980; Langlands et al., 1981; Wittenberg and Devlin, 1988) or it depended upon the amount consumed (Dick, 1969; Suttle, 1977; Kincaid, 1980; van Ryssen and Stielau, 1981).

In sheep, intravenous administrations of 100 mg ^{99}Mo decreased ($P < .01$) the proportion of Cu primarily in the nuclear fraction of liver and increased it ($P < .01$) mainly in cytosol (Kelleher and Ivan, 1986). Dosing with 50 mg Mo decreased ($P < .01$) the proportion of Cu in light mitochondria but otherwise had little effect on overall Cu distribution among fractions (nuclear, heavy mitochondria, microsomal, cytosolic). The majority of ^{99}Mo appeared in the nuclear fraction (Kelleher and Ivan, 1986).

Wang et al. (1988) showed that in calves fed a diet low in Cu without additional Mo and S, a decrease in liver Cu occurred in all fractions (nuclear, mitochondrial and cytosolic), but in the pathological decline associated with Mo (35 ppm) and S (.3%) supplementation, the nuclear fraction was particularly affected. In the control group, Cu concentrations increased very rapidly, especially in the nuclear fraction, after moderate supplementation of the diet with 5 ppm Cu (Wang et al., 1988). Gooneratne et al. (1979), found that almost all extra Cu entering hepatic cells of sheep was concentrated in nuclei.

The effect of Mo in the presence of S on tissue Cu is so strong that the incidence of Cu toxicosis in sheep has been prevented or reduced by intravenous injections of ammonium tetrathiomolybdate (Gooneratne et al., 1981; Humphries et al., 1986; Howell and Kumaratilake, 1990), by oral drenches of tetrathiomolybdate (Kincaid and White,

1988) or by increasing dietary Mo and S concentrations (Pierson and Aanes, 1958; Ross, 1966; Hogan et al., 1968; Kline et al., 1971; Hidiroglou et al., 1984). An early review on Mo already suggested its use in controlling large hepatic Cu levels (Marston, 1952).

Another consistent observation regarding increased intake of Mo is the elevation of a Cu fraction in plasma which is insoluble in trichloroacetic acid solution (Smith and Wright, 1975a,b; Pitt et al., 1980; Suttle, 1983b; Hynes et al., 1984; Wittenberg and Devlin, 1987; Wang et al., 1988). This fraction also contains Mo (Smith and Wright, 1975a).

The major effect of Mo on Cu absorption occurred in large intestine of cattle (Golfman and Boila, 1990), where Cu seems to be mainly absorbed in ruminants.

Molybdenum Excretion

Notable species differences in Mo excretion exist. Under most conditions, urine is the major route of excretion of Mo in pigs, rats and man, but apparently not in cattle or in sheep on low SO_4 intakes (Underwood, 1976). Over 75% of both orally or intravenously administered ^{99}Mo was excreted in urine of swine in 120 hours. In contrast, fecal excretion was the main route of excretion of both oral and intravenous doses of ^{99}Mo in cattle, with only 15% excreted in urine in 168 hours by intravenously dosed cattle (Bell et

al., 1964). In calves, ^{99}Mo appeared in urine much sooner after a single abomasal dose than when administered in feed, but even when the rumen was bypassed, urine was still not the primary route of excretion. When ^{99}Mo was administered daily, for 7 days, urine was the primary excretion route when it entered directly into the abomasum, but fecal excretion predominated when the radioisotope was placed in rumen (Miller et al., 1972). Intraruminally administered ^{99}Mo in calves peaked from 24 to 48 hours after dosing, with approximately 15% of the dose appearing in feces (Fischer et al., 1976b). Urinary excretion of ^{99}Mo administered intraruminally in calves was first detectable in the 24 hour sample and, in general, maximal urinary excretion of ^{99}Mo occurred between 24 and 48 hours, with approximately 2% of the dose (Fischer et al., 1976b). Cumulative fecal excretion of an intraruminally administered dose of ^{99}Mo in calves at 144 hours after dosing varied from 65% to 68% of the dose and cumulative urinary excretion at 144 hours after dosing from 7.4% to 13.9% (Fischer et al., 1976b). Absorbed Mo from molybdenum trioxide was rapidly excreted in urine of guinea pigs (Fairhall et al., 1945). In sheep and cattle on low Mo intakes, little Mo is excreted and most of that excreted is in urine (Scaife, 1956; Weber et al., 1983; Lesperance et al., 1985). When additional Mo is provided, the proportion excreted increases (Lesperance et al., 1985) and most of it appears in feces (Weber et al., 1983).

Molybdenum excretion depends on dietary S levels. When SO_4 intake was raised, Mo excretion in urine increased (Dick, 1953b). Grace and Suttle (1979) observed, however, a decrease in urinary Mo excretion following increases in S intake. The same authors also reported a decline in Mo absorption and in endogenous Mo excretion in feces and a rise in Mo retention. At the renal level, there is a mutual inhibition of molybdate and sulfate for tubular reabsorption (Bishara and Bray, 1978).

Concentration of Mo in milk is extremely susceptible to changes in dietary Mo intake (Underwood, 1976). Milk is one of the major excretion routes for Mo when dietary Mo concentrations are small (Wittenberg and Devlin, 1987). It represented 16% of Mo intake when the diet contained .6 ppm Mo. Although milk excretion accounted for only 2.5% of Mo intake of cows consuming 19.3 or 34.8 ppm Mo in their diets, milk Mo concentration and total daily excretion of Mo increased significantly with each increment in dietary Mo concentration (Wittenberg and Devlin, 1987). Milk of ewes consuming similar dietary Mo concentrations, had much greater Mo concentrations than that of cattle. Excretion of Mo in milk of ewes represented 24.3%, 11.7% and 10.5% of total daily Mo intake, for females fed .9, 18.4 and 40.7 ppm Mo (Wittenberg and Devlin, 1988). Oral administration of molybdate caused rapid rises of Mo concentrations in milk of cows and goats, but it did not affect xanthine oxidase

activity of milk of either species (Hart et al., 1967). Increases in Mo concentrations in milk after dietary Mo supplementation have also been observed by Vanderveen and Keener (1964) and Huber et al. (1971) in lactating cows. In lactating beef cows, daily Mo excretion in milk declined with decreasing milk production (Wittenberg and Devlin, 1987), while in ewes, despite a reduced milk production over time, daily milk Mo excretion was maintained or even increased for Mo-supplemented animals, because Mo concentrations in milk increased (Wittenberg and Devlin, 1988).

Huber et al. (1971) reported a 10-fold increase in milk Cu concentrations when 53 ppm Mo was fed to dairy cows, but such an effect was not found by Vanderveen and Keener (1964) in dairy cows, by Wittenberg and Devlin (1987) in beef cows or by Wittenberg and Devlin (1988) in ewes.

Molybdenum is excreted in milk in an absorbable form. When lactating beef cows were fed .6, 19.3 or 34.8 ppm Mo or when lactating ewes were fed .9, 18.4 or 40.7 ppm Mo, plasma concentrations of Mo increased in calves and lambs (Wittenberg and Devlin, 1987, 1988).

Biological Roles of Molybdenum

The unique role of Mo in biological systems is exemplified by nitrogenase, the enzyme that converts nitrogen into ammonia at room temperature and normal

pressure (Schrauzer, 1976), the significance of which is only realized when the tremendous amounts of pressure and heat necessary to produce ammonia industrially from atmospheric nitrogen are taken into account.

All known biological functions of Mo are attributable to molybdoenzymes (Rajagopalan, 1984). Molybdenum is a component of at least five distinct enzymes that catalyze diverse and unrelated reactions, namely nitrogenase, nitrate reductase, xanthine oxidase, aldehyde oxidase and sulfite oxidase (Nicholas, 1975).

Molybdenum-dependent enzymes have been identified in all species of living systems and facilitate such processes as conversion of nitrogen and nitrate to ammonia in plants and lower organisms, growth of microorganisms on compounds such as nitrate, purines and pyridines as carbon and nitrogen sources, utilization of nitrate as an electron sink, and conversion of sulfite to sulfate in animals (Rajagopalan, 1984).

The discovery that Mo content of the diet had a marked influence on xanthine oxidase activity of rat tissues (de Renzo et al., 1953a,b; Richert and Westerfeld, 1953) was the first indication that Mo could have a role in animal metabolism. The identification of the metal in rabbit liver aldehyde oxidase (Mahler et al., 1954) established Mo as a functional trace element in animals (Rajagopalan, 1984).

Cohen et al. (1971) documented the presence and function of Mo in sulfite oxidase.

In animals, there are three kinds of Mo-containing enzymes: xanthine oxidase-dehydrogenase, aldehyde oxidase and sulfite oxidase. These enzymes have a common cofactor termed molybdopterin (an alkyl phosphate-substituted pterin) to which Mo is ligated by two S atoms (Anke and Risch, 1989). Sulfite oxidase has been isolated from bovine liver (Cohen et al., 1971). This enzyme catalyzes oxidation of sulfite to sulfate (Shamberger, 1979). In lower animals xanthine oxidase functions in utilization of purine as a nitrogen source. In higher organisms, however, its true physiological function is not clear. The most studied reaction of this enzyme is the conversion of xanthine into uric acid (Anke and Risch, 1989).

Molybdenum Deficiency and Essentiality

Under naturally occurring conditions, an uncomplicated Mo deficiency has not been reported in ruminants (Wittenberg and Devlin, 1987).

Teresi et al. (1942) did not observe any growth improvement in rats fed milk providing .5 μg Mo/day with addition of sodium molybdate in their drinking water. Chicks fed synthetic diets low in Mo, with added tungstate, a competitive inhibitor of Mo, developed an apparent Mo deficiency, but rats did not (Higgins et al., 1956).

Studies with chicks and turkey poults indicated that addition of Mo to purified diets resulted in 14% to 19% increase in growth over unsupplemented groups (Reid et al., 1956).

Ellis et al. (1958) observed larger gains ($P < .01$) and increased ($P < .01$) cellulose digestibility in lambs fed additional Mo in a semipurified diet, but Ellis and Pfander (1960) and Anke and Risch (1989) could not confirm these findings, whereas Martinez and Church (1970), using washed suspensions of rumen microorganisms, observed an increase in cellulose digestion with addition of 10 to 100 ppm Mo to the medium. Shariff et al. (1990) found that 10 ppm supplemental Mo fed to steers had negative effects on in situ dry matter disappearance of barley and corn grains when a high-grain diet was fed; however, it had positive effects on dry matter disappearance of roughages and corn grain when a high roughage diet was fed to cattle.

The observation of Tillman et al. (1965), in sheep, that low Mo diets increased ruminal nitrate and blood nitrate concentrations but decreased blood nitrite, suggest that nitrate reductase activity of ruminal microorganisms may be influenced by Mo. However, Buchman (1966) indicated that as little as .9 ppm dietary Mo was adequate to maintain normal activity of nitrate and nitrite reductases of ruminal microorganisms of cattle.

Xanthine oxidase promotes conversion of hypoxanthine, a sub-product of purine metabolism, into the intermediary compound xanthine, which is further converted to uric acid. In parts of New Zealand where soil Mo concentrations were very small, deaths of sheep were associated with occurrence of kidney stones. Analysis of renal calculi indicated that they were formed by xanthine. It was suggested that xanthine precipitated in kidney in consequence of inadequate levels of xanthine oxidase, because of small concentrations of dietary Mo (Bell, 1953; Askew, 1958), but on similar low-Mo pastures in many parts of the world no xanthine calculi formation in sheep has been observed (Anke and Risch, 1989).

Anke et al. (1978), in a series of experiments using semipurified diets containing less than .06 ppm Mo developed Mo deficiency in goats. Weight gains, feed intake and birth rates were smaller and kid mortality was greater in Mo-deficient groups, compared to control animals. Anke and Risch (1989) described a 10-year study with growing, pregnant and lactating goats fed semisynthetic diets which provide information on essentiality of Mo.

Under practical feeding conditions, the problem that generally occurs in consequence of low dietary Mo levels is the induction of Cu toxicosis (Dick, 1956a; Hidiroglou et al., 1984) a condition relatively common in housed lambs, because of the generally small concentrations of Mo in concentrates (Ross, 1966; Pope, 1971; Todd, 1972).

Molybdenum Requirements

No minimal requirements of Mo are suggested or recommended for beef cattle, sheep or dairy cattle by the NRC (NRC, 1984, 1985, 1989).

Anke et al. (1978), based on development of Mo deficiency signs in goats fed semipurified diets, suggested a minimal dietary concentration for ruminants of 60 ppb Mo. In subsequent work, Mo requirement of growing, pregnant and lactating goats was estimated to be less than 100 ppb in the diet (Anke and Risch, 1989). Therefore, there is not a big difference in these suggested values.

Molybdenum Toxicity

The NRC (1980) estimated maximal tolerable Mo concentrations for several animal species at 5 to 10 ppm. The NRC (1984) listed 6 ppm Mo as the maximal tolerable level for beef cattle. For sheep and dairy cattle 10 ppm Mo were indicated as the maximal tolerable level (NRC, 1985, 1989).

Suttle (1988) suggested the terms "molybdenosis" and "hypocuprosis" to be distinguished, reserving the first for pathological defects uniquely attributable to Mo containing anions and explainable by tissue biochemical events independent of Cu. The author however emphasized that there is little evidence in this respect.

Dietary levels of Mo are affected by parent soil material, soil pH, forage type and forage maturity (Reid and Horwath, 1980; Gupta and Lipsett, 1981). Molybdenum in alkaline soils is more available to plants than is that in acid soils (Beeson and Matrone, 1976).

Molybdenum-induced Cu deficiency is an endemic problem in ruminants (Ward, 1978). Cattle are particularly susceptible to antagonistic effects of dietary Mo (Underwood, 1981; Wittenberg and Devlin, 1988). Species differences in tolerance to Mo are substantial; cattle are by far the least tolerant, followed by sheep, while horses and pigs are the most tolerant of farm livestock (Underwood, 1976). Sheep were rarely affected and swine and horses did not show any susceptibility to elevated Mo concentrations, in areas where cattle, especially young animals, suffered severe diarrhea and emaciation (Ferguson et al., 1943; Britton and Goss, 1946; Cunningham, 1946).

Harmful effects of excess dietary Mo have been described for cattle and sheep under natural conditions (Ferguson et al., 1938, 1943; Cunningham, 1946; Cunningham et al., 1953; Kretschmer and Beardsley, 1956; Cunningham and Hogan, 1959; Becker et al., 1965; Alloway, 1973; Kubota, 1975), under experimental conditions (Chapman and Kidder, 1963; Lesperance and Bohman, 1963; Vanderveen and Keener, 1964; Marcilese et al., 1969, 1970; Huber et al., 1971; Kincaid, 1980; van Ryssen and Stielau, 1980a; Humphries et

al., 1983; Phillippo et al., 1987a; Wittenberg and Devlin, 1987, 1988; Van Niekerk and van Niekerk, 1989) and environmental contamination (Gardner and Hall-Patch, 1962; Sas, 1987). Toxic effects have been observed in cows grazing pastures containing up to 160 ppm of Mo (dry basis) (Becker et al., 1965) or fed rations containing 100 to 220 ppm Mo (Huber et al., 1971; Vanderveen and Keener, 1964). No toxicosis was reported in swine continuously fed a diet containing 1000 ppm Mo for 3 months (Davis, 1950; Underwood, 1977).

In diets containing small Cu concentrations, but adequate S, Mo concentrations as little as 1 to 2 ppm are sufficient to induce a severe Cu deficiency in calves (Fischer et al., 1976a; Bremner et al., 1987; Humphries et al., 1988b) and sheep (Dick, 1954b; Suttle, 1973b).

Phillippo et al. (1982), based on a survey investigation, implicated excess Mo intake as a cause of poor reproductive performance in heifers. Soon after, in planned experiments, Mo supplementation caused a significant delay in age at first ovulation of heifers and a significant decrease in pregnancy rate, compared to control animals, Fe supplemented animals or a control group receiving the same amount of feed as that consumed by Mo-treated females (Phillippo et al., 1985). A second experiment confirmed the effects of elevated Mo consumption in delaying puberty of heifers and decreasing conception rate (Phillippo et al.,

1987a). In addition, a significant reduction in pulsatile release of luteinizing hormone was observed. This is thought to be a direct toxic effect of Mo, not a result of a secondary Cu deficiency (Phillippo et al., 1985; 1987a).

In male dairy calves fed increasing amounts of Mo for 129 days the most striking observation was the lack of libido exhibited by the animals (Thomas and Moss, 1951). Histological examination of the testis indicated marked damage to interstitial cells and germinal epithelium with little or no spermatogenesis occurring. Testicular growth and development as determined by weight and size was retarded in calves on a diet containing 295 ppm Mo and 11 ppm Cu for 42 days (Campbell et al., 1976). Van Ryssen et al. (1990), however, concluded that a large Mo intake did not have a deleterious effect on fertility or growth of young rams when fed for up to 14 weeks.

Phillippo et al. (1987b) observed marked effects of Mo supplementation on performance and clinical condition of cattle, including reduced rate of live weight gain, decreased feed intake and reduced feed efficiency.

Bone deformities or connective tissue lesions caused by Mo toxicosis have been described in cattle (Davis, 1950; Cook et al., 1966; Fischer et al., 1976a) and sheep (Hogan et al., 1971b; Pitt et al., 1980; van Ryssen et al., 1986). Molybdenum concentrations in bones of wethers fed 10 to 12 ppm Mo for 7 months in a diet containing 6.1 ppm Cu and .22%

S increased ($P < .01$) relative to control sheep (Hidiroglou et al., 1982). Intraruminal infusion of molybdate in sheep increased concentration of sulfate in ruminal fluid and decreased reduction rate of sulfate to sulfide by 50% (Gawthorne and Nader, 1976).

High level Mo supplementation (34.8 ppm) for 9 weeks, resulted in reduced milk yield of beef cows (Wittenberg and Devlin, 1987). Molybdenum toxicity is increased by additional sulfate (Vanderveen and Keener, 1964; Cunningham and Hogan, 1959; Huber et al., 1971).

Internal administration of ammonium molybdate, through a stomach tube, in a single dose of 1000 mg Mo/kg live weight resulted in elevated Mo concentrations in tissues of sheep, especially in kidney, lung and liver (Arzumanyan, 1986).

Copper x Molybdenum x Sulfur Interactions

The existence of an antagonism between Cu and Mo was first noticed by Ferguson et al. (1943), in cattle grazing pastures with large Mo concentrations. Soon after, Dick and Bull (1945) showed that, when intake of Mo by sheep fed a normal diet was increased by 10 or 100 mg/day, Cu concentration in liver was significantly reduced. Dick (1954b) observed that Mo had a severely limiting effect on Cu accumulation in liver, but this effect was only manifested when the diet also contained a sufficient

quantity of SO_4. The complexity of the Cu x Mo x S interrelationship was summarized 35 years ago by Dick (1956a):

> Although for any particular diet there is a quantitative relationship between the Mo content of the diet and the amount of Cu stored by the animal and for a given Cu intake sheep could be either in positive or negative Cu balance, depending on Mo intake, the magnitude of the effect of Mo is, in turn, dependent on the amount of inorganic sulfate in the diet. (1956a, p. 233)

The extreme range in Cu concentration of plants is probably from 1 to 20 ppm (dry matter) and under most circumstances Cu concentration of animal diets is from 7 to 14 ppm (Todd, 1976). On the other hand, Mo concentration of plants, although generally in the range of 1 to 3 ppm, can vary from less than .1 to 100 ppm (Todd, 1976). In many disorders which are related to Cu status of the animal, the dominant feature is the Mo status of the animal's feed, particularly in ruminants, and other constituents of the feed, mainly S, play an important role (Todd, 1976).

The important features of the Cu x Mo interaction are that it occurs only in ruminant animals, its incidence is dependent on an adequate dietary S supply, and no major disturbances in Cu metabolism occur if molybdate is administered parenterally or is introduced into the gastrointestinal tract distal to the rumen (Bremner and Mills, 1986). The interaction is physiologically significant only in ruminants, for it is in these animals

that oxidized forms of organic and inorganic S in the diet are converted to sulfide by the activity of microorganisms before reaching the intestines (Gawthorne, 1987).

There is a peculiar dose-dependence of the interaction and an apparent existence of separate syndromes at small and large Mo intakes (Bremner and Mills, 1986; Gawthorne, 1987).

At small dietary Mo concentrations (1 to 5 ppm) there are gradual reductions in plasma and tissue Cu concentrations and in activities of Cu-dependent enzymes. Clinical lesions resemble those found in simple Cu deficiency. These effects are prevented by Cu supplementation (Bremner and Mills, 1986).

The second syndrome, typified by "teart scours," is associated with much greater Mo intakes, usually > 25 ppm Mo in diet, and is characterized by rapid onset of severe diarrhea, growth failure and severe debilitation. Although the condition is ameliorated by administration of large amounts of Cu, signs appear before Cu reserves are depleted (Bremner and Mills, 1986). In this case, Mo seems to exert toxic effects in its own right, in addition to secondary effects of Cu deficiency (Gawthorne, 1987).

Copper metabolism may be affected even by very small dietary Mo concentrations, as long as adequate S is present (Gawthorne, 1987). Concentrations as little as 1 ppm dietary Mo affected Cu metabolism in both cattle (Simpson et al., 1981) and sheep (Suttle, 1973b). Dick (1953a) found

that when S intake of sheep was 2 g/day and Cu 7 to 10 mg/day, as little as .5 mg Mo/day significantly limited Cu storage. In pastures containing .15% S and less than 5 ppm Cu, 1.8 ppm Mo affected Cu metabolism in young cattle (Fisher et al., 1976a). Supplementation of 2 ppm Mo in a diet containing 4 ppm Cu and .28% S was sufficient to severely reduce tissue Cu reserves of calves (Bremner et al., 1987). Addition of 2 ppm Mo to a diet containing 4 ppm Cu, .1 ppm Mo and .28% S reduced growth rate of calves by 15% and reduced liver and plasma Cu to levels indicative of severe Cu deficiency (Humphries et al., 1988b).

Van Ryssen and Stielau (1980b) pointed out that differences in dietary S concentrations could explain why different ratios of Cu:Mo, for example, 2:1 (Miltimore and Mason, 1971), 5:1 (Alloway, 1973) and 7:1 (Case, 1974) have been suggested as safe limits against molybdenosis.

Severity of Cu deficiency is greater if concentrations of both dietary Mo and S are increased (Bremner and Davies, 1980). An increased intake of S alone, however, can reduce liver and blood Cu concentrations in sheep (Wynne and McClymont, 1956; Hill and Williams, 1965; Spais et al., 1968; Suttle, 1974b; Suttle and McLauchlan, 1976).

Ivan et al. (1986) reported greater concentrations of Cu in plasma and liver in fauna-free rams than in faunated rams. Absorption and retention of Cu was 38% to 50% greater in fauna-free than in faunated sheep and the authors

suggested that rumen ciliate protozoa increased ruminal production of sulfide, which complexed part of the Cu, making it unavailable for absorption and utilization. Ivan (1988) observed that faunated lambs had smaller ($P < .01$) final hepatic Cu concentrations, smaller ($P < .01$) total liver Cu per unit of Cu intake and smaller ($P < .01$) ruminal Cu solubility, than fauna-free rams, when fed both low and high Cu diets.

Sulfur compounds are broken down extensively by ruminal microflora to yield sulfide (Lewis, 1954; Anderson, 1956; Spais et al., 1968) and it has been suggested that subsequent formation of insoluble Cu sulfide in rumen decreased availability of dietary Cu (Dick, 1954b; Hartmans and Bosman, 1970). It was first thought that only inorganic S interacted with Cu and Mo (Dick, 1953a,b, 1954a,b) but Cook et al. (1966) and Suttle (1973a, 1974b, 1975b) indicated that both organic and inorganic S were equally effective in the antagonism. Suttle (1974b) observed that both inorganic and organic S supplementation increased ruminal sulfide considerably ($P < .001$) in sheep and to a similar extent. Ruminal sulfide (S^{--}) concentrations increased after ingestion of a single meal, exceeding those of continuously fed animals; there was a negative relationship between response in plasma Cu and "resting" ruminal sulfide (Suttle and Peter, 1985). According to Hartmans and Bosman (1970), Mo promotes reduction of S

compounds to sulfide in rumen, but Gawthorne and Nader (1976) reported an increase in sulfide concentration in ruminal fluid in spite of a 50% decrease in rate of sulfite production, when molybdate was infused into the rumen. According to these authors, the slower rate of sulfide production did not lead to a smaller concentration of sulfide because it was compensated by a second action of molybdate to inhibit rate of apparent absorption of sulfide from rumen. Huisingh et al. (1975) found that dietary Mo (50 ppm) inhibited production of sulfide from sulfate in the rumen of sheep fed purified diets, but enhanced production of sulfide from methionine.

Several hypotheses involving the Cu x Mo and the Cu x Mo x S relationship have been proposed over the last 35 years. Mason (1978), Suttle (1980), Mason (1982), Mills (1985), Bremner and Mills (1986), Mason (1986, 1990) and Suttle (1991) reviewed the subject.

Dick (1956b) proposed that in ruminants transport of Mo across membranes was blocked by SO_4 and that Cu transport was impaired during this process. Dowdy and Matrone (1968a,b) hypothesized that Mo formed complexes with Cu and that Cu bound in this state was biologically inactive, but this did not take into account the effect of S. Miller et al. (1970) agreed with this hypothesis and suggested that the complex was formed in the rumen. Matrone (1970) hypothesized that it involved an interaction between the Cu-

Mo complex and an unspecified intermediate of S metabolism in the rumen, which formed a triple unabsorbable complex of Cu, Mo and S. Formation of CuS in the animal, possibly in the gastrointestinal tract, was proposed by Dick (1954b), Hartmans and Bosman (1970) and Suttle (1974b). Cupric sulfide was found to be largely unavailable for rats (Schultze et al., 1936) and sheep (Suttle, 1974b). However, there is no direct evidence that appreciable amounts of Cu are present in the alimentary tract as CuS (Bremner and Davies, 1980). Huisingh et al. (1973) proposed that Cu became unavailable through formation of cupric molybdate and cupric sulfide. Suttle (1974c) proposed that Cu is made unavailable by a Cu x Mo x S complex, cupric thiomolybdate, in rumen, after sulfate has been converted to sulfide by ruminal bacteria. Finally, Dick et al. (1975) studied properties of di-, tri- and tetrathiomolybdates in vitro and in vivo with rabbits, rats and sheep. The authors proposed that the mechanism of action whereby molybdate and sulfate in the diet limit absorption and storage of Cu in liver of ruminant animals is, first, reduction of sulfate to sulfide by ruminal microorganisms; secondly, reaction of this sulfide with molybdate to form thiomolybdates which in turn combine with Cu to form insoluble Cu thiomolybdates, thereby limiting absorption of dietary Cu. Copper combined with thiomolybdates is not available for absorption. Also, thiomolybdate not combined with Cu in the rumen is absorbed

and mobilizes tissue Cu, giving rise to elevated blood Cu values. This complex is insoluble in TCA and its magnitude, for any given Cu intake, is related to Mo intake (Dick et al., 1975). This has been known as the "thiomolybdate hypothesis."

The proposition of Dick et al. (1975) did not explain the Cu x Mo x S interaction in non-ruminant species, because of its limited capacity for sulfide production in the gastrointestinal tract. So, it remained for Mason (1978), to propose that thiomolybdate might be formed in tissues.

The thiomolybdate hypothesis depended upon confirmation of ruminal synthesis and absorption of thiomolybdates and an enormous research effort has been placed in this direction. Duodenal administration of ^{99}Mo-molybdate in sheep was followed by rapid absorption and excretion, via urine, of most of the dose administered resembling the pattern seen in orally dosed non-ruminant animals (Mason et al., 1978). Passage of ^{99}Mo-molybdate through the rumen profoundly modified absorption and excretion patterns. Absorption was decreased and delayed. Particularly at large S concentrations, most radioactivity remained in feces (Mason et al., 1978). Mills et al. (1978) failed to detect thiomolybdate formation in cultures of ruminal microorganisms through spectrophotometric examination. Clarke and Laurie (1980) indicated that under physiological conditions, di- and trithiomolybdates would form in the

rumen more readily than tetrathiomolybdate. In rats given Mo as molybdate (MoO_4^{--}), either alone or with S^{--}, no effect on absorption of orally administered ^{64}Cu was detected (Mills et al., 1981). In contrast, in rats administered an equivalent quantity of Mo as tetrathiomolybdate (MoS_4^{--}) there was a marked decrease ($P < .001$) in ^{64}Cu absorption (Mills et al., 1981). Mason et al. (1982a), using ruminal infusion, intravenous injection and addition to plasma of ^{99}Mo-labelled molybdate and ^{99}Mo-labelled tetrathiomolybdate, demonstrated with gel filtration and chromatography thiomolybdate synthesis and absorption in vivo in sheep. However, they failed to detect ^{99}Mo-tetrathiomolybdate in any of plasma samples examined after ^{99}Mo-molybdate infusion and most ^{99}Mo in plasma were identified as di-and trithiomolybdate. In rats, in contrast to the effects of tetrathiomolybdate, neither dithiomolybdate ($MoO_2S_2^{--}$) nor trithiomolybdate ($MoOS_3^{--}$) inhibited Cu absorption from the digestive tract or induced biochemical or clinical signs of Cu deficiency (Bremner et al., 1982). Spectrophotometric examination of aqueous supernatants of in vitro cultures of bovine ruminal contents incubated with molybdate and potential sources of S^{--}, and samples drawn directly from the rumen of steers fed high Mo diets yielded evidence of presence of thiomolybdates, but tetrathiomolybdate rather than trithiomolybdate was the predominant thiomolybdate species present in the aqueous phase (El-Gallad et al.,

1983). Tri- and tetrathiomolybdate were rapidly absorbed from the rumen and small intestine to circulate in plasma in a protein-bound and in a TCA-insoluble form (Kelleher et al., 1983). Suttle and Field (1983) found that, at large dietary Mo and S concentrations, sufficient tetrathiomolybdate was formed in the rumen of lambs and complexed with Cu to impair its absorption, but formation of dithiomolybdate did not play any important role in the Cu and Mo antagonism. Intravenously infused ^{99}Mo- and ^{35}S-labelled tri- and tetrathiomolybdates were identified in plasma of sheep bound to the Cu fraction insoluble in TCA and most ^{99}Mo and ^{35}S were associated with albumin (Hynes et al., 1984). Hynes et al. (1985) detected di- and trithiomolybdates, labelled with ^{99}Mo, in plasma of cattle after administration of molybdate into the rumen. The proportion of soluble Mo in the rumen of lambs was much greater for a high Mo diet than for a low Mo diet, however both Mo and Cu became predominantly insoluble in lambs fed diets supplemented with cupric sulfate (Ivan and Veira, 1985). Price and Chesters (1985) showed that factors limiting Cu availability were largely associated with digesta solids. After molybdate injection directly into rumen of sheep, thiomolybdates displaced from ruminal, duodenal and ileal solids were predominantly tri- and tetrathiomolybdates (Price et al., 1987). Whether injected into the rumen as molybdate or tetrathiomolybdate, forms

that appeared in plasma were mainly di- and trithiomolybdate (Price et al., 1987). The authors suggested that Cu absorption is likely inhibited by tri- and tetrathiomolybdate, while post-absorptive effects on Cu metabolism are probably caused by di- and trithiomolybdates. These results (Price et al., 1987) provide good direct evidence for ruminal synthesis of thiomolybdates under conditions similar to those found in the field since sheep were fed a diet containing 6.2 ppm Mo and .43% S.

The solid phase of ruminal digesta consisting of microorganisms and plant material plays a major role in the interaction among Cu, Mo and S, and thiomolybdates may be intermediate reactants only, in the formation of macromolecular Cu chelators (Allen and Gawthorne, 1987).

Allen and Gawthorne (1988) proclaimed that the "thiomolybdate hypothesis" needed to be modified and postulated that formation of protein-tetrathiomolybdate complexes distorts equilibrium between physiological Cu chelators of transport processes and apoenzymes. According to the authors, Cu is diverted away from metabolically useful forms in gut and tissues, and into abnormal protein-tetrathiomolybdate-Cu complexes that accumulate in the kidney or are excreted in the gut.

Recent results indicate that there are probably two sites of interaction, gut and tissues. At the first site, tetrathiomolybdate and trithiomolybdate are likely to be

involved in reducing Cu absorption, whereas in body tissues trithiomolybdate is probably the most important species (Mason, 1990).

The principal mechanism by which the interaction depletes grazing animals of Cu is formation of unabsorbable complexes with trithiomolybdate and tetrathiomolybdate in rumen and their irreversible binding to solid phase of digesta. When Mo is present in excess of Cu in S-rich diets, sufficient trithiomolybdate may be absorbed to inhibit cuproenzyme activity in gut and peripherally (Suttle, 1991).

Copper and Molybdenum Bioavailability Studies

While bioavailability of Fe and Zn has received considerable attention in recent years, that of Cu has been relatively neglected (Anonymous, 1986). This is, in part, because of a lack of techniques for assessing Cu bioavailability (Anonymous, 1986). Bioavailability of Mo has not even been mentioned, most certainly because no deficiencies have been detected in practical-type diets. A few references found in the literature regarding Mo sources are a repetition of a statement found in Underwood (1977), concerning absorption of ammonium and sodium molybdate. A thorough study of Mo bioavailability has not been made (Winston et al., 1985).

Definition and Expression of Bioavailability

Many different definitions of bioavailability can be found in the literature and there is a great deal of disagreement among many of them, so that recently Ammerman et al. (1989) stated that the study of mineral bioavailability has been hindered through the years due to the lack of agreement concerning the definition of bioavailability and a suitable method of determination.

Within some of the definitions found in the literature, two distinctive groups appear evident, one that demands the measurement of nutrient utilization, the other not necessarily. Utilization is the process of transport, cellular assimilation and conversion to a biologically active form (O'Dell, 1984a).

Some of the definitions in the first group are those of Thompson (1965), Fox et al. (1981), Fairweather-Tait (1987) and O'Dell (1989, 1990b) and in the second group, Miller (1980), Miller (1981), Forbes and Erdman (1983), Suttle (1986), Bender (1989) and Corah and Ives (1991).

In the study of mineral metabolism it is generally recognized that total content of an element in a particular dietary source has little significance unless it is qualified by a factor indicating biological utilization of the element (Thompson, 1965). Bioavailability is a quantitative measure of utilization of a nutrient under specified conditions to support an organism's normal

structure and physiological processes (Fox et al., 1981). The definition of bioavailability proposed for all nutrients is that it is a measure of the proportion of the total in a feedstuff or diet that is digested, absorbed and metabolized by normal pathways (Fairweather-Tait, 1987). Bioavailability refers to the proportion of an ingested nutrient that is absorbed and utilized (O'Dell, 1984a, 1989, 1990b).

Bioavailability of a mineral element implies the availability of that element to some organism for body use (Miller, 1980). Bioavailability of trace elements refers to that portion which can be utilized by the animal to fulfill the functions for which the element is needed (Miller, 1981). Forbes and Erdman (1983) considered bioavailability to reflect the efficiency with which consumed elements are absorbed from the gastrointestinal tract and are thus available for storage or use. Availability is the proportion of dietary Cu which is absorbed (Suttle, 1986). Bender (1989) defined bioavailability as the proportion of a nutrient capable of being absorbed and available for use or storage, or, more briefly, the proportion of a nutrient that can be utilized. Bioavailability is defined as the absorbability of the nutrient by the animal (Corah and Ives, 1991).

Bender (1989) criticized Fairweather-Tait's (1987) definition in that it is not clear why both terms, digestion

and absorption are included. If the nutrient is present in an absorbable form it does not need digestion and materials that are hydrolysed in the intestine are not necessarily absorbed (Bender, 1989). Substances which are absorbed are not necessarily metabolized (Bender, 1989). If an individual is unable to metabolize the nutrient for genetic reasons or because of a disorder or interference by drugs this is an individual physiological effect and not a property of the food (Bender, 1989).

Bender (1989) also brought into consideration that if the definition of bioavailability includes utilization, this means that above a certain intake of protein there is an absolute limit to its bioavailability since amino acids in excess of immediate use are wasted by oxidation to provide energy.

Many techniques and indices are employed in assessing bioavailability, and quantitative data may be expressed either on an absolute basis or with reference to a standard nutrient source of high availability (Forbes and Erdman, 1983). In general, bioavailability of an element in a particular source of that element is determined relative to its functional availability from a standard source (Miller, 1980). Fox et al. (1981) defined relative bioavailability value as the bioavailability of a test source of a nutrient, expressed as a percentage of the value obtained when the

nutrient is fed in the form of a reference material, a substance that contains the nutrient of interest.

Factors that Affect Bioavailability

Both intrinsic and extrinsic factors affect mineral bioavailability (O'Dell, 1983). Intrinsic factors are animal species, genotype, age, sex, metabolic function, physiological stress, intestinal microflora, infection and physiological state (Miller, 1980; Miller, 1981; O'Dell, 1983; Kincaid, 1988a). Extrinsic factors are chemical form, solubility of the element species, state of oxidation of the element, particle size, concentrations of interacting elements in diet, chelation and processing (Fritz, 1976; Miller, 1980; O'Dell, 1983; Kincaid, 1988a). Also, adsorption of minerals to macronutrients, binding of minerals to other components, reduction and oxidation reactions may take place (van Dokkum, 1989).

Methods of Determining Bioavailability

Fox et al. (1981) referred to primary and secondary indices of response. Primary indices of response include quantifiable levels of morphology or physiological function that indicate health status, like measures of growth (height, weight, head circumference, etc.), skeletal development (bone size, conformation, and mineralization), hematopoiesis, circulatory function, etc. (Fox et al.,

1981). Secondary indices of response are quantifiable responses that are not direct measures of health status but must be correlated with primary indices under defined conditions, like whole body retention of elements or the levels of inorganic elements, metabolites, enzymes, or hormones in tissue, body fluids, or excretory products (Fox et al., 1981).

According to Miller (1980), bioavailability of a mineral element from a basal ingredient or from supplemental forms of the element may be measured by total body retention, specific tissue incorporation or specific compound synthesis. Ideally, a full assessment of mineral availability from a diet or food should include the measurement of total content, an estimate of the proportion of the total that is in an absorbable form, the actual amount absorbed and, finally, the proportion of the total which is utilized by the body (Southon et al., 1988).

Research results on bioavailability of different Cu compounds present a confused and often conflicting picture; a major reason for this centers around methodology difficulties (Miller, 1981). Most researchers agree that conventional balance techniques are inadequate for determination of Cu bioavailability (Dick, 1969; Mills and Williams, 1971; Suttle, 1974a; Miller, 1981; Bedi and Chesters, 1982; Suttle, 1983a; Lo et al., 1984). This stems from the fact that, of the amount of Cu ingested daily by

sheep, at least 95% is excreted in feces, and virtually none is excreted in urine (Dick, 1969). Urinary output may account for only 1% to 2% of total excreted in sheep and relatively minor errors in determination of fecal Cu content may affect the decision as to whether an animal is at zero, negative or positive Cu balance (Mills and Williams, 1971).

Similar technical problems are associated with attempts to determine endogenous excretion of some trace metals into the gastrointestinal tract using isotopic techniques (Mills and Williams, 1971). Smith et al. (1968) described such difficulties during attempts to determine endogenous excretion of ^{64}Cu into the gastrointestinal tract of sheep fed low and high Mo diets.

The radioactive isotopes of Cu used most frequently are ^{64}Cu and ^{67}Cu. The half-life of ^{64}Cu is only 12.8 hours, so that use of this isotope is limited to short-term studies (less than 4 days). The other isotope, ^{67}Cu, has a longer half-life, 61.7 hours, permitting studies of slightly longer duration, of up to 18 days (Allen and Solomons, 1984). This last isotope, ^{67}Cu, has a somewhat longer half-life, but it is expensive and has been available from only very few suppliers (Danks, 1988). In studies with ruminants, the short half-lives of radio isotopes of Cu are a distinct disadvantage, because biological half-lives of some aspects of Cu metabolism greatly exceed physical half-life (Gooneratne et al., 1989a). For example, half-life of Cu

stored in liver was found to be 72 days and 111 days in two trials with dairy cows, as determined with use of stable isotope ^{65}Cu (Buckley et al., 1985).

Use of stable isotopes of Cu eliminates the problem of short physical half-life of Cu radioisotopes. Unfortunately, natural abundance of ^{65}Cu in foods and therefore in biological samples is 31%, so that a large dose of ^{65}Cu is needed relative to dietary Cu in order to produce a measurable change in the ratio of added isotope to naturally occurring isotope (Allen and Solomons, 1984). Also, mass spectrometry determinations are very expensive.

Absorption is one way to estimate bioavailability and sometimes bioavailability has been defined as the amount absorbed (Suttle, 1986; Corah and Ives, 1991). The element must be absorbed before it can be used in a functional way, but absorption is not synonymous with bioavailability (Miller, 1981; Southgate, 1989). An example of such a case is 3,5-diiodosalicylic acid. Iodine from this compound is well absorbed by cattle and sheep but it is poorly utilized (Aschbacher et al., 1963, 1966; Aschbacher, 1968; Aschbacher and Feil, 1968). Also, some Fe chelates are well absorbed but do not contribute to metabolism or nutritional status (Southgate, 1989).

In a case like that of Cu, in which percentage of absorption is small, Miller (1981) recommended measurement of tissue concentrations. Concentrations of inorganic

elements in tissue and body fluids often provide the most versatile means of assessing status and bioavailability over a wide range of intakes, from deficiency to beyond requirement (Fox et al., 1981). Hepatic concentration and total content of Cu has been the main index of response used in determination of bioavailability of Cu sources. Even O'Dell (1990b), who has so emphatically indicated the need of measurement of the nutrient's utilization when determining its bioavailability admitted that liver serves as the chief storage organ for Cu and thus liver Cu concentration is probably one of the best indexes of Cu status. Lee et al. (1988) started their paper on Cu bioavailability in rats declaring that response indexes such as growth, hemoglobin concentrations, whole body Cu retention, liver and serum Cu concentrations do not represent utilization of Cu and that parameters that might be useful in measuring Cu bioavailability are activities of serum ceruloplasmin and liver Cu-Zn superoxide dismutase. The authors, however, ended up stating that, to avoid the more time consuming liver superoxide dismutase assay, liver Cu could be used to assess Cu status.

Amine et al. (1972) described a slope-ratio assay for estimation of biological availability of dietary Fe sources for rats and chicks. Statistical procedures have been discussed by Finney (1978). The same slope ratio technique has been used by Lo et al. (1984), for Cu from soybean

protein, in rats; Wong-Valle (1988), for manganese from inorganic sources, in chicks and sheep; Baker et al. (1991), for Cu sources in chicks; and Ledoux et al. (1991), for Cu from inorganic sources, in chicks.

Another point of disagreement in Cu availability studies is dietary level of the nutrient at which its bioavailability should be determined. According to Miller (1980), in all determinations of relative bioavailability the basal diet must be low in the test element and O'Dell (1990b) stated that Cu bioavailability is best determined by use of criteria that effectively assess nutritional status when intake is somewhat limiting. Biological availability of Cu to non-ruminants has frequently been assessed from responses of initially Cu-deficient animals to repletion with different sources of Cu (Suttle, 1974a; Lo et al., 1984).

Techniques for measuring biological availability of Cu using a Cu-depletion followed by a Cu-repletion have been proposed by Suttle (1974a), in sheep, and Price and Chesters (1985), in rats. The latter study involved determination of increase of cytochrome oxidase in duodenal mucosa of rats to assess distribution of available Cu of different sources in rumen, duodenal and ileal digesta of sheep. Depletion periods prior to Cu supplementation were also used in cattle by Wittenberg and Boila (1988) and Wittenberg et al. (1990). The repletion technique, however, has the drawback that it

can only be used on hypocupraemic animals and it is not certain that response of animals which are marginally deficient in a trace element will always be representative of animals of normal metal status (Bremner and Davies, 1980).

Black et al. (1984a,b), proposed a technique for estimating manganese bioavailability based on tissue uptake during short-term and high-level dietary supplementation. The technique has already been applied to Cu, in chicks (Ledoux et al., 1989a, 1991). One reason to use large dietary Cu levels with sheep may be the large variation in hepatic Cu concentrations noticed in both cattle and sheep (Miltimore et al., 1978; van Ryssen and Stielau, 1980a; Ivan and Veira, 1985; Kincaid et al., 1986; Ledoux, 1987; Kincaid, 1988a,b) which prevented detection of small treatment differences (Kincaid, 1988a,b; Ivan et al., 1990). The greater the amount of Cu in diets, the greater the hepatic Cu concentration of lambs (Dick, 1954b; Goodrich and Tillman, 1966b; Saylor and Leach, 1980; Buckley and Tait, 1981; Zervas et al., 1990), therefore differences among sources may be more easily detected at greater dietary concentrations.

Bioavailability of Copper Sources

According to Nelson (1988), there is a lack of meaningful data concerning biological availability of Cu

sources. Much more information on Cu availability exists for non-ruminants than for ruminants. Copper bioavailability studies in poultry, rats and swine were recently reviewed by Ledoux (1987).

Several Cu sources have been tested in ruminants. Oral administrations of cupric carbonate, cupric chloride, cupric nitrate and cupric sulfate were somewhat similarly effective in elevating blood and plasma Cu concentrations in sheep (Lassiter and Bell, 1960) and cattle (Chapman and Bell, 1963), but cuprous and cupric oxide powder were much less available forms (Lassiter and Bell, 1960; Chapman and Bell, 1963; Kincaid, 1988b). Cupric sulfate was as effective as Cu-glycinate, a chelated Cu-EDTA, a Cu-amino acid chelate, or a Cu- proteinate in ability to change hepatic Cu concentrations in sheep and cattle (MacPherson and Hemingway, 1968; Miltimore et al., 1978; Kincaid, 1988b; Wittenberg et al., 1990).

Lassiter and Bell (1960) performed a classical work of Cu availability for sheep using ^{64}Cu-labelled inorganic compounds. Sources evaluated in three experiments were cupric sulfate, cupric chloride, cupric nitrate, cupric carbonate, cuprous and cupric oxide powder, cupric oxide needles and copper wire. After oral or intravenous administration of the compounds, radioactivity was measured in whole blood, plasma, urine and feces. The authors indicated a tissue preference for the sulfate form over the

chloride and nitrate. Chapman and Bell (1963), also by radioisotope technique, evaluated absorption and excretion of cupric carbonate, cupric nitrate, cupric sulfate, cupric chloride, cupric oxide (powder and needles), cuprous oxide and copper wire, by cattle. Cupric carbonate had the greatest rate of absorption, but also the greatest rate of excretion in urine and feces. Cupric sulfate ranked third in absorption rate, sixth in urinary excretion and fifth in fecal excretion, indicating a favorable retention in tissues, a finding similar to that of Lassiter and Bell (1960) in sheep. Kincaid et al. (1986), in a comparison of cupric sulfate and a Cu-proteinate, using calves, observed that animals fed proteinate had greater ($P < .05$) amounts of Cu in liver at 12 weeks of feeding than those fed the sulfate (325 and 220 ppm, respectively), but neither of those groups differed from unsupplemented animals (238 ppm). Kincaid (1988a) evaluated Cu availability from cupric sulfate, a Cu-amino acid chelate and a Cu-proteinate, measuring liver Cu uptake in calves fed Mo in different forms (hay with low Cu:Mo ratio, ammonium molybdate in feed, tetrathiomolybdate, and ammonium molybdate in water). Cupric sulfate was effective in promoting hepatic Cu uptake, except when Mo was in forage. The Cu-proteinate seemed to be more effective than cupric sulfate, but the Cu-amino acid chelate was not. Kincaid (1988b) reported a Cu bioavailability study from cupric sulfate, cupric oxide and

a Cu-amino acid chelate, in calves, concurrently fed additional Mo. Cupric sulfate was more effective for maintaining plasma TCA-soluble Cu than cupric oxide, when tetrathiomolybdate was simultaneously fed. When inorganic Mo was concurrently fed, cupric sulfate was comparable to the chelate for maintaining plasma and liver Cu concentrations. Cupric sulfate supplementation increased ($P < .05$) liver Cu concentrations for steers consuming a high Mo diet, containing either small or large S concentrations, relative to steers receiving no Cu supplement, cupric oxide needles or injectable cupric oxide (Wittenberg and Boila, 1988). Charmley and Ivan (1989), reported hepatic Cu content increases of 48%, 57% and 61% in wethers fed 29 ppm additional Cu as cupric acetate, cupric sulfate and cupric chloride, respectively, but differences were not significant. Ivan et al. (1990), reported that both cupric sulfate and cupric chloride added to barley or grass silage diets increased ($P < .05$) final hepatic Cu concentrations in cows and there were no differences ($P > .05$) between sources. Poole et al. (1990) found that Cu from pig slurry and cupric sulfite incorporated into the diet of sheep were equally available, as evaluated from increase in hepatic Cu concentrations, but they were less available than Cu from cupric sulfate. Wittenberg et al. (1990) compared cupric sulfate and a Cu-proteinate in Cu-depleted steers fed corn silage and barley concentrate diets with added Mo. No

differences were found between these two Cu supplements for rate of liver Cu repletion or ability to reduce plasma and liver Mo concentrations.

Copper Availability versus Age of Sheep

The development of a functional rumen results in a marked reduction in dietary Cu utilization in lambs (Suttle, 1975a). Availability of Cu was apparently almost complete in lambs shortly after birth, declining to about 30% if milk-feeding was continued to 50 days of age (Suttle, 1975a). The mean availability of Cu to milk-fed and early-weaned lambs was 75% and 8%, respectively (Suttle, 1975a). Mean Cu availability decreased from 71% to 47% immediately before weaning and to 10.8% 15 days after weaning (Suttle, 1975a). Copper availability was greater than 90% when young lambs were fed a milk replacer; as the amount of pasture eaten was increased availability of Cu gradually decreased to 9%, for an all pasture diet (Grace and Watkinson, 1988). When rumen was by-passed by abomasal dosing, mean Cu availability in lambs was 21%, compared with 3.7% for lambs dosed intraruminally (Suttle, 1975a).

Modifications of dietary components by microbial activity in ruminal fluid is the major factor governing change in availability of Cu (Wittenberg et al., 1990). Price and Chesters (1985) demonstrated that relative availability of Cu for rats in undigested dried grass (75%)

was substantially greater than in ruminal digesta (12%) of sheep consuming the same grass.

Effects of Molybdenum and Sulfur on Copper Availability

Small increases in herbage Mo and S concentrations will cause major reductions in Cu availability (Suttle, 1986). Dietary Cu can react with other elements in rumen resulting in reduced availability. Two well-studied interactions are the interaction of sulfide with Mo in rumen to produce thiomolybdate, which reacts with Cu to produce an insoluble complex, and formation of insoluble CuS in rumen (Mason, 1981). The Cu x Mo x S interaction is described in a separate section.

Bioavailability of Molybdenum

Bioavailability of Mo would be best represented by a blank page. No specific studies on determination of biological availability of Mo sources have been reported in the literature. According to Winston (1981), essentially nothing is known of availability and fate of forms of Mo in food.

The only studies in which more than one Mo source were used are those of Fairhall et al. (1945) and Cook et al. (1966). Fairhall et al. (1945) evaluated toxicity of Mo from molybdenite, molybdenum trioxide, ammonium molybdate and calcium molybdate fed to rats, guinea pigs and rabbits.

Cook et al. (1966) compared organic and inorganic Mo fed to rabbits.

There is a lack of convenient and inexpensive radioisotopes of Mo that would be suitable for long-term studies (Mason et al., 1989). Molybdenum-99 is a short-lived and relatively inconvenient radioisotope (Mason et al., 1989).

CHAPTER 3
RELATIVE BIOAVAILABILITY VALUES OF COPPER IN INORGANIC SALTS AND IN A COPPER-LYSINE COMPLEX FOR LAMBS: EXPERIMENTS 1, 2 AND 3

Introduction

Copper is well defined as an essential element and, as such, it is involved in a great number of metabolic processes. Copper deficiencies in grazing ruminants have been found worldwide (Underwood, 1981; McDowell, 1985). Concurrently, Mo-induced Cu deficiencies in cattle related to environmental contamination with Mo near Mo processing factories (Hornick et al., 1977; Parada, 1981), mining activities (Vlek and Lindsay, 1977), motor oil spills (Sas, 1987), or in the vicinity of oil refineries (Gardner and Hall-Patch, 1962) or to pelleted feed containing high levels of Mo (Lloyd et al., 1976) have also been described. Molybdenum induced hypocuprosis under natural conditions on pasture has been reported for sheep (Ferguson et al., 1938, 1943; Hogan et al., 1971b; Alloway, 1973) and cattle (Cunningham, 1946; Cunningham et al., 1953; Becker et al., 1965).

Copper compounds have been used worldwide as dietary supplements to overcome deficiencies caused by naturally low

Cu concentrations in feeds or forages or deficiencies induced by elevated dietary Mo levels.

The relative biological availability of the desired element in a compound or supplement is one of the major considerations in selection of a suitable source of the element (Ammerman and Miller, 1972). Little attention, however, has been given to Cu bioavailability, in part because of the lack of techniques for its determination (Anonymous, 1986).

A bioavailability assay, using tissue mineral uptake during short-term high-level elemental supplementation as the bioassay criterion, was suggested by Black et al. (1984a,b), for inorganic Mn sources. Ledoux et al. (1989b), working with chicks, indicated that hepatic Cu uptake could be a useful bioassay criterion for estimation of Cu bioavailability from inorganic sources.

The accumulation of Cu in the liver of sheep fed high dietary Cu concentrations for 10 days was linear; therefore, Ledoux (1987) suggested a 10-day bioassay to estimate relative bioavailability of Cu sources for sheep, using slope ratio comparisons.

The association of Cu to amino acids, in the form of chelates or complexes, has been claimed to increase the availability of Cu (Kirchgessner and Grassmann, 1970; Ashmead et al., 1985; Kratzer and Vohra, 1986), but there are conflicting reports regarding their advantage compared

to cupric sulfate for ruminants (Miltimore et al., 1978; Kincaid, 1988a,b; Wittenberg et al., 1990) and chicks (Baker et al., 1991). Copper-amino acid chelates or complexes have been available in the market in recent years. Since their cost is greater than the commonly used inorganic sources, the inclusion of a copper-lysine complex in the study was thought to be appropriate.

The present study was undertaken to determine the relative biological availability of inorganic Cu compounds and a copper-lysine complex, based on their hepatic Cu accumulation following short-term high-level feeding to lambs. In the first experiment, cupric carbonate, cupric oxide and cupric sulfate were compared to the standard source, cupric chloride. The second experiment was almost a replication of the first experiment, except for the substitution of cupric carbonate with cupric acetate. In the third experiment, a copper-lysine complex was compared to cupric sulfate.

Materials and Methods

Experiment 1

Thirty-five Texas crossbred wether lambs weighing from 36 to 48 kg (average ± standard deviation = 40.8 ± 2.7 kg), initially, were randomly assigned to one of the following seven treatments:

A. control, fed the basal diet with no additional Cu;

B. basal diet + 60 ppm Cu, as cupric chloride;

C. basal diet + 120 ppm Cu, as cupric chloride;

D. basal diet + 180 ppm Cu, as cupric chloride;

E. basal diet + 120 ppm Cu, as cupric carbonate;

F. basal diet + 120 ppm Cu, as cupric oxide;

G. basal diet + 120 ppm Cu, as cupric sulfate.

Five animals were assigned to each treatment.

The cupric chloride ($CuCl_2 \cdot 2H_2O$) was a reagent grade source and the cupric carbonate, cupric oxide and cupric sulfate were feed grade compounds. The Cu sources were added to the basal diet on an as-fed basis at the expense of corn starch. The basal diet (Table 3-1) contained 8.3 ppm Cu and 1.6 ppm Mo (dry matter basis), by analysis, and it was formulated to meet the nutrient requirements of sheep (NRC, 1985). The Cu content of the experimental diets was verified by chemical analysis.

Initially, all lambs were housed in two large covered floor pens and group-fed for 14 days a corn-soybean meal-cottonseed hulls basal diet at an amount selected to ensure an average daily intake of about 1 kg/animal.

After the adaptation period to the basal diet, lambs were weighed and liver biopsy samples were taken from all animals using a laparotomy technique. Animals were tranquilized with xylazine (50 μg/kg body weight). After shearing, the right paracostal area was prepared by antiseptic scrub and a subcutaneous line block was made with

TABLE 3-1. BASAL DIET COMPOSITION (EXPERIMENTS 1, 2 AND 3)

Ingredient	Percent (as-fed basis)[a]		
	Exp. 1	Exp. 2	Exp. 3
Ground yellow corn	58	56.95	56.95
Cottonseed hulls	21	21	21
Soybean meal, 48% CP	12	12	12
Alfalfa meal	3	3	3
Corn oil	3	3	3
Cornstarch[b]	1.45	2.28	2.28
Ground limestone	.55	.55	.55
Trace mineralized salt	1	1	1
Vitamins[c]	+	+	+
Sodium Sulfate (Na_2SO_4)	---	.22	.22
Ethoxyquin	.0125	.0125	.0125
Sodium selenite (Na_2SeO_3)[d]	+	+	+
Mineral concentration[e]			
Ca, %	.55	.41	.43
Mg, %	.15	.16	.15
P, %	.27	.29	.28
S, %	.14	.22	.22
Cu, ppm	8.3	12.6	9.5
Mo, ppm	1.6	1.1	.9
Fe, ppm	96	116	131
Mn, ppm	46	49	42
Zn, ppm	65	25	52

[a] Diet dry matter = 89.2% (Exp. 1), 87.9% (Exp. 2), 88.1% (Exp.3).

[b] Copper supplements added on an air-dry basis at the expense of equivalent weight of corn starch.

[c] Vitamin A palmitate, 2200 USP units/kg; vitamin D_3, 440 USP units/kg; vitamin E, 15 IU/kg.

[d] Equivalent to .2 ppm Se.

[e] Dry matter basis.

2% lidocaine. A 5 cm incision was made to access the liver and a .29 ± .13 g (mean ± standard deviation, dry weight) sample of liver tissue was removed from the caudal part of the right lobe with the aid of Babcock forceps and scissors. The incision was closed in all layers (peritoneal, muscular and skin) with nonabsorbable suture. Liver biopsy samples were frozen for further mineral analyses.

After the surgical procedure, animals were placed in individual, wooden raised, expanded metal floor pens in an open-sided barn and fed the basal diet for a further 7-day period, to allow animals time to adjust to pens and recover from surgery. At the end of adjustment period, animals were assigned to experimental diets, which were fed for 10 days, from November 1 through 10, 1988. Feed intake was restricted to 1 kg/animal daily and tap water was available ad libitum. Water contained .02 ppm Mo and no detectable Cu. On day 11 of the experimental period, animals were weighed, stunned with a captive bolt shot, killed by exsanguination and had the liver removed, which was frozen for mineral analyses. Animals were classified as lambs at slaughter.

Approximately 5 g (dry weight) of liver tissue were removed in duplicate from the ventral part of the left lobe of the organ. Liver biopsies, liver samples and feed samples were digested in nitric acid (Fick et al., 1979). Concentrations of Cu in tap water; Cu, Fe, Mn, Zn, Ca and Mg in Cu sources and in basal diet; Cu in experimental diets;

and Cu, Fe, Mn, and Zn in liver were determined by flame atomic absorption spectrophotometry on a Model 5000 spectrophotometer with an AS-50 autosampler (Perkin-Elmer Corp., Norwalk, CT; Anonymous, 1982). Phosphorus in Cu sources and in basal diet was determined colorimetrically (Harris and Popat, 1954). Sulfur concentration in basal diet was determined at the University of Minnesota, with a Model S-132 sulfur analyzer (Leco, Warrendale, PA). Molybdenum in tap water, basal diet and Cu sources was determined by graphite furnace with a Perkin-Elmer Zeeman/3030 atomic absorption spectrophotometer with an AS-60 autosampler (Anonymous, 1984). Standards were matched for macroelement and acid concentrations as appropriate and standard reference material (bovine liver 1577a) from the National Institute of Standards and Technology (Gaithersburg, MD) was included with samples.

Relative solubility in water, .4% HCl, 2% citric acid and neutral ammonium citrate (Watson et al., 1970) and magnetic susceptibility (Watson et al., 1971) of Cu sources were determined and X-ray diffraction patterns were interpreted.

Initial and final live body weights, difference between final and initial weights, initial (biopsy) and final liver mineral concentrations and their differences were analyzed by one-way analysis of variance, with PROC GLM of SAS (1988), using the model:

$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$, where

Y_{ij} = j^{th} observation on i^{th} treatment,

μ = overall mean of all observations,

τ_i = added effect of the i^{th} treatment measured as a deviation from μ, and

ϵ_{ij} = random error.

Treatment differences, when present, were separated by Duncan's (1955) procedure.

Final liver Cu, Fe, Mn and Zn concentrations were also analyzed by multiple linear regression using the GLM procedure of SAS (1988), with initial concentration (biopsy value) as a covariate. Logarithmic (log) transformation (base 10) was performed on final tissue mineral concentrations, to reduce variance heterogeneity.

Slopes and their standard errors were estimated by fitting the following multiple linear regression model:

$Y = \beta_0 + \beta_1(\text{biopsy}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2 + \beta_5 LS_3 + \beta_6 LS_4$, where

Y = log final liver mineral concentration, ppm dry matter (DM) basis;

β_0 = intercept;

β_1, β_2, β_3, β_4, β_5 and β_6 = slopes;

biopsy = initial liver mineral concentration, ppm DM basis;

L = added dietary Cu level, ppm as-fed basis;

S_1 = 1 if source 1 (cupric chloride), 0 otherwise;

S_2 = 1 if source 2 (cupric carbonate), 0 otherwise;

S_3 = 1 if source 3 (cupric oxide), 0 otherwise;

S_4 = 1 if source 4 (cupric sulfate), 0 otherwise.

Thus in the model used in regression analysis, all sources had the same intercept. The multiple linear regression equations, therefore, were the following:

1.) for source 1 (cupric chloride),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + \beta_2 L + \beta_3 L S_1 \text{ or, since } S_1 = 1$$

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_3) L$$

2.) for source 2 (cupric carbonate),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_4) L,$$

3.) for source 3 (cupric oxide),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_5) L,$$

4.) for source 4 (cupric sulfate),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_6) L,$$

where $(\beta_2 + \beta_3)$, $(\beta_2 + \beta_4)$, $(\beta_2 + \beta_5)$ and $(\beta_2 + \beta_6)$ are the slopes for source 1, source 2, source 3 and source 4, respectively.

The relative bioavailability values (RBV) of the Cu sources were estimated from the ratio of the log transformed slopes, with cupric chloride as the reference source. The RBV's were expressed as percentages.

Slope ratios and their standard errors were estimated for final liver Cu concentrations using the method of error propagation as described by Kempthorne and Allmaras (1965). The resulting formula for standard error is the same as equation 7.6.7 in Finney (1978).

In PROC GLM of SAS, the model statement was "final mineral concentration = biopsy + level + level x source," with "source" as class statement.

It is emphasized that the estimated relative bioavailability value based on the ratio of slopes of log transformed data has a different interpretation from that based on the ratio of slopes of the actual data. In this instance, the linear or near-linear relationship applies to log transformed data--not data on the original scale. Therefore, the slopes that are being compared measure the rate of change of log ppm Cu, rather than ppm Cu itself. A linear relationship for the log transformed data implies an exponential relationship for the original data, so that the rate of change of ppm Cu will vary with the added dietary Cu concentration.

Experiment 2

Forty-two Texas crossbred wether lambs, weighing from 43 to 56 kg (average ± standard deviation = 44.7 ± 5.7 kg), initially, were assigned randomly to one of the following treatments:

A. control, fed the basal diet with no additional Cu;

B. basal diet + 60 ppm Cu, as cupric chloride;

C. basal diet + 120 ppm Cu, as cupric chloride;

D. basal diet + 180 ppm Cu, as cupric chloride;

E. basal diet + 120 ppm Cu, as cupric acetate;

F. basal diet + 120 ppm Cu, as cupric oxide;

G. basal diet + 120 ppm Cu, as cupric sulfate.

Six animals were assigned to each treatment. Cupric chloride ($CuCl_2 \cdot 2H_2O$) and cupric acetate [$Cu(C_2H_3O_2)_2 \cdot H_2O$] were reagent grade sources; cupric oxide and cupric sulfate, feed grade sources.

The basal diet (Table 3-1) was formulated to meet nutrient requirements of lambs (NRC, 1985). It contained 12.6 ppm Cu and 1.1 ppm Mo (dry matter basis), by analysis. The Cu sources were added to the basal diet on an as-fed basis, at the expense of corn starch. The Cu content of experimental diets was confirmed by chemical analysis.

Initially, all lambs were housed in two large covered floor pens and group-fed for 7 days a commercial corn-soybean meal-cottonseed hulls mixture at an amount selected to ensure an average daily intake of about 1.2 kg/animal.

On November 5, 1990, lambs were weighed and on November 8 they were subjected to liver biopsy, using the technique described in Exp. 1. A sample of .65 ± .26 g (mean ± standard deviation, dry weight) liver tissue was removed. Liver biopsy samples were frozen for subsequent mineral analyses.

After the surgical procedure, animals were placed in individual, wooden raised pens, with expanded metal floors, in an open-sided barn. Recovery from surgery and adaptation to cages and to the basal diet lasted 17 days. The basal

diet fed during this period did not contain additional S. Experimental diets were fed for 10 days, starting on November 25. Animals were fed 1 kg of the diet daily. Tap water containing .02 ppm Mo and no detectable Cu was available ad libitum.

Blood samples were taken from all lambs by jugular puncture at the beginning (day 1) and at the end (day 10) of the experimental period. Blood was centrifuged for 30 minutes at 2500 x g, 20 minutes after collection. An equal volume (2 ml) of 10% (weight:volume) trichloroacetic acid (TCA) was added to serum samples, mixed and centrifuged (25 minutes at 2500 x g). The supernatant was saved and precipitate was resuspended into 5% TCA (w:v) and the mixing and separation procedure repeated. Supernatant removed from both processes was combined for TCA-soluble Cu analysis.

Animals were slaughtered on day 11 of the experiment for collection of livers, which were immediately frozen for subsequent mineral analyses. All animals were classified as lambs at slaughter.

Mineral analyses in tap water, Cu sources, basal diet, experimental diets, liver biopsies and liver samples were determined as described for Exp. 1. Copper and Zn in serum were read directly in undiluted and undigested samples by flame atomic absorption spectrophotometry. Relative solubility, magnetic susceptibility and X-ray diffraction patterns of Cu sources and statistical analyses of the data

were performed as described for Exp. 1. The multiple linear regression model was as shown in previous experiment,

$Y = \beta_0 + \beta_1(\text{biopsy}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2 + \beta_5 LS_3 + \beta_6 LS_4$,

the difference being source 2, which was cupric acetate in present experiment. The multiple linear regression equations were the following:

1.) for source 1 (cupric chloride),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_3)L$$

2.) for source 2 (cupric acetate),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_4)L$$

3.) for source 3 (cupric oxide),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_5)L$$

4.) for source 4 (cupric sulfate),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_6)L, \text{ where}$$

$(\beta_2 + \beta_3)$, $(\beta_2 + \beta_4)$, $(\beta_2 + \beta_5)$ and $(\beta_2 + \beta_6)$ are the slopes for source 1, source 2, source 3 and source 4, respectively. Relative bioavailability values of the Cu sources, slope ratios and their standard errors were estimated as described for Exp. 1.

Experiment 3

Forty-two Texas crossbred wether lambs, weighing from 34 to 45 kg (average ± standard deviation = 39.4 ± 2.8 kg), initially, were assigned randomly to one of the following seven treatments:

A. control, fed the basal diet with no additional Cu;

B. basal diet + 60 ppm Cu, as a copper-lysine complex;

C. basal diet + 120 ppm Cu, as a copper-lysine complex;

D. basal diet + 180 ppm Cu, as a copper-lysine complex;

E. basal diet + 60 ppm Cu, as cupric sulfate;

F. basal diet + 120 ppm Cu, as cupric sulfate;

G. basal diet + 180 ppm Cu, as cupric sulfate.

Six animals were assigned to each treatment.

Copper-lysine was a feed grade source and cupric sulfate, reagent grade ($CuSO_4 \cdot 5H_2O$). The Cu sources were added to the basal diet on an as-fed basis at the expense of corn starch. The basal diet (Table 3-1) contained 9.5 ppm Cu and .9 ppm Mo (dry matter basis), by analysis, and it was formulated to meet nutrient requirements of lambs (NRC, 1985). The Cu content of the experimental diets was confirmed by chemical analysis.

Initially, all lambs were housed in two large covered floor pens and group-fed for 3 months a commercial corn-soybean meal-cottonseed hulls mixture containing 11 ppm Cu (dry matter basis), by analysis. Lambs were fed an amount selected to ensure an average daily intake of about 1.2 kg/ animal.

They were weighed on August 20, 1990, subjected to liver biopsy on August 22 and placed in individual, wooden raised cages with expanded metal floors in an open-sided barn on August 23. It was not possible to collect a liver biopsy sample from two of the 42 lambs. Therefore, those

two were assigned to the control group. Liver biopsy samples were taken using the technique described previously. Samples of liver tissue removed averaged .50 ± .28 g (mean ± standard deviation, dry weight). Liver biopsy samples were frozen for further mineral analysis. Recovery from surgery and adaptation period to cages and to the basal diet lasted 15 days.

Animals were fed individually 1.2 kg of the respective experimental diet once daily, for 10 days. Tap water was available ad libitum and contained .02 ppm Mo and no detectable Cu. Lambs were weighed just before slaughter on day 11 of the experiment and livers were collected and immediately frozen for subsequent mineral analysis. Animals were classified as lambs at slaughter.

Mineral analyses of tap water, Cu sources, basal diet, experimental diets, liver biopsies and liver samples; relative solubility, magnetic susceptibility and statistical analyses were accomplished as described earlier in Exp. 1. Slopes and their standard errors were estimated by fitting the following multiple linear regression model:

$$Y = \beta_0 + \beta_1(\text{biopsy}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2, \text{ where}$$

Y = log final liver mineral concentration, ppm dry matter (DM) basis;

β_0 = intercept;

β_1, β_2, β_3, β_4 = slopes;

biopsy = initial liver mineral concentration, ppm DM basis;

L = added dietary Cu level, in ppm;

S_1 = 1 if source 1 (copper-lysine), 0 otherwise;

S_2 = 1 if source 2 (cupric sulfate), 0 otherwise.

The multiple linear regression equations were as following:

1.) for source 1 (copper-lysine),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + \beta_2 L + \beta_3 LS1 \text{ or, since } S_1 = 1,$$

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_3)L, \text{ and}$$

2.) for source 2 (cupric sulfate),

$$Y = \beta_0 + \beta_1(\text{biopsy}) + (\beta_2 + \beta_4)L, \text{ where}$$

$(\beta_2 + \beta_3)$ and $(\beta_2 + \beta_4)$ are slopes for source 1 and source 2, respectively. Relative bioavailability values of the Cu sources, slope ratios and their standard errors were estimated as described in Exp. 1, except for the reference source, which was cupric sulfate in Exp. 3.

Results

Experiment 1

Chemical and physical characteristics of Cu sources are presented in Table 3-2. Copper concentrations of the chloride, carbonate, oxide and sulfate compounds were 37.3%, 54.6%, 74.1% and 25.1%, respectively. The reagent grade cupric chloride contained small amounts of Zn, Mg and P. Iron, Mn, Zn, Mg and P were detected in the feed grade carbonate and oxide. In the feed grade cupric sulfate only small amounts of Mg and P were found.

TABLE 3-2. CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE COPPER SOURCES (EXPERIMENT 1)

Item	Copper source			
	Chloride, reagent grade	Carbonate, feed grade	Oxide, feed grade	Sulfate, feed grade
Chemical constituents, as-fed basis				
Cu, %	37.3	54.6	74.1	25.1
Fe, %	---[a]	.27	.54	---
Mn, ppm	---	23	37	---
Mo, ppm	---	---	---	---
Zn, ppm	82	577	718	---
Ca, ppm	---	---	---	---
Mg, ppm	13	250	2100	24
P, ppm	931	1742	1658	866
Particle size, % [b]				
> 850 μm	46.9	---	---	---
850 to 150 μm	53.1	80.4	48.3	96.3
150 to 75 μm	---	3.5	36.2	3.5
< 75 μm	---	16.1	15.5	0.2

TABLE 3-2. CONTINUED

Item	Copper source			
	Chloride, reagent grade	Carbonate, feed grade	Oxide, feed grade	Sulfate, feed grade
Physical appearance	Greenish, crystals	Light green, fine powder	Black, fine powder	Light blue, crystals
Relative solubility, %[c]				
Water	90.4	---	---	98.9
Neutral ammonium citrate	99.9	98.7	4.9	98.8
HCl, .4%	100	97.7	59.1	95.5
Citric acid, .2%	100	97.9	8.3	100
Magnetic susceptibility, %	---	---	---	---
Interpretation of X-ray patterns[d]	Not available	$Cu_2(OH)_2CO_3$	CuO plus minor Cu_2O	$CuSO_4 \cdot 5H_2O$

[a] Not detected.

[b] Retained by a No. 20 sieve (U.S. Bureau of Standards), passed through a No. 20 but retained by a No. 100, passed through a No. 100 and retained by a No. 200 and passing a No. 200, respectively. Corresponding sieve sizes are 850μm, 150μm and 75μm, respectively.

[c] Solubility of .1 g in 100 ml solvent at 37°C for 1 hour, with constant stirring, expressed as a percentage of total copper concentration.

[d] Courtesy of Pitman-Moore, Mundelein, IL.

All four sources had particles that predominantly passed the 20 sieve and were retained by the 100 sieve, which correspond to 850 μm and 150 μm, respectively, however, 47% of the chloride was retained by the 20 sieve and therefore had particles larger than 850 μm.

Cupric chloride and cupric sulfate were highly soluble in water; in contrast, cupric carbonate and cupric oxide were completely insoluble. Except for cupric oxide, all sources were highly soluble in neutral ammonium citrate, .4% HCl and 2% citric acid solutions. As indicated in Table 3-2, cupric oxide was the least soluble source and was only 60% soluble in .4% HCl.

None of the mineral sources had any magnetic susceptibility, except the natural adhering property of the oxide and the carbonate to the weighing paper, which could be confounded with magnetic susceptibility.

The X-ray diffraction patterns of the feed grade sources indicated a high degree of purity for the carbonate and the sulfate, while the oxide contained mainly cupric oxide (CuO) with minor quantities of cuprous oxide (Cu_2O).

Most animals consumed the provided amount of feed within a few hours after feeding, although 13 lambs from Cu-supplemented groups had orts varying from 10 g/day to 240 g/day, on the average (1% to 24% of feed offered): two on treatment B, four on treatment C, three on treatment D, three on treatment F and one on treatment G. The greatest

amounts of refused feed were from two lambs on treatment F, cupric oxide, 134 and 237 g/day, respectively. Eleven of the 13 animals left less than 5% of the amount offered. Livers were completely healed at biopsy site at the end of the experiment.

There was no difference in live body weight among treatments at the beginning ($P = .93$) or at the end ($P = .93$) of the 10-day feeding period, neither did final body weight differ from initial body weight ($P = .98$).

Two lambs had diarrhea on day 6 of the feeding period, one on treatment C and one on treatment D.

The initial and final liver Cu concentrations are presented in Table 3-3. Initial liver Cu concentrations varied from 68 to 499 ppm, averaging 238 ppm. There was no difference among treatments ($P = .93$). Final liver Cu concentrations varied from 110 to 681 ppm and these were affected by dietary Cu treatments ($P < .0001$). Lambs fed the Cu-supplemented diets had higher final liver Cu values than animals fed the control diet ($P < .05$).

The increase in liver Cu concentration varied from 9 to 473 ppm during the 10-day feeding period for animals receiving supplementary Cu. In wethers fed the control diet, concentration change varied from -26 to +101 ppm Cu (dry matter basis). The increase in liver Cu concentration was highly affected by dietary treatment ($P < .0001$). Within the Cu-supplemented groups, cupric oxide resulted in

TABLE 3-3. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC COPPER CONCENTRATION OF LAMBS (EXPERIMENT 1)

Copper source	Added Cu (ppm)[a]	Liver Cu concentration (ppm DM basis)		
		Initial	Final	Increase
Control	0	187 ± 29[b]	216 ± 48[g]	29 ± 22[g]
Chloride[c]	60	278 ± 60	458 ± 59[ef]	180 ± 26[ef]
Chloride[c]	120	247 ± 71	454 ± 68[ef]	207 ± 39[e]
Chloride[c]	180	217 ± 32	510 ± 58[ef]	293 ± 42[e]
Carbonate[d]	120	240 ± 23	493 ± 51[ef]	253 ± 38[e]
Oxide[d]	120	248 ± 48	337 ± 29[f]	89 ± 28[f]
Sulfate[d]	120	246 ± 19	587 ± 29[e]	341 ± 36[e]

[a] Basal diet contained 8.3 ppm Cu, DM basis, by analysis.

[b] Mean ± standard error of the mean (n = 5).

[c] Reagent grade.

[d] Feed grade.

[e, f, g, h] Means within a column not sharing a common superscript differ ($P < .05$). Statistical analyses based on log (base 10) transformed data.

the least hepatic Cu uptake. There was no difference among the other three sources.

Multiple linear regression equations and coefficients of determination (R^2) for several alternative models are presented in Table 3-4. The greatest improvement in the R^2 value was obtained when liver biopsy Cu concentrations were used as a covariate in the model. Log transformation of the dependent variable did not improve R^2 values, neither did the use of total dietary Cu concentration (added Cu concentrations plus basal) or Cu concentration adjusted for feed intake as the independent variable or inclusion of the initial liver Cu level x Cu source interaction in the model. However, log transformation decreased variance heterogeneity, consequently, this response variable was used in final statistical analyses.

Table 3-5 shows the slopes and their standard errors. The steepest slope was that of cupric sulfate, but it did not differ ($P = .23$) from that of cupric carbonate, which in turn did not differ from the slope of cupric chloride ($P = .31$). Cupric oxide had the lowest slope and it was lower ($P < .01$) than that of the other three sources.

The relative bioavailability values, based on slope ratios, using cupric chloride as the reference source, are also shown in Table 3-5. As shown by the 95% confidence intervals, and corroborating the slope analyses, cupric sulfate had the greatest relative biological availability.

TABLE 3-4. ALTERNATIVE MULTIPLE LINEAR REGRESSION MODELS AND EQUATIONS OF HEPATIC COPPER ON DIETARY COPPER CONCENTRATIONS (ADDED, TOTAL OR ADJUSTED) WITH AND WITHOUT INITIAL VALUE AS COVARIATE (EXPERIMENT 1)

Models and equations[a]	R^2	RMSE	CV	P-value
MODEL = Level + Level*Source				
LIVCU = $277.8 + 1.46x_2 + 1.796x_3 + .489x_4 + 2.576x_5$	.475	119.3	27.3	.0005
DIFF = $54.4 + 1.364x_2 + 1.654x_3 + .285x_4 + 2.391x_5$	.670	76.2	38.3	.0001
LOGLIV = $2386.7 + 1.999x_2 + 2.477x_3 + 1.118x_4 + 3.165x_5$	.474	.2	5.6	.0005
LOGDIFF = $1566 + 5.671x_2 + 6.803x_3 + 1.854x_4 + 7.987x_5$	.508	.4	17.5	.0003
MODEL = Biocu + Level + Level*Source				
LIVCU = $70.3 + .928x_1 + 1.371x_2 + 1.664x_3 + .300x_4 + 2.404x_5$	.787	77.2	17.7	.0001
LOGLIV = $2140 + 1.102x_1 + 1.891x_2 + 2.321x_3 + .893x_4 + 2.961x_5$	.764	.1	3.8	.0001
MODEL = Biocu + Totlev + Totlev*Source				
LIVCU = $147 + .866x_1 + .854x_2 + 1.084x_3 - .185x_4 + 1.728x_5$	.804	75.5	17.3	.0001
MODEL = Biocu + Totlev + Totlev*Source + Biocu*Source				
LIVCU = $140 + 1.751x_2 + 1.377x_3 + 1.023x_4 + 3.491x_5$	.837	74.4	17.0	.0001
MODEL = Biocu + Adjlev + Adjlev*Source				
LIVCU = $66 + .927x_1 + 1.470x_2 + 1.707x_3 + .400x_4 + 2.477x_5$	.798	75.3	17.2	.0001
MODEL = Biocu + Adjlev + Adjlev*Source + Biocu*Source				
LIVCU = $72 + 2.292x_2 + 1.935x_3 + 1.753x_4 + 4.250x_5$	.826	75.2	17.2	.0001

TABLE 3-4. CONTINUED

[a] Models as described in model statement (class = source) in PROC GLM of SAS; R^2 = coefficient of determination; RMSE = square root of mean square error; CV = coefficient of variation; LIVCU = final liver Cu concentration, ppm dry matter (DM) basis; DIFF = change in liver Cu concentration (final - initial), ppm DM basis; LOGLIV = log (base 10) of LIVCU, regression coefficients times 10^3; LOGDIFF = log (base 10) of change in liver Cu concentration, regression coefficients times 10^3; Biocu = x_1 = initial liver Cu concentration, based on liver biopsy samples, ppm DM basis; Totlev = total dietary Cu levels (added + basal), ppm as-fed basis; Adjlev = dietary Cu adjusted for feed intake, ppm as-fed basis; x_2 = ppm Cu, as cupric chloride; x_3 = ppm Cu, as cupric carbonate; x_4 = ppm Cu, as cupric oxide; x_5 = ppm Cu, cupric sulfate; examples of regression equations are as following: $Y = \beta_0 + \beta_1(\text{Biocu}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2 + \beta_5 LS_3 + \beta_6 LS_4$, where $S_1 = 1$ if cupric chloride (0 otherwise), $S_2 = 1$ if cupric carbonate (0 otherwise), $S_3 = 1$ if cupric oxide (0 otherwise) and $S_4 = 1$ if cupric sulfate (0 otherwise); since S_1, S_2, S_3 and $S_4 = 1$, β_2 is added to β_3, β_4, β_5 and β_6, respectively, resulting the coefficients for each of the sources; or $Y = \beta_0 + \beta_1(\text{Biocu}) + \beta_2(\text{Totlev}) + \beta_3 LS_0 + \beta_4 LS_1 + \beta_5 LS_2 + \beta_6 LS_3 + \beta_7 LS_4 + \beta_8 BS_0 + \beta_9 BS_1 + \beta_{10} BS_2 + \beta_{11} BS_3 + \beta_{12} BS_4$, where S_0 = control (not included), B = Biocu, $[(\beta_1 + \beta_9) + (\beta_2 + \beta_4)]$, $[(\beta_1 + \beta_{10}) + (\beta_2 + \beta_5)]$, $[(\beta_1 + \beta_{11}) + (\beta_2 + \beta_6)]$ and $[(\beta_1 + \beta_{12}) + (\beta_2 + \beta_7)]$ are the slopes for source 1, 2, 3 and 4, respectively.

TABLE 3-5. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF HEPATIC COPPER CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF COPPER SOURCES (EXPERIMENT 1)

Cu source	Slope ± SE[a, b]	RV ± SE[c]	Confidence limits (95%)
Chloride, b_2	.001891 ± .000335[d]	100	---
Carbonate, b_3	.002320 ± .000488[dg]	122.7 ± 23.7	76.3 - 169.1
Oxide, b_4	.000893 ± .000489[e]	47.2 ± 22.7	2.8 - 91.7
Sulfate, b_5	.002961 ± .000489[fg]	156.6 ± 26.4	104.8 - 208.3

[a] Slope ± standard error (log ppm Cu, dry basis/ppm Cu, as-fed basis; slopes obtained from the multiple linear regression of log (base 10) transformed hepatic Cu concentrations (ppm, dry basis), on initial hepatic Cu (ppm, dry basis), added dietary Cu (ppm Cu, as-fed basis) and Cu level * Cu source; intercept = 2.140497; initial value coefficient = .001102.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .31$, $b_4 - b_2 = .019$, $b_5 - b_2 = .012$, $b_4 - b_3 = .007$, $b_5 - b_3 = .23$, $b_5 - b_4 = .0001$.

[c] Relative value and standard error; RV equals ratio of slopes, with chloride as the reference source; expressed in percentage.

[d, e, f, g] Slopes with different superscripts differ; P-values given in footnote "b".

Cupric carbonate did not differ from cupric chloride. The lowest relative value was that of cupric oxide.

Initial and final liver Fe, Mn and Zn concentrations are shown in Table 3-6. There were no differences among treatment groups in initial Fe (P = .31), Mn (P = .26) and Zn (P = .11) values. Also, there were no differences among treatments in final Fe (P = .70), Mn (P = .61) and Zn (P = .14) concentrations. However, when initial values were used as a covariate, final liver Zn concentrations were affected by dietary Cu level (P = .0049), but the effect seems to be erratic, since no systematic change can be inferred from the means (Table 3-6).

Experiment 2

Chemical and physical characteristics of the four Cu sources used in this experiment are shown in Table 3-7. Copper concentrations were 37.3%, 32.1%, 74.1% and 25.1%, for the chloride, acetate, oxide and sulfate sources, respectively. The reagent grade chloride and acetate compounds and the feed grade sulfate contained minor amounts of other elements, especially P, but in the oxide form several other minerals were found, mainly Fe, Mg and P. The four Cu sources had particles that passed a 20 sieve and were retained by a 100 sieve, predominantly.

Solubility tests performed on the Cu sources indicated that the chloride, the acetate and the sulfate compounds

TABLE 3-6. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC ZINC, IRON, AND MANGANESE CONCENTRATIONS IN LAMBS (EXPERIMENT 1)

Copper source	Added Cu (ppm)	Liver concentration (ppm, DM basis)					
		Zinc		Iron		Manganese	
		Initial	Final	Initial	Final	Initial	Final
Control	0	156 ± 21[a]	120 ± 5	200 ± 41	241 ± 45	15.5 ± 2.1	13.1 ± .4
Chloride	60	140 ± 11	128 ± 8	206 ± 27	178 ± 19	15.5 ± 1.3	13.7 ± .7
Chloride	120	108 ± 7	117 ± 5	141 ± 27	276 ± 49	11.8 ± 1.2	13.8 ± .7
Chloride	180	135 ± 6	130 ± 6	161 ± 29	277 ± 66	11.8 ± 1.2	12.7 ± 1.0
Carbonate	120	121 ± 4	130 ± 7	275 ± 69	192 ± 21	13.4 ± 2.3	12.5 ± .5
Oxide	120	124 ± 6	136 ± 13	145 ± 10	248 ± 68	12.0 ± .7	12.3 ± .5
Sulfate	120	177 ± 44	170 ± 29	176 ± 22	214 ± 26	14.6 ± .9	13.5 ± .8

	P-values[b]		
Initial value	< .0001	.0723	.0701
Cu Level	.0049	.6828	.7073
Level * source	.1995	.4448	.5903

[a] Mean ± standard error of the mean (n = 5).

[b] Statistical analyses based on log (base 10) transformed data.

TABLE 3-7. CHEMICAL AND PHYSICAL CHARACTERISTICS OF COPPER SOURCES (EXPERIMENT 2)

Item	Copper source			
	Chloride, reagent grade	Acetate, reagent grade	Oxide, feed grade	Sulfate, feed grade
Chemical constituents, as-fed basis				
Cu, %	37.3	32.1	74.1	25.1
Fe, %	---[a]	---	.54	---
Mn, ppm	---	---	37	---
Mo, ppm	---	---	---	---
Zn, ppm	82	---	718	---
Ca, ppm	---	---	---	---
Mg, ppm	13	15	2100	24
P, ppm	931	1034	1658	866
Particle size, %[b]				
> 850 μm	46.9	1.1	---	---
850 to 150 μm	53.1	97.5	48.3	96.3
150 to 75 μm	---	1.2	36.2	3.5
< 75 μm	---	.3	15.5	.2

TABLE 3-7. CONTINUED

Item	Copper source			
	Chloride, reagent grade	Acetate, reagent grade	Oxide, feed grade	Sulfate, feed grade
Physical appearance	Greenish, crystals	Dark blue, fine crystals	Black, fine powder	Light blue, crystals
Relative solubility, %[c]				
Water	90.4	95.2	---	98.9
Neutral ammonium citrate	99.9	98.8	4.9	98.8
HCl, .4%	100	99.9	59.1	95.5
Citric acid, 2%	100	100	8.3	100
Magnetic susceptibility, %	---	---	---	---
Interpretation of X-ray patterns[d]	---	$Cu(C_2H_3O_2)_2 \cdot H_2O$	CuO plus minor Cu_2O	$CuSO_4 \cdot 5H_2O$

[a] Not detected.

[b] Retained by a No. 20 sieve (US Bureau of Standards), passed through a No. 20 but retained by a No. 100, passed through a No. 100 and retained by a No. 200, and passing a No. 200, respectively. Corresponding sieve sizes are 850 μm, 150 μm and 75 μm, respectively.

[c] Solubility of .1 g in 100 ml solvent at approximately 37°C for 1 hour, with constant stirring, expressed as a percentage of total Cu concentration.

[d] Courtesy of Pitman-Moore, Mundelein, IL.

were highly soluble in water, neutral ammonium citrate, .4% HCl and 2% citric acid solutions. In contrast, cupric oxide was insoluble in water, almost insoluble in neutral ammonium citrate and 2% citric acid solutions and only 60% soluble in .4% HCl solution. None of the Cu sources showed any magnetic susceptibility.

Most animals consumed the provided amount of feed (1 kg/day) within a few hours after feeding. No feed refusal occurred. At slaughter, the site of liver where biopsy was taken was completely healed. Initial and final live body weights did not differ among treatments (P = .9974 and P = .9974, respectively). There was no difference between the initial and final body weights (P = .9480).

In Table 3-8, initial, final and change in liver Cu concentrations during the 10-day feeding period are presented. Initial values varied from 76 to 354 ppm Cu, averaging 166 ppm. There were no differences among treatments at the beginning of the experiment (P = .70). Final liver Cu concentrations had a minimum of 155 ppm Cu and a maximum of 882 ppm Cu and these values were affected by treatments (P = .045). Groups fed oxide and 60 ppm Cu as chloride did not differ from those fed the control diet. Change in liver Cu concentration in Cu-supplemented lambs during the 10-day feeding period varied from 59 to 528 ppm. In animals fed the control diet it varied from -2 to 59 ppm Cu. Change in liver Cu concentrations was highly influenced by dietary treatments (P < .0001).

TABLE 3-8. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC COPPER CONCENTRATION OF LAMBS (EXPERIMENT 2)

Copper source	Added Cu (ppm)[a]	Liver Cu concentration (ppm, DM basis)		
		Initial	Final	Increase
Control	0	194 ± 22[b]	225 ± 20[f]	31 ± 11[g]
Chloride[c]	60	181 ± 43	395 ± 112[ef]	214 ± 70[ef]
Chloride[c]	120	149 ± 21	368 ± 63[e]	220 ± 45[ef]
Chloride[c]	180	150 ± 8	412 ± 20[e]	262 ± 24[e]
Acetate[c]	120	188 ± 25	414 ± 37[e]	226 ± 28[ef]
Oxide[d]	120	163 ± 23	296 ± 17[ef]	133 ± 22[f]
Sulfate[d]	120	136 ± 13	350 ± 38[e]	214 ± 27[ef]

[a] Basal diet contained 12.6 ppm Cu, DM basis, by analysis.

[b] Mean ± standard error of the mean (n = 6).

[c] Reagent grade.

[d] Feed grade.

[e, f, g] Means within a column not sharing a common superscript differ ($P < .05$); statistical analyses based on log (base 10) transformed data.

In Table 3-9 several alternative equations are presented. The greatest improvement in R^2 resulted when initial liver Cu value was used as a covariate. Logarithmic transformation of final liver Cu values improved R^2 slightly, however, its greatest effect was reducing variance heterogeneity, consequently all further statistical analysis on final liver Cu concentrations were done using log of final Cu level with initial value as a covariate.

Slopes of the regression equation are shown in Table 3-10. The slopes of chloride, acetate and sulfate did not differ ($P > .05$). The lowest slope was that of the oxide compound. Relative bioavailability values of the Cu sources, based on the slope ratios, are also shown in Table 3-10. As indicated by the 95% confidence intervals, cupric oxide had the lowest relative value. The relative values of the acetate and sulfate compounds did not differ from that of chloride source.

Serum initial total Cu concentrations did not differ among treatments ($P = .22$), nor did final concentrations ($P = .69$). However, when initial values were used as a covariate, final serum Cu concentrations (Table 3-11) were affected by dietary Cu concentrations ($P = .033$) and there was a level x source interaction ($P = .0525$).

Initial and final serum TCA-soluble Cu concentrations are shown in Table 3-11. There were no differences among

TABLE 3-9. ALTERNATIVE MULTIPLE LINEAR REGRESSION MODELS AND EQUATIONS OF HEPATIC COPPER ON DIETARY COPPER CONCENTRATIONS (ADDED, TOTAL OR ADJUSTED), WITH AND WITHOUT INITIAL VALUE AS COVARIATE (EXPERIMENT 2)

Models and equations[a]	R^2	RMSE	CV	P-value
MODEL = Level + Level*Source				
LIVCU = $270 + .888x_2 + 1.201x_3 + .212x_4 + .666x_5$	.163	133.1	37.9	.1494
DIFF = $77 + 1.161x_2 + 1.241x_3 + .464x_4 + 1.140x_5$	.348	94.8	51.1	.0027
LOGLIV = $2379 + 1.371x_2 + 1.916x_3 + .729x_4 + 1.257x_5$	.266	.14	5.7	.0195
LOGDIFF = $1707 + 4.530x_2 + 5.246x_3 + 3.204x_4 + 5.028x_5$	.507	.25	11.6	.0001
TOTLIVCU = $46.0 + .125x_2 + .203x_3 + .022x_4 + .110x_5$	.152	21.8	37.4	.1798
MODEL = Biocu + Level + Level*Source				
LIVCU = $-75 + 1.789x_1 + 1.376x_2 + 1.273x_3 + .663x_4 + 1.513x_5$	.675	84.1	23.9	.0001
LOGLIV=$1998 + 1.976x_1 + 1.910x_2 + 1.997x_3 + 1.227x_4 + 2.193x_5$	.738	.09	3.4	.0001
TOTLIVCU = $-9.8 + .289x_1 + .204x_2 + .214x_3 + .095x_4 + .247x_5$	.661	14.0	24.0	.0001
MODEL = Biocu + Totlev + Totlev*Source				
LIVCU = $25 + 1.797x_1 + .598x_2 + .382x_3 - .161x_4 + .606x_5$	.731	77.6	22.1	.0001
MODEL = Biocu + Level + Level*Source + Biocu*Source				
TOTLIVCU = $-8.4 + .474x_2 + .583x_3 + .444x_4 + .503x_5$	.806	11.2	19.2	.0001

TABLE 3-9. CONTINUED

[a] Models as described in model statement (class = source) in PROC GLM of SAS; R^2 = coefficient of determination; RMSE = square root of mean square error; CV = coefficient of variation; LIVCU = final liver Cu concentration, ppm dry matter (DM) basis; DIFF = change in liver Cu concentration (final - initial), ppm DM basis; LOGLIV = log (base 10) of LIVCU, regression coefficients times 10^3; LOGDIFF = log (base 10) of change in liver Cu concentration, regression coefficients times 10^3; Biocu = x_1 = initial liver Cu concentration, based on liver biopsy samples, ppm DM basis; Totlev = total dietary Cu levels (added + basal), ppm as-fed basis; TOTLIVCU = total Cu in liver, mg DM basis; x_2 = ppm Cu, as cupric chloride; x_3 = ppm Cu, as cupric carbonate; x_4 = ppm Cu, as cupric oxide; x_5 = ppm Cu, as cupric sulfate; examples of regression equations are as following: $Y = \beta_0 + \beta_1(\text{Biocu}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2 + \beta_5 LS_3 + \beta_6 LS_4$, where $S_1 = 1$ if cupric chloride (0 otherwise), $S_2 = 1$ if cupric carbonate (0 otherwise), $S_3 = 1$ if cupric oxide (0 otherwise) and $S_4 = 1$ if cupric sulfate (0 otherwise); since S_1, S_2, S_3 or $S_4 = 1$, β_2 is added to β_3, β_4, β_5 and β_6, respectively, resulting the coefficients for each of the sources; or $Y = \beta_0 + \beta_1(\text{Biocu}) + \beta_2(\text{Totlev}) + \beta_3 LS_0 + \beta_4 LS_1 + \beta_5 LS_2 + \beta_6 LS_3 + \beta_7 LS_4 + \beta_8 BS_0 + \beta_9 BS_1 + \beta_{10} BS_2 + \beta_{11} BS_3 + \beta_{12} BS_4$, where S_0 = control (not included in equation shown), B = Biocu, $[(\beta_1 + \beta_9) + (\beta_2 + \beta_4)]$, $[(\beta_1 + \beta_{10}) + (\beta_2 + \beta_5)]$, $[(\beta_1 + \beta_{11}) + (\beta_2 + \beta_6)]$ and $[(\beta_1 + \beta_{12}) + (\beta_2 + \beta_7)]$ are the slopes for source 1, 2, 3 and 4, respectively.

TABLE 3-10. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF HEPATIC COPPER CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF COPPER SOURCES (EXPERIMENT 2)

Cu source	Slope ± SE[a, b]	RV ± SE[c]	Confidence limits (95%)
Chloride, b_2	.001910 ± .000273[d]	100	---
Acetate, b_3	.001997 ± .000386[df]	104.6 ± 18.1	69.2 - 139.9
Oxide, b_4	.001227 ± .000390[ef]	64.3 ± 17.4	30.2 - 98.4
Sulfate, b_5	.002193 ± .000403[d]	114.8 ± 18.3	79.0 - 150.7

[a] Slope ± standard error (log ppm Cu, dry basis/ppm Cu, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed hepatic Cu concentration (ppm, dry basis), on initial hepatic Cu (ppm, dry basis), added dietary Cu level (ppm Cu, as-fed basis) and Cu level * Cu source; intercept = 1.998145; initial value coefficient = .001976.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .80$; $b_4 - b_2 = .043$; $b_5 - b_2 = .40$; $b_4 - b_3 = .068$; $b_5 - b_3 = .65$; $b_5 - b_4 = .022$.

[c] Relative value and standard error; RV equals ratio of slopes, with chloride as reference source; expressed in percentage.

[d, e, f] Slopes with different superscripts differ; P-values given in footnote "b".

TABLE 3-11. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON SERUM TOTAL COPPER AND ZINC, AND ON SERUM TCA-SOLUBLE COPPER IN LAMBS (EXPERIMENT 2)

Copper source	Added Cu (ppm)	Serum Cu (ppm)		TCA-soluble Cu(ppm)		Serum Zn (ppm)	
		Initial	Final	Initial	Final	Initial	Final
Control	0	1.08 ± .06[a]	.84 ± .03	.96 ± .05	.71 ± .04	.88 ± .07	.89 ± .02
Chloride	60	1.00 ± .05	.88 ± .03	.83 ± .05	.74 ± .03	.89 ± .02	.91 ± .02
Chloride	120	.94 ± .06	.88 ± .08	.78 ± .06	.74 ± .07	.90 ± .03	.90 ± .04
Chloride	180	1.11 ± .10	.96 ± .05	.94 ± .10	.77 ± .04	.87 ± .05	.83 ± .04
Acetate	120	1.00 ± .06	.92 ± .05	.86 ± .08	.79 ± .05	.77 ± .03	.80 ± .04
Oxide	120	1.10 ± .08	.87 ± .06	.96 ± .08	.73 ± .06	.85 ± .06	.88 ± .05
Sulfate	120	.90 ± .05	.85 ± .05	.76 ± .04	.74 ± .06	.83 ± .03	.86 ± .03
		P-values[b]					
Initial[c]		---	< .0001	---	< .0001	---	< .0001
Cu level		.6307	.0330	.5720	.1069	.2316	.2014
L * S[d]		.2047	.0525	.2737	.0653	.1577	.7238

[a] Mean ± standard error of the mean (n = 6).

[b] Based on log (base 10) transformed data.

[c] Initial value.

[d] Level * source interaction.

treatments at the beginning of the experiment (P = .38). Final serum TCA-soluble Cu concentrations were not affected by dietary treatments (P = .78). When the initial value was used as a covariate, effect in the model was high (P < .0001); however, final TCA-soluble Cu values still did not differ (P > .05), even though there was a tendency for a level x source interaction (P = .0653).

The slope of the multiple regression equation for final serum Cu (Table 3-12) for the oxide source was lower (P < .02) than those of the other sources. These did not differ (P > .05). As indicated by slopes (Table 3-13), lambs fed acetate and sulfate had greater TCA-soluble Cu than those fed oxide (P < .02). Animals fed chloride tended to have greater serum TCA-soluble Cu than those on oxide (P = .058). Zinc concentrations in serum (Table 3-11) were not affected by dietary Cu treatments (P > .05).

Table 3-14 indicates initial and final liver Fe, Mn and Zn concentrations. Initial values did not differ among treatments (Fe, P = .92; Mn, P = .49 and Zn, P = .32). When the statistical analyses was run without initial value as a covariate, final hepatic Fe and Mn concentrations were not affected by dietary treatments (P = .86 and P = .58, respectively), but for Zn there was a treatment effect (P = .0325). Duncan's procedure for separation of means indicated that lambs fed oxide and acetate had smaller liver Zn concentrations than those fed 60 and 120 ppm Cu as

TABLE 3-12. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF FINAL SERUM COPPER CONCENTRATION ON ADDED DIETARY COPPER CONCENTRATION, WITH INITIAL COPPER AS COVARIATE (EXPERIMENT 2)

Cu source	Slope ± SE[a, b]
Chloride, b_2	.00026730 ± .00010768[c]
Acetate, b_3	.00041123 ± .00015721[c]
Oxide, b_4	-.00005242 ± .00015832[d]
Sulfate, b_5	.00036313 ± .00016135[c]

[a] Slope ± standard error (log ppm Cu, fresh basis/ppm Cu, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed final serum Cu concentration (ppm, fresh basis), on added dietary Cu concentration (ppm, as-fed basis) and Cu level * Cu source interaction, with the initial Cu value as a covariate.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .30$; $b_4 - b_2 = .02$; $b_5 - b_2 = .50$; $b_4 - b_3 = .007$; $b_5 - b_3 = .78$; $b_5 - b_4 = .02$.

[c, d] Slopes with different superscripts differ; P-values given in footnote "b".

TABLE 3-13. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF TCA-SOLUBLE COPPER CONCENTRATION ON ADDED DIETARY COPPER, WITH INITIAL VALUE AS COVARIATE (EXPERIMENT 2)

Cu source	Slope ± SE[a, b]
Chloride, b_2	.00022884 ± .00014602[ce]
Acetate, b_3	.00044873 ± .00021286[c]
Oxide, b_4	-.00013018 ± .00021434[de]
Sulfate, b_5	.00045306 ± .00021856[c]

[a] Slope ± standard error (log ppm Cu, fresh basis/ppm Cu, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed final serum TCA-soluble Cu (ppm) on added dietary Cu level (ppm, as-fed basis) and Cu level * Cu source interaction, with initial serum TCA-soluble Cu as a covariate.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .24$; $b_4 - b_2 = .058$; $b_5 - b_2 = .24$; $b_4 - b_3 = .013$; $b_5 - b_3 = .99$; $b_5 - b_4 = .016$.

[c, d, e] Slopes with different superscripts differ; P-values given in footnote "b".

TABLE 3-14. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC ZINC, IRON, AND MANGANESE CONCENTRATIONS IN LAMBS (EXPERIMENT 2)

Copper source	Added Cu (ppm)	Liver concentration (ppm, DM basis)					
		Zinc		Iron		Manganese	
		Initial	Final	Initial	Final	Initial	Final
Control	0	106 ± 5[a]	113 ± 3	271 ± 26	186 ± 21	13.6 ± .3	11.2 ± .8
Chloride	60	112 ± 5	121 ± 4	314 ± 58	190 ± 34	15.3 ± 1.1	12.7 ± 1.0
Chloride	120	102 ± 2	121 ± 3	309 ± 49	227 ± 27	15.5 ± .8	12.6 ± .7
Chloride	180	99 ± 2	117 ± 2	309 ± 24	208 ± 37	15.2 ± 1.1	13.2 ± .8
Acetate	120	102 ± 6	107 ± 3	297 ± 19	193 ± 17	14.9 ± .8	12.4 ± .3
Oxide	120	100 ± 3	111 ± 4	270 ± 37	182 ± 32	13.8 ± .4	11.5 ± 1.0
Sulfate	120	102 ± 4	114 ± 3	298 ± 18	184 ± 18	15.9 ± .8	12.2 ± 0.8

	P-values[b]		
Initial value	.9918	< .0001	.0008
Cu Level	.3584	.3055	.5273
Level * source	.2608	.5565	.5657

[a] Mean ± standard error of the mean (n = 6).

[b] Based on log transformed data.

chloride. Control lambs did not differ from the Cu-supplemented groups. When the initial values were used as a covariate, no differences were detected in final liver Fe, Mn and Zn concentrations (Table 3-14).

Experiment 3

Chemical and physical characteristics of Cu-lysine and cupric sulfate are shown in Table 3-15. The Cu-lysine contained 7.59% Cu and the cupric sulfate 25.4% Cu, by analysis. The reagent grade cupric sulfate contained only small amounts of Mg and P; however, the lysine source had several other minerals present, mainly Fe, Zn and Ca, which were probably constituents of the carrier. Both sources had particle size distributions predominantly passing a 20 sieve and being retained by a 100 sieve, therefore having sizes between 150 μm and 850 μm. Both sources were highly soluble in water, .4% HCl, 2% citric acid and neutral ammonium citrate solutions. Copper-lysine was somewhat less soluble in water. Neither Cu-lysine nor cupric sulfate was found to have any magnetic susceptibility.

The concentration of lysine in the Cu-lysine source provided by the manufacturer was 16%. Animals allocated to treatment D, the highest level, therefore consumed an additional amount of 2.58 g lysine, compared with controls, representing .5 g N, or less than 2% of the daily crude

TABLE 3-15. CHEMICAL AND PHYSICAL CHARACTERISTICS OF COPPER SOURCES (EXPERIMENT 3)

Item	Copper source	
	Lysine, feed grade	Sulfate, reagent grade
Chemical constituents, (as-fed basis)		
Cu, %	7.59	25.4
Fe, %	.38	---[a]
Mn, ppm	50	---
Mo, ppm	---	---
Zn, ppm	665	---
Ca, %	1.69	---
Mg, ppm	436	8
P, ppm	1084	961
Particle size, %[b]		
> 850 μm	.1	1.4
850 to 150 μm	87.5	79.3
150 to 75 μm	8.9	18.0
< 75 μm	3.5	1.3

TABLE 3-15. CONTINUED

Item	Copper source	
	Lysine, feed grade	Sulfate, reagent grade
Physical appearance	Dark green, fine granules plus powder	Light blue, crystals
Relative solubility, %[c]		
Neutral ammonium citrate	92.0	95.6
Citric acid, 2%	96.2	100
HCl, .4%	98.2	100
Water	80.8	100
Magnetic susceptibility	---	---

[a] Not detected.

[b] Retained by a No. 20 sieve (U.S. Bureau of Standards), passed through a No. 20 but retained by a No. 100, passed through a No. 100 and retained by a No. 200 and passing a No. 200, respectively. Corresponding sieve sizes are 850 μm, 150 μm and 75 μm, respectively.

[c] Solubility of .1 g in 100 ml solvent at 37°C for 1 hour, with constant stirring, expressed as a percentage of total copper concentration.

protein (% N x 6.25) requirements of 40 kg lambs (NRC, 1985).

All lambs consumed the amount of feed provided daily (1.2 kg), in general, within a few hours after the early morning feeding. There were no differences in live body weights among treatments at the beginning ($P = .9997$) or at the end ($P = .98$) of the 10-day feeding period. Final body weight did not differ from initial body weight ($P = .93$). At termination of the experiment, liver of animals was completely healed at biopsy site.

Initial, final and increase in liver Cu concentrations during the 10-day experimental period are presented in Table 3-16. Initial liver Cu concentrations varied from 90 to 377 ppm (dry matter basis), averaging 204 ppm. There was no difference among treatments in initial Cu value ($P = .56$). Final liver Cu concentrations varied from 100 to 676 ppm and these were influenced by Cu dietary treatments ($P < .0001$). Change in liver Cu level during the 10-day feeding period varied from 110 to 377 ppm in Cu-supplemented animals. In the group fed the control diet, change varied from 20 to 143 ppm Cu. Liver Cu increase was highly affected by dietary treatment ($P < .0001$). Final liver Cu concentration and its increase during the 10-day feeding period were not different within the Cu-supplemented groups, but they were greater than in lambs fed the basal diet.

TABLE 3-16. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC COPPER CONCENTRATION OF LAMBS (EXPERIMENT 3)

Copper source	Added Cu (ppm)[a]	Liver Cu concentration (ppm, DM basis)		
		Initial	Final	Increase
Control	0	180 ± 32[b]	218 ± 78[f]	69 ± 52[f]
Lysine[c]	60	189 ± 77	362 ± 110[e]	173 ± 42[e]
Lysine[c]	120	227 ± 77	436 ± 110[e]	209 ± 61[e]
Lysine[c]	180	252 ± 74	484 ± 137[e]	232 ± 84[e]
Sulfate[d]	60	184 ± 66	380 ± 82[e]	197 ± 57[e]
Sulfate[d]	120	202 ± 30	446 ± 69[e]	244 ± 86[e]
Sulfate[d]	180	186 ± 68	451 ± 58[e]	266 ± 32[e]

[a] Basal diet contained 9.5 ppm Cu, DM basis, by analysis.

[b] Mean ± standard error of the mean [n = 4, for control (initial value and increase), n = 6 for others].

[c] Feed grade.

[d] Reagent grade.

[e, f] Means within a column not sharing a common superscript differ ($P < .05$); statistical analyses based on log (base 10) transformed data.

Several alternative models for multiple linear regression were tested. Equations, R^2 values and coefficients of variation are shown in Table 3-17. The greatest improvement in R^2 value resulted when initial liver Cu value was used as a covariate. Use of total dietary Cu concentrations (added plus basal) as the independent variable did not improve the fit. Log transformation of the independent variable did not improve coefficient of determination but it reduced variance heterogeneity, so that further statistical analysis was run on final liver Cu log transformed data.

In Table 3-18 are presented the slopes for the regression equation. The equation representing cupric sulfate had a greater slope than Cu-lysine ($P < .0585$).

The relative bioavailability value (Table 3-18), based on the slope ratio, was 68% for Cu-lysine, when compared to the reference source, cupric sulfate. The 95% confidence interval of the relative value of Cu-lysine confirmed the result of the test for difference in slopes.

Initial and final liver Fe, Mn and Zn concentrations are presented in Table 3-19. There was no difference among treatments in initial Fe ($P = .66$), Mn ($P = .53$) and Zn ($P = .72$) concentrations and the same occurred with final Fe ($P = .29$), Mn ($P = .34$) and Zn ($P = .81$) concentrations. Addition of initial value to the model, as a covariate, did not alter statistical significance.

TABLE 3-17. ALTERNATIVE MULTIPLE LINEAR REGRESSION MODELS AND EQUATIONS OF HEPATIC COPPER ON DIETARY COPPER CONCENTRATIONS (ADDED, TOTAL OR ADJUSTED), WITH AND WITHOUT INITIAL VALUE AS COVARIATE (EXPERIMENT 3)

Models and equations[a]	R^2	RMSE	CV	P-value
MODEL = Level + Level*Source				
LIVERCU = 270.1 + 1.267Lys + 1.196Sulf	.404	96.176	24.2	.0001
DIFF = 118.1 + .690Lys + .920Sulf	.398	63.162	30.8	.0001
LOGLIVCU = 2405.7 + 1.634Lys + 1.643Sulf	.422	.123	4.8	.0001
LOGDIFF = 1994.6 + 2.261Lys + 2.732Sulf	.401	.190	8.4	.0001
MODEL = Biocu + Level + Level*Source				
LIVERCU = 96.7 + 1.121Biocu + .641Lys + .908Sulf	.695	63.584	15.6	.0001
LOGLIVCU = 2246.3 + 1.200Biocu + .777Lys + 1.147Sulf	.690	.073	2.8	.0001
MODEL = Biocu + Totlev + Totlev*Source				
LIVERCU = 128.6 + 1.131Biocu + .368Lys + .628Sulf	.731	60.557	14.8	.0001
MODEL = Biocu + Totlev + Totlev*Source + Biocu*Source				
LIVERCU = 136.3 + 1.112Lys + 1.756Sulf	.742	61.086	14.9	.0001

[a] Models as described in model statement (class = source) in PROC GLM of SAS; R^2 = coefficient of determination; RMSE = square root of mean square error; CV = coefficient of variation; LIVERCU = final liver Cu concentration, ppm dry matter (DM) basis; DIFF = change in liver Cu concentration (final - initial), ppm DM basis; LOGLIVCU = log (base 10) of LIVERCU, regression coefficients times 10^3; LOGDIFF = log (base 10) of change in liver Cu concentration, regression coefficients times 10^3; Biocu = initial liver Cu concentration, based on liver biopsy samples, ppm DM basis; Totlev = total dietary Cu levels (added + basal), ppm as-fed basis; Lys = ppm Cu, as Cu-lysine; Sulf = ppm Cu, as cupric sulfate example of regression equation is as following: $Y = \beta_0 + \beta_1(\text{Biocu}) + \beta_2 L + \beta_3 LS_1 + \beta_4 LS_2$, where $S_1 = 1$ if Cu-lysine (0 otherwise), $S_2 = 1$ if cupric sulfate (0 otherwise); since S_1 or $S_2 = 1$, the coefficients for the sources are $(\beta_2 + \beta_3)$ and $(\beta_2 + \beta_4)$, respectively.

TABLE 3-18. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF HEPATIC COPPER CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF COPPER SOURCES (EXPERIMENT 3)

Cu source	Slope ± SE[a]	RV ± SE[b]	Confidence limits, 95%
Sulfate	.001147 ± .000217[c]	100	---
Lysine	.000777 ± .000230[d]	67.8 ± 15.7	36.9 - 98.6

[a] Slope ± standard error (log ppm Cu, dry basis/ppm Cu, as-fed basis); slopes obtained from the multiple linear regression of the log (base 10) of final liver Cu concentration (ppm, dry basis) on initial liver Cu (ppm, dry basis), added dietary Cu level (ppm, as-fed basis) and dietary Cu level * source interaction; intercept = 2.246323; coefficient of initial value = .001200.

[b] Relative value and standard error; RV equals ratio of slopes, with sulfate as reference source; expressed in percentage

[c, d] Slopes differ (P = .0585).

TABLE 3-19. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF COPPER ON HEPATIC IRON, MANGANESE AND ZINC CONCENTRATIONS IN LAMBS (EXPERIMENT 3)

Copper source	Added Cu (ppm)	Liver concentration (ppm, DM basis)					
		Zinc		Iron		Manganese	
		Initial	Final	Initial	Final	Initial	Final
Control	0	85 ± 5[a]	114 ± 11	193 ± 14	186 ± 9	9.9 ± 1.2	12.5 ± 1.0
Lysine	60	92 ± 5	112 ± 4	294 ± 83	204 ± 14	10.8 ± .6	13.6 ± .6
Lysine	120	94 ± 4	106 ± 3	206 ± 15	182 ± 18	11.8 ± 1.3	12.9 ± .4
Lysine	180	91 ± 5	105 ± 2	263 ± 57	178 ± 13	12.9 ± .5	14.1 ± .4
Sulfate	60	85 ± 5	112 ± 4	221 ± 33	174 ± 6	11.5 ± 1.2	13.9 ± .6
Sulfate	120	93 ± 6	113 ± 2	182 ± 21	170 ± 9	10.9 ± .6	12.4 ± .8
Sulfate	180	86 ± 8	107 ± 6	241 ± 36	164 ± 5	11.6 ± .9	13.6 ± .7

	P-values[b]		
Initial value	.7855	.4901	.0075
Cu level	.5241	.1792	.3850
Level * source	.3886	.1869	.7002

[a] Mean ± standard error of the mean (control, n = 4; lysine and sulfate, n= 6).

[b] Statistical analyses based on log (base 10) transformed data.

Discussion

Chemical and Physical Characteristics of the Copper Sources

Chemical and physical characteristics of three of the Cu sources (carbonate, oxide and sulfate) used in this study are in close agreement with those obtained by Ledoux (1987). Some minor differences in values for the "contaminants" may be due to the way calculations were performed, besides the normal analytical error, since carbonate, oxide and sulfate came from the same lot, in both cases. In the present study, mineral concentrations in Cu sources were adjusted for the blanks.

The Cu-lysine complex used in Exp. 3 was found to have 7.59% Cu in this study, which is similar to the value reported by Baker et al. (1991), 7.5% Cu. The Cu-lysine was analyzed in the present study with and without ashing, at 600°C, overnight, before solubilization in hydrochloric acid. When no ashing was done, a Cu concentration of 8.0% resulted, which is similar to the Cu concentration (8.39%) obtained in another determination done in this laboratory in which sources were refluxed in concentrated HNO_3:HCl (vol:vol) for 4 hours (P. R. Henry and C. B. Ammerman, personal communication).

The relative solubilities of carbonate, oxide and sulfate in water, neutral ammonium citrate, .4% HCl and 2%

citric acid in the present study were similar to those obtained by Ledoux (1987).

Contrary to what was found for the Cu sources used in the present study, Ledoux (1987) reported some magnetic susceptibility for the oxide and the carbonate, but this may have resulted from interpretation of the natural adhering capacity of those two sources to the weighing paper as magnetic susceptibility, in the latter case.

Feed Intake and Body Weight

Diarrhea has been described as one of the signs of acute Cu toxicosis (Todd, 1969; Luckey and Venupogal, 1977; Osweiler et al., 1985). Although both diarrheic lambs in Exp. 1 were fed diets supplemented with Cu (120 and 180 ppm Cu as chloride), it is not attributed to an excess of Cu, because it disappeared as suddenly as it appeared.

In Exp. 1, only lambs fed supplemental Cu had orts, but those fed cupric carbonate consumed all the feed provided. The feed refusal seems to be more related to stress and to individual variation in adjustment to cages than to the presence of additional Cu in the diet, since animals fed cupric carbonate consumed all feed offered and no feed refusal was noted on other experiments. An effect due to Cu source may also be discarded, since carbonate, as discussed later, was as available as chloride and sulfate, but it was more available than oxide, and this treatment had three

animals with some feed refusal. However, an effect due to another factor, like taste, may have been present.

Ledoux (1987), in a 15 and 30-day period experiment, in which 15, 30 and 45 ppm Cu were added to a basal diet of similar composition as that used in the present study, observed no feed refusal, as was the case in Experiments 2 and 3 in the present study, and animals in Ledoux's study even had a slight increase in body weight. In the same study (Ledoux, 1987), when 20, 40, 60 or 80 ppm Cu were added to a similar basal diet and fed for 10 or 20 days to Cu-depleted sheep, animals consumed all feed provided and no changes in body weight were observed.

Ability of sheep to maintain intake and body weight at dietary Cu concentrations in excess of the maximal tolerable level (25 ppm) suggested by the NRC (1980) has previously been reported (Dick, 1954b; Hill and Williams, 1965; Todd, 1969; van der Berg et al., 1983; Ledoux, 1987). The same phenomenon has been observed in cattle (Skipper, 1951). However, feed intake ($P < .05$) and daily weight gain ($P < .01$) of lambs fed diets with 20 ppm added Cu were reduced compared with values for lambs without supplemental Cu (Pond, 1989). In this instance, the trial lasted 12 weeks.

Copper in Liver

The liver has long been recognized as the main organ involved in storage of Cu (Comar et al., 1948; Todd, 1962)

and as the key organ in metabolism of Cu (Gooneratne et al., 1989a). It is known at least since the early 1930s that liver contains a much greater concentration of Cu than any other organ (Cunningham, 1931; Bremner, 1980). Dick (1954b) found that almost 80% of total Cu content in the body of sheep is in liver.

The Cu content of liver was the best indicator for comparative measurements of absorption rates from different Cu compounds (Kirchgessner and Weser, 1965). Radioactivity of an intravenous injection of ^{64}Cu was completely cleared from circulating blood within 15 minutes of injection and 75% of the dose was retained by liver (Neethling et al., 1968). Amongst several indicators, including 18 blood components, Buckley and Tait (1981) considered only plasma aspartate aminotransferase and liver Cu concentration as useful indicators of high Cu status in lambs. Feeding high-Cu diets had little effect on Cu concentrations in muscle or serum, but it caused a marked increase in Cu content of liver (Cromwell et al., 1981). Rosa et al. (1986) observed increases in serum and liver Cu concentrations of mature wethers, but not in kidneys or spleen, when 40 ppm Cu were added to a basal diet containing 8 ppm Cu. Ledoux (1987) determined hemoglobin in serum and Cu in bone, kidney, liver, serum and spleen of sheep fed from 0 to 80 ppm added dietary Cu for 10 or 20 days, and concluded that liver was the most sensitive tissue to increased dietary Cu

concentrations. Concentrations of liver Cu may be regarded as the most accurate method in determining Cu status of sheep (van Niekerk and van Niekerk, 1989). Whereas liver and kidney of Cu-dosed lambs and goats contained large excess of Cu, concentrations in other organs (brain, heart, muscle and spleen) were only slightly elevated above those in animals fed the basal diet (Zervas et al., 1990). Rate of Cu accumulation in liver can be used as a comparative measure of dietary Cu availability in that it represents Cu absorbed in excess of net requirements (Zervas et al., 1990).

Initial hepatic Cu concentrations in the present experiment were estimated from samples obtained from biopsies. Even though the biopsy sample is a very small proportion of whole liver, the distribution of Cu was found to be uniform throughout the liver of sheep (Hogan et al., 1971a; Osborn et al., 1983; Woolliams et al., 1983; Puls, 1988), and Dick (1944) and Hogan et al. (1971a) demonstrated that Cu determinations of liver biopsy samples were in good agreement with values determined on bulk samples taken post mortem. Donald et al. (1984), however, reported that hepatic Cu concentration in sheep was overestimated by the biopsy method by approximately 5%.

Initial average of liver Cu concentrations in lambs of the three experiments were within the range (100 to 400 ppm) considered normal by Underwood (1981) and Davis and Mertz

(1987), and are in close agreement with those reported for lambs fed diets containing about 10 ppm Cu and 1 ppm Mo (Buckley and Tait, 1981), but are lower than those reported by Charmley and Ivan (1989), who used a basal diet containing 6.4 ppm Cu and .47 ppm Mo. The initial values of all three experiments were also within limits (25 to 100 ppm Cu, wet weight; approximately 80 to 330 ppm Cu, dry weight) for adequate dietary Cu concentrations (5 to 10 ppm Cu) referred to by Puls (1988).

Ledoux (1987), using sheep fed a basal diet of a similar composition as that used in the present study, reported hepatic Cu concentrations which were similar (one experiment) and greater (two experiments) than initial values observed in lambs in the present study.

Final hepatic Cu concentrations in Cu supplemented animals in the present study were not as great as those reported by van Ryssen (1979), Saylor and Leach (1980), Buckley and Tait (1981), Charmley and Ivan (1989) and Zervas et al. (1990). Van Ryssen (1979) fed a basal diet with 13.5 ppm Cu, .38 ppm Mo and .26% S; when Cu as cupric sulfate was added to increase dietary Cu to 60 ppm, hepatic Cu concentrations increased from 282 ppm in 2-year-old wethers fed the basal diet to 878 ppm in the supplemented group, after 157 days. In lambs previously maintained for 50 days on a low-Cu diet and then fed 2.2, 11.3 or 47.0 ppm Cu as cupric sulfate for further 60 days, Cu concentrations in

liver were 58, 191 and 280 ppm Cu, fresh tissue (Saylor and Leach, 1980). Lambs fed for 10 weeks diets containing 1 ppm Mo, .18% S and 10.9, 17.8 or 25.2 ppm Cu as cupric sulfate had hepatic Cu concentrations of 239, 454 and 721 ppm, (Buckley and Tait, 1981). When 9.1 ppm Cu, 1.6 ppm Mo and .26% S or 37 ppm Cu, 1.3 ppm Mo and .19% S were fed to another group of lambs for 11 weeks, hepatic Cu concentrations were 372 and 1109 ppm, respectively (Buckley and Tait, 1981). Copper supplementation (as chloride, acetate or sulfate), to elevate Cu concentrations from 6.4 to 34 ppm in diets containing .4 to .5 ppm Mo fed to lambs for 87 days, increased hepatic Cu concentrations from 512 ppm in lambs fed the basal diet to 1040 ppm (acetate), 1050 ppm (sulfate) and 1170 ppm (chloride) (Charmley and Ivan, 1989). Zervas et al. (1990) fed diets having 7.2, 33.5 or 67.5 ppm Cu, as cupric sulfate, .9 ppm Mo and .18% S; after 91 days, hepatic Cu concentrations of lambs were approximately 330, 2750 and 4000 ppm.

Final overall average hepatic Cu concentrations (433, 503 and 542 ppm) in sheep fed a similar diet (Ledoux, 1987) as the one used in the present study, but with smaller concentrations of supplemental Cu, were somewhat greater than the averages observed in Exp. 1, 2 and 3 in this study (436, 351 and 398 ppm, respectively). Final Cu concentrations in liver did not reach values reported by Gooneratne and Howell (1983) and Hidiroglou et al. (1984) in

sheep that died from Cu toxicosis. One factor that explains the differences is certainly the length of exposure to high dietary Cu concentrations. Also, greater initial hepatic Cu values or breed of sheep might be involved.

Suttle (1983b) speculated that changes in Cu absorption may be underestimated by hepatic Cu retention when liver Cu values reach 1000 ppm, because of increased endogenous losses. In the present three experiments, no such high hepatic Cu concentrations were observed in any of the lambs.

Increases in hepatic Cu concentrations as a consequence of increases in dietary Cu concentrations are well known and have been reported by Cunningham (1944), Dick (1954b), Hill and Williams (1965), Goodrich and Tillman (1966b), Felsman et al. (1973), van Ryssen (1979), Saylor and Leach (1980), Buckley and Tait (1981), Woolliams et al. (1985a,b), Rosa et al. (1986), Ledoux (1987), Charmley and Ivan (1989), Pond (1989), Ivan et al. (1990) and Zervas et al. (1990).

In the present three experiments, data were analyzed using the initial liver Cu values as a covariate and statistical analysis of several models has shown this to be the most appropriate (Tables 3-4, 3-9 and 3-17). Donald et al. (1984) compared the residual standard deviation from analysis of covariance in which initial sample values were treated as the covariate, and from an analysis of differences between Cu concentration in liver of biopsy samples taken at the start and at the end. Residual

standard deviation was reduced by approximately 50% by covariance analysis. Goodrich et al. (1968) recommended use of covariance analysis for blood and plasma data where both initial and final concentrations were measured. They found that final value adjusted for initial value was a more efficient measure of treatment response than change or uncorrected final level.

Use of covariance analysis for liver Cu concentrations, the covariate being represented by the initial value, seems to be the most appropriate, since it was demonstrated in experiments 1, 2 and 3 that the initial value markedly influenced ($P < .0001$) the final liver Cu concentration (Table 3-20). Dependence of final liver Cu concentrations on initial concentrations have been previously referred to by Ledoux (1987).

Covariance analysis would probably have been able to overcome the problems of large variations in hepatic Cu concentrations reported by numerous researchers, in sheep (van Ryssen and Stielau, 1980a; Ivan and Veira, 1985; Ledoux, 1987; Ivan et al., 1990) and in cattle (Miltimore et al., 1978; Kincaid et al., 1986; Kincaid, 1988a,b). The large variation in hepatic Cu concentrations of sheep may be a consequence of differences in absorption. Turner et al. (1987), in everted sacs of sheep jejunum in vitro, observed an extremely large variation among sheep in rates of Cu uptake, which reached a 10-fold difference between the

TABLE 3-20. ANALYSES OF VARIANCE OF LOGARITHMIC (BASE 10) TRANSFORMED FINAL HEPATIC COPPER CONCENTRATION, WITH INITIAL VALUE AS COVARIATE

Source[a]	df[b]	SS[c]	MS[d]	F-value	P-value
		Experiment 1			
Model	5	.943199	.188640	18.75	.0001
Error	29	.291739	.010060		
Biopsy	1	.357115	.357115	35.50	.0001
Level	1	.339470	.339470	33.74	.0001
L * S	3	.164502	.054834	5.45	.0043
		Experiment 2			
Model	5	.764320	.152864	20.25	.0001
Error	36	.271763	.007549		
Biopsy	1	.489057	.489057	64.78	.0001
Level	1	.321092	.321092	42.53	.0001
L * S	3	.046389	.015463	2.05	.1244
		Experiment 3			
Model	3	.423231	.141077	26.77	.0001
Error	36	.189705	.005270		
Biopsy	1	.203188	.203188	38.56	.0001
Level	1	.120668	.120668	22.90	.0001
L * S	1	.018858	.018858	3.58	.0666

[a] Source of variation.

[b] Degrees of freedom.

[c] Sum of squares.

[d] Mean squares.

greatest and smallest values. Large differences between groups of sheep in response to oral Cu repletion, assessed from changes in plasma Cu concentrations, have been attributed to differences in efficiency of Cu absorption (Wiener et al., 1978). In some cases it was recognized that these large variations prevented detection of treatment differences (Kincaid 1988a,b; Ivan et al., 1990). Wiener and Field (1969) observed that Cu concentration in liver was three times as variable, in terms of coefficient of variation, as in blood. Variations in liver Cu concentrations within treatments tended to increase with increasing Cu intakes (van Ryssen and Stielau, 1980a).

Use of covariance analysis obviously requires estimate of liver Cu concentrations at the start of the experiment. Some researchers (van Ryssen and Stielau, 1980a,b; van Ryssen and Stielau, 1981; Ledoux, 1987 - Exp. 4; Charmley and Ivan, 1989) used values of a small group slaughtered at the beginning of experiments to determine initial hepatic Cu concentrations. This procedure, however, does not take into account the high variation generally observed in liver Cu concentrations and dependence of the final value on initial value.

The increase in liver Cu concentration as a result of increasing concentrations of Cu in the diet was linear in Exp. 1, 2 and 3. Linear or near linear increases in liver Cu of lambs or sheep with respect to dietary Cu have been

reported by Dick (1954b), Goodrich and Tillman (1966b), Saylor and Leach (1980), Buckley and Tait (1981), Woolliams et al. (1985a,b) and Zervas et al. (1990).

Dick (1954b) found a linear relationship between change in Cu content of liver and total Cu intake per day (2.5 to 20 mg Cu). Goodrich and Tillman (1966b) fed 5.5, 55 and 550 ppm Cu as cupric carbonate with .2% S to lambs for 56 days. Final Cu concentrations in liver were 218, 248 and 918 ppm, respectively, when sulfate was the S source, and 235, 458 and 1173 ppm, respectively, when elemental S was provided. Saylor and Leach (1980), in lambs fed 2.3, 11.3 or 47.0 ppm Cu as cupric sulfate observed hepatic Cu concentrations of 58, 191 and 298 ppm, respectively. Buckley and Tait (1981) used dietary Cu concentrations of 10.9, 17.8 and 25.2 ppm, from cupric sulfate; the corresponding hepatic Cu concentrations were 239, 454 and 721 ppm, after a 10-week feeding and the increase was linear. Woolliams et al. (1985b), fed 5, 10, 22 and 28 ppm Cu, as cupric sulfate and observed a linear increase in the change in Cu concentrations in liver over a 12-week feeding period. Zervas et al. (1990) provided 0, 30 and 60 ppm added Cu as cupric sulfate for 3 months; the resulting Cu concentrations in liver were 330, 2750 and 4000 ppm. Also, amount and concentration of Cu in liver increased in direct proportion to total Cu consumed during the feeding period, 92 or 182 days, but the increase occurred independently of daily

amount of Cu consumed (van Ryssen and Stielau, 1980a). A linear uptake of Cu over a range of Cu concentrations from 0 to 100 μM (6.4 ppm) was observed in vitro by Turner et al. (1987), using everted sacs of sheep jejunum. Liver Cu concentrations of 12-week-old lambs fed for 137 days 33.5 or 67.5 ppm Cu as cupric sulfate were 8.9 and 16 times greater than controls fed 7.2 ppm Cu (Zervas et al., 1989). In goats, when 38 or 69.5 ppm Cu of the same source was fed for 91 days, the increase in hepatic Cu concentrations was 4.4 and 8.5 times greater, respectively, than in control animals fed 7.8 ppm Cu (Zervas et al., 1989).

On the other hand, several reports indicated a lack of dependence of the response on dietary Cu concentration or a non-linear relationship (Hill and Williams, 1965; Felsman et al., 1973; Woolliams et al., 1983; Stoszek et al., 1986; Ledoux, 1987; Ivan, 1988).

Hill and Williams (1965) recorded a marked increase in storage of Cu in liver when intake of Cu of lambs was increased from 11 to 37 mg/day, but little further increase in lambs consuming 53 mg/day, for 7 weeks. Ivan (1988) observed higher hepatic Cu uptake by rams fed 7.3 ppm Cu than by those fed 15.4 ppm Cu, while Woolliams et al. (1983) reported a curvilinear relationship between hepatic and dietary Cu (4 to 29 ppm Cu). Felsman et al. (1973) fed 0, 125, 250 and 500 ppm added dietary Cu to calves for 3 months in one experiment, and 0, 300, 600 and 900 ppm Cu in another

experiment; final liver Cu concentrations were higher due to dietary Cu addition, but there was no difference among added concentrations in each of the experiments. Dick (1954b) observed a smaller storage of Cu in liver at the highest Cu intake (33.5 mg/day).

When liver Cu concentrations of cattle fed daily up to 1000 mg Cu were examined using linear and nonlinear multiple regression, the linear model yielded a coefficient of determination (R^2) of .659 and a coefficient of variation (CV) of 42.5%, while the exponential model resulted in a R^2 of .740 and a CV of 37.5% (Stoszek et al., 1986). The authors concluded that the better fit of the exponential model indicated that rate of increase in liver Cu concentration diminished at high Cu supplementation levels. Indeed, the increase in R^2 from .66 to .74 is very little and the decrease in CV is not substantial. Standard errors for the linear and exponential models were very close, 13.8 and 12.2 ppm, respectively. Ledoux (1987) observed an increase ($P < .01$) in liver Cu concentration of sheep fed 15, 30 and 45 ppm added dietary Cu for 15 and 30 days, but there was no difference ($P > .10$) in liver Cu concentration of the sheep fed supplemental Cu.

In a recent review, Suttle (1987) indicated that liver Cu concentration in response to increasing dietary Cu differs by animal species. Sheep and cattle show a steady increase in liver Cu concentrations, whereas in laying hens,

pigs and rats no change happens until the dietary Cu concentrations reach at least 100 ppm. Underwood (1977) already noticed that ruminant and nonruminant animals differ in their response to elevated dietary Cu intake, but little supporting information was given. Differences among species (cattle, sheep and rats) in liver Cu accumulation in response to high dietary Cu levels have also been referred to by Stoszek et al. (1986). Ledoux et al. (1989a) reported a quadratic increase in hepatic Cu concentrations in liver of chicks fed 0 to 300 ppm added dietary Cu for 22 days. In this experiment (Ledoux et al., 1989a), there was no change in liver Cu concentration from 0 to 100 ppm added Cu, but a marked linear increase occurred thereafter. In a subsequent experiment (Ledoux et al., 1989b), when the same added dietary concentrations were used, for 1, 2 and 3 weeks, the same quadratic effect was noticed for the two latter periods. Recently, Baker et al. (1991) reported having obtained linear increases in hepatic Cu accumulation between 250 and 500 ppm dietary Cu, in chicks and pigs. In pigs, it has been shown by Cromwell et al. (1981) that high concentrations of dietary Cu increased hepatic Cu concentrations curvilinearly, with very little change occurring from 0 to 125 ppm added dietary Cu. This was true when cupric sulfate was fed, but not cupric oxide (CuO) (Cromwell et al., 1989).

An additional factor in the relationship between liver and dietary Cu concentrations seems to be the length of time of feeding, as found by Ledoux (1987). The regression of liver Cu concentration of Cu-depleted sheep on added dietary Cu (0, 20, 40, 60 and 80 ppm) by time indicated that the increase in liver Cu observed in animals fed diets for 10 days was linear ($P < .01$) while that observed in sheep fed for 20 days was quadratic ($P < .01$).

The question of whether the relationship between dietary Cu and hepatic Cu is linear or not also seems to depend upon the form in which the relationship is expressed. Abdellatif (1968) found a linear regression ($P < .01$) when total intake of Cu (400 to 2800 mg Cu) over a period of 39 and 54 days was plotted against change in liver Cu during the same period. The same was reported by van Ryssen and Stielau (1980a). In Exp. 1 and 2, when dietary Cu, as cupric chloride, in ppm or ultimately in total added amount fed per day, in milligrams, was plotted against final liver Cu concentration, the line was curvilinear. When dietary Cu was plotted versus increase or change in hepatic Cu concentration (final minus initial value), the relationship was linear. Yet in Exp. 3, linearity was less evident when added dietary Cu concentrations were plotted against change in hepatic Cu concentrations than against final hepatic Cu concentrations. In Exp. 2, no improvement resulted when total liver Cu accumulation was regressed on total Cu intake

during the 10-day feeding period. The resultant R^2 value was 39.4% and CV, 46.7%. When regression analysis was run on log transformed data, a R^2 of 63.8% and a CV of 54.9% resulted, and these are not better than the R^2 of 73.8% and CV of 3.4% obtained with the model chosen in the present study (Table 3-9).

Genetic effects on relationship between dietary and liver Cu concentrations are probably another factor that may explain part of the discrepancies found in these reports. The relationship between dietary Cu concentrations from 5.1 to 26.7 ppm and change in hepatic Cu concentrations was linear in Welsh Mountain sheep over a range of five dietary Mo + S combinations, but in Scottish Blackface ewes the relationship was linear or curvilinear, depending on dietary Mo and S concentrations (Woolliams et al., 1985a). Genetic variations in mineral metabolism have been reported by Wiener and Field (1969), Wiener et al. (1969), Wiener (1971, 1979) and van der Berg et al. (1983), in sheep, and by Smart and Christensen (1985), in cattle. Large differences among breeds of sheep in response to oral Cu repletion were mainly attributable to differences in efficiency of Cu absorption (Wiener et al., 1978). Van der Berg et al. (1983) reported not only sheep breed and crossbred differences in hepatic Cu concentrations but also in the percentage of supplemented Cu retained in liver. The relationship between dietary Cu concentration (4 to 29 ppm) and rate of hepatic Cu

accumulation (mg Cu/kg daily) in three breeds of sheep was different for each breed (Woolliams et al., 1983). In lines of sheep genetically selected for high concentration of Cu in plasma (high-line) and low plasma Cu concentrations (low-line) (Wiener et al., 1985), the concentration of Cu in liver was greater ($P < .01$) in high-line than in low-line of sheep and it increased linearly with Cu concentration of the diet but at a greater rate in the high-line (Woolliams et al., 1985b).

Last but not least, dietary and metabolic interactions among Cu, Mo and S must be mentioned. It has been shown that when diets of sheep contain elevated levels of Mo (Dick and Bull, 1945; van der Schee et al., 1980; Harrison et al., 1987) or of Mo and S (Dick, 1953a, 1954a; Wynne and McClymont, 1956; Goodrich and Tillman, 1966a; Suttle, 1977; Weber et al., 1983), Cu absorption and metabolism is impaired. In Scottish Blackface ewes, the relationship between dietary Cu concentrations from 5.1 to 26.7 ppm and change in hepatic Cu concentrations was linear when dietary Mo and S were 4.5 ppm and .24%, or 4.5 ppm and .40%, respectively; it tended to be curvilinear when dietary Mo and S were 2.0 ppm and .24%, or 4.5 ppm and .13%, respectively; and it was curvilinear when dietary Mo and S were .7 ppm and .24%, respectively (Woolliams et al., 1985a).

Liver and Dietary Copper Relationship as Influenced by Copper Absorption

Copper-depleted adult wethers retained more radioactivity from ^{64}Cu in blood, 4 hours after a 1 mg Cu intravenous injection, than did non-depleted sheep (Neethling et al., 1968). Also, there was a marked difference in the amount of radioactivity in blood, following an intraruminal injection of 1 mg Cu between Cu-depleted and normal sheep (Neethling et al., 1968). On the other hand, intraruminal administration of up to 1000 mg of Cu in a single dose did not increase absorption of the element, even in Cu-depleted sheep (Neethling et al., 1968). Suttle et al. (1978), however, noticed some evidence for the existence of a homeostatic control in Cu absorption and retention in lambs at high Cu intakes (up to 47 ppm Cu). Woolliams et al. (1983) suggested a saturation of the absorptive mechanism of sheep or an increase in endogenous Cu loss in bile as liver Cu concentrations were increased.

The capacity of liver to resist changes in Cu concentrations until dietary Cu exceeds a certain threshold level has been referred to in chickens (Smith, 1969; Norvell et al., 1975; Jensen and Maurice, 1979; Johnson et al., 1985, Ledoux et al., 1989a,b; Baker et al., 1991). On the other hand, Corbett et al. (1978) suggested that the close relationship between dietary Cu and liver Cu indicated the

existence of very little homeostatic control over Cu absorption by sheep.

Van Ryssen and Stielau (1980a) commented, based on the observations by Neethling et al. (1968), that true Cu availability estimates calculated with the use of a Cu depletion-repletion technique (Suttle 1974a) may not give a true reflection of Cu availability under natural conditions.

Relative Biological Availability

O'Dell (1984a, 1989) defined bioavailability as the proportion of an ingested nutrient that is absorbed and utilized. Many researchers emphasized that utilization of the nutrient is a key factor in determining availability (Thompson, 1965; Fox et al., 1981; Miller, 1981; Fairweather-Tait, 1987; Lee et al., 1988). Others are less restrictive and define or estimate bioavailability as the proportion which is absorbed (Suttle, 1986; Corah and Ives, 1991) or the proportion taken up by certain tissues (Ammerman et al., 1989).

Forbes and Erdman (1983) considered bioavailability to reflect the efficiency with which consumed elements are absorbed from the gastrointestinal tract and are available for storage and use. Bender (1989) stressed this latter point, by defining bioavailability as the proportion of a nutrient capable of being absorbed and available for use or storage, or the proportion that can be utilized.

Ammerman et al. (1989) stated recently that the study of mineral bioavailability has been hindered through the years due to the lack of agreement concerning the definition of bioavailability and a suitable method of determination. Indeed, from the definition of bioavailability to the actual measurement a big gap exists, mainly because of difficulties in determining mineral utilization. So, as far as Cu is concerned, its accumulation in liver has been used as the primary criterion of Cu bioavailability in sheep (MacPherson and Hemingway, 1968; Charmley and Ivan, 1989; Poole et al., 1990); in cattle (Miltimore et al., 1978; Kincaid et al., 1986; Kincaid, 1988a,b; Wittenberg and Boila, 1988; Ivan et al., 1990; Wittenberg et al., 1990); and in chicks (Ledoux et al., 1989a,b; Baker et al., 1991; Ledoux et al., 1991).

Inorganic Sources

Definitions of cupric carbonate ($CuCO_3$), cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$), cupric oxide (CuO) and cupric sulfate penta-hydrate ($CuSO_4 \cdot 5H_2O$) were adopted in 1975 (Association of American Feed Control Officials, 1991), and all four are "Generally Recognized As Safe" (Association of American Feed Control Officials, 1968).

Cupric sulfate

In all three experiments, cupric sulfate had the greatest or tended to have the greatest slope. The slopes of sulfate in Exp. 1, .002961 ± .000489 ppm Cu (dry

basis)/ppm Cu (as-fed basis), and in Exp. 2, .002193 ± .000403 ppm Cu (dry basis)/ppm Cu (as-fed basis), were of about the same magnitude.

The difference between the RBV of cupric sulfate and cupric oxide was greater in Exp. 1 (157% vs. 47%) than in Exp. 2 (115% vs. 64%). A difference between oxide and sulfate from Exp. 1 to Exp. 2 can be inferred from increases in hepatic Cu concentrations from both sources. In Exp. 1, the average increase for oxide was 89 ppm, while it was almost four-fold greater for sulfate, 341 ppm. In Exp. 2, the increase in Cu concentrations in liver caused by oxide supplementation (133 ppm) was greater and by sulfate (214 ppm) smaller than in Exp. 1. The increase due to cupric sulfate supplementation was less than two-fold that due to cupric oxide in Exp. 2. The increases in hepatic Cu concentrations when 60, 120 and 180 ppm Cu as chloride and the control diet were fed were quite similar in both experiments. The less marked increase in liver Cu concentration in Exp. 2 for sulfate may be related to the greater dietary S concentration in Exp. 2, .22%, against .14% in Exp. 1. It is known that increases in dietary S concentrations can decrease Cu absorption in sheep (Underwood, 1981) and that elevated dietary Mo and S can decrease hepatic Cu storage (Davis and Mertz, 1987). When sheep were fed .40% S instead of .04% S, in diets containing 5.2 ppm Cu and .8 ppm Mo, a decrease in hepatic Cu

concentrations occurred (Wynne and McClymont, 1956). Addition of sulfate to a high-Cu normal-Mo diet decreased the increment in hepatic Cu concentrations of lambs (Hill and Williams, 1965). In initially hypocupraemic ewes, additional S to diets containing 4 or 8 ppm Cu reduced responses in plasma Cu by 39% to 56% (Suttle, 1974b). Since cupric sulfate is more soluble than cupric oxide, it probably reacts faster and more extensively with S than cupric oxide in the gastrointestinal tract.

Within several Cu sources (cupric carbonate, cupric chloride, cupric nitrate, cupric sulfate, cuprous oxide, cupric oxide powder, cupric oxide needles and Cu wire), cupric sulfate ranked third in absorption rate, sixth in urinary excretion and fifth in fecal excretion, indicating a favorable retention in body tissues (Chapman and Bell, 1963). In spite of this and of several reports indicating the efficacy of cupric sulfate as a Cu dietary supplement for sheep (Suttle, 1974b; Dalgarno and Mills, 1975; Suttle and Price, 1976; Buckley and Tait, 1981; Woolliams et al., 1985b; van Ryssen and Barrowman, 1987; Charley and Ivan, 1989), and cattle fed Cu deficient diets (Stoszek et al., 1979; Wittenberg and Boila, 1988), or cattle grazing high-Mo pastures (Ferguson et al., 1943; Cunningham, 1944; Cunningham et al., 1953), the effectiveness of oral doses of cupric sulfate in raising the Cu reserves of sheep has been questioned (Judson et al., 1984; Ivan and Veira, 1985;

Trengove and Judson, 1985; Kincaid et al., 1986). A possible explanation for these apparently contradicting findings may be differences in the dietary Mo and S concentrations, because the effectiveness of cupric sulfate as a Cu supplement depended on pasture Mo and S concentrations (Boila et al., 1984). Higher Mo and S levels required additional Cu supplementation (Boila et al., 1984). Because sulfate is reduced to sulfide in the rumen (Lewis, 1954), Todd et al. (1962) suggested that Cu from cupric sulfate may be precipitated as its insoluble sulfide.

Dietary supplementation with cupric sulfate increased plasma Cu concentrations (Suttle, 1974b; Dalgarno and Mills, 1975; Suttle and Price, 1976), and liver Cu concentrations (MacPherson and Hemingway, 1968; Dalgarno and Mills, 1975; Buckley and Tait, 1981; Woolliams et al., 1985a,b; van Ryssen and Barrowman, 1987; Charmley and Ivan, 1989) in lambs and sheep, and increased both plasma and liver Cu concentrations of cattle (Stoszek et al., 1979; Wittenberg and Boila, 1988). In contrast, cupric sulfate was ineffective in raising Cu reserves of sheep (Judson et al., 1984; Ivan and Veira, 1985; Trengove and Judson, 1985), and calves (Kincaid et al., 1986).

Cupric sulfate supplementation increased ($P < .05$) liver Cu concentrations for steers consuming a high Mo diet (10 ppm) containing either low (.13% S) or high sulfate concentration (.37% S), relative to steers receiving no Cu

supplementation, cupric oxide needles or injectable Cu (Wittenberg and Boila, 1988). These researchers also observed that plasma Cu was greatest ($P < .05$) with cupric sulfate-treated steers, but differences in ceruloplasmin activity in plasma among treatments were not significant ($P > .05$).

Hepatic Cu concentrations of grazing lambs dosed once weekly with a drench containing 70 mg Cu as cupric sulfate, Cu-glycine or Cu-EDTA during 12 months were substantially increased compared with the animals fed no supplement, but there was no difference in the effectiveness among the three Cu salts (Macpherson and Hemingway, 1968).

Cupric acetate

The slope of acetate in Exp. 2 was higher than that of oxide, but it did not differ from that of sulfate or chloride. This contrasts with findings of Todd et al. (1962), who observed that cupric acetate appeared to be more toxic than cupric sulfate, in that signs of toxicosis developed sooner and, therefore, when much smaller amounts of Cu had been ingested. Signs of toxicosis developed in the group fed cupric acetate within 7 to 14 weeks, whereas the first case in the cupric sulfate group occurred after 17 weeks. The same amount of Cu was administered to each sheep daily, .25 g Cu, as acetate or sulfate (Todd et al., 1962).

Cupric chloride

The slope of chloride was greater than that of oxide and was lower than that of sulfate in Exp. 1. In Exp. 2

the slope of chloride did not differ from that of sulfate. Also, it did not differ from carbonate in Exp. 1 or from acetate in Exp. 2. The slopes of chloride in Exp. 1 and 2 were similar, .001891 ± .000335 ppm Cu (dry basis)/ppm Cu (as-fed basis) and .001910 ± .000273 ppm Cu (dry basis)/ppm Cu (as-fed basis), respectively.

Cupric chloride labelled with ^{64}Cu promoted higher blood and plasma activity of Cu than cupric sulfate or cupric nitrate, in both orally and intravenously administered mature wethers, however, significantly more ^{64}Cu was excreted in urine from intravenously-administered chloride (Lassiter and Bell, 1960). Addition of cupric sulfate or cupric chloride, in aqueous solutions, to barley silage or grass silage, containing 6.1 ppm Cu and 4.3 ppm Cu, respectively, to increase Cu concentration in the diet to 20 ppm, increased ($P < .05$) final concentration of Cu in liver of cows, in a 4-months study (Ivan et al., 1990). Differences between sulfate and chloride were not significant ($P > .05$, Ivan et al. 1990).

Cupric carbonate

Carbonate in Exp. 1 had a higher slope than oxide, but it did not differ from chloride and from sulfate. Cupric carbonate had the highest rate of absorption in cattle, compared with cupric nitrate, cupric sulfate, cuprous oxide or cupric oxide, but it also had the highest rate of excretion in urine and feces (Chapman and Bell, 1963).

Zanetti et al. (1991), in chicks fed conventional dietary amounts, obtained a relative bioavailability value of 66% ($P < .05$) for a feed grade cupric carbonate, when compared to a reagent grade cupric acetate. The same carbonate and acetate sources were used in Exp. 1 and 2, but no direct comparison can be made, because in the present study the two sources were used in different experiments. Cupric carbonate resulted in a relative value of 123% in Exp. 1 and cupric acetate in 105%, in Exp. 2, both compared with cupric chloride. There were no differences ($P > .05$) between carbonate and chloride in Exp. 1 or acetate and chloride in Exp. 2.

Cupric oxide

Oxide in Exp. 1 and 2 had the lowest slope. It was lower than chloride, sulfate, acetate and carbonate. The slopes for oxide obtained in both experiments were quite similar, .000893 ± .000489 ppm Cu (dry basis)/ppm Cu (as-fed basis) in Exp. 1 and .001227 ± .000390 ppm Cu (dry basis)/ppm Cu (as-fed basis) in Exp. 2.

Even though cupric oxide is the most widely used Cu supplement in animal feed (Miller, 1983; Nelson, 1988), it was generally found to be the less available Cu source (Lassiter and Bell, 1960; Chapman and Bell, 1963; Kincaid, 1988b). Copper supplied as carbonates, sulfates and chlorides has been reported to be well utilized, while lower

bioavailability has been reported for either cupric or cuprous oxide (Fritz, 1976).

Wethers fed approximately 29 ppm supplemental Cu as cupric chloride, cupric acetate or cupric sulfate in a corn silage-based diet, for 86 days, had their liver Cu content increased ($P < .05$) by 61%, 48% and 57%, respectively, while in lambs fed the basal diet, which contained 6.7 ppm Cu, it decreased 20% (Charmley and Ivan 1989), compared with the average of liver Cu content of eight animals slaughtered at the beginning of the experiment. It was concluded that chloride, acetate or sulfate salts were equally available to lambs (Charmley and Ivan 1989), which is in agreement with the finding in Exp. 2.

In a 10-day feeding experiment, 20, 40, 60 or 80 ppm added dietary Cu from cupric acetate or 60 ppm from oxide, sulfate or carbonate did not affect ($P > .10$) final liver Cu concentrations of Cu-depleted sheep (Ledoux, 1987). However, when the ratios of final liver Cu to initial liver Cu concentrations were evaluated, a numeric increase ($P > .10$) was observed with increasing dietary Cu concentrations. At 60 ppm added dietary level, ratios were 1.52, 1.28, 1.21 and 1.13 for carbonate, acetate, sulfate and oxide, respectively. The final/initial ratio of animals fed the control diet was 1.10. Since liver biopsy samples were taken at the beginning of the experiment, regression analysis using the initial value as covariate would probably

have been more sensitive. The final/initial ratios in the present study were much higher than in Ledoux's experiment. In animals fed 60 ppm additional Cu as cupric chloride, they were 1.65, 1.92 and 2.18, in Exp. 1, 2 and 3, respectively. Ledoux (1987) used cupric acetate, but cupric acetate and cupric chloride did not differ in Exp. 2.

One factor that seems important in Ledoux's (1987) experiment is the high initial hepatic Cu concentration, in spite of previous treatment with intravenous injections of ammonium tetrathiomolybdate, to induce Cu-depletion. It is not known whether it was useful or not, because no estimate of the Cu status of the sheep prior to this treatment has been done, but reductions in hepatic Cu concentrations of sheep in consequence of intravenous administrations of tetrathiomolybdate have been reported (Gooneratne et al., 1981, 1989b; Howell and Kumaratilake, 1990). The average for the various groups ranged from 369 to 532 ppm in Ledoux's experiment, with an overall mean of 446 ppm, while in the present study the average initial value was generally lower than 250 ppm Cu, the overall means being 238, 166 and 204 ppm for Exp. 1, 2 and 3, respectively.

Ledoux et al. (1991) working with chicks, obtained relative Cu bioavailability values of .54% for cupric oxide, 88.5% for cupric sulfate, and 54.3% for cupric carbonate, when compared with cupric acetate. In the present study, if acetate were taken as the standard in Exp. 2, the relative

bioavailabilities of oxide and sulfate would be 52% and 119%, respectively. The value for sulfate is not so much different, but that for oxide is not as low in sheep as it was in chicks. If sulfate were used as the standard, the relative values of oxide, acetate and carbonate of Ledoux et al. (1991) would be .6% , 113% and 61% , respectively; of oxide and acetate from Exp. 2, 56% and 91%; and of oxide and carbonate from Exp. 1, 30% and 78%. Again, the values for acetate and carbonate obtained with lambs are more or less comparable to those of chicks, but oxide seems to be of much lower availability in chicks than in sheep.

A very low biological value of cupric oxide relative to cupric sulfate in chicks was also reported by Baker et al. (1991). A lack of response of liver Cu to high dietary concentrations of cupric oxide (up to 720 ppm) was observed by Norvell et al. (1974, 1975) in chicks, and by Cromwell et al. (1989) in weanling pigs (up to 500 ppm).

Cupric compounds are more soluble than cuprous compounds (van Dokkum, 1989). In this respect, it is somewhat surprising that cuprous oxide (Cu_2O) resulted in a relative value of 92.5% compared to cupric sulfate ($P > .05$) in chicks while for cupric oxide it was -1.7% ($P < .01$) (Baker et al., 1991). Neethling et al. (1968) did not observe differences in percentage of recovery of ^{64}Cu in feces, blood, bile and urine when administered intravenously and intraruminally as the cuprous (Cu_2Cl_2) or the cupric

form ($CuCl_2$). The authors stated that apparently the cuprous ion was rapidly oxidized to the higher valence state in rumen or in liver of sheep. Oxidation in rumen, however, would be quite surprising, considering the highly reducing environment present in this organ.

Copper in Serum and Relative Values of the Copper Sources

Wethers from Exp. 2 had initial and final serum Cu concentrations within .6 and 1.5 ppm, the normal range in sheep, and even within .8 and 1.2 ppm, in which a high proportion of the values is generally found (Underwood, 1981).

Lassiter and Bell (1960) reported greater blood and plasma Cu concentrations in sheep following oral administration of cupric chloride, relative to cupric sulfate and cupric carbonate, and these three compounds resulted in greater concentrations than those of cupric oxide. Steers treated with cupric carbonate had the highest level of ^{64}Cu in whole blood, followed by those fed cupric nitrate, cupric sulfate, cuprous oxide and cupric oxide (Chapman and Bell, 1963). The concentrations of plasma ^{64}Cu from steers fed cupric carbonate and cupric nitrate were not different ($P > .05$), but both were greater ($P < .05$) than other sources. Also, levels of ^{64}Cu in plasma were not significantly different for steers fed cupric sulfate and cupric chloride (Chapman and Bell, 1963).

In Exp. 2 of the present study, only oxide could be separated from other sources, in terms of the size of the slope. Plasma is a poor indicator of Cu status in sheep (Wiener et al., 1976) and cattle (Smart and Christensen, 1985). Plasma Cu concentrations are held remarkably constant (Suttle, 1987) and in cattle they only drop below .6 to .9 ppm after hepatic Cu concentrations are smaller than about 40 ppm (Haag and Adams, 1958; Hartmans, 1969; Claypool et al., 1975; Stoszek et al., 1986). A similar picture was found in red deer. Plasma Cu values below .5 ppm were only observed when liver Cu concentrations fell below 20 ppm, (Freudenberger et al., 1987).

Of three Cu sources (chloride, nitrate and sulfate) used in an experiment with wethers by Lassiter and Bell (1960), sulfate had the lowest solubility and exhibited the lowest amount of ^{64}Cu in blood, plasma, urine and feces from intravenously administered doses. The authors suggested that this was indicative of tissue preference for the sulfate form of Cu over chloride and nitrate.

After 2 months of feeding cupric acetate, cupric chloride or cupric sulfate, to increase dietary Cu concentration from 6.7 to 35 ppm, plasma Cu concentrations in wethers were lower ($P < .01$) in lambs fed the sulfate compound than in the control group and those fed acetate and chloride (Charmley and Ivan, 1989).

TCA-Soluble Copper

Kincaid (1988b) observed that cupric sulfate was more effective for maintaining plasma TCA-soluble Cu than cupric oxide, when diammonium tetrathiomolybdate (16 mg Mo/day) was concurrently fed to calves. In Exp. 2 of the present study a similar effect was observed, the sulfate promoting a higher slope ($P < .02$) than oxide in serum TCA-soluble Cu.

Copper-Lysine

Copper-lysine tended to be less available ($P = .0585$) than cupric sulfate, in Exp. 3. This does not agree with Forth et al. (1973), Kirchgessner and Grassmann (1970), Grassmann and Kirchgessner (1974), Ashmead and Christy (1985), Ashmead et al., 1985; Kincaid et al. (1986) and Kincaid (1988a).

Forth et al. (1973), in isolated intestinal segments of rats in vitro, observed a three-fold increase ($P < .05$) in the transfer of ^{64}Cu, from cupric sulfate, when it was administered simultaneously with histidine, alanine or phenylalanine, compared with controls without amino acids. Kirchgessner and Grassmann (1970) reported a higher ($P < .05$) Cu content of liver of rats (μg/total liver) when Cu was added as an amino acid, peptide or polypeptide complex, than as cupric sulfate. Fifteen different L-amino acid complexes were compared to cupric sulfate in rats by Kirchgessner and Grassmann (1970), liver Cu storage being on

the order of 80% to 140% for the Cu amino acid complexes relative to cupric sulfate; Cu-lysine resulted in 27.0 ± 5.6 μg Cu in the liver, versus 22.9 ± 3.9 μg for cupric sulfate ($P < .05$). Liver Cu concentrations in rats were higher ($P < .05$) when Cu-leucinate was fed (38.0 ± 2.7 ppm) than when cupric sulfate was fed (34.9 ± 5.5 ppm) and both were higher ($P < .05$) than in control rats (Grassmann and Kirchgessner, 1974). Ashmead and Christy (1985), citing privately printed or unpublished literature, claimed that Cu from an amino acid chelate was absorbed 2.8 times better than that from cupric carbonate, three times better than that from cupric oxide and 4.1 times better than that from cupric sulfate. Calves fed for 12 weeks a Cu-proteinate supplement had greater ($P < .05$) hepatic Cu concentrations (325 ppm) than those fed cupric sulfate (220 ppm), but not than the control group fed the basal diet (238 ppm); calves fed cupric sulfate did not differ from control (Kincaid et al., 1986). Kincaid (1988a) noticed that a Cu-proteinate appeared more effective than cupric sulfate in promoting increase in liver Cu concentrations of calves.

In cattle, however, a Cu-amino acid chelate (Kincaid, 1988a,b) and a Cu-proteinate (Wittenberg et al., 1990) were comparable to cupric sulfate, or were less effective than cupric sulfate (Miltimore et al., 1978). Kincaid (1988a,b) found that cupric sulfate was comparable to a Cu-amino acid chelate for maintaining plasma and liver Cu in calves when

dietary inorganic Mo was concurrently fed. Wittenberg et al. (1990) did not observe differences between cupric sulfate and a Cu-proteinate for rate of liver Cu repletion or for ability to reduce plasma and liver Mo concentrations in cattle consuming a high Mo diet (10 ppm added Mo). A chelated form of Cu did not increase liver Cu concentrations in cattle, compared with cupric sulfate, even when the chelated form was fed at five-fold the recommended levels (Miltimore et al., 1978). Ward and Spears (1991) compared Cu solubility in ruminal fluid of steers fed a corn silage diet supplemented with 5 ppm Cu from Cu-lysine or cupric sulfate and did not detect differences between the two Cu sources.

The absorption of Cu by everted sacs of sheep jejunum in vitro, in presence of histidine, lysine or glutamine, followed the same kinetic pattern as the absorption of ionic Cu and with rates not significantly different (Turner et al., 1987). The authors, however, stressed the possibility of amino acids or other Cu-binding ligands affecting the transfer of Cu from the mucosal cell to the blood stream and this could not be discerned in their experimental conditions.

In chicks, using 0 to 150 ppm added dietary Cu , Baker et al. (1991) did not observe significant differences between a Cu-lysine complex (same as used in Exp. 3) and cupric sulfate in the ability to increase hepatic Cu

concentrations. Baker et al. (1991), using the linear regression slope-ratio, obtained a relative availability value of 115.5% for the Cu-lysine complex, compared with the standard, reagent grade cupric sulfate ($P > .05$). In this laboratory, the relative value of Cu-lysine for chicks was 101.5% of that of cupric sulfate (P. R. Henry and C. B. Ammerman, personal communication).

With single amino acids as ligands, the rate of Cu absorption depends on the type of amino acid, its configuration, and the degree of polymerization (Underwood, 1977). The author suggested that such differences could partially explain variations in Cu availability arising from diets containing different amounts and types of protein. Amino acids have usually been assumed to facilitate Cu absorption from the gastrointestinal tract, but definitive studies are lacking and Cu-amino acid double-labelling experiments have not been reported (Ettinger, 1984). Amino acids modify Cu absorption, either enhancing it or depressing it, below absorption of cupric sulfate, depending upon the nature of the complex they form with Cu (Delhaize et al., 1987). In ruminant animals, pregastric fermentation may bring about changes in the forms of Cu that are not possible in other species (Gawthorne, 1987).

So, considering the results obtained in Exp. 3 and data and statements from the literature, the frequent claim that chelated minerals are absorbed and(or) metabolized more

efficiently than inorganic compounds (Kirchgessner and Grassmann, 1970; Kratzer and Vohra, 1986; Ashmead, 1970; Ashmead et al., 1985; Manspeaker et al., 1987) needs to be confirmed, at least for Cu, in ruminants, since most of the work has been done with rats (Kirchgessner and Grassmann, 1970) and in vitro (Ashmead 1970, Ashmead et al., 1985). A difference may exist between chelates and complexes, but there is not enough evidence to support this hypothesis. Even in rats the superiority of chelated Cu forms is not definitive. Marceau et al. (1970) reported that administration of Cu to rats in a protein or amino acid-bound form did not change initial rate of absorption from gastrointestinal tract, which was the same as for ionic ^{64}Cu.

An intriguing observation is that apparent absorption of Cu increased ($P < .05$) in adult wethers when urea was added to corn silage (Ivan et al., 1983). The crude protein concentration of silage with or without urea was not reported, but the authors suggested that a greater availability of Cu due to a higher amino acid content possibly resulted in the urea-added corn silage. The effect of urea may not be related to a greater amino acid content at the intestinal level, because Goodrich and Tillman (1966b) observed that lambs fed protein diets absorbed less Cu than those fed urea, and these authors suggested

formation of a Cu-ammonium complex, in urea-treated animals, which could have favored Cu absorption.

Solubility Tests versus Relative Bioavailability Tests

In general, solubility values obtained in neutral ammonium citrate, .4% HCl and 2% citric acid are in good agreement with relative bioavailability values obtained from the ratio of the slopes of hepatic Cu concentration in lambs. The solubility test indicated cupric oxide to be a very insoluble source of Cu and the same was observed for its relative bioavailability value. Acetate, carbonate, chloride and sulfate differed very little in their solubility values in neutral ammonium citrate, .4% HCl and 2% citric acid and so did their relative bioavailability. Copper-lysine tended to be less soluble in neutral ammonium citrate, 2% citric acid and .4% HCl. Its solubility in water was lower than that of cupric sulfate. These values are in good agreement with the relative bioavailability values obtained with lambs. There are situations, however, where mineral solubility can be misleading, one example being the availability of Cu from cupric sulfate in the presence of increased dietary Mo (Madsen and Johnson, 1989).

Particle Size and Relative Bioavailability Values

Particle size of the Cu sources does not seem to have affected their relative bioavailability values. Cupric

chloride had the greatest proportion (47%) of particles greater than 850 μm but its relative value was similar to that of acetate and carbonate, which had 98% and 80% of particles smaller than 850 μm, respectively. Cupric oxide had a larger proportion of particles smaller than 150 μm (52%), nonetheless it had a lower relative bioavailability value than cupric chloride, which had particles greater than 150 μm. The distribution of particle sizes of Cu-lysine and cupric sulfate was of about the same magnitude.

Effect of Copper Intake on Hepatic Concentrations of Iron, Manganese and Zinc

In Exp. 1, only Zn concentrations in liver were affected ($P < .005$) by dietary Cu even though there was no systematic change that can be related to dietary Cu concentrations. In Exp. 2 and 3, no influence of dietary Cu on hepatic Fe, Mn and Zn was observed.

Zinc and Fe concentrations in liver of sheep dosed with a cupric sulfate solution were markedly elevated (Gooneratne et al., 1979), but those sheep had a very high Cu intake and were treated until hemolytic crisis developed. In a 15 and 30-day period study, addition of 15, 30, and 45 ppm Cu to the diet of sheep did not affect hepatic Fe and Mn concentrations, but Zn concentrations in liver were greater ($P < .01$) in animals slaughtered on day 0 than in Cu supplemented animals at the end of the trial (Ledoux, 1987). Van Ryssen and Stielau (1980a) reported an increase in

hepatic Fe concentrations as Cu intakes decreased from 70 mg/day to 25 mg/day and greater ($P < .01$) amounts of Zn were present in liver of sheep fed high Cu treatments (70 mg Cu/day). Concentrations of Mn and Zn in liver were not affected ($P > .05$) by dietary Cu level (10 or 30 ppm), but Fe concentrations were increased ($P < .01$) by high Cu concentrations, fed for 12 weeks (Pond, 1989).

Zinc concentration in liver of calves fed 26 ppm supplemental Cu, as a proteinate or cupric sulfate, was similar to animals fed the control diet containing 11 ppm Cu (Kincaid et al., 1986). Liver concentrations of Fe, Mn and Zn were not affected ($P > .01$) in initially Cu-depleted sheep fed 0, 20, 40, 60 and 80 ppm added dietary Cu concentrations for 10 or 20 days (Ledoux, 1987), or in Cu-depleted sheep fed added Cu concentrations at 20, 40, 60 and 80 ppm from cupric acetate or 60 ppm from cupric oxide, cupric sulfate or cupric carbonate (Ledoux, 1987).

In view of the results obtained in the present study and reports in the literature, no consistent effects of high dietary Cu levels on hepatic Fe and Zn concentrations especially during short term studies can be drawn. According to O'Dell (1984a), the nutritional effect of excessive Zn on Cu metabolism is much stronger than vice versa.

Cost of Available Copper from Cupric Oxide and Cupric Sulfate

Cupric oxide is the Cu compound used most widely in animal feed supplements (Miller, 1980; Nelson, 1985, 1988). Even though the cost per unit weight of Cu from cupric sulfate is greater than that of cupric oxide, the cost of available Cu from sulfate is less than that of cupric oxide.

Using prices listed by Nelson (1988), $ 2.73/kg of cupric oxide and $ 1.01/kg of cupric sulfate, cost of elemental Cu from the oxide, considering 74% Cu in the compound, would be $ 3.63/kg Cu, and from sulfate, using 25% Cu in the compound, $ 4.03/kg Cu. In Exp. 1, oxide was 30% as available as sulfate; and in Exp. 2, 56%. Even if the larger value is taken, 56%, the cost of oxide per kilogram available Cu is 60% greater than from sulfate. This does not take into account the additional cost of shipping and handling of the compound to, in and from the feed mill. Nelson (1988) already recognized that the additional cost of using cupric sulfate as a Cu source is offset by its greater biological availability. There was not a big change in the prices of these two Cu compounds over the last 3 years. According to J. Nelson (personal communication), the cost of cupric oxide in September, 1991, was $ 2.97/kg and of cupric sulfate, $ 1.08/kg, therefore 8.8% and 6.9% greater than in 1988, so that the calculations based on the old prices remain essentially the same.

Summary and Conclusions

Three experiments were conducted to determine the relative biological availability of Cu from inorganic sources and a copper-lysine complex, using crossbred wether lambs. After a variable adaptation period to a ground corn-soybean meal-cottonseed hulls basal diet and to cages and for recovery from a liver biopsy procedure, lambs were assigned randomly to dietary treatments and fed for 10 days. Treatments were the basal diet (control), supplemented with 60, 120 or 180 ppm Cu as reagent grade cupric chloride, or 120 ppm Cu from feed grade cupric carbonate, cupric oxide or cupric sulfate (Exp. 1); 60, 120 or 180 ppm Cu as reagent grade cupric chloride, or 120 ppm Cu from reagent grade cupric acetate or feed grade cupric oxide or cupric sulfate (Exp. 2); or 60, 120 or 180 ppm Cu as feed grade Cu-lysine complex or reagent grade cupric sulfate (Exp. 3). On day 11 of the feeding period, animals were euthanized and liver collected. Copper, Fe, Mn and Zn concentrations were determined in the biopsy samples (initial value) and in samples of the whole liver (final value). Logarithmic transformed data were analyzed by multiple linear regression of hepatic mineral concentrations on added dietary Cu concentrations and Cu level x Cu source interaction, with initial value as covariate. Relative bioavailability values of Cu sources were calculated using the slope ratio method. Increases in dietary Cu concentrations resulted in linear

increases in hepatic Cu concentrations. In Exp. 1, bioavailability values for carbonate, oxide and sulfate were 123%, 47% and 157%, relative to cupric chloride. In Exp. 2, the relative biological availability values for chloride, acetate, oxide and sulfate were 100%, 105%, 64% and 115%, respectively. In Exp. 3, Cu from Cu-lysine complex was 68% as available as that from cupric sulfate. Iron, Mn and Zn concentrations in liver were not affected by added dietary Cu.

The relative bioavailability values for cupric acetate, cupric carbonate, cupric chloride and cupric sulfate were of similar magnitudes. Cupric oxide was less available for lambs than cupric acetate, cupric carbonate, cupric chloride and cupric sulfate. The biological availability value of the Cu-lysine complex was lower than that of cupric sulfate.

Substitution for cupric oxide in mineral mixtures or concentrates with a more bioavailable Cu source such as cupric sulfate, should be considered by the animal feed industry. Based on hepatic Cu accumulation in lambs, there seems to be little reason for using a Cu-lysine complex in this species, in substitution to cupric sulfate.

CHAPTER 4
EFFECT OF HIGH DIETARY MOLYBDENUM CONCENTRATIONS AND LENGTH OF FEEDING TIME ON MOLYBDENUM AND COPPER STATUS OF LAMBS: EXPERIMENT 4

Introduction

Molybdenum is an essential element but Mo requirements of animals are extremely low (Underwood, 1981). A Mo deficiency in farm animals under practical feeding conditions has not been reported to date.

The importance of Mo in animal nutrition stems from its interaction with Cu, in association with S, mainly in ruminants, either because of its effect of inducing Cu deficiency or because of its potential to avoid Cu toxicosis. The latter situation is the main interest in this study.

Acute and chronic toxicosis in ruminants, especially in sheep, have been reported all over the world, particularly in intensive indoor rearing of lambs fed concentrate feeds high in Cu and relatively low in Mo (Adamson et al., 1969; Tait et al., 1971; Todd, 1976; Niederman et al., 1987). Swine feed containing cupric sulfate as a growth promoter has been a frequent cause of Cu poisoning when fed to sheep or calves (Todd, 1976). Contamination of a commercial sheep feed with ingredients intended for swine has also been

reported (Stahr et al., 1989). Clegg et al. (1986) described in sheep Cu intoxication which originated from Cu pipes used for drinking water. Acute or chronic Cu poisoning has also been observed in calves (Mylrea et al., 1974), dairy cows (Stogdale, 1978) and goats (Belford et al., 1989).

Outbreaks of Cu toxicosis have been effectively controlled through intravenous (Gooneratne et al., 1981, 1989b; Humphries et al., 1986), subcutaneous (Humphries et al., 1988a) or oral (Kincaid and White, 1988) administration of ammonium tetrathiomolybdate, but thiomolybdate preparations are not readily available.

Dietary Mo or Mo and S supplementation of commercial concentrate sheep diets have been suggested as a means of preventing or controlling hepatic Cu accumulation (Harker, 1976; Suttle, 1977). Olson et al. (1984) suggested addition of Mo and SO_4 to high-Cu broiler litter fed to ewes. Addition of high concentrations of Mo and S to diets for limited periods of time has also been suggested to decrease liver Cu concentrations in lambs (van Ryssen et al., 1986).

The addition of Mo plus SO_4 to concentrate diets has been effective in decreasing hepatic Cu concentrations of lambs fed high-Cu diets under experimental conditions (Hogan et al., 1968; Ross, 1966; Harker, 1976; Suttle, 1977) or to decrease death losses of Cu-poisoned lambs under practical

conditions (Pierson and Aanes, 1958; Hidiroglou et al., 1984; Niederman et al., 1987).

The objective of the present experiment was to estimate the effect of length of time and concentration of Mo supplementation on Cu and Mo excretion and tissue uptake in lambs, to determine the best tissue or excretion to collect and optimal length of time for a high-level short-term dietary Mo supplementation as a method to compare inorganic Mo sources.

Materials and Methods

Forty Texas crossbred wether lambs, weighing from 33 to 47 kg (average ± standard deviation = 40.2 ± 3.9 kg), were assigned randomly to a 4 x 2 factorial arrangement of treatments which included 0, 15, 30 or 45 ppm added dietary Mo fed for 14 and 28 days. The Mo source was sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), reagent grade, containing 39.6% Mo, by analysis, and was added to the corn-soybean meal-cottonseed hulls basal diet (Table 4-1) on an air-dry basis, at the expense of corn starch. Dietary Mo concentrations were verified by chemical analysis. The basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S (dry basis), by analyses, and was formulated to meet the nutrient requirements of lambs (NRC, 1985).

For the duration of the experiment lambs were housed in metallic individually raised pens with expanded metal floor,

TABLE 4-1. COMPOSITION OF THE BASAL DIET (EXPERIMENT 4)

Ingredients	Percent (as-fed basis)[a]
Ground yellow corn	56.95
Cottonseed hulls	21
Soybean meal, 48% CP	12
Alfalfa meal	3
Corn oil	3
Cornstarch[b]	2.28
Ground limestone	.55
Trace mineralized salt	1
Vitamins[c]	+
Sodium sulfate (Na_2SO_4)[d]	.22
Sodium selenite (Na_2SeO_3)[e]	+
Ethoxyquin	.0125
Mineral concentration[f]	
Ca, %	.55
Mg, %	.16
P, %	.26
S, %	.22
Cu, ppm	11
Mo, ppm	1.2
Fe, ppm	119
Mn, ppm	43
Zn, ppm	61

[a] Diet dry matter 88.9%.

[b] Molybdenum supplements added on an air-dry basis at expense of equivalent weight of corn starch.

[c] Vitamin A palmitate, 2200 USP units/kg; vitamin D_3, 440 USP units/kg; vitamin E, 15 IU/kg.

[d] Provided .05% additional S.

[e] Equivalent to .2 ppm Se.

[f] Dry matter basis.

in a ventilated barn. During a 7-day adjustment period all lambs were fed the basal diet. Animals were individually fed daily 1 kg of the respective experimental diet and tap water containing .02 ppm Mo was available ad libitum. No Cu was detected in tap water.

Feces and urine were collected daily during the last 5 days of each period. Feces were collected in canvas bags, and urine was collected individually into plastic buckets containing 100 ml 25% HCl, added daily. Total daily fecal and urinary output were measured and sampled on a 10% basis. Fecal aliquots were composited in plastic bags and kept frozen until dried at 60°C for 72 hours for grinding. Urinary aliquots were composited in plastic bottles and maintained in a refrigerator, filtered through a No. 40 Whatman paper into acid-washed plastic bottles and frozen before analyses.

On days 0, 13 and 27 of the experiment, blood samples were taken by jugular vein puncture. Blood was centrifuged for 30 minutes at 2500 x g, 20 minutes after collection. An equal volume (2 ml) of 10% (weight/volume) trichloroacetic acid (TCA) was added to serum samples, mixed and centrifuged at 2500 x g, for 25 minutes. The supernatant was saved and precipitate was resuspended into 5% TCA, and the mixing and separating procedures repeated. Supernatant removed from both processes was combined for TCA-soluble Cu analysis.

At the end of each period animals were slaughtered for collection of bile, liver, kidney and muscle (sterno mandibularis), which were frozen for subsequent mineral analyses. All animals were classified as lambs at slaughter.

Samples of kidney, liver, muscle, bile, feces, urine and feed were digested in nitric acid (Fick et al., 1979). Bile and urine were evaporated and the residue ashed at 600°C before wet solubilization. Copper concentrations in basal diet, tissues, excretions, serum and TCA-supernatant, and Fe, Mn and Zn concentrations in liver and kidney were determined by flame atomic absorption spectrophotometry on a Model 5000 spectrophotometer with an AS-50 autosampler (Perkin-Elmer Corp., Norwalk, CT; Anonymous, 1982). TCA-insoluble Cu in serum was calculated by subtracting TCA-soluble Cu from total Cu in serum. Molybdenum concentrations in feces, urine, kidney, liver, muscle, bile and feed were determined in digested samples by graphite furnace with a Perkin-Elmer Zeeman/3030 atomic absorption spectrophotometer with an AS-60 autosampler (Anonymous, 1984). Molybdenum readings of tissue, feces, urine, bile and feed in graphite furnace were run with three firings of deionized water in between samples or after the Mo standard, to decrease carryover effects. Carryover or memory effects on Mo determinations with graphite furnace atomic absorption were mentioned by Ericson et al. (1987) and Loya (1989).

Serum for Mo analysis was digested with HNO_3 and $HClO_4$ and diluted to 25 ml, followed by a complexation and coprecipitation procedure. The precipitate was dissolved with HNO_3 and diluted to 3 ml, which was run by inductively coupled plasma spectrometry (ICP) for Mo. Serum samples from unsupplemented lambs had to be combined within length of feeding period to obtain readings greater than the detection limit. This was done on an equal volume basis. Molybdenum determinations in serum were performed at the Environmental Trace Substance Research Center, of the University of Missouri, Columbia, MO. Phosphorus in basal diet was determined colorimetrically (Harris and Popat, 1954). Sulfur concentration in the basal diet was determined at the University of Minnesota, with a Model S-132 sulfur analyzer (Leco, Warrendale, PA). Standards were matched for macroelement and acid concentrations as appropriate and standard reference material from the National Institute of Standards and Technology (Gaithersburg, MD) was run with samples.

Animals' initial and final body weights and the difference between final and initial weights were analyzed by two-way analysis of variance and all laboratory data by multiple linear regression using the general linear model procedure (PROC GLM) of SAS (1988).

The statistical model was:

$Y = \beta_0 + \beta_1(\text{Mo Level}) + \beta_2(\text{Time}) + \beta_3(\text{L x T})$, where

Y = log mineral concentration in tissue or mineral excretion/day;

β_0 = intercept; β_1, β_2 and β_3 = slopes;

Mo Level = L = added dietary Mo concentrations, in ppm;

Time = T = length of time of feeding (14 or 28 days).

Presence of quadratic and cubic effects of Mo level was checked by introducing a quadratic and cubic term in the model when Mo level was significant. Logarithmic (log) transformation (base 10) was performed on the data (mineral concentrations in tissues or mineral excretion/day) in order to reduce variance heterogeneity.

In the statistical analysis of Mo in serum, each animal fed the basal diet, within feeding period, was assigned the same value, because serum samples were combined for analyses.

Results and Discussion

Feed Consumption

Most animals consumed the amount of feed provided (1 kg/day) in a few hours after feeding. Three lambs, one from the group fed the control diet for 14 days, one fed 15 ppm added Mo for 14 days, and one fed 45 ppm added Mo for 28 days, consumed only 93.4%, 83.4% and 68.8% of the total amount of feed offered. Feed refusal of those three lambs did not seem to be related to dietary Mo concentrations, but

rather to nervousness and stress or lack of adjustment to cages.

Both lack of response and negative responses in feed consumption in cattle and sheep to elevated dietary Mo concentrations were reported in literature. Heifers fed 100 ppm Mo for 12 months consumed less ($P < .05$) feed daily than unsupplemented animals, however, differences were not significant when feed intake was calculated on an equal body weight basis (Lesperance and Bohman, 1963). Lactating beef cows fed a diet containing 6 ppm Cu, .13% S and 0, 19 or 35 ppm Mo, for 9 weeks consumed all of the daily allotment of feed (Wittenberg and Devlin, 1987). There was no evidence of differences in feed consumption in lambs fed a high-Cu diet (45.3 ppm Cu) supplemented with 0, 2, 4, 8 or 16 ppm Mo, for 27 weeks (Suttle, 1977). Dietary Mo concentrations (.9, 18.4 or 40.7 ppm Mo) did not influence ($P > .05$) dry matter intake in ewes fed these Mo concentrations for 6 weeks (Wittenberg and Devlin, 1988).

Forage containing 3 or 4.3 ppm Mo fed to steers for 14 days resulted in lower ($P < .01$) intake than forage containing .6 ppm Mo (Buchman, 1966). Suttle and Field (1968a) observed a reduction in intake of about 50% in ewes fed for 45 days semipurified diets containing less than 1.5 ppm Cu, .2 ppm Mo and .07% S, supplemented with 50 ppm Mo plus 1% SO_4. Intake of lambs fed 75 mg Cu/day for 50 days was depressed in a further 12-day period when they were fed

140 mg Mo plus 2 g S/day or 140 mg Mo plus 2 g S/day and 4.3 mg Cu/day (van Ryssen et al., 1986). It seems therefore that greater concentrations or longer times than that used in Exp. 4 are required to affect feed intake.

Lambs' Body Weight

Initial and final live weights of lambs did not differ among treatments (P = .989 and P = .819, respectively). Final body weights did not differ from initial body weights (P = .933).

Weight gains of calves fed diets containing 100 and 200 ppm added Mo were not different from those fed the control diet (Skipper, 1951). Feed consumption of adult wethers fed for 160 days diets containing either 12 to 14 ppm Cu or 50 ppm additional Mo plus .4% SO_4 was not altered (Marcilese et al., 1969). Lactating beef cows fed 0, 19 or 35 ppm supplemental Mo in a diet containing 6 ppm Cu, .6 ppm Mo and .13% S, for 9 weeks, gained .4 kg/day (Wittenberg and Devlin, 1987). No evidence of differences in growth rate was seen in lambs fed a diet containing 45.3 ppm Cu and supplemented with 0, 2, 4, 8 or 16 ppm Mo, for 27 weeks (Suttle, 1977). No differences (P > .05) were observed in final weight of sheep fed .6, 20.8, 38.4 or 58.5 mg Mo/day in a basal diet providing 82 mg Cu and 3.7 g S/day (van Ryssen and Stielau, 1981). Body weight change of ewes fed diets containing 4.5 to 4.9 ppm Cu, .14% to .15% S and .9,

18.4 or 40.7 ppm Mo for 6 weeks was not influenced ($P > .05$) by dietary treatment (Wittenberg and Devlin, 1988). Final body weight of initially 5-month-old rams fed a basal diet containing 13 ppm Cu, 1.6 ppm Mo, .45% S and 38 ppm supplemental Mo for 14 weeks was slightly but not lower ($P > .05$) than body weight of unsupplemented control animals (van Ryssen et al., 1990).

However, when heifers were fed 6.0 or 9.2 ppm Cu, .2% S and 100 ppm Mo, at the end of 60 days there was a reduction ($P < .05$) in weight gain, compared to control animals fed a diet with .6 ppm Mo; the difference was greater ($P < .01$) at 120 days and remained so until the end of a 12-month period (Lesperance and Bohman, 1963). High levels of Mo administration (1.5 or 3.0 mg Mo/kg body weight/day), for 100 days, reduced ($P < .01$) body weights of yearling steers grazing pastures containing 8.2 ppm Cu, 1.3 ppm Mo and .32% S (Cook et al., 1966). This was equivalent to about 400 and 800 mg Mo/animal daily. Lambs fed 9.8 ppm Cu, .5 ppm Mo, .08% S and 25 ppm supplemental Mo for 30 weeks grew more slowly than unsupplemented animals, but there were no significant differences between growth rates of lambs fed additional 25 ppm Mo plus .5% SO_4 and the animals not fed additional Mo (Bremner and Young, 1978). Average daily gain of wethers fed 5 ppm Cu and 8.4 ppm Mo for 221 days was smaller ($P < .01$) than in lambs fed 5.4 ppm Cu and .4 ppm Mo in the diet (Ivan and Veira, 1985). Intravenous

administration of tetrathiomolybdate (2.6 mg/kg) in young lambs for about 10 weeks resulted in slower ($P < 0.1$) growth (Allen and Gawthorne, 1986).

As for feed intake, Mo effect on weight change seems to depend on dietary concentration and length of time of feeding. In addition, Cu and S dietary concentrations play an important role.

Fecal Molybdenum Excretion

Molybdenum excretion in feces increased with increasing dietary Mo concentrations in a cubic fashion ($P < .0001$; Tables 4-2, 4-3, 4-4). Time itself had no effect ($P = .65$) in the linear model (not shown in table) and was therefore excluded. The coefficient of determination (R^2) increased from .798 for the original model (Table 4-4) to .980 when the quadratic term was included. The addition of the cubic term resulted in a R^2 of .992 (Table 4-4), which is little improvement over the previous value. The coefficient of variation (CV) decreased from 8.7% in the linear to 1.8% in the cubic model. The means (Table 4-2) suggested a level * time interaction. When this term was included in the cubic model, it had an effect ($P = .05$, Table 4-4). Although there was a cubic effect ($P < .001$; Table 4-4), the linear effect of Mo level accounted for most of the variation. Type I sum of squares (SS) of the linear effect represented 80% of the total SS. Most of the remaining variation was

TABLE 4-2. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER EXCRETION IN THE FECES OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a]				
		0	15	30	45	Mean
Molybdenum	14	.60 ± .03[b]	12.32 ± .48	34.47 ± 3.22	50.87 ± 1.92	24.57 ± 4.55
	28	.61 ± .05	16.42 ± .48	33.91 ± 1.22	36.07 ± 2.95	21.75 ± 3.38
	Mean	.61 ± .03	14.37 ± .75	34.19 ± 1.62	43.47 ± 2.97	23.16 ± 2.80
Copper	14	6.3 ± .28	6.3 ± .22	6.6 ± .15	6.2 ± .50	6.3 ± .15
	28	6.6 ± .20	6.1 ± .10	6.6 ± .24	6.5 ± .27	6.4 ± .11
	Mean	6.4 ± .17	6.2 ± .12	6.6 ± .13	6.3 ± .28	6.4 ± .09

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in mg/day (n = 5).

TABLE 4-3. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER EXCRETION IN THE FECES OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	2.780988	123.72	.0001	.022477
	Level	.142786	24.31	.0001	.005874
	Levsqr[d]	-.003793	-11.20	.0001	.000339
	Levcub[e]	.000035	7.10	.0001	.000005
	L * T[f]	-.000115	-2.02	.0513	.000057
Copper	Intercept	.79972	22.96	.0001	.03483
	Level	-.00042	-.34	.7359	.00124
	Time	.00021	.13	.8941	.00157
	L * T	.00002	.36	.7211	.00006

[a] Based on log (base 10) of μg Mo/day and mg Cu/day.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Quadratic term of Mo level.

[e] Cubic term of Mo level.

[f] Level * time interaction.

TABLE 4-4. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (μg/day) AND COPPER (mg/day) EXCRETION IN FECES (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1, Mo	3	17.5691		.0001	.798	8.7
Error	36	4.4548				
Level	1		17.5232	.0001		
Time	1		.0004	.9551		
L * T[f]	1		.0455	.5481		
Model 2, Mo	4	21.8471		.0001	.992	1.8
Error	35	.1768				
Level	1		17.5232	.0001		
Levsqr[g]	1		4.0487	.0001		
Levcub[h]	1		.2546	.0001		
L * T	1		.0206	.0513		
Model, Cu	3	.0011		.8889	.017	5.2
Error	36	.0624				
Level	1		$4*10^{-8}$	.9964		
Time	1		.0009	.4840		
L * T	1		.0002	.7211		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

[g] Quadratic term of Mo level.

[h] Cubic term of Mo level.

due to a quadratic effect of Mo level (18%). The cubic term, however, accounted only for 1% of the total SS, as did the level * time interaction. Therefore, for practical purposes, a linear model would probably be adequate to describe the relationship between added dietary Mo concentrations and fecal Mo excretion in lambs.

The principal route of excretion of an oral dose of ^{99}Mo in rats and pigs is generally via urine, whereas in sheep and cattle it is via feces (Bell et al., 1964; Miller et al., 1972). Fecal excretion of ^{99}Mo in 7 days by steers averaged 92.4% of an oral dose and 30.2% of an intravenous dose, whereas in swine 15% of an oral dose and only .6% of an intravenous dose were excreted in feces in 5 days (Bell et al., 1964). Duodenal administration of molybdate in sheep resulted in rapid absorption and excretion, via urine, of most of the dose administered; however, after ruminal administration most of it remained in feces (Mason et al., 1978). When Mo intake of wethers was small, less than 10% of the ingested amount was excreted from the body and most of this was in urine; when additional dietary Mo was provided (5, 10, 15 or 25 mg Mo/day), 50% to 60% of the dose was excreted, with approximately 65% of that excreted appearing in feces, (Weber et al., 1983). An increase ($P < .01$) in excretion of Mo in feces was noticed by Lesperance et al. (1985) in weanling heifers fed 0 or 100 ppm Mo for 12 months. The addition of .5% SO_4 reduced ($P < .05$) fecal Mo

excretion in animals fed 100 ppm Mo (Lesperance et al., 1985). This disagrees with earlier studies by Dick (1956a), who reported an increase in the amount of Mo excreted in feces of sheep by the administration of sulfate, suggesting a reduction in Mo absorption from gut.

Fecal Copper Concentrations

Ewes fed diets based on dried-grass meal, ground straw and barley, containing 6 to 7 ppm Cu, excreted 2.9 to 4.6 mg Cu/day in feces (Stevenson and Unsworth, 1978). Fecal Cu excretion in lambs fed the basal diet (Table 4-2) was larger, but their dietary Cu was also greater. Fecal Cu excretion (Table 4-2, 4-3, 4-4) was not affected by added dietary Mo concentration ($P = .74$) or by length of time of feeding ($P = .89$). No level * time interaction was detected ($P = .72$).

Hidiroglou et al. (1984) reported an increase in fecal Cu excretion, in Cu-poisoned lambs, 4 days after initiation of molybdate plus sulfate supplementation. It persisted throughout the monitoring period (approximately 5 weeks). Golfman and Boila (1990) reported in steers a tendency toward greater ($P = .07$) fecal excretion of Cu with the addition of 10 ppm Mo to diets fed for 21 days.

Urinary Molybdenum Excretion

Urinary excretion of Mo (Table 4-5) was markedly raised by increasing dietary concentrations of the element. The

curve that best described the increase was cubic ($P < .0001$; Table 4-6, 4-7). Length of time of feeding did not affect urinary Mo excretion when the linear model was run ($P = .82$) neither was there a level * time interaction ($P = .72$). The R^2 was .675 for the original model (Table 4-7) and it increased to .894 when the quadratic term was incorporated. The addition of the cubic term raised R^2 to .954 (Table 4-7). The CV was 17.2% in the linear model, 9.7% in the quadratic model and 6.5% in the cubic model. Most of the total SS was represented by the linear effect of Mo level (69%; Table 4-7), but the quadratic term of Mo level accounted for 22% and the cubic term for 6% of total SS. A linear model, therefore, seems inappropriate to describe the dietary Mo concentration and daily urinary Mo excretion relationship.

Urine is a major route of excretion of Mo in pigs and rats, but not in cattle or sheep on low sulfate intakes (Underwood, 1976, 1977; Mills and Davis, 1987). Urinary excretion of Mo in cattle was less than .5% of an oral dose of ^{99}Mo and less than 3% of an intravenous dose during the first 24 hours; at the end of 7 days, urinary excretion averaged 4.5% of the orally administered dose and 9.5% of the intravenous dose (Bell et al., 1964). In contrast, both oral and intravenously administered doses of ^{99}Mo were rapidly excreted in urine of swine; in the first 24 hours 53% to 86% of the oral or intravenous dose had been excreted

TABLE 4-5. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER EXCRETION IN THE URINE OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Molybdenum	14	101 ± 52[b]	4948 ± 461	5839 ± 739	9157 ± 891	5011 ± 796
	28	48 ± 8	5392 ± 683	6501 ± 681	11414 ± 1763	5839 ± 1035
	Mean	75 ± 26	5170 ± 396	6170 ± 487	10286 ± 1004	5425 ± 648
Copper	14	556 ± 154	507 ± 137	573 ± 61	590 ± 79	556 ± 53
	28	652 ± 196	783 ± 175	497 ± 113	447 ± 167	595 ± 82
	Mean	604 ± 119	645 ± 114	535 ± 62	519 ± 90	576 ± 48

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in μg/day (n = 5).

TABLE 4-6. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER EXCRETION IN THE URINE OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	1.70969	25.32	.0001	.06753
	Level	.24266	14.05	.0001	.01728
	Levsqr[d]	-.00885	-8.69	.0001	.00102
	Levcub[e]	.00010	6.83	.0001	.00002
Copper	Intercept	2.49165	12.95	.0001	.19245
	Level	.00963	1.40	.1689	.00686
	Time	.01124	1.29	.2042	.00869
	L * T[f]	-.00051	-1.66	.1060	.00031

[a] Based on log (base 10) of μg Mo/day and μg Cu/day.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Quadratic term of Mo level.

[e] Cubic term of Mo level.

[f] Level * time interaction.

TABLE 4-7. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (μg/day) AND COPPER (μg/day) EXCRETION IN URINE (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1, Mo	3	24.0678		.0001	.675	17.2
Error	36	11.6008				
Level	1		24.0224	.0001		
Time	1		.0027	.9278		
L * T[f]	1		.0428	.7177		
Model 2, Mo	3	34.0267		.0001	.954	6.5
Error	36	1.6419				
Level	1		24.0224	.0001		
Levsqr[g]	1		7.8779	.0001		
Levcub[h]	1		2.1264	.0001		
Model, Cu	3	.1608		.3983	.078	8.5
Error	36	1.9048				
Level	1		.0151	.5962		
Time	1		.0002	.9520		
L * T	1		.1455	.1060		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

[g] Quadratic term of Mo level.

[h] Cubic term of Mo level.

and during the 5-day collection period swine cleared more than 75% of both intravenous and oral doses (Bell et al., 1964). Urinary excretion of Mo in sheep was increased by elevated S intake (Dick, 1956b; Weber et al., 1983).

Supplementation of the diet of ewes with 4 ppm Mo increased ($P < .001$) urinary excretion of Mo from 562 μg/day in unsupplemented control animals to 1314 μg/day (Suttle, 1975b). When sheep were fed a diet that provided 5.5 mg Cu, .7 mg Mo and 1.1 g S daily, less than 10% of the ingested Mo was excreted from the body and most of this was in urine. When additional dietary Mo was fed (5, 10, 15 or 25 mg/day), 50% to 60% of the dose was excreted, with approximately 65% of that excreted appearing in feces (Weber et al., 1983). Urinary Mo excretion was greater ($P < .01$) in weanling heifers fed a diet containing 100 ppm Mo, compared with animals not fed additional Mo (Lesperance et al., 1985).

Urinary Copper Excretion

Urinary Cu excretion of ewes fed a diet based of ground straw, ground barley and dried-grass meal, containing 6 to 7 ppm Cu, varied from .08 to .09 mg/day (Stevenson and Unsworth, 1978). Copper excretion in urine of wethers in Exp. 4 (Table 4-5) was about seven times greater. Differences may have been due in part to Cu intake.

Copper excretion in urine (Table 4-5) was not affected by added dietary Mo concentrations ($P = .17$) or by length of

time of feeding (P = .20; Table 4-6), but there was a tendency towards a level * time interaction (P = .11; Table 4-6, 4-7).

In mature wethers fed a semipurified diet containing 10.8 ppm Cu, addition of 25 ppm Mo and .5% SO_4 increased urinary Cu excretion, relative to unsupplemented sheep (Smith et al., 1968). Excretion of Cu in urine of sheep increased (P < .05) in response to supplemental Mo (0 to 25 mg/day) only when additional S (1.1 g/day) was provided and no significant differences were observed among 5, 10, 15 or 25 mg/day added Mo (Weber et al., 1983). The basal diet provided a daily intake of 5.5 mg Cu, .7 mg Mo and 1.1 g S. Molybdate plus sulfate supplementation of Cu-poisoned lambs did not affect urinary excretion of Cu (Hidiroglou et al., 1984).

Urinary Cu excretion of all wethers of Exp. 4 varied from 187 to 1352 μg/day, averaging 576 μg/day. In unsupplemented lambs it varied from 187 to 1223 μg/day, with an average of 604 μg/day. Total daily urinary Cu excretion, over a 40-day collection period, of wethers fed for 160 days a basal diet containing 12 to 14 ppm Cu, .03% SO_4 and < 1 ppm Mo ranged from 87 to 455 μg Cu, with an average of 208 μg Cu/day (Marcilese et al., 1970). These values are smaller than those of lambs fed the control diet of Exp. 4. When the same amount of Cu was fed with 50 ppm Mo and .4% SO_4, however, urinary Cu excretion increased from 200 μg to

1580 µg/day (Marcilese et al., 1970), amounts that are almost three times greater than those of lambs in the present study. The difference may be explained by the length of feeding in both studies.

Marcilese et al. (1970) also reported an elevated urinary Cu excretion associated to a four to ninefold increase in daily urine volume of wethers fed additional 50 ppm Mo and .4% SO_4, compared with sheep fed the basal diet. Increases in urinary Cu excretion in lambs after tetrathiomolybdate administration were observed by Gooneratne et al. (1989c) and these were partly caused by higher urine volumes. No change in urine volume was observed by Mason et al. (1988) after trithiomolybdate was administered to adult wethers. In the present experiment, no differences (P = .28) in urine volume were observed during the 5-day collection period due to Mo concentration, but the values were quite variable, both among and within treatments. Daily urine volume varied from .6 l to almost 12 l, with an average of 2.9 l.

Liver Molybdenum Concentrations

Normal Mo concentrations in liver of sheep suggested by Anke et al. (1985) are 2.20 ± 1.13 ppm, or 2 to 4 ppm (Underwood, 1977, 1981), which are somewhat smaller than the values found in lambs fed the basal diet (Table 4-8). Normal hepatic Mo concentrations in sheep, as indicated by

Puls (1988), vary from 1.5 to 6.0 ppm. Mo concentrations in liver of lambs fed the control diet were comparable to the largest value of this wide range, but Mo-fed lambs (Table 4-8) did not reach the toxic concentrations (30 to 60 ppm) given by Puls (1988) or the 20 to 30 ppm of animals fed high-Mo, low-S diets (Underwood, 1981).

Molybdenum concentrations in liver (Table 4-8) were affected by added dietary Mo concentrations ($P = .06$), but not by length of time of feeding ($P = .54$; Table 4-9). There was no level * time interaction ($P = .77$; Table 4-9, 4-10). When a quadratic model was fitted, there was no quadratic effect ($P = .59$).

The increases ($P < .01$) in liver Mo concentrations reported by van Ryssen and Stielau (1981) in sheep fed 82 mg Cu, 3.77 g S and .6, 20.8, 38.4 and 58.5 mg Mo/day, for 193 days, were more striking than those in Exp. 4: 2.5, 5.6, 11.0 and 21.2 ppm, respectively. This also occurred in a study by van Ryssen and Barrowman (1988), in which Ile de France and Mutton Merino sheep consuming 10 ppm Cu and 8, 23 or 45 mg Mo/day for 64 days had hepatic Mo concentrations of 6, 27 and 58 ppm (Ile de France) or 7, 27 and 34 ppm (Mutton Merino), respectively. Length of time of feeding could be one of the explanations for the differences. However, in ewes fed diets containing 4.5 to 4.9 ppm Cu, .14% to .15% S and .9, 18.4 or 40.7 ppm Mo, for 6 weeks, there was only a trend toward greater ($P < .10$) final hepatic Mo

TABLE 4-8. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE LIVER OF LAMBS (EXPERIMENT 4)

	Period	Added dietary molybdenum concentrations (ppm)[a]				
Mineral	(day)	0	15	30	45	Mean
Molybdenum	14	5.88 ± .27[b]	9.36 ± .31	8.21 ± .61	12.07 ± .46	8.87 ± .55
	28	6.31 ± .40	5.97 ± .19	11.85 ± .98	10.60 ± 1.12	8.68 ± .69
	Mean	6.10 ± .24	7.66 ± .59	10.03 ± .81	11.33 ± .62	8.78 ± .44
Copper	14	293 ± 21	273 ± 21	353 ± 72	366 ± 19	321 ± 20
	28	356 ± 15	276 ± 20	346 ± 114	224 ± 9	301 ± 30
	Mean	325 ± 16	275 ± 14	350 ± 64	295 ± 26	311 ± 18

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm, dry basis (n = 5).

TABLE 4-9. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON HEPATIC MOLYBDENUM AND COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	.829836	10.94	.0001	.075878
	Level	.005343	1.98	.0558	.002704
	Time	-.002098	-.61	.5443	.003428
	L * T[d]	.000036	.29	.7711	.000122
Copper	Intercept	2.33390	22.14	.0001	.10542
	Level	.00887	2.36	.0238	.00376
	Time	.00723	1.52	.1378	.00476
	L * T	-.00045	-2.68	.0111	.00017

[a] Based on log (base 10) of ppm Mo and ppm Cu, dry basis.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-10. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (ppm) AND COPPER (ppm) IN LIVER (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model, Mo	3	.4220		.0001	.588	9.8
Error	36	.2961				
Level	1		.4180	.0001		
Time	1		.0033	.5320		
L * T[f]	1		.0007	.7711		
Model 1, Cu	3	.1365		.0501	.193	5.1
Error	36	.5716				
Level	1		.0051	.5746		
Time	1		.0176	.2999		
L * T	1		.1138	.0111		
Model 2, Cu	3	.1037		.1228	.146	5.2
Error	36	.6043				
Level	1		.0051	.5851		
Levsqr[g]	1		.0039	.6343		
L * T	1		.0948	.0229		
Model 14 d	1	.0354		.1106	.135	4.5
Error	18	.2261				
Model 28 d	1	.0835		.0515	.195	5.6
Error	18	.3455				

[a] Degrees of freedom. [b] Sum of squares.

[c] Type I sum of squares. [d] Coefficient of determination.

[e] Coefficient of variation. [f] Level * time interaction.

[g] Quadratic term of Mo level.

concentrations with increasing dietary Mo concentrations (Wittenberg and Devlin, 1988).

Concentrations of Mo in liver of calves fed 9.9 ppm Cu and 400 ppm Mo did not differ from those in calves fed 200 ppm (Cox et al., 1960). The authors suggested that a saturation in liver occurred at the lower level of intake. Liver Mo gradually increased in weanling heifers fed 6.0 or 9.2 ppm Cu, .6 ppm Mo, .2% S and 100 ppm supplementary Mo for 12 months (Lesperance and Bohman, 1963). Significantly larger amounts of Mo were found in liver after 60 days and the difference was highly significant at 120 days (Lesperance and Bohman, 1963). Heifers fed 0, 5, 10, 20 or 50 ppm supplemental Mo had increased liver Mo concentrations, 6, 10, 11, 22 and 22 ppm Mo, respectively, at 75 days of feeding (Vanderveen and Keener, 1964). At 150 days, those concentrations were 5, 10, 13, 21 and 30 ppm; no changes occurred thereafter until 300 days (Vanderveen and Keener, 1964). In yearling steers, grazing pastures containing 8.2 ppm Cu, 1.3 ppm Mo and .32% S and administered 1.5 and 3.0 mg Mo/kg body weight/day, liver Mo concentrations at 50 days of feeding had only been increased ($P < .05$) by the high dietary level; at 100 days of feeding, there were no differences ($P > .05$) among treatments, although the initial trend remained (Cook et al., 1966). Hepatic Mo concentrations increased ($P < .05$) in lambs fed 8 ppm supplemental Mo compared to those fed 2 ppm Mo but the

increase depended upon dietary Cu (10 or 40 ppm) and S (.1% or .4%) (Goodrich and Tillman, 1966a). Hepatic concentrations of Mo increased five to 10-fold in dairy cows fed 6.4 ppm Cu and from 53 to 300 ppm added Mo, compared with unsupplemented cattle (Huber et al., 1971). Hepatic Mo concentration increases were directly related to Mo intake but they decreased with addition of SO_4 to diets (Huber et al., 1971). Hepatic Mo concentrations in rams fed a basal diet containing 9.8 ppm Cu, .5 ppm Mo, .08% S and additional 25 ppm Mo or 25 ppm Mo plus .5% SO_4 were 7.6 and 3.0 ppm (fresh tissue), respectively, while in lambs fed the control diet Mo concentration in liver was 1.0 ppm (Bremner and Young, 1978). Molybdenum concentrations in liver of 2-year-old wethers increased ($P < .01$) from 4 ppm in a group fed 13.5 ppm Cu, .38 ppm Mo and .26% S, to 35.6 and 39.5 ppm, when dietary Mo was elevated to 45 ppm or to 48 ppm Mo plus .48% S, respectively (van Ryssen, 1979). Concentrations of Mo in liver of mature wethers grazing a molybdate sprayed pasture containing 6.0 to 8.7 ppm Cu and 5.5 to 12.5 ppm Mo for 74 weeks or 33.5 ppm Mo for the last 2 weeks, after lime application, were greater ($P <.01$) than in sheep grazing an unfertilized pasture containing .4 to 1.5 ppm Mo and similar Cu concentrations (6 versus 2 ppm, fresh weight, respectively) (Pitt et al., 1980). In young wethers, grazing those same pastures for 13 weeks, these figures were 6.4 and 2.4 ppm ($P < .01$), respectively (Pitt et al., 1980).

Molybdenum applications (0, 200, 600 and 2000 g Mo/ha) on pastures resulted in large increases ($P < .05$) in hepatic Mo concentrations of sheep grazing those pastures (Langlands et al., 1981). Copper concentrations in forage varied from 6 to 12 ppm, S from .20% to .41% and Mo concentrations were increased by fertilization from .4 to 1.8, 3.5 and 10.5 ppm, on the average, for the three Mo applications (Langlands et al., 1981). The increase in dietary Mo content to provide 35 or 70 mg additional Mo/day for 46 days resulted in elevated ($P < .01$) hepatic Mo concentrations of lambs (van Ryssen et al., 1986). In goats fed 1000 ppm Mo, hepatic Mo increased ($P < .001$) from 1.2 to 70 ppm (Anke and Risch, 1989). Feeding 38 ppm additional Mo in a diet with 13 ppm Cu, 1.6 ppm Mo and .45% S to 5-month-old rams for 14 weeks resulted in elevated ($P < .01$) concentrations of Mo in their livers (18.3 ppm), compared with those in rams without additional Mo (4.4 ppm) (van Ryssen et al., 1990). Molybdenum concentrations in liver of pregnant ewes was elevated five- and ninefold after oral administrations of 54 or 162 mg tetrathiomolybdate/day, respectively (Kincaid and White, 1988).

Liver stores of Mo indicated that this organ is not a site of mass storage, even though increases in liver Mo were highly significant (Lesperance and Bohman, 1963). Most studies in the literature confirm this earlier finding, as do the results of this experiment.

Hepatic Copper Concentrations

Liver Cu concentrations of lambs on the basal diet and of Mo-fed animals (Table 4-8) are within the wide range (100 to 400 ppm) considered normal in sheep (Underwood, 1981).

Added Mo dietary concentrations influenced Cu concentrations in liver (P = .02), length of time of feeding had no effect (P = .14; Table 4-9), but there was a level * time interaction (P = .01; Table 4-9, 4-10). A quadratic term had no effect (P = .63, Table 4-10). Analysis of the data by time indicated an effect (P = .05) of dietary Mo level at 28 days, but not at 14 days (P = .11; Table 4-10). At 28 days of feeding, liver Cu concentrations were reduced (P = .05) by increasing dietary Mo concentrations, but not at 14 days (P = .11).

Decreases in hepatic Cu concentrations as a consequence of increases in dietary Mo concentrations have been observed in cattle (Cox et al., 1960; Chapman and Kidder, 1963; Lesperance and Bohman, 1963; Vanderveen and Keener, 1964; Huber et al., 1971; Kincaid, 1980; Phillippo et al., 1985; Bremner et al., 1987; Phillippo et al., 1987a,b; Humphries et al., 1988b; Kincaid, 1988b; Wang et al., 1988; Kleczkowski, 1989) and in sheep (Dick and Bull, 1945; Dick, 1954b; Wynne and McClymont, 1956; Goodrich and Tillman, 1966a; Marcilese et al., 1969; Ross, 1970; Harker, 1976; Suttle, 1977; van Ryssen, 1979; Pitt et al., 1980; van Ryssen and Stielau, 1981; Hidiroglou et al., 1984; Harrison

et al., 1987; Van Ryssen et al., 1986; Suttle et al., 1988; van Ryssen and Barrowman, 1988; Moshtaghi-Nia et al., 1989; van Niekerk and van Niekerk, 1989; White et al., 1989; van Ryssen et al., 1990).

Copper concentrations in liver of heifers decreased ($P < .01$) in groups fed 250 mg Mo/animal daily, alone or in combination with Co, for 279 days (Chapman and Kidder, 1963). Marked decreases in liver Cu concentrations were observed in lactating dairy cows fed a basal diet containing 6 ppm Cu to which from 53 to 300 ppm Mo were added (Huber et al., 1971). Hepatic Cu concentrations of lambs fed 24.4 ppm Cu, .42% SO_4 and 1.8, 4.2 or 7.7 ppm Mo were 549, 509 and 361 ppm, respectively (Harker, 1976). Liver Cu concentrations of 6-week-old calves were reduced when the diet was supplemented with 10 ppm Mo or when calves were given 100 mg of diammonium tetrathiomolybdate three times per week, both treatments supplying 16 mg Mo/day for 12 weeks (Kincaid, 1988b). Elevated Mo concentration (.3 or 5 ppm) in the diet (6.4 or 10 ppm Cu, .35% or .47% S) of 18-month-old bulls, decreased the level of Cu in liver after 3 months of feeding (Kleczkowski, 1989). Liver Cu concentrations of sheep were much smaller when an additional 50 ppm Mo plus .4% SO_4 were fed instead of the basal diet only (12 to 14 ppm Cu and .03% SO_4) or basal diet plus .4% SO_4, for 160 days (Marcilese et al., 1969). Oral administration of 100 mg ammonium molybdate and 1 g sodium

sulfate for 13 weeks caused a significant decrease from 1576 to 805 ppm in hepatic Cu concentrations of Cu-loaded lambs, while in untreated lambs they increased from 1188 to 1369 ppm (Ross, 1970). Hepatic Cu concentrations of 2-year-old wethers fed 58 ppm Cu, 46 ppm Mo and .27% S or 56 ppm Cu, 48 ppm Mo and .48% S for 157 days were smaller ($P < .01$) than in sheep fed 60 ppm Cu, .42 ppm Mo and .27% S (van Ryssen, 1979). In lambs fed 75 mg Cu/day for 50 days, an intake of 5.4 mg Cu, 69 mg Mo and 3.7 g S/day, for a further 46-day period, decreased ($P < .001$) hepatic Cu concentrations about 40% relative to animals fed 5.7 mg Cu, .4 mg Mo and 2 g S, from approximately 1000 to 600 ppm (van Ryssen et al., 1986). Consumption of 5 mg Cu, 42 mg Mo and 3.9 g S/day resulted in a reduction ($P <.05$) of 33% in hepatic Cu concentrations compared with lambs fed the low-Mo diet in the same study (van Ryssen et al., 1986). Van Ryssen and Barrowman (1988) observed that reduction in liver Cu concentrations caused by Mo dietary supplementation (0, 25 or 50 ppm) was more pronounced ($P < .05$) in Ile de France than in Mutton Merino sheep. When lambs were fed, for 16 weeks, diets containing 7.6 ppm Cu, 11.2 ppm Mo and .33% S, or 16.6 ppm Cu, 11.9 ppm Mo and .33% S, liver Cu concentrations decreased, while in lambs fed 11.5 ppm Cu, 2.8 ppm Mo and .18% S (control group), those concentrations increased. In animals fed 24.9 ppm Cu, 12.5 ppm Mo and .32% S, hepatic Cu concentrations declined during the first 4

weeks and increased from week 4 to week 16, but did not reach the levels of the control group (Moshtaghi-Nia et al., 1989). Molybdenum supplementation (10 ppm) of the mineral mixture of ewes starting 1 month prior to breeding, and of the creep feed of their lambs, starting at birth, caused a decrease ($P < .05$) in hepatic Cu concentrations of lambs at 10 weeks after birth, relative to groups of lambs fed 10 ppm additional Cu or no supplement (White et al., 1989). Dietary Mo supplementation (38 ppm) of a diet with 13 ppm Cu, 1.6 ppm Mo and .45% S, for 14 weeks, reduced ($P < .01$) Cu concentrations in liver of 5-month-old rams, compared with the unsupplemented group (van Ryssen et al., 1990).

Some studies have clearly indicated the need of simultaneous addition of S, for Mo to be effective or to potentiate its efficacy in decreasing hepatic Cu concentrations (Dick, 1952, 1953b; Wynne and McClymont, 1956; Bremner and Young, 1978; van Ryssen et al., 1990). When ewes were fed for 6 months different combinations of lucerne hay and oaten hay, all sheep having the same daily intake of Cu (15 mg) and Mo (10.7 mg), hepatic and blood Mo concentrations markedly increased as the proportion of oaten hay increased in the diet (Dick, 1952). The author suggested that there was a "factor" present in lucerne responsible for decreasing liver and blood Cu concentrations. Indeed, it was soon identified as sulfate (Dick, 1953b). Sheep maintained for 50 weeks on a diet

containing 5.2 ppm Cu and both Mo and SO_4 supplements, to increase dietary concentrations from .83 ppm Mo and .04% SO_4 to 5.14 ppm Mo and .40% SO_4, showed a progressive fall in hepatic Cu concentrations, larger than when Mo only was added (Wynne and McClymont, 1956). The addition of 25 ppm Mo plus .5% SO_4 to the basal diet (9.8 ppm Cu, .5 ppm Mo and .08% S) of lambs for 30 weeks reduced hepatic Cu concentrations, compared with animals fed the basal diet (84 ppm versus 229 ppm, respectively), but Mo alone had no effect (180 ppm, fresh tissue) (Bremner and Young, 1978). When Mo (20 mg/sheep/day for 25 days; 48 mg/sheep/day for additional 37 days; and 65 mg/sheep/day for further 28 days) and S (5.4 g/kg) were included in the diet (16 ppm Cu, .21% S) of 12-month-old rams, liver Cu concentrations were smaller ($P < .05$) than when the same amounts of Mo only were included (van Ryssen et al., 1990).

Nonetheless, high dietary Mo did not influence liver Cu concentrations in steers (Cook et al., 1966) and in sheep (Langlands et al., 1981). Cook et al. (1966) administered 1.5 and 3.0 mg Mo/kg body weight to cattle grazing pastures having 8.2 ppm Cu, 1.3 ppm Mo and .32% S; based on a dry matter intake of 2.5% of the average body weight, this is equivalent to about 60 and 120 ppm dietary Mo. Dick (1969) observed in sheep that the maximal fall in liver Cu content occurred at dietary Mo concentrations of 15 to 20 ppm and, as it built up beyond 50 ppm, it became progressively less

effective, until at about 100 ppm dietary Mo, it had no apparent effect on liver Cu storage. A similar effect was noticed by van Ryssen and Stielau (1981), in sheep fed high Cu (84 mg/day) and high S (3.7 g/day). In this study, addition of up to 38 mg Mo/sheep/day resulted in marked reductions ($P < .001$) in liver Cu concentrations, but at 58 mg Mo/sheep/day, elevated Cu was again observed, greater than in the group fed 21 mg Mo/sheep/day (van Ryssen and Stielau, 1981). Also, in ewes fed diets containing .9, 18.4 or 40.7 ppm Mo, for 6 weeks there was only a tendency ($P < .10$) for final hepatic Cu concentrations to be greater with each increment in dietary Mo (Wittenberg and Devlin, 1988). The authors attributed this lack of response to Mo to the relatively low dietary S concentrations (.15%), and suggested that reduced Cu absorption due to thiomolybdate formation in the digestive tract might have been minimal. The generalization of Dick (1969) referred to earlier in the paragraph, however, conflicts with results obtained by Huber et al. (1971), Suttle (1977), Kincaid (1980) and van Ryssen et al. (1986).

Huber et al. (1971), in cows fed diets containing 53 ppm Mo for 2 months, and 53 or 173 ppm Mo for 4 additional months, observed a transient rise in liver Cu concentrations for about 1 month, after which a rapid and continuous decline occurred. Retention (% of ingested) and concentration of Cu in liver of lambs fed a high-Cu diet

(45.3 ppm) and 0, 2, 4, 8 and 16 ppm supplemental Mo, for 18 to 20 weeks, decreased logarithmically as dietary Mo increased (Suttle, 1977). The first Mo increment reduced liver Cu retention by 50% (Suttle, 1977). Copper concentration in liver of calves was reduced when 50 ppm Mo were added to drinking water (270 mg Mo/day) but not 1 ppm (4.8 mg Mo/day) or 10 ppm (50 mg Mo/day) (Kincaid, 1980). Those apparently conflicting findings are probably related to dietary Cu and S levels. Molybdenum plus sulfate can increase or decrease the Cu status of animals, depending on their intakes relative to that of Cu (Underwood, 1976). Suttle (1983b) concluded that Cu absorption in ewes was inhibited most by 4 to 6 ppm dietary Mo and that inhibition of rumen sulfide (S^{--}) production at greater Mo concentrations gave rise to a recovery in Cu absorption.

Oral or intravenous administration of tetrathiomolybdate seems to produce a response similar to oral administration of Mo (Kincaid and White, 1988; Gooneratne et al., 1989b). Liver Cu concentrations were reduced ($P < .05$) in pregnant ewes by oral administrations of tetrathiomolybdate (Kincaid and White, 1988). Tetrathiomolybdate intravenous administrations tended to reduce liver Cu concentration in lambs from 377 to 287 ppm (Gooneratne et al., 1989b).

The reviewed papers indicate that response of liver Cu concentration to elevated dietary Mo is quite variable. It

certainly depends upon dietary S (Dick, 1956a,b; Cunningham and Hogan, 1959; Cunningham et., 1959; Kline et al., 1971; Underwood, 1977; Bremner et al., 1978; Mason et al., 1978; van Ryssen and Stielau, 1980b; van Ryssen et al., 1986; van Ryssen et al., 1990), dietary Cu (Mason et al., 1978; Mills and Davis, 1987; Moshtaghi-Nia et al., 1989) and time of exposure, as indicated by this experiment and by Wynne and McClymont (1956), Huber et al. (1971) and Moshtaghi-Nia et al. (1989). As seen in the previous discussion, however, the response most likely to be expected to elevated intakes of Mo would be a decrease in liver Cu concentrations.

Molybdenum Concentrations in Muscle

Concentrations of Mo in muscle in normal cattle are .45 ppm (Penumarthy and Oehme, 1978). Values of lambs fed the basal diet (Table 4-11) were smaller.

Molybdenum concentrations in muscle (Table 4-11) increased as added dietary Mo concentrations increased ($P = .001$), but there was no effect of length of time of feeding ($P = .62$; Table 4-12). There was no level * time interaction ($P = .99$; Table 4-12, 4-13).

Increases in concentrations of Mo in muscle as a result of rises in dietary levels of the element have been reported by Dick (1956a), Huber et al. (1971) and van Ryssen and Stielau (1981). Molybdenum content in muscle of sheep increased when dietary Mo was elevated from .3 to 20.8

TABLE 4-11. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE MUSCLE OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a]				
		0	15	30	45	Mean
Molybdenum	14	.07 ± .02[b]	.21 ± .04	.41 ± .04	.42 ± .05	.28 ± .04
	28	.08 ± .01	.24 ± .01	.30 ± .01	.57 ± .07	.30 ± .04
	Mean	.08 ± .01	.22 ± .02	.36 ± .02	.49 ± .05	.29 ± .03
Copper	14	9.7 ± .8	7.6 ± .8	7.3 ± .8	6.2 ± .2	7.7 ± .4
	28	9.3 ± 1.3	6.5 ± .8	7.5 ± .4	7.2 ± .7	7.6 ± .5
	Mean	9.5 ± .7	7.0 ± .6	7.4 ± .4	6.7 ± .4	7.6 ± .3

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm, dry basis (n = 5).

TABLE 4-12. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE MUSCLE OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	1.875786	13.28	.0001	.141249
	Level	.018099	3.60	.0010	.005033
	Time	.003228	.51	.6160	.006381
	L * T[d]	-.000003	-.01	.9902	.000227
Copper	Intercept	1.015920	12.14	.0001	.083703
	Level	-.006093	-2.04	.0484	.002983
	Time	-.003977	-1.05	.2999	.003781
	L * T	.000159	1.18	.2457	.000135

[a] Based on log (base 10) of ppm Cu and ppb Mo.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-13. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (ppb) AND COPPER (ppm) IN MUSCLE (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2}[d]	CV[e]
Model, Mo	3	3.6809		.0001	.782	7.2
Error	36	1.0261				
Level	1		3.6613	.0001		
Time	1		.0196	.4121		
L * T[f]	1		$4*10^{-6}$	.9902		
Model, Cu	3	.0996		.0306	.216	11.5
Error	36	.3603				
Level	1		.0853	.0060		
Time	1		.0003	.8608		
L * T	1		.0139	.2457		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

mg/day, but response to Mo depended on the amount of dietary SO_4, Mo in muscle being greater at the lower SO_4 level (Dick, 1956a). Molybdenum concentrations in muscle of lactating dairy cows was elevated from .5 ppm in animals fed the control diet to 6.4 and 14.0 ppm in cows fed 53 and 173 ppm added Mo, respectively, for 6 months (Huber et al., 1971).

Molybdenum concentrations in longissimus dorsi of sheep fed daily .6, 20.8, 38.4 or 58.5 mg Mo, 82 mg Cu and 3.7 g S, for 193 days, increased ($P < .01$) markedly from the two smallest (.18 and .15 ppm) to the two greatest dietary concentrations (.44 and .94 ppm, respectively; van Ryssen and Stielau, 1981).

Copper Concentrations in Muscle

Copper concentrations in muscle (Table 4-11) decreased with increasing concentrations of added dietary Mo ($P = .05$), but length of time of feeding had no effect ($P = .30$; Table 4-12) neither was there a level * time interaction ($P = .25$; Table 4-12, 4-13).

The muscle (longissimus dorsi) of 2-year-old wethers, fed 13.5 ppm Cu, .38 ppm Mo and .26% S, contained 6.7 ppm Cu (van Ryssen, 1979). Increases of dietary Cu to 60 ppm, Cu and Mo to 58 ppm and 46 ppm, respectively, or Cu, Mo and S to 56 ppm, 48 ppm and .48%, respectively, did not change ($P > .05$) Cu concentrations in muscle (van Ryssen, 1979). Van

Ryssen and Stielau (1981) reported differences ($P < .05$) in Cu concentrations of longissimus dorsi of sheep fed .2, .6, 20.8, 38.4 or 58.5 mg Mo/day, but response was irregular; values were greater at 58.5 mg/day (6.1 ppm) and .2 mg/day (6.2 ppm) than at 20.8 mg/day (4.6 ppm) and .6 mg/day (5.0 ppm); the value resulting from 38.4 ppm dietary Mo (5.6 ppm) did not differ from the others.

Renal Molybdenum Concentrations

Molybdenum concentrations in kidney of lambs fed the basal diet (Table 4-14) were greater than the "normal" concentrations (1.25 ± .50 ppm) suggested by Anke et al. (1985), the normal range (1.5 to 1.6 ppm) suggested by Puls (1988) or the normal value (1.43 ppm) reported by Gooneratne et al. (1989b). Groups having supplemental Mo (Table 4-14) had Mo concentrations in kidney that were close to the lowest value of the range (7.4 to 275 ppm) given by Puls (1988) for sheep having elevated renal concentrations of Mo. This latter range (Puls, 1988) is an indication of the marked variability that can be found in renal Mo concentrations of sheep.

No effect of added dietary Mo concentrations ($P = .51$) or of length of time of feeding ($P = .19$) on Mo concentrations in kidney was detected with the linear model (Table 4-15). The numeric values of the means, however, increased with increasing dietary concentrations of Mo. The

statistical analysis of the untransformed data resulted in a R^2 of 70%, but coefficient of variation (CV) was 38%. When log transformed data were used, the R^2 increased to 80% and CV decreased to 16%. The F-value of type I sum of squares (SS; Table 4-16), however, indicated that each of the terms in the linear model affected Mo concentrations in kidney (level, $P < .0001$; time, $P = .0645$; L * T, $P = .004$). When a quadratic term for Mo level was included in the model, it did affect the response ($P = .0484$; Table 4-16), but the type I SS of the squared term accounted for only about 2% of total SS, so that for practical reasons it could be excluded.

There was a level * time interaction ($P = .004$; Table 4-15, 4-16) and PROC GLM run by time indicated that Mo level affected renal Mo concentrations both at 14 ($P < .0001$) and 28 days ($P < .0001$) and its type I SS represented most of the total SS, especially at 28 days (Table 4-16).

Variances of Mo concentrations in kidney were quite heterogeneous and increased with increasing concentrations of Mo in the diet (Table 4-14). Variances in untransformed data were .212 and .115 in groups fed the basal diet for 14 and 28 days, respectively, and increased to 13.71 and 15.02 in the 45 ppm added dietary Mo groups for 14 and 28 days, respectively. Log transformation of the data reduced variance heterogeneity considerably, but there still was a five- to sevenfold difference between 30 or 45 ppm and 0 ppm

TABLE 4-14. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN KIDNEY OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Molybdenum	14	3.42 ± .21[b]	4.01 ± .44	5.32 ± .85	9.67 ± 1.66	5.60 ± .71
	28	2.84 ± .15	3.62 ± .22	9.01 ± 1.40	14.39 ± 1.73	7.46 ± 1.18
	Mean	3.13 ± .15	3.81 ± .24	7.16 ± .99	12.03 ± 1.38	6.53 ± .70
Copper	14	24 ± 1.2	24 ± 1.8	23 ± 1.0	24 ± 1.4	24 ± .6
	28	22 ± 1.2	23 ± 1.3	27 ± 1.8	30 ± 3.2	26 ± 1.2
	Mean	23 ± .9	23 ± 1.1	25 ± 1.2	27 ± 1.9	25 ± .7

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Cu and .22% S.

[b] Mean ± standard error of the mean, in ppm, dry basis (n = 5).

TABLE 4-15. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE KIDNEY OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	.57094	5.64	.0001	.10129
	Level	.00239	.66	.5112	.00361
	Time	-.00605	-1.32	.1942	.00458
	L * T[d]	.00050	3.07	.0040	.00016
Copper	Intercept	1.43280	27.58	.0001	.05948
	Level	-.00357	-1.93	.0616	.00185
	Time	-.00365	-1.56	.1283	.00235
	L * T	.00024	2.82	.0078	.00008

[a] Based on log (base 10) of ppm Mo and ppm Cu.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-16. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (ppm) AND COPPER (ppm) IN KIDNEY (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model, Mo	4	2.1233		.0001	.818	15.8
Error	35	.4713				
Level	1		1.8754	.0001		
Levsqr[f]	1		.0563	.0484		
Time	1		.0533	.0645		
L * T[g]	1		.1382	.0029		
Model 14 d	1	.4976		.0001	.635	18.1
Error	18	.2864				
Model 28 d	1	1.5160		.0001	.863	15.0
Error	18	.2412				
Model, Cu	3	.0575		.0055	.293	4.5
Error	36	.1388				
Level	1		.0215	.0238		
Time	1		.0054	.2463		
L * T	1		.0307	.0078		
Model 14 d	1	.0004		.7092	.008	3.9
Error	18	.0511				
Model 28 d	1		.0517	.0044	.371	5.0
Error	18		.0877			

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Quadratic term of Mo level.

[g] Level * time interaction.

added Mo, so that some of the P-values obtained from a ratio of these variances were less than .05. A great deal of this variation was caused by analytical problems. Several times day-to-day and rack-to-rack variation was observed and many times the same sample did result in different values in the same rack. Variability in plasma or serum Mo concentrations determined by graphite furnace atomic absorption caused by analytical problems has been referred to by Nielsen and Mertz (1984) and lack of sensitivity and interference effects by Morrice et al. (1989). Ericson et al. (1987) mentioned carryover effects in Mo determinations with graphite furnace and Loya (1989), memory effects.

Kidney concentrations of Mo in lactating dairy cows increased from 1.7 ppm in animals fed the control diet to 42.3 and 62.6 ppm when 53 or 173 ppm Mo were added to their diets, respectively (Huber et al., 1971). Molybdenum concentrations in kidney of lambs treated for 30 weeks with 25 ppm Mo or 25 ppm Mo plus .5% SO_4 increased (8.6 and 4.6 ppm Mo, respectively), relative to unsupplemented animals (.6 ppm Mo) (Bremner and Young, 1978). Mature wethers grazing pasture sprayed with molybdate and containing 5.5 to 12.5 ppm Mo throughout a period of 74 weeks and 33.5 ppm Mo for an additional 2-week period had greater ($P < .01$) Mo concentrations in kidney (6.8 ppm Mo, fresh weight) than sheep grazing unsprayed pasture containing .4 to 1.5 ppm Mo (.4 ppm Mo, fresh weight); for young wethers grazing the

same pastures for 13 weeks these values were 8.1 and .5 ppm Mo (fresh weight), respectively ($P < .01$) (Pitt et al., 1980). Molybdenum concentrations in kidney of ewes grazing pastures fertilized with 0 to 2000 g Mo/ha were markedly increased ($P < .001$) (Langlands et al., 1981). Oral daily administrations of 54 or 162 mg tetrathiomolybdate to pregnant ewes resulted in a 17 and 27-fold increase in Mo concentration in kidney, respectively (Kincaid and White, 1988). Goats exposed to 1000 ppm dietary Mo showed an increase ($P < .001$) in Mo concentrations in kidney, from .44 to 111 ppm (Anke and Risch, 1989). Intravenous administrations of tetrathiomolybdate in young lambs increased Cu concentrations in kidney to 160 ppm, compared with a mean concentration of 19 ppm in 83 other lambs from the same flock (Gooneratne et al., 1989b). Feeding 38 ppm supplemental Mo to 5-month-old rams, for 14 weeks, resulted in greater ($P < .01$) concentrations of Mo in kidney cortex, relative to those in the unsupplemented group (van Ryssen et al., 1990). Kidney and spleen of cows fed high dietary Mo concentrations (53 to 300 ppm) concentrated Mo to a greater degree than any of the other tissues measured--blood, liver, brain, muscle and intestine (Huber et al., 1971). Dietary Mo intakes of .6, 20.8, 38.4 or 58.5 mg/day markedly increased ($P < .01$) Mo concentrations in kidney cortex of sheep (1.7, 6.5, 54.7 and 136.5 ppm, respectively) and in kidney medulla (1.4, 2.3, 7.1 and 24.1 ppm, respectively)

(van Ryssen and Stielau, 1981). From the tissues analyzed (liver, kidney cortex, kidney medulla, lungs, spleen, longissimus dorsi, heart muscle and wool), the most pronounced increase in Mo concentrations due to Mo supplementation was in kidney cortex (van Ryssen and Stielau, 1981).

Renal Copper Concentrations

Copper concentrations in kidney (Table 4-14) were affected by added dietary Mo concentrations (P = .06) and not by length of time of feeding (P = .13; Table 4-15, but there was a level * time interaction (P =.008; Table 4-15, 4-16). When PROC GLM was run by time, there was no Mo level effect at 14 days (P = .71), but there was at 28 days (P = .0044; Table 4-16). As indicated in Table 4-14, renal Cu levels increased with elevated dietary Mo concentrations, at 28 days of feeding.

Renal Cu concentrations were increased in several studies with sheep fed high Mo concentrations (Marcilese et al., 1970; Langlands et al., 1981; van Ryssen and Stielau, 1981; Kincaid and White, 1988; van Ryssen et al., 1990). Wethers fed 50 ppm additional Mo and .4% SO_4 had three to five times more (P < .01) Cu in kidney than sheep fed the basal diet (12 to 14 ppm Cu, .03% SO_4 and < 1 ppm Mo) or those fed supplemental SO_4 only (Marcilese et al., 1970). The dietary Cu concentration used in the latter experiment

was similar to those used in Exp. 4, but kidney Cu concentrations reported by Marcilese et al. (1970) for wethers fed additional molybdate and sulfur were much greater than those observed in Exp. 4. Reasons for this difference may be duration of feeding, which lasted from 120 to 180 days in the studies by Marcilese et al. (1970).

Kidney Cu concentrations increased ($P < .001$) in ewes grazing pastures fertilized with 0, 200, 600, and 2000 g Mo/ha (Langlands et al., 1981). In sheep fed .6, 20.8, 38.4 or 58.5 mg Mo/day for 193 days, Cu concentrations increased ($P < .01$) in both kidney cortex and kidney medulla, but especially in cortex (van Ryssen and Stielau, 1981). Kidney Cu concentrations were elevated ($P < .05$) in pregnant ewes given oral administration of tetrathiomolybdate (Kincaid and White, 1988). Copper concentrations in kidney cortex of initially 5-month-old rams fed 35 ppm supplemental Mo for 14 weeks was elevated ($P < .01$) relative to those in animals fed the basal diet (van Ryssen et al., 1990).

Responses of kidney Cu concentrations in sheep to dietary Mo concentrations have been found to depend on Mo concentration (Suttle, 1977), breed (Suttle, 1977), sheep age (Pitt et al., 1980) and dietary S concentrations (Bremner and Young, 1978; van Ryssen et al., 1990). Concentrations of Cu in kidney of lambs fed a diet containing 45.3 ppm Cu and supplemented with 0, 2, 4, 8 or 16 ppm Mo decreased ($P < .05$) at the two greater Mo

concentrations in Scottish Blackface lambs (24.4, 27.2, 19.9 and 18.2 ppm, respectively), but tended to increase in Finnish Landrace lambs (18.0, 17.0, 20.6 and 21.5 ppm, respectively) (Suttle, 1977). Copper concentrations in kidney of young wethers but not mature wethers was increased ($P < .01$) when grazing pastures fertilized with molybdate, compared with sheep consuming untreated pasture (Pitt et al., 1980). Copper concentrations in kidney of lambs fed 25 ppm added dietary Mo plus .5% SO_4 for 30 weeks were elevated (9.6 ppm, fresh tissue), but not of those fed 25 ppm supplemental Mo only (6.0 ppm, fresh tissue), relative to lambs fed the basal diet (5.0 ppm, fresh tissue) (Bremner and Young, 1978). Kidney Cu concentrations in 12-month-old rams were greater ($P < .01$) when Mo and S were included in the diet than when Mo only was added (van Ryssen et al., 1990).

Two studies with cattle do not give a clear indication of the effect of Mo on kidney Cu concentrations (Huber et al., 1971; Kleczkowski, 1989). High dietary Mo concentrations fed for 6 months tended only to increase kidney Cu concentrations in lactating dairy cows (Huber et al., 1971). Kleczkowski (1989) reported a decrease ($P < .05$) in Cu concentrations in kidney of 18-month-old bulls fed for 3 months a diet containing 5 ppm Mo, compared with cattle fed .3 ppm Mo.

According to Allen and Gawthorne (1988), Cu is diverted away from metabolically useful forms in gut and tissues, and into abnormal protein-tetrathiomolybdate-Cu complexes that accumulate in kidney or are excreted in gut.

Biliary Molybdenum Concentrations

Molybdenum concentrations in bile (Table 4-17) were not altered by dietary Mo concentrations (P = .48) or by length of time of feeding (P = .77; Table 4-18) and no level * time interaction occurred (Table 4-18, 4-19). No data were found in the literature on biliary Mo concentrations in ruminants.

Biliary Copper Concentrations

Copper concentrations in bile (Table 4-17) were influenced by added dietary Mo concentrations (P = .06) and by length of time of feeding (P < .04; Table 4-18) but there was a level * time interaction (P = .008; Table 4-18, 4-19). When GLM analysis was run by time, no effect of dietary Mo level on biliary Cu concentrations was observed at 14 days (P = .71), but there was at 28 days (P = .004; Table 4-19). It is quite surprising that Cu concentrations in bile decreased during the 28-day period, with increasing dietary Mo concentrations. An increase in concentrations of Cu in bile would be the expected response, since Cu concentrations were decreased in liver of animals fed supplemental Mo for 28 days, and bile is considered the major route of Cu

TABLE 4-17. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE BILE OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Molybdenum	14	.026 ± .011[b]	.017 ± .002	.028 ± .006	.035 ± .007	.026 ± .004
	28	.024 ± .012	.019 ± .005	.024 ± .005	.036 ± .010	.026 ± .004
	Mean	.025 ± .007	.018 ± .002	.026 ± .004	.036 ± .006	.026 ± .003
Copper	14	1.3 ± .18	1.8 ± .15	1.3 ± .14	1.6 ± .26	1.5 ± .10
	28	2.5 ± .38	1.5 ± .28	1.1 ± .10	1.3 ± .25	1.6 ± .18
	Mean	1.9 ± .28	1.7 ± .16	1.2 ± .09	1.4 ± .18	1.6 ± .10

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm, fresh bile (n = 5).

TABLE 4-18. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE BILE OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	1.232440	4.97	.0001	.248182
	Level	.006223	.71	.4846	.008811
	Time	-.003328	-.29	.7700	.011292
	L * T[d]	.000039	.10	.9229	.000399
Copper	Intercept	2.984508	24.58	.0001	.121406
	Level	.008283	1.91	.0635	.004326
	Time	.011915	2.17	.0365	.005484
	L * T	-.000544	-2.78	.0085	.000195

[a] Based on log (base 10) of ppb Mo and ppb Cu.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-19. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (ppb) AND COPPER (ppb) IN BILE (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model, Mo	3	.5648		.1102	.156	22.3
Error	35	3.0500				
Level	1		.5526	.0165		
Time	1		.0114	.7195		
L * T[f]	1		.0008	.9229		
Model, Cu	3	.2743		.0104	.266	4.6
Error	36	.7580				
Level	1		.1110	.0276		
Time	1		.0002	.9214		
L * T	1		.1631	.0085		
Model 14 d	1	.0025		.7062	.008	4.1
Error	18	.3070				
Model 28 d	1	.2716		.0040	.376	5.0
Error	18	.4510				

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

excretion (Underwood, 1977; Allen and Solomons, 1984; Mills and Davis, 1987). Also, no change was observed in Cu excreted in urine or feces. If an increase in fecal Cu concentrations had been observed, the decrease in bile excretion of Cu would be less contradictory, since excretion of Cu directly through the intestinal wall is also a major pathway of Cu elimination (van Campen, 1971). Gooneratne et al. (1989c) described in lambs an elevated excretion of endogenous fecal Cu due to tetrathiomolybdate administration, free of bile, which was assumed to be associated to other gastrointestinal secretions, but their nature was not identified. In cattle, increasing dietary Cu, Mo and S concentrations resulted in elevated biliary Cu excretion (Gooneratne et al., 1988). In lambs and sheep, intravenous administrations of tetrathiomolybdate increased biliary Cu concentrations and total amount of Cu excreted in bile in 24 hours (Gooneratne et al., 1985, 1989b, 1989c; Ke and Symonds, 1989). Increased biliary Cu excretion was also observed by Gooneratne et al. (1989c,d), after intraduodenal administrations of tetrathiomolybdate.

Biliary Cu concentration by itself, without knowledge of the daily production of bile, may be of little meaning, since total amount excreted in a day might change according to the volume of bile secreted, however, Gooneratne et al. (1989b) did not notice changes in biliary flow of lambs due to dietary Cu concentrations or intravenous

tetrathiomolybdate administration. Neethling et al. (1968) observed that urinary excretion of Cu in adult wethers was quantitatively more important than the biliary route, when single doses of radioactive Cu in excess of normal daily intake were administered, by intra-abomasal or by intraruminal injection. Also, Binnerts (1978) found that the contribution of bile in the endogenous fecal excretion of Cu was small and variable in dairy cows.

Copper concentrations in bile of lambs fed 2.2, 11.3 or 47 ppm Cu was .2, .5 and 1.5 ppm, but the difference was not significant (Saylor and Leach, 1980), possibly because of a small sampling number. In lambs and sheep the amount of Cu excreted daily in bile was not related to liver Cu concentrations or to dietary Cu concentrations (Gooneratne et al., 1985; Charmley and Ivan, 1989; Gooneratne et al., 1989b). In cattle, however, the amount of Cu secreted in bile fell markedly as liver Cu concentrations were decreased by reducing Cu from 12 to 4 ppm in the diet (Phillippo and Graça, 1983), or increased when dietary Cu levels were raised (Symonds et al., 1983; Gooneratne et al., 1988).

Concentrations of Cu excreted in bile of hypocupraemic ewes, repleted for 35 days with a semipurified diet supplemented with 6 ppm Cu, were not affected by Mo supplementation (Suttle, 1975b). Biliary Cu concentrations in this study were much smaller (87 ppb in ewes fed the basal diet) than those observed in Exp. 4. Caple and Heath

(1978) reported a Cu concentration of .033 ± .034 ppm in bile of sheep. In dairy cows, a biliary Cu concentration of .3 to .4 ppm was observed (Binnerts, 1978). Copper concentration in bile of steers was .12 ppm before an intravenous infusion of cupric sulfate and it increased ($P < .05$) to .20 ppm during the infusion and to .23 ppm ($P < .01$) after the infusion (Charmley and Symonds, 1985), but a decrease in Cu secretion in bile was observed in sheep when Cu intake was raised from 6.8 to 17.8 mg/day (Grace and Gooden, 1980).

Biliary Cu concentrations in sheep varied from 16 to 32 ppm (fresh weight) (Schenkel and Krehl, 1983), and from 162 to 261 ppb in Cu supplemented and thiomolybdate-treated sheep (Gooneratne et al., 1985). Biliary Cu excretion increased 1.5 to threefold after intravenous thiomolybdate injection and the peak of Cu concentration in bile was reached 3 hours after administration (Gooneratne et al., 1985). Charmley and Ivan (1989) found .4 ppm Cu in bile of lambs fed a control diet containing 6.7 ppm Cu; when 29 ppm Cu were added to the diet, as chloride, acetate or sulfate, Cu in bile increased to 1.2, 1.7 and 1.4 ppm, but the change was not significant. Gooneratne et al. (1989b) reported a Cu concentration of .35 ppm in bile of lambs, prior to thiomolybdate administration. All these reported values are much smaller than those of Exp. 4. However, after thiomolybdate administration biliary Cu concentrations

increased ($P < .05$) to 1.0 ppm (Gooneratne et al., 1989b), concentrations that are closer to those obtained in Exp. 4. Caple and Heath (1978) observed that concentrations of Cu in bile were always less than in serum and were between 1/100 and 1/1000 of the concentration in liver. In the lambs used in Exp. 4, Cu concentration in bile was greater than in serum, even in wethers fed the basal diet. The same relationship between biliary and hepatic Cu, however, was observed in Exp. 4.

Serum Molybdenum Concentrations

Initial serum Mo concentrations in pooled samples were .009, .03, .01, .009 ppm in groups fed 0, 15, 30 and 45 ppm added Mo for 14 days, and .007, .01, .01 and .01 ppm in groups fed these amounts of Mo for 28 days, averaging .01 ppm. These and final Mo concentrations in serum of lambs fed the basal diet (Table 4-20) were similar to plasma concentrations referred to as normal by Puls (1988). Similar values were reported in unsupplemented lambs (Suttle, 1977) and sheep (Pitt et al., 1980; Weber et al., 1983). Supplemented wethers (15 and 30 ppm Mo, Table 4-20) had serum Mo concentrations within the range of .2 and .6 ppm indicated as high by Puls (1988), but none of the supplemented groups (Table 4-20) reached the toxic concentrations (2.3 to 3.0 ppm) shown in the same reference, neither the high concentrations (8.2 and 11.5 ppm) reported

by Wittenberg and Devlin (1988), in ewes supplemented with 20 and 40 ppm Mo, but dietary Cu and S in this study were low (4.5 to 4.9 ppm Cu and .14% to 15% S) and it lasted 6 weeks.

Molybdenum concentrations in serum (Table 4-20) increased linearly with added dietary Mo concentrations (Table 4-21). Length of time did not affect the response ($P = .69$; Table 4-21) and there was no level * time interaction ($P = .48$; Table 4-21, 4-22). The averages (Table 4-20) suggest a greater response at 28 days than at 14 days, which is consistent with observations of Lesperance and Bohman (1963), Pitt et al. (1980) and Wittenberg and Devlin (1988).

Quadratic and cubic effects of Mo concentration were tested. When the regression model included only Mo level and its quadratic term, the R^2 increased to .954, the CV decreased to 7.9% and there was an effect of the square term ($P < .0001$). There was also a cubic effect ($P < .0001$), but this term did not greatly increase the R^2 (.971) or decrease the CV (6.3%). Type I sum of squares (SS) of the quadratic term accounted for 14.6% of total SS and the cubic term only 1.8% (Table 4-22). Therefore, for practical purposes, the linear model seems adequate to explain the relationship. In the cubic model, there was a level * time interaction ($P = .0465$, Table 4-22), but type I SS of this term accounted only for .3% of total SS.

TABLE 4-20. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE SERUM OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Molybdenum (Final)	14	.009[b]	.20 ± .03[c]	.41 ± .05	.58 ± .07	.30 ± .05
	28	.008	.24 ± .05	.49 ± .08	.82 ± .10	.40 ± .08
	Mean	.008	.22 ± .03	.45 ± .05	.70 ± .07	.35 ± .06
Copper (Initial)	14	1.04 ± .02	1.22 ± .16	.83 ± .09	1.06 ± .04	1.04 ± .05
	28	1.05 ± .04	1.08 ± .07	1.16 ± .13	1.06 ± .11	1.09 ± .04
	Mean	1.05 ± .02	1.15 ± .09	.99 ± .09	1.06 ± .06	1.06 ± .03
Copper (Final)	14	.95 ± .05	1.04 ± .07	.93 ± .03	1.02 ± .10	.98 ± .03
	28	.83 ± .04	1.00 ± .08	1.07 ± .11	1.34 ± .05	1.06 ± .05
	Mean	.89 ± .03	1.02 ± .05	1.00 ± .06	1.18 ± .07	1.02 ± .03

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Result of reading of combined serum samples, in ppm.

[c] Mean ± standard error of the mean, in ppm, fresh basis (n = 5).

TABLE 4-21. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON MOLYBDENUM AND COPPER CONCENTRATIONS IN THE SERUM OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Molybdenum	Intercept	1.365358	4.64	.0001	.294157
(Final)	Level	.033277	3.19	.0030	.010443
	Time	-.005423	-.41	.6878	.013384
	L * T[d]	.000341	.72	.4756	.000472
Copper	Intercept	3.025913	39.93	.0001	.075786
(Initial)	Level	-.002130	-.79	.4354	.002701
	Time	.000078	.02	.9818	.003424
	L * T	.000081	.66	.5121	.000122
Copper	Intercept	2.911966	42.02	.0001	.069297
(Final)	Cu-Initial	.124979	2.85	.0072	.043812
	Level	-.003187	-1.79	.0827	.001784
	Time	-.004617	-2.06	.0470	.002242
	L * T	.000266	3.30	.0022	.000080

[a] Based on log (base 10) of ppb Mo and ppb Cu.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-22. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF MOLYBDENUM (ppb) IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1	3	18.3694		.0001	.811	16.1
Error	35	4.2848				
Level	1		18.2956	.0001		
Time	1		.0102	.7746		
L * T[f]	1		.0637	.4756		
Model 2	4	22.0746		.0001	.974	6.0
Error	34	.5796				
Level	1		18.2956	.0001		
Levsqr[g]	1		3.3075	.0001		
Levcub[h]	1		.3988	.0001		
L * T	1		.0728	.0465		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Standard error of the estimate.

[f] Level * time interaction.

[g] Quadratic term of Mo level.

[h] Cubic term of Mo level.

One of the most pronounced biochemical changes in sheep and cattle to high Mo intakes is the elevation in Mo concentrations in blood or plasma, as shown by Dick (1956b, 1969), Lesperance and Bohman (1961), Huber et al. (1971), Suttle (1975b, 1977, 1983b), Bremner and Young (1978), Pitt et al. (1980), Weber et al. (1983) and Wittenberg and Boila (1988). At Mo intakes from .4 to 95.9 mg/day, Mo concentrations in blood of sheep were proportional to Mo intakes, but inversely dependent on dietary SO_4 concentration (Dick, 1956b, 1969). Plasma Mo concentrations increased approximately 100-fold ($P < .01$) during the first 30 days of feeding 100 ppm added dietary Mo to weanling heifers, but was relatively constant thereafter (Lesperance and Bohman, 1961). Blood Mo concentrations increased five to ten times above pretreatment values in dairy cows fed 53 and 173 ppm additional Mo and increases were directly related to Mo intake (Huber et al., 1971). Plasma Mo concentrations in initially hypocupraemic ewes fed a semipurified diet containing 6 ppm Cu and no supplementary Mo or 4 ppm Mo, for 35 days, were increased ($P < .001$) by 1 to 2 ppm in the Mo supplemented groups (Suttle, 1975b). The effect was totally eliminated by simultaneously adding organic or inorganic S to the diet (.3% S) (Suttle, 1975b). Plasma Mo concentrations of lambs fed a diet containing 45.3 ppm Cu, .6 ppm Mo and .2% S, supplemented with 0, 2, 4, 8 or 16 ppm Mo, increased linearly with dietary Mo

concentrations; at 12 weeks of feeding, concentrations were 10, 23, 38, 75 and 171 ppb, respectively (Suttle, 1977). Plasma Mo concentrations of hypocupraemic ewes increased with Mo concentrations in three different kinds of diets in a predominantly linear relationship ($P < .01$) (Suttle, 1983b). One was a semipurified diet containing 7.2 ppm Cu, .35% S and .5, 2.5, 4.5 or 8.5 ppm Mo, which was fed for 65 days. The second were hays containing 7 to 8 ppm Cu, .30% to .34% S and .4, 2.8, 4.3, 14.2 or 18.7 ppm Mo, fed for 21 days. The third were pastures containing 6.4 ppm Cu, .27% S and .7, 3.5, 5.9 or 12.4 ppm Mo (Suttle, 1983b). In lambs fed 25 ppm additional Mo or 25 ppm Mo plus .5% SO_4, plasma Mo concentrations over a period of 4 to 17 weeks were 16.3 and .9 ppm, respectively, while in lambs fed the control diet values were less than .2 ppm (Bremner and Young, 1978). In sheep fed no additional S, the relationship between blood Mo and Mo intake (0 to 50 mg Mo/day) was linear and the R^2 was .95 (Weber et al., 1983). Molybdenum concentration in blood decreased with additional S (1.1 g/day), relative to the group fed only supplemental Mo, especially at greater Mo intakes (Weber et al., 1983). Plasma Mo concentrations were greater ($P < .05$) with each increment in dietary Mo (.9, 18.4 or 40.7 ppm) fed to ewes for 6 weeks (Wittenberg and Boila, 1988).

Increases in blood, plasma or serum Mo concentrations as a consequence of elevated dietary Mo concentrations have

also been reported by Vanderveen and Keener (1964) and Cook et al. (1966) in cattle, by Goodrich and Tillman (1966a) and van Ryssen et al. (1990) in lambs, by van Ryssen and Stielau (1981) and van Ryssen and Barrowman (1988) in sheep, and by Anke and Risch (1989) in goats. Serum Mo concentrations in heifers increased (.07, .17, .36, .78, 2.33 ppm) at 75 days of feeding, when 0, 5, 10, 20 or 50 ppm Mo were added to their diets (Vanderveen and Keener, 1964). Plasma Mo concentrations in yearling steers reflected ($P < .01$) increasing Mo intakes (1.5 or 3.0 mg Mo/kg body weight/day), at 25 days of feeding, but, at 100 days, plasma Mo of the two groups administered additional Mo did not differ (Cook et al., 1966). Concentrations of Mo in plasma increased ($P < .01$) in lambs when dietary Mo was elevated from 2 to 8 ppm (Goodrich and Tillman, 1966a). At higher Mo intakes, Mo concentrations in plasma of sheep increased ($P < .01$), the difference being greater between 38.4 and 58.5 mg/day than between .6 and 20.8 mg Mo/day (van Ryssen and Stielau, 1981). Plasma Mo concentrations were elevated in Cu-loaded sheep when their diets, containing 10 ppm Cu, were supplemented with 25 or 50 ppm Mo for 64 days, compared with unsupplemented animals (van Ryssen and Barrowman, 1988). Molybdenum concentration in plasma of initially 5-month-old rams fed a diet having 38 ppm added Mo for 14 weeks (1.14 ppm Mo) was greater ($P < .01$) than in sheep fed the basal diet (.09 ppm Mo) (van Ryssen et al., 1990). All organs and

blood serum of goats accumulated Mo when the animals were fed 1000 ppm Mo; the greatest increase ($P < .001$) was in serum, from .018 to 8.7 ppm (Anke and Risch, 1989).

Lesperance and Bohman (1961) considered plasma Mo the best immediate criterion to evaluate excess Mo intake, but over a long period of time Mo in liver was more indicative of toxicosis. Results from the present experiment confirm the first part of their conclusion.

Serum Copper Concentrations

Copper concentrations in serum of wethers from Exp. 4 (Table 4-20) were within the range considered normal (Underwood, 1981). There was no difference among serum Cu concentrations (Table 4-20) of the various groups at the beginning of the experiment ($P = .67$; Table 4-23). Length of time of feeding affected final Cu concentrations in serum ($P = .047$), while dietary Mo concentrations only tended to do so ($P = .08$; Table 4-21), but there was a level * time interaction ($P = .02$; Table 4-21, 4-23). When the GLM analysis was run by time, no effect of Mo level was noted at 14 days ($P = .53$), but at 28 days Mo level increased ($P < .0001$) serum Cu concentrations (Table 4-23).

Both cattle and sheep show contrasting responses of total Cu in blood, plasma or serum to elevated dietary Mo concentrations. Increases in plasma Cu concentrations have been observed in sheep and cattle (Smith et al., 1968; Smith

TABLE 4-23. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF COPPER (ppb) IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1[f]	3	.0128		.6723	.041	3.0
Error	36	.2954				
Level	1		.0021	.6147		
Time	1		.0070	.3603		
L * T[g]	1		.0036	.5121		
Model 2[h]	4	.1427		.0001	.537	2.0
Error	35	.1231				
Initial-Cu	1		.0356	.0031		
Level	1		.0654	.0001		
Time	1		.0033	.3418		
L * T	1		.0384	.0022		
Model 14 d	2	.0139		.1608	.193	2.0
Error	17	.0579				
Initial-Cu	1		.0125	.0725		
Level	1		.0014	.5301		
Model 28 d	2	.1227		.0001	.655	2.0
Error	17	.0647				
Initial-Cu	1		.0219	.0282		
Level	1		.1008	.0001		

[a] Degrees of freedom. [b] Sum of squares.

[c] Type I sum of squares. [d] Coefficient of determination.

[e] Coefficient of variation. [f] Initial copper in serum.

[g] Level * time interaction. [h] Final copper in serum.

and Wright, 1975a,b; Bremner and Young, 1978; van Ryssen and Stielau, 1981; van Ryssen et al., 1986; van Ryssen and Barrowman, 1988; Wang et al., 1988; van Ryssen et al., 1990).

Supplementation of the diet (10.8 ppm Cu) of wethers with 25 ppm Mo and .5% SO_4 for 50 days increased ($P < .001$) total Cu in plasma from 1.3 to 1.8 ppm (Smith et al., 1968). Smith and Wright (1975b) observed an increase ($P < .01$) in total Cu concentration in plasma of wethers given additional dietary Mo concentrations of 16 and 24 ppm, but those fed 8 ppm Mo did not differ from sheep fed the basal diet, neither did 16 ppm differ from 24 ppm. Total Cu concentrations in plasma of lambs fed 25 ppm additional Mo or 25 ppm Mo plus .5% SO_4, for 30 weeks, increased, relative to the group fed the basal diet (Bremner and Young, 1978). Increases ($P < .01$) in total Cu concentrations in plasma of sheep were observed in medium- (38.4 mg Mo/day) and high-Mo (58.5 mg Mo/day) treatments when compared with low-Mo (20.8 mg Mo/day) and the group not fed additional Mo (.6 mg Mo/day) (van Ryssen and Stielau, 1981). In lambs fed 75 mg Cu/day for 50 days and then switched to diets providing daily 5 mg Cu and 35.7 or 140 mg supplemental Mo and 1, 2 or 4 g added S, concentrations of Cu in plasma on day 13 were greater ($P < .01$) than in lambs fed no supplemental Mo and S (van Ryssen et al., 1986). Plasma Cu concentrations were elevated in sheep with addition of 25 or 50 ppm Mo to their

diets (10 ppm Cu), for 64 days, relative to the unsupplemented group. Plasma Cu concentrations decreased abruptly after withdrawal of Mo, reaching within 16 days the concentrations found in sheep fed the basal diet (van Ryssen and Barrowman, 1988). Copper concentrations in plasma of calves fed a diet supplemented with 35 ppm Mo and .3% S increased compared with animals fed the control diet, and this was particularly marked over the first 4 weeks of feeding (Wang et al., 1988). Total Cu values in plasma of initially 5-month-old rams fed a diet containing 38 mg additional Mo for 14 weeks were greater ($P < .05$) than in the group fed the basal diet (van Ryssen et al., 1990).

On the other hand, decreases in plasma or serum Cu concentrations have been reported in cattle by Vanderveen and Keener (1964), Humphries et al. (1988b) and Kleczkowski (1989), and in lambs by Dowdy and Matrone (1968a). The addition of 0, 5, 10, 20 or 50 ppm Mo to the diet of heifers decreased ($P < .05$) serum Cu concentrations in all Mo treatments, at 300 days of feeding (Vanderveen and Keener, 1964). Plasma Cu concentrations were reduced ($P < .001$) in calves fed, for 41 weeks, 2 ppm Mo or 2 ppm Mo plus 150 ppm Fe in a basal diet containing 4 ppm Cu, 100 ppm Fe, .1 ppm Mo and .28% S (Humphries et al., 1988b). These calves had been reared until 10 to 12 weeks old on a low Cu milk-substitute diet (Humphries et al., 1988b). Increase of dietary Mo concentration from .3 to 5 ppm reduced ($P < .05$)

concentrations of Cu in serum in cattle (Kleczkowski 1989). Lambs fed a purified diet containing 1 ppm Cu and varying concentrations of Mo (0 to 4 ppm) and S (.03% to .12%) exhibited decreasing plasma concentrations of Cu (Dowdy and Matrone, 1968a). A decrease in plasma Cu concentrations occurred in all lambs, regardless of the diet fed, but the decline was sharper and faster in animals fed 4 ppm Mo and .12% S (Dowdy and Matrone, 1968a).

In several studies with cattle and sheep, no response of Cu concentration in blood or plasma to increasing dietary Mo concentrations was observed (Wynne and McClymont, 1956; Cook et al., 1966; Huber et al., 1971; Suttle, 1977; Suttle et al., 1988; Wittenberg and Devlin, 1988). Blood Cu concentrations of lambs and sheep fed 4.3 ppm supplemental Mo did not differ ($P > .05$) from those of animals fed the basal diet (.83 ppm Mo, 5.2 ppm Cu and .04% S); however, when SO_4 was also added, to increase dietary concentrations to .40%, blood Cu concentrations were reduced ($P < .05$), from 1.08 ppm in the group fed the basal diet to .64 ppm in the group fed Mo plus SO_4 (Wynne and McClymont, 1956). Plasma Cu concentrations were not affected by Mo administrations (1.5 or 3.0 mg Mo/kg body weight) in yearling steers, for 100 days (Cook et al., 1966). Blood Cu concentrations of cows fed from 53 to 300 ppm supplemental Mo were not altered compared with animals on the control diet (Huber et al., 1971). Plasma Cu concentrations of

lambs fed 18 to 27 weeks a diet containing 45.3 ppm Cu, supplemented with 0, 2, 4, 8 or 16 ppm Mo, were unaffected by dietary treatment, except when unsupplemented animals were approaching a hemolytic crisis (Suttle, 1977). Molybdenum supplementation (5 ppm) of the diet of lambs for 9 weeks did not affect plasma Cu concentrations (Suttle et al., 1988). Plasma Cu concentrations of ewes fed diets with .9, 18.4 or 40.7 ppm Mo increased ($P < .01$) during the 6-week feeding period, but they were not influenced ($P > .05$) by dietary Mo concentrations (Wittenberg and Devlin, 1988).

In some circumstances response of plasma Cu to dietary Mo depended on the concentration of dietary Mo (Kincaid, 1980; Wittenberg and Devlin, 1987) or on age of the animals (Pitt et al., 1980). Addition of 0, 1, 10 or 50 ppm Mo to the drinking water of calves, providing < 1, 4.8, 50 and 270 mg Mo/day, elevated ($P < .05$) plasma Cu concentrations only at the greatest Mo concentration (Kincaid, 1980). A dietary Mo increase from .6 to 19.3 ppm did not affect ($P > .05$) plasma Cu of lactating beef cows, however, a further dietary increase to 35 ppm resulted in a decrease ($P > .05$) of plasma Cu concentrations (Wittenberg and Devlin, 1987). Total Cu in plasma of mature wethers was not altered ($P > .05$) when grazing for 76 weeks pastures containing .4 to 1.5 ppm Mo or 5.5 to 12.5 ppm Mo throughout a period of 74 weeks and 33.5 ppm Mo for the final 2 weeks after lime application, but they were changed ($P < .01$) in young

wethers grazing these pastures for the final 13 weeks (Pitt et al., 1980).

TCA-Soluble Copper

Initial serum TCA-soluble Cu did not differ among treatments (P = .90; Table 4-24, 4-25, 4-26). Final serum TCA-soluble Cu (Table 4-24) did not show any effect of added dietary Mo level (P = .43), neither did the difference between initial and final values (P = .28; analysis without initial value, not shown in tables). When the initial value was used as a covariate of final value, Mo level still was ineffective (P = .57), even though length of time tended to affect final values (P = .07; Table 4-25). No level * time interaction occurred (P = .56; Table 4-25, 4-26).

As with total Cu in plasma, responses of TCA-soluble Cu to elevated dietary Mo concentrations varied in different studies. No significant change in TCA-soluble Cu concentrations of sheep resulted from 0 to 24 ppm Mo supplementation (Smith and Wright, 1975a,b). TCA-soluble Cu concentrations were greater (P < .05) in lambs fed 25 ppm supplemental Mo plus .5% SO_4 for 30 weeks than in lambs fed the basal diet; when only 25 ppm Mo was added, values did not differ from lambs fed the basal diet (Bremner and Young, 1978). TCA-soluble Cu concentrations in hypocupraemic ewes decreased markedly when dietary Mo was elevated up to 4 ppm, but it increased when Mo in diet was further elevated up to

TABLE 4-24. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-SOLUBLE COPPER IN SERUM OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Copper (Initial)	14	.83 ± .03[b]	.99 ± .18	.79 ± .04	.84 ± .04	.86 ± .05
	28	.84 ± .03	.90 ± .06	.98 ± .12	.86 ± .10	.90 ± .04
	Mean	.84 ± .02	.94 ± .09	.89 ± .07	.85 ± .05	.88 ± .03
Copper (Final)	14	.77 ± .05	.80 ± .06	.61 ± .04	.77 ± .06	.74 ± .03
	28	.62 ± .04	.69 ± .06	.61 ± .10	.73 ± .11	.66 ± .04
	Mean	.70 ± .04	.74 ± .04	.61 ± .05	.75 ± .06	.70 ± .02

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm (n = 5).

TABLE 4-25. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-SOLUBLE COPPER IN THE SERUM OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Copper (Initial)	Intercept	2.939932	41.92	.0001	.070127
	Level	-.001189	-.48	.6370	.002499
	Time	-.000007	-.01	.9982	.003168
	L * T[d]	.000050	.45	.6584	.000113
Copper (Final)	Intercept	2.736514	28.71	.0001	.095327
	Cu-Initial	.000256	3.65	.0009	.000070
	Level	-.001452	-.57	.5704	.002535
	Time	-.006000	-1.87	.0692	.003201
	L * T	.000068	.59	.5579	.000114

[a] Based on log (base 10) of ppb Cu.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-26. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF TCA-SOLUBLE COPPER (ppb) IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1[f]	3	.0041		.9003	.016	2.8
Error	36	.2529				
Level	1		.0002	.8679		
Time	1		.0025	.5560		
L * T[g]	1		.0014	.6584		
Model 2[h]	4	.1316		.0044	.344	3.0
Error	35	.2509				
Initial-Cu	1		.0899	.0011		
Level	1		$1*10^{-5}$	.9699		
Time	1		.0392	.0253		
L * T	1		.0025	.5579		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Initial TCA-soluble copper in serum.

[g] Level * time interaction.

[h] Final TCA-soluble copper in serum.

24 ppm (Suttle, 1983b). This quadratic response was thought to be related to changes in sulfide metabolism in the rumen (Suttle, 1983b). TCA-soluble Cu in lactating beef cows was unaffected ($P > .05$) by a dietary Mo increase from .6 to 19.3 ppm; however, a further increase in dietary Mo to 34.8 ppm resulted in a decline ($P < .05$) in this plasma Cu fraction (Wittenberg and Devlin, 1987). In ewes fed similar dietary Mo concentrations, no such decline occurred (Wittenberg and Devlin, 1988). Oral administration of tetrathiomolybdate (54 or 162 mg/day), for 5 months, reduced ($P < .05$) TCA-soluble Cu in plasma of pregnant ewes (Kincaid and White, 1988). TCA-soluble Cu decreased ($P < .01$) in rams fed for 14 weeks a diet with 38 ppm supplemental Mo relative to a group fed the basal diet (van Ryssen et al., 1990).

TCA-Insoluble Copper

TCA-insoluble Cu in serum at the beginning of the experiment did not differ among lambs allocated to the eight treatment groups ($P = .58$; Table 4-27, 4-28, 4-29). Final TCA-insoluble Cu was not affected by Mo dietary concentrations ($P = .26$) or length of time of feeding ($P = .75$; Table 4-28) but there was a level * time interaction ($P = .01$; Table 4-28, 4-29). The addition of the initial value as a covariate to the model did not improve P-values. However, the regression analysis performed by time indicated

TABLE 4-27. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-INSOLUBLE COPPER CONCENTRATIONS IN SERUM OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Copper (Initial)	14	.206 ± .019[b]	.227 ± .015	.186 ± .011	.216 ± .024	.211 ± .010
	28	.209 ± .022	.180 ± .026	.181 ± .008	.201 ± .018	.193 ± .010
	Mean	.208 ± .014	.204 ± .016	.183 ± .006	.209 ± .014	.202 ± .007
Copper (Final)	14	.174 ± .009	.238 ± .021	.315 ± .050	.250 ± .061	.244 ± .022
	28	.206 ± .011	.314 ± .032	.460 ± .079	.608 ± .076	.397 ± .044
	Mean	.190 ± .009	.276 ± .023	.388 ± .050	.429 ± .075	.321 ± .027

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm (n = 5).

TABLE 4-28. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-INSOLUBLE COPPER CONCENTRATIONS IN THE SERUM OF SHEEP (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Copper (Initial)	Intercept	2.360232	30.40	.0001	.077650
	Level	-.000047	-.02	.9867	.002784
	Time	-.002944	-.84	.4067	.003504
	L * T[d]	.000002	-.02	.9847	.000125
Copper (Final)	Intercept	2.396077	13.26	.0001	.180723
	Cu-Initial	-.626639	-1.07	.2930	.586371
	Level	-.005064	-1.15	.2581	.004400
	Time	.001793	.32	.7497	.005573
	L * T	.000540	2.72	.0102	.000198

[a] Based on log (base 10) ppb Cu.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-29. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF TCA-INSOLUBLE COPPER (ppb) IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model 1[f]	3	.0169		.5848	.055	4.0
Error	34	.2913				
Level	1		.0002	.8851		
Time	1		.0167	.1720		
L * T[g]	1		$3*10^{-6}$	.9847		
Model 2[h]	4	1.0499		.0001	.598	6.0
Error	33	.7057				
Initial-Cu	1		.1059	.0330		
Level	1		.4565	.0001		
Time	1		.3288	.0004		
L * T	1		.1587	.0102		
Model 14 d	2	.0484		.4863	.092	7.6
Error	15	.4796				
Initial-Cu	1		.0142	.5160		
Level	1		.0342	.3172		
Model 28 d	2	.5890		.0001	.723	4.5
Error	17	.2257				
Initial-Cu	1		.0212	.2237		
Level	1		.5678	.0001		

[a] Degrees of freedom. [b] Sum of squares.

[c] Type I sum of squares. [d] Coefficient of determination.

[e] Coefficient of variation. [f] Initial TCA-insoluble Cu.

[g] Level * time interaction. [h] Final TCA-insoluble Cu.

that at 14 days Mo level had no effect ($P = .32$) on TCA-insoluble Cu, but it did ($P < .0001$) at 28 days (Table 4-29).

Smith et al. (1968) reported an additional Cu fraction in plasma, about 42% of total Cu in plasma, from Mo supplemented sheep, which was neither direct reacting Cu nor ceruloplasmin. Suttle and Field (1968a) noticed a third fraction in plasma Cu, besides ceruloplasmin and direct reacting Cu, which was increased by Mo plus SO_4 supplementation. The rise in Cu concentrations in plasma after Mo supplementation in presence of adequate S occurs because of formation of firmly bound Cu and Mo and it corresponds to the TCA-insoluble fraction (Suttle, 1980). Hynes et al. (1984) demonstrated in sheep that circulating thiomolybdates lead to a decrease in TCA-soluble fraction of Cu in plasma and to an increase in the portion of Cu bound to albumin, identified as TCA-insoluble Cu. When thiomolybdate is administered intravenously there is an increase in plasma Cu, with a Cu-thiomolybdate complex formed which is mostly TCA-insoluble (Gooneratne et al., 1981, 1985).

TCA-insoluble Cu in serum has been shown to consistently increase as a result of Mo supplementation (Smith and Wright, 1975a,b; Pitt et al., 1980; Suttle, 1983b; Wittenberg and Devlin, 1987; Wang et al., 1988). This has happened even with smaller concentrations of

dietary Mo (0 to 24 ppm) (Smith and Wright, 1975b), and shorter time (12 days) (Smith and Wright, 1975b) than in the first period of Exp. 4. TCA-insoluble Cu in serum of wethers increased ($P < .01$) with increasing dietary Mo supplementation, but 0 ppm and 8 ppm Mo did not differ, neither did 16 and 24 ppm (Smith and Wright, 1975b). TCA-insoluble Cu increased in mature ($P < .002$) and in young ($P < .01$) wethers grazing pasture sprayed with molybdate, relative to sheep grazing untreated pasture (Pitt at al. 1980). TCA-insoluble Cu of hypocupraemic ewes increased with Mo concentrations in the diet (0 to 24 ppm) in a predominantly linear relationship ($P < .01$) (Suttle, 1983b).

The most striking effect ($P < .05$) of Mo (35 ppm) and S (.3%) supplementation in calves was the appearance of Cu in plasma which was insoluble in trichloracetic acid and this was particularly evident over the first 4 weeks of supplementation (Wang et al., 1988). Smith and Wright (1975b) observed that changes which occurred in plasma Cu distribution of wethers took place within the first 12 days of feeding diets with added concentrations of Mo of 0, 8, 16 or 24 ppm; observations were continued for 203 days. No TCA-insoluble Cu was present in plasma of wethers fed 25 ppm supplemental Mo but no additional S, despite their elevated plasma Mo concentrations; the basal diet contained .08% S (Bremner and Young, 1978).

TCA-Soluble and TCA-Insoluble Copper Expressed as a Percentage of Total Copper in Serum

When TCA-soluble Cu was expressed as a percentage of total Cu, it accounted for all Cu in plasma in groups receiving 0 and 8 ppm supplemental Mo, but in groups receiving 16 and 24 ppm Mo, percentage TCA-soluble Cu decreased ($P > .001$) to 64% and 60%, respectively (Smith and Wright, 1975b). Wittenberg and Devlin (1987) observed a decrease ($P < .05$) from 81.1% TCA-soluble Cu in cows fed the basal diet, to 75.1% in animals fed 40 ppm additional Mo, but the group fed 20 ppm added dietary Mo did not differ from the unsupplemented group. In ewes (Wittenberg and Devlin, 1988), those Mo concentrations did not have any effect ($P > .05$) on TCA-soluble Cu calculated as a percentage of total Cu (91.7%, 93.0% and 88.9%, for 0, 20 and 40 ppm added Mo, respectively).

In Exp. 4, when final TCA-soluble Cu was expressed as a percentage of total Cu in serum (Table 4-30) neither Mo level ($P = .46$) nor time ($P = .56$) had any effect (Table 4-31), but a level * time interaction was observed ($P = .06$; Table 4-31, 4-32). PROC GLM performed by time indicated a decrease of the percentage soluble Cu fraction in serum, at 28 days ($P = .004$), but not at 14 days ($P = .19$; Table 4-32).

When TCA-insoluble Cu was expressed as a percentage of total Cu in plasma, it was greater ($P < .01$) in sheep having

TABLE 4-30. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-SOLUBLE AND INSOLUBLE COPPER IN SERUM OF LAMBS, EXPRESSED AS A PERCENTAGE OF TOTAL COPPER IN SERUM (EXPERIMENT 4)

TCA-copper	Period	Added dietary molybdenum concentrations (ppm)[a]				
(%)	(day)	0	15	30	45	Mean
Soluble	14	81.4 ± 1.4[b]	77.0 ± 1.7	66.3 ± 5.0	76.5 ± 4.4	75.3 ± 2.0
	28	74.7 ± 2.2	68.5 ± 2.4	57.1 ± 5.8	54.0 ± 6.7	63.6 ± 2.9
	Mean	78.1 ± 1.7	72.8 ± 2.0	61.7 ± 3.9	65.3 ± 5.3	69.4 ± 2.0
Insoluble	14	18.5 ± 1.4	23.0 ± 1.7	33.7 ± 5.0	23.5 ± 4.4	24.7 ± 2.0
	28	25.3 ± 2.2	31.5 ± 2.4	42.9 ± 5.8	46.0 ± 6.7	36.4 ± 2.9
	Mean	21.9 ± 1.7	27.3 ± 2.0	38.3 ± 3.9	34.7 ± 5.3	30.5 ± 2.0

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean (n = 5).

TABLE 4-31. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON TCA-SOLUBLE AND TCA-INSOLUBLE COPPER IN THE SERUM OF LAMBS, EXPRESSED AS A PERCENTAGE OF TOTAL COPPER IN SERUM (EXPERIMENT 4)

TCA-copper	Regression equation[a]				
(%)	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Soluble	Intercept	1.930062	21.26	.0001	.090792
	Initial-Cu	-.000136	-.16	.8707	.000832
	Level	.001578	.75	.4577	.002101
	Time	-.001562	-.59	.5597	.002651
	L * T[d]	-.000187	-1.98	.0558	.000095
Insoluble	Intercept	1.157136	7.16	.0001	.161571
	Initial-Cu	.002180	.40	.6910	.005437
	Level	-.001463	-.36	.7216	.004071
	Time	.007495	1.46	.1546	.005144
	L * T	.000260	1.42	.1658	.000183

[a] Based on log (base 10) transformed data.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-32. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF TCA-SOLUBLE COPPER EXPRESSED AS A PERCENTAGE OF TOTAL COPPER IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model	4	.1470		.0002	.460	3.8
Error	35	.1722				
Initial-Cu	1		$6*10^{-5}$	.9123		
Level	1		.0640	.0010		
Time	1		.0637	.0010		
L * T[f]	1		.0193	.0558		
Model 14 d	2	.0066		.3830	.107	3.0
Error	17	.0551				
Initial-Cu	1		.0006	.6769		
Level	1		.0060	.1913		
Model 28 d	2	.0760		.0141	.394	4.6
Error	17	.1168				
Initial-Cu	1		$2*10^{-5}$	.9543		
Level	1		.0760	.0040		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

16 or 24 ppm Mo added in their diets than in animals fed the basal diet or in those fed 8 ppm supplemental Mo (Smith and Wright, 1975b) and it was greater ($P < .05$) in cows consuming 34.8 ppm Mo than .6 or 19.3 ppm Mo (Wittenberg and Devlin, 1987). In the first study, TCA-insoluble Cu was about 40% of total Cu in plasma of sheep fed 16 or 24 ppm supplemental Mo, while it was almost devoid in the other two groups (Smith and Wright, 1975b). In the second study (Wittenberg and Devlin, 1987), these values varied from 18.9% and 20.6% in cows fed the basal diet and 20 ppm Mo supplemented cows, to 24.9% in animals fed 40 ppm additional Mo. In ewes, TCA-insoluble Cu as a percentage of total Cu in plasma was not influenced ($P > .05$) by 0, 20 or 40 ppm supplemental Mo fed for 6 weeks; values were 8.3%, 7.0% and 11.1%, respectively (Wittenberg and Devlin, 1988).

In Exp. 4, when TCA-insoluble Cu was expressed as a percentage of total Cu in serum (Table 4-30), there were no effects of Mo level ($P = .72$) and length of time ($P = .15$) and there was no level * time interaction ($P = .17$; Table 4-31, 4-33). The data, however, suggested a level * time interaction and regression analysis done by time resulted in an effect ($P = .0016$) of added dietary Mo concentrations on the percentage insoluble Cu at the 28-day feeding period, but at 14 days it was not changed ($P = .33$; Table 4-33).

TABLE 4-33. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF TCA-INSOLUBLE COPPER EXPRESSED AS A PERCENTAGE OF TOTAL COPPER IN SERUM (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model	4	.5382		.0002	.472	9.3
Error	33	.6021				
Initial-Cu	1		.0107	.4499		
Level	1		.1900	.0028		
Time	1		.3008	.0003		
L * T[f]	1		.0366	.1658		
Model 14 d	2	.0394		.4644	.097	11.5
Error	15	.3662				
Initial-Cu	1		.0149	.4470		
Level	1		.0245	.3319		
Model 28 d	2	.1895		.0055	.458	7.5
Error	17	.2244				
Initial-Cu	1		.0052	.5401		
Level	1		.1844	.0016		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

Iron, Manganese and Zinc Concentrations in Liver and Kidney

Concentrations of Fe (P = .86), Mn (P = .50) and Zn (P = .65) in liver (Table 4-34, 4-35) were not affected by Mo level, but there was a tendency for an increase in liver Zn concentrations (Table 4-34) due to length of time of feeding (P = .07; Table 4-35). Length of time of feeding did not affect Fe (P = .50) and Mn (P = .55) concentrations in liver (Table 4-34, 4-35). No level * time interaction was detected in hepatic Fe, Mn and Zn values (Table 4-35, 4-36). Concentrations of Fe, Mn and Zn in kidney (Table 4-37) were not affected by dietary Mo concentrations or by length of time of feeding (Table 4-38, 4-39). This is in agreement with Harrison et al. (1987) and van Niekerk and van Niekerk (1989). Iron, Mn and Zn concentrations in liver and kidney cortex of lambs fed 0, 2, 4 and 8 mg Mo/day for 125 days were variable but no differences were observed owing to dietary treatment (Harrison et al., 1987). Concentrations of Fe, Mn and Zn in kidney and liver of ewes and rams fed for 12 and 24 weeks, respectively, a diet containing 3.4 ppm Cu, 40 ppm Mo and .22% S or 3.6 ppm Cu, 38 ppm Mo and .34% S did not differ from sheep consuming 8 ppm Cu, 1.3 ppm Mo and .22% S in their diets (van Niekerk and van Niekerk, 1989).

Chapman and Kidder (1963) observed an increase in Fe concentrations in liver of heifers fed 250 mg Mo/day. Cattle fed supplementary Mo (1.5 or 3.0 mg Mo/kg body weight/day) had more (P < .05) Fe in liver than controls at

TABLE 4-34. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON IRON, MANGANESE AND ZINC CONCENTRATIONS IN THE LIVER OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Iron	14	196 ± 33[b]	190 ± 19	168 ± 8	192 ± 25	186 ± 11
	28	196 ± 18	209 ± 18	176 ± 12	180 ± 13	190 ± 8
	Mean	196 ± 18	200 ± 13	172 ± 7	186 ± 14	188 ± 7
Manganese	14	12.0 ± .9	11.3 ± .5	10.4 ± .9	11.7 ± .5	11.3 ± .4
	28	10.8 ± .6	11.7 ± .7	11.3 ± .5	11.2 ± .4	11.2 ± .3
	Mean	11.4 ± .6	11.5 ± .4	10.8 ± .5	11.4 ± .3	11.3 ± .2
Zinc	14	109 ± 4	115 ± 6	110 ± 2	117 ± 7	113 ± 2
	28	120 ± 6	134 ± 8	135 ± 19	121 ± 6	128 ± 5
	Mean	115 ± 4	125 ± 6	122 ± 10	119 ± 4	120 ± 3

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% S.

[b] Mean ± standard error of the mean, in ppm (n = 5).

TABLE 4-35. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON IRON, MANGANESE AND ZINC CONCENTRATIONS IN THE LIVER OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Iron	Intercept	2.229549	27.60	.0001	.080079
	Level	.000524	.18	.8567	.002879
	Time	.002504	.69	.4971	.003650
	L * T[d]	-.000061	-.47	.6410	.000130
Manganese	Intercept	1.078077	22.51	.0001	.047904
	Level	-.001170	-.69	.4974	.001707
	Time	-.001298	-.60	.5525	.002164
	L * T	.000052	.68	.5034	.000077
Zinc	Intercept	1.982285	39.83	.0001	.049766
	Level	.000822	.46	.6456	.001773
	Time	.004165	1.85	.0721	.002248
	L * T	-.000027	-.34	.7375	.000080

[a] Based on log (base 10) of ppm Fe, ppm Mn or ppm Zn.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-36. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF IRON (ppm), MANGANESE (ppm) AND ZINC (ppm) IN LIVER (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model, Fe	3	.0111		.7570	.032	4.3
Error	36	.3357				
Level	1		.0065	.4089		
Time	1		.0025	.6083		
L * T[f]	1		.0021	.6410		
Model, Mn	3	.0016		.9213	.013	5.4
Error	36	.1180				
Level	1		$6*10^{-5}$	.8895		
Time	1		$3*10^{-5}$	.9236		
L * T	1		.0015	.5034		
Model, Zn	3	.0259		.0800	.169	2.9
Error	36	.1274				
Level	1		.0007	.6530		
Time	1		.0248	.0120		
L * T	1		.0004	.7375		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

TABLE 4-37. EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON IRON, MANGANESE AND ZINC CONCENTRATIONS IN THE KIDNEY OF LAMBS (EXPERIMENT 4)

Mineral	Period (day)	Added dietary molybdenum concentrations (ppm)[a] 0	15	30	45	Mean
Iron	14	228 ± 41[b]	179 ± 16	97 ± 6	178 ± 15	170 ± 16
	28	189 ± 12	210 ± 20	108 ± 12	186 ± 10	173 ± 11
	Mean	209 ± 21	194 ± 13	102 ± 7	182 ± 8	172 ± 9
Manganese	14	5.0 ± .2	5.0 ± .3	4.8 ± .2	4.8 ± .2	4.9 ± .1
	28	4.7 ± .2	4.9 ± .3	4.9 ± .4	4.5 ± .4	4.8 ± .2
	Mean	4.8 ± .2	4.9 ± .2	4.9 ± .2	4.6 ± .2	4.8 ± .1
Zinc	14	100 ± 3	98 ± 4	97 ± 4	104 ± 5	100 ± 2
	28	101 ± 4	110 ± 3	103 ± 4	106 ± 7	105 ± 2
	Mean	100 ± 3	104 ± 3	100 ± 3	105 ± 4	102 ± 2

[a] Basal diet contained 11 ppm Cu, 1.2 ppm Mo and .22% s.

[b] Mean ± standard error of the mean, in ppm (n = 5).

TABLE 4-38. REGRESSION ANALYSES OF THE EFFECT OF ADDED DIETARY MOLYBDENUM CONCENTRATIONS AND FEEDING PERIOD ON IRON, MANGANESE AND ZINC CONCENTRATIONS IN THE KIDNEY OF LAMBS (EXPERIMENT 4)

	Regression equation[a]				
Mineral	Parameter	Estimate	T[b]	P > |T|	STDERR[c]
Iron	Intercept	2.310182	18.64	.0001	.123928
	Level	-.006142	-1.34	.1879	.004573
	Time	-.001583	-.28	.7786	.005587
	L * T[d]	.000145	.71	.4812	.000204
Manganese	Intercept	.711008	15.71	.0001	.045255
	Level	-.000474	-.29	.7707	.001613
	Time	-.001004	-.49	.6264	.002044
	L * T	.000001	.02	.9871	.000073
Zinc	Intercept	1.967220	55.84	.0001	.035231
	Level	.000391	.31	.7572	.001255
	Time	.001724	1.08	.2858	.001592
	L * T	-.000008	-.14	.8925	.000057

[a] Based on log (base 10) of ppm Fe, ppm Mn or ppm Zn.

[b] T-value for the null hypothesis that parameter = zero.

[c] Standard error of the estimate.

[d] Level * time interaction.

TABLE 4-39. ANALYSES OF VARIANCE--LOGARITHM (BASE 10) OF IRON (ppm), MANGANESE (ppm) AND ZINC (ppm) IN THE KIDNEY (EXPERIMENT 4)

Source	df[a]	SS[b]	SS-I[c]	P-value	R^{2d}	CV[e]
Model, Fe	3	.1139		.1750	.130	6.7
Error	35	.7598				
Level	1		.0981	.0407		
Time	1		.0048	.6408		
L * T[f]	1		.0110	.4812		
Model, Mn	3	.0041		.7041	.038	8.0
Error	36	.1053				
Level	1		.0023	.3848		
Time	1		.0019	.4290		
L * T	1		$8*10^{-7}$	.9871		
Model, Zn	3	.0053		.4027	.077	2.1
Error	36	.0638				
Level	1		.0006	.5676		
Time	1		.0047	.1118		
L * T	1		$3*10^{-5}$	.8925		

[a] Degrees of freedom.

[b] Sum of squares.

[c] Type I sum of squares.

[d] Coefficient of determination.

[e] Coefficient of variation.

[f] Level * time interaction.

100 days of feeding (Cook et al., 1966). Concentrations of Fe in liver, but not kidney, increased ($P < .05$) by oral administration of tetrathiomolybdate (54 mg or 162 mg/day) in pregnant ewes (Kincaid and White, 1988). Molybdenum supplementation (5 ppm) for 32 weeks, increased ($P < .01$) liver Fe concentrations in heifers, particularly between 20 and 28 weeks of feeding (Phillippo et al., 1987b).

As opposed to findings reported by Chapman and Kidder (1963), Cook et al. (1966), Kincaid and White (1988) and Phillippo et al. (1987b), hepatic Fe concentrations were not affected in the present experiment by dietary Mo concentrations, which is in agreement with Lesperance and Bohman (1961), Huber et al. (1971), Harrison et al. (1987), and van Niekerk and van Niekerk (1989). Liver Fe concentrations were not altered in heifers fed 100 ppm added dietary Mo, for one year (Lesperance and Bohman, 1961). High intakes of Mo (from 53 to 300 ppm added concentrations) had no effect on liver Fe concentrations of lactating dairy cows (Huber et al., 1971).

A lack of effect of dietary Mo on hepatic Zn concentrations was also reported by Kincaid (1980), van Ryssen et al. (1986) and Phillippo et al. (1987b). Zinc concentrations in liver of calves having a daily intake of less than 1, 4.8, 50 or 270 mg Mo in drinking water for 21 days increased slightly, but the increase was not significant (Kincaid, 1980). Diets providing daily about 5

mg Cu and 0, 35, 70 or 140 mg supplemental Mo, with 0, 1, 2 or 4 g added S, had no significant effect on the concentrations of Zn in liver or kidney of ewes that had previously been on a high Cu intake (75 mg Cu/day) (van Ryssen et al., 1986). There were no significant changes in liver Zn concentrations of heifers due to 5 ppm Mo supplementation for 32 weeks (Phillippo et al., 1987b).

Some influence of Mo on Zn, however, has been noticed (Parada, 1981; Hidiroglou et al., 1982; Kincaid and White, 1988; Gooneratne et al., 1989b). Parada (1981) observed clinical signs ascribable to Zn deficiency in a group of cows grazing pasture industrially poisoned with Mo and containing 420 ppm Mo, 14 ppm Cu and 30 ppm Zn. Zinc content of bones of wethers increased ($P < .01$) following Mo supplementation (10 to 12 ppm Mo), for 7 months, relative to unsupplemented sheep (Hidiroglou et al., 1982). Zinc concentrations in liver, but not kidney, were reduced ($P < .05$) by daily oral administrations for about 5 months of 162 mg tetrathiomolybdate, but not by 54 mg in pregnant ewes (Kincaid and White, 1988). Iron and Zn concentrations were not affected in kidney of lambs after intravenous administration of tetrathiomolybdate (Gooneratne et al., 1989b).

General Discussion

On the basis of the coefficient of determination, feces and urine Mo variations were highly explained by changes in dietary Mo and their CV's were relatively small, however, these statistics are based on a cubic equation. The linear equation for feces resulted in a R^2 of .798, with a CV of 8.7% . For urine, the R^2 and CV of the linear equation was .675 and 17.2%, respectively. Of these two, only the R^2 of feces is comparable to those of muscle and kidney, .782 and .797, respectively, which were adequately described by a linear model. For kidney Mo, at 14 days, the R^2 and CV were .635 and 18.1% respectively; and at 28 days, .863 and 15.0%, respectively. The greatest R^2 with a linear model occurred in serum, .811. The R^2 for liver was somewhat smaller, .588. The less distinct capacity of the liver to indicate Mo status was already stated by Masaoka et al. (1989). Much earlier, Lesperance and Bohman (1963) indicated that the liver is not a site of massive storage of Mo, but the liver seems to be a better indicator for long term high Mo intake (Lesperance et al., 1985).

Of all tissues and excretions analyzed in this short term experiment, serum seems to be the tissue of choice for a comparison of Mo sources. The question that arises is that of serum Mo being a reliable measure of Mo availability. If Mo is bound to Cu in plasma in an insoluble form, this is certainly not true. But if a source

of Mo that will reduce the chance of Cu toxicosis is being sought, it is acceptable, because that is the effect which Mo is expected to do in this instance, reduce Cu availability in tissues. On low or normal S diets this is probably not the case, because of little thiomolybdate synthesis, and serum Mo is apparently a good estimate of relative bioavailability of inorganic Mo sources. At low dietary S levels (.05%), the minimal concentration of Mo in diet to start the Cu x Mo x S interference in lambs was around 3 ppm (Lamand et al., 1988). Dietary concentrations of Mo tested in this study were 2.4, 4.8, 7.2 and 9.6 ppm and thiomolybdate synthesis was inferred from TCA-insoluble Cu detection in plasma. It is not known how much Mo was complexed to S and Cu in the lambs in the present experiment, but a certain degree of complexation certainly occurred, based on the increase in the serum Cu fraction insoluble in TCA. If tissue uptake of uncomplexed molybdate is the objective, then a lower dietary S concentration is probably more appropriate.

The present study indicated that 45 ppm Mo in a diet containing adequate S (.22%) and fed for 28 days was necessary to reduce hepatic Cu concentrations in lambs fed a normal Cu concentration, but 15 ppm fed for 14 days were sufficient to increase serum Mo concentrations. Suttle (1977) suggested that it would generally be unnecessary to add more than 4 ppm Mo to limit the accumulation of Cu in

lambs. This probably refers to long-term feeding periods. Van Ryssen et al. (1986) recommended a dietary supplement of 70 mg Mo/day, to reduce liver Cu concentrations in lambs, provided dietary Cu concentrations were simultaneously reduced.

Summary and Conclusions

An experiment was conducted to determine the effect of elevated dietary concentrations of Mo and length of time of feeding on the excretion and tissue concentrations of Mo and Cu in individually fed housed lambs. Forty lambs were randomly assigned to 0, 15, 30 or 45 ppm added dietary Mo, fed for 14 or 28 days. Molybdenum, as sodium molybdate, reagent grade, was added to a ground corn-soybean meal-cottonseed hulls basal diet, containing 11 ppm Cu, 1.2 ppm Mo and .22% S. Total feces and urine collections were performed daily during the last 5 days of each period and sampled on a 10% basis. On day 0, 13 and 27, blood samples were taken by jugular vein puncture. Part of the serum was mixed with trichloroacetic acid solution, centrifuged and the supernatant saved for Cu analysis. On day 14 and 28, lambs were euthanized for collection of bile, liver, kidney and muscle. Concentrations of Mo and Cu were analyzed in feed, feces, urine, bile, liver, kidney, muscle and serum; of Cu in the TCA-supernatant; and of Fe, Mn and Zn in liver and kidney. Logarithmic transformed data were analyzed by

multiple linear regression of mineral concentration in tissues or fluids or daily mineral excretion in feces or urine on added dietary Mo concentrations, time of feeding and the interaction term.

Increases in dietary Mo concentrations resulted in cubic increases of daily Mo excretion in feces and urine and in linear increases of Mo concentration in muscle, kidney and serum. Dietary Mo supplementation decreased Cu concentrations in liver, muscle, and bile; increased Cu concentrations in kidney; and increased total and TCA-insoluble Cu in serum. Added dietary Mo did not affect biliary Mo concentrations, daily fecal and urinary Cu excretion, TCA-soluble Cu concentration in serum and Fe, Mn and Zn concentrations in liver and kidney. Length of time of feeding had no effect on Mo excretion or tissue concentrations. Length of time of feeding and the level * time interaction affected or tended to affect hepatic Cu concentrations and total, TCA-soluble and TCA-insoluble Cu in serum. Hepatic Cu was decreased by added dietary Mo at 28 days of feeding and total and TCA-insoluble Cu was increased at 28 days of feeding.

It was concluded that Mo concentration in serum would be the most appropriate determination for a comparison of Mo inorganic sources and that 14 days would be sufficient to measure Mo dietary effect on serum and tissue Mo concentrations. A period of 28 days would be adequate time

to estimate effects of dietary Mo on Cu tissue concentrations.

Added dietary Mo concentrations up to 45 ppm on an adequate S diet were able to decrease liver Cu concentrations of lambs and the addition of Mo, plus sulfur, if necessary, to practical diets, may therefore be a useful procedure to control or prevent Cu toxicosis in sheep on high dietary Cu concentrations.

CHAPTER 5
RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM FROM INORGANIC SOURCES FOR LAMBS: EXPERIMENT 5

Introduction

Although Mo is an essential element, animal requirements are extremely small (Underwood, 1981) and primary Mo deficiencies in farm animals fed practical diets or under grazing have not been reported to date. The importance of Mo in animal nutrition stems from its interaction with Cu and S, primarily in cattle and sheep. At a given dietary Cu concentration, animals can experience Cu deficiency or Cu toxicosis, depending on the dietary Mo and S concentrations. Copper toxicosis in ruminants, especially in sheep, has been observed worldwide, mainly in housed lambs fed diets containing elevated Cu and relatively small Mo concentrations.

Addition of Mo and S to commercial sheep diets, continuously at low concentrations (Harker, 1976; Suttle, 1977) or for a limited period of time (van Ryssen et al., 1986) to avoid hepatic Cu accumulation has been suggested. No studies on Mo bioavailability or comparisons of the efficacy of Mo sources to decrease tissue Cu concentrations have been reported in the literature.

The objective of the present experiment was to determine, based on Mo excretion or tissue uptake in lambs, bioavailability values for ammonium molybdate, molybdenum trioxide and molybdenum metal, compared to sodium molybdate, taken as the standard source. At the same time, effects of added dietary concentrations of Mo on the excretion and tissue concentration of Cu were measured.

Materials and Methods

Forty Texas crossbred wether lambs, weighing from 38 to 52 kg (average ± standard deviation = 44 ± 3 kg), initially, were assigned randomly to one of the following seven treatments:

A. control, fed the basal diet with no added dietary Mo;

B. basal diet + 15 ppm Mo, as sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$);

C. basal diet + 30 ppm Mo, as sodium molybdate;

D. basal diet + 45 ppm Mo, as sodium molybdate;

E. basal diet + 30 ppm Mo, as ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)];

F. basal diet + 30 ppm Mo, as molybdenum trioxide (MoO_3);

G. basal diet + 30 ppm Mo, as molybdenum metal.

Six animals were assigned to treatments A, C, E, F and G and five animals to treatments B and D. One animal on treatment A needed to be removed from the experiment on day 6, because of low feed consumption.

Molybdenum sources, except Mo metal, were reagent grade and were added to the basal diet (Table 5-1) on an as-fed basis, at the expense of corn starch. The basal diet was formulated to meet nutrient requirements of lambs (NRC, 1985). It contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S (dry matter basis), by analyses. Molybdenum content of experimental diets was verified by chemical analysis.

Animals were kept in individual, raised pens with expanded metal floors, from October 13 until November 16, 1989. Adaptation to cages and the basal diet lasted 6 days. Feeding of experimental diets started on October 19, 1989, and lasted 28 days. Animals were individually fed 1 kg of the diet once a day. Tap water, containing .02 ppm Mo and no detectable Cu, was available ad libitum.

Feces and urine were collected daily during the last 5 days of the experiment. Feces were collected in cotton cloth bags. Urine was collected individually into plastic buckets containing 100 ml of 25% HCl, added daily. Total daily fecal and urinary output was measured and sampled on a 10% basis. Both feces and urine samples of each animal from the 5-day period were mixed in one composite sample. Feces were kept frozen in plastic bags until grinding and mixing in a blender with stainless steel blades and subsampling for laboratory analyses. Urine samples were maintained in plastic bottles in refrigerator, filtered with No. 40

TABLE 5-1. COMPOSITION OF THE BASAL DIET (EXPERIMENT 5)

Ingredients	Percent (as-fed basis)[a]
Ground yellow corn	56.95
Cottonseed hulls	21
Soybean meal, 48% CP	12
Alfalfa meal	3
Corn oil	3
Cornstarch[b]	2.28
Ground limestone	.55
Trace mineralized salt	1
Vitamins[c]	+
Sodium sulfate (Na_2SO_4)[d]	.22
Sodium selenite (Na_2SeO_3)[e]	+
Ethoxyquin	.0125
Mineral concentration[f]	
Ca, %	.43
Mg, %	.15
P, %	.28
S, %	.21
Cu, ppm	11.5
Mo, ppm	1.1
Fe, ppm	115
Mn, ppm	42
Zn, ppm	65

[a] Diet dry matter 88.9%.

[b] Molybdenum supplements added on an air-dry basis at expense of equivalent weight of corn starch.

[c] Vitamin A palmitate, 2200 USP units/kg; vitamin D_3, 440 USP units/kg; vitamin E, 15 IU/kg.

[d] Provided .05% additional S.

[e] Equivalent to .2 ppm Se.

[f] Dry matter basis.

Whatman paper into acid washed plastic bottles and frozen until analyzed.

Blood samples were taken on day 27 of the experiment, by jugular vein puncture. Blood was centrifuged for 30 minutes at 2500 x g. An equal volume (2 ml) of 10% (weight:volume) trichloroacetic acid (TCA) was added to serum samples, mixed and centrifuged for 25 minutes, at 2500 x g. Supernatant was saved and precipitate was resuspended into 5% (w:v) TCA, and mixing and separation procedure repeated. Supernatants removed from both processes were combined for TCA-soluble Cu analysis.

Animals were slaughtered on November 16, for collection of bile, kidney, liver and muscle (sterno mandibularis), which were immediately frozen for subsequent mineral analyses. Animals were classified as lambs at slaughter.

Samples of kidney, liver, muscle, bile, feces, urine and feed were digested in nitric acid (Fick et al., 1979). Bile was evaporated and residue pre-digested in HNO_3 and ashed at 600°C prior to solubilization. The same was done with urine, except for pre-digestion. Mo sources were refluxed in concentrated HNO_3:HCl (vol:vol) for 4 hours. Copper concentrations in water, animal tissues, excretions and TCA-supernatant; Cu, Fe, Mn, Zn, Ca and Mg in Mo sources and in basal diet; and Fe, Mn and Zn in liver and kidney were determined by flame atomic absorption spectrophotometry on a Model 5000 spectrophotometer with an AS-50 autosampler

(Perkin-Elmer Corp., Norwalk, CT; Anonymous, 1982). Serum TCA-insoluble Cu was obtained from the difference between total Cu in serum and TCA-soluble Cu. Molybdenum concentrations in feed, water, animal tissues, excretions and Mo sources were determined by graphite furnace with a Perkin-Elmer Zeeman/3030 atomic absorption spectrophotometer with an AS-60 autosampler (Anonymous, 1984). Molybdenum readings in tissues, excretions and feed in graphite furnace were run with three firings with deionized water in between samples or after the Mo standard to decrease carryover effects. Serum for Mo analysis was digested with HNO_3 plus $HClO_4$ and diluted to 25 ml, followed by a complexation and coprecipitation procedure. Precipitate was dissolved with HNO_3 and diluted to 3 ml, which was run by inductively coupled plasma spectrometry (ICP) for Mo. Serum samples of unsupplemented lambs had to be combined to get readings greater than detection limit. This was done on an equal volume basis. Molybdenum determinations in serum were performed at the Environmental Trace Substances Research Center, of the University of Missouri, Columbia, MO. Phosphorus in Mo sources and basal diet was determined colorimetrically (Harris and Popat, 1954). Sulfur concentration in basal diet was determined at the University of Minnesota, with a Model S-132 S analyzer (Leco, Warrendale, PA). Standards were matched for macroelement and acid concentrations as appropriate and standard

reference material from the National Institute of Standards and Technology (Gaithersburg, MD) was run with samples.

Relative solubility in water, .4% HCl, 2% citric acid and neutral ammonium citrate (Watson et al., 1970) and magnetic susceptibility (Watson et al., 1971) of Mo sources were determined. X-ray diffraction patterns were obtained and interpreted.

Initial and final live body weights and difference between final and initial body weights were analyzed by one-way analysis of variance, with PROC GLM of SAS (1988) using the model

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}, \quad \text{where}$$

Y_{ij} = j^{th} observation on i^{th} treatment;

μ = overall mean of all observations;

τ_i = added effect of the i^{th} treatment measured as deviation from μ;

ϵ_{ij} = random error.

Mineral data were analyzed by multiple linear regression using the GLM procedure of SAS (1988). Logarithmic (log) transformation (base 10) was performed on tissue or fluid mineral concentration or daily mineral excretion, to reduce variance heterogeneity.

Slopes and their standard errors were estimated by fitting the following multiple linear regression model:

$Y = \beta_0 + \beta_1 L + \beta_2 LS_1 + \beta_3 LS_2 + \beta_4 LS_3 + \beta_5 LS_4$, where

Y = log mineral concentration in tissue (ppm dry matter basis) or excretion (μg/day);

β_0 = intercept; β_1, β_2, β_3, β_4 and β_5 = slopes;

L = added dietary Mo concentration, ppm as-fed basis;

S_1 = 1 if source 1 (sodium molybdate), 0 otherwise;

S_2 = 1 if source 2 (ammonium molybdate), 0 otherwise;

S_3 = 1 if source 3 (molybdenum trioxide), 0 otherwise;

S_4 = 1 if source 4 (molybdenum metal), 0 otherwise.

Multiple linear regression equations, therefore, were the following:

1.) for source 1 (sodium molybdate),

$Y = \beta_0 + \beta_1 L + \beta_2 LS_1$, or, since $S_1 = 1$,

$Y = \beta_0 + (\beta_1 + \beta_2) L$;

2.) for source 2 (ammonium molybdate),

$Y = \beta_0 + (\beta_1 + \beta_3) L$;

3.) for source 3 (molybdenum trioxide),

$Y = \beta_0 + (\beta_1 + \beta_4) L$;

4.) for source 4 (molybdenum metal),

$Y = \beta_0 + (\beta_1 + \beta_5) L$;

where, $(\beta_1 + \beta_2) = b_2$, $(\beta_1 + \beta_3) = b_3$, $(\beta_1 + \beta_4) = b_4$ and $(\beta_1 + \beta_5) = b_5$ are slopes for source 1, source 2, source 3 and source 4, respectively.

Relative bioavailability values (RBV) of the Mo sources were estimated from the ratio of log transformed slopes, with sodium molybdenum as the reference source. The RBV's

were expressed as percentages. Slope ratios and their standard errors were estimated for Mo sources using the method of error propagation as described by Kempthorne and Allmaras (1965). Resulting formula for standard error is the same as equation 7.6.7 in Finney (1978). In the case of feces, reciprocals of log transformed slopes were used to calculate slope ratios.

In the statistical analyses of Mo in serum the result of combined samples were assigned to each of the animals of the group fed the basal diet. Because of greater volume available, detection limit in the ICP determination was lower.

In PROC GLM of SAS, the model statement was "mineral (in tissue, excretion) = level + level * source," with source as class statement.

It is emphasized that the estimated relative bioavailability value based on the ratio of slopes of log transformed data has a different interpretation from that based on the ratio of slopes of the actual data. In this instance, the linear or near-linear relationship applies to log transformed data--not data on the original scale. Therefore, the slopes that are being compared measure the rate of change of log ppm Cu, rather than ppm Cu itself. A linear relationship for the log transformed data implies an exponential relationship for the original data, so that the

rate of change of ppm Cu will vary with the added dietary Cu concentration.

Results and Discussion

Chemical and Physical Characteristics of Molybdenum Sources

Chemical and physical characteristics of Mo sources are shown in Table 5-2. The sodium compound contained 39.6% Mo; the ammonium, 54.5% Mo; the trioxide, 66.3% Mo; and the metal, 98.2% Mo. No major mineral contaminants were found in chemical compounds, as should be expected from reagent grade sources, except for some Mg and relatively high quantities of P.

Sodium molybdate (sodium), ammonium molybdate (ammonium) and molybdenum trioxide (trioxide) had particles that passed a 20 sieve and were retained by a 100 sieve, predominantly, therefore having particle sizes between 150 μm and 850 μm. All Mo metal (metal) passed a 200 sieve, therefore its particle size was less than 75 μm.

Relative solubility of Mo sources was quite variable. The most soluble source in solvents tested was ammonium molybdate, which was completely soluble in water and .4% HCl solution, almost completely soluble in neutral ammonium citrate and less soluble in 2% citric acid. In contrast, Mo metal was almost completely insoluble in those four solvents. The sodium form was almost completely soluble in water and .4% HCl, but much less soluble in neutral ammonium

TABLE 5-2. CHEMICAL AND PHYSICAL CHARACTERISTICS OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Item	Molybdenum sources			
	Sodium, reagent grade	Ammonium, reagent grade	Trioxide, reagent grade	Metal
Chemical constituents, as-fed basis				
Mo, %	39.6	54.5	66.3	98.2
Cu, ppm	---[a]	---	---	---
Fe, ppm	---	---	---	---
Mn, ppm	---	---	---	---
Zn, ppm	---	---	---	---
Ca, ppm	---	---	---	---
Mg, ppm	22	12	25	12
P, ppm	2350	2079	3468	538
Particle size, %[b]				
> 850 μm	4.5	---	5.1	---
850 to 150 μm	84.7	95.1	93.1	---
150 to 75 μm	10.0	4.0	1.7	---
< 75 μm	.8	.8	.1	100

TABLE 5-2. CONTINUED

Item	Molybdenum sources			
	Sodium	Ammonium	Trioxide	Metal
Physical appearance	White, fine crystals	White, fine crystals	Light gray, fine powder	Black, fine powder
Relative solubility, %[c]				
Water	89.9	100	47.0	.4
Neutral ammonium citrate	35.6	85.5	80.7	.2
HCl, .4%	91.3	99.0	19.9	.1
Citric acid, 2%	37.6	73.8	54.9	.2
Magnetic susceptibility, %	---	---	---	---
Interpretation of X-ray patterns[d]	$Na_2MoO_4 \cdot 2H_2O$	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	MoO_3	Mo-metal

[a] Not detected.

[b] Retained by a No. 20 sieve (U.S. Bureau of Standards), passed through a No. 20 but retained by a No. 100, passed through a No. 100 and retained by a No. 200 and passing a No. 200, respectively. Corresponding sieve sizes are 850μm, 150 μm and 75 μm, respectively.

[c] Solubility of .1 g in 100 ml solvent at 37°C for 1 hour, with constant stirring, expressed as a percentage of total Cu concentration.

[d] Courtesy of Pitman-Moore, Mundelein, IL.

citrate and 2% citric acid. The trioxide was 81% dissolved in neutral ammonium citrate, but was less soluble in 2% citric acid and water and much less in .4% HCl. Underwood (1977), and Mills and Davis (1987) referred to molybdenum trioxide as an insoluble compound. Based on results presented in Table 5-2, this is certainly true when solvent is .4% HCl, but not so with water, at least not close to physiological temperature, as used in the present study, 2% citric acid and, especially, neutral ammonium citrate. Molybdenum trioxide, from industrial emission of a Mo containing catalyst, was 28.6% soluble in water (Gardner and Hall-Patch, 1962).

None of the sources indicated any magnetic susceptibility. Interpretation of X-ray patterns was consistent with the labels as provided by suppliers.

Feed Consumption

Feed offered (1 kg/day) in general was eaten shortly after the morning feeding by most animals. Three lambs, however, one in the group fed the basal diet, one in treatment B and one in treatment G, did not consume an average of 17.6%, 24.6% and 5.7%, respectively, of total feed offered. Some feed refusal probably not related to the addition of Mo to diets also happened in the previous experiment.

Feed intake was unaffected in various studies with sheep (Suttle, 1977; Wittenberg and Devlin, 1988) and cattle (Lesperance and Bohman, 1963; Wittenberg and Devlin, 1987), when elevated dietary Mo concentrations were fed, but it was in others (Buchman, 1966; Suttle and Field, 1968a; van Ryssen et al., 1986).

Animals' Body Weight

Neither initial (P = .96) nor final body weight (P = .52) differed among treatments nor was there a difference between initial and final body weights (P = .50). Lack of influence of high dietary Mo concentrations on body weight has been observed in Exp. 4 and has been reported previously, in cattle (Skipper, 1951; Chapman and Kidder, 1963; Wittenberg and Devlin, 1987), and sheep (Suttle, 1977; van Ryssen and Stielau, 1981; Wittenberg and Devlin, 1988; van Ryssen et al., 1990). However, high Mo intakes reduced body weights of heifers (Lesperance and Bohman, 1963), steers (Cook et al., 1966), and lambs (Bremner and Young, 1978) or dressed carcass weights of lambs (Suttle et al., 1988).

Effects of Dietary Molybdenum on Molybdenum and Copper Excretion and Tissue Concentrations

Effects of increasing dietary concentrations of Mo on Cu and Mo concentration in tissues and fecal and urinary excretion in sheep and cattle have been discussed in detail

in Exp. 4, therefore, less emphasis will be given to this matter in the present experiment.

Fecal molybdenum excretion

Fecal Mo excretion (Table 5-3) was influenced greatly by added concentrations of dietary Mo ($P < .0001$), but there was no level * source interaction ($P = .20$). The amounts of Mo excreted in feces followed the increase in Mo intake, as was seen in the previous study, and by Lesperance et al. (1985). Daily Mo excretion in feces of wethers fed the control diet was comparable to that observed in Exp. 4. The same happened with lambs fed 45 ppm Mo as sodium, but those fed 15 and 30 ppm Mo as sodium had somewhat less Mo in feces than animals fed the same amounts for the same period of time in Exp. 4.

Fecal copper excretion

Fecal Cu excretion (Table 5-3) increased with increasing concentrations of Mo in diet ($P = .001$). There was a tendency for a level * source interaction ($P = .13$).

The daily amount of Cu excretion in feces was affected by Mo dietary concentrations in Exp. 5, in contrast to that observed in Exp. 4, even though dietary Cu and S concentrations were similar. Lambs fed 15, 30 and 45 ppm additional Mo, as sodium, excreted about the same amounts of Cu in both experiments, while the group fed the basal diet in Exp. 5 seems to have a little less Cu excretion than that from Exp. 4. Increases in fecal Cu excretion due to

TABLE 5-3. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON DAILY FECAL MOLYBDENUM AND COPPER EXCRETION OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Fecal excretion (mg/day)	
		Mo	Cu
Control	0	.52 ± .05[b]	5.5 ± .4
Sodium[c]	15	11.10 ± 1.26	6.0 ± .6
Sodium[c]	30	25.47 ± 1.36	6.3 ± .3
Sodium[c]	45	37.28 ± .54	6.7 ± .3
Ammonium[c]	30	25.09 ± 1.38	6.9 ± .4
Trioxide[c]	30	24.28 ± 1.17	7.3 ± .3
Metal	30	28.46 ± 1.20	6.6 ± .2
		ANOVA[d]	
P-values	Mo Level	< .0001	.0011
	Level * source	.2032	.1324
R^{2e}		.847	.310
CV (%)[f]		5.8	7.5

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[c] Reagent grade.

[d] Multiple linear regression analyses of log (base 10; μg Mo/day) or log (mg Cu/day) on added dietary Mo concentrations and level * source interaction.

[e] Coefficient of determination.

[f] Coefficient of variation.

elevated dietary Mo concentrations have been observed previously in cattle (Golfman and Boila, 1990) and sheep (Hidiroglou et al., 1984).

Trioxide tended to induce the greatest fecal Cu excretion, however the only difference in slopes (Table 5-4) was between the trioxide and sodium forms (P = .02). All remaining comparisons had a P-value of .19 or greater.

Urinary molybdenum excretion

Urinary excretion of Mo (Table 5-5) increased as concentrations of the element were raised in diet (P < .0001) and a level * source interaction occurred (P < .0001). Urinary excretion of Mo was proportional to Mo consumption in the present and, to a lesser extent, in the previous study. The amount of Mo eliminated daily by the group fed the basal diets from Exp. 4 and 5 were similar. In lambs fed the sodium form, however, urinary Mo increased more markedly with dietary Mo additions in Exp. 5 than in Exp. 4. Increases in urinary Mo excretion following elevated Mo intake was observed also by Suttle (1975b) and Lesperance et al. (1985).

Urinary copper excretion

Excretion of Cu in urine (Table 5-5) was not affected by Mo dietary concentrations (P = .49) nor was there a level * source interaction (P = .87).

Urinary Cu excretion was also not affected by Mo intake in Exp.4, but values seem to be a little smaller in Exp. 5

TABLE 5-4. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF DAILY FECAL COPPER EXCRETION ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

Source	Slopes ± SE[a, b]
Sodium molybdate, b_2	.001872 ± .000801[c]
Ammonium molybdate, b_3	.003094 ± .001114[ce]
Molybdenum trioxide, b_4	.004100 ± .001114[de]
Molybdenum metal, b_5	.002582 ± .001114[ce]

[a] Slope ± standard error (log mg Cu.day^{-1}/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed daily fecal Cu excretion on added dietary Mo concentrations and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .20$; $b_4 - b_2 = .019$; $b_5 - b_2 = .46$; $b_4 - b_3 = .39$; $b_5 - b_3 = .66$; $b_5 - b_4 = .19$.

[c, d, e] Slopes with different superscripts differ; P-values given in footnote "b".

TABLE 5-5. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON DAILY URINARY MOLYBDENUM AND COPPER EXCRETION OF LAMBS (EXPERIMENT 5).

Molybdenum Source	Added Mo (ppm)[a]	Urinary excretion (μg/day)	
		Mo	Cu
Control	0	61 ± 4[b]	370 ± 83
Sodium[c]	15	3930 ± 420	222 ± 32
Sodium[c]	30	8606 ± 1283	491 ± 148
Sodium[c]	45	16314 ± 2677	468 ± 168
Ammonium[c]	30	6092 ± 563	318 ± 72
Trioxide[c]	30	7556 ± 920	410 ± 99
Metal	30	55 ± 78	410 ± 92
		ANOVA[d]	
P-values	Mo Level	< .0001	.4902
	Level * source	< .0001	.8709
R^{2e}		.890	.036
CV (%)[f]		10.6	10.1

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[c] Reagent grade.

[d] Multiple linear regression analyses of log (base 10; μg Mo/day) or log (μg Cu/day) on added dietary Mo concentrations and level * source interaction.

[e] Coefficient of determination.

[f] Coefficient of variation.

than those obtained in Exp. 4. Lack of response of urinary Cu to increased Mo intake was reported by Weber et al. (1983) and Hidiroglou et al. (1984). However, increases in urinary Cu excretion in sheep in response to additional dietary Mo were observed by Marcilese et al. (1970) and Weber et al. (1983), when additional S was also provided.

Hepatic molybdenum

Hepatic Mo concentrations of lambs fed the basal diet (Table 5-6) were found to be within normal limits of 2 to 4 ppm suggested by Underwood (1977, 1981), but those of lambs fed additional Mo were not as large as the range of 20 to 30 ppm of animals fed high-Mo low-S diets (Underwood, 1981). Hepatic Mo concentration (Table 5-6) increased with additional Mo in the diet ($P < .0001$) and a level * source interaction ($P = .0035$) occurred. Apparently hepatic Mo concentrations started to level off around 30 ppm added dietary Mo. Therefore, the analysis was also run without the greatest added value, 45 ppm, but no big change occurred in P-values. For Mo level it was $< .0001$ and for the level * source interaction, .0067. A similar phenomenon was observed in the previous experiment, although less evident, at 28 days of feeding.

Hepatic concentrations of Mo in the present experiment responded to raised Mo intake, and in sodium-fed animals it did so more clearly than in Exp. 4. In the present experiment, Mo concentrations in liver of wethers fed sodium

TABLE 5-6. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON HEPATIC MOLYBDENUM AND COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Liver concentrations (ppm)[b]	
		Mo	Cu
Control	0	3.34 ± .36[c]	390 ± 24
Sodium[d]	15	6.00 ± 1.12	350 ± 88
Sodium[d]	30	7.52 ± .46	373 ± 30
Sodium[d]	45	7.77 ± .61	300 ± 60
Ammonium[d]	30	8.38 ± .35	280 ± 51
Trioxide[d]	30	9.24 ± .52	366 ± 89
Metal	30	5.51 ± .35	369 ± 50
		ANOVA[e, f]	
P-values	Mo Level	< .0001	.3335
	Level * source	.0035	.7270
R^{2g}		.628	.061
CV (%)[h]		13.7	8.4

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Dry matter basis.

[c] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[d] Reagent grade.

[e] Multiple linear regression analyses of log (base 10) ppm Mo or log ppm Cu on added dietary Mo concentrations and level * source interaction.

[f] When hepatic Mo values of lambs fed 45 ppm Mo were excluded from the analysis, P-values were < .0001 (Mo Level) and .0067 (Level * source)

[g] Coefficient of determination.

[h] Coefficient of variation.

did not reach concentrations observed in the previous experiment, possibly because initial values were smaller in animals from Exp. 5, based on values of lambs fed the basal diet.

Raises in concentration of Mo in liver due to increase in Mo consumption have been observed in many studies with sheep (Bremner and Young, 1978; van Ryssen, 1979; van Ryssen and Stielau, 1981; van Ryssen et al., 1986; van Ryssen and Barrowman, 1988; van Ryssen et al., 1990) and cattle (Lesperance and Bohman, 1961; Vanderveen and Keener, 1964; Huber et al., 1971).

Hepatic copper

Hepatic Cu concentrations (Table 5-6) of wethers in the present experiment were found to be within the normal range of 100 to 400 ppm for sheep (Underwood, 1981). Hepatic Cu concentrations (Table 5-6) were not changed by additional dietary Mo ($P = .33$), nor was there a level * source interaction ($P = .73$).

Copper concentrations in liver were not affected by dietary Mo concentrations in animals from Exp. 5, as opposed to results from the former experiment. Numerical values of averages, however, decreased as dietary concentrations of Mo increased, but there was a large variation within treatments, which certainly affected level of significance. A covariance analysis, if initial liver Cu values were available, possibly would have been useful, as already

discussed in chapter 3. A similar situation was reported by Ivan and Veira (1985), in which large variation of liver Cu concentrations among individual lambs hindered the effect of Mo supplementation. In general, values from Exp. 4 and 5 are comparable, especially when the usually observed great variability in liver Cu concentrations, already referred to in discussion of Exp. 1, 2 and 3, is considered.

Molybdenum in muscle

Concentrations of Mo in muscle (Table 5-7) increased with increasing concentrations of Mo in diet ($P < .0001$) and a level * source interaction was observed ($P = .0002$).

In both Exp. 4 and 5, Mo concentrations in muscle responded to dietary changes in Mo concentrations. Values seem to be a little greater in Exp. 5, than in Exp. 4, for the same Mo source, what may be related to a possible greater initial value in animals of Exp. 5, taking into account concentrations of the group fed the basal diet.

Elevated Mo concentrations in muscle in response to Mo supplementation have been reported by Dick (1956a); Huber et al. (1971) and van Ryssen and Stielau (1981).

Copper in muscle

Copper concentrations in muscle (Table 5-7) in animals from Exp. 5 were not affected ($P = .55$) by dietary Mo concentrations, but in Exp. 4 they decreased ($P = .048$) as a consequence of increasing dietary amounts of Mo. No level * source interaction was observed ($P = .14$).

TABLE 5-7. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON MUSCLE MOLYBDENUM AND COPPER CONCENTRATION OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Muscle concentrations (ppm)[b]	
		Mo	Cu
Control	0	.11 ± .01[c]	8.3 ± .7
Sodium[d]	15	.28 ± .02	6.8 ± .3
Sodium[d]	30	.44 ± .02	7.6 ± .4
Sodium[d]	45	.67 ± .04	7.7 ± .4
Ammonium[d]	30	.49 ± .04	6.7 ± .4
Trioxide[d]	30	.56 ± .08	7.3 ± .4
Metal	30	.27 ± .04	8.3 ± .7
		ANOVA[e]	
P-values	Mo Level	< .0001	.5517
	Level * source	.0002	.1428
R^{2f}		.826	.151
CV (%)[g]		4.7	7.7

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Dry matter basis.

[c] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[d] Reagent grade.

[e] Multiple linear regression analyses of log (base 10) ppb Mo or log ppm Cu on added dietary Mo concentrations and level * source interaction.

[f] Coefficient of determination.

[g] Coefficient of variation.

In general, values from Exp. 4 and 5 for animals fed the sodium form are comparable. Inconsistent effects of increasing dietary Mo on Cu levels in muscle have been reported by van Ryssen and Stielau (1981).

Renal molybdenum

Renal Mo concentrations (Table 5-8) were clearly elevated ($P < .0001$) by dietary concentrations of the element in Exp. 5, as they were in the prior study. There was a Mo level * source interaction ($P < .0001$).

Values found in Exp. 5 are smaller than those observed in Exp. 4, for the same period of time and Mo source. The group fed the basal diet had renal Mo concentrations within the range considered normal by Puls (1988). Rises in concentration of Mo in kidney in consequence of additions of Mo to the diet have been noticed by Huber et al. (1971), Bremner and Young (1978), Pitt et al. (1980) and Langlands et al. (1981)

Renal copper

Kidney Cu concentrations (Table 5-8) were elevated as dietary concentrations of Mo increased ($P < .0001$). A level * source interaction occurred ($P = .003$).

Concentrations of Cu in kidney of lambs increased with intake of Mo in the previous work. However, in Exp. 5 there was a sharper increase, when sodium was fed, even though kidney in animals fed the control diet contained the same

TABLE 5-8. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON RENAL MOLYBDENUM AND COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Kidney concentrations (ppm)[b]	
		Mo	Cu
Control	0	1.64 ± .16[c]	21.9 ± 1.1
Sodium[d]	15	2.80 ± .45	23.3 ± .6
Sodium[d]	30	4.30 ± .95	25.3 ± 1.3
Sodium[d]	45	10.64 ± 1.62	39.3 ± 1.5
Ammonium[d]	30	6.05 ± .74	28.9 ± 1.3
Trioxide[d]	30	6.90 ± 1.18	32.9 ± 2.5
Metal	30	1.70 ± .16	23.3 ± 1.0
		ANOVA[e]	
P-values	Mo Level	< .0001	< .0001
	Level * source	< .0001	.0032
R^{2f}		.769	.624
CV (%)[g]		28.0	4.4

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Dry matter basis.

[c] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[d] Reagent grade.

[e] Multiple linear regression analyses of log (base 10) ppm Mo or Cu on added dietary Mo concentrations and level * source interaction.

[f] Coefficient of determination.

[g] Coefficient of variation.

amounts in both experiments and dietary Cu and S were similar.

As seen in the discussion of the previous experiment, the effect of added Mo to the diet of sheep and cattle on kidney Cu concentrations has been irregular. However, if increased intakes of Mo induced a decrease in hepatic Cu concentrations, as clearly observed in Exp. 4 and an increase in serum Cu concentrations, as in both Exp. 4 and 5, then an increase in Cu concentrations in kidney can be expected, since Cu removed from liver seems not to have been eliminated in urine or in bile and there was only a small increase in feces. The latter may be only due to unabsorbed Cu, but it may also have originated from excretion of Cu directly through the intestinal wall (van Campen, 1971).

Slopes for the multiple regression of Cu concentration in kidney are shown in Table 5-9. Slopes for sodium, ammonium and trioxide did not differ ($P > .05$), but these were steeper than the slopes for the metal ($P \leq .01$). Trioxide tended to promote a larger increase in kidney Cu concentration than sodium ($P = .10$) and ammonium ($P = .16$).

Biliary molybdenum

Molybdenum concentrations in bile (Table 5-10) were elevated by additional concentration of Mo in diet ($P < .0001$) and this was dependent on Mo source ($P = .0025$).

The build-up in concentrations of Mo in bile due to increased Mo consumption was not so evident in Exp. 4 as it

TABLE 5-9. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF RENAL COPPER CONCENTRATIONS ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

Source	Slopes ± SE[a, b]
Sodium molybdate, b_2	.005229 ± .000836[c]
Ammonium molybdate, b_3	.005156 ± .001162[c]
Molybdenum trioxide, b_4	.006864 ± .001162[c]
Molybdenum metal, b_5	.002054 ± .001162[d]

[a] Slope ± standard error (log ppm Cu, dry basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed renal Cu concentration on added dietary Mo concentrations and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .94$; $b_4 - b_2 = .099$; $b_5 - b_2 = .0014$; $b_4 - b_3 = .16$; $b_5 - b_3 = .01$; $b_5 - b_4 = .0001$.

[c, d] Slopes with different superscripts differ; P-values given in footnote "b".

TABLE 5-10. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON BILIARY MOLYBDENUM AND COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Bile concentrations (ppm)[b]	
		Mo	Cu
Control	0	.010 ± .002[c]	.73 ± .08
Sodium[d]	15	.021 ± .006	.55 ± .12
Sodium[d]	30	.037 ± .009	.92 ± .21
Sodium[d]	45	.069 ± .013	.63 ± .06
Ammonium[d]	30	.050 ± .008	.67 ± .06
Trioxide[d]	30	.046 ± .010	.76 ± .13
Metal	30	.011 ± .003	.47 ± .04
		ANOVA[e]	
P-values	Mo Level	< .0001	.7453
	Level * source	.0025	.2338
R^{2f}		.546	.117
CV (%)[g]		23.8	6.1

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Dry matter basis.

[c] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[d] Reagent grade.

[e] Multiple linear regression analyses of log (base 10) ppb Mo or log ppb Cu on added dietary Mo concentrations and level * source interaction.

[f] Coefficient of determination.

[g] Coefficient of variation.

was in Exp. 5. Values in Exp. 5 were smaller in lambs fed the basal diet and greater in Mo-supplemented animals, than in the former study. One of the possible reasons for differences is sample preparation. In the present experiment, bile was pre-digested in nitric acid, prior to ashing, while it was not in Exp. 4 and some of the samples suflated (puffed) in furnaces, especially those with originally greater volume and therefore more organic residue after evaporation.

Biliary copper

Biliary Cu concentrations (Table 5-10) were not changed by concentrations of Mo in diet ($P = .74$). No interaction between Mo level and Mo source was found ($P = .23$). In contrast, in the previous study there was a decrease in biliary Cu concentrations when various concentrations of Mo were fed for 28 days.

Copper concentrations in bile obtained in Exp. 5 were smaller than those observed in Exp. 4. Intravenous administration of tetrathiomolybdate increased daily biliary Cu excretion in lambs (Gooneratne et al., 1989b,c,d) and sheep (Gooneratne et al., 1985; Ke and Symonds, 1989).

Serum molybdenum

Added dietary Mo concentrations markedly affected Mo concentrations in serum (Table 5-11; $P < .0001$). A level * source interaction was observed ($P < .0001$). Lambs fed the basal diet in Exp. 4 and 5 had comparable Mo concentrations

TABLE 5-11. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON SERUM MOLYBDENUM AND COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	Serum concentrations (ppm)[b]	
		Mo	Cu
Control	0	.007[c]	.86 ± .04[d]
Sodium[e]	15	.28 ± .05	1.09 ± .05
Sodium[e]	30	.48 ± .05	1.07 ± .06
Sodium[e]	45	1.20 ± .12	1.34 ± .04
Ammonium[e]	30	.85 ± .08	1.30 ± .10
Trioxide[e]	30	.81 ± .09	1.19 ± .08
Metal	30	.01 ± .00	1.04 ± .07
		ANOVA[f]	
P-values	Mo Level	< .0001	< .0001
	Level * source	< .0001	.1679
R^{2}[g]		.908	.449
CV (%)[h]		12.4	2.0

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Fresh basis.

[c] Result of combined serum samples.

[d] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[e] Reagent grade.

[f] Multiple linear regression analyses of log (base 10) ppb Mo or log ppb Cu on added dietary Mo concentrations and level * source interaction.

[g] Coefficient of determination.

[h] Coefficient of variation.

in serum. Wethers fed the sodium source in Exp. 5, however, reached greater Mo concentrations in serum, especially at 45 ppm added Mo, than at the 28-day period of Exp. 4. No explanation has been found for this difference, since all dietary constituents were similar.

Increases in Mo concentrations in plasma of sheep fed supplemental sodium molybdate have been described by Weber et al. (1983) and ammonium molybdate by Suttle (1983b), van Ryssen et al. (1986) and Wittenberg and Devlin (1988).

Serum copper

Concentrations of Cu in serum (Table 5-11) in the present study were elevated ($P < .0001$) when larger amounts of Mo were consumed, which is in agreement with observations in the former experiment, when sodium was provided for 28 days. There was no level * source interaction ($P = .17$). Of all data, from both Exp. 4 and 5, the best accordance in response to elevated Mo intake was probably seen in serum Cu concentrations.

Elevated concentrations of Cu in serum of sheep as a consequence of elevated dietary Mo have been observed by Smith et al. (1968), Smith and Wright (1975a,b), Bremner and Young (1978), van Ryssen and Stielau (1981), van Ryssen et al. (1986), van Ryssen and Barrowman (1988) and van Ryssen et al. (1990).

The only slopes for total serum Cu (Table 5-12) that differed were those of ammonium and metal ($P = .024$).

TABLE 5-12. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF SERUM TOTAL COPPER CONCENTRATIONS ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

Source	Slopes ± SE[a, b]
Sodium molybdate, b_2	.003723 ± .000826[cd]
Ammonium molybdate, b_3	.004953 ± .001148[c]
Molybdenum trioxide, b_4	.004115 ± .001148[cd]
Molybdenum metal, b_5	.002245 ± .001148[d]

[a] Slope ± standard error (log ppm Cu, fresh basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed serum Cu concentration on added dietary Mo concentrations and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .21$; $b_4 - b_2 = .69$; $b_5 - b_2 = .13$; $b_4 - b_3 = .48$; $b_5 - b_3 = .024$; $b_5 - b_4 = .12$.

[c, d] Slopes with different superscripts differ; P-values given in footnote "b".

Slopes for trioxide (P = .12) and sodium (P = .13) tended to be higher than that of metal.

Serum TCA-soluble copper

Concentrations of Cu soluble in trichloroacetic acid solution (Table 5-13) were not influenced (P = .29) by the amounts of Mo in diet, but there was a level * source interaction (P = .04). The same lack of response to Mo level was found in Exp. 4. In general, values from both experiments were comparable. Unresponsiveness of TCA-soluble Cu in serum to dietary Mo supplementation has been also reported by Smith and Wright (1975b). Expression of TCA-soluble Cu as a percentage of total Cu in serum (Table 5-14), however, indicated an effect (P < .0001) of Mo dietary concentration. The value was decreased as Mo in diet was increased. Such a transformation did not indicate any effect of Mo in ewes fed 0 to 40 ppm supplemental Mo for 6 weeks (Wittenberg and Devlin, 1988), neither did it in Exp. 4. Decreases in TCA-soluble Cu calculated as a percentage of total Cu in serum were observed by Smith and Wright (1975b), in sheep, and Wittenberg and Devlin (1987), in cattle.

There was no difference (P > .55) among slopes for TCA-soluble Cu for sodium, ammonium and trioxide (Table 5-15), but these were lower (P < .015) than the slope of metal. Sodium, ammonium and trioxide decreased the soluble fraction of Cu in serum, while metal did not.

TABLE 5-13. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON SERUM TCA-SOLUBLE AND INSOLUBLE COPPER CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum source	Added Mo (ppm)[a]	TCA-Cu (ppm)	
		Soluble	Insoluble
Control	0	.78 ± .05[b]	.09 ± .02
Sodium[c]	15	.87 ± .03	.22 ± .05
Sodium[c]	30	.79 ± .05	.27 ± .07
Sodium[c]	45	.68 ± .04	.67 ± .07
Ammonium[c]	30	.73 ± .06	.53 ± .08
Trioxide[c]	30	.73 ± .05	.45 ± .10
Metal	30	.94 ± .08	.10 ± .01
		ANOVA[d]	
P-values	Mo Level	.2940	< .0001
	Level * source	.0434	.0003
R^{2e}		.244	.627
CV (%)[f]		2.6	11.38

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[c] Reagent grade.

[d] Multiple linear regression analyses of log (base 10) ppb Cu on added dietary Mo concentrations and level * source interaction.

[e] Coefficient of determination.

[f] Coefficient of variation.

TABLE 5-14. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON TCA-SOLUBLE AND INSOLUBLE COPPER IN SERUM OF LAMBS, EXPRESSED AS A PERCENTAGE OF TOTAL COPPER IN SERUM (EXPERIMENT 5)

Molybdenum sources	Added Mo (ppm)[a]	TCA-copper (%)	
		Soluble	Insoluble
Control	0	89.8 ± 2.9[b]	10.2 ± 2.9
Sodium[c]	15	80.4 ± 3.8	19.6 ± 3.8
Sodium[c]	30	75.4 ± 5.7	24.6 ± 5.7
Sodium[c]	45	50.6 ± 4.4	49.4 ± 4.4
Ammonium[c]	30	58.7 ± 4.8	41.3 ± 4.8
Trioxide[c]	30	63.4 ± 6.3	36.6 ± 6.3
Metal	30	90.0 ± 1.5	10.0 ± 1.5
		ANOVA[d]	
P-values	Mo Level	< .0001	< .0001
	Level * Source	.0007	.0004
R^{2e}		.584	.593
CV (%)[f]		4.2	18.5

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[c] Reagent grade.

[c] Multiple linear regression analyses of log (base 10) % TCA-Cu on added dietary Mo concentrations and level * source interaction.

[e] Coefficient of determination.

[f] Coefficient of variation.

TABLE 5-15. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF TCA-SOLUBLE AND TCA-INSOLUBLE COPPER CONCENTRATIONS ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

	Slopes ± standard error[a]	
Molybdenum source	TCA-soluble Cu[b]	TCA-insoluble Cu[c]
Sodium molybdate, b_2	-.001507 ± .000992[d]	.019960 ± .003574[f]
Ammonium molybdate, b_3	-.002203 ± .001380[d]	.027612 ± .004968[f]
Molybdenum trioxide, b_4	-.002103 ± .001380[d]	.024384 ± .004968[f]
Molybdenum metal, b_5	.001496 ± .001380[e]	.003928 ± .004968[g]

[a] Slopes (log ppm Cu, fresh basis/ppm Mo, as-fed basis) obtained from the multiple linear regression of log (base 10) transformed TCA-soluble and TCA-insoluble Cu concentrations (ppm) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .55$; $b_4 - b_2 = .61$; $b_5 - b_2 = .011$; $b_4 - b_3 = .94$; $b_5 - b_3 = .01$; $b_5 - b_4 = .012$.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .07$; $b_4 - b_2 = .30$; $b_5 - b_2 = .0002$; $b_4 - b_3 = .53$; $b_5 - b_3 < .0001$; $b_5 - b_4 < .0001$.

[d, e] Slopes with different superscripts differ; P-values given in footnote "b".

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

Expression of TCA-soluble Cu as a percentage of total Cu in serum (Table 5-16) did result in different P-values for differences among slopes, but the ranking of sources did not change, compared to the log transformed values expressed in concentration (Table 5-15).

Serum TCA-insoluble copper

Serum TCA-insoluble Cu (Table 5-13) was elevated ($P < .0001$) as a consequence of increasing dietary Mo concentrations, as it was in the prior study at 28 days of feeding. Response to Mo was highly dependent on its source ($P = .0003$). There was good agreement in values from both experiments. Serum Cu fraction insoluble in a TCA solution calculated as a percentage of total serum Cu (Table 5-14) had the same change as its correspondent log transformed values expressed in concentration. Dietary concentrations of Mo affected the insoluble Cu when expressed as a percentage of total in serum ($P < .0001$). Increases in TCA-insoluble Cu in serum following Mo supplementation have been observed in several studies with sheep (Smith and Wright, 1975b; Pitt et al., 1980; Suttle, 1983b) and cattle (Wittenberg and Devlin, 1987; Wang et al., 1988). The increase in TCA-insoluble Cu is responsible for the increase observed in serum total Cu.

Slopes for the multiple regression equation (Table 5-15) for sodium, ammonium and trioxide did not differ ($P > .05$) and these where higher than the slope of metal ($P =$

TABLE 5-16. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF TCA-SOLUBLE AND TCA-INSOLUBLE COPPER CONCENTRATIONS AS A PERCENTAGE OF TOTAL COPPER IN SERUM ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

	Slopes ± standard error[a]	
Molybdenum source	TCA-soluble Cu[b]	TCA-insoluble Cu[c]
Sodium molybdate, b_2	-.005230 ± .001034[d]	.016237 ± .003253[f]
Ammonium molybdate, b_3	-.007157 ± .001437[d]	.022658 ± .004522[f]
Molybdenum trioxide, b_4	-.006218 ± .001437[d]	.020268 ± .004522[f]
Molybdenum metal, b_5	.000749 ± .001437[e]	.001683 ± .004522[g]

[a] Slopes (log % Cu/ppm Mo, as-fed basis) obtained from the multiple linear regression of log (base 10) transformed TCA-soluble and TCA-insoluble Cu percentages on added dietary Mo concentrations (ppm Mo, as-fed basis) and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .12$; $b_4 - b_2 = .94$; $b_5 - b_2 = .0003$; $b_4 - b_3 = .53$; $b_5 - b_3 < .0001$; $b_5 - b_4 = .0003$.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .096$; $b_4 - b_2 = .30$; $b_5 - b_2 = .0002$; $b_4 - b_3 = .61$; $b_5 - b_3 < .0001$; $b_5 - b_4 = .0001$.

[d, e] Slopes with different superscripts differ; P-values given in footnote "b".

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

.0002). The regression coefficient of ammonium tended to be greater than that of sodium (P = .07). Conversion of TCA-insoluble Cu values into percentages of total Cu in serum (Table 5-16) resulted in the same pattern as obtained with the slopes based on log transformed values expressed in concentration (Table 5-15).

Summary of dietary molybdenum effects on molybdenum and copper

Table 5-17 contains a summary of the effects of added dietary Mo concentrations on concentrations of Mo and Cu in bile and tissues and on daily amount excreted in feces and urine from Exp. 4 and 5. The effects on Mo concentrations in tissues or on the amounts excreted closely agree between Exp. 4 and 5 and these are in harmony with most findings reported in the literature. The effects on Cu tissue concentrations or daily excretions did not show such good agreement between experiments and with data found in the literature, but the most common observations reported in most papers, i.e., increase in Cu concentrations in serum and increase in TCA-insoluble Cu, the first as a consequence of the latter, have been seen in both experiments 4 and 5. The same refers to renal Cu concentrations. Lack of agreement may be related to length of time of feeding, initial Cu and Mo status and(or) dietary levels of Cu, Mo and S.

TABLE 5-17. SUMMARY OF EFFECTS OF MOLYBDENUM DIETARY CONCENTRATIONS ON MOLYBDENUM AND COPPER TISSUE CONCENTRATIONS AND DAILY FECAL AND URINARY EXCRETION (EXPERIMENTS 4 AND 5)

Tissue or excretion	Exp. 4		Exp. 5		Expected[a]	
	Mo	Cu	Mo	Cu	Mo	Cu
Feces	↑[b]	No effect	↑	↑	↑	↑
Urine	↑	No effect	↑	No effect	↑	?[c]
Liver	↑	↓ (28 d)	↑	No effect	↑	↓
Kidney	↑	↑ (28 d)	↑	↑	↑	↑
Muscle	↑	↓	↑	No effect	↑	?
Bile	No effect	↓ (28 d)	↑	No effect	---	↑
Serum	↑	↑ (28 d)	↑	↑	↑	↑
TCA-sol[d]	---[e]	No effect	---	No effect	---	?
TCA-ins[d]	---	↑ (28 d)	---	↑	---	↑
TCA-sol[f]	---	No effect	---	↓	---	↓
TCA-ins[f]	---	No effect	---	↑	---	↑

[a] Most frequent response found in the literature.

[b] ↑ or ↓ indicate increase or decrease, respectively.

[c] Irregular effect.

[d] TCA-soluble or TCA-insoluble Cu, in ppm.

[e] Not analyzed or no information available.

[f] TCA-soluble or TCA-insoluble Cu, as a percentage of total Cu in serum.

Hepatic and Renal Ash

The average ash percentage of liver was 4.48% (standard error of the mean = .145) and of kidney 6.28% (standard error = .04). Neither liver (P = .16) nor kidney (P = .82) ash percentages were affected by Mo dietary treatments.

Iron, Manganese and Zinc in Liver and Kidney

Iron, Mn and Zn concentrations in liver (Table 5-18) and kidney (Table 5-19) of animals from the present experiment were not affected by Mo intake (P > .25), as was true in Exp. 4. However, a level * source interaction (P = .05) was seen for Zn in liver.

In both kidney and liver, Fe, Mn and Zn concentrations of animals from Exp. 5 were quite close to those found in the previous study.

Slopes of the multiple linear regression (Table 5-20) indicated that metal increased liver Zn relative to sodium and trioxide (P < .02), but it did not differ from ammonium (P > .05). However, when Duncan's (1955) procedure was run on all means (log transformed data), hepatic Zn concentrations of animals fed the control diet did not differ from metal or from any other treatment (P > .05). No initial value is available to determine if there was indeed an increase in hepatic Zn concentration in this group or if these lambs had already greater concentrations than the remaining in the experiment.

TABLE 5-18. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATIONS OF MOLYBDENUM ON HEPATIC ZINC, IRON AND MANGANESE CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum Source	Added Mo (ppm)[a]	Hepatic concentrations (ppm)[b, c] Zn	Fe	Mn
Control	0	111 ± 2[efg]	169 ± 9	10.3 ± .8
Sodium[d]	15	114 ± 5[efg]	203 ± 41	10.2 ± .6
Sodium[d]	30	119 ± 2[ef]	166 ± 9	11.4 ± .3
Sodium[d]	45	103 ± 3[g]	164 ± 25	9.9 ± .3
Ammonium[d]	30	116 ± 2[ef]	173 ± 14	10.5 ± .6
Trioxide[d]	30	111 ± 3[fg]	184 ± 18	9.9 ± .3
Metal	30	126 ± 8[e]	186 ± 12	10.0 ± .4

		ANOVA[h]		
P-values	Mo Level	.9901	.8802	.6829
	Level*Source	.0547	.7686	.6680
R^{2i}		.205	.039	.044
CV (%)[j]		1.9	4.4	5.1

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[c] Dry matter basis.

[d] Reagent grade.

[e, f, g] Means not sharing a common superscript differ ($P < .05$; Duncan's test).

[h] Multiple linear regression analyses of log (base 10) ppm Zn, Fe or Mn on added dietary Mo concentrations and level * source interaction.

[i] Coefficient of determination.

[j] Coefficient of variation.

TABLE 5-19. EFFECT OF SOURCE AND ADDED DIETARY CONCENTRATION OF MOLYBDENUM ON RENAL ZINC, IRON AND MANGANESE CONCENTRATIONS OF LAMBS (EXPERIMENT 5)

Molybdenum Source	Added Mo (ppm)[a]	Renal concentrations (ppm)[b]		
		Zn	Fe	Mn
Control	0	93 ± 3[c]	210 ± 27	4.9 ± .2
Sodium[d]	15	99 ± 9	122 ± 22	5.4 ± .2
Sodium[d]	30	98 ± 5	173 ± 32	5.1 ± .2
Sodium[d]	45	85 ± 5	172 ± 44	4.7 ± .3
Ammonium[d]	30	110 ± 9	171 ± 7	5.2 ± .4
Trioxide[d]	30	99 ± 4	152 ± 22	4.7 ± .1
Metal	30	99 ± 6	164 ± 27	4.8 ± .1
		ANOVA[e]		
P-values Mo Level		.7570	.5922	.2861
Level * source		.1385	.9012	.6591
R^{2f}		.151	.031	.073
CV (%)[g]		3.1	9.1	7.1

[a] Basal diet contained 11.5 ppm Cu, 1.1 ppm Mo and .21% S.

[b] Dry matter basis.

[c] Mean ± standard error of the mean (n = 5 for control, 15 ppm and 45 ppm Mo as sodium; n = 6 in remaining treatments).

[d] Reagent grade.

[e] Multiple linear regression analyses of log (base 10) ppm Zn, Fe or Mn on added dietary Mo concentrations level * source interaction.

[f] Coefficient of determination.

[g] Coefficient of variation.

TABLE 5-20. SLOPES OF THE MULTIPLE LINEAR REGRESSION OF LIVER ZINC CONCENTRATIONS ON ADDED DIETARY MOLYBDENUM CONCENTRATIONS (EXPERIMENT 5)

Source	Slopes ± SE[a, b]
Sodium molybdate, b_2	-.000550 ± .000517[c]
Ammonium molybdate, b_3	.000055 ± .000719[ce]
Molybdenum trioxide, b_4	-.000608 ± .000719[c]
Molybdenum metal, b_5	.001131 ± .000719[de]

[a] Slope ± standard error (log ppm Zn, dry basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log (base 10) transformed liver Zn concentration on added dietary Mo concentrations and level * source interaction.

[b] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .32$; $b_4 - b_2 = .92$; $b_5 - b_2 = .006$; $b_4 - b_3 = .38$; $b_5 - b_3 = .15$; $b_5 - b_4 = .02$.

[c, d, e] Slopes with different superscripts differ; P-values given in footnote "b".

Molybdenum Bioavailability

Sodium molybdate is the only Mo compound listed in the 1991 Official Publication of the Association of American Feed Control Officials (AAFCO) and its definition was proposed in 1987 (Association of American Feed Control Officials, 1991). Apparently sodium molybdate has not yet been accepted as a GRAS (Generally Recognized as Safe) compound, but according to A. R. Hanks (personal communication; Purdue University, East Lafayette, IN) sodium molybdate seems to be a case where the Food and Drug Administration has accepted the compound by not objecting to the AAFCO definition.

No specifically designed studies to determine bioavailability values of inorganic sources of Mo have been found in the literature. This makes the discussion of this part of the results of the experiment particularly difficult. The only work recovered in which more than one Mo compound was used is that of Fairhall et al. (1945). In this study, molybdenum trioxide (MoO_3), ammonium molybdate [$(NH_4)_6Mo_7O_2 \cdot 4H_2O$], calcium molybdate ($CaMoO_4$) and molybdenite ($MoS_2$) were administered orally to white rats, in daily amounts of 10, 100 and 500 mg Mo. Molybdenite did not induce any signs of toxicosis, with any of the amounts, when fed for 44 days. In contrast, animals receiving the other three compounds lost appetite and weight and their fur became harsh and rough. No statistical information was

provided, but hepatic and renal concentrations of Mo seemed to have been similarly increased by the ammonium and the trioxide form. For example, when the trioxide compound was fed at 100 mg Mo daily, liver and kidney concentrations were .24 and .14 mg Mo/10 g fresh tissue, while for ammonium those figures were .16 and .16 mg, respectively.

Livestock readily and rapidly absorb Mo from most diets and many inorganic forms of the element (Underwood 1977, 1981; Mills and Davis, 1987). Molybdenum in animal tissues reflects Mo intake, but many factors influence resulting tissue concentrations, particularly intakes of Cu and S in ruminants (Mills and Davis, 1987). Other factors influencing Mo metabolism, in addition to Cu and S, include dietary levels of Mn, Zn, Fe, Pb, W, ascorbic acid, methionine, cysteine and protein (NRC, 1980).

Underwood (1977) and Mills and Davis (1987), based on work of Ferguson et al. (1943), stated that Mo of high-Mo herbage is particularly well absorbed by ruminants. Findings by Cunningham et al. (1953), confirm this statement, but a study conducted by Cook et al. (1966), with rabbits, does not. Molybdenosis was produced experimentally in cattle with daily oral administrations of ammonium molybdate, but more elemental Mo than found in toxic forage was required to induce the disorder in barn-fed cattle (Cunningham et al., 1953). The difference may be due to the availability of both forms of Mo, but also to other factors,

like S concentration in diet, which has been shown to affect Mo retention (Dick, 1953b). Cook et al. (1966) compared the effect of two forms of Mo, each at two levels, in alfalfa hay, one considered "organic," resulting from application of inorganic Mo as a fertilizer to the crop, and the other inorganic, resulting from addition of a Mo compound (not specified), at levels similar to those found in Mo fertilized alfalfa, to the control forage, containing 1 ppm Mo. Concentrations of Mo in hay that resulted from this procedure were 20 and 222 ppm in "organic" form, and 18 and 206 ppm, in the inorganic form. In addition, hay containing the high organic Mo was mixed with control hay, until Mo concentration was reduced to 30 ppm. The six hays were fed to rabbits for 150 days and the following Mo concentrations resulted, for the control, 20, 30 and 222 ppm organic Mo and 18 and 206 ppm inorganic Mo, respectively: in plasma, .11, .25, .35, 2.53, .32 and 3.30 ppm; in liver, .42, .50, .59, 1.58, .48 and 1.93 ppm; and in bones, 1.08, 3.18, 5.04, 30.6, 3.56 and 33.3 ppm. Molybdenum concentration in plasma, liver and bones in rabbits fed high-inorganic and high-organic Mo concentrations were greater ($P < .01$) than in other groups. Plasma Mo in the high-inorganic group was greater ($P < .01$) than in the high-organic group, but Mo concentration in liver and bones of those two groups did not differ ($P > .05$). Differences in Mo concentrations in plasma, liver and bones among control animals, 20 and 30 ppm organic and 18 ppm inorganic were not significant ($P > .05$).

Ammonium and sodium molybdate induced severe diarrhea in cows after a few days of oral administration of high doses (Ferguson et al., 1943). These tests were run separately, on different cows and at different times.

In most studies with sheep and cattle in which tissue concentrations of Mo were measured after dietary supplementation of inorganic forms of the element, either ammonium molybdate or sodium molybdate was used. In the majority of the cases, it was not specified whether reagent grade compounds were used, although it can be assumed that they were. Very little information is available on molybdenum trioxide and none on Mo metal.

Ammonium molybdate--cattle studies

Daily oral administrations of ammonium molybdate, equivalent to 150 ppm Mo in diet, induced signs of Mo toxicosis in cattle 4 days after the first dose and it became more severe on the 14^{th} day (Cunningham, et al., 1953). Plasma Mo concentrations of cows fed for 9 weeks diets containing 6 ppm Cu and .14% S, with additional ammonium molybdate to increase dietary Mo concentrations from .6 to 19.3 or 34.8 ppm, were .24, 1.96 and 4.22 ppm ($P < .05$) respectively (Wittenberg and Devlin, 1987). The correspondent figures in milk were .10, .51 and 1.19 ppm, respectively.

Ammonium molybdate--sheep studies

Supplementation of the diet of pregnant ewes with 50 ppm Mo, plus 1% SO_4, for 46 days, and 25 ppm Mo plus .53% SO_4, for an additional 53 days, before parturition, increased ($P < .01$) Mo concentration in blood of ewes (16 ppb) and their lambs (7 ppb), at parturition, in the unsupplemented group, to 309 (ewes) and 47 ppb (lambs), respectively (Suttle and Field, 1968b). Molybdenum concentrations in liver of lambs were elevated from 1.4 to 6.8 ppm (Suttle and Field, 1968b). Plasma Mo concentrations in initially hypocupraemic ewes fed a semipurified diet containing 6 ppm Cu, no supplementary Mo or 4 ppm Mo and no supplementary S or .3% S, for 35 days, were increased by 1 to 2 ppm in Mo supplemented groups ($P < .001$) (Suttle, 1975b). The effect was totally eliminated by simultaneously adding .3% organic or inorganic S to the diet Suttle, 1975b). Molybdenum supplementation for 30 weeks of a diet containing 9.8 ppm Cu, .5 ppm Mo and .08% S, to provide either 0 or 25 ppm added Mo, increased hepatic Mo concentration of lambs from 1.0 ppm in the unsupplemented group to 7.6 ppm (fresh tissue) in the supplemented group (Bremner and Young, 1978). Additional S (.5% SO_4) depressed Mo increase in liver to 3.0 ppm (Bremner and Young, 1978). Ammonium molybdate was absorbed rapidly and efficiently in sheep after duodenal administration, but absorption was decreased and delayed when the compound was administered

into the rumen (Mason et al., 1978). Ammonium molybdate, added to the diet of lambs for 98 days, to elevate Mo concentration from .4 to 16 ppm, increased hepatic Mo concentrations from 1.7 ppm in animals fed no additional Mo and 13 ppm Cu, to 4.3 ppm when dietary Cu concentration was 13 ppm and to 6.8 ppm when dietary Cu was 29 ppm (Van der Schee et al., 1980). Addition of ammonium molybdate to the diets of wethers to provide daily intakes of about 20 mg Mo for 182 days or 40 mg Mo for 92 days, combined to three levels of Cu intake (approximately 20, 40 or 70 mg/day), with S fixed at about 2 g/day, resulted in elevated ($P < .01$) hepatic Mo concentrations (van Ryssen and Stielau, 1980a). Molybdenum concentrations in liver were 2.9 ppm in pre-experimental animals, 9.5 to 9.9 ppm in sheep fed 20 ppm Mo for 182 days and 24.4 to 28.1 ppm in wethers fed 40 ppm Mo for 92 days; there was no difference ($P < .01$) due to dietary Cu concentrations (van Ryssen and Stielau, 1980a). Plasma Mo concentrations in wethers fed for 92 days approximately 40 ppm Mo, were greater ($P < .01$) than those of sheep fed approximately 20 ppm; average values varied from 2.5 to 3.7 ppm Mo in sheep fed 20.5 to 22.2 ppm Mo and from 6.4 to 7.5 ppm in animals fed 37.7 to 39.7 ppm Mo; no value for unsupplemented animals was reported (van Ryssen and Stielau, 1980a). Supplementation of the diets of 1-year-old Cu-loaded sheep for 193 days with ammonium molybdate, to elevate dietary Mo from .6 ppm (control) to

20.8, 38.4 and 58.5 ppm, increased ($P < .01$) hepatic Mo concentrations (2.5, 5.6, 11.0 and 21.2 ppm, respectively), and kidney cortex Mo (1.7, 6.5, 54.7 and 136.5 ppm, respectively) (van Ryssen and Stielau, 1981). Increases ($P > .01$) in other tissues (kidney medulla, lung, spleen, skeletal muscle, heart muscle, wool and plasma) were less remarkable. Addition of ammonium molybdate, to raise Mo concentration of a diet containing .22% S and 6.1 ppm Cu, from .4 ppm to 10 to 12 ppm Mo, for 7 months, increased ($P < .01$) Mo concentrations in bones of wethers from 1.7 ppm in the group fed the basal diet to 48 ppm in supplemented animals (Hidiroglou et al., 1982). Hypocupraemic ewes fed for 21 days a diet containing 7.3 ppm Cu, .35% S and supplemented with 0, 2, 4 or 8 ppm Mo, had their plasma Mo concentrations increased with Mo concentrations in the diet in a predominantly linear relationship ($P < .01$) (Suttle, 1983b). Hepatic Mo concentrations of Cu-poisoned sheep fed daily .1 g ammonium molybdate (approximately 50 ppm Mo, if 1 kg feed intake is assumed) plus 1 g sodium sulfate (raised to 2 g on 15th day) increased ($P < .01$) from 2.2 ppm to 3.9 ppm about 2 months later (Hidiroglou et al., 1984). Ammonium molybdate, added to a diet of wethers to increase Mo concentration from .4 to 8.4 ppm, increased ($P < .01$) hepatic Mo concentrations from 1.3 to 2.6 ppm; when additional Cu was provided, to elevate dietary concentration from 5 to 11 ppm, the rise ($P < .01$) in hepatic Mo

concentration was from 1.3 to 3.5 ppm for low and high-Mo groups, respectively (Ivan and Veira, 1985). Incorporation of ammonium molybdate to the diets of 10-month-old lambs, to raise daily Mo intake, with additional S, increased ($P < .01$) plasma Mo concentration after 2 weeks, from .002 ppm in the lambs with a daily intake of .4 mg Mo, 2 g S and 5.7 mg Cu, to 1.54 ppm in the group consuming 42 mg Mo, 3.9 g S and 5 ppm Cu, and to 2.87 ppm when intake was 99 mg Mo, 3.6 g S and 4.3 mg Cu (van Ryssen et al., 1986). Addition of 0, 2, 4 and 8 ppm Mo to the diets of Ile de France (IF) and South African Mutton Merino (MM) lambs resulted in hepatic Mo concentrations of 3.4, 3.7, 4.7 and 9.7 ppm in IF lambs and 3.2, 3.7, 4.3 and 10.2 ppm in MM lambs, respectively; only the greatest dietary Mo concentration caused a significant ($P < .01$) increase in Mo concentration in liver (Harrison et al., 1987). Ammonium molybdate, included in diets (5 ppm Cu, .14% S) of ewes, for 6 weeks, at amounts to raise Mo dietary concentration to 18.4 and 40.7 ppm, promoted an increase ($P < .05$) in plasma Mo concentrations from .73 ppm (control group) to 8.16 and 11.54 ppm, respectively (Wittenberg and Devlin, 1988). Ammonium molybdate, added to the diet (containing 13 ppm Cu and .45% S) of 5-month-old rams, at the concentration of 38 ppm Mo, increased ($P < .01$) Mo concentration in liver from 4.4 ppm in the group fed the basal diet to 18.3 ppm in the supplemented group; increases ($P < .01$) in testis varied from .9 to 3.7 ppm, and in kidney

cortex from 2.5 to 51.0 ppm, respectively (van Ryssen et al., 1990).

Sodium molybdate--cattle studies

Sodium molybdate added to the diet (0, 5, 10, 20 and 50 ppm Mo) of heifers led to an increase in Mo concentration of milk (.02, .09, .09, .32 and .68 ppm Mo, respectively), serum (.03, .23, .39, 1.07 and 2.07 ppm Mo, respectively) and liver (5, 10, 13, 21, 30 ppm Mo, respectively), at 150 days of feeding (Vanderveen and Keener, 1964). Molybdenum, administered orally to steers, at concentrations of 0, 1.5 or 3.0 mg/kg body weight (approximately equivalent to 60 and 120 ppm Mo, assuming 2.5% of dry matter intake of average body weight) promoted an increase ($P < .01$) in plasma Mo concentration, from .05 to 1.00 and 2.80 ppm, respectively, at 25 days of feeding (Cook et al., 1966). Oral administration of sodium molybdate to cows, in order to increase daily Mo intake from 5 to 50 mg or to 100 mg, for 10 days, and to goats, in order to elevate daily Mo intake from 1.1 mg to 13.0 mg, for 7 days, was followed by an immediate rise in concentration of Mo in milk, from 26 ppb (pretreatment value) to 44 and 61 ppb in supplemented cows, respectively, and from 12 ppb (pretreatment value) to 70 and 72 ppb in two groups of supplemented goats (Hart et al., 1967). Sodium molybdate, when fed to cows at the dietary Mo concentrations of 53 ppm for 4 months or 53 ppm for 2 months and then 173 ppm for 2 more months, increased Mo

concentrations in blood from "traces" (pretreatment level) to 2.66 and 7.47 ppm, respectively, and in milk from .03 ppm (pretreatment) to 1.03 and 1.83 ppm, respectively (Huber et al., 1971). Sodium molybdate, fed to lactating dairy cows at a concentration of 53 ppm Mo for 6 months or of 53 ppm Mo for 2 months and then 173 ppm Mo for 4 more months, increased Mo concentrations, relative to unsupplemented cows in blood (traces, 3.3 and 7.3 ppm, respectively), liver (3.6, 10.4 and 32.4 ppm, respectively), kidney (1.7, 42.3, 62.6 ppm, respectively), brain (traces, 1.7 and 5.1 ppm, respectively) and muscle (.5, 6.4 and 14 ppm, respectively) (Huber et al., 1971).

Sodium molybdate--sheep studies

Hepatic Mo concentrations of growing lambs fed 2 or 8 ppm Mo, in combination with 10 or 40 ppm Cu and .10% or .40% S, increased ($P < .01$) from 3.7 to 6.0 ppm; the largest Mo concentration in liver was observed at 8 ppm Mo in combination with 10 ppm Cu and .40% S (Goodrich and Tillman, 1966a). Wethers fed sodium molybdate to provide 5, 10, 15, 25 or 50 mg Mo/day, showed a linear increase ($R^2 = .95$) in blood Mo concentrations, from approximately .1 ppm in unsupplemented animals, to approximately 2.2 ppm in the group fed 50 mg Mo/day; when 1.1 g additional S was administered, Mo increase was less marked, especially at the greater Mo intakes (Weber et al., 1983).

Molybdenum trioxide

Molybdenum trioxide is well absorbed by guinea pigs, rabbits and rats (Fairhall et al., 1945). Cattle grazing pasture contaminated with molybdenum trioxide from industrial emissions and containing 18 to 34 ppm Mo had blood Mo concentrations varying from .12 to .47 ppm (Gardner and Hall-Patch, 1962).

From the studies reviewed, it can be concluded that both ammonium and sodium molybdate are well absorbed in both sheep and cattle.

Relative Bioavailability Values of Molybdenum Sources

Molybdenum in feces

Slopes of the multiple linear regression of fecal Mo excretion (Table 5-21) did not differ at the 5% probability value. However, the P-value for the difference between slopes of metal and sodium was .06, indicating a larger Mo excretion when metal was fed, compared to sodium. Neither ammonium ($P = .69$) nor trioxide ($P = .62$) differed from metal, while there was a tendency for a larger Mo excretion of Mo from ammonium ($P = .16$) and trioxide ($P = .20$), relative to sodium.

Relative bioavailability values (Table 5-21) of Mo sources obtained from the quotient of reciprocals of slopes of fecal Mo excretion indicated that only the metal was smaller ($P < .05$) than the sodium form, but not than the ammonium and the trioxide.

TABLE 5-21. SLOPES OF MULTIPLE LINEAR REGRESSION FOR DAILY FECAL MOLYBDENUM EXCRETION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.039915 ± .003222[f]	100	---
Ammonium, b_3	.045239 ± .004479[fh]	88.2 ± 7.7	73.2 - 103.3
Trioxide, b_4	.044796 ± .004479[fh]	89.1 ± 7.8	73.8 - 104.4
Metal, b_5	.047112 ± .004479[gh]	84.7 ± 7.2	70.7 - 98.8

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log μg Mo.day^{-1}/ppm Mo, as-fed basis); slope obtained from the multiple linear regression of log of daily fecal Mo excretion (μg/day) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = 3.038874.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .16$; $b_4 - b_2 = .20$; $b_5 - b_2 = .06$; $b_4 - b_3 = .92$; $b_5 - b_3 = .69$; $b_5 - b_4 = .62$.

[d] Relative value and standard error; RV equals quotient of reciprocal of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g, h] Slopes with different superscripts differ; P-values given in footnote "c".

Molybdenum in urine

Slopes of the regression of urinary Mo excretion on dietary concentration of Mo (Table 5-22) did not differ ($P > .50$) for sodium, ammonium and trioxide. These were all higher than the slope of metal ($P < .0001$). Urinary Mo excretion was increased when sodium, ammonium and trioxide were included in diets, while there was little change when metal was the Mo source, probably indicating its low absorption.

Relative values of sodium, ammonium and trioxide (Table 5-22) did not differ based on their respective confidence limits, but all three were greater than that of metal.

Molybdenum in liver

Slopes of the multiple linear regression for liver Mo concentrations are shown in (Table 5-23). Trioxide had the steepest slope and metal the smallest, but there was no difference between slopes for trioxide and ammonium ($P = .52$). There was a tendency for a difference between sodium and metal ($P = .12$).

The relative values of Mo sources (Table 5-23), compared to the reference source, sodium, were 141%, 157% and 68%, for ammonium, trioxide and metal, respectively. As indicated by the 95% confidence limits, trioxide had a greater relative value than sodium, while ammonium did not, neither did the value of ammonium differ from metal.

TABLE 5-22. SLOPES OF MULTIPLE LINEAR REGRESSION FOR DAILY URINARY MOLYBDENUM EXCRETION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.050445 ± .004585[f]	100	---
Ammonium, b_3	.051199 ± .006374[f]	101.5 ± 10.8	80.3 - 122.7
Trioxide, b_4	.054147 ± .006374[f]	107.3 ± 10.9	85.9 - 128.8
Metal, b_5	-.017221 ± .006374[g]	-34.1 ± 14.6	-62.7 - -5.6

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log μg Mo.day^{-1}/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of daily urinary Mo excretion (μg/day) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = 2.236713.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .89$; $b_4 - b_2 = .50$; $b_5 - b_2 < .0001$; $b_4 - b_3 = .66$; $b_5 - b_3 < .0001$; $b_5 - b_4 < .0001$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

TABLE 5-23. SLOPES OF MULTIPLE LINEAR REGRESSION FOR HEPATIC MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.008410 ± .001473[f]	100	---
Ammonium, b_3	.011863 ± .002047[g]	141.1 ± 23.3	95.4 - 186.8
Trioxide, b_4	.013227 ± .002047[g]	157.3 ± 24.8	108.6 - 205.9
Metal, b_5	.005680 ± .002047[f]	67.5 ± 20.4	27.5 - 107.6

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppm Mo, dry basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of hepatic Mo concentration (ppm) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = .565383.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .048$; $b_4 - b_2 = .006$; $b_5 - b_2 = .12$; $b_4 - b_3 = .52$; $b_5 - b_3 = .004$; $b_5 - b_4 = .0004$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

When slopes were obtained from regression analyses in which the values from lambs fed 45 ppm added Mo were excluded (Table 5-24), P-values of differences between slopes for ammonium and sodium (P = .55), trioxide and sodium (P = .20), and metal and sodium (P = .014) changed dramatically compared to those shown in Table 5-23. Similarly, the relative values changed considerably and, based on the 95% confidence intervals, only metal had a smaller relative value than that of the sodium form.

Molybdenum in muscle

Slopes of the multiple regression equation for Mo in muscle (Table 5-25) were not different for sodium and ammonium (P = .22) and sodium and trioxide (P = .56). These were higher than the slope of metal (P < .0001), and trioxide was higher than sodium (P = .05). Trioxide, ammonium and sodium increased concentrations of Mo in muscle, while this was much less evident for metal.

Bioavailability values of ammonium, trioxide and metal, relative to the standard source, sodium, (Table 5-25) were 113%, 121% and 61%, although only the last, metal, was smaller than the standard source (P < .05).

Molybdenum in kidney

Slopes of the regression equation for renal Mo concentrations are presented in Table 5-26. There was no difference among slopes for sodium, ammonium and trioxide (P > .05) but these were higher than the slope of metal (P <

TABLE 5-24. SLOPES OF MULTIPLE LINEAR REGRESSION FOR HEPATIC MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES, WHEN VALUES FROM LAMBS FED THE GREATEST ADDED CONCENTRATION WERE EXCLUDED FROM ANALYSIS (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.011887 ± .002187[f]	100	---
Ammonium, b_3	.013096 ± .002085[f]	110.2 ± 18.0	75.0 - 145.4
Trioxide, b_4	.014461 ± .002085[f]	121.7 ± 19.3	83.9 - 159.4
Metal, b_5	.006914 ± .002085[g]	58.2 ± 14.6	29.6 - 86.8

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppm Mo, dry basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of hepatic Mo concentration (ppm) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = .528372.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .55$; $b_4 - b_2 = .20$; $b_5 - b_2 = .014$; $b_4 - b_3 = .51$; $b_5 - b_3 = .003$; $b_5 - b_4 = .0003$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

TABLE 5-25. SLOPES OF MULTIPLE LINEAR REGRESSION FOR MUSCLE MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.017658 ± .001570[f]	100	---
Ammonium, b_3	.019954 ± .002182[fi]	113.0 ± 10.8	91.7 - 134.2
Trioxide, b_4	.021295 ± .002182[gi]	120.6 ± 11.1	98.9 - 142.3
Metal, b_5	.010738 ± .002182[h]	60.8 ± 10.4	40.4 - 81.2

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppb Mo, dry basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of muscle Mo concentration (ppb) on added dietary Mo concentrations (ppm Mo, as-fed basis) and level * source interaction; intercept = 2.081864.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .22$; $b_4 - b_2 = .05$; $b_5 - b_2 = .0002$; $b_4 - b_3 = .56$; $b_5 - b_3 < .0001$; $b_5 - b_4 < .0001$

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g, h, i] Slopes with different superscripts differ; P-values given in footnote "c".

TABLE 5-26. SLOPES OF MULTIPLE LINEAR REGRESSION FOR KIDNEY MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.016980 ± .002141[f]	100	---
Ammonium, b_3	.019900 ± .002976[f]	117.2 ± 15.5	86.7 - 147.7
Trioxide, b_4	.021278 ± .002976[f]	125.3 ± 15.9	94.1 - 156.5
Metal, b_5	.001718 ± .002976[g]	10.1 ± 16.9	-22.9 - 43.2

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppm Mo, dry basis/ppm Mo, as-fed basis; slopes obtained from the multiple linear regression of log of kidney Mo concentration (ppm) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = .168286.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .25$; $b_4 - b_2 = .09$; $b_5 - b_2 < .0001$; $b_4 - b_3 = .66$; $b_5 - b_3 < .0001$; $b_5 - b_4 < .0001$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

.0001). Trioxide tended to have a higher slope than sodium (P = .091). According to Mo concentrations in kidney, relative values of ammonium, trioxide and metal (Table 5-26), compared to the reference source, sodium, were 117%, 125% and 10%, respectively. Based on the confidence limits of relative values, metal had a smaller value than the remaining sources.

Molybdenum in bile

There were no differences among slopes for sodium, ammonium and trioxide (Table 5-27, P > .20), which were greater than the slope of metal (P < .001).

Relative values, based on biliary Mo concentration, of ammonium and trioxide (Table 5-27) were 131% and 120%, but these were not different from the standard source, sodium (P > .05). The relative value of metal was 18%, and it was smaller (P < .05) than that for ammonium and sodium. The upper level of the 95% confidence limit of metal is so close to the lower confidence limit of trioxide, that for practical reasons the relative value of trioxide can also be considered greater than metal.

Molybdenum in serum

Slopes for Mo concentrations in serum (Table 5-28) were steeper when ammonium and trioxide were the Mo sources. Slopes for ammonium and trioxide did not differ (P = .88) and these were greater than the slope for sodium (P < .02). All these three sources had slopes greater than metal (P <

TABLE 5-27. SLOPES OF MULTIPLE LINEAR REGRESSION FOR BILIARY MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.020856 ± .004337[f]	100	---
Ammonium, b_3	.027373 ± .006028[f]	131.2 ± 26.8	78.8 - 183.7
Trioxide, b_4	.025137 ± .006028[f]	120.5 ± 25.9	69.8 - 171.2
Metal, b_5	.003775 ± .006028[g]	18.1 ± 27.0	-34.9 - 71.1

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppb Mo, fresh basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of biliary Mo concentration (ppb) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = .858489.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .21$; $b_4 - b_2 = .41$; $b_5 - b_2 = .0009$; $b_4 - b_3 = .72$; $b_5 - b_3 = .0002$; $b_5 - b_4 = .0007$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g] Slopes with different superscripts differ; P-values given in footnote "c".

TABLE 5-28. SLOPES OF MULTIPLE LINEAR REGRESSION FOR SERUM MOLYBDENUM CONCENTRATION AND ESTIMATED RELATIVE BIOAVAILABILITY VALUES OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Mo source[a]	Slope ± SE[b, c]	RV ± SE[d]	Conf. int.[e]
Sodium, b_2	.046199 ± .003735[f]	100	---
Ammonium, b_3	.056780 ± .005192[g]	122.9 ± 10.1	103.0 - 142.8
Trioxide, b_4	.055937 ± .005192[g]	121.1 ± 10.1	101.3 - 140.8
Metal, b_5	-.007114 ± .005192[h]	-15.4 ± 12.0	-38.8 - 8.0

[a] Sodium molybdate (RG), ammonium molybdate (RG), molybdenum trioxide (RG) and Mo metal; RG = reagent grade.

[b] Slope ± standard error (log ppb Mo, fresh basis/ppm Mo, as-fed basis); slopes obtained from the multiple linear regression of log of serum Mo concentration (ppb) on added dietary Mo concentrations (ppm, as-fed basis) and level * source interaction; intercept = 1.213434.

[c] P-values of differences between slopes (Z-test, 2-sided): $b_3 - b_2 = .017$ $b_4 - b_2 = .014$; $b_5 - b_2 < .0001$; $b_4 - b_3 = .88$; $b_5 - b_3 < .0001$; $b_5 - b_4 < .0001$.

[d] Relative value and standard error; RV equals ratio of slopes, with sodium as reference source; in percentage.

[e] 95% confidence interval of relative values.

[f, g, h] Slopes with different superscripts differ; P-values given in footnote "c".

.0001). The slope for metal can be considered zero and, indeed, the Mo level in serum of lambs fed Mo metal was practically identical to that of the group fed the basal diet. The relative value of ammonium, 123%, and trioxide, 121%, were of the same magnitude and greater than the reference source, sodium. Metal, as measured by Mo concentration in serum, was essentially unavailable.

Overall discussion

Table 5-29 shows the arrangement of slopes of Mo daily excretion or tissue concentration in increasing order (fecal excretion) or decreasing order (all others), based on log transformed values of slopes independently of statistical difference. Only in bile, feces and serum the trioxide was not first in ranking, but in these three, trioxide was similar to ammonium. Molybdenum trioxide was considered an insoluble compound (Underwood, 1977; Mills and Davis, 1987), but it was stated that it is well absorbed by rabbits and guinea pigs when fed in high doses, while ammonium and sodium were said to be well absorbed in ruminants (Underwood, 1977; Mills and Davis, 1987).

As already referred to previously, almost no work was done with Mo and there are no results available to compare with those obtained in Exp. 5. The trioxide was more efficient than the sodium source in increasing Mo concentrations in muscle and serum, and it was always comparable to ammonium, in all measurements made. Metal was

always less effective in all tests performed, except in feces, in which it did not differ from ammonium and trioxide. Feces may not be a good indicator, because Mo excretion in bile increased with additional Mo.

If fecal excretion of Mo were representative of Mo absorption, then the sodium source should have resulted in the largest Mo urinary excretion or tissue uptake, but this did not occur. Fecal excretion was the main route of oral and intravenous doses of Mo in cattle (Bell et al., 1964) and of oral doses in cattle (Miller et al., 1972) and sheep (Weber et al., 1983).

Suttle (1978) reported that tetrathiomolybdate did not greatly accelerate removal of Cu already accumulated in liver and that its primary effect would be located in the gastrointestinal tract, involving a reduction in absorption of Cu. In such a case, molybdenum trioxide seems to be the Mo source of choice.

Effects of Mo sources on Cu excretion and tissue concentration were not as evident and consistent as they were on Mo excretion and tissue concentrations. When slopes were ranked according to their log transformed values, independently of statistical difference (Table 5-30), trioxide was first for fecal Cu excretion and kidney Cu, but ammonium was first in serum Cu fractions.

TABLE 5-29. ARRANGEMENT OF MOLYBDENUM SOURCES IN DECREASING (FECAL EXCRETION) OR INCREASING ORDER (ALL OTHERS), BASED ON VALUES OF SLOPES OF MOLYBDENUM DAILY EXCRETION OR TISSUE CONCENTRATIONS (EXPERIMENT 5)

Excretion/tissue	Order of slopes[a, b]			
Fecal excretion	Sodium[c]	Trioxide[ce]	Ammonium[ce]	Metal[de]
Urinary excretion	Trioxide[c]	Ammonium[c]	Sodium[c]	Metal[d]
Liver	Trioxide[d]	Ammonium[d]	Sodium[d]	Metal[c]
Muscle	Trioxide[df]	Ammonium[cf]	Sodium[c]	Metal[e]
Kidney	Trioxide[c]	Ammonium[c]	Sodium[c]	Metal[d]
Bile	Ammonium[c]	Trioxide[c]	Sodium[c]	Metal[d]
Serum	Ammonium[d]	Trioxide[d]	Sodium[c]	Metal[e]

[a] Order is based on value of log transformed slope, independent of statistical difference.

[b] Slopes of sources not followed by the same superscript within rows differ ($P \leq .06$); letters assigned to liver are based on regression analysis in which values of lambs fed 45 ppm added Mo were excluded.

TABLE 5-30. ARRANGEMENT OF MOLYBDENUM SOURCES IN DECREASING ORDER, BASED ON VALUES OF SLOPES OF COPPER DAILY EXCRETION OR TISSUE CONCENTRATIONS (EXPERIMENT 5)

Excretion/tissue	Order of slopes[a, b]			
Fecal excretion	Trioxide[de]	Ammonium[ce]	Metal[ce]	Sodium[c]
Urinary excretion	..... No effect of molybdenum source			
Liver	..... No effect of molybdenum source			
Muscle	..... No effect of molybdenum source			
Kidney	Trioxide[c]	Sodium[c]	Ammonium[c]	Metal[d]
Bile	..... No effect of molybdenum source			
Serum	Ammonium[c]	Trioxide[cd]	Sodium[cd]	Metal[d]
TCA-soluble Cu	Ammonium[c]	Trioxide[c]	Sodium[c]	Metal[d]
TCA-insoluble Cu	Ammonium[c]	Trioxide[c]	Sodium[c]	Metal[d]

[a] Order is based on value of log transformed slope, independent of statistical difference.

[b] Slopes of sources not followed by the same superscript within rows differ ($P \leq .07$).

A summary of relative values of the four Mo sources is shown in Table 5-31. The numerical relative values of trioxide and ammonium were quite similar in all tissues or excretions, but especially in liver (when 45 ppm Mo was excluded), muscle, kidney, bile and serum. The numerical relative value of trioxide was always greater than that of sodium, except for feces. Metal had the greatest variation, from -34%, in urine, to 85%, in feces.

Summary and Conclusions

Thirty-nine lambs were used to estimate the relative bioavailability values of inorganic Mo sources and their effects on Cu fecal and urinary excretion and tissue concentration. After a 6-day adaptation period to cages and to the ground corn-soybean meal-cottonseed hulls basal diet, containing 11.5 ppm Cu, 1.1 ppm Mo and .21% S, animals were assigned randomly to dietary treatments. Treatments were the basal diet (control), supplemented with 15, 30 or 45 ppm Mo as sodium molybdate (reagent grade, RG) or 30 ppm Mo as ammonium molybdate (RG), molybdenum trioxide (RG), or metal. Lambs were individually fed for 28 days. Total feces and urine were individually collected daily during the last 5 days of the experiment, sampled on a 10% basis and composited by lamb. Blood samples were taken at the end of the experiment. Part of blood serum was mixed with trichloroacetic acid (TCA) and the supernatant saved. On

TABLE 5-31. SUMMARY OF RELATIVE BIOAVAILABILITY VALUES AND CORRESPONDENT 95% CONFIDENCE INTERVALS OF MOLYBDENUM SOURCES (EXPERIMENT 5)

Item	Trioxide[a]	Ammonium[a]	Sodium[a]	Metal[a]
Feces	89.1[b]	88.2	100	84.7
	(73.8 - 104.4)[c]	(73.2 - 103.3)		(70.7 - 98.8)
Urine	107.3	101.5	100	-34.1
	(85.9 - 128.8)	(80.3 - 122.7)		(-62.7 - -5.6)
Liver[d]	121.7	110.2	100	58.2
	(83.9 - 159.4)	(75.0 - 145.4)		(29.6 - 86.8)
Muscle	120.6	113.0	100	60.8
	(98.9 - 142.3)	(91.7 - 134.2)		(40.4 - 81.2)
Kidney	125.3	117.2	100	10.1
	(94.1 - 156.5)	(86.7 - 147.7)		(-22.9 - 43.2)
Bile	120.5	131.2	100	18.1
	(69.8 - 171.2)	(78.8 - 183.7)		(-34.9 - 71.1)
Serum	121.1	122.9	100	-15.4
	(101.3 - 140.8)	(103.0 - 142.8)		(-38.8 - 8.0)

[a] Molybdenum trioxide, ammonium molybdate, sodium molybdate and Mo metal.

[b] Bioavailability value based on ratio of slopes relative to sodium molybdate.

[c] 95% confidence interval.

[d] Based on regression analysis with values of lambs fed 45 ppm Mo excluded.

day 28, lambs were euthanized, for collection of bile, liver, muscle and kidney. Concentrations of Mo and Cu were determined in Mo sources, feed, feces, urine, bile, liver, kidney, muscle and serum; of Cu in TCA-supernatant; and of Fe, Mn and Zn in liver and kidney. Urinary and fecal Mo and Cu were expressed as daily amount excreted. Logarithmic transformed data were analyzed by multiple linear regression of mineral concentrations in tissues and fluids or daily mineral excretion in urine and feces on added dietary Mo concentration. Relative bioavailability values of Mo sources were calculated using the slope ratio method, with sodium molybdate as the reference source. For feces, the ratio of reciprocals of the slopes was used.

Added dietary Mo concentrations increased fecal and urinary Mo excretion, but Mo source had little effect on fecal Mo excretion. Increases in Mo intake led to elevation in Mo concentrations in liver, muscle, kidney, bile and serum and the effect depended on Mo source. Additional Mo in the diet increased fecal but not urinary Cu excretion. Trioxide tended to promote the greatest fecal Cu excretion. Supplemental Mo did not change Cu concentration in liver, muscle and bile but it did elevate Cu concentrations in kidney and serum. TCA-soluble Cu concentration in serum was not affected by additional Mo intake, but the insoluble fraction was increased. When TCA-soluble Cu was expressed as a percentage of total Cu in serum, a decrease was noticed

in this fraction. Trioxide, ammonium and sodium promoted or tended to promote a larger increase in renal and serum total Cu and serum TCA-insoluble Cu concentration than metal.

Relative bioavailability values (RBV) based on ratio of slopes of the multiple linear regression varied for the various excretions or tissues analyzed. Ammonium had RBV from 88% (feces) to 131% (bile); metal, from -15 (serum) to 85% (feces); trioxide, from 89% (feces) to 125% (kidney). Based on the previous study and on information from the literature, serum seems to provide the most reliable RBV and these were -15.4% (metal), 100% (sodium), 121.1% (ammonium) and 122.9% (trioxide). Slopes for trioxide were always similar to those of ammonium and slopes of equations of the regression of Mo concentrations in muscle and serum on added dietary Mo concentration were greater for trioxide than for sodium. The slope of metal from Mo urinary excretion and Mo concentrations in muscle, kidney, bile and serum was smaller than that of the other three sources.

Ammonium molybdate and molybdenum trioxide had similar relative bioavailability values and these were greater than the relative value of sodium molybdate, when judging from the slope ratio of Mo concentration in serum. The RBV of metal was always smaller than the value of sodium.

Molybdenum metal had a low biological availability, compared to sodium molybdate. Molybdenum trioxide is a highly available Mo source. Molybdenum trioxide tended to

be the most effective source in increasing fecal Cu excretion.

Ammonium and sodium molybdate and molybdenum trioxide were equally potent in affecting renal and serum Cu concentrations, and serum TCA-soluble and insoluble Cu concentrations.

CHAPTER 6
GENERAL SUMMARY AND CONCLUSIONS

Five experiments were conducted, including three to estimate relative biological availability of Cu from inorganic sources and a copper-lysine complex, one to determine the effect of elevated dietary concentrations of Mo and length of time of feeding on the excretion and tissue concentrations of Mo and Cu, and one to estimate relative bioavailability values of Mo inorganic sources and the effects of added dietary Mo on fecal and urinary excretion and tissue concentrations of Cu.

Thirty-five (Exp. 1), 42 (Exp. 2 and 3), 40 (Exp. 4) and 39 (Exp. 5) crossbred wether lambs, weighing from 40 to 45 kg on the average, were housed in individual cages. After a variable adaptation period (6 to 17 days) to a ground corn-soybean meal-cottonseed hulls basal diet and to cages and for recovery from a surgical biopsy procedure (Exp. 1, 2 and 3), lambs were assigned randomly to dietary treatments, fed for 10 days (Exp. 1, 2 and 3), 14 or 28 days (Exp. 4) or 28 days (Exp. 5). The basal diet contained 8.3 to 12.6 ppm Cu, .9 to 1.6 ppm Mo and .14% to .22% S.

Treatments were the basal diet, supplemented with 60, 120 or 180 ppm Cu as reagent grade (RG) cupric chloride, or

120 ppm Cu from feed grade (FG) cupric carbonate, cupric oxide or cupric sulfate (Exp. 1); 60, 120 or 180 ppm Cu as RG cupric chloride, or 120 ppm Cu from RG cupric acetate, FG cupric oxide and cupric sulfate (Exp. 2); 60, 120 or 180 ppm Cu as FG copper-lysine complex or RG cupric sulfate (Exp. 3); 0, 15, 30 or 45 ppm added dietary Mo from RG sodium molybdate (Exp. 4); and 15, 30 or 45 ppm Mo as sodium molybdate (RG) or 30 ppm Mo as ammonium molybdate (RG), molybdenum trioxide (RG), or Mo metal (Exp. 5).

Total feces and urine were individually collected (Exp. 4 and 5) during the last 5 days of each period (Exp. 4) or of the 28-day period of Exp. 5, sampled on a 10% basis and individually composited. On day 0, 13 and 27 (Exp. 4) or 27 (Exp. 5), blood samples were taken by jugular vein puncture. Part of the serum was mixed with trichloroacetic acid (TCA) solution, centrifuged and supernatant saved for Cu analysis.

On day 11 (Exp. 1, 2 and 3), 14 and 28 (Exp.4) and 28 (Exp. 5) of the feeding period, lambs were euthanized for collection of liver (Exp. 1, 2 and 3) or bile, liver, kidney and muscle (Exp. 4 and 5).

Concentrations of Cu, Fe, Mn and Zn were determined in biopsy samples (initial value) and in samples of whole liver (final value); of Cu, Fe, Mn, Zn, Mo, Ca, Mg and P in feed and Cu sources; and S in feed (Exp. 1, 2 and 3). Concentrations of Mo and Cu were analyzed in feces, urine, bile, liver, kidney, muscle and serum; of Cu in TCA-

supernatant; of Fe, Mn and Zn in liver and kidney; of Mo, Cu, Fe, Mn, Zn, Ca, Mg, P and S in feed (Exp. 4 and 5); and of Mo, Cu, Fe, Mn, Zn, Ca, Mg and P in Mo sources (Exp. 5). Urinary and fecal Mo and Cu were expressed as daily amount excreted.

Logarithmic (base 10) transformed data were used in all statistical analyses. In Exp. 1, 2 and 3, hepatic mineral concentrations were regressed on added dietary Cu levels and Cu level * Cu source interaction, with initial value as covariate. In Exp. 4, data were analyzed by multiple linear regression of mineral concentration in tissues or fluids or daily mineral excretion in feces or urine on added dietary Mo concentrations, length of time of feeding and the interaction term. In Exp. 5, data were analyzed by multiple linear regression of mineral concentrations in tissues and fluids or daily mineral excretion in urine and feces on added dietary Mo concentration and Mo level * Mo source interaction. Relative bioavailability values (RBV), expressed in percentage, of Cu and Mo sources were calculated using the slope ratio method.

In Exp. 1, bioavailability values of the carbonate, oxide and sulfate were 123%, 47% and 157%, relative to cupric chloride (100%). In Exp. 2, RBV of the chloride, acetate, oxide and sulfate were 100%, 105%, 64% and 115%, respectively. In Exp. 3, Cu from Cu-lysine was 68% as

available as from cupric sulfate. Iron, Mn and Zn levels in liver were not affected by added dietary Cu.

Statistical evaluation revealed that the relative bioavailability values of cupric acetate, cupric carbonate, cupric chloride and cupric sulfate were of similar magnitudes. Copper from oxide was less available for lambs than that from acetate, carbonate, chloride and sulfate. The biological availability value of the Cu-lysine was smaller than that of cupric sulfate.

Substitution for cupric oxide in mineral mixtures or concentrates with a more bioavailable Cu source such as cupric sulfate, should be considered by the animal feed industry. Based on hepatic Cu accumulation in lambs, there seems to be little reason for using a Cu-lysine complex in this species, in substitution to cupric sulfate.

In Exp. 4, increases in dietary Mo concentrations resulted in cubic increases of daily Mo excretion in feces and urine and in linear increases of Mo concentration in muscle, kidney and serum. Dietary Mo supplementation decreased Cu concentrations in liver, muscle and bile; increased Cu concentrations in kidney; and increased total and TCA-insoluble Cu in serum. Added dietary Mo did not affect biliary Mo concentrations, daily fecal and urinary Cu excretion, TCA-soluble Cu concentration in serum and Fe, Mn and Zn concentrations in liver and kidney. Length of time of feeding had no effect on Mo excretion or tissue

concentrations. Hepatic Cu was decreased by added dietary Mo at 28 days of feeding and total and TCA-insoluble Cu in serum was increased at 28 days of feeding. It was concluded that Mo concentrations in serum would be the most appropriate determination for a comparison of Mo inorganic sources and that 28 days would be the adequate time to estimate the effect of dietary Mo on Cu tissue concentrations.

Added dietary Mo concentrations up to 45 ppm on an adequate S diet were able to decrease liver Cu concentrations of lambs and the addition of Mo, plus sulfur, if necessary, to practical diets, may therefore be a useful procedure to control or prevent Cu toxicosis in sheep on high dietary Cu concentrations.

In Exp. 5, added dietary Mo concentrations increased fecal and urinary Mo excretion, but Mo source had little effect on fecal Mo excretion. Increases in Mo intake led to increases in Mo concentrations in liver, muscle, kidney, bile and serum and the effect depended on Mo source. Additional Mo in the diet increased fecal but not urinary Cu excretion. Trioxide tended to induce the greatest fecal Cu excretion. Supplemental Mo did not change Cu concentration in liver, muscle and bile, but it did elevate Cu concentrations in kidney and serum. TCA-soluble Cu concentration in serum was not affected by additional Mo intake, but the insoluble fraction was increased. When TCA-

soluble Cu was expressed as a percentage of total Cu in serum, a decrease was noticed in this fraction. Trioxide, ammonium and sodium promoted or tended to promote a larger increase in renal and serum total Cu and serum TCA-insoluble Cu concentration than metal.

Relative bioavailability values varied for the various excretions or tissues analyzed. Ammonium had a RBV from 88% (feces) to 131% (bile); metal, from -15% (serum) to 85% (feces); trioxide, from 89% (feces) to 125% (kidney). Based on the previous study and on information from the literature, serum seemed to provide the most reliable RBV and these were -15.4% (metal), 100% (sodium), 121.1% (ammonium) and 122.9% (trioxide). Slopes of trioxide were always similar to those of ammonium and slopes of equations of regression of Mo concentrations in muscle and serum on added dietary Mo concentration were greater for trioxide than for sodium. The slope of metal was smaller than that of the other three sources in urine, muscle, kidney, bile and serum.

Ammonium molybdate and molybdenum trioxide had similar relative bioavailability values and these were greater than the relative value of sodium molybdate, based on the slope ratio of Mo concentration in serum. The RBV of metal was always smaller than the value of sodium. Molybdenum metal is a source of low biological availability, compared to

sodium molybdate. Molybdenum trioxide is a highly available Mo source.

Ammonium and sodium molybdate and molybdenum trioxide were equally effective in increasing renal and serum Cu concentrations and serum TCA-insoluble Cu concentrations.

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BIOGRAPHICAL SKETCH

Edison Beno Pott was born in Panambi, Rio Grande do Sul (RS), Brazil, on May 31, 1950. He completed his high school education in 1969. In 1971 he entered the College of Veterinary Medicine at the Universidade Federal do Rio Grande do Sul, in Porto Alegre, RS, where he graduated in December, 1974. During 1975 and 1976 he worked for the Master of Science degree at the Department of Animal Science of the Universidade Federal do Rio Grande do Sul, concentrating in the area of animal nutrition.

During his first year on the master's program he was hired by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), the Brazilian national agricultural research institute. In January, 1977 he moved to Corumbá, Mato Grosso do Sul, in the very western area of the central region of Brazil, to work at EMBRAPA's research unit, with responsibility for research with beef cattle in the Brazilian Pantanal region. From January, 1978 to October, 1982 he was the vice-director of EMBRAPA's research unit in Corumbá, being involved mainly in administration. He started working in research after 1982, mainly on mineral surveys and mineral supplementation of beef cattle in the Pantanal region. In Corumbá, he was responsible for the

Animal Nutrition Laboratory from March, 1983 to April, 1986; coordinator of the research unit publications committee from February, 1979 to March, 1983; and a member of this committee from March, 1983 to May, 1986. He was also a member of the committee that evaluated the transformation of Corumbá's research unit into a regional research center, with broader activities.

He is a member of the American Society of Animal Science, Colégio Brasileiro de Nutrição (Brazilian Nutrition Society), Gamma Sigma Delta Honor Society of Agriculture and Sociedade Brasileira de Zootecnia (Brazilian Society of Animal Science).

He arrived in Gainesville, FL, in December, 1987 to work on a Ph.D. program in animal science, which he expects to conclude in December, 1991.

He married the former Marlene Maria May in 1974 and they have two sons, Edison and Madison, and a daughter, Aline.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Clarence B. Ammerman, Chairman
Professor of Animal Science

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Joseph H. Conrad, Cochairman
Professor of Animal Science

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Alfred M. Merritt
Professor of Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard D. Miles
Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John E. Moore
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P. V. Rao
Pejaver V. Rao
Professor of Statistics

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1992

Dean, College of Agriculture

Dean, Graduate School