Presumed primary and secondary hepatic copper accumulation in cats

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Objective—To determine signalments, clinical features, clinicopathologic variables, imaging findings, treatments, and survival time of cats with presumed primary copper-associated hepatopathy (PCH) and to determine quantitative measures and histologic characteristics of the accumulation and distribution of copper in liver samples of cats with presumed PCH, extrahepatic bile duct obstruction, chronic nonsuppurative cholangitis-cholangiohepatitis, and miscellaneous other hepatobiliary disorders and liver samples of cats without hepatobiliary disease.

Design—Retrospective cross-sectional study.

Animals—100 cats with hepatobiliary disease (PCH [n = 11], extrahepatic bile duct obstruction [14], cholangitis-cholangiohepatitis [37], and miscellaneous hepatobiliary disorders [38]) and 14 cats without hepatobiliary disease.

Procedures—From 1980 to 2013, cats with and without hepatobiliary disease confirmed by liver biopsy and measurement of hepatic copper concentrations were identified. Clinical, clinicopathologic, and imaging data were compared between cats with and without PCH.

Results—Cats with PCH were typically young (median age, 2.0 years); clinicopathologic and imaging characteristics were similar to those of cats with other liver disorders. Copper-specific staining patterns and quantification of copper in liver samples confirmed PCH (on the basis of detection of > 700 µg/g of liver sample dry weight). Six cats with PCH underwent successful treatment with chelation (penicillamine; n = 5), antioxidants (5), low doses of elemental zinc (2), and feeding of hepatic support or high-protein, low-carbohydrate diets, and other hepatic support treatments. One cat that received penicillamine developed hemolytic anemia, which resolved after discontinuation of administration. Three cats with high hepatic copper concentrations developed hepatocellular neoplasia.

Conclusions and Clinical Relevance—Results suggested that copper accumulates in livers of cats as primary and secondary processes. Long-term management of cats with PCH was possible. (J Am Vet Med Assoc 2014;244:68–77)

Copper is an essential micronutrient and cofactor involved in the function of numerous enzymes and biochemical reactions because of its 2 redox states (Cu+ [reduced] and Cu2+ [oxidized]). A complex network of proteins regulates enteric uptake, systemic circulation and distribution, hepatic accumulation, and biliary excretion of copper; such mechanisms maintain copper balance for a broad range of dietary intake conditions.1,2

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Accumulated hepatic copper, especially Cu2+, is a potential cellular toxin that increases oxidant stress in hepatocytes. The oxidative impact of excessive intracellular copper compromises availability of glutathione and damages nucleic acids, proteins, and lipids.1,2 These effects interrupt the function of metabolic pathways, generation of energy, and structural components of cells.1,2

The most completely characterized primary copper storage hepatopathy is Wilson’s disease in humans, which is caused by > 300 mutations in the ATPase Cu2+-transporting ATPase β-polypeptide gene (ATP7B) that impair biliary copper excretion and cellular copper use.3 Specific mutations may affect copper sensing, copper-mediated trafficking, and copper transport, contributing to diverse disease phenotypes.3 Some humans with the disease are clinically affected at a very young age with severe hepatic copper accumulation, whereas...
Results of histochemical detection of copper in liver samples can indicate a primary causal role (eg, Wilson's disease) or an epiphenomenon of cholestasis. During PCH, the pattern of initial lobular accumulation of copper in liver differs between humans and dogs. In humans with Wilson's disease, copper first accumulates in the periporal region, then accumulates in the intermediate and centrilobular regions where liver injury leads to tissue remodeling (ie, hepatitis, regenerative nodules, formation, and cirrhosis). Conversely, in dogs with PCH, copper initially accumulates in the centrilobular region, then accumulates in intermediate and periportal regions. With the formation of regenerative nodules, deposition of fibrillary connective tissue, parenchymal extinction, and bridging fibrosis, the lobular pattern of distribution of copper becomes indistinguishable. In 2 cats with presumed PCH, copper was predominantly distributed in the centrilobular region of the liver in one but was panlobular in the other with marked architectural remodeling (ie, regenerative nodules and cirrhotic liver). Compared with humans, dogs seem to be resistant to hepatocellular copper accumulation as an epiphenomenon of cholestasis; however, the frequency of development and pattern of secondary hepatic copper accumulation in cats are not known. The authors and others investigators have detected copper accumulation in the periportal regions of livers in some cats with nonsuppurative cholangiohepatitis.

Unfortunately, a paucity of information is available regarding the pathological accumulation of copper in liver tissue in cats. The upper limit of the reference range for hepatic copper concentration in cats is < 180 µg/g of liver sample dry weight. In cats, copper concentrations in the liver, rather than other tissues or plasma, reflect dietary copper intake; results reflect copper concentrations in the liver, rather than other tissues or plasma, reflect dietary copper intake; results indicate that liver is the preferential site of copper accumulation in this species. In a recent retrospective study of the results of histologic examination of liver samples in another study; procedures in that study were approved by the Institutional Animal Care and Use Committee of Cornell University. Diagnoses of presumed PCH were made on the basis of detection of markedly high hepatic copper concentrations (> 700 µg/g of liver sample dry weight) with either diffuse copper granule distribution or marked centrilobular and intermediate zone copper accumulation without any additional cholestatic disorders. Diagnoses of EHBDO were made on the basis of findings of hyperbilirubinemia associated with ultrasonographically or visually confirmed major bile duct obstruction, distended irregular tortuous large bile ducts with mixed inflammatory periductal and intraductal infiltrates, circumferential periductal edema and fibrosis, and biliary epithelial hyperplasia. Diagnoses of CCHS were made on the basis of detection of lymphocytic or lymphoplasmacytic periportal and portal infiltrates with or without progressive small duct destruction. Cholecytisitis was diagnosed by means of detection of lymphoplasmacytic...
ic or neutrophilic inflammation in the lamina propria of gallbladder mucosa. Diagnoses of hepatic lymphosarcoma were determined on the basis of findings of monomorphic neoplastic populations of lymphocytes infiltrating the portal tract, perportal tissue, or hepatic sinusoids.\textsuperscript{15} Diagnoses of hepatic lipidosis were made on the basis of detection of more than 80% of hepatocytes with cytosolic expansion with lipid vacuoles.\textsuperscript{16} The discrimination of fat from glycogen-like vacuoles was determined on the basis of findings of mild lymphocytic infiltration and fibrosis of hepatitis indicates individual hepatocyte (piecemeal) necrosis can be detected in only the most peripheral portion of the limiting plate but without individual hepatocyte necrosis or destruction of the limiting plate.\textsuperscript{17} Interface hepatitis indicates individual hepatocyte (piecemeal) necrosis of the limiting plate accompanied by a variable degree of lymphocytic infiltration and fibrosis of the portal region that destroys the limiting plate.\textsuperscript{17} Diagnoses of portosystemic vascular anomaly were made on the basis of results of gross or ultrasonographic examination indicating a congenital portosystemic shunt and the typical features of portal hyopoperfusion and hepatic atrophy in liver biopsy samples. Diagnoses of ductal plate malformation (eg, polycystic and congenital hepatic fibrosis phenotypes) were made on the basis of findings of malformed bile ducts or small cystic biliary structures embedded in an expanded extracellular matrix without associated inflammatory lesions.\textsuperscript{18} Medical records of cats with and without hepatobiliary disease were reviewed to determine signalment (age, sex, and breed), clinical signs, clinicopathologic variables (CBC, serum biochemical analysis, urinalysis, FeLV antigen testing, and FIV antibody testing results), coagulation test results (prothrombin time, activated partial thromboplastin time, and fibrinogen concentration), and abdominal ultrasonographic findings. Abdominal ultrasonographic examinations were performed by board-certified radiologists or experienced small animal internists. For cats with presumed PCH, responses to copper chelation treatment and survival times were recorded.

**Statistical analysis**—Data were evaluated for normality with box-and-whisker plots and the Kolmogorov-Smirnov test. Age, clinicopathologic variables, and hepatic copper concentrations had non-Gaussian distributions and were therefore reported as median (range) values. Clinical variables (breed, sex, and clinical signs) and imaging findings were enumerated for cats in each diagnostic category and reported as proportions for each group; variables were then compared between groups in 2 × 2 tables. Data were tested for significance with a Fisher exact test; values of \( P < 0.05 \) were considered significant. Clinicopathologic variables of cats with presumed PCH were compared with those of cats with EHBDO, CCHS, and miscellaneous hepatobiliary disorders, first by use of a Kruskal-Wallis ANOVA to detect differences between groups, then by use of a Wilcoxon rank sum test to compare significantly different variables between cats with PCH and those with other diseases; a Bonferroni correction was applied (values of \( P < 0.017 \) were considered significant) to account for multiple comparisons. All statistical analyses were performed with commercially available software.\textsuperscript{7}

**Results**

The number, age, relevant clinicopathologic variables, and hepatic copper concentrations of cats in each diagnosis category were summarized (Table 1). The miscellaneous hepatobiliary disorders category included cats with the following diseases: hepatic lymphosarcoma (\( n = 9 \)), hepatic lipidosis (8), portal hepatitis (7), polycystic liver disease or ductal plate malformation (5), cholecystitis and choledolithiasis (4), suppurrative cholangitis (2), congenital portosystemic shunt (1), biliary hyperplasia without inflammation (1), and hepatic metastasis of pulmonary carcinoma (1). Cats without hepatobiliary disease included 10 healthy cats with hematologic and serum biochemical analysis variables within reference intervals that underwent liver biopsy as control animals in another study\textsuperscript{14} and 4 cats without histologic evidence of hepatobiliary disease (1 each with systemic lymphosarcoma, colonic adenocarcinoma, lipogranulomas without affiliated inflammation or hepatocyte necrosis, and inflammatory bowel disease). All cats tested for FeLV (\( n = 103 \)) had negative results. One cat in the miscellaneous hepatobiliary disorders group had positive results for FIV antibodies.

The 11 cats with presumed PCH were significantly (\( P < 0.001 \) for all comparisons) younger than other cats with or without hepatobiliary disease. There were 7 neutered males and 4 neutered females with presumed PCH, 8 neutered males and 6 neutered females with EHBDO, 21 neutered males and 16 neutered females with CCHS, 24 males (3 sexually intact and 21 neutered) and 14 females (1 sexually intact and 13 neutered) with miscellaneous hepatobiliary disorders, and 7 neutered males and 7 females (6 sexually intact and 1 neutered) without hepatobiliary disease. No sex predisposition was detected for any disease category (\( P > 0.3 \) for all comparisons). No significant breed predisposition was
were CCHS included weight loss (n = 24 [65%]), hyporexia 3 days to 6 months. Clinical signs for the 37 cats with duration of illness for cats with EHBDO ranged from argy (11), vomiting (7), and abdominal effusion (1); jaundice (n = 13), vomiting (7), and abdominal effusion (2), and diarrhea (2). Duration of illness prior to determination of a definitive diagnosis ranged from 3 weeks to 5 years for these cats. Cats with CCHS typically had chronically high transaminase (ALT and AST) or cholestatic (alkaline phosphatase and γ-glutamyltransferase) enzyme activities. The diverse types of disease processes in cats with miscellaneous hepatobiliary diseases and the absence of clinical signs in cats without hepatobiliary disease precluded comparison of clinical features for such cats with those for cats in other disease groups.

A significantly (P < 0.001) higher median hepatic copper concentration was found for cats with presumed PCH, compared with that for cats in all other diagnosis categories (Figure 1; Table 1). All cats with PCH had hepatic copper concentrations > 700 µg/g dry weight of liver sample. Eight cats with CCHS, 1 cat with EHBDO, and 1 cat in the miscellaneous hepatobiliary disorders group also had hepatic copper concentrations > 700 µg/g dry weight. All cats without hepatobiliary disease had hepatic copper concentrations < 190 µg/g dry weight; however, hepatic copper concentrations < 190 µg/g of dry weight were also detected for some cats with EHBDO (5/14), CCHS (7/37), and miscellaneous hepatobiliary disorders (23/38). Of the 15 cats with miscellaneous hepatobiliary disorders and hepatic copper concentrations > 190 µg/g of dry weight, 7 had portal hepatitis (2 of these cats had enteric lymphosarcoma), 3 had hepatic lymphosarcoma, 2 had severe ductal plate malformation, and 1 each had septic cholecystitis, suppurative cholangiohepatitis, and hepatic lipidosis.

Cats with presumed PCH had rhodamine-stained copper granules that were detected in the highest density in centrilobular liver regions but also in intermediate
and portal regions (Figure 2). The ability to identify the zonal distribution of copper in liver tissue was preserved in all cats except 1 cat with a hepatocellular carcinoma and 2 cats undergoing terminal diffuse liver necrosis. Cats with high hepatic copper concentrations secondary to cholestatic disorders had rhodanine-stained copper granules in portal and intermediate regions in close proximity to lymphocytic or lymphoplasmacytic infiltrates, near portal tracts expanded by biliary ductal hyperplasia and fibrosis, or associated with ductopenia. Cats with hepatic copper concentrations <190 µg/g of dry weight rarely had microscopically visible rhodanine-stained copper granules. When granules were observed for such cats, they had random zonal distribution.

Two cats with presumed PCH that had hepatic copper concentrations >2,000 µg/g of dry weight and 1 cat with CCHS that had a hepatic copper concentration >1,000 µg/g of dry weight had primary hepatocellular neoplasia; each of these cats had marked hepatic inflammation. Of those 2 cats with presumed PCH, one had hepatocellular carcinoma and the other hepatocellular adenoma. The cat with CCHS had hepatocellular adenoma. Additionally, 1 cat in the miscellaneous hepatobiliary disorder group had hepatocellular adenoma, but the hepatic copper concentration for that cat was only 66 µg/g of dry weight; this cat had no other hepatobiliary lesions. Digital scanning of rhodamine-stained sections of liver samples obtained from the cat with presumed PCH that had hepatocellular carcinoma allowed comparison of tissue copper concentrations between neoplastic and nonneoplastic regions; substantially higher tissue copper concentration was found in neoplastic tissue (8,369 µg/g of dry weight) than in adjacent nonneoplastic tissue (3,342 µg/g of dry weight; Figure 4).

No clinicopathologic variables could be used to reliably distinguish cats with presumed PCH from those with other disorders (Table 1). Cats with PCH had significantly (P = 0.002) higher median serum ALT activity, compared with cats with CCHS or miscellaneous liver disorders and cats without hepatobiliary disease, and significantly (P = 0.001) higher serum total bilirubin concentration, compared with cats without hepatobiliary disease. Cats with EHBDO had significantly higher serum γ-glutamyl transferase activity (P < 0.001) and total bilirubin concentration (P = 0.001) than cats with PCH. For cats with presumed PCH that underwent assessment of coagulation variables (8/11), marked prolongation (ie, time longer than the upper limit of the reference interval) of prothrombin time and activated partial thromboplastin time was detected for 2 cats with acute hepatic failure; prolonged prothrombin time was found for 2, and prolonged activated partial thromboplastin time was found for 1. Of the cats with EHBDO that underwent assessment of coagulation variables (12/14), 5 had prolonged prothrombin time or activated partial thromboplastin time; 3 of those 5 cats had prolonged times for both of these variables. Of the cats with CCHS that underwent assessment of coagulation variables (19/37), 1 had prolonged prothrombin time and activated partial thromboplastin time, 1 had prolonged prothrombin time, and 1 had prolonged activated partial thromboplastin time. Of the cats with miscellaneous hepatobiliary disorders that underwent assessment of coagulation variables (29/38), 3 (10%) had prolonged prothrombin time and activated partial thromboplastin time, 8 (28%) had prolonged prothrombin time, and 7 (24%) had prolonged activated partial thromboplastin time. No significant differences in development of prolonged prothrombin time or activated partial thromboplastin time were detected among groups.

![Figure 1](image1.png)

Figure 1—Graph indicating hepatic copper concentrations for cats with presumed PCH (n = 11), EHBDO (14), CCHS (37), or miscellaneous hepatobiliary disorders (38) or without hepatobiliary disease (14). The value of a hepatic copper concentration for a cat with PCH that is higher than the upper limit of the scale is indicated at the top of the graph.

![Figure 2](image2.png)

Figure 2—Photomicrograph of a section of a liver sample obtained from a cat with PCH demonstrating dense red-orange staining specific for copper in the centrilobular region. Notice the low density of granules with positive staining for copper in other liver regions (eg, portal tract in the left portion of the photomicrograph). Rhodanine stain; bar = 300 µm.
Abdominal ultrasonographic examinations were performed for 10 of 11 cats with presumed PCH, 14 of 14 cats with EHBDO, 33 of 37 cats with CCHS, and 36 of 38 cats with miscellaneous hepatobiliary disorders. Of the 10 cats with presumed PCH, liver size was determined to be normal in 9 and small in 1; hepatic nodules were detected in 4, and coarse parenchymal ultrasonographic texture was detected in 2. The hepatocellular carcinoma in 1 cat with presumed PCH measured 3.5 cm in diameter. Neither cat with hepatocellular adenoma (1 with presumed PCH and 1 with CCHS) had nodules detected by means of ultrasonographic examination. Two cats with presumed PCH had multiple small nodules (<3.5 mm) that were histologically determined to be regenerative. Ultrasonographically normal echogenicity of liver parenchyma was detected for 6 of 14 cats with EHBDO; the other 8 cats had hyperechoic liver parenchyma. Cats with hyperechoic liver parenchyma also often had numerous small (<1 cm) parenchymal nodules and scalloped liver margins; these findings suggested a nodular contour of the liver surface. Two cats with EHBDO had a small volume of abdominal effusion; fluid samples were not obtained for either cat. A distended tortuous common bile duct and cystic duct (commonly with a thickened wall) and enlarged gallbladder, with or without dilated intrhepatic bile ducts, were typically identified for cats with EHBDO. Five cats had choleliths (gallbladder \(n = 3\), common bile duct \(1\) and many small choleliths in intrhepatic bile ducts \(1\)). Five cats had dilated pancreatic ducts and a hypoechoic pancreas. Three cats had major bile duct obstruction associated with gallbladder fibrosis and fibrosing choleodochitis. Of 33 cats with CCHS, 12 (36%) had hepatomegaly, 11 (33%) had coarse parenchymal echogenicity, 8 (24%) had diffuse hyperechoic hepatic parenchyma, 6 (18%) had distinct parenchymal nodules, 3 (9%) had irregular liver margins, and 2 (6%) had abdominal effusion. Of 36 cats in the miscellaneous hepatobiliary disorders category, 13 (36%) had hepatomegaly, 1 (3%) had a small liver, 10 (28%) had hyperechoic hepatic parenchyma, 4 (11%) had coarse parenchymal echogenicity, 11 (31%) had parenchymal nodules (3 hypoechoic and miliary \(<3\) mm), 2 with single hypoechoic cystic lesions, 4 with polycystic malformations, 1 with hyperechoic nodules, and 1 with mass lesion confirmed to be hepatocellular adenoma), 3 (8%) had irregular liver margins, 3 (8%) had abdominal effusion, 9 (25%) had mesenteric lymphadenopathy, and 9 (25%) had small intestinal walls with a thickness >3 mm.

The gross appearance of the liver in cats with presumed PCH was variable and not useful for distinguishing this diagnosis from other causes of liver disease. Liver samples obtained from 9 of 11 cats with PCH were available for histologic examination by the authors. Descriptions of histologic features determined by board-certified veterinary pathologists were available for the other 2 cats; although these 2 cats had hepatic copper concentrations measured at the time of diagnosis, tissue blocks and slides had been discarded by personnel of the pathology service. The magnitude of hepatic inflammation was variable among cats with presumed PCH. Typically, multifocal mixed neutrophilic, lymphocytic, and histiocytic centrilobular hepatitis was identified. Varying
proportions of macrophages, lymphocytes, and plasma cells and few neutrophils were detected around central veins. Granulomatous foci were not observed. Eosinophilic cytoplasmic granules in zone 3 hepatocytes could commonly be identified in H&E-stained liver samples, but accurate assessment of the amount and distribution of copper could be made only by means of examination of rhodanine-stained sections. Copper accumulation seemed to be highest in centrilobular hepatocytes with variable involvement of hepatocytes in the intermediate and rare involvement of periportal hepatocytes. Severe diffuse hepatic necrosis was identified as the cause of death (determined by means of necropsy) for 2 cats. Interestingly, all cats with presumed PCH had hepatocyte vacuolation consistent with glycogen accumulation; this was confirmed for 1 cat by means of examination of periodic acid–Schiff–stained liver samples with and without amylase digestion. Glycogen accumulation was suspected on the basis of the finding of wispy cytosolic vacuoles and a central location of cell nuclei, in contrast to the discrete clear vacuoles characteristic of lipid accumulation; macrovesicular lipidosis often displaced the cell nucleus, but microvesicular lipidosis may not. The degree of glycogen-type vacuolar change ranged from mild to severe. Results of evaluation of Masson trichrome–stained liver samples confirmed deposition of fibrillar collagen in the periportal region with collagen tendrils extending along the space of Disse from central veins. Examination of reticulin-stained sections of liver samples of 7 cats with presumed PCH indicated mild parenchymal collapse around central veins. For 1 cat, remodeling with portal-to-portal and portal-to-central bridging fibrosis developed but was not accompanied by regenerative nodules.

Three cats in other diagnostic categories had centrilobular copper distribution in liver samples that was similar to the distribution for cats with presumed PCH. Liver sample copper concentrations in these cats were 522, 621, and 1,110 µg/g of dry weight. One of these cats had concurrent lymphoma and biliary cystadenoma that was diagnosed when the cat was 16.2 years old, 1 had mild lymphocytic portal hepatitis with microvesicular lipidosis that was diagnosed when the cat was 1.4 years old, and 1 had a choleodochal cyst and small duct destructive cholangitis that was diagnosed when the cat was 10 years old. Because 2 of these cats were substantially older than cats with presumed PCH, none had centrilobular hepatitis, and copper might accumulate in livers during other types of liver disorders, we determined such cats should not be allocated to the presumed PCH diagnosis category. These cats may have represented variations of the PCH phenotype, especially considering their high hepatic copper concentrations and accompanying diffuse hepatocellular vacuolation. Of the 11 cats with presumed PCH, that diagnosis was determined prior to death for 7 and after death for 4. Cats with PCH for which the diagnosis was determined after death developed acute hepatic failure at a young age (when they were 1, 1.4, 1.6, and 4 years old); 3 of these cats had no antecedent illness. One of these cats had acute hemolytic anemia associated with marked Heinz body formation and methemoglobinemia, seemingly attributable to severe diffuse hepatic necrosis and systemic release of copper. Presumed PCH was not identified during initial examination of liver samples for 2 cats but was identified during a second-opinion examination by personnel at the College of Veterinary Medicine at Cornell University.

Methods of management of cats with presumed PCH varied among clinicians. Treatments included various combinations of chelation by means of administration of penicillamine (n = 5; 10 to 15 mg/kg [4.5 to 6.8 mg/lb], PO, q 12 h) or elemental zinc (2 to 4 mg, PO, q 12 to 24 h), feeding of hepatic support diets, or a high-protein, low-carbohydrate diet, and administration of antioxidants (n = 5; eg, vitamin E [10 U/kg, PO, q 24 h], S-adenosylmethionine [20 mg/kg [9.1 mg/lb], PO, q 24 h], or S-adenosylmethionine [90 mg/cat] combined with silibinin-phosphatidylcholine [silybin A and B, 9 mg/ cat, PO, q 24 h]). Although dietary copper restriction was recommended for all cats, copper content in commercially available diets relevant to copper maintenance requirements for cats is not known. Prednisolone was administered to 2 cats to control hepatic and systemic inflammation. Of 5 cats that received penicillamine, 1 had long-term remission (duration of drug administration, 9.5 years). For another cat with PCH that received penicillamine (diagnosis determined when it was 3.6 years old), values of clinicopathologic markers of liver disease improved for 3 months, but the health status of that cat worsened after cessation of treatment. Hepatocellular carcinoma complicated that cat’s condition. One cat with PCH (diagnosis determined when it was 5 months old) had clinical remission with penicillamine treatment for 8 months at the last follow-up time; chelation treatment was combined with administration of a generic hepatic support formula (15 mg of silybin with 30 mg of phosphatidylcholine and 1 mg of elemental zinc/d PO) and feeding of a hepatic support diet. Another cat with PCH (diagnosis determined when it was 2.5 years old) developed hemolytic anemia 3 months after initiation of penicillamine administration (12.5 mg/kg [5.7 mg/lb], PO, q 12 h). After discontinuation of penicillamine administration, hemolytic anemia resolved during the subsequent 6 weeks; zinc sulfate (2.3 mg of elemental zinc in suspension/cat, PO, q 12 h) was subsequently administered to that cat to reduce enteric copper uptake. Interestingly, that cat’s owner’s property was discovered to have previously been the location of a copper mine. One cat had received penicillamine for only 1 week at the time of the last follow-up. Of the 4 cats with PCH that did not receive penicillamine, 1 (PCH diagnosis determined when it was 10.5 years old) received prednisolone (1 mg/kg [0.5 mg/lb], PO, q 24 h), ursodeoxycholic acid (15 mg/kg, PO, q 24 h), and vitamin E (10 U/kg, PO, q 24 h); that cat died when it was 13.2 years old because of progressive liver disease and pancreatic adenocarcinoma. Three cats with PCH (diagnosis determined when they were 0.7, 1.0, and 3.0 years old) were treated with hepatic support diets, S-adenosylmethionine, and silibinin for 1, 5, and 11 months, respectively, at the time of the last follow-up.

Discussion
In the present study, we used the term PCH to indicate hepatocellular damage resulting from patho-
logical copper accumulation attributable to either environmental exposure (ie, excessive amount of copper in food or water) or genetic factors. The term secondary copper-associated hepatopathy was used to indicate hepatocellular copper accumulation with concurrent or antecedent hepatic cholestasis or other pathological processes. Clarification of such terminology is important because excessive dietary copper intake in some dogs seems to cause PCH when copper accumulates and initiates tissue injury. We suggest that the term hepatocellular copper accumulation be used to describe the PCH syndrome. Despite high hepatic copper concentrations in animals with PCH, some affected dogs and cats lack necroinflammatory liver lesions at the time of initial detection of the problem.

Limited information has been published regarding PCH in cats; this lack of information may compromise identification and treatment of the disease in cats. Thus, the goal of this study was to determine clinical data for cats with PCH and information regarding copper accumulation in feline liver tissue. To the authors' knowledge, only 2 case reports of presumed PCH in cats have been published: 1 for a cat with acute liver failure and 1 for a cat with chronic cholestasis and abdominal effusion. Hepatic copper concentrations in those cats were approximately 4,000 μg/g of dry weight. Information in 2 other anecdotal reports of cats with suspected PCH has not helped characterize this syndrome. In 1 study regarding hepatic copper accumulation in cats with spontaneously developing hepatobiliary disorders, only qualitative characteristics of copper distribution but not quantitative measures of copper concentrations were determined. In that study, 140 liver biopsy samples of cats were examined; copper was identified by means of staining of tissue slides, with distribution determined for cats with stable copper (12 [11.5%]). However, in the present study, we quantitatively and morphologically characterized accumulation of copper in liver samples obtained from 100 cats with spontaneous hepatobiliary disorders and 14 cats without hepatobiliary disease. Findings for this diverse population of cats suggested characteristics of a clinical syndrome of presumed feline PCH and successful management strategies. Results also suggested that cats can accumulate copper in the liver secondary to other hepatobiliary disorders, particularly those with a cholestatic effect (ie, CCHS and EHBDO).

For the 11 cats with presumed PCH in the present study, that disease was typically identified when patients were young (≤2.0 years old); no breed or sex predispositions were identified that distinguished such cats from the overall hospital population. Detection of high serum liver enzyme activities (especially ALT) commonly led to further examination of cats and identification of this disorder. Although markedly high serum ALT activity and hyperbilirubinemia were commonly detected for cats, clinicopathologic variables could not be reliably used to distinguish cats with PCH from those with other hepatobiliary diseases. Similarly, results of abdominal ultrasonography could not be used to differentiate cats with PCH from those with other hepatobiliary diseases, with the exception of evidence of biliary tree obstruction or lesions consistent with disorders such as CCHS, cholelithiasis, or cholecystitis.

Hepatic copper concentrations without consideration of results of histologic examination of liver samples could not be used to differentiate cats with primary hepatic copper accumulation from those with secondary hepatic copper accumulation. Although cats with presumed PCH typically had hepatic copper concentrations >1,000 μg/g of dry weight (reference range, <190 μg/g of dry weight), 4 of 37 (11%) cats with CCHS and 1 of 14 (7%) cats with EHBDO also had hepatic copper concentrations >1,000 μg/g of dry weight. In contrast to humans with Wilson's disease, in whom copper initially accumulates in periportal regions, cats with presumed PCH first have hepatic deposition of copper in centrilobular areas followed by panlobular deposition. This centrilobular hepatic copper distribution in cats resembles the pattern of hepatic copper accumulation in dogs with PCH. In dogs with PCH, genetic factors and dietary copper overload can lead to hepatic copper accumulation, parenchymal damage, and development of dissecting fibrillar connective tissue. Multifocal small granulomas develop in areas of liver where copper-laden macrophages accumulate and neutrophils and lymphocytes aggregate in response to hepatocellular necrosis. Similar granulomas were not observed in cats with presumed PCH in this study. In addition to the centrilobular (progressing to panlobular) distribution of copper in livers of cats with presumed PCH, such cats also had glycogen-like vacuolation of cells similar to findings for dogs with vascular hepatopathy. Similar findings of a cellular vacuolar appearance have been found for 2 cats with presumed copper-associated hepatic disease. Identification of a discrete hepatic copper concentration cutoff value for determination of a diagnosis of presumed PCH in cats is difficult, as it is for dogs. Hepatic copper accumulation has been increasing identified for dogs, likely secondary to dietary copper supplementation since 1997, which has complicated selection of treatments. Rather than using a hepatic copper concentration cutoff value for selection of treatments, we advocate collaboration between clinicians and pathologists for determination of the importance of hepatic copper concentrations in consideration of the location of lobular injury, other concurrent liver injury or inflammation, and values of clinicopathologic markers of liver disease (especially serum ALT activity). For determination of a diagnosis of PCH for cats, we recommend consideration of not only the hepatic copper concentration (>700 μg/g of dry weight), but also identification of a centrilobular copper distribution and unique hepatocellular vacuolar change.

In contrast to cats with PCH in the present study, cats with cholestatic liver disorders accumulated copper in periportal hepatocytes adjacent to inflammatory infiltrates, with only small amounts of copper accumulating in centrilobular regions. This pattern of copper accumulation also was identified for some cats with ductal plate malformations and hepatic lymphosarcoma. These findings were similar to the histologic pattern of secondary copper accumulation in humans with cholestatic disorders (eg, drug-induced, ductopenic, neoplastic, and chronic necroinflammatory conditions associated with regenerative nodules and dis-
Cats with CCHS in this study that had accumulated large quantities of copper in hepatic parenchyma also typically had destructive cholangitis resulting in small duct dropout (ie, involution of small bile ductules) with large numbers of rhodamine-stained granules located in periportal hepatocytes adjacent to non supplicative inflammatory infiltrates. Whether cholangitis in cats with secondary copper accumulation might reduce hepatocellular injury is unknown, to the authors’ knowledge.

Responses of cats in this study with presumed PCH to treatments suggested that chelation for removal of copper may be indicated. However, 1 cat developed apparent drug-induced hemolytic anemia. Administration of antioxidants is recommended for PCH cats because of the pathological transition metal association with hepatic copper accumulation. Although restriction of copper intake is advisable, the level of dietary copper intake that should be recommended for cats is unknown. For gestating queens, a diet with 5.8 mg of copper sulfate/kg of food has been recommended to meet physiologic needs.24 Dietary copper concentrations in diets25–27 fed to several cats with PCH in the present study ranged from 0.14 to 0.50 mg/100 kcal (dry and wet diet formulations). Importantly, the form of copper in a particular diet determines its bioavailability (eg, copper oxide has lower bioavailability than copper chelates). Studies regarding the bioavailability of selected forms and amounts of dietary copper have not been conducted for determination of adult cat dietary maintenance requirements. To determine the effect of dietary copper in cats, it would be necessary to determine copper concentrations in liver rather than copper concentration in plasma or other organs.12,24 Measurement of plasma copper concentrations likely would have limited use as a diagnostic test or treatment-monitoring method for cats with PCH. This is consistent with the fact that hemolysis secondary to marked hepatocellular copper release and high plasma copper concentrations has only rarely been identified in Bedlington Terriers with copper-associated panlobular hepatic necrosis.23

In this study, hepatocellular carcinoma and adenoma were detected in 2 cats with PCH (hepatic copper concentrations, 2.792 and 2.183 µg/g of dry weight); similar findings have not been previously reported, to the authors’ knowledge. Interestingly, an association between high hepatic copper concentrations and hepatocellular carcinoma has been recently identified for humans with Wilson’s disease.9,26 and Long-Evans cinnamon rats that develop PCH.27 Long-Evans cinnamon rats with chronically high hepatic copper concentrations develop hepatocellular carcinoma as they age.27,28 Humans with Wilson’s disease that have a poor quality of medical management or late-age diagnosis have higher risk for development of hepatocellular carcinoma versus other patients with this disease.27,28

Results of this study determined characteristics of cats with presumed PCH and potential treatments based on the experience of multiple clinicians. Findings indicated that hepatic copper accumulation also may develop secondary to various liver disorders in cats and may contribute to liver injury.

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From this month’s AJVR

Evaluation of thermal antinociceptive effects and pharmacokinetics after intramuscular administration of butorphanol tartrate to American kestrels (*Falco sparverius*)

David Sanchez-Migallon Guzman et al

**Objective**—To evaluate antinociceptive effects and pharmacokinetics of butorphanol tartrate after IM administration to American kestrels (*Falco sparverius*).

**Animals**—Fifteen 2- to 3-year-old American kestrels (6 males and 9 females).

**Procedures**—Butorphanol (1, 3, and 6 mg/kg) and saline (0.9% NaCl) solution were administered IM to birds in a crossover experimental design. Agitation-sedation scores and foot withdrawal response to a thermal stimulus were determined 30 to 60 minutes before (baseline) and 0.5, 1.5, 3, and 6 hours after treatment. For the pharmacokinetic analysis, butorphanol (6 mg/kg, IM) was administered in the pectoral muscles of each of 12 birds.

**Results**—In male kestrels, butorphanol did not significantly increase thermal thresholds for foot withdrawal, compared with results for saline solution administration or the baseline value. However, at 1.5 hours after administration of 6 mg of butorphanol/kg, the thermal threshold was significantly decreased, compared with the baseline value. Foot withdrawal threshold for female kestrels after butorphanol administration did not differ significantly from that after saline solution administration. However, compared with the baseline value, withdrawal threshold was significantly increased for 1 mg/kg at 0.5 and 6 hours, 3 mg/kg at 6 hours, and 6 mg/kg at 3 hours. There were no significant differences in mean agitation-sedation scores, except for males at 1.5 hours after administration of 6 mg/kg.

**Conclusion and Clinical Relevance**—Butorphanol did not cause thermal antinociception suggestive of analgesia in American kestrels. Sex-dependent responses were identified. Further studies are needed to evaluate the analgesic effects of butorphanol in raptors. (Am J Vet Res 2014;75:11–18)