SYMPOSIUM: Trace Minerals

Recent Developments in Cobalt and Copper in Ruminant Nutrition: A Review [1]

C. B. AMMERMAN
Department of Animal Science
University of Florida, Gainesville 32601

Abstract

Cobalt and copper have been recognized as dietary essentials for ruminants since the 1930's, and deficiencies of both minerals have occurred under natural grazing conditions in many countries of the world. Deficiencies are infrequent with drylot feeding, particularly if grains serve as a portion of the diet. Cobalt in the form of vitamin B_{12} is interrelated with iron and copper in hematopoiesis and, thus, indirectly involved with molybdenum. Vitamin B_{12} may function in the formation of excretion products of selenium and thereby reduce the animal's susceptibility to selenium toxicity. Copper metabolism is influenced by many dietary factors, some of which include sulfate sulfur, molybdenum, zinc, protein level, and protein source. Because of the many factors influencing copper metabolism, it is difficult to determine precise dietary copper requirements and to predict potentially toxic levels of copper for ruminants under different feeding programs. Cobalt is comparatively less toxic than copper. Sheep and young cattle are more susceptible to copper toxicity than are mature cattle and may suffer from poisoning even when dietary copper levels are considered to be in the normal range. Several forms of supplemental cobalt and copper are effective in assuring adequate dietary intakes under deficient conditions.

Introduction

The purpose of this paper is to summarize the more recent research relating to cobalt and copper in ruminant nutrition. Research with cobalt for ruminants has been reviewed by Smith and Loosli (88), Davis (29), Underwood (105), and Smith (87); and research with copper has been reviewed by Underwood (105) and Adelstein and Vallee (2). The early history of cobalt and copper nutrition in cattle, particularly in Florida, has been summarized by Becker et al. (14). These earlier reviews have established the dietary need for cobalt and copper and have summarized information on their requirements and metabolism. In general, references included in these papers will not be examined in the present paper except as they may contribute to a specific discussion.

Cobalt

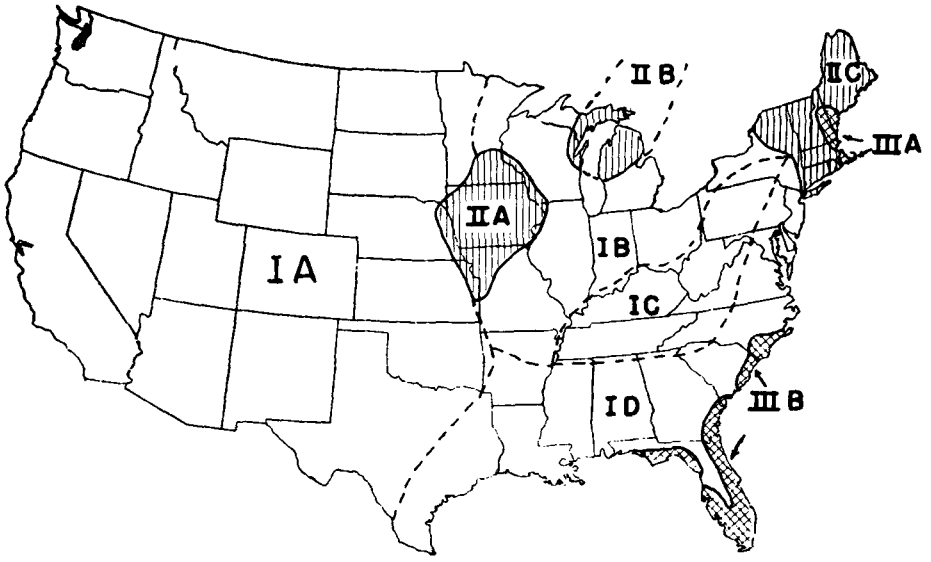
Cobalt deficiency. Cobalt is deficient, particularly under grazing conditions, for the production of ruminants in many parts of the world. The cobalt-deficient areas of the United States, based on soil and forage analyses, have been presented in a recent review by Kubota (59) and are shown in Figure 1. The most severely deficient areas include portions of New England and the lower Atlantic Coastal Plain. Moderately deficient areas include New England, northern New York, northern Michigan, and parts of the Central Plains. That cobalt-deficient areas are not restricted to the United States is emphasized by Latteur (62), who described the deficiency in countries including Australia, New Zealand, East Africa, and Norway. Reports have recently indicated a benefit from supplemental cobalt to cattle in Katanga (91), and cattle suffering from a "lack-of-appetite" disease in Brazil had liver cobalt below 0.05 ppm on a dry tissue basis (103). In other areas of Brazil, however, no effect on growth rates of cattle was obtained from added cobalt (12).

In general, cobalt deficiencies are infrequent when high levels of mixed concentrates are fed. Research at the Oklahoma Station (80), how-

[1] Presented at the Sixty-fourth Annual Meeting of the American Dairy Science Association, University of Minnesota, Minneapolis, June 25, 1969.
Florida Agricultural Experiment Stations, Journal Series no. 3487.

ever, showed a significant increase in the body weight gain of finishing steers when cobalt was added to "all-barley" diets. Earlier workers (94) had produced a similar response by adding a mixture of trace elements, including cobalt, to diets high in barley for finishing heifers.

Cobalt is considered to function in metabolism as vitamin B_{12} and the symptoms of the vitamin B_{12} deficiency have been described as basically those of starvation (29, 88). With regard to the sequence of deficiency symptoms, research with sheep (42) indicated that a reduction in feed intake preceded the onset of a normocytic, normochromic anemia. The anemia intensified during three weeks of vitamin B_{12} therapy before beginning to improve; however, during the same time feed intake had doubled. Reduced liver stores of cobalt and vitamin B_{12} (29, 105) are indicative of a cobalt deficiency, and research with sheep by Jones and Anthony (57) suggests that fecal vitamin B_{12} may indicate cobalt intake. In their studies with sheep, fecal vitamin B_{12} decreased before weight loss and other symptoms of cobalt deficiency oc-



LEGEND

	GENERAL LEVEL OF CO IN LEGUMES (PPM)	MAP SYMBOL	GENERAL REGION
AREAS OF ADEQUATE CO	0.1 TO 0.2	I A	WESTERN UNITED STATES AND GREAT PLAINS STATES
		I B	NORTH CENTRAL STATES
		I C	RIDGE AND VALLEY
		I D	PIEDMONT AND UPPER COASTAL PLAIN
AREAS OF MODERATELY LOW CO	0.1	II A	CENTRAL LOESS AND DRIFT PLAINS
		II B	NORTHERN LAKE STATES
		II C	NEW ENGLAND AND NORTHERN NEW YORK
AREAS OF LOW CO	< 0.07	III A	NEW ENGLAND: MERRIMAC R.–SACO R. DRAINAGE BASIN
		III B	LOWER ATLANTIC COASTAL PLAIN

FIG. 1. Regional distribution of low, moderately low, and adequate areas of cobalt in the United States, Kubota (59).

curred. Supplementation with cobalt increased cobalt in the pigmented hair of dairy cows (9), and Anke (11) suggested that 0.05 ppm cobalt in the black hair of dairy cattle indicates a borderline dietary supply of this element. Perhaps the level of cobalt in the diet plus a positive response from supplemental cobalt will continue to serve as the most satisfactory way of confirming a cobalt deficiency.

Cobalt sources. Since many forages and some concentrate feeds do not supply 0.10 ppm, which may be considered as an adequate dietary allowance (88, 105), there is need for supplemental cobalt compounds. Although only limited research has been done concerning the biological availability of inorganic compounds, the carbonate, chloride, and sulfate forms of cobalt have been proposed as satisfactory dietary sources of the mineral (28). Dewey et al. (30) described an orally administered heavy pellet made of cobalt oxide plus clay which remains in the reticulorumen for several months. Dairy cattle treated with cobalt pellets increased in milk production (85), and ewes treated similarly during pregnancy produced colostrum and milk higher in vitamin B_{12} than that produced by untreated ewes (72). In the same study, lambs from treated ewes had higher liver stores of vitamin B_{12} at birth. Ewes grazing cobalt-deficient pastures with and without oral cobalt oxide pellets produced milk containing 10.3 and 2.5 μg/liter vitamin B_{12} (45). Calves from cows given cobalt pellets during pregnancy were no heavier at birth but gained faster during the first 7 weeks of life than did calves from untreated cows (84). Chapman and Kidder (23) obtained improved performance when pellets were administered to grazing steers and beef cows not exposed to a mineral mixture containing cobalt. The pellets have the limitation of being lost frequently through regurgitation or of becoming ineffective because of the formation of a surface coating (105).

Cobalt and other dietary factors. The interrelationship of vitamin B_{12} with iron and copper in hematopoiesis has been recognized for some time (105), but few other relationships between vitamin B_{12} or cobalt with other dietary factors have been clearly established. An interrelationship has been suggested between cobalt and selenium. Cobalt-deficient sheep have been more susceptible to selenium toxicity than sheep with adequate cobalt nutrition (41). Andrews et al. (6) obtained definite responses to selenium supplementation in areas of New Zealand known to have soils deficient in cobalt. In further studies by these researchers, sheep grown on cobalt-deficient diets had a significantly higher concentration of selenium in the kidney. Wise et al. (112) demonstrated a metabolic relationship in sheep between cobalt and selenium in the presence of white muscle disease. The cobalt concentration of the kidney, other organs, and skeletal muscle was unaffected by selenium intake unless signs of white muscle disease were evident. When lesions of white muscle disease were present, the cobalt concentration of the kidney was significantly lowered. These workers suggest that the cobalt-selenium interrelation may involve that of vitamin B_{12} in the metabolism of dimethyl selenide. Since vitamin B_{12} functions in the metabolism of methyl groups, it may be involved in the formation of dimethyl selenide as well as other excretion products of selenium. Thus, if cobalt aids in the excretion of selenium, this hypothesis would help to explain the increased susceptibility of cobalt-deficient sheep to selenium toxicity (41).

The interaction of supplemental cobalt and molybdenum was studied in growing cattle by Chapman and Kidder (22). The administered cobalt (8 mg daily) resulted in higher blood hemoglobin and packed cell volume and significantly higher copper and lower iron levels in the liver. Increased dietary molybdenum (250 mg daily) resulted in decreased blood hemoglobin, decreased packed cell volume, decreased liver copper stores, and increased iron in the liver. Cobalt partially but not completely alleviated the decrease in liver copper when molybdenum was administered.

Copper

Copper deficiency. The deficiency of copper in ruminant animals under natural grazing conditions occurs in many countries including Australia, New Zealand, United States, Great Britain, and The Netherlands (105). While cobalt deficiency results primarily from an inadequate dietary intake, copper deficiency can result from a multiplicity of factors only one of which is a dietary shortage of copper. As with cobalt, however, copper deficiencies in ruminants occur mostly under grazing conditions, with gross symptoms of the deficiency rare when concentrates are fed. Under certain conditions, forages which cause copper deficiency when grazed will not do so when harvested and fed dry. Factors involved in "physiological" or "conditioned" copper deficiency have been reviewed by Abdellatif (1). The same factors which influence the appearance of deficiency symptoms also affect the dietary requirement for copper, making it difficult to establish required dietary levels satisfactory for the same species under varied nutritional environments. A genetic

influence on copper metabolism in sheep has been shown by Wiener et al. (110, 111). In a flock involving three breeds and the crosses among them, the level of copper in blood and liver and the susceptibility of newborn lambs to swayback were related significantly to breed.

In some areas, the deficiency of copper results from vegetation low in copper, but in many locations it may result from one of several factors in the forage. That the presence of molybdenum in the forage is one of the predominant factors interfering with copper metabolism has been reviewed by Underwood (105). Forages grown on the organic soils of Florida, for example, are high in molybdenum, and their consumption results in molybdenosis or hypocuprosis in grazing cattle (14). Recent research in the Irish Republic and the United Kingdom (95, 96, 108) has shown a strong positive correlation between the regional distribution of molybdenum in stream sediments and the incidence of hypocuprosis in livestock. Molybdenum toxicity in grazing animals in Oregon was closely related to the molybdenum content of the soils (60).

Symptoms of copper deficiency in cattle may include slow growth, loss of body weight, bleached hair, and anemia. Lesions of the heart may be seen on autopsy and the development of weak, fragile long bones which break easily have been reported (28). Liver copper levels are influenced by the amounts and relative proportions of dietary copper, molybdenum, and inorganic sulfate and have been suggested by Florida researchers (28) as the most effective way of diagnosing copper deficiency in cattle. Cattle in Florida in an adequate state of copper nutrition have liver copper of 100 to 300 ppm on a dry tissue basis and do not show deficiency symptoms consistently until the level reaches 25 ppm. In research by Poole (75) many dairy cows clinically normal had liver copper of less than 5 ppm on a dry matter basis. Liver cytochrome oxidase activity decreases during copper deficiency (69, 76) and has been suggested as an index of the copper status of the animal. Liver iron increases greatly in cattle which are extremely copper-deficient.

As a simplified method of detecting copper deficiency, Anke (11) has proposed that 7 ppm copper in the black hair of dairy cows is a borderline level for copper intake. These workers found an average of 7.8 ppm copper and 0.25 ppm molybdenum in 671 samples of hair from cattle. Extensive work by Chauvaux et al. (24), however, involving a total of 536 hair samples obtained three times per year from 31 farms, including 10 with severe copper deficiencies, suggested that copper levels in the hair were not effective for diagnostic purposes. In studies with humans supposedly consuming adequate and inadequate diets, only small differences (11.3 versus 12.0 ppm) in copper concentration of hair were observed (81). That copper content of the hair may be influenced by dietary copper intake is suggested by Seekles and Claessens (83), who reported a positive correlation between blood copper and hair copper in cattle. The copper content of hair varies with season, perhaps as it relates to shedding (8, 73), and is influenced by pregnancy (8) and hair color (73). The necessity of proper cleaning and preparation of hair samples to remove contaminating minerals and to avoid leaching of minerals has been emphasized (7, 47, 73). In comparing the response of hair copper to other criteria of copper adequacy, liver, kidney, blood, and hair responded in decreasing order of sensitivity to dietary copper intake (10). Binot et al. (16) found that the copper content of hair and blood in cattle varied directly with the copper in the forage and inversely with the amount of iron, calcium, lead, zinc, and sulfate in the forage. Anke (11) obtained a positive correlation between the molybdenum content of the pasture and the black hair of cattle and reported that symptoms of molybdenosis resulted when molybdenum in the hair exceeded 1 ppm.

Copper toxicity. Copper toxicity in ruminants is an important nutritional problem, with sheep being more susceptible than cattle to copper poisoning (105). In a recent review by Todd (97), chronic copper poisoning was divided into two distinct phases including a) a period of passive accumulation of copper in the tissues (primarily the liver) during which no symptoms of toxicity are exhibited and b) the toxic phase, which is an acute illness frequently referred to as the "hemolytic crisis," which can cause death within a few hours but which more generally results in deaths in 2 to 4 days. Jaundice, methemoglobin, and hemoglobinuria are evident during the latter phase. These results are compared with the findings of McCosker (64), who made a detailed study on blood copper in chronic copper poisoning in sheep. During Stage I there was a transient elevation in red blood cell copper. In Stage II the level of whole blood copper essentially doubled from increases in red blood cell copper and also direct-reacting copper. Plasma color changed from the presence of bilirubin, and there was a reduction in hematocrit. The excess copper of as much as 190 μg per 100 ml in the blood during the "hemolytic crisis" (Stage III)

appeared first in the plasma as direct-reacting copper and later in the red blood cells. The acute toxic effect of copper per se was considered the immediate cause of death in some animals, with anemia and uremia considered as contributing factors in the death of others. Similar findings with calves subjected to chronic copper poisoning have been reported by Todd and Thompson (102) and Weiss and Baur (109). In addition, they (109) reported dystrophy and cirrhosis of the liver and levels of copper in the liver at death between 898 and 2,091 ppm on a dry tissue basis. Copper, accumulated at high levels in the liver, in combination with other stress factors, produces liver cell necrosis, which releases copper into the blood stream resulting in hemoglobinemia and methemoglobinemia. Serum glutamic oxaloacetic transaminase (SGOT) activity may aid in diagnosing and evaluating the treatment of copper poisoning (82, 100). Concentrations of SGOT increased rapidly during approximately 4 weeks before death and decreased in relation to the effectiveness of the copper toxicity treatment. The authors emphasize the value of plasma arginase as an aid in differential diagnosis, since this enzyme increases in liver damage while SGOT concentrations increase with either liver or other tissue damage. A single dose of intravenously injected copper (80 to 160 mg per head) caused death in sheep within a few hours to several days after injection and resulted in symptoms similar to gastroenteritis rather than chronic copper poisoning (101). Repeated injections of smaller amounts of copper resulted in characteristic symptoms of copper toxicity.

The copper toxicity of sheep under natural grazing conditions in Western Australia has been suggested by Beck and Bennetts (13) to result from factors including native plants high in copper and hepatoxic principles of fungal origin. Other toxic principles causing liver degeneration and, thus, predisposing the animal to copper toxicity have been discussed by Underwood (105) and more recently by Clemetson (25), McCosker (63), and Pryor (79).

The form in which copper is administered can influence the degree of toxicity. Chapman et al. (20) administered as much as 12 g of $CuSO_4$ per head daily to steers either dry in a gelatin capsule or in a water drench. This amount of $CuSO_4$ in the dry form did not cause toxic symptoms in more than 1 year of administration. Death from the liquid drench resulted within approximately 60 days.

Copper, sulfate, and molybdenum interrelationships. Since the early work of Dick (31, 32), the interrelationship of copper, sulfate, and molybdenum has received considerable attention. Studies were conducted by Vanderveen and Keener (106) to evaluate this interrelationship in Holstein heifers. Heifers receiving diets low in copper containing as much as 50 ppm molybdenum and no added sulfate developed no symptoms of molybdenum toxicity. Identical results were observed with diets containing 5 to 20 ppm molybdenum and 0.3% added sulfate sulfur. Heifers receiving 50 ppm molybdenum plus 0.3% sulfate sulfur developed severe symptoms of molybdenosis which were corrected by the addition of copper. Research with growing lambs (44) indicated that body weight gains were reduced by increasing the sulfate-sulfur level from 0.10 to 0.40% in diets which contained 2 ppm molybdenum and 10 ppm copper. This did not occur, however, when the diets contained 8 ppm molybdenum. Liver copper was lowest when 0.4% sulfate sulfur was fed in combination with 8 ppm molybdenum. Working with older sheep, Marcilese et al. (66, 67) reported a response with 0.4% dietary sulfate as sodium sulfate similar to that with the control diet as measured by liver copper accumulation, plasma clearance, and urinary excretion of intravenously injected radioactive copper. The sulfate plus 50 ppm molybdenum, however, resulted in reduced liver copper accumulation, reduced ceruloplasmin formation, a delayed plasma clearance of the injected copper, and increased urinary excretion of both stable and radioactive copper. Similar results with sulfate plus molybdenum have been reported by Suttle and Field (92) and by Smith et al. (86). Hogan et al. (52), in a study with sheep, concluded that under conditions which facilitate excessive storage of liver copper, molybdenum plus sulfate depresses storage. Based on research with both ruminants and nonruminants, Dowdy and Matrone (34, 35) have proposed the presence of a copper-molybdenum complex which is unavailable to the animal for metabolism. Hartmans and Van Der Grift (49) reported that when yearling cattle were fed extra sulfur as $CaSO_4 \cdot 2\ H_2O$ or Na_2SO_4 in the powder form in drylot, the copper content of the livers from both groups decreased to the same degree and significantly more than with control animals. The molybdenum content of the diet is not known, however. Increased dietary sulfate depressed the secretion of molybdenum in milk of sheep (51) which otherwise was directly related to molybdenum content of the forage.

Lambs born from ewes grazing "copper-deficient" pastures often develop enzootic ataxia or swayback. Mills and Fell (70) reported this

condition when ewes were maintained on diets high in sulfate and molybdenum. Suttle and Field (93) obtained liver and brain copper concentrations in the newborn lamb similar to those found with ataxia by feeding either a low copper diet or a molybdenum plus sulfate supplement to the pregnant ewe. Effects were more severe when the two treatments were combined. Other researchers (18, 53) failed in attempts to produce the disease by feeding high dietary sulfate and molybdenum. Differences in the basal diet and breed of sheep were suggested as an explanation for the dissimilar results. The oral administration of large amounts of molybdate and sulfate by Butler et al. (19) to young lambs with initial low copper status produced some stiffness and incoordination of the hind limbs with nonspecific lesions of the central nervous system similar to those associated with ataxia.

Copper and other dietary factors. The research of Standish et al. (90) has shown that excessive levels of dietary iron depress liver copper in cattle, whereas that by Chapman and Kidder (23) indicates that iron accumulates in the liver when copper is deficient. Rats fed a copper-deficient diet accumulated excess liver iron, and those fed an iron-deficient diet stored high levels of copper in the liver (89). Copper is required for the utilization of iron in hemoglobin formation (105), and on the basis of other research with rats, Marston and Allen (68) postulated that copper is required for the release of iron from its storage sites in the liver.

Dynna and Havre (39) have observed a complex zinc-copper deficiency in cattle under both grazing and stall-fed conditions. The symptoms are typical of those expected for both zinc and copper, and the condition is aggravated by the administration of zinc only. A positive response is observed when supplemental zinc and copper are provided.

Bosman (17) reported that highly digestible crude protein combined with a low starch equivalent increases the sulfide content of rumen liquor of grazing cows. Thus, under these conditions the possibility is increased for the formation of CuS, which is poorly absorbed. The author suggests that high blood urea, associated with a surplus of organic sulfur in the rumen, may indicate the presence of high concentrations of sulfide in the rumen and serve as evidence of a copper deficiency in the animal. Increasing dietary protein resulted in reduced levels of liver copper in sheep (5, 65, 107) presumably by decreasing copper absorption, and soybean meal had a greater depressing effect on liver copper than did casein. Similar results have been reported with swine by Combs et al. (26). Goodrich and Tillman (43) and van Walleghem (107) obtained higher liver copper with sheep when urea served as the nitrogen supplement than with purified soybean protein.

Research in The Netherlands by Hartmans and Bosman (48) indicates the copper in dried forage is more available to the animal than that in fresh forage and that, despite having a lower copper content, mature grass is more effective in maintaining liver copper of cattle than young grass.

Copper in milk. One of the more important problems relating to copper in the dairy industry has been oxidized flavor from small amounts of copper in the milk. Copper contamination has been controlled most effectively by the elimination of this metal and its alloys in dairy plant equipment (74, 105). Hartmans (46) has called attention to another source of copper contaminating milk in The Netherlands. It came from topdressing pastures with $CuSO_4$ and improper washing of the cow's udder before milking. It has been proposed (58, 74) that the naturally occurring copper in milk, as it acts in combination with the iron normally present, may aid in catalyzing the oxidation of the lipids. The naturally occurring copper is associated predominantly with the fat phase of the milk (15, 54, 58). Underwood (105) reported copper in normal cows' milk of 0.6 mg/liter and that there was a mammary barrier which allowed dietary copper to influence milk copper only at subnormal levels. Others (113) reported 0.44 mg/liter and 0.66 for cows grazing near a copper ore works compared with 0.41 mg/ liter for cows located in other areas of the same country (104). Colostrum is highest in copper, and the content in milk decreases progressively throughout the lactation (78, 105). The estrous cycle of cows had no effect on the natural copper content of milk or its susceptibility to oxidized flavor (36).

Dunkley et al. (38) injected cows with a copper glycinate suspension and increased both blood and milk copper for at least four weeks, but increased the incidence of spontaneous oxidized flavor only within the first 24 hours after injection. In further research Dunkley et al. (37) significantly increased milk copper by feeding supplemental copper as $CuSO_4 \cdot 5\ H_2O$ and $CuNa_2EDTA$, but obtained no significant changes in the oxidative stability of the milk. Copper in the milk ranged from 0.04 to 0.06 mg/liter.

Supplemental sources of copper. Several copper compounds have served as dietary supple-

ments of the element for ruminants. Lassiter and Bell (61) reported greater blood intake of radioactive copper in sheep when radioactive cupric chloride was given orally than when cupric sulfate or cupric nitrate was administered. The copper from cupric oxide needles was less well absorbed. In another phase of the same study, cupric carbonate was better utilized than was cupric oxide powder or needles. In research with steers using radioactive compounds (21), the copper from cupric nitrate, cupric sulfate, and cupric chloride was absorbed to a similar extent. Cupric carbonate compared favorably with these compounds in absorption. More radiocopper was absorbed into the blood stream from powdered cupric oxide than from the same compound in the needle form. The authors concluded, from both the physical characteristic of the compound and the biological value of the copper, that cupric sulfate was the most suitable source of those tested for use as a dietary copper supplement.

In 1959 Allcroft and Uvarov (4) published results on an injectable copper compound in the form of glycinate for cattle. The material was injected subcutaneously in the area of the brisket. Two or three injections a year should maintain normal copper nutrition, even under conditions of severe copper deficiency. These studies indicated a fairly wide margin of safety with this method of copper supplementation. While this method of copper administration is apparently not widely used outside of Great Britain, other research reports (3, 27, 33, 38) have indicated its use. Injectable copper glycinate and also CuCa-EDTA were effective in maintaining normal blood copper in pregnant ewes (50). The injectable Cu-EDTA complexes currently available generally result in less localized reaction than occurs with copper glycinate but may have a greater toxicity potential than the glycinate form (56, 77, 98).

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Metabolism of Iron and Manganese [1]

J. W. THOMAS
Dairy Department, Michigan State University, East Lansing 48823

The common "trace minerals" originate from metals of the first transition series. Some ideas about the similarity of these metals are depicted in Table 1, in which they are compared to calcium.

Metallo Compounds—Mode of Action

As these transition metals increase in atomic number from scandium to copper, one or two typical valence electrons are in the outermost or 4s orbital while the sixth or 3d orbital is being filled with electrons (Table 1). The 3d and 4s levels are very close in energy and changes within the next to the outer orbital of the atom have a limited effect on chemical properties, so that one cannot easily predict atomic behavior with a change of one 3d electron. Ions of the transition elements may accept electrons from donor atoms into the 3d orbitals which may completely (or partially) fill this orbital. The electronic structure of these atoms can then correspond to that of another element and the electronic structure of several resulting pairs of ions are similar, i.e., Cr^{+2} and Mn^{+3}, Mn^{+2} and Fe^{+3}, Fe^{+2} and Co^{+3}.

Stability and action of chelated compounds depend on many properties of the metal ion, the chelating agent and the chelate formed. There is little difference in complexing power between cations having similar ionic radius, electron geometry, and electronegativity. Such cations may be substituted for one another in many in vitro enzyme systems where these metals act only in chelation, but not when they act as electron transfer agents (25, 52–55, 97, 134, 163, 166).

There are several instances where one of these metal ions can substitute for (or antagonize) another. Manganese has been found to be incorporated into the heme molecule in vivo and in vitro. The time sequence of synthesis and turnover of this manganese porphyrin is indistinguishable from that of iron porphyrin. This manganese porphyrin has been identified as a normal constituent in human and rabbit reticulocytes. The proportion is increased in animals given high amounts of manganese and in anemic rats (18, 46). This is interpreted as biological substitution of manganese for iron. Rats and cattle fed high doses of manganese

[1] Published with the approval of the Director of the Agricultural Experiment Station as Journal Article no. 4882.