INTERRELATIONSHIPS OF COPPER, SULFUR, AND NITROGEN IN RUMINANTS

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

INTRODUCTION

Copper is an essential nutrient for all farm animals; however, the amount that must be supplied to meet requirements is affected by many factors. The affects of molybdenum, sulfate, zinc, and various trace minerals on the copper status of ruminants have been extensively investigated and reviewed (Underwood, 1962), but their effects, alone and in combinations, are not clearly understood.

Sheriha (1962) found that 1000 ppm of copper in the purified diet of Oltjen et al. (1962) did not induce toxicity in lambs during a 45-day experiment. The sulfur in this diet was furnished by sulfate and the nitrogen by urea. Lewis (1954) and Anderson (1956) found that sulfide is a normal metabolic product of sulfate reduction in the rumen, thus it is possible that the formation of copper sulfide could decrease the absorption of copper. Reilly (1961) reported that copper-ammonium complexes are readily formed when ammonia and copper ions are present. The purified diet of Oltjen et al. (1962) would be expected to present optimum conditions for the formation of copper-ammonium complexes as well as the insoluble copper sulfide since urea is rapidly hydrolyzed to ammonia and carbon dioxide and a large amount of sulfate is present. These experiments were conducted for the purpose of determining the effects of sulfur and nitrogen sources and copper levels on growth and retention of copper, nitrogen, sulfur, phosphorus, and calcium in lambs.

REVIEW OF LITERATURE

Considerable research has been done on copper and its application to animal nutrition and health. Several workers (Elvehjem, 1935; Schultze, 1940; Marston, 1952; Davis and Loosli, 1954; Allcroft and Lewis, 1957; Davis, 1957; Matrone, 1960; Underwood, 1962) have prepared extensive summaries and reviews on copper. As many of the research papers in these reviews are old, it was felt that a rather complete review of the subject would be valuable, in that recent findings may clarify past controversies.

The Chemistry of Copper

Copper is characterized by its yellow color although it appears green when viewed by light transmitted through a thin film of the metal (Babor and Lehrman,1956). It stands near the bottom of the activity series of the metals, thus is easily reduced from its compounds. Dry air has no action on copper but in moist air, containing carbon dioxide, a green coating of copper hydroxyl carbonate is formed. Copper does not liberate hydrogen from hydrochloric or sulfuric acids, but nitric acid dissolves the metal rapidly, forming cupric nitrate and oxides of nitrogen. Hot concentrated sulfuric acid is reduced by copper, producing copper sulfate and sulfur dioxide. Ammonia solution acts on copper in the presence of air to form a deep blue solution containing a complex ion $[Cu(NH_3)_4{}^{++}]$.

Copper forms two series of salts, the cupric (oxidation state of 2+) and the cuprous (oxidation state of 1+). The cupric salts are blue when hydrated, whereas the cuprous salts are colorless. In general, the cupric salts are water soluble, while many of the cuprous salts are insoluble in water. An exception is cupric sulfide (CuS).

Corwin (1950) studied the ability of copper to react with nitrogen, oxygen, and sulfur. In the dry state the affinity of sulfur and nitrogen for copper was less than that of oxygen while in aqueous solution the order of affinity was sulfur, nitrogen, and oxygen. In a lipid media, the affinity of these elements for copper was similar to that in air. Thus, in biological systems that are both aqueous and fatty, a variety of complexing reactions take place.

The copper-ammonium complex that is formed when cupric ions combine with ammonia is illustrated by the following formula:

$$Cu^{++} + 4NH_3 \rightleftharpoons Cu(NH_3)_4^{++}$$

This compound is an ionic one in which the $Cu(NH_3)_4^{++}$ is the positive ion and various other ions such as Cl^-, $SO_4^=$, or $PO_4^{\equiv}$ form the negative species. Reilley (1961) showed that the addition of ammonia to a solution of cupric salt lowers the concentration of Cu^{++} and increases the concentration of $Cu(NH_3)_4^{++}$. These complexes are dissociated in acid solution but completely associated under basic conditions.

Sources of Copper and Their *In Vivo* Availabilities

Inorganic Sources

Several workers have investigated the absorption and excretion of copper from wire (Cu), cupric sulfide (CuS), cupric oxide (CuO),

cuprous oxide (Cu_2O), cupric chloride ($CuCl_2$), cupric carbonate ($CuCO_3$), cupric sulfate ($CuSO_4$), cupric hydroxide [$Cu(OH)_2$], cupric iodide (CuI), and cupric nitrate [$Cu(NO_3)_2$]. Cupric sulfide is apparently a poor source of copper. Schultze et al. (1936a) found that CuS was unavailable to rats, but that $Cu(OH)_2$ and CuI were rapidly absorbed. Barber et al. (1961) reported that feeding pigs 250 ppm of copper as CuS did not increase their liver stores of copper. Bowland et al. (1961) reported that 5.1% of the copper in an oral dose of $CuSO_4$ was absorbed from the gastrointestinal tract of swine, but that only 1.7% of the copper in CuS was utilized. Using five animals per treatment, Dick (1954a) found that sheep fed no copper had average liver copper contents of 376 ppm on a dry matter basis. Lambs fed 30 mg. of copper as $CuSO_4$ had 1495 ppm of copper in their livers while those fed an equal amount of copper as CuS had 517 ppm.

Chapman and Bell (1963) used several compounds containing ^{64}Cu in an experiment with steers. The ^{64}Cu content of the blood was used as a measure of absorption. The blood values of steers fed the various copper sources by the above workers ranked as follows: $CuCO_3$ > $Cu(NO_3)_2$ > $CuSO_4$ > $CuCl_2$ > Cu_2O > CuO powder > CuO needles > Cu wire. Approximately 2.4% of the copper fed as $CuCO_3$ was found in the urine while less than 0.13% appeared from the other compounds. Lassiter and Bell (1960), using materials containing ^{64}Cu also found that $CuCO_3$, $CuCl_2$, $Cu(NO_3)_2$, and $CuSO_4$ were readily available to sheep. Copper oxide was less available than the compounds listed above. Bunch et al. (1961) reported that CuO was equal to $CuSO_4$ for growth and hemoglobin formation in swine, but higher liver copper values resulted from feeding $CuSO_4$ than when feeding CuO. The availabilities

of $CuSO_4$, $CuCO_3$, and CuO were found to be similar in swine by Buescher et al. (1961) and Allen et al. (1961). Other workers (Schubert et al., 1948; Kulwich et al., 1953; Mahoney et al., 1955) found ^{64}Cu acetate, chloride, nitrate, and sulfate to be available as measured by absorption, tissue concentration, and excretion.

Hart et al. (1928) found that liver ash treated with ammonia was as active as $CuSO_4$ in overcoming anemia when injected into rats. Harvey and Sutherland (1953) reported that copper ammonium sulfate increased liver storage of copper in ruminants. This limited work indicates that the copper in copper-ammonium complexes is available to animals.

Organic Sources

Mills (1954, 1955, 1956, 1957, 1958) conducted a series of experiments to investigate the availability of naturally occurring organic copper complexes. He found that copper in plants was largely in the form of a copper-glycinate complex. This compound and other soluble complexes in the plant were absorbed intact through the intestinal mucosa and were more biologically useful than supplements of inorganic copper. Mills (1957) stated that feeding freeze-dried herbage to copper-deficient rats gave consistently greater responses in growth, hemoglobin level, and liver copper storage than did dosings with a level of $CuSO_4$ that was equal to the total amount of copper found on analysis of the herbage. Mills (1958) suggested that the formation of water soluble complexes by rumen microorganisms may be necessary before copper can be absorbed. He also stated that it may be difficult for the free ion to be absorbed from the alkaline milieu of the small intestine.

The copper in wheat germ, whole wheat, alfalfa, brewers yeast, pork heart, pork liver, copper caseinate, glycine amide biuret, alanine amide biuret, hemocyanin, cysteine cuprous mercaptide, copper aspartate, copper citrate, copper nucleinate, copper pyrophosphate, and copper sulfate was found (Schultze et al., 1934, 1936a) to be readily available to severely anemic rats for hemoglobin formation. Only copper hematoporphyrin was found to be unavailable. This led Schultze et al. (1936a) to state that the availability of natural forms of copper is apparently of little practical importance. McHargue (1926) also stated that copper in green leaves, seeds, and vital organs of animals has a greater biological potency than an equal proportion of a crystalline salt.

Dye and O'Hara (1959), Fearn and Habel (1961), and Morgan et al. (1962) have found copper glycinate to be a very successful means of supplying copper to ruminants. Oral adminstration and subcutaneous and intravenous injections have all been successfully used with this compound. Twelve copper compounds, namely, sulfate, acetate, citrate, glycinate, oxychloride, borogluconate, naphthenate, oleate, asparaginate, versenate, caseinate, and ammonium sulfate were injected subcutaneously by Harvey and Sutherland (1953). Only the oxime, oxychloride, and naphthenate failed to give satisfactory liver copper storage.

Copper in Biological Systems

Mallory (1925) stated, "The reason that copper is so generally found in minute quantities in human organs is not due to its being a normal constituent of the tissues, but because man is constantly exposed to taking the metal into his system through foods and drinks

contaminated with it." Marston (1952) stated that copper is probably a functional part of all living cells and that the cellular concentration of copper closely resembles that of the metabolic gradient, indicating a relationship between copper and cellular activity.

The Copper Requirements of Cattle and Sheep

A copper content of 4-10 ppm in the total ration has generally been considered to meet the requirements of farm animals. Underwood (1962) summarized the pertinent data and stated that pastures containing less than 4 ppm of copper induced subnormal blood and liver copper levels and the typical copper deficiency symptoms in cattle and sheep; however, 4-6 ppm of copper met requirements of cattle and British breeds of sheep, while 6 ppm in the pasture was necessary for Merino sheep. Slightly higher requirements for sheep have been suggested by other workers. Beck (1941) considered 7-8 ppm to be a minimum requirement. Dunlop et al. (1930) stated that 1% $CuSO_4 \cdot 5H_2O$ in the salt (calculated to be 25 ppm in the total ration using National Research Council (1957) requirements) fed to ewes had a greater beneficial effect than 0.3% (7.3 ppm) in preventing swayback in lambs.

The National Research Council (N.R.C., 1958) set the copper requirements of beef cattle between 4 and 8 ppm. Harvey et al. (1961) found that 4 ppm of copper maintained liver stores in cattle, while Jamieson and Russell (1946) reported a copper deficiency in cattle fed a diet containing 2.5 ppm of copper. Human copper requirements (De, 1949) are about the same as those for cattle and sheep, but horses have a much lower requirement (Schutte, 1964).

The Absorption, Excretion, and Transport of Copper.

Little is known about the mechanism by which copper is absorbed or the site(s) of absorption. However, Mills (1956, 1958) reported that neutral or anionic organic copper complexes were more readily available than $CuSO_4$. The iron-copper-nucleoprotein complex isolated from fish muscle also produced greater regeneration of hemoglobin than ionic iron or copper (Saha and Guha, 1941). These limited observations suggest that copper is absorbed as a complex.

In contrast to iron (Bothwell et al., 1958; Pirzio-Biroli and Finch, 1960), the absorption of copper from the gastrointestinal tract is not regulated by body stores (Adelstein and Vallee, 1962). Tompsett (1940) observed that calcium prevents and hydrochloric acid enhances the absorption of copper. Greatest absorption of copper was obtained when the intestinal pH was less than 4.0. This led Tompsett to state that copper is absorbed from the upper part of the small intestine where the contents are still very acid. Sachs et al. (1943) stated that $CuSO_4$ introduced into Thiry's fistula loops established in the intestines of dogs was absorbed only from those originating from the upper levels of the jejunum and little or none from the distal and middle segments. Adelstein and Vallee (1962), however, suggested that the metal may be absorbed from the stomach. This postulation is supported by work reported by Earl et al. (1954), Cartwright et al. (1954a,b) and Bearn and Kunkel (1955). These workers observed a prompt rise in plasma radioactivity following the oral administration of ^{64}Cu which indicates absorption from the upper alimentary tract.

Copper is unlike iron not only in its absorption, but also in its excretion. Once iron is absorbed it is lost only in minute quantities, except through bleeding, while copper is continually lost, at a slow rate, through the stool. Gubler (1956) suggested that copper homeostatis in man is accomplished by an adjustment of the rate of excretion to that of absorption. However, Boughton and Hardy (1934) stated that in sheep, copper is eliminated at a rate so slow that animals continued to die from chronic copper poisoning for five months after the source of copper was removed. Beck (1963) observed similar results with sheep, but found that rabbits lost the metal very rapidly from the liver. Rats are apparently similar to sheep in their ability to store copper (Boyden et al., 1938). These differences in ability to excrete copper from the body may explain species and breed differences in susceptibility to copper toxicity. However, Beck (1961) reported that the high liver copper of the duck was not due to an inability to regulate storage, as injected copper was rapidly excreted.

Comar et al. (1948) used ^{64}Cu to estimate the amount of copper absorbed by ruminants. Seventy-five percent of an oral dose of copper was found in the feces and 5% in the urine. A similar retention of copper (20%) has been reported by Chapman and Bell (1963). The possibility that a considerable proportion of the copper in the feces may have been absorbed and later excreted into the intestine has been excluded by the observations of Comar et al. (1948) that only 3% of an intravenous dose was found in the stool.

Bile always contains copper and is the main vehicle for its removal from the liver (Flinn and Inouye, 1929). The importance of

the liver, kidney, and intestinal wall in the excretion of ^{64}Cu from dogs was investigated by Mahoney et al. (1955). The bile duct was ligated and the flow of bile diverted into the urinary bladder. About 7-10% of an intravenously administered dose of copper was excreted in the bile, about 1.5% passed directly through the intestinal wall, and about 0.6% was excreted in the urine. Leverton and Binkley (1944) also suggested that the intestinal wall permits only one-way passage of copper. Scoular (1938), Comar (1950), Porter (1951), and Beck (1956) stated that urine is not a major excretory pathway for copper and that normally less than 3-4% of the dietary copper will be found in the urine.

Other pathways of copper excretion have been investigated. Mitchell and Hamilton (1949) found a negligible quantity of copper in human perspiration. Milk is low in copper with values between 0.15 and 0.60 ppm being reported (Cox and Mueller, 1937; Elvehjem et al., 1929a; Grebennikov et al., 1964). Eggs are low in copper (0.5-0.7 ppm) and do not offer a major pathway of excretion for poultry (Elvehjem et al., 1930; Smith and Gray, 1948).

Gubler et al. (1953a) reported that 82-96% of the copper in plasma is protein bound and will not react with diethyldithiocarbamate solution. Starcher et al. (1964b) found that in normal sheep, swine, and chickens; 84, 74, and 30%, respectively, of the plasma copper was in this form. The copper-protein fraction of plasma was first isolated by Mann and Keilin (1938) and is termed ceruloplasmin. It contains about 0.34% copper (eight atoms per molecule), has a molecular weight of 151,000, and is an α_2-globulin (Holmburg and Laurell, 1947, 1948, 1951; Aisen and Morell, 1964).

The administration of copper, either orally or by injection, produces a slow rise in plasma copper, followed by a rapid fall in about 4-5 hours and a slower secondary rise (Earl et al., 1954; Bearn and Kunkel, 1955). Much of the copper in the initial rise is loosely bound to albumin and reacts directly with diethyldithiocarbamate, while that in the secondary rise is in the ceruloplasmin form (Gubler et al., 1953a). It has been suggested (Adelstein and Vallee, 1962) that the loosely bound albumin copper is a transport form and is easily deposited in the liver and other organs. Red blood cell copper remains constant and is not greatly affected by the copper status of the individual (Cartwright et al., 1948; Wintrobe et al., 1953; Bush et al., 1956).

Copper Levels in Animal Organs, Tissues, and Fluids.

Table I shows the copper content of the liver, heart, lungs, spleen, and kidney of several species of animals. The heart, lungs, spleen, and kidney are quite uniform in copper content and differences between species are small. Liver copper is quite variable and species differences do exist. Beck (1956) reported that the livers of man, rats, rabbits, whales, snakes, cats, dogs, foxes, kangaroos, pigs, emus, crocodiles, and fowl usually contain 10-50 ppm of copper on a dry basis. However, the livers of sheep, cattle, ducks, frogs, and fish contain between 100 and 400 ppm of copper.

Underwood (1962) stated that the pituitary, thyroid, and thymus are examples of glands with a low copper content. The spleen and lungs are organs with medium copper content while the liver, heart, kidneys, hair, and brain, in that order, are highest in the metal. Widdowson (1950) also reported that the liver contained a greater

TABLE I

THE COPPER CONTENT[a] OF ORGANS FROM DIFFERENT SPECIES (CUNNINGHAM, 1931)

Species	Liver	Heart	Lungs	Spleen	Kidney
Human, adult[b]	24.9	---	---	5.2	17.5
Bovine, adult[c]	77.0	15.6	5.3	2.9	19.7
Bovine, newborn	470.0	14.8	4.9	4.8	15.7
Bovine, foetus	262.8	10.4	3.6	5.4	8.5
Sheep, adult	236.6	17.9	9.6	5.0	17.8
Horse, adult[c]	14.8	17.6	6.8	3.2	28.9
Pig, adult[c]	41.3	14.9	5.3	6.0	21.1
Pig, few days old	232.8	12.8	3.4	6.7	14.7
Dog, 9 days old	98.2	17.4	6.2	---	14.2
Cat, adult[c]	25.3	14.4	3.8	5.2	10.1
Guinea-pig, adult	17.0	21.2	9.5	---	19.9
Rabbit, adult	9.2	22.3	8.1	---	13.7
Rat, 90 gm.[c]	10.0	27.8	9.5	8.1	22.6
Badger, adult[c]	21.7	12.8	5.6	3.0	9.4
Domestic fowl, adult[c]	12.4	14.9	2.4	---	11.7

[a] Measured in ppm on a dry basis

[b] Average of 3 series of analyses

[c] Average of 2 series of analyses

proportion of the body's copper than any other tissues measured. In addition to the values reported in Table I, several other workers have reported "normal" values. Schultze et al. (1936b) showed pig liver, spleen, and ribs to contain 41.9, 8.1,and 6.9 ppm, respectively. Bruckmann and Zondek (1939) reported 34.6 ppm as the normal value for human liver, while Ryley et al. (1961) stated that sheep livers contain more than 100 ppm. The copper content of tissues such as the eye (Bowness et al., 1952; Bowness and Morton, 1952) have been studied. These workers reported that the iris of trout contained 105 ppm; whereas, in sheep it contained only 50 ppm.

The various organs of the body vary in their ability to store copper. Lindow et al. (1929b) fed rats 5 mg. of copper per day and observed approximately 1.6-, 2-, 5-, and 20-fold increases in bone, kidney, spleen, and liver copper concentrations, respectively. Little change in heart, brain, muscle, and skin copper was observed. Underwood (1962) stated that conditions of copper excess or deficiency do not greatly affect the copper content of the endocrine glands, muscles, brain, heart, and skin. However, a high copper intake increases the copper in liver, kidney, spleen, and lungs while in deficiency the blood, liver, kidney, spleen, and hair copper contents are rapidly reduced.

Reports showing the copper content of whole blood from various species are summarized in Table II. The average copper level in the blood of pigs, sheep, cattle, and man is about 110 mcg. per 100 ml. Several species, such as the chicken, duck, kangaroo, frog, and salmon have much higher blood copper levels. Cartwright (1950) reported the

TABLE II

THE COPPER CONTENT OF WHOLE BLOOD FROM NORMAL, MATURE INDIVIDUALS OF DIFFERENT SPECIES

Species	mcg./100 ml.	Reference	
Man	114	Kehoe et al.	(1940)
Man	185-229	Tompsett	(1934)
Man	70-117	Lahey et al.	(1953a)
Horse	50	Elvehjem et al.	(1929b)
Horse	179-208	Tompsett	(1934)
Sheep	156-180	Tompsett	(1934)
Sheep	75-135	Beck	(1956)
Sheep	80-200	Albiston et al.	(1940)
Cow	70-170	Beck	(1956)
Cow	180-223	Tompsett	(1934)
Pig	165-181	Tompsett	(1934)
Pig	103	Kehoe et al.	(1940)
Rabbit	139-155	Tompsett	(1934)
Guinea pig	40-58	Beck	(1956)
Guinea pig	179-192	Tompsett	(1934)
Domestic fowl	110-470	Beck	(1956)
Domestic duck	220-450	Beck	(1956)
Kangaroo	270-390	Beck	(1956)
Frog	250-670	Beck	(1956)
Salmon	450-640	Beck	(1956)

following values, in mcg. per 100 ml., for whole blood, plasma, and cellular copper in normal, adult humans: 93.9, 109.5, and 75.4, respectively.

Dempsey et al. (1958) stated that there was a high correlation, although a value was not reported, between plasma and liver copper. Other workers (Loosmore and Allcroft, 1951; Dent et al., 1956; Hill et al., 1962; Steinberg, 1964) have stated that blood and plasma copper do not consistently reflect liver storage. Hill et al. (1962) reported that when serum copper values were below 40-50 mcg. per 100 ml. the liver copper values were below 10-20 ppm, but when serum copper values were above 40-50 mcg. per 100 ml. a wide range in liver stores was found. Hemingway et al. (1962) reported a correlation of 0.64 between blood and liver copper when liver contents were less than 50 ppm. A similar correlation (0.53) was reported by Hemingway et al. (1964) when liver contents were less than 25 ppm. MacPherson et al. (1964) stated that blood copper contents were below 70 mcg. per 100 ml. when liver contents were less than 50 ppm. Normal and high liver copper levels were not highly associated with blood copper levels. Copper storage is uniform throughout the liver (Eggers and Mercedes, 1964). Plasma copper is apparently a better indicator of change in copper status than blood copper since Wintrobe et al. (1953) found that during copper deficiency in pigs the reduction in plasma copper was much greater than the reduction in cellular copper.

Factors Affecting Copper Absorption and Tissue Levels.

Age, certain hormones, pregnancy, and some diseases affect the concentration of copper in tissues and blood. There is apparently no

sex influence on copper storage (Underwood, 1962) other than in the salmon (Beck, 1956). However, pregnancy produces a two- to three-fold increase in the serum copper of women (Johnson, 1961), but there is apparently no change in the blood copper of pregnant ewes (McDougall, 1947b). In contrast, Butler and Barlow (1963) reported lower plasma copper contents of pregnant ewes.

In all species studied, except sheep, the copper content of the liver is higher at birth than in the adult (Wilkerson, 1934; McFarlane and Milne, 1934; Bruckmann and Zondek, 1940). Lorenzen and Smith (1947) reported copper contents of livers, in parts per million, from guinea pigs, rabbits, and rats of 67, 37, and 58 in newborn and 23, 23, and 34, respectively in adults. Nusbaum et al. (1958) reported values on the copper content of human livers at different ages. These values were: 0 to 3 months, 324.9 ppm, and 3 months to 1 year, 23.5 ppm. Sheep are the exception and an average value for newborn lambs, as reported by Cunningham (1946a), is 168 ppm. McDougall (1947a) reported a similar value for newborn lambs and observed that mature sheep had liver values as high as 600 ppm.

Blood copper, urine output of copper, and liver stores of copper are affected when the basal metabolic rate of an animal is altered. Kozelka and Pedrero (1952) stated that hypophysectomized rats and rats with alloxan diabetes have an increased urinary copper output. Robertson and Broome (1957) observed that the administration of thyroxine elevated blood copper by 75% and that thiouracil lowered blood copper by 50% in sheep. Diethylstilbestrol also caused a significant increase in serum copper in swine (Cox and Hale, 1960). Markowitz et al. (1955) and Gubler et al. (1952a) observed a rise in blood

copper, but no increase in red cell copper, in animals with bacterial and turpentine infections. The rise in blood copper was not observed when a low copper diet was fed.

Three maladies are known that produce hypocupremia in humans, but none have been shown in farm animals. These ailments are: Kwashiokor or dysproteinemia (Lahey, 1957; MacDonald, 1961), Wilson-Uzman's disease (Lahey et al., 1953b; Bearn, 1957), and the nephrotic syndrome (Cartwright et al., 1954a). Brenner (1959) stated that worm infestations in dairy calves significantly lowered blood and liver copper. Hypercupremia has been observed in several pathological and nutritional conditions (Locke et al., 1932; Cartwright, 1950). These conditions include leukemia, Hodgkin's disease, virus and microbial infections, myocardial infarctions, thyrotoxicosis, pernicious anemia, sickle cell anemia, diabetes, carcinoma, hyperthyroidism, hemochromatosis, malaria, portal and biliary cirrhosis, iron deficiency anemia, allergic reactions, and arsenic poisoning.

Copper Containing Pigments and Enzymes

Turacin, the red pigment found in the feathers of an African bird, is an example of a copper porphyrin (Rimington, 1939). Coproteins which have been isolated and characterized include hemocyanin (Redfield, 1950), erythrocuprein (Markowitz et al., 1959), hepatocuprein (Mohamed and Greenberg, 1954), and milk copper protein (Dills and Nelson, 1942).

The following enzymes either contain copper or it is associated in their activities; cytochrome oxidase (Keilin and Hartree, 1938; Wainio et al., 1958; Griffiths and Beinert, 1961), uricase (Mahler et al.,

1955), tyrosinase or polyphenol oxidase (Keilin and Mann, 1938; Mallette, 1950; Fritzpatrick et al., 1950; Kertesz and Zito, 1957), glutathione oxidase (Ames and Elvehjem, 1945), butyryl coenzyme A dehydrogenase (Mahler, 1953, 1954), catalase (Schultze and Kuiken, 1941; Adams, 1953), δ-aminolevulinic acid dehydrase (Iodice et al., 1958), ascorbic acid oxidase (Dawson, 1950; Dunn and Dawson, 1951), laccase (Nakamura, 1958), and β-mercaptopyruvate transsulferase (Kun and Fanshier, 1959). Another copper containing protein has been isolated from sheep's hide that catalyzes the aerobic oxidation of L-cysteine (Scaife, 1956b).

Copper Deficiency

Because of their low copper contents, milk and skeletal muscle have been used in rations where it was desired to produce an experimental copper deficiency (Waddell et al., 1929; Foster, 1931; Moore, 1962). Nearly all other food materials normally contain sufficient copper (Lindow et al., 1929a; Greaves and Anderson, 1936) to meet daily requirements. Neal et al. (1931) were the first to demonstrate that a deficiency of copper occurs in livestock. Bennetts (1932, 1933), Bennetts and Chapman (1937), Bennetts and Hall (1939), and Filmer (1933) reported early observations on copper deficiency in Australia. Later reports (Stewart, 1932; Sjollema, 1933, 1938; Innes and Shearer, 1940; Cunningham, 1944; Allcroft, 1946; Davis, 1950; Henderson, 1957; Jensen et al., 1958) have shown that it exists in Great Britain, New Zealand, South Africa, South America, Canada, the Netherlands, Czechoslovakia, Finland, Florida, and Colorado.

Copper deficiency has been referred to by many names. Bush sickness in New Zealand, nakuruitis in Kenya, salt sick in Florida, pine and teart in Scotland, coast disease, enzootic ataxia, and enzootic marasmus in Australia, falling disease in Africa, and licking disease, swayback, swingback, and steely wool in Europe are all terms applied to the disease.

External lesions

Marston (1950) stated that the earliest symptoms of copper deficiency in sheep are those associated with the wool. Copper is responsible for the catalytic oxidation of the -SH group in prekeratin protein to the -SS- group of keratin in wool and hair. The failure of this reaction to take place during a lack of copper produces "steely" wool in sheep. Marston et al. (1948a,b), Marston and Lee (1948), and Lee (1956) have reported that 5 mg. (about 5 ppm) of copper daily was not sufficient to maintain the crimp of Merino sheep wool, although it was enough for normal reproduction. Other workers (Keil and Nelson, 1931; Cunningham, 1949; Hundley, 1950) have reported a lack of pigmentation of the hair coat of cattle, rats, and sheep during copper deficiency. This loss of pigmentation is due to a decreased tyrosinase activity during copper deficiency (Fresch, 1949; Lerner et al., 1950). This enzyme is responsible for the conversion of tyrosine to the pigment melanin.

Other gross deficiency symptoms that have been described (Bennetts et al., 1941; Davis et al., 1946; Allcroft and Parker, 1949; Teague and Carpenter, 1951; Dye and O'Hara, 1959; Dutt and Mills, 1960) are as follows: severe scours, muscular weakness, incoordination, a tucked up

and pained appearance, low milk production, lowered fertility, marked stunting of young stock, rough hair coat, depraved appetites, rickets-like swellings of the long bones, fractured bones in older cattle, lack of rigidity in leg joints, hocks excessively flexed, crooked forelegs, arching of the back, and sudden death. In addition to these symptoms, Davis (1951) stated that up to 10% of the calves born to deficient cows may have enlarged heads, missing bones, and abnormally formed bones.

Internal lesions

Copper analyses of various body tissues and fluids are generally accepted as sensitive indicators of copper deficiency. Cattle deficient in this metal have plasma copper levels of less than 50 mcg. per 100 ml. and liver levels of less than 30 ppm (Bennetts et al., 1948; Jamieson and Allcroft, 1950; Senior et al., 1954; Alexander and Harvey, 1957). Mills et al. (1963) reported blood values of 120 and 50 mcg. per 100 ml. for normal and deficient cattle and liver levels of 152 and 17 ppm on a dry basis for similar animals. It has been stated (Eden, 1941) that ewes with blood copper levels of 70 mcg. per 100 ml. may bear ataxic lambs. Howell and Davison (1959) reported normal values for liver, kidney, frontal pole (brain), cerebellum, caudate nucleus, and cerebral white matter of 109.08, 13.45, 15.53, 15.75, 19.26, and 8.15 ppm, respectively. Copper levels in deficient lambs for respective tissues were 16.14, 15.79 5.37, 4.04, 16.0, and 1.70 ppm. Swine apparently are able to exhibit lower blood copper levels and still appear normal. Schultze et al. (1936c) reported that in pigs a blood copper level of 20 mcg. per 100 ml. is sufficient for slow hemoglobin

formation, but that hematopoiesis stops if the level falls below 10 mcg.

Anemia is always produced during copper deficiency (Elvehjem and Hart, 1929, 1932; O'Dell et al., 1961). This anemia has been described as normocytic, normochromic in dogs (Maass et al., 1944; Van Wyk et al., 1953), macrocytic, hypochromic in ruminants (Marston et al., 1948a), and microcytic, hypochromic in chickens (Hill and Matrone, 1961), rats (Smith and Medlicott, 1944) rabbits (Smith et al., 1944), and swine (Cartwright et al., 1956). A reduced number of cells has been observed in all species.

Several compounds of biochemical interest have been found to be affected by a lack of copper. Cytochrome oxidase (Schultze, 1939; Van Reen, 1954; Mills and Williams, 1962) has been of special interest because of its key role in the electron transport system. Schultze (1941) suggested that the cytochrome oxidase activity of bone marrow is required for hematopoiesis and anemia is produced because copper is essential for the formation and maintenance of cytochrome oxidase. Gubler et al. (1957) reported that the glutathione content of liver was reduced, uric acid and allantoin excretions were increased, total tissue iron was reduced, and heart weight was increased 200% during copper deficiency. Cytochrome a has been found to be greatly reduced and cytochromes b and c slightly reduced during a lack of this metal (Cohen and Elvehjem, 1934). Gallagher et al. (1956a,b) have assayed for a wide variety of enzymes and metabolic end products in copper deficient rats. Phospholipid synthesis was depressed due to failure of acyl CoA to condense with α-glycero-phosphate. Long-chain fatty acid and RNA syntheses were normal. The α hemoglobin fraction was almost completely absent in copper deficient

tissue and the mitochondria were susceptible to aging due to a loss of nucleotides and glutathione. These workers stated that during copper deficiency mitochondria lose the capacity to oxidize any substrate due to low cytochrome oxidase activity. Tsai et al. (1964) also reported that deficient guinea pigs had reduced phosphorus concentrations in the lipid fraction of the brain.

Pathological changes that have been reported during copper deficiency are: spleen parenchyma are atrophied, blood volume is reduced, all tissues are pale, the heart is flabby and lacks tone (Neal et al., 1931); demyelination of the central nervous system, degenerate nuclei, axones, and dendrites of the large motor neurons (Jensen et al., 1958; Fell et al., 1961); abnormally shaped erythrocyte nuclei in birds (Simpson et al., 1963); thickening of Bowman's capsule of the kidney, accumulation of cellular debris in the subcapsular space, and degenerate glomeruli surrounded by fibrous tissue (Blakemore and Venn, 1950). Other workers (Bennetts et al., 1942, 1948) have reported abnormalities of the heart such as: pale myocardium, fibrosis of the myocardium, and atropy of heart fibers. Shields et al. (1962) concluded that copper is needed for the maintenance of the structural elements of the heart and arteries. This is supported by the work of Tsai et al. (1964) and Starcher et al. (1964a) who reported reduced elastin contents of aortas from copper deficient animals.

Copper Toxicity

Copper poisoning is usually not produced by native foods, but by overfeeding of copper salts in worming agents (Boughton and Hardy, 1934), industrial contamination in the vicinity of copper mines (Garner, 1957), and by grazing orchards after spraying with bordeaux mixture (Muth, 1952).

Cattle are much more resistant to copper poisoning than are sheep (Ferguson, 1943; Cunningham, 1946b, 1950). There are also breed differences in sheep, as the British breeds are more prone to copper poisoning than are pure Merinos and Border-Leicesters (Albiston et al., 1940; Marston and Lee, 1948; Marston, 1952). Swine can apparently withstand larger amounts of copper than cattle or sheep. In fact, several workers have reported that the addition of 150 to 250 ppm of copper to the diets of swine had beneficial effects (Mitchell, 1953; Barber et al., 1955a, b; Bowler et al., 1955; Allen et al., 1958, 1961; Hawbaker et al., 1961).

Garner (1957) stated that the toxic single dose of $CuSO_4$ administered orally to sheep was approximately 20 mg. of copper per kg. of body weight, while in cattle this dose is about 200 mg. per kg. of body weight. The daily intake of copper required to ultimately be toxic (chronic copper poisoning) to sheep is very small. Garner (1957) reported that the addition of 25 mg. of available copper to a normal daily ration for sheep can produce dangerous amounts of liver storage. The high resistance of cattle to copper toxicity was illustrated by Kidder (1949) when a 500 lb. steer was fed 5 gm. of $CuSO_4$ daily for 122 days before death occurred.

Several compounds have been tested in an attempt to increase the excretion of copper from human tissue (Walsche, 1956; Cumings, 1962). Two compounds that increase copper excretion via the urine, and may be useful in animal nutrition, are dimercaprol (British antilewisite) and penicillamine. Bull et al. (1956) have also used FeS to reduce copper stores in sheep.

External lesions

Boughton and Hardy (1934) and Rose and Edgar (1936) were among the earliest workers to report observations on copper poisoning. Chapman et al. (1962) reported antemortem symptoms in cattle as: loss of weight, weakness, incoordination, dullness, yellow coloring about the eyes and nostrils, and hemoglobinuria. A bloody nasal discharge (Garner, 1957) is often observed in addition to hemoglobinuria.

Internal lesions

The main post-mortem findings of copper poisoning are generalized icterus and hemolysis. The liver is slightly enlarged, yellow in color, and friable, and the gall bladder is distended with a very viscous liquid (Kidder, 1949; Jones, 1954). The spleen is enlarged, soft, and dark colored, while the kidneys are enlarged and show hemorrhagic mottling when removed from the capsule. The lungs are often poorly collapsed. There may also be excess pericardial fluid and epicardial hemorrhage (Garner, 1957).

Chapman et al. (1962) observed only slight, if any, histopathological changes due to copper toxicity in the esophagus, rumen wall, omasum, abomasum, gall bladder, pancreas , lung, heart, and skeletal muscle. These workers reported liver necrosis and fatty and albuminous degeneration of the liver and kidneys.

The hemolytic crisis that is the cause of death in copper poisoning has been associated with the liberation of large amounts of copper from the liver. The blood copper level may rise above 200 mcg. per 100 ml. a short while before death (Sutter et al., 1958). Todd and Thompson (1963a) did not agree that the elevated blood copper per se

is the cause of hemolysis or methemoglobinurea. These workers reported the blood copper to be 5-20 times normal, blood glutathione about zero, and methemoglobin about 5 gm. per 100 ml. Todd and Thompson (1963b) have also shown that serum-glutamic-oxaloacetate transaminase activity is elevated several weeks prior to the hemolytic crisis.

The analysis of liver is of value in the diagnosis of copper poisoning. Albiston et al. (1940) stated that the liver copper content of normal sheep rarely goes over 500 ppm on a dry weight basis. From liver copper analyses of 12 sheep that had died of copper poisoning, two were under 1000 ppm, two between 1000 and 2000, and eight over 2000 ppm. Shand and Lewis (1957) reported similar values for cattle; as the copper content in dry liver of all deaths was over 1300 ppm.

Interactions of Copper With Other Minerals, Vitamins, and Protein

Iron

The blood of copper deficient animals contains less than normal amounts of hemoglobin (Hart et al., 1928; Lahey et al., 1952) and decreased numbers of erythrocytes (Stein and Lewis, 1933; Smith and Medlicott, 1944). Cassidy and Eva (1958) have also observed lowered hemoglobin contents of pigs fed high copper diets. Hill and Matrone (1961) stated that the main function of copper appears to be in maintaining a normal hematological picture in relation to the production of red blood cells and may have little, if anything, to do with the actual production of hemoglobin. In contrast, Anderson and Tove (1958) reported that copper was needed for the synthesis of "haem" in vitro.

The Utah workers (Cartwright, 1950; Chase et al., 1952a,b; Gubler et al., 1952b,c; Wintrobe et al., 1953) have reported extensive studies

on the influence of copper on iron absorption and metabolism in pigs and rats. Their data have shown that there was no lowering of liver iron in copper deficient rats if iron was injected parenterally; copper deficient swine, fed ample iron, had low total body iron contents; copper alone did not overcome copper deficiency anemia; iron was not absorbed from the intestine if tissue copper was low as the amount of copper in the tissues, but not in the feed, influenced the amount of iron absorbed; plasma iron values increased when copper deficient swine were fed a diet containing copper, but not iron which indicates that copper is concerned with the mobilization of iron from body tissue; and large doses of iron injected parenterally did not overcome the anemia which suggested that copper deficient swine could not properly metabolize or incorporate iron into the hemoglobin molecule. Muntwyler and Hanzal (1933) also suggested that copper was required for the mobilization of iron from body tissue.

Molybdenum and Sulfur

Molybdenum is an essential element in that it is required for xanthine oxidase activity (DeRenzo et al., 1953; Totter et al., 1953). However, in the United Kingdom, Australia, New Zealand, the Netherlands, and Florida conditions exist where the molybdenum level of pastures is high (20-100 ppm, dry basis), while the copper content (5-20 ppm) is low to normal (Ferguson et al., 1938, 1943; Lewis, 1943a,b; Stewart et al., 1946). The condition produced in these areas is referred to as teart in cattle and swayback in lambs. Lewis (1943a,b) stated that acid soils do not produce teart forage even if the molybdenum content is high, and that clovers have a great ability to absorb molybdenum

from the soil. Cattle are more sensitive to molybdenum excess than sheep (Cunningham et al., 1959). Data by Jeter and Davis (1951) and Kratzer (1952) have indicated that non-ruminants (chickens and rats) can stand higher levels of molybdenum than ruminants.

The interaction between molybdenum, copper, and sulfate was first studied by Dick and Bull (1945) and Dick (1952, 1953a,b,c). Dick's observations on gains and blood, and liver copper and molybdenum levels with sheep fed chaffed lucerne and chaffed oaten hays (high and low sulfate, respectively) contributed much background information for further studies in this area. The molybdenum-copper interaction may be quite different, depending on the level of inorganic sulfate in the diet. When the sulfate content of the ration is low, high levels of molybdenum may increase blood and liver copper (Wynne and McClymont, 1955; Allcroft and Lewis, 1956; Miller et al., 1956). With adequate copper and low molybdenum levels, high sulfate rations will result in a depletion of copper from body tissues (Wynne and McClymont, 1955, 1956; Mylrea, 1958; Evans and Davis, 1963). A combination of high molybdenum and sulfate also results in a depletion of blood and liver copper (Cunningham et al., 1959; Harvey et al., 1961). In contrast, Underwood (1962) stated that neither molybdenum nor sulfate alone interferes with copper retention.

Dick (1954b) observed a loss of crimp in the wool of sheep within seven days after feeding high molybdenum and sulfate diets. He also noted that blood copper levels were elevated (Table III), but the copper was apparently not available as indicated by the copper deficient character of the wool. Dick (1956a) summarized the available data by stating that under conditions of high molybdenum and sulfate intake,

TABLE III

THE EFFECT OF MOLYBDENUM AND SULFATE INTAKE ON BLOOD COPPER LEVELS IN SHEEP FED 10 MG. OF COPPER PER DAY (DICK, 1954b)

Molybdenum intake mg./day	Sulfate intake gm./day	Percent increase in blood copper
15	1.1	1.4
15	1.8	1.1
15	3.1	9.7
15	5.7	15.6
30	1.1	5.2
30	1.8	9.0
30	3.1	15.3
30	5.7	16.7
60	1.1	15.3
60	1.8	13.5
60	3.1	26.1
60	5.7	67.1
90	1.1	25.3
90	1.8	30.3
90	3.1	46.1
90	5.7	74.1

the absorption of copper is reduced and tissue stores become depleted to a state of copper deficiency. There is no effect on blood copper unless the molybdenum intake is sufficiently great, that in spite of high sulfate intake, the concentration of molybdenum in the tissues becomes high enough to block copper excretion. When this occurs, the absorption as well as excretion of copper is blocked and copper is mobilized from the animal's reserves. As the concentration of copper in the blood increases, the mobilization of storage copper is stopped by a mass action effect, and despite high blood copper and only slightly lowered liver copper levels, copper deficiency will occur.

As stated above, many of the symptoms produced by high molybdenum-high sulfate diets are similar to those of copper deficiency (Kulwich et al., 1953) and are overcome by adding copper to the diet (Neilands et al., 1948). Other workers, however, have suggested effects of high molybdenum intakes other than on copper metabolism (Britton and Goss, 1946; Van Reen and Pearson, 1954). Mills et al. (1958) have studied metabolic effects of molybdenum toxicity in the rat and found that liver uricase, sulfide oxidase, and kidney alkaline phosphatase activities were depressed, while glucose-6-phosphatase and liver alkaline phosphatase activities were elevated. Femur alkaline phosphatase activity is also depressed (Johnson and Miller, 1961). Cunningham and Hogan (1959) stated, "Since the symptoms associated with molybdenum excess are those of copper deficiency and since it has been shown that there is no evidence of direct toxicity of molybdenum, it would appear that high molybdenum in pasture is likely to be harmful only when conditions are right for molydenum to induce a pathological copper deficiency in grazing sheep."

The work reported above has been followed up by studies to determine the mechanisms underlying these interrelationships. It has been indicated that methionine supplements protect against molybdenum toxicity in sheep (Scaife, 1956a). Van Reen and Williams (1956) suggested that this effect of methionine was due to its oxidation to sulfate, since orally administered sulfate, thiosulfate, cystine, or methionine had protective effects against molybdenum when fed to rats. These compounds reduced the content of molybdenum in the urine and tissues, which suggests that sulfates prevent the absorption of molybdenum. This effect of sulfate on molybdenum absorption has also been reported by Ferguson et al. (1938), Dick (1956b), and Underwood (1962).

Lewis (1954) and Anderson (1956) have shown that sulfide is a normal metabolic product of sulfate reduction in the rumen. Lewis (1954) reported that the normal sulfate-sulfur content in the dry matter of grass is about 0.3%, but may go as high as 1.0%. He suggested that H_2S was an intermediate in the reduction of sulfate and that 70-100% of the sulfate in the rumen is converted to sulfides. Halverson et al. (1960), in an attempt to determine the mechanism of the molybdenum-copper interaction, reported that excess molybdenum depressed growth and produced anemia and diarrhea in rats fed a low copper diet. The addition of copper alleviated the anemia and diarrhea. Cystine (0.94%) led to increased anemia, diarrhea, and deaths when it was added to the low copper diet. These workers suggested that a drop in liver sulfide oxidase, caused by molybdenum, leaves the animal open to sulfide poisoning and that the copper deficiency may be due to the precipitation of CuS. Siegel and Monty (1961) reported that molybdenum

decreased sulfide oxidase activity and that both copper and sulfate returned it to normal. Scaife (1956b) reported the inhibition of two copper containing enzymes by molybdenum and suggested molybdenum may interact with copper by inhibiting copper containing enzymes.

Phosphorus

Davis et al. (1953) found that in Florida, where copper deficiency is complicated by high levels of molybdenum, abnormalities in bone formation occur. He reported that molybdenum causes a loss of phosphorus from the body as well as a depressed absorption of feed phosphorus if the copper content of the ration is low. A similar bone condition has been observed in copper deficient dogs (Baxter, 1951). These typical symptoms of rickets are overcome if the animals are fed copper sulfate (Comar et al., 1949; Arrington and Davis, 1953).

Shirley et al. (1950, 1951), using ^{32}P, ^{99}Mo, and ^{64}Cu, found losses of phosphorus from the bodies of steers and rats to be two to three times normal when the diet contained less than requirement levels of copper and excessively high levels of molybdenum. These bone abnormalities have been associated with altered alkaline phosphatase activities. The activity of this enzyme is apparently increased in liver (Van Reen, 1954) and blood (Comar et al., 1949) during conditions of low copper, high molybdenum intake. These conditions produce a lower enzyme activity in the kidney (Van Reen and Williams, 1956; Mills et al., 1958).

Protein

Copper has a great chelating affinity for amino acids, peptides, and proteins (Chaberek and Martell, 1959; Dawson and Nair, 1950).

Flinn and Inouye (1929) stated that this close affinity is demonstrated by the retention of $CuSO_4$ in tissue following injection and the green staining of the intestinal walls by large oral doses of copper.

White et al. (1951) stated that the addition of casein to a purified diet, to give protein levels above 10%, reduced copper toxicity. McCall and Davis (1961) showed that 17.5% protein in rat rations inhibited the accumulation of toxic levels of copper in the liver, but a 10% protein ration had no protective effect. Ammerman et al. (1963) reported that lambs fed casein had significantly lower liver copper levels than those fed soya. In contrast, Bunch et al. (1961) reported no copper-protein interaction when protein levels of 16 and 22% were fed to baby pigs.

Vitamins A, E, Pantothenic Acid, and Choline

Shirley et al. (1962) observed that when vitamin A supplementation was increased from 150 to 2000 I.U. per head daily there was less copper deposition in the livers of swine. When the daily copper intake was increased from 22 to 172 ppm there was a concurrent increase in the level of vitamin A in the liver. Shirley et al. (1963) also reported that 25,000 I.U. of vitamin A per day significantly decreased copper deposition in the heart of steers. The addition of 250 mg. of vitamin E per day significantly increased the level of copper in the heart. In contrast to these interrelations of copper and vitamin A, it has been shown (Kamstra et al., 1953; Halverson and Hart, 1950; Halverson and Hendricks, 1955) that traces of a copper-iron-cobalt-manganese mixture destroyed vitamin A activity in mixed rations.

The addition of choline to a purified ration (White et al., 1951) increases the absorption of copper. Pantothenic acid has also been

associated with copper in that a deficiency of this vitamin may produce gray hair by blocking copper utilization for hair growth and melanin formation (Hundley and Ing, 1951). Less copper was found in the skin when pantothenic acid was deficient. Singer and Davis (1950) reported that 30 and 40 mcg. daily of calcium pantothenate overcame graying of rats on a copper deficient diet. Unna and Sampson (1940), however, found no response to calcium pantothenate. These copper-vitamin interactions apparently need further investigation.

Zinc

Sutton and Nelson (1937), Smith and Larson (1946), Gray and Ellis (1950), and Davis (1958) reported that zinc levels above 0.5% of the ration produced anemia and subnormal growth that were corrected by the addition of copper. Brink et al. (1959) observed zinc toxicity at 0.2% of the ration in swine fed about 5 ppm of copper. Excess dietary zinc also causes accumulation of zinc in liver and a rapid loss of liver iron and copper (Duncan et al., 1953; Grant - Frost and Underwood, 1958; Cox and Harris, 1960). Dick (1954a) reported that 20 mg. of zinc in the daily ration of sheep did not limit liver copper when the daily ration contained 30 mg. of copper, but a significant limitation was found when the zinc was increased to 100 mg. per day. Data presented by Magee and Matrone (1960) illustrate this copper-zinc interrelationship; with no zinc in the diet, liver copper, iron, and zinc were 15.7, 425.7, and 37.2 ppm, respectively; with 0.75% zinc they were 5.3, 232.5, and 398.7 ppm; and with 1.00% zinc, levels were 4.0, 161.9, and 452.2 ppm for respective minerals. In contrast, Cox and Hale (1962) reported that 0.4% zinc lowered liver iron, but not copper. Zinc at a

level of 0.2% did not effect either metal in swine.

Van Reen (1953) found that a high level of copper in the liver, such as may occur with copper toxicity, resulted in almost complete elimination of zinc from liver tissue. The mechanism of these interrelationships is obscure, but Sastry and Sarma (1958) have suggested that the antagonistic effect of zinc on copper was a reflection of an interference on iron metabolism by zinc. The antagonistic effect of zinc on copper has suggested an interference with copper-associated enzyme systems (Hill and Matrone, 1962). Serum alkaline phosphatase values are increased and cytochrome oxidase and catalase activities are decreased by feeding a high level of zinc (Van Reen, 1953; Luecke et al., 1958; Hoefer et al., 1960).

Zinc deficiency, as well as toxicity, apparently allows some minerals to accumulate at faster or slower than normal rates. Moses and Parker (1964) observed that zinc deficient rats (fed 2.5 ppm zinc) tended to accumulate copper and iron in all tissues, but calcium and magnesium in bone were lowered. These changes were overcome by 10 ppm of zinc.

Other Trace Minerals

Hill et al. (1964) stated that for an ion to be antagonistic to copper, it should have a valence shell that is isoelectric with that of copper, it should have the favored coordination number of four, and form complexes of similar configuration (tetrahedral). Hill et al. (1963a,b), Hill et al. (1964), and Britton and Hill (1964) have reported cadmium and silver to increase the severity of copper deficiency symptoms, such as decreased growth, aortic elastin content, and hemoglobin concentration. Hodgson et al. (1962) stated "There are apparently

other conditioned copper deficiencies in which unidentified factors are responsible for low availability of plant copper to animals."

EXPERIMENTAL PROCEDURE

Trial 1

Forty-nine lambs were randomly allotted, within sex, into nine groups of five and one group of four lambs. All sheep were drenched with a phenothiazine preparation and fed a ration containing urea and sulfate (Table IV) and 5.5 ppm of copper during a 7-day standardization period. At the end of this period and following a 17-hour shrink, the lambs were weighed (average weight, 24 kg.) and randomly placed on their respective treatments. Sulfate was the source of sulfur (0.20% S) and copper levels supplied by cupric carbonate were 0, 5.5, 11, 22, 44, 88, 176, 352, 704, or 1408 ppm of the diet.

The lambs were housed indoors in individual pens on concrete floors. Feed and water were provided free choice. Weights, without shrinking, were taken at 14-day intervals during the experiment and a final shrunk weight, after 17 hours away from feed and water, was taken at the termination of the 56-day growth trial. Initial and final hemoglobin values were determined by use of an AO Spencer Hb meter. Percent packed cells were determined using a micro-hematocrit. The data were analyzed statistically by analysis of variance.

Trial 2

Eighteen lambs with an average initial shrunk weight of 25 kg. were randomly allotted, within sex, to six groups of three lambs

TABLE IV

COMPOSITION OF PURIFIED DIETS

Ingredients	Urea diets		Purified soy diets	
	Sulfate %	Elemental sulfur %	Sulfate %	Elemental sulfur %
Corn starch	29.4	29.4	24.6	24.6
Dextrose	29.4	29.4	24.6	24.6
Cellulose[a]	30.0	30.0	30.0	30.0
Urea[b]	4.2	4.2	--	--
Purified soy protein[c]	--	--	13.6	13.6
Corn oil[d]	1.0	1.0	1.0	1.0
Polyethylene resin[e]	1.0	1.0	1.0	1.0
Choline chloride	0.1	0.1	0.1	0.1
Vitamins A and D[f]	0.02	0.02	0.02	0.02
Minerals	5.0[g]	5.0[h]	5.0[g]	5.0[h]

[a]Solka-Floc. B-W 20. Brown Co., Berlin, N. H.

[b]Crystalline urea. Courtesy John Deere Chemical Co., Pryor, Okla.

[c]Purified soybean protein. Nutritional Biochemicals Corp., Cleveland, Ohio.

[d]Mazola. Santoquin added to give 0.0125% in total ration.

[e]Alathon. E. I. Du Pont de Nemours, Inc., Wilmington, Del.

[f]20,000 I. U. and 2,500 U.S.P. units of vitamins A and D per gram.

[g]Composition of sulfate (0.20%S) diets, %; K_2CO_3, 44.33; $CaHPO_4$, 26.50; $MgSO_4$, 10.00; Na_2SO_4, 5.00, NaCl, 12.50; $FeSO_4$, 0.85; $MnSO_4 \cdot H_2O$, 0.23; $Na_2B_4O_7$, 0.25; $ZnSO_4 \cdot 7H_2O$, 0.30; KI, 0.0003; CaF_2, 0.004; $Cr_2(SO_4)_3$, 0.0008; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $CoCl \cdot 6H_2O$, 0.0009; Na_2SeO_4, 0.0005.

[h]Composition of elemental sulfur (0.20%S) diets, %: elemental sulfur, 4.00; K_2CO_3, 44.33; $CaHPO_4$, 26.50; $MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$, 8.15; Na_2CO_3, 3.70; NaCl, 12.50; $FeCl_2 \cdot 4H_2O$, 1.10; $MnCO_3$, 0.15; $Na_2B_4O_7$, 0.25; $ZnCO_3$, 0.13; KI, 0.0003; CaF_2, 0.004; $Cr_2(C_2H_3O_2)_6 \cdot 2H_2O$, 0.001; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $CoCl_2 \cdot 6H_2O$, 0.0009; Na_2SeO_4, 0.0005.

per treatment. Treatments in this 55-day growth trial consisted of a urea ration containing either sulfate or elemental sulfur and with copper levels of 5.5, 55, and 550 ppm being fed with each sulfur source, thereby providing a 2 x 3 factorial arrangement of treatments. The compositions of the rations are shown in Table IV. Statistical analyses were conducted by analysis of variance and when the F test was significant, orthogonal comparisons among copper levels were made. Plasma copper was determined by the method of Cartwright et al. (1945). Other details of experimental procedure were as described in Trial 1.

Trial 3

Eighteen lambs with initial shrunk weights of about 26 kg. were randomly allotted, within sex, to three per group. A purified soybean protein ration, containing sulfate and 5.5 ppm of copper, was fed during the 7-day standardization period. Treatments in this 56-day growth trial consisted of the purified soybean ration containing sulfate or elemental sulfur (Table IV) and copper levels of 5.5, 55, and 550 ppm being fed with each source of sulfur to give a 2 x 3 factorial arrangement of treatments. At the termination of the feeding trial, a sample of liver was removed by laparotomy and analyzed for copper by the method of Sandell (1959). Other details of procedure were as described in Trial 1.

Trial 4A

Liver samples were obtained for copper analysis from 20 lambs by laparotomy 16 days prior to the start of the trial. During this period, all lambs were fed a standard ration and were injected with

two million units of procaine penicillin G. The lambs were then assigned to treatment groups on the basis of initial liver copper level and sex (3 ewes and 2 wethers per treatment). Shrunk weights were taken and the animals placed directly on their experimental rations. Average initial shrunk weight in this 60-day trial was 30 kg. and the treatments were as follows: Urea with sulfate or elemental sulfur and purified soybean protein with sulfate or elemental sulfur, giving a 2 x 2 factorial arrangement. All rations contained 550 ppm of copper. Compositions of these rations are shown in Table IV, except that 5.0% cottonseed hulls replaced an equal amount of cellulose. Other procedures were as described previously.

Trial 4B

Four approximately equal groups of three wether lambs with average initial weights of 36 kg. were fed the rations described in Trial 4A. The animals were randomly assigned to treatments and placed in metabolism stalls described by Briggs and Gallup (1949) during which time a 20-day adjustment period was followed by successive 10-day preliminary and collection periods. During the adjustment period, each lamb was fed ad libitum and water was available at all times. The daily feed allowance for each animal was held constant during the preliminary and collection periods; the amount of feed given each animal was the same as that voluntarily consumed by that animal during the adjustment period. Feces and urine were collected and prepared for analyses by methods described by Tillman and Swift (1953). Nitrogen and sulfur contents of feeds, feces, and urine were determined by

procedures of A.O.A.C. (1960). Copper was determined by a method of Sandell (1959).

Trial 5

Twenty-four wether lambs having initial weights of about 40 kg. were allotted at random to eight groups of three per group. Treatments included sulfate or elemental sulfur as sulfur sources, urea or purified soybean as nitrogen sources, and copper levels of 5 and 100 ppm in a 2 x 2 x 2 factorial arrangement of treatments. Compositions of the rations are shown in Table IV, with the exception that 5.0% cottonseed hulls replaced an equal amount of cellulose in all rations. Calcium and phosphorus were determined by procedures of A.O.A.C. (1960). All other procedures were as described in Trial 4B, except each animal was fed 700 gm. of his assigned ration daily.

Trial 6

Thirty-two crossbred lambs were blocked into eight groups of four lambs on the basis of initial weight, sex, source, and feeding location. All sheep were wormed with a phenothiazine-lead arsenate bolus, placed in individual pens on slatted floors, and fed a standard ration for 14 days prior to the start of the experiment. Initial shrunk weights were taken after 17 hours off feed and water and the lambs randomly allotted, within blocks, to their respective treatments of: soybean meal, soybean meal plus 1.0% urea, autoclaved soybean meal, and autoclaved soybean meal plus 1.0% urea. The composition of the semipurified rations fed in a 2 x 2 factorial arrangement of treatments are shown in Table V. All rations contained 100 ppm of copper, 0.10% sulfur, and 2 ppm of molybdenum in addition to the levels of each supplied by the soybean

TABLE V

COMPOSITION OF RATIONS FED IN TRIAL 6

Ingredients, %	Soybean Meal		Autoclaved Soybean Meal	
	No urea	1.0% urea	No urea	1.0% urea
Soybean meal	20.0	20.0	20.0	20.0
Dextrose	22.0	21.5	22.0	21.5
Corn starch	22.0	21.5	22.0	21.5
Cellulose[a]	30.0	30.0	30.0	30.0
Corn oil[b]	1.0	1.0	1.0	1.0
Vitamins A and D[c]	0.02	0.02	0.02	0.02
Urea[d]	--	1.0	--	1.0
Minerals[e]	5.0	5.0	5.0	5.0

[a]Solka-Floc. B-W 20. Brown Co., Berlin, N.H.

[b]Mazola. Santoquin added to corn oil to give 0.0125% in total ration.

[c]20,000 I. U. and 2,500 U.S.P. units of vitamins A and D per gram

[d]Crystalline urea. Courtesy John Deere Chemical Co., Pryor, Okla.

[e]Composition of mineral mixture, %: elemental sulfur, 2.00; K_2CO_3, 44.33; $CaHPO_4$, 26.5; $MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$, 8.15; Na_2CO_3, 3.70; NaCl, 12.50; $FeCl_2 \cdot 4H_2O$, 1.10; $MnCO_3$, 0.15; $Na_2B_4O_7$, 0.25; $ZnCO_3$, 0.13; KI, 0.0003, $CoCl_2 \cdot 6H_2O$, 0.0009; CaF_2, 0.004; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $NaSeO_4$, 0.0005; $Cr_2(C_2H_3O_2)_6 \cdot 2H_2O$, 0.001; $CuCO_3 \cdot Cu(OH)_2$ (to give 100 ppm Cu in total ration), 0.35.

meals. Proximate composition of the rations (A.O.A.C., 1960) were 5.8% ash, 2.2% ether extract, and 22.0% crude fiber. The crude protein (N x 6.25) content of rations with added urea was 13.7%, while those without urea contained 10.8%. Protein solubility (Lyman et al., 1953) was 76.8 and 28.2% for nonautoclaved and autoclaved soybean meals, respectively.

All lambs were weighed at 14-day intervals during the experiment and a 17-hour shrunk weight preceded the final weighing. Three blocks of lambs (12 head), all from one source, were weighed off the experiment after 44 days because a lamb fed autoclaved soybean meal and another fed autoclaved soybean meal plus 1.0% urea showed symptoms of copper toxicity. All other lambs were fed for 77 days. Feed and water were available ad libitum. Liver samples were obtained by laparotomy on all animals immediately after they were removed from the trial. Blood samples were taken by jugular puncture prior to the start and at the termination of the trial. Plasma and liver copper were determined by procedures outlined by Cartwright et al. (1945) and Sandell (1959), respectively. Statistical analyses of the data were by analysis of variance.

RESULTS

Trial 1

Although not statistically significant ($P > .05$), animals receiving no copper apparently had lower feed intakes and gains than those which received copper (Table VI). Other differences between copper levels were not significant ($P > .05$) in any response criteria. There were no external signs of copper toxicity during the 56-day trial or during a 45-day period after the trial ended. During the latter period, all animals received a general purpose sheep ration which contained 13% crude protein and 15 ppm of copper; Boughton and Hardy (1934) had reported that sheep continued to die from copper poisoning for five months after the copper level was reduced to normal levels.

Trial 2

Gain, feed consumption, and gain/100 gm. feed (Table VII) were greater ($P < .01$) for sheep fed sulfate than for those fed elemental sulfur. When the results were pooled across both sulfur sources and tested in orthogonal comparisons, the sheep fed 5.5 and 55 ppm of copper had more efficient ($P < .01$) feed conversions than those fed 550 ppm of copper. With the exception of the elemental sulfur ration which contained 55 ppm of copper, gain/100 gm. feed decreased as the copper level increased. Lambs fed elemental sulfur showed little change

TABLE VI

EFFECTS OF VARIOUS COPPER LEVELS ON LAMBS FED A UREA AND SULFATE CONTAINING PURIFIED DIET, TRIAL 1

Copper level, ppm.	0	5.5	11	22	44	88	176	352	704	1408	Standard Error of Treatment Means[a]
No. of lambs	5	5	5	4[b]	5	5	5	5	5	3[b]	
Av. daily gain, gm.	54.4	77.1	63.5	90.7	72.6	81.6	63.5	86.2	68.0	86.2	18.1
Av. daily feed, gm.	717	789	889	993	789	812	807	844	816	844	77.1
Gain/100 gm. feed, gm.	6.80	9.36	6.37	9.15	9.47	9.92	7.32	10.2	8.38	9.13	1.87
Hemoglobin, gm./100 ml. blood											
Av. Initial	13.8	13.4	12.4	12.4	13.3	13.1	12.9	12.6	13.0	12.5	
Av. final	12.6	12.3	11.8	12.5	12.8	12.1	12.0	11.5	11.6	11.5	
Av. change	-1.2	-1.1	-0.6	0.1	-0.5	-1.0	-0.9	-1.1	-1.4	-1.0	0.55
Hematocrit, %											
Av. Initial	40.1	39.5	37.2	35.6	38.4	36.6	37.8	36.1	38.0	37.8	
Av. final	36.0	36.8	36.3	35.7	36.7	36.1	34.9	33.4	34.6	33.4	
Av. change	-4.1	-2.7	-0.9	0.1	-1.7	-0.5	-2.9	-2.7	-3.4	-4.4	1.67

[a]The standard error when three per treatment is the reported standard error times $\sqrt{5/3}$ and four per treatment is the reported standard error times $\sqrt{5/4}$.

[b]One animal removed from each of these treatments because of failure to go on feed.

TABLE VII

EFFECTS OF SOURCES OF SULFUR AND COPPER LEVELS ON LAMBS FED A UREA CONTAINING PURIFIED DIET, TRIAL 2

Sulfur Source	Sulfate (SO_4)			Elemental Sulfur (S)			Standard Error of Treatment Means
Copper Level, ppm.	5.5	55	550	5.5	55	550	
No. of lambs	3	3	3	3	3	3	
Av. daily gain, gm.[a]	127.0	127.0	86.2	-9.1	0.0	-40.8	22.7
Av. daily feed, gm.[b]	1034	1188	1129	572	671	454	159
Gain/100 gm. feed, gm.[c]	12.13	10.37	6.50	-2.20	-0.60	-8.83	1.39
Hemoglobin, gm./100 ml. blood							
Av. Initial	12.7	12.4	14.2	13.2	13.1	13.0	
Av. final	12.6	12.0	13.6	11.4	11.9	11.8	
Av. change	-0.1	-0.4	-0.6	-1.8	-1.2	-1.2	0.63
Hematocrit, %							
Av. Initial	35.0	35.1	38.8	36.6	38.1	37.5	
Av. final	33.5	30.5	35.0	30.9	32.8	31.5	
Av. change	-1.5	-4.6	-3.8	-5.7	-5.3	-6.0	1.39
Plasma copper, mcg./100 ml.							
Av. Initial	116.0	120.1	121.1	126.7	108.9	129.6	
Av. final[d]	72.7	87.8	91.5	117.9	110.2	141.3	
Av. change	-43.3	-32.3	-29.6	-8.8	1.3	11.7	9.9

[a] Gain. $SO_4 > S (P < .01)$.

[b] Consumption. $SO_4 > S (P < .01)$

[c] Efficiency. $SO_4 > S (P < .01)$ and 5.5 and 55 > 550 ($P < .01$).

[d] Loss of plasma copper. $S < SO_4 (P < .01)$.

in plasma copper level while those fed sulfate had a marked decrease, indicating that the sulfate ion depressed copper absorption.

Trial 3

This trial was a replica of Trial 2, except that purified soybean protein was used as the nitrogen source. Lambs fed 550 ppm of copper had higher ($P < .01$) liver copper levels and greater ($P < .05$) changes in plasma copper than those fed 5.5 or 55 ppm of copper (Table VIII). The data indicate a trend toward improved gains and feed efficiencies as copper level increased.

Trial 4A

Table IX exhibits the results of adding 550 ppm of copper to the urea or purified soybean rations, which contained sulfate or elemental sulfur. Tests of simple effects were made by methods described by Steel and Torrie (1960) for factorial analyses. Since the interaction was significant, the results depended on both factors; therefore, only simple effects are presented. Gain, feed consumption, and gain/100 gm. feed were lowered ($P < .01$) when the combination of urea and elemental sulfur was fed; this combination also produced the highest liver copper values ($P < .01$). Liver copper levels were lower ($P < .01$) in lambs fed the combination of isolated soybean protein and elemental sulfur than in those fed urea and elemental sulfur. These results indicate that both sulfate and protein act to prevent absorption of copper.

Initial plasma copper values, which were taken following the removal of initial liver samples, were high (207 mcg./100 ml. plasma) possibly

TABLE VIII

EFFECTS OF SOURCES OF SULFUR AND COPPER LEVELS ON LAMBS FED A PROTEIN CONTAINING PURIFIED DIET, TRIAL 3

Sulfur Source	Sulfate (SO_4)			Elemental Sulfur (S)			Standard Error of Treatment Means
Copper Level, ppm.	5.5	55	550	5.5	55	550	
No. of lambs	3	3	3	3	3	3	
Av. daily gain, gm.	86.2	117.9	131.5	127.0	122.5	149.7	13.6
Av. daily feed, gm.	1170	1184	1256	1279	1225	1397	72.6
Gain/100 gm. feed, gm.	7.53	10.09	10.33	9.76	9.83	10.81	1.31
Final liver copper, ppm. D.M. basis[a]	218	248	918	235	458	1173	172
Hemoglobin, gm./100 ml. blood							
Av. Initial	11.6	12.3	11.5	12.0	11.7	12.2	
Av. final	11.2	12.3	11.3	11.9	12.3	12.1	
Av. change	-0.4	0.0	-0.2	-0.1	0.6	-0.1	0.69
Hematocrit, %							
Av. Initial	32.0	35.4	32.2	32.7	34.7	34.4	
Av. final	32.9	36.1	31.4	34.8	35.9	35.0	
Av. change	0.9	0.7	-0.8	2.1	1.2	0.6	2.64
Plasma Copper, mcg./100 ml.							
Av. Initial	75.3	76.0	69.5	106.9	93.5	85.9	
Av. final	80.1	81.1	100.8	95.5	89.1	118.6	
Av. change[b]	4.8	5.1	31.3	-11.4	-4.4	32.7	11.0

[a] Liver copper. 550 > 55 and 5.5 ($P < .01$).

[b] Change in plasma copper. 500 > 55 and 5.5 ($P < .05$).

TABLE IX

EFFECTS OF SULFUR AND NITROGEN SOURCES ON LAMBS FED 550 ppm OF COPPER IN A PURIFIED DIET, TRIAL 4A

Nitrogen Source / Sulfur Source	Urea SO_4 (A)	Urea S (B)	Purified soy protein SO_4 (C)	Purified soy protein S (D)	Standard Error of Treatment Means[a]
No. of lambs	5	5	5	4[b]	
Av. daily gain, gm.[c]	136.1	-40.8	163.3	176.9	22.7
Av. daily feed, gm.[c]	1551	853	1406	1560	99.8
Gain/100 gm. feed, gm.[c]	8.61	-5.57	11.57	11.26	2.08
Hemoglobin, gm./100 ml. blood					
Av. Initial	9.7	11.1	9.7	9.6	
Av. final	11.7	13.6	11.9	11.1	
Av. change	2.0	2.5	2.2	1.5	0.49
Hematocrit, %					
Av. Initial	28.6	30.6	27.0	26.2	
Av. final	34.9	39.9	34.2	30.9	
Av. change	6.3	9.3	7.2	4.7	1.63
Plasma Copper, mcg./100 ml.					
Av. Initial	203.7	184.5	204.3	219.5	
Av. final	150.4	183.8	144.6	183.6	
Av. change[d]	-53.3	-0.7	-59.7	-35.9	18.9
Liver Copper, ppm, dry basis					
Av. Initial	382	388	383	378	
Av. final[c]	685	1902	630	1003	161

[a] Standard error when four per treatment is reported standard error times $\sqrt{5/4}$.

[b] One lamb removed because of failure to recover from laparotomy to obtain initial liver samples.

[c] Gain, feed consumption, feed efficiency, and final liver copper. Significant ($P < .01$) interaction. Simple effects: A vs. B ($P < .01$), A vs. C (N.S.), D vs. B ($P < .01$), and D vs. C (N.S.).

[d] Change in plasma copper. $SO_4 > S$ ($P < .05$).

because of the surgery performed. Markowitz et al. (1955) and Gubler et al. (1952a) have also observed a significant rise in blood copper level of animals having either bacterial or turpentine infection. The excitation caused by the operation could have also raised the plasma copper level (Robertson and Broome, 1957). However, lambs fed sulfate had greater ($P < .05$) negative changes in plasma copper than those fed elemental sulfur.

Trial 4B

The effects of different sulfur and nitrogen sources upon the digestibilities of dry matter and nitrogen, and the retention of minerals are shown in Table X. The combination of soy protein and sulfate reduced both ($P < .01$) the digestibility of dry matter and retention of sulfur. The urea-containing rations promoted greater ($P < .01$) digestibility and retention of dietary nitrogen, and ($P < .05$) retention of copper. Analysis of simple effects (Steel and Torrie, 1960) indicated that the combination of urea plus elemental sulfur lowered ($P < .01$) sulfur digestibility.

Trial 5

A significant ($P < .05$) copper level by sulfur source interaction was obtained (Table XI) for dry matter digestibility. Analysis for simple effects indicated that the interaction was caused by a depression ($P < .01$) when sulfate rations containing 100 ppm of copper were fed.

Copper level by nitrogen source interactions were obtained for nitrogen digestibility ($P < .01$) and nitrogen retention ($P < .05$).

TABLE X

BALANCE STUDY ON LAMBS FED 550 ppm OF COPPER AND TWO SULFUR AND TWO NITROGEN SOURCES, TRIAL 4B

Nitrogen Source	Urea		Purified Soy Protein		Standard error of treatment means
Sulfur Source	(SO_4) (A)	(S) (B)	(SO_4) (C)	(S) (D)	
No. of lambs	3	3	3	3	
Av. daily intake, gm.					
Feed	1200	636	1200	1067	
Nitrogen	20.92	11.09	20.98	18.65	
Sulfur	2.40	1.27	3.19	2.84	
Copper	0.66	0.35	0.66	0.59	
Dry matter					
Digestibility, %[a]	69.54	65.39	59.44	65.21	1.45
Nitrogen					
Digestibility, %[b]	65.63	68.92	54.85	61.13	2.22
Retention, % of intake[b]	62.10	61.92	50.91	57.45	2.18
Sulfur					
Digestibility, %[c]	65.89	29.62	63.86	44.24	2.85
Retention, % of intake[d]	32.73	24.54	1.68	33.91	8.66
Copper					
Retention, % of intake[e]	7.79	11.35	-11.88	2.58	4.86

[a]Dry matter digestibility. Significant ($P < .01$) Interaction. Simple effects: A vs. B (N.S.), A vs. C ($P < .01$), D vs. B (N.S.), and D vs. C ($P < .05$).

[b]Nitrogen digestibility and retention. Urea > soy ($P < .01$).

[c]Sulfur digestibility. Significant ($P < .01$) Interaction. Simple effects: A vs. B ($P < .01$), A vs. C (N.S.), D vs. B ($P < .01$), and D vs. C ($P < .01$).

[d]Sulfur retention. Significant ($P < .01$) Interaction. Simple effects: A vs. B (N.S.), A vs. C ($P < .01$), D vs. B (N.S.), and D vs. C ($P < .01$).

[e]Copper retention. Urea > soy ($P < .05$).

TABLE XI

BALANCE STUDY USING TWO LEVELS OF COPPER AND TWO SULFUR AND TWO NITROGEN SOURCES, TRIAL 5

Nitrogen Source	Urea				Purified Soy Protein				Standard error of treatment means
Sulfur Source	SO_4		S		SO_4		S		
Copper level, ppm.	5	100	5	100	5	100	5	100	
No. of lambs	3	3	3	3	3	3	3	3	
Av. daily intake									
Feed, gm.	700	689	700	700	700	700	692	664	
Nitrogen, gm.	13.09	12.88	13.09	13.09	13.86	13.86	13.70	13.15	
Sulfur, gm.	1.40	1.38	1.40	1.40	1.86	1.86	1.84	1.77	
Copper, mg.	3.50	68.90	3.50	70.00	3.50	70.00	3.46	66.40	
Calcium, gm.	2.52	2.48	2.43	2.43	2.95	2.95	2.46	2.36	
Phosphorus, gm.	2.10	2.07	1.94	1.94	2.58	2.58	2.01	1.93	
Dry Matter									
Digestibility, %[a]	77.07	73.26	76.35	76.27	80.55	68.85	78.62	77.49	2.25
Nitrogen									
Digestibility, %[b]	68.96	69.99	69.11	72.80	67.94	64.26	70.80	65.35	1.53
Retention, % of intake[c]	64.73	64.66	64.45	67.45	63.98	59.84	67.81	62.68	1.61
Sulfur									
Digestibility, %[d]	71.95	73.11	45.42	50.13	73.25	68.60	55.87	45.34	3.08
Retention, % of intake[e]	20.76	18.03	34.51	38.36	9.14	-2.82	33.55	23.15	5.38
Copper									
Retention, mg./day[f]	-8.67	0.05	-9.43	6.30	-9.33	-1.90	-12.10	0.49	3.13
Calcium									
Retention, % of intake[g]	-39.81	-63.07	-25.40	-8.74	-23.65	-20.38	-5.80	-14.69	9.20
Phosphorus									
Retention, % of intake[h]	9.97	-13.01	1.36	11.17	12.14	1.23	15.69	13.04	6.31

TABLE XI (Continued)

[a]Dry matter digestibility. Significant ($P < .05$) copper level by sulfur source interaction. Significant simple effects: Cu_{100}-SO_4 vs. Cu_5-SO_4 ($P < .01$) and Cu_{100}-SO_4 vs. Cu_{100}- S ($P < .05$).

	SO_4	S
Cu_5	78.81	77.48
	**	*
Cu_{100}	71.06	76.88

[b]Nitrogen digestibility. Significant ($P < .01$) copper level by nitrogen source interaction. Significant simple effects: Cu_{100}-soy vs. Cu_5-soy ($P < .01$) and Cu_{100}-soy vs. Cu_{100}-urea ($P < .01$).

	Urea	Soy
Cu_5	69.04	69.37
	**	**
Cu_{100}	71.40	64.80

[c]Nitrogen retention. Significant ($P < .05$) copper level by nitrogen source interaction. Significant simple effects: Cu_{100}-soy vs. Cu_5-soy ($P < .01$) and Cu_{100}-soy vs. Cu_{100}-urea ($P < .01$).

	Urea	Soy
Cu_5	64.59	65.90
		**
Cu_{100}	66.06	** 61.26

[d]Sulfur digestibility. SO_4 > S and significant ($P < .01$) copper level by nitrogen source interaction. Significant simple effects: Cu_5-urea vs. Cu_5-soy ($P < .01$), Cu_{100}-urea vs. Cu_{100}-soy ($P < .05$), and Cu_{100}-soy vs. Cu_5-soy ($P < .01$).

	Urea	Soy
Cu_5	58.68 **	64.56
		**
Cu_{100}	61.62 *	56.97

[e]Sulfur retention. Urea > soy ($P < .01$) and S > SO_4 ($P < .01$).

[f]Copper retention. 100 ppm > 5 ppm ($P < .01$).

[g]Calcium retention. Soy > urea ($P < .05$) and S > SO_4 ($P < .01$).

[h]Phosphorus retention. Significant ($P < .05$) copper level by sulfur source interaction. Significant simple effects: Cu_{100}-SO_4 vs. Cu_5-SO_4 ($P < .01$) and Cu_{100}-SO_4 vs. Cu_{100}-S ($P < .01$).

	SO_4	S
Cu_5	11.06	8.52
	**	
Cu_{100}	-5.89 **	12.10

These interactions were caused by the depression ($P < .01$) obtained when soybean rations contained 100 ppm of copper.

The digestibility of sulfur, as indicated by simple effect analyses (Steel and Torrie, 1960), was reduced ($P < .01$) by feeding soybean protein rations containing 100 ppm of copper. Main effect analyses indicated that sulfur supplied as sulfate was better digested ($P < .01$) than elemental sulfur. When sulfur retention was the response criteria, urea was superior to soy protein ($P < .01$) and elemental sulfur was superior ($P < .01$) to sulfate.

Copper retentions are reported in mg./day because of the differences in copper intakes. More copper was retained ($P < .01$) when 100 ppm were fed than when lambs consumed rations containing 5 ppm of copper.

More calcium ($P < .05$) was retained when soybean protein rations were fed than when urea was the nitrogen source. Lambs consuming elemental sulfur also retained more calcium than those fed sulfate ($P < .01$). A significant ($P < .05$) copper level by sulfur source interaction was obtained for phosphorus retention. Simple effects showed this measurement to be depressed ($P < .01$) by feeding sulfate rations with 100 ppm of copper.

Trial 6

The results of this experiment are shown in Table XII. Differences in gain, feed consumption, feed efficiency, and changes in hemoglobin, hematocrit, and plasma copper were not significant ($P > .05$); however, final plasma copper values tended to be higher in those lambs fed the autoclaved soybean meal.

TABLE XII

EFFECTS OF PROTEIN SOLUBILITY ON THE PERFORMANCE AND COPPER STATUS OF LAMBS FED 100 ppm OF COPPER

	Soybean Meal		Autoclaved Soybean Meal[a]		Standard Error of Treatment Means
	No Urea	1.0% Urea	No Urea	1.0% Urea	
No. of lambs	8	8	8	8	
Av. initial wt., kg.	28.9	30.5	28.4	28.8	
Av. daily gain, gm.	111.0	118.6	106.1	111.2	14.6
Av. daily feed, kg.	1.10	1.19	1.07	1.14	0.07
Gain/100 gm. feed, gm.	10.15	9.69	10.05	9.80	1.08
Final liver copper, dry basis, ppm.[b]	1480	1300	1755	2120	198
Hemoglobin, gm./100 ml. blood					
Av. Initial	11.7	11.3	11.7	12.1	
Av. final	12.7	13.1	13.6	13.8	
Av. change	1.0	1.8	1.9	1.7	0.52
Hematocrit, %					
Av. Initial	33.2	32.7	33.9	35.6	
Av. final	35.8	37.2	37.8	40.5	
Av. change	2.6	4.5	3.9	4.9	1.61
Plasma Copper, mcg./100 ml.					
Av. Initial	132.7	123.6	129.7	133.2	
Av. final	151.9	137.8	162.1	172.0	
Av. change	19.2	14.2	32.4	38.8	11.8

[a] Autoclaved at 124°C. and 1.27 gm./cm.^{2} pressure for 2 hours.

[b] Autoclaved soybean meal > soybean meal ($P < .025$).

Lambs fed autoclaved soybean meal stored more ($P < .025$) copper than lambs consuming unheated soybean meal rations. Urea had no effect on plasma or liver copper levels. As this level of urea increases the ruminal ammonia level (Lewis, 1957), these data along with those of the previous trials indicate that the presence of ammonia in the rumen does not affect copper absorption.

DISCUSSION

Results of both mineral balance trials showed that the presence of high levels of copper lowered the digestion and retention of protein-nitrogen but not urea-nitrogen. The sheep fed protein rations also absorbed less copper than those fed urea. Since copper chelates with amino acids, peptides, and proteins (Flinn and Inouye, 1929; Dawson and Nair, 1950; Fruton and Simmonds, 1953), the formation of such copper-protein complexes offers a possible explanation of the lowered retentions of both copper and nitrogen in sheep fed high copper-soybean protein rations. White et al. (1951) found that protein levels above 10% reduced copper toxicity while McCall and Davis (1961) showed that 17.5% protein inhibited the accumulation of toxic levels of copper, but a 10% protein ration had no protective effect in rats. Mills (1958) has suggested that copper is normally absorbed in the form of organic complexes that are capable of direct absorption because of their molecular size and solubility; therefore, copper-protein complexes may not be absorbed because of their large size. The results of Trial 6 are in accord with this idea: lambs fed autoclaved soybean meal stored more copper than lambs consuming unheated soybean meal rations. Autoclaving the soybean meal lowered the solubility of its protein and poorly soluble proteins remain unseparated, while soluble proteins are dispersed and surrounded by the medium (Gurd and Wilcox, 1956). The autoclaved soybean meal rations could, therefore, be expected to have

less protein in solution to react with copper for the formation of copper-protein complexes. The results of Ammerman et al. (1963) also bear upon this point. They found that lambs fed soybean meal had higher liver copper values than those fed casein, a highly soluble protein (Chalmers et al., 1954; Chalmers and Synge, 1954).

The present experiments also show that 0.20% sulfur as sulfate, in the presence of 2 ppm of molybdenum, significantly lowered the absorption of copper. This reduction was not found when the same level of sulfur was added as elemental sulfur, indicating that elemental sulfur is not utilized via the formation of a hydrogen sulfide intermediate as was found with sulfate (Lewis, 1954). Several workers (Wynne and McClymont, 1956; Mylrea, 1958; Evans and Davis, 1963) reported that high levels of sulfate reduce liver copper stores in the presence of low levels of molybdenum.

Sulfur digestion and retention data indicate that the highly soluble sulfate was readily digested, but because of large urinary losses the retention of sulfate-sulfur was low. Similar results have been found for nitrogen retention when highly soluble proteins were fed to sheep (Sherrod and Tillman, 1962). In Trial 5, urea significantly improved the retention and 100 ppm of copper with soybean protein significantly lowered the digestion of both sources of sulfur.

Both urea and sulfate significantly reduced the retention of calcium. Pensack et al. (1964) have shown that sulfate in poultry rations increased the absorption of chlortetracycline and suggested that sulfate ions tie up calcium as calcium sulfate, allowing the antibiotic to be absorbed from the duodenum, while the calcium is absorbed in the lower part of the tract. The results of the present

experiments indicate that sulfate lowered the amount of calcium available to lambs. Calcium sulfide is possibly also formed in the rumen.

Phosphorus retention was significantly reduced by sulfate in the presence of 100 ppm of copper. Other workers (Shirley et al., 1950, 1951; Davis et al., 1953) have observed excessive losses of phosphorus and abnormal bone formations when animals were fed low copper-high molybdenum rations. The present results indicate that phosphorus depletion may also occur in high copper rations if sufficient sulfate ions are present.

SUMMARY

A series of growth and mineral balance trials involving 173 individually-fed lambs were conducted to determine the effects of sulfur sources (sulfate and elemental sulfur), nitrogen sources (urea and purified soybean protein), and copper levels on growth, liver storage of copper, and balance of nitrogen, sulfur, copper, phosphorus, and calcium. In addition, the effect of autoclaved and nonautoclaved soybean meals on copper storage was studied. As shown by balance trials and liver analyses, lambs fed purified soybean protein did not retain or store as much copper as those fed urea. Also, the addition of sulfate, but not elemental sulfur, decreased the retention and liver storage of copper.

Nitrogen digestion and retention in lambs fed soybean protein rations were significantly reduced by feeding 100 ppm of copper. The sulfate rations promoted the greatest sulfur digestibility, but because of large urinary losses, the retention of sulfur was greatest in those lambs fed elemental sulfur. Both urea and sulfate lowered the retention of calcium. Phosphorus retention was lowered by feeding 100 ppm of copper with sulfate rations.

Lambs fed autoclaved soybean meal had significantly higher final liver copper values than those fed nonautoclaved meal. Other response criteria were not affected by feeding the two meals, with or without 1% urea.

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