COPPER ASSOCIATED LIVER DISEASE IN THE DOG:  GASTROENTEROLOGY
AN EMERGING PROBLEM OR CHRONIC CONFUSION?
Sharon A. Center, DVM, DACVIM

Introduction
Outside of the Bedlington terrier (BT), the role of excess hepatic copper as an etiologic agent of liver disease remains unclarified. The BT is the only breed to date where a genetic cause for copper storage hepatopathy has been confirmed. Initially, a microsatellite DNA marker (C04107) was located in the area of the copper storage disease allele. This marker was used to predict individual dog phenotype (affected vs. nonaffected). Further work (positional molecular cloning) identified the involved mutation and gene (different from the causal factor of Wilson’s disease in man; Coronado 2003). This is an autosomal recessive trait due to a mutation in the COMMD1 gene (originally called the MURR1 gene). COMMD1 = copper metabolism gene MURR1. The MURR1 is a member of a family of proteins defined by the presence of a conserved and unique motif termed the COMM domain. This domain functions as an interface for protein–protein interactions (nuclear transcription or regulatory effects). COMMD1 is the prototype of a new protein family that plays a role in several important cellular processes, including NF-kappaB signaling, sodium transport, and copper metabolism. The 10 COMMD family members are well conserved between vertebrates. A 13.9 kb deletion in exon 2 of COMMD1 on chromosome 10 was confirmed to cause copper storage hepatopathy in most BT (van de Sluis et al. 1999). However, there are some BT families with copper storage hepatopathy that do not have this mutation.

What About Other Dog Breeds?
Case reports of individual dogs (pure breeds, e.g., Dalmatian, corgi, Labradors, dobermans, others, and mixed breeds) with hepatopathies characterized as chronic hepatitis or cirrhosis associated with high copper (> 1,500 μg Cu/gm liver) are becoming more common. Special circumstances may relate to the West Highland white terrier; it is curious that some of these dogs with very high hepatic copper concentrations have died in old age without any sign of liver injury. Recent manuscripts describing chronic hepatitis in Labrador retrievers have generated much interest. One of these affiliates Labrador hepatitis with excessive liver copper storage. While reported evidence suggests a familial problem, preliminary genetic studies have not identified a candidate region. Labrador hepatitis may be asymptomatic or symptomatic, but is typically associated with increased ALT and ALP activity with the fold increase in ALT > ALP. Dogs presented early only have increased liver enzymes, while those presenting late may demonstrate the full gamut of clinicopathologic features consistent with chronic hepatitis and cirrhosis. Ultrasonographic features are variable. Survival cannot be predicted from clinical or histologic assessments. A similar condition occurs in the doberman pinscher, where copper is found in some but not all dogs with chronic hepatitis. Some of these present in acute fulminant liver failure. We are currently observing an increase in dogs (many breeds) with copper associated inflammatory liver disease despite the fact that we have routinely stained and measured metals in liver biopsies for several decades.

What About Copper Storage Hepatopathy in Cats?
We have seen copper storage hepatopathy in a small number of cats at Cornell, and there are a few published cases consistent with a copper retention problem.

Causes of High Liver Copper Concentrations
Confusion in relating hepatic copper as a cause of liver disease exists because tissue copper values > 1,500 μg/gm dry weight liver may accompany hepatitis or cirrhosis in dogs in a cause OR effect relationship. In health, copper is ingested with food and water, is rapidly absorbed from the alimentary canal, temporarily bound to transport proteins, and circulated to the liver, where it is actively transported into the hepatocyte. A series of enzymes, chaperones, and pump mechanisms distribute copper intracellularly and transport excess copper into canaliculi for biliary excretion. These mechanisms normally maintain a neutral copper balance. Excessive accumulation of copper results in sequestration of copper within lysosomes, resulting in intraorganelle membrane oxidation, autodigestive enzyme release, cell membrane and organelle damage, and eventually, oxidative hepatocellular death. Consequently, excessive hepatocellular copper accumulation can reflect 1) the ingestion of pathologic quantities of copper, 2) an inborn error of hepatocellular copper transporters, or 3) antecedent liver injury and impaired ability to maintain a neutral copper balance (cholestasis, canalicul damage).
Tissue Injury Caused by Copper
Copper, like iron, is a transition metal contributing to oxidative tissue injury (Fenton Reaction, Haber-Weiss Reaction). Each metal plays a pathologic role in a diversity of liver disorders; excessive iron stores commonly accompany excessive copper stores in dogs with necroinflammatory liver lesions. Accumulated copper imparts an oxidative injury, leading to lysosomal degradation, intracellular membrane damage, and dysfunction of enzymes and organelle membranes, leading to mitochondrial damage and cell death (cytolytic and apoptotic). Liver injury, associated with pathologic copper accumulation in hepatocytes, reflects functional and morphologic damage.

Confirming a Diagnosis of Copper Associated Liver Disease
Liver biopsy is the only way to substantiate localization and severity of copper accumulation. While cytologic aspirates can suggest excessive copper stores, aspiration or imprint specimens cannot definitively ascertain this problem. Concern should be given to the morphologic description of liver biopsies implicating copper storage as the cause of chronic disease. The histologic distribution of hepatic copper granules assists in differentiating primary from secondary causes. Copper granules in hepatocytes adjacent to necroinflammatory injury and in hepatocytes distant from these lesions suggest a primary copper role. Primary copper storage in dogs involves Zone 3 (centrilobular) accumulation, but copper also is found in hepatocytes in other zones as well as within regenerative nodules. Secondary copper accumulation is only associated with regions of cell injury. Assessment of tissue copper deposition is complicated with needle biopsies, as these may inadequately detail acinar structure. Laparoscopic or wedge (surgical) biopsies are preferred. Liver biopsies acquired using skin punch methods distort tissue architecture and are difficult to interpret.

Qualitative Copper Stains
With routine light microscopy, hematoxylin and eosin stains copper granules as pink-brown or pink-grey intracytoplasmic refractile inclusions in hepatocytes, macrophages, and lipogranulomatous lesions. Qualitative estimates of copper retention are made on the basis of copper protein-specific stains; rhodanine or rubeanic acid is most commonly used. This allows subjective grading of copper retention, but this assessment must ALWAYS be reconciled with quantitative tissue copper determination (dry weight basis).

Quantitative Copper Measurements
Verify the relevance of Cu as a component of liver injury, considering that copper granules can be observed with tissue copper concentrations > 400 ppm; values > 800 ppm are common in dogs with hepatitis, and values > 1,500 ppm indicate severe accumulation. While copper accumulation can represent an epiphenomenon of chronic inflammatory and cholestatic liver injury, it can nevertheless contribute to liver injury. So, whether the copper appears to be “primary” or “secondary” may not determine the need for chelation.

Plasma Copper or Ceruloplasmin Measurements
These measurements cannot diagnose or monitor copper storage hepatopathy in the dog.

Therapeutic Strategies
Irrespective of the underlying cause, dogs with excessive hepatic copper stores should have restricted copper intake. Tissues with copper concentrations > 1,000 ppm should be chelated with d-penicillamine for at least 6 months. Only tissue biopsy can confirm the extent of tissue copper removal after protracted chelation therapy, although liver enzymes (ALT activity) are used as a surrogate marker. Typically after the first month of treatment, enzyme activity notably declines.

Dietary and Water Copper Allowances
Limiting intake of copper will reduce copper loading but will not facilitate loss of excess copper from the body and must be accompanied by chelation if excessive hepatic copper stores exist. Reducing copper intake is especially important during initial chelation therapy. If a homemade diet is used, it is essential to consult the USDA food tables to ascertain which foods are high in copper. (http://www.nal.usda.gov/fnic/foodcomp/Data/SR18/nutrlist/sr18w312.pdf. This site provides Cu content for most foods and can be sorted alphabetically or by quantitative copper content.) Further diet adjustment (protein, energy, sodium content) can be done using NAT 2 at the University of Illinois (also linked to the USDA food tables; http://nat.crgq.com/mainnat.html). Special diets also can be formulated by veterinary nutritionists at Balance-IT. If a homemade diet is used, AVOID organ meats, shellfish, nuts, and certain cereals rich in copper. Also, avoid vitamins or treats containing copper; these may not be listed on ingredient lists and will require a call to the distributor or
manufacturer. Contact the company Balance-It (BalanceIt.com) for an appropriate vitamin/mineral supplement. Generally, for a BT-sized dog, dietary copper intake is restricted to < 5 mg/kg (ppm, dry matter basis food). An allowance no greater than 0.4 mg copper per day for a BT has been prescribed, as this was reported to maintain a neutral copper balance (10 kg dog, 40 ug/kg per day, unpublished data, Brewer et al., Morris Animal Foundation Study report). Recommendations for humans with copper storage hepatopathy are to restrict copper intake to < 1 milligram per day (approximately 10–14 ug/kg day) and avoid intake of water containing > 1 ug/ copper per liter. It is prudent to avoid domestically softened water passing through copper tubing, which is thought to transfer a higher copper load during initial flushing of pipes each day. The current NRC estimated copper allowance for adult dogs is 0.15 mg/100 kcal (150 ug/100 kcal), while the AAFCO allowance for growth and adult maintenance is a 0.21 mg/100 kcal minimum. Thus, it appears that recommendations for dogs may be too high for dogs with confirmed hepatic copper accumulation, especially if a highly available form of copper is used as the food supplement. Typical puppy and adult dog foods contain between 15–25 mg/kg (ppm) dry matter basis or 0.4–0.5 mg/100 kcal; thus many diets exceed restrictions previously suggested for dogs with copper storage hepatopathy. Because of wide variability among diets in copper content, individual analysis of a specified commercial diet may be necessary to select the most appropriate food. Formulas are constantly “tweaked,” so it is important to check with the company marketing the food or have a new analysis done. The prescription canine liver diet L/d (Hills prescription diet L/d) provides approximately 4.9 mg/kg (ppm, dry matter basis, approximately 0.1 mg copper/100 kcal), similar to the intake proposed for Bedlington terriers with copper hepatopathy. Humans with Wilson’s disease (copper storage hepatopathy), consuming a lactovegetarian diet, almost totally noncompliant with anticopper therapy (chelation), were able to avoid excessive copper accumulation as a result of severe dietary copper restriction (Brewer et al. 1993).

When to Chelate?

Tissue copper concentrations > 1,000 µg/gm should be chelated for at least 6 months. Only histology can confirm the extent of copper removal although ALT activity serves as a surrogate marker. Pathologic copper concentrations in liver tissue cannot be deduced from plasma metals or metal binding proteins (ceruloplasmin). Needle aspirates CANNOT be used to judge response to therapy or to make an initial diagnosis.

There are two drugs used for chelation, d-penicillamine and trientine; there is far more experience with the former in animals. D-penicillamine is considered the gold standard for chelation, as it offers benefits beyond chelation in chronic hepatic inflammation. Trientine may be more potent in producing cupriuresis but has been associated with acute renal failure in a small number of dogs. After chelation, oral zinc therapy is proposed by some (given 30 minutes before meals) to curtail enteric copper uptake. Zinc induces metallothionein, which irreversibly binds copper in the enteric canal, where it is eliminated in feces. Long term use of zinc may (rarely) provoke iron deficiency.

D-Penicillamine

Dimethylcystine: a dose of 10-15 mg/kg PO BID is recommended, given 30 minutes prior to feeding (enhances bioavailability). Treatment is continued for 6 months, and normalization of liver enzymes or re-biopsy is used to prove efficacy. While there is no proven feline dose, the dog dose has worked well in a few cats treated. Chronic maintenance therapy is 25% to 50% of the effective tolerated decoppering dose. It is important to maintain dietary copper restriction during initial chelation and chronic management. Vomiting is a common side effect that may be managed by dividing the total dose into 2 or 3 treatments per day and administering them with a small piece of food.

There is conflicting evidence regarding the decoppering influence of D-penicillamine. In addition to copper chelation, d-penicillamine also provides an immunomodulatory effect. In humans, excess copper is mobilized during the initial treatment year, with urinary excretion being the major route of copper elimination. An improvement in health status occurs before mobilization of substantial copper stores, suggesting that copper detoxification may precede tissue chelation. D-penicillamine sequesters copper in an innocuous state either through direct binding or via induction of copper binding proteins (e.g., metallothioneins). D-penicillamine also has an antifibrotic effect through inhibition of collagen cross-linking and stimulation of collagenase activity. Treatment also has been shown to restore cellular glutathione (an important antioxidant) and to restore function of the mononuclear macrophage system, increasing host defense against antigenic and/or endotoxic insults. An anti-inflammatory effect suppresses activity of leukotrienes and prostaglandins. D-penicillamine also removes zinc and other essential metals from the body and can lead to Fe deficiency anemia. Co-treatment for these possible scenarios will reduce the copper
chelating efficacy. Co-treatment with zinc is strictly contraindicated, as this will negate the benefits of each therapy. If zinc is used, it is only indicated after the decoppering phase is completed.

Side effects include acute hypersensitivity drug reaction in humans (i.e., fever, malaise, rash, pruritis, lymphadenopathy), immune-mediated disease of kidneys and lungs, Lupus-like syndrome, polyarthritis, nephrotic syndrome, anorexia (altered taste sensation, nausea), leukopenia, thrombocytopenia, peripheral neuritis, a myasthenia-like syndrome, aplastic anemia, dermatitis, and hair loss. D-penicillamine imparts an anti-pyridoxine (vitamin B6 inactivation) effect in humans, requiring concurrent pyridoxine supplementation. We supplement dogs with 25 mg pyridoxine per day.

**Tetramine**

An alternative chelator used when d-Penicillamine is not tolerated, tetramine (Syrpine, 250 mg capsules, Merck, Sharp & Dohme; Cuprid) is given at an initial dose of 5–7 mg/kg PO BID, 1–2 hours before a meal (lower dose is safer). Touted to be a more effective cupriuretic/chelating agent than d-penicillamine, tetramine mobilizes copper from a different compartment. Unlike d-penicillamine, it does not remove copper from the CNS. Side effects in humans are similar to the side effects of d-penicillamine. While there were no demonstrated side effects in a limited clinical trials in dogs, I and others have observed acute renal injury in dogs chelated with the initially recommended 10–15 mg/kg per day PO BID dose.

**Zinc Therapy: After Decoppering**

A number of dogs we have treated cannot tolerate zinc therapy at any dose.

*Loading phase:* 5–10 mg/kg of elemental zinc per day given in 2 divided doses, 30 minutes before meals (e.g., 50–200 mg/elemental zinc per day per 10–12 kg divided BID PO in Bedlington & WHW Terriers). Doses should be separated by at least 8 hours and segregated from food by 30–60 minutes. In case of vomiting, give zinc with a small piece of meat. (but food reduces zinc uptake!). Although zinc gluconate, sulfate, or acetate can be used, the latter form is best tolerated. Encapsulated zinc acetate can be acquired as Galzin™ from GATE Pharmaceuticals (Phone: 800-292-GATE; Fax: 215-591-8801).

*Maintenance phase:* 50% of effective loading phase dose, after 2–6 months.

*Life-long treatment.* 2–3 mg/kg elemental zinc per day divided BID doses 30 minutes before meals as chronic supplementation in liver disease and for chronic copper toxicosis therapy. These doses were extrapolated from study of very few dogs. The theoretical advantage of zinc in chronic copper hepatotoxicosis is that interrupted therapy (up to 24 days) is associated with sustained release of zinc from acquired body stores, which provides protective coverage. Chronic therapy may cause iron deficiency, similar to chronic chelation.

*Monitoring:* Plasma zinc concentrations should be determined initially, then periodically, during the first month and then every 2 to 3 months in the initial 6 months of treatment to ensure avoidance of toxic zinc concentrations ( > 800 µg/dl [8–10 ppm] that cause hemolysis). Plasma zinc concentrations DO NOT correlate with tissue zinc values. However, demonstrating an increased plasma zinc concentration with supplementation suggests increased uptake. Target plasma zinc concentrations range between 200–400 µg/dl. While optimal plasma Zn concentrations are undetermined, baseline values should rise slightly.

*Plasma zinc toxicity:* Hemolysis, both intravascular and extravascular, can occur with zinc toxicity as well as formation of heinz bodies and spherocytes. Plasma zinc concentrations usually exceed 1000 µg/dl when zinc toxicity underlies hemolysis. Care must be taken to collect specimens in zinc free containers; contact of serum with some glass or rubber stoppers in vacutainers can increase zinc concentrations by as much as 1.44 mg/L (0.144 mg/dl = 144 µg/dl). Special transport tubes are available (royal blue stopper: trace element free heparinized vacutainer). Venoject™ tubes contribute very small amounts of zinc. Normal plasma zinc concentrations in dogs ranges from 0.7 to 2 ppm (70 to 200 µg/dL).

*Antioxidants:* Excessive hepatic copper causes liver injury, primarily by causing oxidative injury associated with mitochondrial and hepatocyte GSH depletion.
Vitamin E: 10 iu/kg PO per day is recommended for its antioxidant, anti-inflammatory, and antifibrotic effects.

s-Adenosylmethionine (SAMe): 20 mg/kg is recommended as a thiol donor while hepatic injury continues.

Vitamin C: Contraindicated, as it is surmised to enhance tissue injury derived from transition metals.

Glucocorticoids: Co-administration of glucocorticoids is ONLY in an acute hepatic necrosis crisis. In this circumstance, it is argued to improve lysosomal membrane integrity.

Immunomodulatory drugs: Some dogs with copper associated hepatopathy have T cell confirmed inflammatory infiltrates in their liver (e.g., certain Labrador retrievers, other breeds). These dogs require immunomodulatory therapy as prescribed for other immune-mediated necroinflammatory liver disorders (e.g., azathioprine, glucorticoids, mycophenolate).

Antifibrotic drugs: Vitamin E, SAMe, and polyunsaturated phosphatidylcholine (20–50 mg/kg PO per day) may provide benefit. The role of milkthistle (silybin or silibinin) is unresolved.

Summary of Therapeutic Strategies: Copper Storage Disease

1. COPPER CHELATION (removes copper from tissues or blood)
   D-penicillamine or trientine
   Do NOT combine zinc treatment with D-penicillamine chelation: zinc uptake by chelator
2. REDUCE SYSTEMIC CU UPTAKE
   a. limit dietary Cu intake
   b. limit water Cu intake
   c. zinc supplementation: chronic therapy AFTER chelation removal of Cu; efficacy not proven
3. GLUCOCORTICOIDs: ONLY for dogs with crisis hepatocellular necrosis/hemolysis
4. AVOID VITAMIN C SUPPLEMENTATION: may augment transition metal induced tissue injury
5. ANTIOXIDANTS: Vitamin E (alpha- tocopherol) & SAMe.
   a. vitamin E 10 IU /kg/day PO
   b. SAMe (as Denosyl) 20 mg/kg PO/day on an empty stomach
6. URSODEOXYCHOLIC ACID: if cholestatic injury/high bile acids: 10–15 mg/kg/day

References
