INTERRELATIONSHIPS BETWEEN MOLYBDENUM AND
COPPER IN FEMALE RATS

by

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CHAPTER I

INTRODUCTION

Molybdenum (Mo) began to attract attention when it was found to be an essential nutrient in animals (1, 2). The role of Mo in biological function in humans has been related to the three known Mo-containing enzymes: xanthine dehydrogenase/oxidase (XDH), aldehyde oxidase and sulfite oxidase (SOX) (3, 4). The latter is essential in the terminal oxidation of sulfur-containing amino acids. However, whether or not any of the reactions catalyzed by XDH or aldehyde oxidase are necessary for human well-being has yet to be established (5).

In recent years, both epidemiological and experimental studies have shown that Mo may be involved in the prevention of cancer. Data collected from different areas of the world revealed that Mo deficiency and the incidence of cancer in human were highly correlated (6-8). With experimental animals, the incidence of carcinogenesis induced by chemicals was lower in the Mo supplemented groups (7, 9, 10). In spite of the evidence which supports the hypothesis that Mo has inhibitory effect on tumorogenesis, the possible inhibitory mechanism of Mo is still unknown. The biological activity of Mo is influenced by the presence of its antagonist, tungsten (W) which counters the inhibitory effect of Mo on mammary carcinogenesis in rats (9, 10).

Copper (Cu) functions primarily in the form of Cu containing enzymes such as superoxide dismutase (SOD), ceruloplasmin (Cp) and

cytochrome oxidase. It also exhibits some antagonistic effect with Mo. When the Cu stores are normal and dietary Cu is adequate, large amounts of Mo are required to produce toxicity symptoms (4). In Mo toxicity Cu supplementation can overcome its effect. Thus there appears to be a balancing effect between the two minerals, and toxic effects result if the ratio between Cu and Mo is too high or too low (4). Therefore, it has been postulated that Cu may be involved in the biological activities of Mo in the prevention of cancer.

In an attempt to elucidate a possible mechanism of their interactions, this study was conducted to examine the interrelationships between Mo and Cu in female weanling outbred Sprague-Dawley rats by injecting Mo and Cu simultaneously.

CHAPTER II

LITERATURE REVIEW

Molybdenum, atomic number 42, was known to have a biological function in 1930 when it was found to be an essential part of the enzyme nitrogenase which acts as a catalyst in the fixation of nitrogen by a microorganism (11). It was discovered in animal tissues in 1932 (12). An essential role for Mo in animal nutrition was further indicated by the discovery in 1953 that XDH is a Mo containing metalloenzyme which is dependent for its activity on the presence of this metal (1, 2). Subsequently the element was found to be a constituent of various flavin-dependent enzymes, i.e., aldehyde oxidase (13) and SOX (14) in animal tissues.

The biological functions of Mo in animals are related to the formation and activities of the Mo-dependent enzymes. Xanthine dehydrogenase/oxidase and aldehyde oxidase, mainly found in liver, kidney, lung, and milk, are involved in the electron transport chain in cells. Xanthine dehydrogenase/oxidase catalyzes the oxidative hydroxylation of xanthine and hypoxanthine to uric acid. Aldehyde oxidase oxidizes a wide variety of nitrogen-containing heterocyclic compounds. Sulfite oxidase found in liver, kidney, and intestine is essential for detoxification of the sulfite arising from metabolism of sulfur-containing amino acids, from ingestion of bisulfite preservative and from inhalation of sulfur dioxide, an atmospheric pollutant (5). The activities of these enzymes are affected by the dietary intake of Mo.

Under most normal dietary conditions, the Mo content of tissues is quite low (0.1 to 1.0 $\mu\text{g/g}$ wet wt in humans [15] and chickens, with lower amounts in rats [16]). Human liver, kidneys, adrenal, and blood have been found to contain the most Mo in the body, with lesser but still significant quantities in lungs, spleen, and muscles (15). The Mo content of whole blood is about 1.5 $\mu\text{g}/100\text{ ml}$ (or 0.156 $\mu\text{mol/L}$) evenly distributed between the erythrocytes and plasma. Especially in liver, kidney, and bone, the concentration of tissue Mo can be increased or decreased by raising or lowering dietary intake. The richest dietary sources are meats, cereal grains, and legumes (15, 17). No recommended dietary allowance for Mo has been established for humans. The average human mixed diet in the United States has been estimated to contain between 0.1 and 0.46 mg which is within the range of "Estimated Safe and Adequate Daily Dietary Intakes" of 0.15 to 0.5 mg/day for adults (17). Rats and chicks have an extremely low-Mo requirement, and as little as 0.02 ppm Mo in the diet was sufficient to maintain normal growth of rats (16).

Molybdenum is readily and rapidly absorbed from most foodstuffs and other water-soluble inorganic forms of the metal. The hexavalent water-soluble forms, sodium and ammonium molybdate, are particularly well absorbed, 83% in the stomach and the remainder in the small intestine with no absorption occurring in the large intestine (18). Most soluble forms of Mo are excreted in the urine with part via the bile (19). The mechanism whereby Mo is transported in the blood is not yet known. As its absorption is usually more rapid than its excretion, the level of Mo in the blood stream may remain high for

several days after a single dose and so relatively infrequent dosing will ensure a continual high concentration in the animal. The organism has little tendency to retain Mo, so when dosing is discontinued, excretion proceeds until only trace amounts of Mo remain in the tissues (20). Distribution studies with radioactive isotope ^{99}Mo indicate that Mo tends to accumulate preferentially in the liver and kidneys (19).

True Mo deficiency has been impossible to achieve in experimental animals. The nearest approximation to an induced deficiency state has been produced by feeding a Mo antagonist such as tungstate or sulfate to animals (3, 4, 21). In early experiments with rats fed low-Mo diets (essentially no detectable Mo) intestinal and liver XDH activities were reduced to 10% of normal, but neither the growth nor the excretion of uric acid of the depleted animals was affected (22, 23).

Similar experiments with chickens receiving low Mo diets have failed to show consistent changes in growth or development. However, when W was added to diets to give W:Mo ratio of 1000:1, the birds exhibited depletion of tissue XDH, increased excretion of xanthine and hypoxanthine, decreased excretion of uric acid, growth retardation and death (16). Johnson et al. (24) also showed that supplementation of W to rats at levels of 1 to 100 ppm in the drinking water proportionately decreased hepatic SOX activities as well as hepatic XDH activities, and total liver Mo. These effects can be overcome by the administration of sufficient amounts of Mo. The W effect was also observed when the metal was injected

intraperitoneally. Injection of Mo into rats depleted of hepatic SOX and XDH activities by W treatment resulted in significant restoration of both activities within a few hours (24).

No "natural" case of Mo deficiency in humans has been reported. The only case of diet-induced deficiency of this metal has recently been reported by Abumrad and co-workers (25). They described a patient on total parenteral nutrition for 18 months suffered from symptoms of lack of SOX and XDH activities, and displayed low levels of sulfate and uric acid excretion, with high amounts of sulfite, xanthine and hypoxanthine. Treatment with 300 ug ammonium molybdate daily reversed these defects.

Inorganic sulfate also influences Mo metabolism. Miller et al. (26) illustrated that inorganic sulfate reduced Mo retention in the tissues of rats both by reducing intestinal absorption and by increasing urinary excretion. Thus, protein intakes can also be important since this can be a source of endogenous sulfate which can be just as effective as dietary inorganic sulfate (4). Consequently, inorganic sulfate was used to alleviate Mo toxicity in all species studied.

Chronic ingestion of excess amounts of Mo produces a disease state called "teart" in several animal species. The clinical manifestations of Mo toxicity vary among different species, but growth retardation or loss of body weight is an invariable result of high Mo intakes. Certain animals such as cows and sheep show extraordinary sensitivity to dietary Mo levels as low as 2 to 30 ppm while horses and pigs can tolerate a level as high as 1000 ppm (3,4).

Rats, rabbits, guinea pigs, and poultry are in the intermediate. The growth inhibition in rats was observed at the Mo level of 75 to 100 ppm in the diets (26, 27). Liver SOX activity was also depressed in molybdenotic rats (4, 28). Growth retardation and decreased food consumption were observed in rats fed a diet containing 400 to 1200 ppm Mo (29). In many ways, the symptoms of Mo toxicity resemble those of Cu deficiency and treatment with supplemental Cu can overcome or prevent the symptoms (3, 4, 20, 28). This observation indicated that there may be an interaction between Cu and Mo.

Molybdenum toxicity in humans has been reported in the Soviet Union, where excessive dietary intakes of Mo (10 to 15 mg/day) resulted in high serum uric acid levels, elevated blood Mo levels, and increased tissue XDH activities concomitant with a high incidence of a gout-like syndrome (3, 4).

A relationship between Mo deficiency and incidence of esophageal cancer in humans was first reported by Burrell et al. in 1966 (6). Epidemiological evidence suggests that the high incidence of this cancer among Bantu of Transkei in Southern Africa was attributed to the consumption of food grown locally on soil low in Mo. Studies conducted in China indicated that the prevalence of esophageal cancer in both humans and chickens was high in certain areas where Mo was deficient in the soil (7). An epidemiological survey conducted in ninety-four cities in the U.S. also showed a correlation between the incidence of esophageal cancer and low Mo content of water supplies (8). Luo et al. (9) studied the effects of Mo on carcinogenesis induced by N-nitrososarcosine ethyl ester (NSEE) in rats. They found

that the addition of Mo at either 2 or 20 ppm level in the drinking water significantly inhibited NSEE-induced esophageal and forestomach carcinogenesis in male rats. Wei et al. (10) investigated the effects of Mo on N-nitroso-N-methylurea (NMU)-induced mammary carcinogenesis in female Sprague-Dawley rats. Their results indicated that mammary carcinoma incidence of the rats given 10 ppm Mo in the drinking water was significantly lower than that of the rats given no Mo supplementation as observed at 198 days after NMU treatment.

The antagonistic relationship between Mo and Cu was realized when it was discovered that supplemental Cu successfully controlled "teart" in cattle. Thus it was realized that Cu metabolism may be influenced by the ingestion of excessive quantities of Mo. Molybdenum toxicity can be induced by the addition of sodium molybdate to the normal ration and it may be controlled by dosing the animals with copper sulfate (20). Intravenous injection of smaller quantities of copper sulfate has been reported to be equally effective. If copper sulfate is administered together with molybdate, proportionately less of the Mo is excreted in the urine (20); however, whether this is due to decreased absorption or to a stimulation of excretion via the bile is not known. Interaction studies with the radioactive isotopes ^{99}Mo and ^{64}Cu suggest that Mo, when administered to rats, tends to decrease the accumulation of Cu in the liver (19).

Copper interacts metabolically with many elements, such as zinc (Zn), iron (Fe), cadmium (Cd), and Mo. The interaction between Mo

and Cu takes place predominantly in the gut and may involve the formation of an unavailable compound containing Cu and Mo (4). Huisingh et al. (30) proposed that Cu becomes unavailable by two routes: (i) through interaction with Mo to form a biologically unavailable Cu-Mo complex called cupric molybdate to make both Cu and Mo less available, and (ii) through the formation of an insoluble cupric sulfide (CuS) in the rumen, intestine, or tissues. Deosthale and Gopalan (31) showed that the serum Cu concentrations were higher in all human subjects given the diet containing sorghum with a higher content of Mo. The higher serum concentrations of Cu following increased Mo intake suggest mobilization of tissue Cu and increased urinary excretion.

About 25% of ingested Cu is absorbed from the upper small intestine (21). The Cu reaching the liver is either stored in mitochondria or released for incorporation into erythrocyte, Cp, and the numerous Cu-containing enzymes of the cells (4). Bile contains Cu and is probably the main vehicle for its excretion; when injected intravenously a proportion of ^{64}Cu appears in the bile (32) and is voided in the feces. The liver, kidneys, brain, heart, and hair are organs high in Cu.

About 95% of the Cu is bound to Cp, the blue Cu-protein of plasma. Ceruloplasmin functions as a primary Cu transport protein from which Cu atoms are transferred to cytochrome c oxidase and other Cu proteins (33). Ceruloplasmin also has enzyme activity important in the mobilization of Fe into the plasma from Fe storage cells in the liver (34).

Superoxide dismutases are a class of metalloenzymes that are present in all aerobic organisms. Superoxide dismutases catalyze the breakdown of superoxide radicals to oxygen and hydrogen peroxide and protect living cells against the toxic effects of free-radical oxygen (21, 35). Animal species have two forms of SOD. One group is the cupro-zinc enzyme (CuZnSOD) found predominantly in the cytosol. The other group is the mangano-zinc enzyme (MnZnSOD) found predominantly in the mitochondria. Copper deficiency may result in a decrease in the SOD activity of liver cytosol and mitochondria intermembrane space. It has been shown that, after a single subcutaneous injection of rats with CuSO_4 solution (5 mg Cu/kg body wt), liver Cu begins to increase at the 6th h, reaches its maximum at the 24th h, remains at approximately the same level at the 48th h, and decreases to the initial value at the 96th h (36). The SOD activity in both cytosol and mitochondrial fractions was augmented at the 48th h after a single subcutaneous injection of CuSO_4 solution (5 mg Cu/kg body wt). They also observed that the Cu content of the liver submitochondrial fraction was markedly increased 48 h after the Cu injection.

CHAPTER III

MATERIALS AND METHODS

Animals and diet

Three-week-old weanling out-bred female Sprague-Dawley rats purchased from Harlan Sprague-Dawley, Inc., Houston, Texas, were used in this study. They were individually housed in stainless steel, wire-mesh-bottom cages in a room temperature controlled at 22 ± 1 C and a 12-hour light-dark cycle. They were weighed weekly. All animals were given ad libitum AIN-76A diet (Tables 1-3) (37-39) containing 0.026 ppm Mo and 6.3 ppm Cu, and deionized drinking water until they reached an average body weight of 200 g. They were then randomly divided into 5 groups of 10 animals each. Anterodorsal and posterodorsal positions of the rats were shaved for the administration of Cu and Mo solutions.

Experimental design

As shown in Table 4, the animals in group 1 were subcutaneously injected with saline solution and served as a control group. The animals in groups 2 to 5 were simultaneously injected with a saline solution containing 5 mg Cu from cupric sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) per kg body weight, and a saline solution containing 0, 5, 10, or 50 mg Mo from sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) per kg body weight, respectively. At the 48th h after injection, the animals were anesthetized with carbon dioxide and exsanguinated by cardiac puncture.

Table 1. Composition of a semi-purified AIN-76A diet

Ingredient	%
Vitamin free casein	20.0
DL-Methionine	0.3
Cerelose ¹	65.0
Corn oil ¹	5.0
Celufil ²	5.0
AIN-76A vitamin mix (see Table 2)	1.0
AIN-76 mineral mix (see Table 3)	3.5
Choline bitartrate	0.2
Total	----- 100.0

¹Glucose monohydrate.

²Non-nutritive fiber.

Table 2. Composition of AIN-76A vitamin mixture

Ingredient	Amount Provided by 1% in Diet (mg/kg or IU/kg)	Requirements of Rats (mg/kg or IU/kg) ¹
Thiamine Hydrochloride	6.00 mg	1.25 mg
Riboflavin	6.00 mg	2.50 mg
Pyridoxine Hydrochloride	7.00 mg	7.00 mg
Nicotinic Acid	30.00 mg	15.00 mg
Calcium Pantothenate	16.00 mg	8.00 mg
Folic Acid	2.00 mg	-- ²
Biotin	0.20 mg	-- ²
Cyanocobalamin	0.01 mg	0.005 mg
Vitamin A	4,000.00 IU	2,000.00 IU
Vitamin D	1,000.00 IU	1,000.00 IU
Vitamin E	50.00 IU	50.00 IU
Vitamin K	0.50 mg	0.05 mg

¹"Nutrient Requirements of Laboratory Animals," No. 10, Second Revised Edition, 1972. National Academy of Sciences.

²Due to synthesis by the intestinal flora, there is no dietary requirement under conventional conditions.

Table 3. Composition of AIN-76 mineral mixture

Element	Amount Provided by 3.5% in Diet (mg/kg of Diet)	Requirements of Rats (mg/kg of Diet) ¹
Calcium	5,200.0	5,000.0
Phosphorus	4,000.0	4,000.0
Sodium	1,020.0	500.0
Potassium	3,600.0	1,800.0
Magnesium	500.0	400.0
Manganese	54.0	50.0
Iron	35.0	35.0
Copper	6.0	5.0
Zinc	30.0	12.0
Iodine	0.2	0.15
Selenium	0.1	0.04
Chromium	2.0	--
Chloride	1,560.0	500.0
Sulfate	1,000.0	--

¹"Nutrient Requirements of Laboratory Animals," No. 10, Second Revised Edition, 1972. National Academy of Sciences.

Table 4. Experimental design

Group No.	No. of Rats	Diet	Treatments ¹
1	10	AIN-76A	Control
2	10	AIN-76A	5 mg Cu ² /kg B.W.
3	10	AIN-76A	5 mg Cu/kg B.W. + 5 mg Mo ³ /kg B.W.
4	10	AIN-76A	5 mg Cu/kg B.W. + 10 mg Mo/kg B.W.
5	10	AIN-76A	5 mg Cu/kg B.W. + 50 mg Mo/kg B.W.

¹Administered subcutaneously when the average body weight of the rats reached 200 g.

²CuSO₄·5H₂O

³Na₂MoO₄·2H₂O

Sample preparation

A blood sample was collected from each rat into a heparinized syringe; one ml of whole blood was transferred into a 30 ml beaker for the determination of mineral content. The remainder was transferred into a chilled plastic tube and was centrifuged (Model J-21, Beckman Instruments, Palo Alto, CA) at $3,000 \times g$ for 20 min to obtain plasma which was used for the assay of Cp activity. Liver, kidney, and spleen from each rat were removed and rinsed immediately in chilled 0.25 M sucrose solution. These tissues were blotted of excess sucrose solution, dissected free of any attached muscle, peritoneum, or fibrous tissue, weighed, and then frozen at -80°C until assayed.

Enzymatic assay

Xanthine dehydrogenase/oxidase (EC 1.2.1.37, 1.2.3.2) - The activity of hepatic XDH was determined by the formation of uric acid from xanthine by a modification of the procedures of Stirpe and Corte (40). Homogenization of liver was carried out in ice-cold 0.1 M Tris at pH 8.1 (1 part liver + 5 parts buffer) in a Tissumizer (Tekmar Co., Cincinnati, OH). The homogenate was centrifuged at $45,000 \times g$ for 30 min at 2°C . The supernatant solution was dialyzed against 0.1 M Tris at pH 8.1 for 6 h at $3 \pm 1^{\circ}\text{C}$ before assay. The activity of XDH was assayed by spectrophotometer (Model UV-260, Shimadzu Co., Kyoto, Japan) at 293 nm using methylene blue as electron acceptor.

Sulfite oxidase (EC 1.8.3.1) - The activity of hepatic SOX was assayed following the method of Cohen and Fridovich (41).

Spectrophotometric assays of SOX were carried out at 420 nm in terms of the coupled reduction of ferricyanide.

Superoxide dismutase (EC 1.15.1.1) - Superoxide dismutase activity was assayed in terms of its ability to augment aerobic o-dianisidine dihydrochloride (ODD) photooxidation following the method of Misra and Fridovich (42). The product accumulated after a progressive oxidation of ODD was measured spectrophotometrically at 460 nm.

Ceruloplasmin (ferroxidase, EC 1.12.3.1) - The measurement of Cp was performed in terms of its oxidase activity in plasma by use of ODD as substrate (43). Addition of concentrated sulfuric acid to the reaction mixture stopped the enzymatic reaction and formed a stable product that has absorbance at 540 nm.

Determination of mineral content

Five ml concentrated HNO_3 was added to 1 ml of whole blood sample from each rat and the mixture was evaporated on a hot plate at about 130 C to a final volume of 1 ml. One ml 3.7 M $\text{Mg}(\text{NO}_3)_2$ was added and the mixture was heated at 105-110 C for 1 h, followed by a gradual increase to 190 C for another hour. The sample was then placed in a muffle furnace, and heated at 150 C for 30 min; 200 C, 30 min; 250 C, 30 min; and 470 C for at least 6 h. The residue remaining after ashing was cooled to room temperature, and dissolved in hot 0.12 N HCl to make a final volume of 10 ml.

The liver, kidney, and spleen samples were homogenized and dried on a hot plate. The samples were then digested and ashed following

the same procedures described above for the blood.

Molybdenum content was determined by a catalytic polarographic procedure (44) using a polarographic analyzer (Model 174A EG&G, Princeton Applied Research, Princeton, NJ). Copper, Fe, and Zn contents were determined by flame atomic absorption spectrophotometer (Model 5000, Perkin-Elmer, Norwalk, CT).

Statistical analysis

This study was a completely randomized design (CRD) with 10 replications for each treatment. Data were analyzed by the analysis of variance and the mean differences evaluated by Duncan's new multiple-range test at the predetermined alpha level of 0.05 as described by Steel and Torrie (45).

CHAPTER IV

RESULTS

To investigate the interrelationships between Mo and Cu in female rats, the animals were fed the same diet (AIN-76A) and deionized drinking water before receiving the treatments. They were divided into 5 groups of 10 animals each when they reached an average body weight of 200 g, followed by the Cu and Mo injections. At 48 h after the injections the animals were sacrificed for enzyme assays and mineral determinations.

Xanthine dehydrogenase/oxidase activity

As shown in Table 5, the mean activity of hepatic XDH of the Cu-treated animals (groups 2-5) was significantly ($P<0.05$) higher than that of the control group (group 1) without Cu treatment. The animals injected with Mo (groups 3-5) exhibited significantly ($P<0.05$) higher XDH activities than those injected only with Cu (group 2). The XDH activities among three Mo-treated groups, however, were not significantly different ($P>0.05$).

Sulfite oxidase activity

The effect of Cu and Mo treatments on the activity of hepatic SOX is shown in Table 6. A single subcutaneous injection of Cu at 5 mg/kg body wt had no significant effect on hepatic SOX activity. The simultaneous injection of Mo at the level of 5 mg/kg body wt (group 3), significantly raised SOX activity over the control group, but

Table 5. Effect of copper and molybdenum treatments on the activity of hepatic xanthine dehydrogenase/oxidase (XDH) (μ moles uric acid formed/min/g fresh liver) of female rats

Group No.	Treatments	XDH ^{1,2}
1	Control	0.591 $\pm$ 0.073 ^a
2	5 mg Cu/kg B.W.	0.718 $\pm$ 0.057 ^b
3	5 mg Cu/kg B.W. + 5 mg Mo/kg B.W.	0.893 $\pm$ 0.108 ^c
4	5 mg Cu/kg B.W. + 10 mg Mo/kg B.W.	0.890 $\pm$ 0.079 ^c
5	5 mg Cu/kg B.W. + 50 mg Mo/kg B.W.	0.838 $\pm$ 0.058 ^c

¹Values are mean $\pm$ SD of 10 animals.

²Means with different superscripts are significantly (P<0.05) different.

Table 6. Effect of copper and molybdenum treatments on the activity of hepatic sulfite oxidase (SOX) (μ moles ferricyanide reduced/min/g fresh liver) of female rats

Group No.	Treatments	SOX ^{1,2}
1	Control	7.52 $\pm$ 0.67 ^a
2	5 mg Cu/kg B.W.	7.85 $\pm$ 0.52 ^a
3	5 mg Cu/kg B.W. + 5 mg Mo/kg B.W.	8.84 $\pm$ 0.73 ^b
4	5 mg Cu/kg B.W. + 10 mg Mo/kg B.W.	7.98 $\pm$ 0.78 ^a
5	5 mg Cu/kg B.W. + 50 mg Mo/kg B.W.	8.14 $\pm$ 0.53 ^a

¹Values are mean $\pm$ SD of 10 animals.

²Means with different superscripts are significantly (P<0.05) different.

further increase in Mo levels (groups 4 and 5) lowered SOX activity to the level of those receiving no Mo treatment.

Superoxide dismutase activity

The injection of Cu at 5 mg/kg body wt did not significantly increased the activity of hepatic SOD, as shown in Table 7. Significant elevation in SOD activity over the control group was observed only at Mo level of 50 mg/kg body wt.

Ceruloplasmin activity

The effect of Cu and Mo treatments on the activity of plasma Cp is presented in Table 8. The mean activity of plasma Cp in the control group (group 1), receiving no Cu or Mo treatment, was 0.117 μ moles ODD oxidized/min/ml. Injection of 5 mg Cu/kg body wt significantly ($P < 0.05$) elevated plasma Cp activity of all treatment groups. Simultaneous injection of Mo at 5 or 10 mg/kg body wt seemed to have no effect on Cp activity. There appears to be a threshold somewhere between 10 to 50 mg Mo level over which the activity of plasma Cp decreased. This result suggests that the maximal effect of Mo on plasma Cp activity occurred between the 10 to 50 mg Mo level.

Hepatic mineral content

The influence of injected Cu and Mo on hepatic content of Mo, Cu, Fe, and Zn is shown in Table 9. When the animals were sacrificed 48 h after the subcutaneous injections of Cu and/or Mo, a significant ($P < 0.05$) increase in hepatic Mo content was observed in all Mo-

Table 7. Effect of copper and molybdenum treatments on the activity of hepatic superoxide dismutase (SOD) (units SOD/mg fresh liver) of female rats

Group No.	Treatments	SOD ^{1,2}
1	Control	5.38 $\pm$ 0.55 ^a
2	5 mg Cu/kg B.W.	5.73 $\pm$ 0.88 ^{ab}
3	5 mg Cu/kg B.W. + 5 mg Mo/kg B.W.	5.66 $\pm$ 0.83 ^a
4	5 mg Cu/kg B.W. + 10 mg Mo/kg B.W.	5.97 $\pm$ 0.46 ^{ab}
5	5 mg Cu/kg B.W. + 50 mg Mo/kg B.W.	6.32 $\pm$ 0.27 ^b

¹Values are mean $\pm$ SD of 10 animals.

²Means with different superscripts are significantly (P<0.05) different.

Table 8. Effect of copper and molybdenum treatments on the activity of plasma ceruloplasmin (Cp) (μ moles ODD oxidized/min/ml) of female rats

Group No.	Treatment	Cp ^{1,2}
1	Control	0.117 $\pm$ 0.026 ^a
2	5 mg Cu/kg B.W.	0.203 $\pm$ 0.027 ^{bc}
3	5 mg Cu/kg B.W. + 5 mg Mo/kg B.W.	0.229 $\pm$ 0.052 ^{bc}
4	5 mg Cu/kg B.W. + 10 mg Mo/kg B.W.	0.241 $\pm$ 0.090 ^c
5	5 mg Cu/kg B.W. + 50 mg Mo/kg B.W.	0.189 $\pm$ 0.033 ^b

¹Values are mean $\pm$ SD of 10 animals.

²Means with different superscripts are significantly (P<0.05) different.

Table 9. Effect of copper and molybdenum treatments on hepatic mineral content of female rats^{1,2,3}

Group No.	Mo (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)
1	0.285 ± 0.033 ^a	4.6 ± 0.8 ^a	357 ± 80 ^a	21.0 ± 1.3 ^a
2	0.259 ± 0.049 ^a	81.9 ± 11.4 ^b	291 ± 54 ^b	28.1 ± 3.1 ^b
3	0.489 ± 0.043 ^b	78.6 ± 10.9 ^b	337 ± 70 ^{ab}	29.2 ± 5.0 ^b
4	0.528 ± 0.036 ^b	80.8 ± 8.8 ^b	333 ± 44 ^{ab}	28.0 ± 2.7 ^b
5	0.606 ± 0.082 ^c	74.7 ± 8.1 ^b	307 ± 42 ^{ab}	26.4 ± 2.4 ^b

¹Units are based on fresh tissue weight.

²Values are mean ± SD of 10 animals.

³Means with different superscripts are significantly (P<0.05) different.

treated rats (groups 3-5). No significant ($P > 0.05$) difference was observed between group 1 (control) and group 2 receiving Cu treatment only. This observation is consistent with previous reports (46, 47) that increasing Mo supplementation significantly increased hepatic Mo content of female rats fed AIN-76A diet ad libitum.

Hepatic Cu content was significantly ($P < 0.05$) elevated in the Cu-treated groups (groups 2-5), but was not affected by the additional Mo treatment.

No significant difference in hepatic Fe content was observed in the Cu and Mo treated groups (groups 3-5). The animals in group 2, receiving 5 mg Cu/kg body wt only, showed a significant ($P < 0.05$) decrease in hepatic Fe content when compared to the control (group 1) with no Cu and/or Mo treatments. Hepatic Zn storage was elevated significantly by Cu treatment but was not affected by Mo treatment.

Mineral content of kidney

The effect of Cu and Mo treatments on the mineral content of kidney of female rats fed AIN-76A diet ad libitum is presented in Table 10. The Mo content of kidney showed a similar pattern as that of liver (Table 9); increasing Mo levels resulted in increasing Mo content but the significant ($P < 0.05$) elevation of Mo content over the control group ($0.158 \mu\text{g/g}$ fresh kidney) was observed only at the level of 50 mg Mo/kg body wt ($0.711 \mu\text{g/g}$ fresh kidney).

Similar to the result obtained with liver, the Cu content of kidney from the Cu-treated animals (groups 2-5) was significantly ($P < 0.05$) increased over the control group (group 1). The Fe and Zn

Table 10. Effect of copper and molybdenum treatments on mineral content of kidneys of female rats^{1,2,3}

Group No.	Mo (μg/g)	Cu (μg/g)	Fe (μg/g)	Zn (μg/g)
1	0.158 ± 0.031 ^{ab}	12.1 ± 5.2 ^a	85 ± 16	22.3 ± 2.0
2	0.135 ± 0.016 ^a	23.9 ± 5.5 ^b	66 ± 16	23.4 ± 2.6
3	0.205 ± 0.038 ^{ab}	26.2 ± 5.8 ^b	83 ± 33	23.2 ± 2.1
4	0.272 ± 0.078 ^b	22.9 ± 4.9 ^b	76 ± 11	23.2 ± 2.2
5	0.711 ± 0.256 ^c	25.1 ± 3.9 ^b	73 ± 15	22.3 ± 1.1

¹Units are based on fresh tissue weight.

²Values are mean ± SD of 10 animals.

³Means with different superscripts are significantly (P<0.05) different.

content of kidney in all groups remained unaffected by the simultaneous injection of Cu and/or Mo.

Mineral content of spleen

As shown in Table 11, results were similar to those obtained with liver and kidney; a significant ($P < 0.05$) increase in the Mo content of spleen was observed in all Mo-treated animals (groups 3-5). The mean Mo content of the spleen of group 5 receiving 5 mg Cu and 50 mg Mo was 24-fold higher than that of group 2 receiving 5 mg Cu only.

The Cu and Fe content of spleen in all groups remained unaffected by the Cu and/or Mo treatments. There was no significant difference in Zn content among the Cu-treated groups (groups 2-5). The Zn content of group 4 receiving 5 mg Cu and 10 mg Mo was significantly lower than that of the control (group 1).

Mineral content of blood

The effect of Cu and Mo treatments on the mineral content of blood of female rats fed AIN-76A diet ad libitum is summarized in Table 12. Although Mo content in blood of the Mo-treated animals (groups 3-5) was all increased, significant ($P < 0.05$) difference was observed only at the group receiving 50 mg Mo/kg body wt (group 5).

Blood Cu in all groups remained unaffected by Cu and/or Mo treatments, but Fe and Zn content was significantly ($P < 0.05$) decreased in group 2 that received 5 mg Cu/kg body wt only.

Table 11. Effect of copper and molybdenum treatments on mineral content of spleens of female rats^{1,2,3}

Group No.	Mo (μg/g)	Cu (μg/g)	Fe (μg/g)	Zn (μg/g)
1	0.075 ± 0.010 ^a	7.1 ± 4.4	1032 ± 256	19.1 ± 1.0 ^a
2	0.068 ± 0.008 ^a	7.6 ± 1.6	924 ± 227	18.6 ± 0.4 ^{ab}
3	0.339 ± 0.086 ^{ab}	6.7 ± 1.4	862 ± 250	18.6 ± 0.6 ^{ab}
4	0.590 ± 0.213 ^b	5.7 ± 1.8	901 ± 193	18.5 ± 0.5 ^b
5	1.631 ± 0.619 ^c	6.9 ± 1.6	965 ± 194	18.6 ± 0.6 ^{ab}

¹Units are based on fresh tissue weight.

²Values are mean ± SD of 10 animals.

³Means with different superscripts are significantly (P<0.05) different.

Table 12. Effect of copper and molybdenum treatments on mineral content of blood of female rats^{1,2}

Group No.	Mo (ng/ml)	Cu (μg/ml)	Fe (μg/ml)	Zn (μg/ml)
1	14.9 ± 17.5 ^a (6)	1.5 ± 0.9(6)	549 ± 13 ^a (6)	5.3 ± 0.6 ^a (6)
2	8.6 ± 10.7 ^a (7)	1.8 ± 0.6(7)	463 ± 51 ^b (7)	4.6 ± 0.3 ^b (7)
3	52.0 ± 28.3 ^a (7)	2.0 ± 0.7(7)	492 ± 73 ^b (7)	4.9 ± 0.6 ^{ab} (7)
4	64.6 ± 19.4 ^a (6)	1.6 ± 0.8(6)	460 ± 18 ^b (6)	4.6 ± 0.5 ^b (6)
5	177.2 ± 107.8 ^b (6)	1.3 ± 0.4(6)	501 ± 23 ^{ab} (6)	4.7 ± 0.3 ^b (6)

¹Values are mean ± SD of animal numbers shown in the parentheses.

²Means with different superscripts are significantly (P<0.05) different.

CHAPTER V

DISCUSSION

The present study was designed to determine the effect of simultaneous injection of Mo and Cu on the activities of Mo- and Cu-enzymes and accumulation of minerals in the tissues of female rats. Generally in previous studies, Mo was supplemented to rats alone, or with either Cu and/or W in diet or in drinking water. How these elements get into the body may result in a dramatic difference in the fate of the elements. Suggestions that the rumen is the main site of the Cu and Mo interaction (4, 30) have been confirmed by various investigations. Therefore, subcutaneous injection of Cu and Mo as in the present study, may eventually prevent the formation of metabolically inactive "cupricthiomolybdate" in the gastro-intestinal tract but may not prevent the interaction from occurring in tissues. However, by evaluating the data from the current experiment, Tables 9 and 10, in which the Mo content of liver and kidney was increased with increasing Mo levels but Cu content remained unaffected by additional Mo injection. We were unable to establish evidence for the formation of a Cu-Mo complex, instead under the circumstance of the present experiment, these two elements seem to react independent of each other. These findings are in agreement with the results of Comar et al. (19), who reported that Cu has no significant effect on Mo metabolism in rats. Neilands et al. (48) also reported that the simultaneous administration of Cu did not affect Mo distribution in the rat.

The result with the rats which were sacrificed 48 h after simultaneous injection of Cu and Mo suggests that the injected Cu and Mo have reached the body tissues of the rats and consequently increased the Cu and Mo content of the tissues.

The XDH activity was markedly increased by Cu administration, and simultaneous injection of Mo further elevated the enzyme activity (Table 5). This result suggests that Cu at the level of 5 mg/kg body wt may have a promotive action on the activity of this Mo-containing enzyme. Unlike XDH, SOX activity was not affected by Cu administration, and injection of Mo at level of 10 or 50 mg/kg body wt seemed to have no effect on SOX activity. The mean SOX activity of group 3 with 5 mg of both Cu and Mo was significantly higher than that of any other group. Thus, in comparing these two Mo-containing enzymes, XDH appears to be more sensitive to both Cu and Mo treatments than does SOX. XDH is localized in the cytosol, while SOX is localized in the intermembrane space of mitochondria (3, 5). The difference in the localization of the enzyme may make XDH, the cytoplasmic enzyme, more susceptible to the change of Mo concentration in the cell. Alternatively, Johnson et al. (24) mentioned that SOX has a longer half-life than XDH in the liver. Whether this property of SOX contributes to the lower sensitivity to the Cu and Mo treatments in this study remains unknown at the present time.

The administration of Mo at 5, 10, and 50 mg levels significantly elevated hepatic Mo content in rats (Table 9). This is consistent with previous reported findings (3, 4, 16, 24, 46, 47).

However, no significant difference in hepatic XDH activity was observed between any two levels of Mo administration (Table 5). This may indicate that Mo at the level of 5 mg/kg body wt fully saturated the enzyme system in the liver (49).

It has been reported that the injection of Cu markedly augmented hepatic Cu content and SOD activity (36). Our results confirmed these observations. As shown in Table 9, hepatic Cu content was significantly increased by Cu treatment. A similar result also has been obtained in plasma Cp activity (Table 8). The high activity of Cp and high concentration of Cu in the liver of the Cu-treated rats, indicated that these animals had been fully saturated by 5 mg of Cu. However, these results were not in agreement with that of the hepatic SOD activity. By reviewing the hepatic SOD activity shown in Table 7, injection of Cu at the same dosage used by Ljutakova et al. (36) did not markedly augment hepatic SOD activity and only minor change was observed when Mo was applied. The SOD activity of group 5 receiving 5 mg Cu and 50 mg Mo was significantly higher than that of the control (group 1).

High Cu level in the liver of the Cu-treated rats may provide conditions favorable for greater synthesis of SOD. However, hepatic Cu alone does not stimulate SOD activity so that it seems probable that the response of SOD activity after Cu treatment requires not only the appropriate metal ions but also the presence of superoxide radicals (50).

Hepatic Fe content was significantly reduced by Cu administration but restored to a lesser extent by Mo treatment (Table

9). However, Cu treatment did not cause a significant effect on Fe storage in kidneys of all treatment groups (Table 10). Although these observations could be partially explained by the enzyme system involved in liver and kidney, the knowledge concerning the mechanism of Cu and Fe interaction is still obscure. Decreased Fe storage in liver accompanied with increased hepatic XDH activity supports the hypothesis proposed by Topham (51) that XDH is involved in the mobilization of Fe from hepatic Fe stores. Ceruloplasmin, the Cu metalloenzyme ferroxidase, also serves as a control of the mobilization of Fe into the plasma from Fe storage cells in the liver (34). It was surprising, however, that blood Fe concentration in groups 2 to 4 was still significantly lower than that of the control (group 1). Thus, it is evident that factors other than Cp are also involved.

Injected Mo and Cu were independent of each other as demonstrated by their accumulations in tissues. Injection of Mo has no effect on the activity of Cu-containing enzymes, SOD and Cp. The activity of Mo-containing enzyme, XDH, was improved by the simultaneous injection of Cu at the level of 5 mg/kg body wt. In summary, we conclude that under the present experimental conditions Mo has no effect on Cu utilization but Cu seems to improve Mo utilization.

REFERENCES

1. Richard, D.A. and W.W. Westerfeld. Isolation and identification of the xanthine oxidase factor as molybdenum. *J. Biol. Chem.* 203:915, 1953.
2. De Renzo, E.C., E. Kaleita, P.G. Heytler, J.J. Oleson, B.L. Hutchings, and J.H. Williams. Identification of the xanthine oxidase factor as molybdenum. *Arch. Biochem. Biophys.* 45:247, 1953.
3. Hainline, B.E. and K.V. Rajagopalan. Molybdenum in animal and human health. In: *Trace Elements in Health. A Review of Current Issues.* (J. Rose, ed.), pp.150-164, Butterworths & Co. Ltd., London, 1983.
4. Underwood, E.J. Molybdenum. In: *Trace Elements in Human and Animal Nutrition.* 4th ed., pp.109-131, Academic Press, N.Y., 1977.
5. Coughlan, M.P. The role of molybdenum in human biology. *J. Inher. Metab. Dis.* 6 Suppl. 1:70, 1983.
6. Burrell, R.J., W.A. Roach, and A. Shadwell. Esophageal cancer in the Bantu of the Transkei associated with mineral deficiency in garden plants. *J. Natl. Cancer Inst.* 36:201, 1966.
7. Yang, C.S. Research on Esophageal Cancer in China: A Review. *Cancer Res.* 40:2633, 1980.
8. Berg, J.W., W. Haenszel, and S.S. Devesa. Epidemiology of gastrointestinal cancer. *Proc. 7th. Natl. Cancer Conf.* pp.459-464, 1973.
9. Luo, X.M., H.J. Wei, and S.P. Yang. Inhibitory effects of molybdenum on esophageal and forestomach carcinogenesis in rats. *J. Natl. Cancer Inst.* 71:75, 1983.
10. Wei, H.J., X.M. Luo, and S.P. Yang. Effects of molybdenum and tungsten on mammary carcinogenesis in SD rats. *J. Natl. Cancer Inst.* 74:469, 1985.
11. Bortels, H. Molybdenum as a catalyst in the biological fixation of nitrogen. *Arch. Mikrobiol.* 1:333, 1930.
12. Meulen, H.T. Distribution of molybdenum. *Nature* 130:966, 1932.
13. Mahler, H.R., B. Mackler, D.E. Green, and R.M. Bock. Aldehyde oxidase. A molybdoflavoprotein. *J. Biol. Chem.* 210:465, 1954.

14. Cohen, H.J., I. Fridovich, and K.V. Rajagopalan. Hepatic sulfite oxidase. A function role for molybdenum. *J. Biol. Chem.* 246:374, 1971.
15. Schroeder, H.A., J.J. Balassa, and I.H. Tipton. Essential trace metals in man: molybdenum. *J. Chron. Dis.* 23:481, 1970.
16. Higgins, E.S., D.A. Richert, and W.W. Westerfeld. Molybdenum deficiency and tungsten inhibition studies. *J. Nutr.* 59:539, 1956.
17. Food and Nutrition Board, National Research Council. In: Recommended Dietary Allowances. 9th ed., National Academy of Science, Washington, D.C., 1980.
18. Chappell, W.R., R.R. Megler, R. Moure-Eraso, C.C. Solomons, T.A. Tsongas, P.A. Walravens, and P.A. Winston. In: Human Health Effects of Molybdenum in Drinking Water. DPA-600/1-79-006, US Environmental Protection Agency, 1979.
19. Comar, C.L., L. Singer, and G.K. Davis. Molybdenum metabolism and interrelationships with copper and phosphorus. *J. Biol. Chem.* 180:913, 1949.
20. Marston, H.R. Cobalt, copper and molybdenum in the nutrition of animals and plants. *Physiol. Rev.* 32:66, 1952.
21. Li, T.K. and B.L. Vallee. In: Modern Nutrition in Health and Disease. (R.S. Goodhart and M.E. Shils, eds.), 6th ed., pp.408-437, Henry Kimpton Publisher, London, 1980.
22. Richert, D.A. and W.W. Westerfeld. A bioassay for the dietary factor related to xanthine oxidase. *J. Biol. Chem.* 192:49, 1951.
23. Richert, D.A., R.J. Bloom, and W.W. Westerfeld. Purine catabolism in molybdenum deficiency. *J. Biol. Chem.* 227:523, 1957.
24. Johnson, J.L., K.V. Rajagopalan, and H.J. Cohen. Molecular basis of the biological function of molybdenum. Effect of tungsten on xanthine oxidase and sulphite oxidase in the rat. *J. Biol. Chem.* 249:859, 1974.
25. Abumrad, N.N., A.J. Schneider, D. Steel, and L.S. Rogers. Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. *Am. J. Clin. Nutr.* 34:2551, 1981.
26. Miller, R.F., N.O. Price, and R.W. Engel. Added dietary inorganic sulfate and its effect upon rats fed molybdenum. *J. Nutr.* 60:539, 1956.

27. Mills, C.F., K.J. Monty, A. Ichihara, and P.B. Pearson. Metabolic effects of molybdenum toxicity in the rats. *J. Nutr.* 65:129, 1958.
28. Miller, R.F., and R.W. Engel. Interrelations of copper, molybdenum and sulfate sulfur in nutrition. *Fed. Proc.* 19:666, 1960.
29. Van Reen, R. The influence of excessive dietary molybdenum on rat liver enzymes. *Arch. Biochem. Biophys.* 53:77, 1954.
30. Huisingh, J., G.G. Gomez, and G. Matrone. Interactions of copper, molybdenum, and sulfate in ruminant nutrition. *Fed. Proc.* 32:1921, 1973.
31. Deosthale, Y.G. and C. Gopalan. The effect of molybdenum levels in sorghum (*Sorghum Vulgare Pers.*) on uric acid and copper excretion in man. *Br. J. Nutr.* 31:351, 1974.
32. Comar, C.L., G.K. Davis, and L. Singer. The fate of radioactive copper administered to the bovine. *J. Biol. Chem.* 174:905, 1948.
33. Hsieh, H.S. and E. Frieden. Evidence for ceruloplasmin as a copper transport protein. *Biochem. Biophys. Res. Com.* 67:1326, 1975.
34. Frieden, E. Ceruloplasmin. A multifunctional metalloprotein. In: *Trace Element Metabolism in Man and Animals.* (M. Kirchgessner, ed.) 3:36, 1978.
35. Rigo, A., P. Viglino, and G. Rotilio. Polarographic determination of superoxide dismutase. *Analy. Biochem.* 68:1, 1975.
36. Ljutakova, S.G., E.M. Russanov, and S.I. Liochev. Copper increases superoxide dismutase activity in rat liver. *Arch. Biochem. Biophys.* 235:636, 1984.
37. American Institute of Nutrition. Report of the AIN ad hoc committee on standards for nutritional studies. *J. Nutr.* 107:1340, 1977.
38. American Institute of Nutrition. Second report of ad hoc committee on standards for nutritional studies. *J. Nutr.* 110:1726, 1980.
39. American Institute of Nutrition. Guidelines for describing diets for experimental animals. *J. Nutr.* 115:18, 1985.
40. Stirpe, F. and E.D. Corte. The regulation of rat liver xanthine oxidase. *J. Biol. Chem.* 244:3855, 1969.

41. Cohen, H.J. and I. Fridovich. Hepatic sulfite oxidase. *J. Biol. Chem.* 246:359, 1971.
42. Misra, H.P. and I. Fridovich. Superoxide dismutase: a photochemical augmentation assay. *Arch. Biochem. Biophys.* 181:308, 1977.
43. Schosinsky, K.H., H.P. Lehmann, and M.F. Beeler. Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. *Clin. Chem.* 20:1556, 1974.
44. Dunn, C.C., N.S. Wang, and C.H. Chen. Study of catalytic polarography. III. Catalytic current of molybdenum-chlorate in mandelic acid or benzilic acid medium. *Acta. Sci. Natl. Univ. Fudan* 11:197, 1966.
45. Steel, R.G.D. and J.H. Torrie. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed., pp.187-188, McGraw-Hill Book Co., N.Y., 1980.
46. Chao, W.V. Effects of molybdenum and tungsten supplementation on n-methyl-n-nitrosourea-treated female rats. Master's thesis, Texas Tech University, 1986.
47. Yan, L. Effects of molybdenum and tungsten supplementations on growth and mineral metabolism of female rats fed AIN-76A or Lab Chow. Master's thesis, Texas Tech University, 1986.
48. Neilands, J.B., F.M. Strong, and C.A. Elvehjem. Molybdenum in the nutrition of the rat. *J. Biol. Chem.* 172:431, 1948.
49. Pan, Y.W. Effects of molybdenum and tungsten supplementations on molybdenum- and copper-enzymes of female rats fed AIN-76A or Lab Chow. Master's thesis, Texas Tech University, 1986.
50. Dreosti, I.E., I.R. Record, R.A. Buckley, S.J. Manuel, and F.J. Fraser. Ethanol and hepatic superoxide dismutase in rats. In: Trace Element Metabolism in Man and Animals. (J. McC. Howell, J.M. Gawthorne, and C.L. White, eds.) 4:617, 1981.
51. Topham, R.W., M.C. Walker, and M.P. Calisch. Liver xanthine dehydrogenase and iron mobilization. *Biochem. Biophys. Res. Com.* 109:1240, 1982.

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