Copper deficiency and associated diseases

Part 2

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Causes

(1) Primary copper deficiency due to inadequate levels in diet. Grazing in copper deficient soil.

(2) Secondary copper deficiency occurs due to conditioning

factors such as:

1) High molybdenum and or Sulphur intake.

2) Copper absorption and retention is decreased by excessive dietary calcium, zinc, molybdenum and or Sulphur.

Risk factors

Several factors influence the plasma and tissue concentrations of copper, particularly in ruminants, including:

1. Age of animal
2. Demands of pregnancy and lactation
3. Stage of growth
4. Copper sources available to the animals
5. Mineral composition of feed
6. Season of the year
7. Soil characteristics and its mineral composition
8. Breed of animal
9. Concentration of minerals, such as sulfur and molybdenum, which can interfere with the availability of copper.

Epidemiology

- Primarily in **young pastured ruminants** (cattle, sheep, goats, and farmed deer) in spring and summer.
- **Primary deficiency** occurs in sandy soil and heavily weathered areas
- **secondary** in peat or muck soil areas.
- Feed and water supplies may **contain molybdenum**, sulfate and iron salts, which interfere with copper metabolism.
- May be **congenital in newborn lambs** (swayback) if ewes deficient or delayed in nursing lambs (enzootic ataxia). Some breeds of sheep highly susceptible.

Pathogenesis

1. Effects on tissue
2. Chromosomal abnormalities
3. Wool
4. Body weight
5. Diarrhea
6. Anemia
7. Bone
8. Connective tissue
9. heart
10. Blood vessels
11. Pancreas
12. Nervous tissue
13. Reproductive performance
14. Immune system

1. Effects on tissue

consequences of hypocuprosis

Failure of copper metalloenzymes, many of which form part of the antioxidant defense system such as copper/zinc superoxide dismutase (Cu/Zn SOD) and ceruloplasmin.

Copper, as well as other essential trace elements, is an atypical antioxidant because it functions indirectly. Copper is a catalytic cofactor for Cu/Zn SOD and ceruloplasmin. Cu/Zn SOD catalyzes dismutation of the superoxide anion, producing molecular oxygen and hydrogen peroxide, with the latter product usually metabolized by glutathione peroxidase and catalase.

The ferroxidase activity of ceruloplasmin mediates the oxidation of ferrous ions to the ferric state, thereby preventing ferrous ion-dependent formation of hydroxyl radicals via the Fenton reaction

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Thus, in enabling Cu/Zn SOD and ceruloplasmin to function as described, copper can be classified as part of the antioxidant defense system of cells. Copper deficiency can affect the antioxidant defense system resulting in oxidative damage to cellular components.

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The activity of Cu/Zn SOD and glutathione peroxidase is decreased in animals with copper deficiency

- In cattle Copper deficiency associated with:

➢ a decrease in Cu/Zn SOD, ceruloplasmin and cytochrome oxidase activity

➢ increase in lipid peroxidation.

➢ Collectively, this indicates that copper deficiency weakens the antioxidant defense systems.

➢ Ceruloplasmin is the copper-containing enzyme through which copper exerts its physiological function.

➢ The pathogenesis of most of the lesions of copper deficiency has been explained in terms of faulty tissue oxidation because of failure of these enzyme systems.

➢ This role is exemplified in the early stages of copper deficiency by the changes in the wool of sheep.

2. Chromosomal abnormalities

- The association between copper deficiency and DNA damage in cattle has been examined.
- The **Comet assay** is a sensitive, reliable and rapid method for the detection of DNA double- and single-strand breaks and alkali -labile sites detection.
- In naturally-occurring copper deficiency in Aberdeen Angus cattle in Argentina, cytogenetic analysis of peripheral lymphocyte cultures showed a significant increase in the frequency of **abnormal metaphases in moderate to severe copper deficient groups**.
- Thus**, copper deficiency in cattle is associated with an increase in the frequency of chromosomal aberrations (clastogenic effect) as well as in DNA migration**

3. Wool

- The straightness and stringiness of this wool is due to inadequate keratinization, probably due to **imperfect oxidation of free thiol groups.**
- Provision of copper to such sheep is followed by **oxidation of these free thiol groups** and a return to **normal keratinization within a few hours.**

4. Body weight

In the later stages of copper deficiency, the impairment of tissue oxidation causes interference with intermediary metabolism and loss of condition or failure to grow

5. Diarrhea

- The pathogenesis of copper deficiency in causing diarrhea is uncertain and there is little evidence that a naturally-occurring primary copper deficiency will cause diarrhea.
- There are no histological changes in gut mucosa, although villous atrophy is recorded in severe, experimentally produced cases.
- **Diarrhea is usually only a major clinical finding in secondary copper deficiency associated with molybdenosis.**

6. Anemia

- The known importance of copper in the formation of hemoglobin accounts for the anemia in copper deficiency.
- The presence of **hemosiderin deposits** in tissues of copper-deficient animals suggests that copper is necessary for the reutilization of iron liberated from the normal breakdown of hemoglobin.
- There is **no evidence** of excessive hemolysis in copper-deficiency states.

- Anemia may occur in the later stages of primary copper deficiency, but is not remarkable in the secondary form unless there is a marginal copper deficiency, as occurs in peat scours in New Zealand.
- The unusual relationship in New Zealand between copper deficiency and post parturient hemoglobinuria is unexplained.
- **Heinz body** anemia in lambs with deficiencies of copper or selenium and moved from improved pasture to rape (Brassica nap us) has been reported.

7. Bone

- The osteoporosis that occurs in some natural cases of copper deficiency is caused by the depression of osteoblastic activity.
- In experimentally induced primary copper deficiency, the skeleton is osteoporotic and there is a significant increase in osteoblastic activity.
- There is a marked overgrowth of epiphyseal cartilage' especially at costochondral junctions and in metatarsal bones.
- This is accompanied by beading of the ribs and enlargement of the long bones. There is also an impairment of collagen formation.

- There is also an impairment of collagen formation. When the copper deficiency is secondary to dietary excesses of molybdenum and sulfate, the skeletal lesions are quite different and characterized by widening of the growth plate and metaphysis and active osteoblastic activity.
- Copper deficiency in foals causes severe degenerative disease of cartilage, characterized by breaking of articular and growth plate cartilage through the zone of hypertrophic cells, resulting in osteochondrosis of the articular-epiphyseal complex (A-E complex).
- The incidence and severity of osteochondrosis in foals can be decreased by supplementation of the diets of mares during the last 3-6 months of pregnancy and the first 3 months of lactation. Foals from non-supplemented mares have separation of the thickened cartilage from the subchondral bone.

- Clinical, radiographic, and biochemical differences occur between copper-deficient and copper-supplemented foals and there may be a relationship between low copper intakes in rapidly growing horses, inferior collagen quality, biomechanically weak cartilage, and osteochondritis.
- Copper is essential for metalloenzyme lysyl oxidase, which produces aldehydic groups on hydroxylysine residues as a prerequisite for eventual cross-link formation in collagen and elastin. Similar lesions in foals have been attributed to zinc toxicity from exposure of affected animals to pasture polluted by smelters.

- Experimentally, the addition of varying amounts of zinc to the diet of foals containing adequate copper will result in zinc-induced copper deficiency, but there are no effects with zinc intakes up to 580 ppm and it is suggested that 2000 ppm or higher are necessary to affect copper absorption in horses. Similar lesions of osteochondrosis have occurred in young farmed red deer and wapiti X red deer hybrids in New Zealand.

Connective tissue

- Copper is a component of the enzyme lysyl oxidase, secreted by the cells involved in the synthesis of the elastin component of connective tissues and has important functions in maintaining the integrity of tissues such as capillary beds, ligaments, and tendons.

Heart

- The myocardial degeneration of **falling disease** may be a **terminal manifestation of , anemic anoxia**, or be due to **interference with tissue oxidation**.
- In this disease, it is thought that **the stress of calving and lactation** contribute to the development of **heart block and ventricular fibrillation** when there has already been considerable decrease in cardiac reserve. Experimentally induced copper deficiency in piglets causes a marked reduction in growth and hematocrit and cardiac pathology and electrical disturbances.

Blood vessels

- Experimentally produced copper deficiency has also caused **sudden death due to rupture of the heart and great vessels** in a high proportion of pigs fed a copper deficient diet.
- The basic defect is **degeneration of the internal elastic laminae.** There is no record of a similar, naturally occurring disease.
- A similar relationship appears to have been established between serum copper levels **and fatal rupture of the uterine artery at parturition in aged mares**.

Pancreas

- Lesions of the pancreas may be present in normal cattle with a low blood copper status.
- The lesions consist of an increase in dry matter content and a reduction in the concentrations of protein and copper in wet tissue.
- The cytochrome oxidase activity and protein: RNA ratio are also reduced.
- There are defects in acinar basement membranes, splitting, and disorganization of acini, cellular atrophy and dissociation and stromal proliferation.

Nervous tissue

1. Copper deficiency halts the formation of myelin and causes demyelination in lambs, probably by a specific relationship between copper and myelin sheaths.

- Defective myelination can commence

A. as early as the midpoint of the fetus's uterine life.

The focus of lesions in the white matter shifts from the cerebrum in lambs affected at birth (congenital swayback) to the spinal cord in delayed cases, which may reflect respective peaks of myelin development I at those sites at 90 days' gestation and 20 days after birth.

B. The postnatal development of delayed swayback has been confirmed through its control by copper supplementation after birth.

- In experimental animals, it has been shown that copper deficiency does **interfere with the synthesis of phospholipids**.
- While anoxia is a cause of **demyelination, an anemic anoxia** is likely to occur in highly deficient ewes and anemic ewes produce a higher proportion of lambs with enzootic ataxia, there is often no anemia in ewes producing lambs with the more common subacute form of the disease.

- Severely deficient ewes **have lambs affected at birth** and in which **myelin formation** is likely to have been prevented.
- The lambs of ewes less severely deficient have normal myelination at birth and develop demyelination in postnatal life.

Reproductive performance

1. There is no evidence that copper deficiency causes reproductive failure in dairy cows.
2. Copper glycinate given to dairy cattle does not affect the average interval in days between calving and first observed heat, services per conception, or first service conception rate compared with untreated cows in the same population.

3. Experimentally, the addition of **molybdenum to the diet of heifers delayed** the onset of puberty, decreased the conception rate and caused anovulation and anestrus in cattle without accompanying changes in copper status or in live weight gain. Thus, the presence of molybdenum rather than low copper status may affect reproductive performance of cattle.

- Geochemical data indicate that approximately 10% of the cultivated area of England and Wales has soils that may result in forage molybdenum molybdenum concentrations similar to those used in the above experimental diet.
- It appears inadvisable to ascribe poor reproductive performance to subclinical hypocuprosis on the evidence of blood copper analysis alone.
- Other factors, such as management and energy and protein intake, should be examined.

Immune system

1. Copper is an essential trace mineral with an important role in the immune response but the precise mechanism is not well understood.
2. In experimental secondary copper deficiency in cattle induced by molybdenum at 30 ppm and sulfate at 225 ppm, the intracellular copper content of peripheral blood lymphocytes, neutrophils, and monocyte-derived macrophages was reduced between 40% and 70%.

3. In copper deficient animals, the serum ceruloplasmin activity decreased to 50% of control values.

4. Both the copper zinc- superoxide dismutase and the cytochrome c oxidase activities are significantly reduced in leukocytes.

Thus, copper deficiency alters the activity of several enzymes, which mediate antioxidant defenses and ATP formation.

5. These effects may impair cell immune function, affecting the bactericidal capacity and making the animals more susceptible to infection.

6. Copper deficiency results in decreased humoral and cell-mediated immunity, as well as decreased non-specific immunity regulated by phagocytic cells, such as macrophages and neutrophils.

7. The decreased resistance to infection in sheep is amenable to treatment with copper and genetic selection.

8. In lambs genetically selected for low and high concentrations of plasma copper, the mortality from birth to 24 weeks of age in the high line was half that in the low line. Most of the losses were due to a variety of microbial infections.

9. Experimental viral and bacterial infections of cattle can cause a rapid, though transient, increase in serum ceruloplasmin and plasma copper in copper-replete animals, suggesting a major protective role for copper in infectious diseases.

These changes in copper metabolism evolve from an interleukin-1 mediated increase in hepatic synthesis and release of ceruloplasmin, an acute phase protein.

10. Copper concentrations in organs involved in immune regulations such as liver, spleen, thymus, and lung are substantially reduced by copper deficiency, suggesting that copper-deficient animals are at greater risk for infection than copper-adequate animals. However, experimental low copper diets with or without supplemental molybdenum does not alter the specific immunity of stressed cattle.

11. The severity of copper depletion needed for immune dysfunction is less than required to induce clinical signs of copper deficiency and endogenous copper may contribute to the regulation of both non-immune and immune inflammatory responses.
12. Low molecular weight complexes may have an anti-inflammatory effect in animal models of inflammation and it is postulated that the elevation of plasma copper-containing components during inflammatory disease represents a physiological response.
13. In experimental coliform mastitis in dairy cattle, copper in the diet at 20 ppm reduced the clinical response but not duration of the mastitis compared with animals receiving 6.5 ppm beginning 60 days prepartum through 42 days of lactation. 23 Liver copper in the supplemented group was 162 and 33 ppm at calving and 256 and 45 ppm at 42 days postpartum, respectively.

14. Copper deficiency in heifers in Northern India was associated with significant reduction in the candidacidal activity of neutrophils compared with copper supplemented animals.

In experimental copper deficiency in calves,

1. beginning at 6 weeks of age, subclinical and clinical abnormalities appear after the following intervals: hypocupremia at 15 weeks, growth retardation from 15 to 18 weeks, rough hair coat at 17 weeks, diarrhea at 20 weeks and leg abnormalities at 23 weeks.

2. These signs correlate well with the onset of hypocupremia and are indicative of a severe deficiency.

3. Even with these signs of deficiency, the histological abnormalities may be only minor in degree. In experimental primary copper deficiency in calves, beginning at 12 weeks of age, clinical signs of the deficiency may not become apparent for about 6 months

4. Musculoskeletal abnormalities include a stilted gait, a 'knock-kneed' appearance of the forelimbs, overextension of the flexors, splaying of the hooves and swellings around the metacarpophalangeal and carpo-metacarpal joints.
5. Changes in hair pigmentation occur after about 5 months and diarrhea between 5 and 7 months. The diarrhea ceased 12 h after oral administration of a small amount (10 mg) of copper.

Copper-molybdenum-sulfate relationship

- The interaction between copper, molybdenum, and sulfur in ruminant nutrition is unique in its effects on health and production.
- Copper, molybdenum, and sulfur from organic or inorganic sources can combine in the rumen to from an unabsorbable triple complex**, copper tetrathiomolybdate** and deplete the host tissues of copper.
- Secondary or conditioned copper deficiency occurs when the dietary intake of copper is adequate, but absorption and utilization of the copper are inadequate because of the presence of interfering substances in the diet.

- Molybdenum and sulfate alone or in combination can affect copper metabolism.This effect also operates in the fetus and interferes with copper storage in the fetal liver.
- Besides the relationship with molybdenum, an interaction between the absorption of copper and selenium has been demonstrated the administration of selenium to sheep on copper-deficient pastures causing an improvement in copper absorption.
- The toxicity of any level of dietary molybdenum is affected by the ratio of the dietary molybdenum to dietary copper.

- The critical copper:molybdenum ratio in animal feeds is 2 and feeds or pasture with a lower ratio may result in conditioned copper deficiency.
- In some regions of Canada, the copper: molybdenum ratio will vary from 0.1 to 52.7.
- Higher critical ratios closer to **4.1-5 . 1** have been recommended for safety.

- The copper status of growing calves can also be affected to a similar degree by the inclusion of appropriate levels of supplementary iron or molybdenum in the diet.
- Following such inclusion, the liver and plasma concentrations of copper will decline within 12-16 weeks to levels indicating severe copper deficiency.

- The clinical signs of copper deficiency, as indicated by reduced growth rate and changes in the hair texture and color, are evident after 16-20 weeks only in animals supplemented with molybdenum.
- The reduced growth rate was accompanied by a decreased feed intake and reduced efficiency of feed utilization.

- **Copper absorption**
- On the basis of a response to copper injections and no response to copper administered orally to sheep on a high molybdenum intake, it is suggested that interference occurs with the absorption of copper from the gut. It is proposed that thiomolybdates form in the rumen from the reaction of dietary molybdenum compounds with sulfides produced from the reduction of dietary sulfur compounds by rumen bacteria. The thiomolybdates reduce the absorption of dietary copper from the intestine and also inhibit a number of copper-containing enzymes, including ceruloplasmin, cytochrome oxidase, superoxide dismutase and tyrosine oxidase.

- **Copper utilization**
- Sulfate and molybdate can interfere with mobilization of copper from the liver, inhibition of copper intake by the tissues, inhibition of copper transport both into and out of the liver and inhibition of the synthesis of copper-storage complexes and ceruloplasmin. The clinical signs of hypocuprosis (such as steely wool) can occur in sheep on diets containing high levels of molybdenum and sulfate, even though blood copper levels are high. This suggests that under these circumstances copper is not utilizable in tissues and the blood copper rises in response to the physiological needs of the tissues for the element. In pigs, a coppermolybdenum complex can exist in animals and that in this form the copper is unavailable. This would interfere with hepatic metabolism of copper and the formation of copper-protein complexes such as ceruloplasmin.

- **Hepatic storage**
- The copper status of the liver depends on whether the animals are receiving adequate dietary copper. With adequate dietary levels, the liver copper levels are less in the presence of molybdate and sulfate.
- If the animals are receiving a copper deficient diet such that copper is being removed from the liver, then the molybdate plus sulfate animals retain more copper in their liver than copper-deficient animals not receiving sulfate plus molybdate. This supports the hypothesis that molybdate and sulfate together impair the movement of copper into or out of the liver, possibly by affecting copper transport. Sulfate alone exerts an effect. An increase in intake reduces hepatic storage of both copper and molybdenum.

- **Phases of copper deficiency**
- The development of a deficiency can be divided into four phases:
- 1. Depletion
- 2. Deficiency (marginal)
- 3. Dysfunction
- 4. Disease

- During the **depletion phase**, there is loss of copper from any storage site, such as liver, but the plasma concentrations of copper may remain constant. With continued dietary deficiency, the concentrations of copper in the blood decline during the phase of marginal deficiency.
- However, it may be some time before the concentrations or activities of copper-containing enzymes in the tissues begin to decline and it is not until this happens that the phase of dysfunction is reached. There may be a further lag before the changes in cellular function are manifested as clinical signs of disease.

Summary of pathogenesis

The overall effect of these interactions is as follows.

1. Molybdate reacts with sulfides to produce thiomolybdates in the rumen. The subsequent formation of copper thiomolybdate complexes isolates the copper from being biologically available. The thiomolybdates reduce the effectiveness of enzymes containing copper and there are some significant interactions between copper, zinc, and iron. The following is the most important mechanism:

(1)Copper is essential for the synthesis of hemoglobin, reutilization of iron (hemosiderin) liberated from the normal breakdown of hemoglobin, enzymatic activities and tissue oxidation.

(2) Normal copper levels in animals range from 0.5 to 1.5 ug/ml blood, about 90% of copper remains in plasma as ceruloplasmin.

(3) Liver is the main storage organ of copper. High level of copper is observed in liver in number of diseases of man and animals (e.g. Cirrhosis of liver, hepatocellular degeneration and tuberculosis).

(4) Copper deficiency leads to:

1) Faulty in tissue oxidation because copper is essential for function and formation of cytochrome oxidase system so that inadequate keratinization of skin, wool and hair occur due to inability of follicle cells to convert prekeratin to keratin.

2) Decrease Hb synthesis resulting in anemic hypoxia

3) Loss of bone collagen as a result of impaired activity of the copper enzymes (e.g. amine or lysil oxidase causing osteoporosis.

4) Demyelination of CNS as well as necrosis and neuronal degeneration of spinal cord and brain stem.

5) Depigmentation of hairs and wools (achromotrichia) so that blacks hairs turn gray or brown. This occurs because copper interferes with the formation of melanine from tyrosine.

6) Mucosal atrophy in the small intestine resulting in severe diarrhea and malabsorption.

Clinical signs

(1) Microcytic hypochromic anemia due to hindrance in the process of hematopoiesis.

(2) Diarrhea (Scouring) in cattle. This diarrhea is persistent in nature and is defined as ’peat scours’ and ‘teart’ (Table.9).

(3) Bone deformities: Bones become porous (osteoporosis) and there is tendency of spontaneous fracture.

(4) Nervous disorder: Such animal shows nervous manifestations known as neonatal ataxia and sway back, later on paralysis may be occur.

(5) Pigmentary disorder: Black hairs turn grey or brown. There are abnormalities in the growth of hair and wools. Hair color around the eyes is strikingly altered giving glasses like appearance (Spectacle disease).

(6) In sheep: the fine wool becomes limp, gloosy and looses its crimp developing straight and steely wool appearance. Black wool shows depigmentation to white.

(7) Partial or complete alopecia may be occurring.

(8) The coat becomes rough. The red and black coat of cattle changed to a bleached, rusty red.

(9) Myocardial degeneration causing acute heart failure and sudden death (Falling disease).

(10) Infertility, delayed estrus in cattle, dead fetus and abortion may be occurs in all animals.

Disease	Country	Species affected	Copper level in liver	Probable conditioning factor
Swayback	Britain, USA	Sheep	Low	Unknown
Renguerra	Peru	Sheep	Low	Unknown
Teart	Britain	Sheep and cattle	Unknown	Molybdenum
Scouring disease	Holland	Cattle	Unknown	Unknown
Peat scours	New Zealand	Cattle	Low	Molybdenum
Peat scours	Britain	Cattle	Unknown, low level in blood	Unknown
Peat scours	Canada	Cattle	Unknown	Molybdenum
Salt sick	Florida (USA)	Cattle	Unknown	Unknown
'Pine' (unthrifty)	Scotland	Calves	Low	Unknown

Diagnosis

(1) History of diet and clinical findings.

(2) Estimation of Cu level in soil, hair, diet, blood and liver.

(3) Low level of copper reduces hemoglobin level and RBC.

Copper level in body tissues and fluids in primary and secondary copper deficiency

Species and tissue	Normal level	Primary copper deficiency	Secondary copper deficiency
Cattle			
Blood plasma (µg/mL) (convert to SI units by multiplying by 15.7 which gives µmol/L)	1.26 ± 31	<0.5 and as low as 0.1–0.2	<0.5 and as low as 0.2–0.3
Adult liver (µmol/kg DM)	>100 (usually 200)	<20 and as low as 4	<10
Milk (mg/L)	0.05–0.20	0.01–0.02	–
Hair (mg/kg)	6.6–10.4	1.8–3.4	5.5
Sheep			
Blood plasma (µg/mL)	0.7–1.3	0.1–0.2	0.4–0.7
Adult liver (µmol/kg DM)	>200 (usually >350)	20	15–19

Condition	Area	Soil type	Soil copper (mg/kg)	Plant copper (mg/kg DM)
Normal	–	–	18–22	11
Primary copper deficiency	West Australia	Various	1–2	3–5
	New Zealand	Sand	0.1–1.6	3
	New Zealand	Peat	–	3
	Holland	Sand	–	<3
Secondary copper deficiency	New Zealand	Peat	5	7
	Britain	Peat	–	7–20
	Britain	Limestone	–	12–27
	Britain	Stiff clay	–	11
	Ireland	Shale deposits, peat marine, alluvial soils		
	Holland	Sand	–	>5
	Canada	Burned-over peat	20–60	10–25

- **Treatment**

(1) Remove the cause.

(2) Oral copper sulphate 2-4 gm for adult cattle, 1.5 gm for goat and sheep (recovery occurs within hours), repeated weekly interval to prevent reappearance.

(3) Add 5 ppm of copper sulphate to the dry diet or 0.5% copper can be added in mineral mixture.

(4) Very slowly IV injection of diluted 20 mg copper for sheep and 50 mg for cattle are effective for about four months.