Copper Toxicity/Chronic Active Hepatitis

The trace mineral Copper (Cu) is incorporated into several enzymes which catalyze important reactions in the body. Cu is mainly stored in the liver, but is also found in the skeletal system (bone marrow) as well as in muscle, brain and spleen tissue. While unavailable dietary Cu is excreted in the feces, via bile, approximately 40-60% of dietary (ingested) Cu is absorbed into the blood by the mucosa of the small intestine. The serum proteins albumin and transcuprein then transport Cu in the blood to the liver, which is the site of synthesis of the iron-containing protein ceruloplasmin (CEP). CEP transports Cu to target tissues in the body, then returns to the liver. In the liver, CEP is degraded, resulting in Cu being lost in bile salts, which is a main route of excretion. Blockage of the bile ducts will lead to an accumulation of copper in the liver tissue. (Class notes, ANSC 604, Spring 1997).

Other copper-containing enzymes synthesized in the liver are: cytochrome oxidase, which functions in muscles and other tissues to reduce oxygen to water; superoxide dismutase (SOD) which degrades superoxide to yield peroxide and oxygen, also in muscles; and metallothionein. Metallothionein contains many cysteine residues which have sulfhydryl groups that allow it to bind heavy metals, especially copper and zinc. Copper bound to metallothionein is sequestered, thereby decreasing its absorption. Zinc intake regulates the synthesis of metallothionein, and therefore also the absorption rate of copper. Bile salts also inhibit absorption of Cu. Copper-containing enzymes in other parts of the body include dopamine- -hydroxylase in the brain, and lysyl oxidase in the connective tissue (Linder 1991).

Deficiency of copper causes multiple symptoms in animals, including depigmentation, bone problems, spinal cord paralysis and ataxia, cardiac failure, connective tissue abnormalities, and anemia. Deficiencies are more commonly seen than toxicities; however, toxicity of copper also has serious implications for the affected animal. In humans, Wilson's disease has been studied; in dogs, the analogous disease group is chronic active hepatitis (CAH). Both diseases affect the liver, the site of copper storage. The focus of this paper is the role of copper toxicosis in canine CAH.

Levels of copper and other metals stored in the body generally tend to be higher in newborns, decreasing as the individual matures. However, in some animals such as sheep, cattle, and dogs, this trend is not seen. Normal newborn canine hepatic copper concentrations are higher than those seen in mature humans, mice and rats (Keen 1981), and remain fairly constant over the lifetime of the individual (Thornburg 1985), unless the individual is suffering from CAH or some form of toxicosis. Mean concentrations of copper in the liver of normal dogs of any breed is 200-400 parts per million (ppm) on a dry weight (DW) basis (Keen 1984). Dogs with CAH may exhibit concentrations up to 10,000 ppm, with levels of 2,000 generally accepted as being toxic (Thornburg 1985).

There is some speculation as to why canine hepatic copper concentrations are so different from other species. Most likely, this is due to the fact that copper metabolism in dogs is different from other species, with regards to copper-binding proteins. Cu in the liver of dogs is associated not with metallothionein, as it is in other animals, but with other similarly-weighted proteins that have low cysteine concentrations (Keen 1981), making them less likely to bind copper. It has also been found that albumin in canine serum lacks the specific characteristic preferential binding site for Cu(II) that is found in human, bovine, and rat serum albumin (Appleton 1971). Since in other mammalian species albumin transports Cu, the reduced ability of canine albumin to do so presents a possible explanation for the dog's unusual metabolism of Cu. Although the dog has adaptive mechanisms --Cu is transported via gamma globulin in serum-- they may not be sufficient to maintain the efficiency of copper transport afforded by albumin (Appleton 1971).

The defined copper storage diseases that are known, are Wilson's disease in humans, and a similar disorder in Bedlington Terriers. The term "defined" in this case means that copper concentrations are increased up to fifty times normal (Crawford 1985). It is arguable whether other breeds of dogs suffer a storage disorder, such that copper toxicosis is the cause of CAH, or rather if the copper accumulation is a result of the CAH. In Bedlingtons, it is said that dietary copper accumulates in the liver until reaching toxic levels, at which time pathologic changes then develop. The belief is that a genetic defect in the hepatic lysosomal mechanism for copper excretion is responsible for the disease (Crawford 1985). Similarly, in Dobermans, it is suggested that copper toxicosis is a cause rather than a result of liver disease, but not the primary cause, since one of the cases presented demonstrated no copper accumulation at all (Franklin 1988).

CAH is actually a group of hepatic diseases with similar characteristics but different causes. It most often affects certain breeds of dogs, and has been well studied in Bedlington Terriers, West Highland White Terriers, and Doberman Pinschers. In some breeds, CAH is an inherited defect of metabolism involving biliary excretion (Bedlingtons), while in others the cause is not known to be linked to genetics but is thought to be related to a storage defect, perhaps influenced by diet (Dobermans). In Bedlingtons and West Highland White Terriers, an autosomal recessive copper-toxicosis (CT) gene has been linked to the Cu metabolism disorder. A test was developed to assist owners and breeders in identifying carriers of the CT gene earlier in life and without invasive measures. The test involves administering an intravenous dose of the 64Cu radioisotope, and measuring 64Cu excreted in the feces after 48 hours. The method, while not perfect, has been found effective and has enabled diagnosis and preventive treatment to begin before the dog becomes ill, when treatment is most effective (Brewer 1992).

In Dobermans, females of middle-age (approximately 7 years) are by far the most often affected, but the disease is also seen in males. One study suggested a genetic basis due to the high percentage of one breed affected with CAH, in the hospital population (Crawford 1985). Another author concurs, stating that a high frequency of CAH in one breed suggests a genetic basis (Johnson 1982). Further support of this theory is offered, in that the wide range of age of onset (2.5 to 11 years) may indicate the involvement of an environmental agent playing a role in the genetically predetermined host response (Johnson 1982).

Other reported possible causes of CAH in dogs are leptospire infections, adenovirus infections, and primidone therapy (Crawford 1985). A further proposed cause is an immune-mediated reaction from the infectious canine hepatitis virus (Franklin 1988).

Opinions regarding the influence of diet on copper accumulation are varied. One study purported that no evidence of dietary explanation of increased hepatic copper was found (Thornburg 1988). The same author in an earlier paper stated that "the disease is independent of diet; affected dogs accumulate copper when fed a diet that would not result in copper accumulation in a normal dog" (Thornburg 1985). This seems to contradict his previous assertion that "most cases of toxicosis are due to excess copper in the diet" (Thornburg 1983). He also reported that in cases where the dog is suspected to be genetically predisposed to Cu toxicosis, normal dietary levels of copper cannot be tolerated (Thornburg 1983). Su et al speculate that perhaps "commercial dog chows are so laden with copper that dogs are sequestering a continuous overdosage in their livers" (Su 1982).

Although the cause has not been definitively determined, CAH has been studied and much has been learned about its diagnosis and treatment. The disease is progressive, with copper accumulating in the hepatocellular lysosomes as the dog ages, reaching levels that are toxic, and resulting in damage to and eventual failure of the liver (Dill-Macky 1995).

There are three progressive stages of copper toxicosis. During Stage 1, the dog is not clinically ill, but copper levels are accumulating in the liver; values have been reported as high as 1,500 ppm DW (Thornburg 1985). Regardless of breed, the accumulation begins very early in the dog's life. The accumulation progresses at different rates in different breeds, and even differs among individuals within breeds. The copper begins to accumulate in the centrilobular hepatocytes in granules, with the band of granules gradually widening until the midzonal hepatocytes begin to fill as well (Thornburg 1985). The livers of dogs in Stage 1 present no clinical abnormalities, and biopsy at this time will indicate that things are normal. However, if liver tissue is stained, using the methods developed by Thornburg et al. (1985), increased levels of copper are revealed.

Stage 2 begins when the hepatic copper concentration reaches 2,000 ppm DW (Thornburg 1985). The dog is still not clinically ill, but a biopsy at this point will reveal hepatitis, and is the only reliable method of diagnosis. Biochemical and toxicologic findings including elevated serum enzyme levels (Alanine Aminotransferase [ALAT] and Alkaline Phosphatase [AP]) may be seen, but do not specifically indicate copper toxicosis: "Dogs with hepatitis only will have elevated ALAT, but hepatitis never causes clinical disease. The ALAT elevation merely reflects liver damage and is neither characteristic nor diagnostic of inherited copper toxicosis. Hepatitis does not alter the liver function tests" (Thornburg 1988). High ALAT and AP values indicate an episode of necrosis (Thornburg 1984). In a study of Dobermans with CAH, mean ALAT levels of 549 IU/L were seen, with normal being 15 to 50 IU/L, while AP levels were reported at a mean of 661 IU/L, with normal being 20 to 60 IU/L (Crawford 1985). A slightly earlier study, also with Dobermans, reported even higher values for these enzymes: ALAT of 3,045 IU/L (normal 18 to 70 IU/L); and AP of 2,650 IU/L (normal 12 to 70 IU/L) (Thornburg 1984). Increased bilirubin, hypoalbuminemia, anemia, prolonged prothrombin time, low platelet count, increased resting blood ammonia concentrations, raised serum copper content, and increases of both iron and copper content in the liver and kidney tissue were other findings noted in Dobermans, as well as thyroid abnormalities, abdominal effusion, microhepatica, and pleural effusion (Crawford 1985).

Finally, in Stage 3, the dog becomes clinically ill, and may have anorexia, depression, vomiting, abdominal pain, polydipsia, polyuria, icterus, jaundice, ascites, and encephalopathy (Dill-Macky 1995, Franklin 1988). Weight loss is the predominant indicator, and in some dogs, is the only sign. The clinical signs are usually the result of liver necrosis, triggered when copper concentrations exceed 2,000 ppm DW, causing centrilobular hepatocytes to die off and lyse (Thornburg 1985). This event in turn may cause connective-tissue stroma to collapse, leading to formation of ascites (Thornburg 1985). ALAT values are elevated, but then rapidly decrease following necrosis (Thornburg 1985). Bilirubin concentration, and amylase and lipase activities may also be elevated (Dill-Macky 1995).

Interestingly, the concentration of copper in the liver actually decreases after necrosis and repair occur. The cells that die during necrosis are the same ones that are the first to accumulate copper -- the centriolobular hepatocytes. As the cells die, copper is released into the blood and excreted in the urine. As hepatocytes are regenerated, and scar tissue is formed, the overall ppm DW concentration of copper is decreased, since neither of these new cell types contains copper stores (Thornburg 1986).

Because of the progressive nature of the disease, most affected animals are not presented for diagnosis and treatment until the late stages, when clinical symptoms appear. Treatment is mainly supportive, and prognosis is complicated by the fact that diagnosis and treatment come so late in the progression of the

disease. One study revealed that dogs with lower blood glucose levels and prolonged prothrombin time lived less than one week from examination and diagnosis (Dill-Macky 1995). Some Bedlingtons died after one episode of acute necrosis (Thornburg 1985) while most reported cases in Dobermans lived only a few months before succumbing to cirrhosis and liver failure (Cornelius 1989).

In instances where the copper accumulation was detected early on, when the dog was asymptomatic, and treatment began early on, affected individuals seemed to recover and live longer (Cornelius 1989). Some Bedlingtons are routinely biopsied and screened for copper accumulation, with biochemical profiles done annually (Thornburg 1984). Biochemical screening and liver biopsy may permit diagnosis and allow treatment to begin before irreversible damage has occurred (Johnson 1982).

Treatment of CAH may be dietary, medical, or a combination of these methods. Some treatment regimens for Bedlingtons involve a higher-level, "cleansing" dosage of medicine, or a routine, lower-level "maintenance" dosage, in an effort to rid the liver of excess copper and allow the dog a better chance of remaining symptom free (Thornburg 1984). In contrast to these findings, Franklin reported that in Dobermans, prognosis is very poor, since usual methods of medical treatment do not stop disease progression (Franklin 1988). The literature indicates that dietary methods have proved more successful in extending the lives of CAH dogs of this breed.

Dietary modifications suggested for management of dogs with CAH include providing a high quality protein source while lowering the overall level of protein fed. This will act to minimize hyperammonemia and abnormal amino acid ratios; however, if protein restriction is too excessive, it may impair hepatocyte regeneration (Dill-Macky 1995). Suggested protein sources include cottage cheese, eggs, and lean meat (Strombeck 1976, Franklin 1988). Other dietary modifications include providing an easily digestible source of carbohydrates, both to provide calories and to prevent catabolism (Dill-Macky 1995). It is further recommended that dietary fat intake be restricted, to reduce short-chain fatty acids which could contribute to encephalopathy (Hardy 1986).

Dietary supplementation recommended for long-term management of dogs with CAH includes providing a daily balanced vitamin (Dill-Macky 1995), to counteract the pyridoxine deficiency known to be caused by a commonly used drug treatment for CAH [penicillamine] (Thornburg 1984), and supplementation with vitamin C (ascorbic acid) which may help promote copper excretion in the urine (Cornelius 1989). Also, since ascorbic acid is poorly synthesized in dogs with CAH, additional vitamin C in the diet may help to prevent a deficiency (Cornelius 1989). Zinc supplements, in the form of zinc gluconate, may also help to reduce absorption of copper in the intestine (Cornelius 1989) by inducing the synthesis of metallothionein (Schilsky 1993) which would in turn act to bind copper, preventing its absorption. One report mentioned the use of vitamin K as part of

the dietary therapy used to treat the most severely affected of the dogs in the study group (Crawford 1985), presumably to help counteract the prolonged thromoboplastin time seen in these dogs (all Doberman Pinschers, a breed prone to bleeding disorders). Another study of CAH in Doberman Pinschers reported the use of a treament consisting of amino acid supplements and vitamin B12, but did not elaborate as to the reasoning for this particular supplementation (Franklin 1988). Presumably the amino acids would help to stabilize and correct the abnormal rations mentioned by Dill-Macky, while vitamin B12 would help to counteract the drug-induced vitamin deficiency mentioned above.

Medical treatments have two goals: to reduce absorption of copper, perhaps by sequestering it; and to enhance excretion of copper. To this end, chelating agents are commonly used. Chelates are a complex of an organic ligand (electron donor) and a metal (electron acceptor), in a ring structure formed by the positive-negative attraction of the electrons. Certain metals form stronger chelates than others, with Cu forming the strongest, and magnesium the weakest (Class notes, ANSC 604, Spring 1997). Chelates are important in the transport and storage of mineral elements in the body. Therapeutic use of chelating agents has been successful in detoxifying metals from target organs and bones. The chelating agent competes with binding sites in the body for complexing the metals, producing a water-soluble complex which is then excreted in the urine or bile (Kratzner 1986).

The most commonly used chelating agent in the treatment of canine CAH is d-penicillamine, which in addition to mobilizing copper from tissues and promoting its excretion in the urine, seems to increase the synthesis of metallothionein (Schilsky 1993). D-penicillamine also reportedly has anti-inflammatory, immunosuppressive, and antifibrotic effects (Cornelius 1989). Other chelating agents that have been used successfully in the treatment of CAH are trientine (Schilsky 1993) and 2,3,2-tetramine (Dill-Macky 1995).

Aside from chelating agents, CAH is sometimes treated with drugs aimed at reducing the inflammation and resolving hepatic fibrosis. Some commonly used drugs in the treatment of CAH in dogs include glucocorticoids, and corticosteroids, such as prednisone or prednisolone, which can have catabolic and immunosuppressive effects. However, despite the potentially harmful side effects, it has been reported that corticosteroid-treated dogs have a greater long-term survival than untreated dogs (Dill-Macky 1995). Liver enzyme levels may be altered by increasing or decreasing dosages of prednisone, but these results are not conclusive (Crawford 1985) since they are not necessarily indicative of high copper concentrations. Liver copper concentrations fluctuate as a result of the use of steroid drugs such as prednisone; concentrations first decrease dramatically in response to the drug --with greater decreases observed at higher dosages-- then increase again as liver failure develops (Crawford 1985).

Another drug with antifibrotic and anti-inflammatory properties is colchicine. Colchicine may play a role in collagen degradation, yet its benefit has been shown in dogs affected with liver problems. One study reported improvement in a dog treated with colchicine, when earlier treatments with diet modification and prednisolone had failed to produce a favorable response (Dill-Macky 1995).

In summary, while trace amounts of copper are necessary to maintain proper body function, excesses can contribute to the development of CAH in dogs and humans. The exact role of copper cause and effect, i.e. does excess Cu cause CAH, or is excess Cu a result of CAH, remains an unresolved and debated question in the literature. However, most of the studies indicate that CAH is a result of copper accumulation in liver cells. CAH progresses through three stages, culminating in clinical symptoms and usually eventual death of the affected dog. Because diagnosis is usually made in the late stages of the disease, when symptoms finally appear, treatments have a limited efficacy. Treatments center around providing dietary modification and supplementation, in addition to medical methods for removing excess copper from the body. Chelating agents and steroids are commonly used to assist the body in excreting excess copper while fighting inflammation and fibrosis of the liver. CAH cannot be cured; however, if detected in the early stages, treatment may begin immediately, affording the best opportunity to extend the dog's life significantly.

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