There is increasing recognition that inflammatory liver disease in dogs is associated with abnormal hepatic copper (Cu) concentrations. Abnormal hepatic Cu accumulation results as either a primary metabolic defect in Cu metabolism unique to some breeds or as a secondary event associated with chronic hepatic cholestasis resulting in a decrease in biliary excretion of hepatic Cu. Abnormal hepatic copper can also accumulate in the liver secondary to increased dietary intake. Regardless of the cause at a certain concentration Cu contributes to hepatocellular damage.

Normal copper metabolism
Copper is an essential trace element required as a redox co-factor for many different enzymes. Copper enters the body through the diet and approximately 30% is absorbed by the upper small intestine with unabsorbed copper passing through the feces. Although the exact details of intestinal Cu absorption is not completely delineated it is clear that copper is taken up in the intestine through an active transport mechanism shared with zinc. Intestinal Cu is subsequently transported to the liver bound to albumin and transcuprein. The liver is responsible for the uptake and storage of copper, as well as the regulation of excretion of this metal into the bile. Hepatic copper is either complexed to ceruloplasmin, an acute phase reactant protein, and transported to peripheral tissues for utilization, or Cu is redistributed among the various metallothioneins in the liver. Metallothioneins are cysteine-rich, cytosolic proteins capable of binding several metal ions, including copper. Metallothioneins functions are to protect the hepatocyte against the toxicity from free Cu catalyzing oxygen free radicals and also to mediate Cu transport into the bile for removal from the body. The normal hepatic copper concentrations in dogs are maintained at approximately 200-400 µg/g dry weight liver.

Recently there has been characterization of the genetic regulation of copper excretion by the liver. A specific gene in humans ATP7b is a copper-transporting ATPase expressed within the secretory pathway of hepatocytes and plays a critical role in copper excretion and ceruloplasmin production. A second gene encoding COMMD1 (MURR1) is expressed in the liver suggesting that this protein also plays a role in hepatic copper transport and biliary copper excretion. Wilson disease in humans is an inherited mutation in the gene encoding human ATP7b and results in hepatic copper overload and decreased Cu-ceruloplasmin production. Bedlington Terriers also have an inherited disorder of copper homeostasis. These animals have impaired copper excretion into bile but no abnormality in copper incorporation into ceruloplasmin suggesting that the defect occurs distal to the function of ATP7b in intracellular copper transport. This disorder has recently been shown to result from deletion of a gene on dog chromosome 10 encoding a small cytosolic protein termed COMMD1 (MURR1).

Copper hepatotoxicities in dogs
Bedlington terriers
Hepatic copper toxicity was first identified in Bedlington Terriers in 1975. It was subsequently shown that affected Bedlington Terriers have an inherited autosomal recessive defect, which results in reduced biliary excretion of copper with hepatic metallothionein sequestration of Cu in hepatic lysosomes. The pathogenesis of hepatic damage is thought to occur when the metallothionein sequestration ability for Cu becomes exceeded and free copper is released. The mitochondria appear to be the first organelle to become damaged resulting in mitochondrial electron leak initiating lipid membrane peroxidation and eventual cellular death.

The excess hepatic copper is sequestered in lysosomes bound to metallothionein proteins. Routine stained histological sections may show abundant golden-brown refractile hepatocellular lysosomal granules that contain the sequestered Cu. These granules are nonspecific for copper, but may indicate abnormal copper accumulation. A more reliable semi-quantitative estimation involves histochemical staining for hepatic Cu. Reliable tissue bound copper stains include rhodanine and rubenic acid. The copper tends to accumulate in a centrilobular location. A grading system of 1-4 estimating the quantity of Cu granules correlates roughly with quantitative determination of hepatic Cu when the values approach >750 µg/g dry liver weight.

Definitive determination of the amount of hepatic Cu requires a quantitative analysis of tissue Cu. Hepatic copper content is measured using atomic absorption spectroscopy and can be determined on a full 16 g needle biopsy sample, although larger samples provide better accuracy. Samples for analysis should be placed in a Cu free container (such as a serum blood tube) for analysis. Normal canine hepatic Cu concentrations are less than 400 µg/g dry weight liver. The concentration at which abnormal hepatic Cu contributes to hepatic damage is unknown. It is possible at least at my Diagnostic Laboratory, to take adequate size biopsy sample embedded in paraffin for histology and de-paraffinize the sample to obtain a quantitation of copper.
Morphologic evidence of inflammatory hepatic injury in Bedlington Terriers begins when concentrations reach approximately 2,000 µg/g dry weight although sub-cellular morphologic changes are found with lower Cu concentrations. Homozygous affected dogs have increased copper concentrations but should be older than one year of age prior to having a biopsy. This is because the heterozygous carrier dogs normally increase in copper concentrations out of the normal range until around 6-9 months of age before concentrations fall back into the normal range. Genetic testing (VetGen.com) is also available for Bedlington Terriers to determine if they are free of disease. A liver biopsy is required to completely confirm if the dog is phenotypically affected.

**Doberman pinscher**

Doberman hepatitis is a form of chronic hepatitis. The incidence is unknown but may occur in as high as 4 to 6% of dogs. The high percent suggests a genetic predisposition. Females seem to be over-represented. The disease begins in young dogs (1-3 years) with increased ALT concentrations and having sub-clinical hepatitis. Clinical evidence of liver disease usually begins around 4-7 years of age with chronic hepatitis and cirrhosis. Copper appears to be associated with the disease and recent studies suggest that copper is often increased prior to development of clinical hepatitis. Cu$^{64}$ isotope studies demonstrate affected dogs have an impaired biliary excretion of copper. Copper chelator therapy in sub-clinical dogs normalized copper concentrations with improvement in the grade of histological damage. In affected dogs the copper concentrations generally range from 1000-2000 µg/g DW liver. At this point no specific gene has been identified for this disease to determine the mode of genetic transmission. The above evidence suggests a primary defect in copper metabolism in the breed but awaits further conformation. An autoimmune mechanism is also suggested but this too requires further investigation. It appears that the hepatocyte may have abnormal MHC class II complex expression stimulating activation of CD4 T cells and an immune disease.

**Dalmatians**

A retrospective study summarizes 10 Dalmatians suspected of having hepatic copper toxicosis. Two of the dogs were related and all presented for gastrointestinal clinical signs, had elevated liver enzymes and necroinflammatory hepatic changes associated with copper-laden hepatocytes most prominent in a centrilobular location. The mean hepatic copper concentration was 3,197µg/d dry weight liver. In 5 of these 9 dogs, hepatic copper concentrations exceeded 2,000 µg/d DW liver with several dogs having copper levels as high as those observed in Bedlington Terriers. These findings support the hypothesis that a primary metabolic defect in copper hepatic metabolism occurs in the Dalmatian breed. Some of these dogs also have renal glycosuria suggesting a Fanconi-like effect. The mechanism and genetic basis of this condition is under further study.

**West Highland white terriers**

The “Westie” breed has been associated with liver disease and hepatic copper accumulation. The clinical findings appear to be different than other breeds associated with copper accumulation. Dogs reported showed evidence of hepatitis or cirrhosis and had increased hepatic copper ranging from 1000-3000 µg/g dry weight liver. Twenty-four dogs described ranged from 3-7 years of age. Some dogs in this report had high copper concentrations but no evidence of liver disease while others did. While the Bedlington Terrier tends to accumulate Cu with age it was not apparent in this group of dogs. Affected dogs that were bred produced offspring with elevated copper concentrations supporting a genetic defect. Several dogs were treated with zinc therapy and showed reduction in hepatic copper concentrations.

**Labrador retrievers**

Chronic hepatitis is reported to be common in this breed and there is evidence that copper accumulation is associated with some, but not all the cases. There has been extensive work on this syndrome in the Netherlands and they document it to be inherited and in fact, asymptomatic relatives of affected dogs also contain copper in their livers. We find females are more commonly affected and the diagnosis is generally made between 2 to 7 years of age. Hepatic copper concentrations generally range between 750 to 2000 µg/g dry weight liver. The histological location of the Cu being centrilobular suggests that Cu elevation is probably not secondary to cholestasis. It appears that copper chelation is beneficial in some dogs with hepatitis and copper accumulation.

Researchers demonstrated that the copper accumulation in these dogs is controlled using short-term penicillamine therapy followed by feeding a low copper diet. They further found dietary copper in commercially available dog food can influence hepatic copper concentrations and can be a risk factor for the development of copper-associated hepatitis in Labrador retrievers with a genetic susceptibility to copper.

**Other breeds and cats**

The Skye Terrier, Anatolian Shepherd, and possibly the Keeshond as well as other breeds have also been reported with liver disease and increased copper accumulation. The exact mechanism or extensive description in specific breeds is lacking. We will occasionally identify cats with increased copper and evidence of hepatitis and thus cats with evidence of inflammatory liver disease should be investigated for increased copper concentrations.

**Treatment considerations**

Regardless of the cause of hepatic Cu accumulation it has been shown that unbound Cu plays a role in hepatocellular damage. The treatment possibilities are threefold: (1) to decrease further absorption of copper from the gastrointestinal tract by feeding a Cu deficient diet or blocking dietary Cu uptake, (2) to enhance hepatic Cu removal using specific chelator therapy and/or (3) to protect the
liver from copper catalyzed oxidative damage using antioxidant agents. A specific therapeutic plan requires careful case evaluation and individual formulation.

We now speculate that a number of other dogs may have the inability to handle dietary copper resulting in hepatic copper accumulation. This theory comes about because the normal hepatic copper concentration for dogs has been increasing over the years and the fact that canine commercial diets are over supplementation with copper (if you compare that to copper requirements for humans). Further, in a study investigating feral dogs that were unlikely to have ever eaten commercial dog food were found to have significantly lower hepatic copper concentrations compared with normal control dogs eating a commercial diet. Consequently, we believe some dogs taking in excessive copper may have the inability to handle the high copper will develop copper associated hepatitis.

Diets low in copper are recommended for the dogs that have copper associated liver disease based on liver biopsy. However the restriction of dietary copper may do little to lower hepatic copper concentrations in diseased dogs having already large amounts of hepatic copper but diet will lessen further absorption of the metal. It is difficult to limit dietary copper because most commercial dog foods contain supplemental copper that likely exceeds the dog’s actual dietary requirements. Most formulated “liver diets” have lower copper concentrations and are recommended. Homemade diets can also be prepared so that they do not to contain excess copper. These diets should exclude liver, shellfish, organ meats and cereals that are all high in copper content. Vitamins or mineral supplements should not contain copper or iron. The company www.BalanceIt.com makes a copper free dietary vitamin mineral supplement that can be used with homemade diets. They also have formulations for a homemade diet.

If the liver biopsy of a dog with chronic hepatitis indicates significant abnormal hepatic copper accumulation, a low copper diet and copper chelation or zinc therapy should be started. I believe hepatic copper levels of greater than 750 μcg/g dry weight (dw) liver (normal <400 μcg/g dw) requires therapy to reduce copper concentrations. Animals having greater than 1,500 μcg/g dw should all have chelator therapy because that is a concentration considered to definitely be toxic to hepatocytes.

Zinc given orally as the acetate, sulfate, gluconate or other salt has been shown to be effective in preventing hepatic copper absorption from the GI tract in Wilson’s disease patients that have been previously decoppered with penicillamine. Oral zinc therapy works by causing an induction of the intestinal copper-binding protein metallothionein. Dietary copper binds to the metallothionein with a high affinity that prevents transfer from the intestine into the blood. When the intestinal cell dies and is sloughed, the metallothionein bound copper becomes excreted through the stool. I will sometimes use zinc after a course of chelation therapy or as a primary therapy in a dog having modest hepatic copper accumulation or when the client can not afford penicillamine therapy. An initial induction dose of 5-10 mg/kg body weight divided BID of elemental zinc. Following one to 3 months of induction period the dose can be reduced in approximately half. The goal is to get serum zinc concentrations greater than 200μg/dl but less than 500 so I will often check serum zinc concentrations several times during a course of therapy. The zinc must be administered on an empty stomach and has the frequent side effect of vomiting. Zinc also has anti-fibrotic and hepatoprotective properties as well.

Chelator treatment using penicillamine is the primary therapy for copper associated liver disease. Penicillamine binds with copper and then promotes copper removal through the kidneys. Penicillamine is the most frequent copper chelator recommended for use in dogs. The dose is 10-15 mg/kg bid given on an empty stomach. Side effects include anorexia and vomiting but can be managed by starting at a lower dose and then increasing the dose over time or by giving a small amount of food with the drug. Therapy using penicillamine is a slow and prolonged process taking months to cause a substantial reduction in hepatic copper concentrations. Penicillamine also has been shown to have a protective effect in the liver beyond chelation therapy. It is believed penicillamine induces a hepatic copper binding protein, metallothionein, thus binding and sequestering copper in a nontoxic form in the liver. The length of chelation therapy is variable but based on past experience some general recommendations can be made. Ideally repeat liver biopsies should be obtained to determine success of the chelation and to direct duration of therapy. The following is only a general recommendation; if copper is less than 1000 I will generally treat for 3-4 months, if 1000-2000 I treat for 4-6 months and if greater than 2000 6-8 months. I monitor ALT levels and if they become normal I often discontinue therapy, maintain on a low copper diet and will in some cases consider zinc supplementation as well. Ideally repeat liver biopsies with copper quantitation is the gold standard to direct therapy. Recently Cupramine has gone up in significant cost and therefore compounding formulations or DePen out of Canada is an option.

Antioxidant therapy is also indicated. Cu accumulation is thought to catalyze the formation of reactive free radicals causing membrane peroxidation. In another in vivo study using laboratory animals it was demonstrated that vitamin E therapy (d-alpha tocopherol) had protective properties against Cu hepatotoxicity. Vitamin E, a major membrane bound intracellular antioxidant, functions to quench membrane lipid peroxidation damage when free radicals are formed. Based on these preliminary studies it is suggested that vitamin E therapy may have a protective benefit in affected dogs with abnormal hepatic Cu concentrations and oxidative damage. A dose range of 100 to 400 IU of d-alpha tocopherol given daily is suggested. The supplement appears to be safe and free of side effects in this dose range. Other antioxidants or glutathione may also be beneficial however vitamin C has been shown to act as a pro-oxidant in the presence of increased concentrations of copper and should not be supplemented.
Summary
The abnormal accumulation of hepatic Cu can occur as a primary disease or secondary to cholestatic liver disease. Because of Cu’s both direct and indirect effects on hepatocellular morphology and function, attempts should be directed at depleting abnormal hepatic Cu by either blocking Cu absorption or through chelation therapy.

References